CHAPTER TWO

ASTROCYTES IN THE AMYGDALA

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Abstract
The amygdala has received considerable attention because of its established role in specific behaviors and disorders such as anxiety, depression, and autism. Studies have revealed that the amygdala is a complex and dynamic brain region that is highly connected with other areas of the brain. Previous works have focused on neurons, demonstrating that the amygdala in rodents is highly plastic and sexually dimorphic. However, our more recent work explores sex differences in nonneuronal cells, joining a rich literature concerning glia in the amygdala. Prior investigation of glia in the amygdala can generally be divided into disease-related and hormone-related categories, with both areas of research producing interesting findings concerning glia in this important brain region. Despite a wide range of research topics, the collected findings make it clear that glia in the amygdala are sensitive and plastic cells that respond and develop in a highly region specific manner. © 2010 Elsevier Inc.
I. THE AMYGDALA

Located in the temporal lobes, the cluster of nuclei comprising the amygdala is involved in several important behaviors, including social behavior, fear, and anxiety (Adolphs et al., 2005; Bishop et al., 2004; LeDoux, 1998; Mah et al., 2007; Spezio et al., 2007; Truitt et al., 2007; Williams et al., 2006). Tract-tracing work along with recent resting-state fMRI data has demonstrated that the amygdala is reciprocally connected via direct and indirect connections to important homeostatic and cognitive centers such as the hypothalamus, hippocampus, and much of the sensory cortex (Kilpatrick et al., 2006). Given these extensive connections, it is not surprising that the amygdala has been implicated in many behavioral disorders and diseases, including schizophrenia, drug addiction, anxiety disorders, depression, and autism.

Much of the knowledge regarding amygdala function stems from its role in fear and anxiety. Conditioning experiments have identified the amygdala as crucial to the fear response, and additional methodologies have mapped the amygdala’s expanded role in emotional memory and motivation (Bechara et al., 1995; Cahill, 2000; reviewed in Cardinal et al., 2002). Within these fields, it is generally acknowledged that cellular plasticity within the amygdala plays a vital role in shaping behavior. This plasticity has most often been investigated in the context of long-term potentiation (LTP) associated with fear conditioning (Rogan and LeDoux, 1995; Yu et al., 2008). However, neuroendocrine researchers have also been intrigued by amygdala plasticity because of the region’s dense concentrations of steroid hormone receptors (Simerly et al., 1990), its well-documented collection of functional and morphological sex differences (Cahill et al., 2004; Hines et al., 1992; Johnson et al., 2008; Kilpatrick et al., 2006; Rubinow and Juraska, 2009), and its responsiveness to circulating gonadal hormones throughout the lifespan (Cooke and Woolley, 2005; Cooke et al., 1999, 2003; Martinez et al., 2006; Morris et al., 2008). These characteristics strongly suggest that the sex differences seen in amygdala anatomy may underlie sex biases in behavior and disease susceptibility, and that amygdala plasticity may be a key player in the regulation of such attributes.

The exploration of amygdala anatomy has mostly been with rodent models although feline and nonhuman primates have contributed significantly (Rees and Michael, 1984; reviewed in McDonald, 1998). In common laboratory species and in humans, the amygdala is divided into central, medial, cortical, lateral, basal, and accessory basal divisions which are strongly interconnected, although the direction and strength of these connections does not appear uniform among the subregions (Alheid et al., 1995; Grove, 1988a,b; Shammah-Lagnado et al., 2000).
Plasticity within the various amygdala subregions and in the amygdala as a whole has been examined through many paradigms and has been covered in numerous reviews (Cooke, 2006; Sah et al., 2008; Samson et al., 2005; Kinsley and Lambert, 2008). The human literature has examined plasticity in amygdala volume and functionality (Giedd et al., 1997; Killgore and Yurgelun-Todd, 2004) and the rodent literature has repeatedly demonstrated plasticity of amygdala neurons based on fear-related LTP (reviewed in Sigurdsson et al., 2007, see also Sah et al., 2008) and by manipulating hormones (Cooke et al., 1999; Morris et al., 2008). Undoubtedly, the examination of neuronal plasticity in the amygdala, whether in the context of hormones or LTP, has greatly expanded our understanding of the function of this region. However, recently another component of amygdala anatomy, glial cells, has been explored, which may fill many of the gaps in our understanding of this dynamic region. This review examines the current findings concerning glia in the amygdala.

