

Mast cells on the mind: new insights and opportunities

Rae Silver^{1,2,3} and James P. Curley²

¹ Department of Psychology, Barnard College, 3009 Broadway, New York, NY 10027, USA

² Department of Psychology, Columbia University, 1190 Amsterdam Avenue, New York, NY 10027, USA

³ Department of Pathology and Cell Biology, Columbia University Medical Center, 630 West 168th Street, New York, NY 10032, USA

Mast cells (MCs) are both sensors and effectors in communication among nervous, vascular, and immune systems. In the brain, they reside on the brain side of the blood–brain barrier (BBB), and interact with neurons, glia, blood vessels, and other hematopoietic cells via their neuroactive prestored and newly synthesized chemicals. They are first responders, acting as catalysts and recruiters to initiate, amplify, and prolong other immune and nervous responses upon activation. MCs both promote deleterious outcomes in brain function and contribute to normative behavioral functioning, particularly cognition and emotionality. New experimental tools enabling isolation of brain MCs, manipulation of MCs or their products, and measurement of MC products in very small brain volumes present unprecedented opportunities for examining these enigmatic cells.

Mast cell history and heterogeneity

MCs were first characterized in 1878 by the Nobel-prize winning immunologist Paul Ehrlich, who described the histochemical properties and distinct morphological phenotypes of these small (6–12 μm) cells [1]. MCs originate in the bone marrow arising, in humans, from CD34⁺/CD117⁺ pluripotent progenitor cells [2]. MCs circulate in the blood as committed precursors and complete their differentiation in tissues where they reside. They are infamous and well studied for their role in immunoglobulin type E (IgE)-associated allergic and inflammatory disorders, but have not been examined much in the brain. Efficient activation of MCs is via IgE binding to Fc ϵ RI (high-affinity surface receptors for the Fc region of IgE), although MC activation can also occur through ligand binding to other receptors, including Fc γ RIIa, TrkA, complement component receptors, Toll-like receptors (TLRs), and interleukin (IL) receptors [3,4]. The latter are especially significant in non-mammalian species that lack IgE [5].

Classically, MCs have been categorized into distinct subtypes based on their tissue of residence (namely mucosal, serosal, or brain subtype), but they may also be classified by their granule content. Thus, in humans, MCs can be distinguished based upon whether they contain tryptase (MCT) or tryptase and chymase (MCTC) [4].

Complicating such classifications, mature MCs remain plastic and can adjust their phenotype dependent upon their local cellular environment. For instance, some intracerebral MCs lack the c-kit receptor [receptor for stem cell factor (SCF), the main MC growth and survival factor] and have low Fc ϵ RI, pointing to such tissue-specific characteristics [6,7].

MC granules bear numerous preformed and newly synthesized reactive chemicals, termed ‘MC mediators’ (Figure 1), many of which are active in the central nervous system (CNS) or peripheral nervous system (summarized in Table 1). Rapid release of potent preformed mediators is quickly followed by synthesis of lipid mediators and a slower, *de novo* synthesis of cytokines and chemokines [8,9]. The mediator released upon MC activation is specific to the activating signals [4]. Once activated, MC granular content can be released directionally and, in the brain, the extent of spread can be over tissue volume-orders of magnitude greater than synaptic clefts (see Figure S1 in [10], showing MC granule remnants at a distance of more than 50 μm from the cell of origin).

MC relations with blood vessels, glia, neurons, and nerves

In all vertebrates studied, including humans, MCs are found in the brain, on the brain side of the BBB and in the leptomeninges [11,12]. They lie in close proximity to the basal side of the blood vessel wall, in Virchow-Robin perivascular spaces and, thus, are able to communicate with neurons, glia, microglia, extracellular matrix, and blood vessels (Figure 2). It is difficult to establish the absolute numbers of MCs in the brain because they vary with species, age, and the experimental methodologies used for such assessments. Nonetheless, under baseline conditions, in the absence of stress, disease, or trauma, their total is considerably smaller than the numbers of neurons, microglia, and other brain-resident cells (Table 2). Despite their small numbers, activated MCs can impact all components of the neurovascular unit (encompassing vascular, microvascular, and perivascular structures), neurons, microglia, and macroglia. The multiphasic response pattern of MCs, wherein they release preformed granular material within minutes, and newly synthesized mediators for the next several hours, enables their actions as catalysts that amplify and prolong numerous vasoactive, neuroactive, and immunoactive cellular and molecular responses.

