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Evolutionary Perspective on Microglial/Neuronal Coupling with Special Relevance to Psychiatric Illnesses

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Abstract

Microglia have selectively evolved as a morphologically and chemically distinct class of immuno-competent CNS resident cells with potent bidirectional signaling capabilities linked to induction of a macrophage-like phenotype following metabolic, microbiological, or viral insults. It has been empirically determined that a conserved set of shared chemical messengers connects a communication network mediating reciprocal exchange of regulatory information between immune, central nervous, and neuroendocrine systems. From an evolutionary perspective, the pluripotent neuro-protective capabilities of invertebrate microglia have been extended and amplified in classes of mammalian microglia. The state-dependent plasticity of microglia has provoked considerable empirical investigation into their functional/regulatory roles in mediating innate immune surveillance and neural protection within the CNS. Upon pathophysiological dysregulation, aberrant microglial activities may provide significant contributory factors in the etiology and persistence of major neurological, degenerative, and psychiatric disorders. Within this context, invertebrate microglia appear to represent highly appropriate model systems to investigate underlying cellular and molecular mechanisms involved in higher order neuroimmune regulation of multiple CNS activities by mammalian microglia.

Keywords: Mitochondria; Microglia; Nitric oxide; Nitrite; Morphine; Hypoxia

Introduction

Selective evolutionary pressure has provided the CNS with a morphologically and chemically distinct class of immuno-competent "vigilante" cell, microglia, with potent bidirectional signaling capabilities linked to induction of a macrophage-like phenotype [1-3]. Concerted developmental studies have established that adult microglia are derived from primitive myeloid precursors within the embryonic yolk sac that subsequently proliferate into mature microglia and seed the brain during later embryonic and perinatal stages [4-10]. During the postnatal period, mature unstimulated microglia maintain a branched or ramified morphology that is transformed into an activated macrophage-like amoeboid state following microbiological or pathophysiological insults [11-13]. As discussed in depth below, the intrinsic, state-dependent, plasticity of microglia has provoked considerable empirical investigation into their functional/regulatory roles in mediating innate immune surveillance and neural protection within the CNS. When dysregulated, it appears that aberrant microglial activities represent significant contributory factors in the etiology and persistence of major neurological, degenerative, and psychiatric disorders [11,12,14,15].

Common Set of Shared Signal Molecules

The history of neurobiology has demonstrated the value of the invertebrate nervous system as a model for neural phenomena. The giant squid axon and GABAnergic systems are well known examples [16,20]. Since the invertebrate center of our review is the bivalve mollusk *Mytilus edulis,* the examples of evolutionarily conserved chemical signaling will be restricted to this organism. In this regard, monoaminergic neurotransmission is present in this animal, e.g., dopamine, norepinephrine, serotonin, etc. [21]. Cholinergic processes are also present [22]. These reports also demonstrate, on a pharmacological and biochemical basis, that the corresponding receptors are present as well, along with the intracellular second messenger communication processes [23-26]. Opioid peptides and opiate alkaloids (e.g., enkephalins, morphine) are also present with their respective receptors and biosynthetic pathways [27-31]. From a neuroimmune perspective, the same chemical messengers and their receptors have been found on invertebrate immunocytes, including nitric oxide coupling, allowing neurons to communicate with the immune cells, including microglia [32-42] (Figures 1 and 2). RIA and HPLC studies also identified cytokine messengers in these same tissues, e.g., interleukin-1, -6, -10 and tumor necrosis factor along with their receptors [43-51]. Utilizing more modern technologies, like microarray, we have validated the presence of these signaling systems in invertebrate tissues by examining their gene expression patterns [22-52]. There are many more examples of this phenomenon that, for the sake of the review's focus, we will not mention. Thus, the communication between the same cell and other cell types becomes evident, and clearly demonstrates this phenomenon originated much earlier in evolution than previously thought. Hence, the phenomenon of creating networking/pathways of intra-nervous system communication becomes the new evolutionary development advance. It has been well documented within the biomedical literature that both conservation and enhancement of function of common sets of chemical messenger compounds has been exponentially amplified by positive evolutionary pressure. Accordingly, the elucidation of basic mechanistic information regarding diverse mechanistic roles of common sets of chemical messenger molecules has tremendous predictive value within biomedical model systems. Activated macrophages have been demonstrated to synthesize, enzymatically process, and release a variety of pro-inflammatory cytokines, some of which have been shown to influence monoaminergic neural function [27,53]. For example, administration of interleukin-1 (IL-1) was found to enhance *in vivo* release of dopamine and its acidic metabolite dihydroxyphenylacetic acid from rat hypothalamus [54]. *In vivo* observations were confirmed by a complementary *in vitro*