II. Glia

As in most of the central nervous system, several types of glia are present in the amygdala, including astrocytes, oligodendrocytes, and microglia. However, much of the exploration of glia in the limbic system has focused either on astrocytes specifically or on glia in general. Thus, reports examining microglia or oligodendrocytes in the amygdala are rare, but these cells are undoubtedly included in counts of total glial within the amygdala. This circumstance makes attributing specific effects or phenomenon to specific glial subtypes sometimes difficult, but perhaps future investigations will alleviate some of this confusion.

Astrocytes are perhaps the most thoroughly investigated glia subtype in the central nervous system and certainly in the amygdala. Astrocytes were first described in detail in 1895 when Cajal gave one type of glia the name “astrocytes,” based upon their extensive star-like arbors. Cajal (1909) suggested that astrocytes were functioning to insulate neural connections while Golgi (1885), who was also investigating glia, proposed that astrocytes provide a link between neurons and the blood supply based upon their predisposition to contact blood vessels. Unfortunately, after these initial descriptions and hypotheses, astrocytes were mostly ignored. It was not until the identification of the astrocyte inflammatory response that these cells once again received attention.

Numerous reports demonstrate that astrocytes rapidly respond to all manner of brain trauma, including injury, disease, and genetic disorders with a rapid synthesis of glial fibrillary acidic protein (GFAP) at the site of
injury. This reliable, complex response has been carefully examined and reviewed in detail (Eddleston and Mucke, 1993; Eng and Ghirnikar, 1994). The information derived from studies of reactive gliosis has been vital in understanding brain injury and should not be minimized, but as Cotter et al. (2001) described it, “glia were typecast, their crucial role in other cortical function overlooked.”

Fortunately, the past two decades have seen a dramatic increase in attention to glia. Much of the interest stems from findings that astrocytes exhibit rapidly propagating calcium waves, suggesting that they may form a second path of communication working in conjunction with the neuronal system (Guthrie et al., 1999). More recent work suggests that propagating calcium waves are extremely rare in vivo (Wang et al., 2006), but astrocytes are still considered active and dynamic cells. Mature astrocytes generally have large arborsh extending from their cell bodies, and in the cortex, it has been estimated that each astrocyte connects on average to approximately four neurons, 300–600 dendrites, and 1,000,000 synapses (Halassa et al., 2007). Astrocyte processes are closely involved in the formation of new synapses and these processes are highly mobile and extend or retract to modulate contact between neurons (Hatton, 2002; Nishida and Okabe, 2007; Ullian et al., 2001). Interestingly, increased branch complexity in astrocytes has been associated with an increase in functional synapses in some regions (Elmariah et al., 2005; Pfrieger and Barres, 1997) but has also been associated with decreases in dendritic spines (Mong et al., 2001). Thus, it may be that astrocytes have opposing roles under different conditions, promoting and maintaining synapses in some cases, while promoting their elimination in others. In many ways, a dramatic sensitivity to varying conditions has become the hallmark of glia, and their responsiveness to various disease states, which we review next, clearly illustrates this point.

III. Amygdala Glia and Disease

Glia are known to be important metabolic factories for normal brain functioning, responding quickly and topographically to stimuli with rapid increases in metabolic activity (Schummers et al., 2008). The nature of the glial response to disease has been puzzling, with long-standing questions about whether this response is beneficial and neuroprotective (Thippeswamy et al., 2005; Yamamuro et al., 2003) or is actually detrimental to repair (Mukhopadhyay et al., 1994; also see Mena and García de Yébenes, 2008). Furthermore, in several of the diseases and disorders, we describe next, it is not at all certain if the effects seen in glia are merely a response to disease or a contributing cause. As in most cases, that dichotomy may be an illusion, with glia likely playing both roles at various times and in
various instances. However, what is clear is that the extreme sensitivity of glia to brain dysfunction makes them powerful indicators of abnormal brain function and has guided the examination of amygdala glia in several disease models.