Corresponding author: Silver, R. (QR@Columbia.edu).

Keywords: mast cells; neuroimmune interactions; psychiatric disease; multiple sclerosis; ischemia; NGF.

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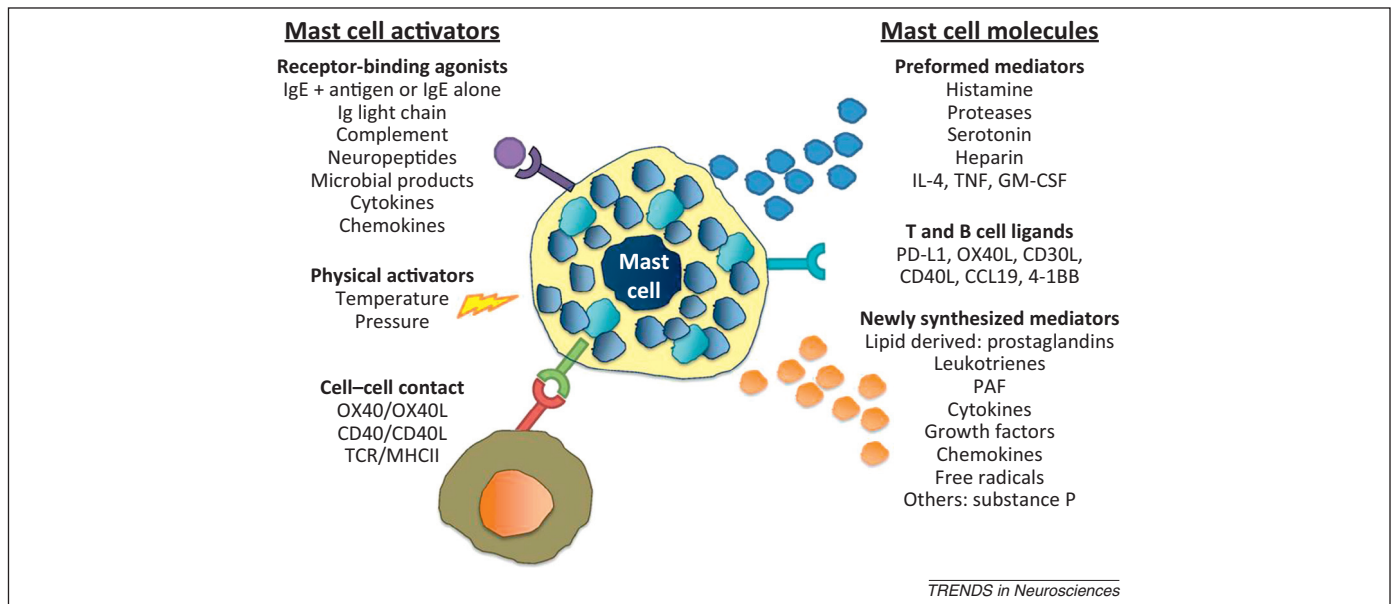


Figure 1. The schematic depicts the multitude of mast cell (MC) activators and modes of response, accounting for their impact on a variety of processes. MCs can be activated by receptor-binding agonists, physical activators, and cell-cell contact. The time course of activation is multiphasic, with an initial release of preformed mediators within minutes, quickly followed by synthesis of lipid mediators and slower, *de novo* synthesis of cytokines and chemokines [8,9]. Activated MCs can also recruit other MCs and can increase the expression of ligands that mediate cell interactions with T and B cells. Reproduced, with permission, from [71]. Abbreviations: CCL19, C-C motif chemokine 19; CD30L (also called CD153), ligand for CD30 (member of the TNF receptor superfamily); CD40 and CD40L, CD40 and its ligand CD40L (also called CD154; member of the TNF receptor superfamily); GM-CSF, granulocyte-macrophage colony-stimulating factor; IgE, immunoglobulin E; IL-4, interleukin 4; MHCII, major histocompatibility complex II; OX40/OX40L, OX40 (also called CD134) and its binding partner, OX40L (also called CD252; member of the tumor necrosis factor receptor superfamily); PAF, platelet-activating factor; PD-L1, programmed cell death 1 ligand 1; TCR, T cell receptor; TNF, tumor necrosis factor; 4-1BB: type 2 transmembrane glycoprotein of the TNF superfamily.

