PNAS COMMENTARY

Shaping functionality in the brain

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Over the last two decades, microglia have emerged as "master regulators" of critical events in the central nervous system (CNS). While their original description focused on their roles in pathological contexts (1–3), more recent literature has described them as surveillants of activity in the CNS (4) and active participants in the shaping of synapses (5, 6), mostly through removing inactive ones. The paper by Gallo et al. in PNAS (7) describes a role for microglia in synaptogenesis during early postnatal development, which implies their importance in the establishment of appropriate functional neuronal circuits.

Microglia are present in the CNS in the early prenatal period (8, 9) before the development and maturation of astrocytes and oligodendrocytes, and during that time they serve as the sole surveillants of events such as phagocytosis of unwanted/unnecessary neurons and remodeling of neural circuits. Similarly, in early postnatal development, as microglia mature they act to eliminate synapses through the interaction of synaptic C3 with its receptor CD11b expressed by the microglia (6). A more recent function of microglia was described for pyramidal excitatory neurons in layers 2/3 in the developing somatosensory cortex (SSC), where contact with microglia generates Ca^{2+} transients that result in spine formation (10). In a reciprocal way, the activation of microglia and their morphology were reported to be regulated by ionotropic glutamatergic and GABAergic neurotransmission (11).

Gallo et al. (7) now extend these findings in GABAergic interneurons and focus specifically on chandelier cells (ChCs), a specialized type of interneurons that form synapses on pyramidal neuron axon initial segments (AIS) (12) and thus control the site where action potentials are generated and regulate synchrony and oscillation of network activity (13). Dysfunctional connectivity between ChCs and pyramidal neurons has been implicated in neuronal developmental abnormalities including epilepsy and schizophrenia (13).

Gallo et al. show in mice that microglia wrap around the proximal length of the AIS in pyramidal neurons in layers 2/3 SSC, covering ∼50% of the AIS (7) during the second postnatal week, and denote them as AXIS microglia (axon initial segment-associated microglia). Since that is the area where ChCs are supposed to interact with and innervate pyramidal neurons, they utilize Nkx2.1-CreER$^{+/-}$;Ai9 $^{+/-}$ mice to visualize the proximity and interactions between the three types of cells along the AIS. They find that this tripartite interaction coincides with increased cartridge length and numbers of boutons but that the interaction is transient and decreases in adult animals. The authors elegantly show that the presence of and tight contact with microglia does not result in increased phagocytosis of ChC material and that microglia are critical components of the tripartite complex, as in their absence both cartridge length and bouton numbers decrease, specifically affecting gephyrin+ GABAergic synapses. It is in fact most likely the levels of GABA that drive the interaction with the $GABA_B$ receptor–expressing microglia. Interestingly, the authors also show that activation of microglia with lipopolysaccharide in the second week of postnatal development, or use of a mouse model of Alzheimer's disease that exhibits robust microglial activation at 14 mo of age, results in the cartridge length and numbers of boutons being decreased as well as the AIS gephyrin+ puncta (7).The take-home message is that AXIS microglia promote early postnatal synaptogenesis by regulating axo-axonic synapse formation by ChCs on pyramidal neuron AIS cartridges, which are characterized by the presence of specific cytoskeletal proteins such as ankyrin G and β4-spectrin.

The timing of this microglial synaptogenic function is important in that it coincides with innervation of pyramidal neurons by ChCs during early postnatal development. Aberrant connections have been associated with the establishment of dysfunctional circuitry in the mammalian cortex. In the current study, such operative dysfunction is not explicitly demonstrated (electrophysiologically or behaviorally), and it would be interesting to examine such outcomes. Moreover, it is not known whether the microglial synaptogenic activity is dynamic or whether it results in permanent dysfunction of the circuitry. In other paradigms of microglial activity, the return of animals to normal chow results in rapid repopulation of the CNS by microglia (14), and thus it would be interesting to examine cartridge length and bouton numbers in the repopulated brains.

One also wonders what the molecular signature of AXIS microglia is and how they differ from regular microglia. The authors show that AXIS microglia use the $GABA_{B1}$ receptor to facilitate the axo-axonic synapse formation, but is this what drives the synaptogenic behavior? It is possible that additional factors may be involved in the process. If not, are these microglia different from other non-AXIS microglia? As no specific markers for AXIS microglia have been determined, approaches that involve single-cell RNA-sequencing profiling may be useful in identifying if these cells are distinct from other parenchymal microglia.

The findings in Gallo et al. (7) point to a critical role for this tripartite connection in early postnatal development.

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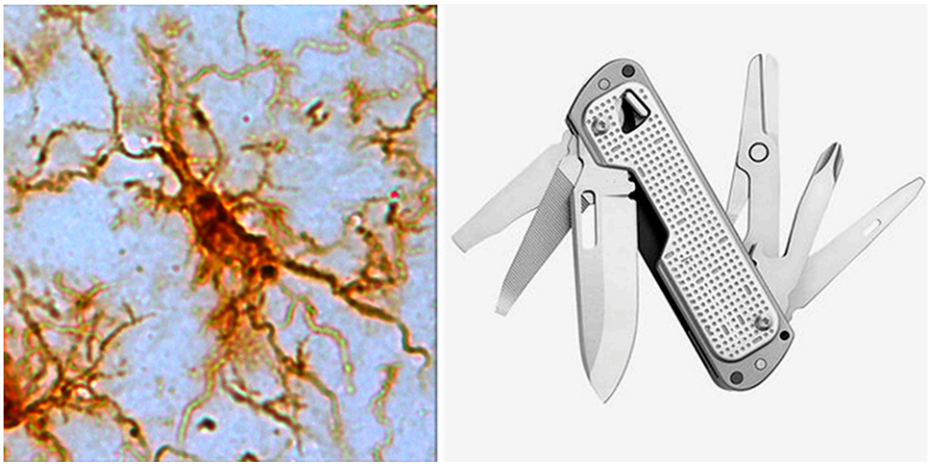

Fig. 1. Microglia as a multifunctional tool. Their different activities during the lifespan of an organism suggest that microglia are dynamic and versatile cells that regulate a diverse range of processes to facilitate proper function in the mammalian brain.

Traditionally, the interactions between ChCs and pyramidal cells are thought to stabilize during this period and persist throughout life. If the interaction with AXIS microglia is a dynamic event, it might be possible that these interactions change in aging as the nature of microglia change and the cells become dystrophic (15). Will the preexisting synapses remain, will they get phagocytosed, and will new synapses (potentially aberrant) be formed?

The results presented by Gallo et al. (7) elegantly introduce a role for microglia in the shaping and fine-tuning of neuronal circuits in the mammalian brain. They also open a plethora of new questions about the function of these microglia cells, and they certainly add to the gamut of functions of microglia in the brain through the lifetime of an organism (Fig. 1). We have come a long way from old descriptions in textbooks in the mid-1990s where the citation for microglia was that "they arise from macrophages and are physiologically and embryologically unrelated to the other cells of the nervous system. We shall therefore not consider the microglia further" (16)!

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