A. Epileptic states

Investigation of patients with temporal lobe epilepsy demonstrates glial satellitosis, the accumulation of glia around neurons, in the lateral amygdala (Faber-zuschratter et al., 2009) and interesting glial phenomena have been detailed in the mouse amygdala as well. Kainic acid, an excitotoxin regularly used to investigate the brain’s response to seizure-like conditions, induces several responses in glia in rodents. After kainic acid injection, microglia become immunopositive for cyclin-dependent kinase 4 and cyclin D1, important regulators of apoptotic cell death (Ino and Chiba, 2001). In amygdala astrocytes, kainic acid injection results in prolonged expression of metallothioneins, a family of proteins involved in neuroprotection (Kim et al., 2003). The apolipoproteins, a family including apolipoprotein E, are primarily expressed in astrocytes under normal conditions. Grootendorst et al. (2000) found, however, that in cases when damage induced by kainic acid extended beyond the injection site into regions such as the amygdala, apolipoprotein E expression was also found in neurons. In cases of mild damage, this effect was not seen, but increases in GFAP-immunoreactivity and microgliosis were seen. The authors conclude that in severe cases, damage to the glial network is large enough to allow accumulation of apolipoproteins from damaged glia in the surrounding neurons, while in mild cases the reactive glia response may ameliorate this outcome (Grootendorst et al., 2000).

Amygdala-kindled seizures also promote astrocyte proliferation in the piriform cortex of rats (Vessal et al., 2004), and kindling of the olfactory bulb, a major source of input for the amygdala, results in astrocyte proliferation in the basolateral amygdala in addition to the piriform cortex (Woldbye et al., 1996). These two studies together suggest that over stimulation of the amygdala, be it through direct kindling or though kindling of a primary input source, can have long-reaching effects on other parts of the brain. The authors suggest that astrocytes become activated in response to the formation of a new synaptic pathway brought on by the hyperexcitability of the epileptic discharge and conclude that astrocytes may support the formation of the kindling pathway (Vessal et al., 2004, 2005). Jung et al. (2009) also found amygdala astrocyte proliferation in a lithium–pilocarpine injection model, and surgical specimens from patients suffering from temporal lobe epilepsy demonstrate an inverse correlation between the extent of astrocytic–reactive gliosis and inhibitory synapses on GAD–positive projection neurons (Yilmazer–Hanke et al., 2007). Again,
these results suggest that astrocyte activity may serve to promote a “rewiring” of seizure sensitive regions.

Astrocytes in the piriform cortex become immunoreactive for nestin, an embryonic intermediate neurofilament protein, after amygdaloid kindling, providing further evidence that astrocyte morphology is affected by seizures (Umeoka et al., 2001). Relatedly, while astrocyte reactivity in the hippocampus and amygdala after amygdala–kindled seizure was equivalent in S-100β knockouts compared to controls, the knockout animals kindled more rapidly and exhibited more severe seizures, suggesting changes in astrocytic structural proteins are related to seizure progression (Dyck et al., 2002). However, as S-100β is expressed only in a subtype of adult astrocytes around blood vessels (Hachem et al., 2005), only some astrocytes would be involved. Investigators have also begun to explore whether astrocytes help to preserve neurons after seizures. Vascular endothelial growth factor (VEGF) protects neurons from cell death (Newton et al., 2003), and astrocytes in the amygdala upregulate VEGF protein expression approximately 24 h after seizure induction. This VEGF upregulation in astrocytes may benefit surrounding neurons (Nicoletti et al., 2008).

Finally, seizure-like states are also examined for their beneficial effects. Electroconvulsive seizures are used for the treatment of major depression, and Wennström et al. (2004) examined Wistar rats given five electroconvulsive treatments and injected with bromodeoxyuridine (BrdU) to monitor cell proliferation. As the seizure literature would predict, the authors report a proliferation of glial cells in the amygdala after electroconvulsive treatment. Specifically, the most dramatic increase was in oligodendrocytes, a proliferation that lasted 3 weeks after treatment and led to the establishment of new mature oligodendrocytes. Interestingly, this effect was region and cell-type specific with oligodendrocyte proliferation being found in the central, lateral, and basal subregions and microglia proliferation found in the medial subregion. The authors speculate that the formation of these new cells may play a role in regulating synaptic function (Wennström et al., 2004).

B. Depression

As the previous report suggests, glia are also implicated in major depressive disorder and bipolar disorder. For example, social exploratory behavior is sometimes used as a model of depression, and Lee et al. (2007) found that blocking glutamate uptake in the amygdala resulted in a dose-dependent reduction in social exploratory behavior and altered circadian activity patterns, reminiscent of depressive states in humans. Importantly, selectively blocking glutamate uptake by astrocytes with dihydrokainic acid in the basolateral amygdala also reduced social exploratory behavior, an effect that could be reversed by simultaneous injection of the NMDA receptor

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antagonist AP5. These results led the authors to conclude that impaired uptake and metabolism of glutamate by astrocytes may be critically involved in depression (Lee et al., 2007).