Table 1. Functional roles of MC mediators in the central and peripheral nervous systems

Class	Mediator	Physiological effect	Refs
Preformed	Histamine	Modulates vascular and BBB permeability	[19]
		Sensitizes nociceptors and activates trigeminocervical and lumbosacral pain pathways	[84,85]
		Potentiates NMDA mediation of glutamatergic neurotransmission in hippocampal neurons	[94]
		Facilitates MC–neuron adhesion	[29]
		Neuroprotective effects following neuron injury after cerebral ischemia	[95]
		Regulates MC–astrocyte interactions	[41,42]
		Modulates mouse behavior; e.g., anxiety-like behavior or locomotor activity	[56,57]
	Serotonin	Promotes hippocampal neurogenesis and supports hippocampal-dependent learning and memory	[58]
	Serine protease: tryptase	Activates sensory neurons	[22,24,25]
		Promotes the release of BDNF from, and the expression of P2X4R in, microglial cells	[40]
Induces release of pro-inflammatory mediators from microglia		[38]	
Regulates permeability of BBB following inflammation		[19]	
Serine protease: chymase	Neuroprotective effects following brain lesion trauma	[20]	
	Regulates permeability of BBB following inflammation	[19]	
Cytokines	TNF- α (both preformed and newly synthesized)	Reduces nerve firing thresholds	[32]
		Facilitates neutrophil recruitment during inflammation to modulate BBB permeability	[76,96]
		Regulates thermoregulatory responses following sepsis induction	[10]
	Regulates MC–astrocyte interactions	[41,42]	
Interleukins; e.g., IL-6, IL-13	Regulate microglial activity	[3]	
Growth factors	e.g., NGF	Promotes survival and differentiation of neurons; reduces neuronal firing thresholds	[31]
		Promotes neuroprotective effect of vasoactive intestinal peptide on reversing motor defects in animal models of Parkinson's disease	[97]

Blood vessels

There is substantial evidence that MCs penetrate the BBB and alter its architecture. Mature MCs themselves can migrate from blood to brain [13]. Perivascular MCs in the skin acquire IgE by extending cell processes across the

blood vessel wall to capture luminal IgE [14], revealing a novel mechanism for monitoring the blood content of critical molecules. Intramuscular injection of the MC degranulator, compound 48/80 (c48/80), results in vastly increased Evans Blue tracer leakage in MC-rich, but not