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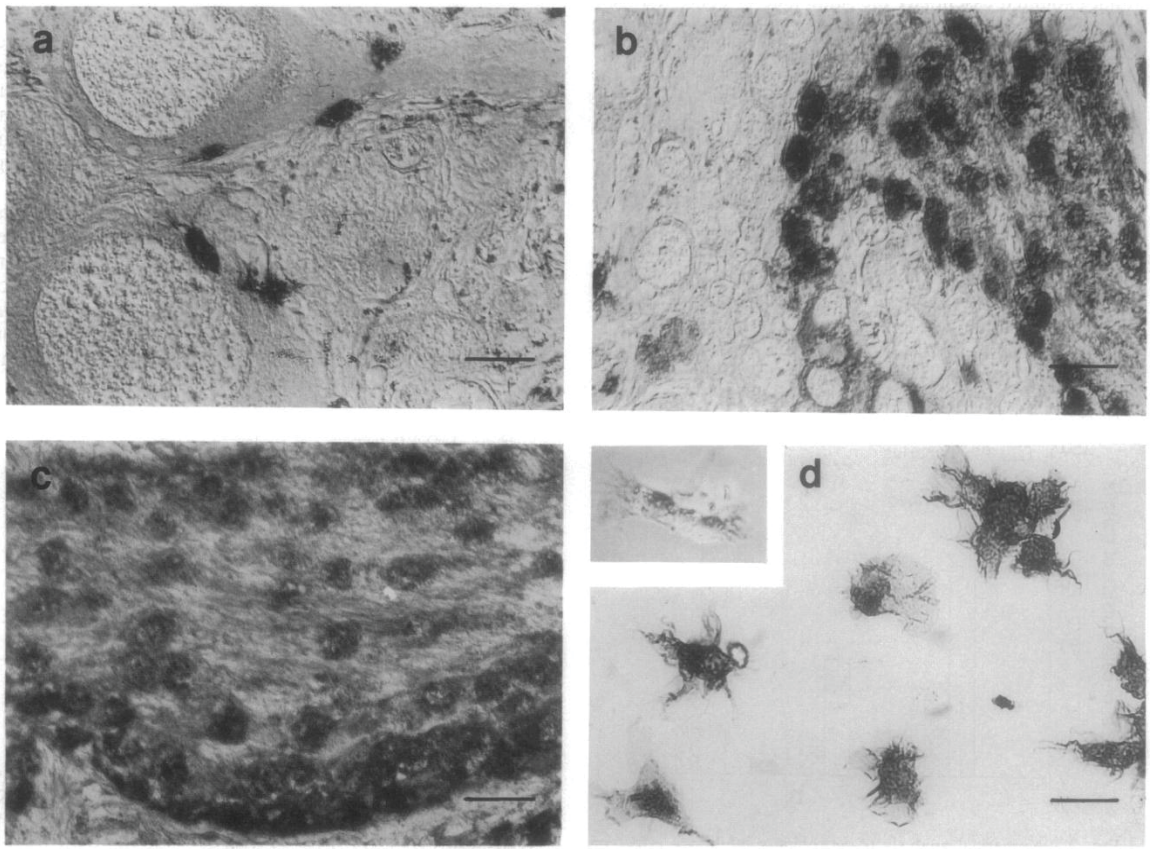

Figure 1: Micrographs of excised visceral ganglia of *Planorbarius*, demonstrating egress of microglial cells selectively immunostained for the presence of ACTH (Nomarski interference). (a) Microglial cells in close contact with ACTH-negative giant neurons, fixed immediately after excision. (b) Fixed after 24 hr of incubation in culture medium; microglia accumulating in neuropilar region. (c) Accumulation of migrating cells at nerve stump. (d) Ameboid conformation of microglial cells in extraganglionic area. (Inset) Abolition of immunoreaction by omission of the primary antibody. (Bars = 20 um.) From, PNAS, 1994, (62).