Gliaal numbers in the amygdala are also affected in people with major depressive disorder. Bowley et al. (2002) examined the amygdala from patients with major depressive and bipolar disorder postmortem and found that glia density and glia/neuron ratio were greatly reduced compared to controls. Interestingly, this reduction was mostly seen in the left hemisphere and was not due to a change in the number of neurons. This reduction in glia was also found in untreated bipolar cases but not in cases treated with either lithium or valproate, suggesting these medications may reduce the loss of glia in the amygdala (Bowley et al., 2002). While a 4-week lithium treatment of adult males rats reduced oligodendrocyte proliferation compared to controls (Orre et al., 2009), the authors note that they did not look for effects of lithium on the survival of cells.

Since the identification of reduced glial numbers in the amygdala in major depressive disorder, other reports have examined this phenomenon in more detail. One study examined the expression of glial markers in the basolateral amygdala in Wister Kyoto (WKY) rats, often used as a model for anxiety and depression due to their exaggerated responses to stressors and depressive-like performance in behavioral tests (Armario et al., 1995; De La Garza and Mahoney, 2004). Gosselin et al. (2009) found a significant deficit in the number of GFAP-immunopositive cells (a common marker for astrocytes) in the basolateral amygdala of WKY rats compared with Sprague-Dawley control animals, a result confirmed by Western analysis of GFAP expression levels. However, the use of a second astrocyte marker, S–100β, revealed no differences in expression between controls and WKY rats. Furthermore, while in control animals GFAP and S–100β were colocalized, in WKY rats a population of S–100β-positive cells was found that were devoid of GFAP immunoreactivity. This led the authors to suggest that in the WKY model, GFAP expression by astrocytes is altered, not the number or density of astrocytes per se (Gosselin et al., 2009).

Another report found similar results using a maternal deprivation paradigm, a model of early-life stress, which is a major risk factor in the etiology of depressive disorders. The maternally deprived animals exhibited a significant reduction in GFAP-immunoreactive astrocyte density in the basolateral amygdala and several other brain regions. This reduction in immunoreactivity was greater than the overall reduction in cell density, which suggests, as in the previous report, that GFAP expression, rather than the number of astrocytes, was altered in this animal model (Leventopoulos et al., 2007). However, it is also clear that early stress can alter cell numbers in the amygdala. Using a prenatal stress paradigm, Kraszpulski et al. (2006) found altered glial numbers in different amygdala subregions at different developmental time points. For example, the number of glia in the lateral
Amygdala nucleus was reduced in prenatally stressed animals at postnatal day 7 only, whereas glia numbers were reduced in the basolateral and central nuclei at postnatal day 25. Neuron number exhibited the same pattern and the authors speculate that these reductions in cell number may underlie the enhanced anxiety seen in prenatally stressed animals in adulthood (Kraszpulski et al., 2006). Unfortunately, how reductions of GFAP expression and/or reduced glia number in the amygdala relate to depression is still unclear.

Despite several reports that astrocytes are affected in the amygdala of both human cases and in animal models of major depressive disorder, other evidence suggests that astrocytes may not be altered during depression. Hamidi et al. (2004) labeled tissue from patients diagnosed with major depressive disorder for S-100β, human leukocyte antigen, and stained for Nissl. Using S-100β, the group reported no significant difference in astrocyte density in depressed patients compared to controls. Moreover, human leukocyte antigen was used to identify microglia and revealed no difference. However, oligodendrocyte density (identified by their compact deeply stained nuclei in Nissl staining) was significantly lower in this population of depressed patients. The authors concluded that the reduction of glia previously reported in the amygdala of patients suffering from major depressive disorder was due to a reduction in oligodendrocytes. These results are difficult to interpret because Gosselin et al. (2009) found a dissociation of S-100β and GFAP-labeled cells in their WKY rat model of depression. Perhaps, if Hamidi and colleagues had also utilized GFAP immunoreactivity as their astrocytic marker, they might have seen a reduction in the number of immunopositive astrocytes as well. So, it is possible that both astrocytes and oligodendrocytes are reduced in the amygdala of depressive disorder cases. Adding to the complexity is an interesting report examining S-100A1, a protein closely related to S-100β that also colocalizes with GFAP. S-100A1 knockout mice appear to develop normally and show no obvious differences in brain development. However, S-100A1 knockout males exhibit reduced anxiety based on an approach–avoidance paradigm (Ackermann et al., 2006). Reduced anxiety in knockouts suggests S-100A1 may somehow facilitate fear and anxiety, two behaviors often elevated in models of major depressive disorder. Regardless of what changes occur to amygdala glia, a clear cause–effect relationship remains elusive, and the question of whether changes in glial numbers in the amygdala are a response to or a cause of depression remains unanswered (see Hercher et al., 2009).