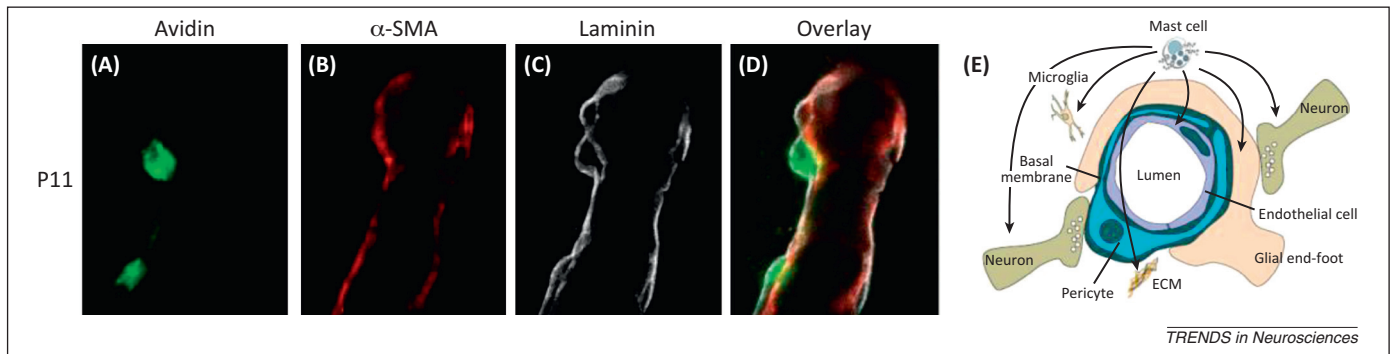


Figure 2. The photomicrograph depicts the cellular localization of thalamic mast cells (MCs) relative to the smooth muscle of the blood vessel and its basal lamina, in a study using triple-label immunocytochemistry. MCs (green) are located between the smooth muscle actin-positive cell (red) and the laminin-delineated basal lamina (white). Thalamic MCs (A) are associated with large blood vessels with smooth muscle containing α -smooth muscle actin (α -SMA) (B). Laminin (C) is present in the basal lamina of the smooth muscle and surrounds the MC. (D) Overlay of (A–C). (E) shows the close proximity of the MC to other cellular elements in nervous tissue, and points to its ability to signal blood vessel components, neurons, glia, and microglia. Abbreviation: ECM, extracellular matrix. Reproduced from [12] (A–D). Adapted from [19] (E).

Table 2. MC diversity in the brain: examples of how MC distribution and number can vary by species and developmental age

Species	MC distribution	MC number	Refs
Common toad, African clawed frog and green frog	MCs are located in the pia mater and choroid plexus juxtaposed with blood capillaries and ventricular ependymal. They are rarely located in the brain parenchyma and, in the perennially aquatic African clawed frog, are confined solely to the pia mater	Doubles from pro-metamorphic stages to the climax of metamorphosis, ranging from 4 to 290 in the African clawed frog, 50 to 1600 in green frogs and 400 to 2200 in toads	[98]
Ring dove	Immature MCs first appear in the pia mater on embryonic day 13–14. 4–5 days post-hatching MCs migrate along blood vessels from the pia mater to the telencephalon and finally mature in the medial habenula of the thalamus in adults	Numbers peak at an average of 1400 MCs in the brain at puberty and decline thereafter	[99]
Rat	MCs mature outside of the brain and first appear in the pia and choroid plexus, peaking in numbers at day PN11 before declining thereafter. MCs infiltrate the parenchyma, including the thalamus, hippocampus, and less extensively the hypothalamus after PN8 and expand in number up to adulthood, being most clustered within the thalamus. Numbers decline in the brain with ageing; 96% of MCs are inside the BBB and over 90% are in contact with blood vessels	Total estimates vary considerably across rat studies likely due to variable experimental methodologies. Early estimates suggested that whole-brain MC numbers peak in the range of 35 000–45 000, although recent studies suggest that this number is much smaller. For instance, [12] found that pial MCs peaked at 5550 and thalamic MCs peaked at 1566	[12,51,100]
Mouse	MCs are most commonly located in the meninges on the brain side of the BBB, as well as the hippocampus and thalamus. The number of intracranial MCs increases steadily from birth to adulthood	Although numbers are strain dependent, intracranial brain MC numbers appear to peak at only approximately 600. Brain regions with relatively high MC numbers, such as the hippocampus and thalamus, have fewer than 100 MCs	[58,101]
Human	Most abundant in young individuals under the age of 19, generally decline with age and can be found in greatest numbers in the pituitary stalk, pineal gland, area postrema, choroid plexus, and areas around the third ventricle, hypothalamus, and thalamus	Reliable whole-brain estimates of total MC numbers are lacking	[102,103]

in MC-lacking brain regions bearing fenestrated capillaries. These findings have been confirmed with various techniques in several species [15,16]. MC effects on vascular permeability are blocked by MC stabilizers and are absent in MC-deficient W/W^v mice [17]. Several possible mediators likely underlie MC effects on the BBB given that MCs secrete numerous potent vasodilators, including heparin, histamine, serotonin, nitric oxide, vasoactive intestinal peptide, calcitonin gene-related peptide (CGRP), vascular endothelial growth factor, and cytokines, including tumor necrosis factor alpha (TNF- α), which in turn induces the expression of intercellular adhesion molecule-1 (ICAM-1) and permits leukocyte entry into the affected tissues [18–20]. Notably, MCs can contribute approximately 90% of thalamic histamine and up to 50% of total brain

histamine in rats [21]. Furthermore, serine proteases, such as the MC-specific protease tryptase, cause not only microvascular leakage, but also neuronal hyperexcitability and inflammation through protease-activated receptors [22–25]. Finally, there may be a further supportive role of MCs: in some cancer tumors, MCs promote angiogenesis [26], although this aspect has not been studied in normal brain development.

Nerves and neurons

Although the functional relation between MCs and neurons *in vivo* is not yet well characterized, communication between MCs and peripheral nerves has been examined and may inform CNS roles [27]. In most tissues, MCs and nerves are apposed with spatial gaps of 20 nm or less.