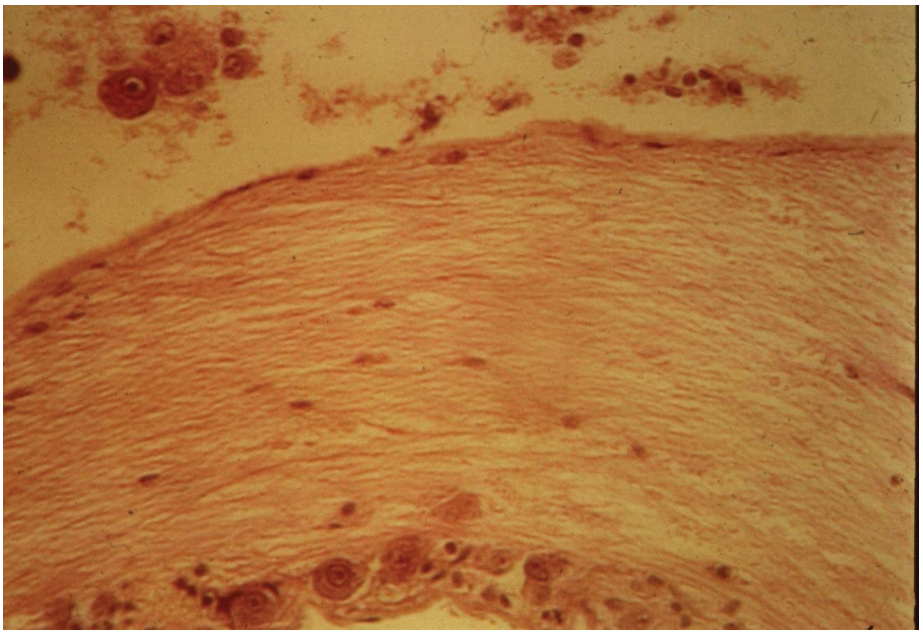

Figure 2: Section of branchial nerve emerging from *Mytilus edulis* visceral ganglia. Note the macrophage-like cells both free and above the nerve trunk and within it, illustrating their ability to freely penetrate this open nervous system. From Prog. Neurobiology, 1989.

study demonstrating evoked release of dopamine and norepinephrine from male rat hypothalamic tissues following administration of IL-1b [55]. Accordingly, a major macrophage/microglial secretory product, i.e., IL-1, appears to represent a key regulatory factor underlying neurophysiological functioning of CNS monoaminergic signaling pathways responsible for integration of complex behavioral paradigms. The significance of dysregulated monoaminergic mechanisms in the etiology of major psychiatric illnesses has been documented for the past 40 years, and includes studies on cortically-mediated intractable pain [56-58]. In sum, macrophage/microglial-derived secretory products include molecules capable of selectively altering diverse neuronal activities and must be included in any list of putative etiological factors involved in major psychiatric disorders. Under normative physiological conditions, microglia provide essential surveillance and intra/inter-cellular communication processes subsumed under innate immunological activities. In contrast, an increased number of activated microglia displaying macrophage-like amoeboid morphologies and chemokinetic properties are visualized in affected CNS areas following trauma or physiological stress [59,60]. Following excessive chemical or microbiological insults, it is apparent that activated microglia may promote inappropriately deleterious release of signal molecules that can excite, inhibit or damage neurons in their respective vicinity [60]. In sum, aberrant coupling of microglial-neuronal communication may promote severely dysfunctional behavioral consequences often leading to the manifestation of major psychiatric disorders.

Evolutionary Origin of Microglial/Neuronal Coupling

Throughout invertebrate and mammalian species, the regulatory activities of immuno-competent microglial cells within nervous structures are deemed of critical importance to normative neuronal function. Accordingly, the resident CNS macrophage appears to represent a significant developmental prototype for intercellular mediation of immune-neuro communication processes in animals that evolved at least 500 million years before humans. For example, one of the activities linked to invertebrate glia is functionally associated with the blood-brain barrier integrity of insects, as well as maintenance and repair of invertebrate nerve cells [61]. In earlier reports, invertebrate immuno-competent glial cells have been demonstrated to possess similar properties as previously described for mammalian microglia including a shared set of chemical messengers such as interleukins and opioid peptides [61-64]. These morphological and biochemical similarities suggest an evolutionarily driven functional convergence of immuno-competent glial cells [62-64]. In this regard, our group and others have demonstrated compelling anatomical and biochemical linkages between invertebrate immunocytes/microglia and mammalian monocytic/microglial/macrophage lineages that include functional utilization of a shared set of chemical messengers [27,43,44,51,65-67]. Briefly, the listed functional similarities include taken from these reports includes: 1) expression of immunocyte-responsive cytokine-like molecules closely resembling those found in higher animals; 2) cross activation of human immunocytes by invertebrate cytokine-like molecules; 3) initiation of a cytokine-like cascade mechanism induced by lipopolysaccharide (LPS) administration; 4) functional involvement of opioid peptide and opioid receptor mechanisms in cytokine production and release related to manifestation of neural trauma; 5) similarities in the metabolic enzyme pathways responsible for the degradation of peptidergic signal molecules; 6) utilization of nitric oxide (NO) as a major regulatory molecule in immunological and neurological tissues [44,68-70] 7) regulated biosynthesis and utilization of endogenous morphine and its stereo-selective mu receptor subtypes as regulatory factors in neural, immune and neuroimmune signaling [45,71-73].