C. Proteinopathies

Glia are also being investigated in other diseases. In Parkinson’s disease, α-synuclein fibrils aggregate and eventually lead to cellular dysfunction, and the presence of α-synuclein-immunoreactive inclusions in neurons has been an important disease marker in research on this disease...
Astrocytic α-synuclein-immunoreactive inclusions have also been described in the human amygdala (Terada et al., 2003), and these astrocytic inclusions appear in the amygdala at stage 3 of the disease, after their initial appearance in neurons, and their immunoreactivity increases in the latter stages of the disease (Braak et al., 2007). In fact, astrocyte inclusion bodies generally follow the progression of neuronal inclusions throughout the brain, including the amygdala, as the disease progresses (Braak et al., 2007). Astrocytes are not thought to produce α-synuclein themselves, so they may take up the altered α-synuclein molecules from afflicted neurons, likely explaining why glia are also eventually affected by disease. Whether these α-synuclein-immunoreactive glia influence the progression of disease symptoms is unclear, but based upon how astrocytes respond to trauma in the undiseased brain, diseased astrocytes in Parkinson’s disease may cause a loss of neuronal connections, effectively severing the amygdala from other brain regions in the latter stages of the disease (Braak et al., 2007). Given that α-synuclein-immunoreactive astrocytes and astrocytes positive for other disease markers (tau and ubiquitin inclusion bodies) have been found in the amygdala of non-Parkinson’s patients who were diagnosed with diffuse neurofibrillary tangles with calcification (DNNTC) dementia (Hashimoto et al., 2003; Yokota et al., 2002), this disease may share pathophysiological mechanisms with Parkinson’s and other proteinopathies (Yokota et al., 2002; also see Jellinger, 2008).

Glia also appear to be affected in Alzheimer’s disease (AD), and astroglialosis in the amygdala has been reported in many AD cases (Brockhaus, 1938; Corsellis, 1970). Scott et al. (1992) investigated the cortical and basal amygdala subregions in brains of AD patients postmortem. They counted glia, dividing them into two categories: large, which the authors presumed to be astrocytes, and small, presumed to be oligodendrocytes and microglia, based on size. When examining glial cell density (cells per mm²), they found that the overall glia density was greater in AD cases in both the basal and cortical regions. However, this was attributable mostly to increased density of large glia. When the data were corrected for structural atrophy, total numbers of glia were reduced in AD cases in both size categories in the basal amygdala. In the cortical region, only the number of small glia was reduced (Scott et al., 1992). Additional reports found an increase in the number of astrocytes expressing peroxiredoxin 6, an antioxidant enzyme, in the amygdala of AD cases compared to controls. Peroxiredoxin 6 staining, which is not found in oligodendrocytes or microglia and only at very low levels in neurons, was found mostly in astrocytes associated with amyloid-β plaques, suggesting the plaques produce reactive oxygen species via astrocytes (Power et al., 2008). Clearance of plaques in AD may not be the responsibility solely of astrocytes, however. Kaku et al. (2003) found that a mutant mouse (osteopetrotic mice), known to have reduced number of microglia, also have greater fibrillary plaques in the amygdala and other regions
compared to controls, suggesting microglia regulate plaque formation and/or clearance. Additional reports confirm that activated microglia follow plaque formation in the amygdala, and activated astrocytes form around these plaques later on (Dudal et al., 2004).

One difficulty in measuring astrocyte proliferation in any age-related disease such as AD is the well-documented increase in GFAP production as the brain ages (Kohmana et al., 1995; Linnemann and Skarsfelt, 1994; Schipper, 1996). Interestingly, this increase in GFAP can be attenuated in several brain regions, including the amygdala, by systemic administration of the steroid pregnenolone in aging animals (Legrand and Alonso, 1998). This result implies that reduced hormone levels during aging may cause widespread increases in GFAP production, and this may contribute to the increased incidence of many neural disorders with age. This finding might not be surprising because pregnenolone is a precursor to several other steroid hormones, and glia are known to be highly sensitive to steroid hormones as discussed below.