These intimate interactions are maintained via transhomophilic binding of cell adhesion molecule-1 (CADM1), which is expressed on both sensory neurons and MCs and promotes a microenvironment for efficient communication resembling synaptic junctions [23,28]. Interesting recent work has identified four isoforms of CADM1 that are expressed differentially in various brain regions across development. One of these, CADM1d, is expressed by mature hippocampal neurons and is the isoform that MCs most strongly adhere to *in vitro* [29]. Other cell-adhesion molecules, such as nectin-3, can also facilitate MC–neuron interaction [30]. MCs and nerves also share common activating signals and receptors, suggesting potential bidirectional communication. MC mediators can act on neurites: for example, MC tryptase binds to proteinase-activated receptors, directly activating sensory neurons [25], MC TNF- α and nerve growth factor (NGF) lower sensory nerve firing thresholds [31,32] and NGF is also required for nerve differentiation and survival [23]. Furthermore, ATP released from MCs can activate neighboring sensory nerves [33]. Likewise, neuropeptides released from neurites, such as CGRP and substance P (SP), bind to their receptors on MCs and can stimulate degranulation [34,35]. *In vitro*, it has been shown that SP-stimulated hypothalamic MCs can have levels of TNF- α up to 14 times higher than unstimulated cells [36].

Finally, MC-derived material can enter adjacent cells, resulting in subcellular insertion of granule contents into neurons. This MC process, termed ‘transgranulation’, has been described in CNS neurons as well as in other tissues [36,37]. Although phagocytosis of cellular debris or pathogens is known for other stationary and mobile cells, this is unlikely to be the function of transgranulation of MC-derived material in the CNS and remains a mystery. Taken together, bidirectional interactions between the nervous system and MCs are established at least for sensory neurons, with increasing detail regarding their interactions in CNS becoming known.

Microglia and macroglia

Several molecular mechanisms for potential interactions between MCs and microglia have been determined *in vitro* [3]. For instance, activation of purinergic P2 receptors on microglia by ATP stimulates the release of IL-33, which binds to MC receptors, triggering the release of IL-6, IL-13, and monocyte chemo-attractant protein 1, which in turn may regulate microglia activity. Similarly, MC tryptase activates microglial protease-activated receptor 2 (PAR2) receptors, facilitating the release of pro-inflammatory mediators, such as TNF- α , IL-6, and reactive oxygen species (ROS), which consequently upregulate the expression of PAR2 receptors on MCs [38,39]. Furthermore, MC activation leads to the upregulation of purinergic receptor P2X, ligand-gated ion channel, 4 (PR2X4) receptors on microglia, resulting in the release of brain-derived neurotrophic factor (BDNF) [40]. Numerous other molecules and receptors, such as complement component 5a receptor (C5aR), chemokine (C-X-C motif) receptor 4/12 (CXCR4 and CXCL12) and TLRs, provide even more opportunities for microglia–MC interaction (reviewed in [3]) suggesting that MCs have a large role in the normative functioning of

microglia, as well as modulating host responses to infection, inflammation, trauma, and stress.

Bidirectional crosstalk between MCs and glia is not limited to microglia. MCs are apposed to astrocytes in the perivascular space, particularly along the thalamic border region of the habenula in the brain [41]. *In vitro* astrocytes can support rat serosal MC viability and, when MCs and astrocytes are co-cultured, MCs are stimulated to release histamine, leukotrienes, and cytokines, likely via activation of CD40–CD40 ligand (CD40L) interactions [41,42]. Significantly, astrocytes have histamine receptors [43] and release cytokines that are capable of inducing MC degranulation [44], further demonstrating the potential for MC–astrocyte crosstalk. Additional *in vitro* work has shown that activated MCs (specifically MC proteases) can induce demyelination and apoptosis of oligodendrocytes, whereas myelin itself stimulates MC degranulation [45]. Although important for normative brain function, these interactions are particularly relevant during neuroinflammation (see later).