Additional Functional Commonalities

As described above, the responsiveness of invertebrate and vertebrate immuno-competent microglia to a common set of signal molecules including interleukins, NO, opioid peptides, catecholamines and endogenous morphine is functionally linked to physiologically driven innate immunological activities. As documented by video time lapse microscopy [62,74], this phenomenon is visualized by stationary microglia becoming amoeboid, macrophage-like, and mobile following traumatic stimuli in invertebrate ganglia [62-64,75]. By functional criteria, noted earlier, activated or polarized macrophages represent a potent immune cell type with the potential to secrete numerous neuroactive signal molecules that permit free penetration of the vertebrate the blood-brain barrier. Operationally, macrophages appear to be sentinels of the immune and nervous systems and may exert the same type of surveillance in other systems as well [14]. Invertebrate ganglia contain microglia and macrophage-like cells in their open ganglionic nervous system [33]. Additionally, selected cytokine-like secretory molecules have been observed to evoke neurophysiological changes in invertebrate neurons in a receptor mediated manner [66,76-78]. In sum, the presence of a common set of signal molecules in comparative animal groups, and their innate immunological stimulating activity functionally linked to the induction of significant morphological cellular changes, strongly suggests conservation of this cellular association along with its operational properties.

Retention of Chemical Messengers: Underlying Rationale

We contend that a likely mechanistic driving force underlying the phenomenon of chemical messenger retention during evolution resides in stereo-selective recognition of enantiomeric compounds within multiple stereo-selective enzyme and receptor signaling pathways [79]. Accordingly, the basic preservation of essential chemical information required for recognition and activation by distinct classes of enzyme and receptors within discrete signaling pathways provides the molecular basis for retention of shared sets of chemical compounds in diverse plant and animal phyla [80,81]. Hence stereo-selective conformational matching in a multiple enzyme or multiple receptor mediated pathway presents a systemic driving force to retain basic chemical identities across animal and plant phyla [66,79,82-85] and in remarkably different cell types. Another common chemical feature of retained signal molecules is the widely expressed precursor to product relationship that allows temporal release of biologically active chemical compounds and peptide sequences from biologically inactive prohormone-like molecules, notably via the action of endo-proteolytic cleavage enzymes [31,86,87]. Thus, macrophage secretory products appear to alter well-established monoaminergic signaling pathways responsible for regulating basic physiological functions, as well as integration of complex behavioral paradigms in both vertebrates and invertebrates. The significance of normative and aberrant monoaminergic mechanisms in the etiology of major psychiatric illnesses has been documented and includes studies on cortically-mediated intractable pain [88,89]. In sum, secretory molecules derived from activated macrophage-like microglia possess potent capabilities for selectively altering a wide variety neuronal activities and must be included in any list of putative etiological factors involved in major

psychiatric as well as CNS-presenting metabolic disorders [90-93].

Conclusions

An evolutionary, retroactively-directed, blueprint for elucidation of neural-immune bidirectional communication mechanisms of higher animals may be gleaned from examination of neural and immune processes of invertebrate microglia. Unifying principles responsible for normative bidirectional neural-immune communication across invertebrate and vertebrate species reside in common anatomical and biochemical substrates. The original need for this relationship may reside in the fact that immune and neural cells require a diversity of sensory inputs aimed at survival and longevity regardless of where the respective organism is positioned within the evolutionary tree. Functional maintenance of a shared set of common messenger molecules, as well as other life maintaining chemical processes, resides in an expansive adaptation of stereoselective/conformational matching processes. By evolutionary criteria, the open circulatory system of invertebrates lent itself to the origin of neuroimmune cooperative events, whereby macrophage-like immune cells gained the capabilities for penetration and residence within "privileged" neuronal compartments. Mammalian microglia have evolved as resident immuno-competent guardians against metabolic, microbiological or viral insults to populations of CNS cells via morphological transformation into active macrophages. Adaptation processes underlying exponential expansion of complex cognitive behaviors in higher animals strongly suggest that dysregulation of microglial-direction neuroimmune processes represents a likely contributing factor to the etiology and persistence of major psychiatric disorders afflicting human populations.

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