D. Other pathologies

In addition to what is known about glia in epilepsy, depression, and various proteinopathies, glia also figure into other areas of disease and disorder research. For example, myo-inositol, a glial marker and second messenger in intracellular calcium regulation, is reduced in the amygdala of narcoleptic patients compared to controls, suggesting glia involvement in sleep disorders (Poryazova et al., 2009). Furthermore, Yokota et al. (2008) found several interesting glial abnormalities in the amygdala during their investigation of cases of neurofibromatosis type 1, a disease in which nervous tissue grows to form tumors. Yokota and colleagues conclude that the glial clusters and satellitosis may be due to altered astrocyte growth regulation. Finally, interleukin-β, which is released during infection and is responsible for inducing fever and behavioral changes, promotes the phosphorylation of Erk1/2 in astrocytes in the amygdala and other regions (Nadjar et al., 2005).

These reports suggest that glia in the amygdala may be involved in, or at least be a sensitive indicator of, a broad range diseases and disorders. Due to their sensitivity and prominent role in maintaining brain homeostasis, the use of glia as indicators of neurological trauma has great potential for disease research. Furthermore, given their role in the formation of synapses and facilitation of neuronal communication, exploration of glia in disease models may lead to novel treatments focusing on restoring lost communication pathways.
Klüver and Bucy (1939) were perhaps the first to identify the amygdala’s involvement in sexual behavior. Given that sex is intimately linked with gonadal hormones, neuroendocrinologists have been investigating how gonadal hormones shape this region both in adulthood and during development. This line of research has revealed an array of sexual dimorphisms within the amygdala (reviewed in Hamann, 2005; Stefanova and Ovtscharoff, 2000) and these sex differences have important implications beyond sexual behavior, as most of the diseases associated with the amygdala, such as depression and autism, exhibit strong sex biases in the population. While it is abundantly clear that gonadal hormones are key components in the early establishment of sex differences and of CNS plasticity, the mechanisms behind hormone-induced alterations in neuroanatomy are still not clear. Glia are responsive to gonadal hormones (Mong et al., 1999, 2001) and investigators are now asking how glia may be involved in the ontogeny of sex differences, and may mediate hormone-induced plasticity in adulthood. This line of questioning is beginning to provide a much more detailed and rich picture of the amygdala.

The evidence for gonadal hormone influence on neurons is well established (reviewed in Woolley, 2007). However, glia also respond to gonadal hormones signals. Estrogen receptors are upregulated in reactive astrocytes after injury (Blurton-Jones and Tuszynski, 2001) and estrogen influences astrocyte morphology in the hypothalamus (Mong et al., 1996), hippocampus (Milner et al., 2000), and in primary culture (Garcia-Segura et al., 1989). Estrogen appears to influence glia in the amygdala as well. The scaffold protein, MNAR/PELP1, is important coactivator in estrogen’s nongenomic activity and has been found in glia in the amygdala (Khan et al., 2006), suggesting glia may respond directly to estrogen through nongenomic means.

### A. Gonadal hormones and amygdala glia during developmental

Since the initial discovery gonadal hormone-sensitive glia, neuroendocrine research has begun to explore glia–hormone interactions across the lifespan, and evidence suggests that hormones may influence glia in the amygdala early in development. For example, a single dose of estrogen during neonatal development increased the number of proliferating glia in the basolateral nucleus 3 days later in male rats but not in females (Dmitar et al., 1995). A slightly more complex pattern of results was found in the medial, cortical,
and central nuclei. In estrogen-treated male rat pups, the percentage of BrdU-labeled glia was increased in all three nuclei, based upon light microscopy identification, whereas in females, estrogen increased the number of labeled glia only in the medial nucleus, while decreasing their numbers in the cortical nucleus and having no effect in the central nucleus (Drekic et al., 1995). Glia also appear responsive to hormones during puberty, another critical period in development. Using BrdU labeling, Ahmed et al. (2008) demonstrated that new cells, including GFAP-positive cells, are added to the medial amygdala during puberty in rats. This pubertal addition was sexually dimorphic with males having more BrdU-positive cells in the medial amygdala than females, a finding that fits with the larger adult volume of the male medial amygdala in rats. Interestingly, the addition of BrdU-positive cells in males was eliminated by prepubertal castration, strongly suggesting that the addition of new cells in the amygdala during puberty, including GFAP-positive cells, is modulated by gonadal hormones. Thus, while gliogenesis in the amygdala appears to be regulated by gonadal hormones during development, the level of responsiveness is both sex- and subregion-specific. This specificity is also found in the adult amygdala in response to gonadal hormones.