The MC–brain–behavior link in health

Fluctuating populations of brain MC

There is substantial evidence that the brain and meningeal population of degranulating MCs fluctuates dynamically during the normal course of behavioral and physiological events, and during development. In all species investigated, MC numbers, distribution, and activation state dramatically shift in response to a wide variety of environmental stimuli, such as sexual and courtship interactions, exposure to opposite sex odors, chronic subordination stress, restraint stress, isolation stress, social housing, aggression, and tactile stimulation, likely in response changes in internal physiological and neurohormonal states [15,16,46–48]. Indeed, it is known that MC number and degranulation are regulated by hormones, such as gonadal steroids, corticotropin-releasing hormone (CRH) [49], various other neuropeptides [50], and nerve growth factors [51]. Furthermore, in some loci, MCs express receptors for estrogen, progesterone [15,52], and CRH [53], as well as having the capacity to synthesize hormones, such as gonadotropin-releasing hormone [47] and CRH [54]. Importantly, MCs in their neural loci are constantly degranulating to some extent (Figure 3) and, when a greater number of brain MC is present, there is an increase in the proportion that are degranulating [55]. These developmental and induced changes in brain MC population and state of degranulation enable targeted delivery of MCs and their mediators to specific brain regions, altering BBB permeability and modulating normal brain functioning [56].

Behavior-modulating role of MCs

Given their potent mediators and ability to signal multiple CNS elements, it is not surprising that MCs are implicated in many behaviors. The most compelling evidence derives from convergent results of studies using various experimental methods such as MC-deficient or -overexpressing animals, pharmacological manipulation of central MC populations, and multiple behavioral measures. For instance, MC-deficient *Kit*^{W-sh/W-sh} (*Wsh/Wsh*) mice exhibited elevated anxiety-like behavior [56]. Importantly, this

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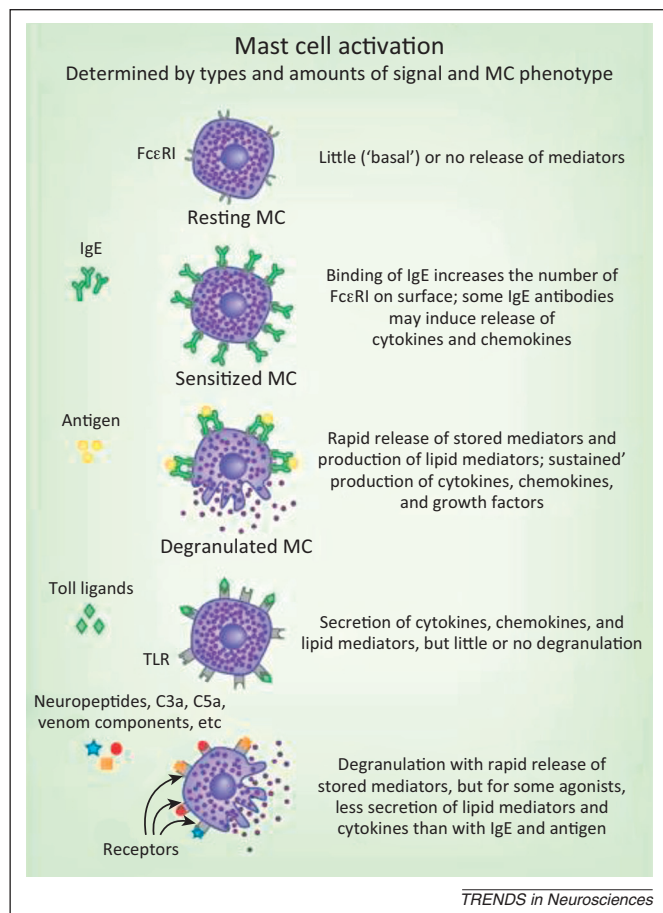


Figure 3. The schematic depicts the patterns of functional activation of mast cells (MCs). MCs can be activated by crosslinking of immunoglobulin E (IgE) bound to high-affinity receptors for IgE (FcεRI) on the cell surface or by antigens, resulting in rapid exocytosis of the cytoplasmic granules (degranulation) followed by production of lipid mediators and then by cytokines, chemokines, and growth factors. Other signals, such as Toll ligands, neuropeptides and so on, can elicit different patterns of mediator release in various MC populations (from [104]). Similar to the data depicted in this generalized schematic, brain MCs studied at the electron microscopic (EM) level display distinct states, including (i) resting; (ii) initiation of degranulation; (iii) fully degranulated; (iv) piecemeal secretion; and (v) resynthesizing [55]. The EM evidence indicates that the MC population is never entirely at rest, and that some MC degranulation occurs even under basal conditions. Abbreviation: TLR, Toll-like receptor.