B. Gonadal hormones and amygdala glia during adulthood

In addition to influencing amygdala glia during development, several reports have documented glial sensitivity to fluctuations in adult hormone levels within the amygdala of adult animals. For example, Blutstein et al. (2006) report that estradiol treatment of mice produces an increase in glutamine synthase gene expression in the medial amygdala of adults, an interesting effect given that neurons are incapable of synthesizing glutamate and are dependent upon glia to replenish their glutamine supply. Additionally, treatment of meadow voles with either testosterone propionate or estradiol benzoate, but not 5alpha-dihydrotestosterone, resulted in a significant increase in BrdU-labeled cells, 35% of which were glia, in the amygdala but not in the dentate gyrus or ventromedial hypothalamus (VMH) (Fowler et al., 2003). Interestingly, estradiol benzoate seems to have this effect in meadow voles but not in prairie voles (Fowler et al., 2005). The presence of an effect on glia in the amygdala, a region closely linked to sexual behavior, in a polygamous rodent species (meadow voles) but not in the monogamous rodent species (prairie voles), suggest hormone-dependent changes in the number of amygdala glia may be involved in reproductive behaviors, which may contribute to changes in female mating behavior.

The medial amygdala is involved in mating behavior in several species and glia within this region seem particularly responsive to changes in adult hormone levels. For example, multiparous females given postpartum contact
with pups exhibit reduced numbers of GFAP-positive cells in the medial amygdala compared to pup-exposed primiparous females (Featherstone et al., 2000), a difference which may be due to greater estrogen exposure in postpartum dams. In addition, the density of GFAP-immunoreactivity is higher in the medial subregion during the proestrus phase compared to the other phases of the estrous cycle in rats (Martinez et al., 2006), and in ovariectomized females, injection of estradiol, alone or with progesterone, increased GFAP-immunoreactive density in portions of the medial amygdala (Martinez et al., 2006).

Similarly, testosterone increases glial cell numbers (based on their appearance in Nissl staining) in the medial posterodorsal amygdala (MePD) of ovariectomized adult females compared to ovariectomized controls (Morris et al., 2008), a pattern of results strongly suggesting estrogenic metabolites of testosterone may lead to glial proliferation in some parts of the adult medial amygdala. In the anteroventral medial amygdala (MePV), by contrast, Carrillo et al. (2007) found no changes in glial number across the estrous cycle, despite changes in volume and other elements. Thus, even within subregions of the medial amygdala (MePD compared to MePV), glia may vary in their responsiveness to changes in steroid hormone levels.

In addition to hormone sensitivity in adulthood, dramatic sex differences can be found in adult amygdala glia. For example, total glia numbers based on Nissl staining are sexually dimorphic in the MePD of adult rats with males having more glia than females (Morris et al., 2008). GFAP-ir density measures also suggest a sex difference in astrocytes within the MePD and MePV regions with females having greater GFAP-ir density than males (Rasia-Filho et al., 2002), which may be related to the smaller volume of this nucleus in females.

Recent work in our lab has further explored sex differences in astrocytes and the role gonadal hormones play in shaping these cells. Using unbiased stereology, we found that male rats have more astrocytes than females in the MePD and that testicular feminized mutant (TFM) males, which have dysfunctional androgen receptors, were feminine in this regard. These results suggest that MePD astrocytes are dependent upon functional androgen receptors to reach masculine numbers (Johnson et al., 2008). Interestingly, this sex difference was found only in the right hemisphere, suggesting androgens may act on MePD astrocytes in a hemisphere-dependent manner. In the same groups of animals (wild-type males, females, and TFM males), we also found that MePD astrocytes in males have more complex arbor than do MePD astrocytes in either females or TFM males, but in this case, only in the left hemisphere. Again, these results suggest an androgen receptor-dependent masculinization of MePD astrocyte morphology in a hemisphere-dependent manner.

Of course, gonadal hormones can influence astrocytes through several possible pathways. In the VMH, data suggest that estrogens influence the
complexity of astrocyte arbors via indirect actions through neurons (Mong et al., 2002). Neurons in the amygdala, especially the medial regions, are rich in steroid hormone receptors, suggesting that a similar indirect pathway may occur in the MePD. Alternatively, steroid hormones may act directly on glial cells. Astrocytes are known to express both androgen and estrogen receptors, although this is highly region- and species-specific (Azcoitia et al., 1999; DonCarlos et al., 2006; Finley and Kritzer, 1999). However, as far as we are aware, no report has confirmed the presence of either estrogen or androgen receptors in amygdala astrocytes in vivo, leaving one to speculate that the majority of hormone effects on astrocytes in the amygdala are through indirect pathways—that steroids directly affect neurons, which then affect astrocytes. It is important to note that the lack of a direct pathway for hormone action does not diminish the importance of investigating steroid hormone influence on glia. If anything, an indirect mechanism, with gonadal hormones affecting neurons which then affect astrocytes, offers a means of studying neuron–astrocyte interactions. Although the mechanisms are unclear, these reports clearly indicate that hormones are involved in the modulation of amygdala glia throughout the lifespan and only continued investigation will lead to an understanding of the mechanism through which such modulation may influence amygdala-based behaviors.

C. Other agents and amygdala glia

In addition to gonadal hormones, glia in the amygdala are responsive to a broad range of nongonadal hormones, neurotransmitters and toxins. For example, Zhang et al. (2009) found that a tryptophan restrictive diet resulted in increased astrocyte arbor size and branching in the mouse amygdala, suggesting sensitivity to serotonin. Given that serotonin receptors have been found in GFAP-positive cells in the amygdala of rats (Xu and Pandey, 2000), serotonin may influence astrocyte morphology directly.

In addition, Glass et al. (2002) noted that although adrenergic receptors are mostly found on neuronal processes, some are present on glial processes in the rat central amygdala. Similarly, although principally neuron-based, glucocorticoid receptors have also been identified in glial processes of the lateral amygdala in rats (Johnson et al., 2005), and Banisadr et al. (2002) note that CCR2, the receptor for chemokine monocyte chemoattractant protein-1 (MCP-1/CCL2) is found on glia in the amygdala, suggesting these cells are involved in the immunological response at inflammation sites.

Other reports note intense glial reactivity in the amygdala in response to soman, a potent and destructive nerve agent (Collombet et al., 2005a,b). A second study demonstrates that some regrowth/migration of cells appears to occur in the amygdala following soman poisoning and that treatment with cytokines causes these cells to differentiate into astrocytes (Collombet
et al., 2005a,b). Additionally, the hyperalgesic effects of bradykinin administration into the amygdala can be blocked by the glial metabolic inhibitor fluorocitrate among other agents (Dalmolin et al., 2007), suggesting glia metabolism contributes to the hyperalgesia. Finally, while we know that glia are involved in the formation of interamygdala connections in the rat brain during early development (Cooke and Simerly, 2005), glial cell division in the amygdala is also very sensitive during development in some species. Kent and Harman (1998) found that slight elevations in body temperature in developing Wallabies result in reduced glial cell division in the amygdala. Although it is not clear what the behavioral consequences of these early changes are, given other work in the field, they may lead to lasting psychosocial changes in the adult animals.

Collectively, these studies hint at the involvement of glia in numerous processes beyond the well-known diseases, disorders and hormone-based findings in which they are currently being aggressively investigated. Perhaps with continued investigation, it will become clear how these results can be integrated into a better understanding of the amygdala and the dynamic cells within it.

V. Conclusions

While the above findings present a scattering of evidence that make a cohesive report of glia function in the amygdala difficult to portray, it is clear that glia in general have offered a wealth of intriguing and informative findings within the past several decades. Several excellent reviews have summarized glia-based findings in various brain regions and in the brain as a whole (Barres, 2008; Eng, Ghirnikar and Lee, 2000; Freeman and Doherty, 2006; Garcia-Segura and Melcangi, 2006). In this review, we have gathered findings regarding glia in the amygdala and it is our hope that this collection of findings will promote continued investigation of glia in this critical brain region. A deeper understanding of glia will be informative for measures of amygdala functionality such as fMRI, a technique closely linked to astrocyte activity (Schummers et al., 2008), which is often used to investigate this region. As this review suggest, it is clear that these cells respond and develop in a highly region specific manner, making further study of glia a necessity. Likewise, the sensitivity and plasticity of glia may make them abundantly useful in diagnosis and treatment of various disorders and diseases, especially those exhibiting strong sex biases in the population. Glia are clearly involved in several disorders and diseases and the amygdala’s prominent sex differences suggest glia in this region may be linked to sex differences in the vulnerability to disorders such as depression, schizophrenia, anxiety disorders, and autism.
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REFERENCES