was not a nonspecific effect because MC deficiency did not alter baseline activity or sensory responses to tactile, olfactory, vestibular, auditory, or pain stimuli. These mice also had significantly reduced whole-brain levels of histamine compared with controls. In wild type mice, central, but not peripheral injections of cromolyn disodium cromoglycate (cromolyn), a MC-stabilizing agent, increased anxiety-like behaviors [56]. By contrast, MC degranulation with c48/80 induced anxiety-like behavior in wild type mice but not in MC-deficient W/W^v mice, a finding that was dependent upon the activation of histamine H1 receptors [57].

Pharmacological studies of rodent models support a link between MCs and cognitive behaviors. MC-deficient Wsh/Wsh mice are significantly impaired in hippocampus-dependent tasks of spatial learning and memory [58]. These impairments are associated with a loss of hippocampal neurogenesis specifically in the dentate gyrus. Both brain and behavioral deficits in Wsh/Wsh mice are restored

by elevating central serotonin levels by chronic treatment with fluoxetine (a selective serotonin reuptake inhibitor) [58]. Furthermore, mice induced with an asthmatic or food allergy learnt to avoid a compartment of an apparatus that had been previously associated with the allergen, a result that could be abolished by pretreatment with the MC degranulator inhibitor cromolyn [59].

The behavioral role of MCs extends beyond emotional and learning behaviors. For instance, the intensity of locomotor activity has been associated with degranulation of meningeal MCs, possibly mediated by the actions of histamine released by MCs on its H1 receptor [60]. Congruently, blocking MC degranulation with cromolyn inhibits other aspects of locomotor activity, such as naloxone-induced withdrawal jumping behavior in morphine-dependent mice [61]. There is also evidence that MC degranulation inhibits food intake in both rats and neonatal chicks [62,63]. Furthermore pretreatment of rats with cromolyn before a restraint stress protected them from exhibiting reduced exploratory and social behavior post stress, suggesting that MCs modulate both behavioral and physiological responses to stress, as well as some social behaviors [64].

Taken together, there is widespread evidence for a functional role of MCs in modulating behavior. However, in most cases, it remains to be discovered which mediator or mediators released upon degranulation are responsible for particular behavioral changes. Another important question is to what extent MCs regulate behavior by altering brain physiology in the short-term following exposure to a particular triggering stimulus versus their impact in shaping long-term behaviorally relevant structural changes to the brain (e.g., modulation of neurogenesis), both developmentally and during adulthood. Finally, MCs may both augment and attenuate the same behaviors (e.g., anxiety). This likely points to a wider principle of MC biology, which is that their effects are strain, tissue, and stimulus specific [65]. Indeed, MC-associated changes in behavior are likely the consequence of a shift in the central balance of numerous mediators and thus, a simple linear relation between MC number, degranulation, and behavioral endpoints is not expected.

Disease and injury

Given their heterogeneity, first-responder capability, and positioning to signal each of the elements of brain, it is perhaps not surprisingly that MCs have been considered a participant in almost all major CNS disease state, including autism, Parkinson's, and Alzheimer's diseases [20,66]. Humans and rodents with allergic rhinitis (which occurs in part as a consequence of MC degranulation) express fatigue, elevated anxiety, and mood changes [67,68]. Significantly, animal studies indicate that some of these allergy-induced behavioral alterations are MC dependent [59]. Patients with mastocytosis, who produce an overabundance of MCs, report several psychopathological symptoms including headaches, increased pain sensitivity, lethargy, cognitive dysfunction, and depression [69,70]. In this section, we summarize findings in three diseases from among the many that have been implicated in MC function: multiple sclerosis (MS) and experimental autoimmune

encephalomyelitis (EAE), migraine and pain sensitivity, and cerebral ischemia.

MS and EAE

Significant accumulations of MCs are found in the white matter and demyelinated lesions and/or plaques of patients with MS, and MC tryptase is elevated in their cerebrospinal fluid (CSF; reviewed in [71]). The most commonly used animal model of MS is EAE. This paradigm parallels MS in that significant demyelination and axonal transection occurs due to a T cell-mediated autoimmune response against proteins in the myelin sheath following an influx of immune cells into the CNS through the BBB [72]. Rats and mice with EAE exhibit increased degranulation of their brain MCs [41] and elevated levels of the demyelinating MC protease [73]. Perivascular MCs may also be the source of vasoactive and proinflammatory mediators that increase the permeability of the BBB, which typically precedes the development of pathological features of MS or EAE [74]. Pharmacological studies confirm a role for MCs in regulating EAE pathophysiology. The severity of EAE can be reduced by preventing activation of MCs by administration of the MC stabilizer picroximil, the H1 receptor antagonist hydroxyzine, or intracisternal administration of c48/80 before immunization (reviewed in [18]).

Research on MC-deficient mice has generally, but not universally, supported this facilitatory role [18]. MC-deficient W/Wv mice displayed significantly reduced levels of primary progressive EAE symptomology compared with controls, using a myelin oligodendrocyte glycoprotein (MOG)-induced model of EAE. This was abrogated following reconstitution of the MC population, even though reconstituted MCs are not necessarily restored to the brain in this mouse line. Significantly, a reduced autoreactive T cell response was also observed using this EAE model in W/Wv mice, but this response was restored and associated with normal T cell activity following MC reconstitution [75]. Given the numerous molecular mechanisms through which MCs are able to influence T-cell functioning, these data suggest that peripheral MC activation is a significant contributor to T cell activation and subsequent EAE disease pathogenesis [18]. Furthermore, following EAE induction in this model, MCs in the meninges were activated to release TNF- α , which recruits neutrophils and leads to increased permeability of the BBB to inflammatory cells, resulting in myelin damage [76]. Accumulating thalamic MCs following EAE induction may also interact with astrocytes to facilitate further BBB breaches [41].

Therefore, it has been argued that MCs regulate both the induction and effector phases of EAE via their involvement in the autoimmune response in peripheral lymphoid organs and their gatekeeping role at the BBB [18]. Interestingly, recent work with W/Wv mice of the SJL strain demonstrated that genetic background can influence the time course and severity of EAE and that MCs are also key modulators of relapse-remitting EAE as well as primary progressive EAE [77]. By contrast, this important regulatory role of MCs is questioned by researchers using other EAE models in which no differences in, or even exacerbated, EAE pathologies were found in W/Wv, Wsh/Wsh and

Box 1. Outstanding questions

- Elegant *in vitro* work has revealed bidirectional crosstalk between MCs and microglia and macroglia, and between MCs and sensory neurons. Future work needs to confirm and extend these findings *in vivo* as well as exploring the interactions between MCs and neurons of the brain.
- One exciting new technical innovation is the ability to isolate MCs directly from the brain. This will open new possibilities for studying MCs in the brain [105].
- More needs to be addressed with regard to how MCs can modulate behavioral phenotypes. Behaviors studied to date are limited in the main to emotional and cognitive domains, and other behaviors need to be investigated. Questions yet to be determined include: why and how do MCs both attenuate and augment the same behaviors? Which mediators are likely to be the key modulators? Do MCs exert both short-term and long-term effects following degranulation? Are MCs more critical for shaping behavior during development or during adulthood?
- In various animal models of neuroinflammation (e.g., MS) and CNS damage (e.g., ischemia), MCs and their mediators have been found to be damaging to the brain, neuroprotective, or even neutral in their effects. Therefore, there is a need to further understand across these various diseases what the commonalities and differences are in the role of MCs and how they act to both augment and attenuate pathologies.
- Some recent studies have challenged the specificity and efficacy of traditional pharmacological compounds (cromolyn and c48/80) for studying MCs. The full significance of these findings has yet to be evaluated for MCs in the brain [106,107].
- Until recently, studies using MC-deficient mice have typically utilized mice (e.g., W/Wv or Wsh/Wsh) that have abnormal expression of the c-kit gene, which is necessary for the development and survival of MCs (see [108] for an excellent in-depth recent review). However, these mice also have several other abnormalities associated with altered c-kit expression. A new generation of MC-deficient mice has been generated in which the expression of Cre recombinase is placed under the control of MC-specific gene promoters and, therefore, is independent of the c-kit mutation and associated complications. These new-generation MC-deficient mice are promising tools for future studies, as is the creation of inducible depletion MC mouse models [109,110].
- New techniques, such as capillary electrophoresis and/or mass spectrometry, along with whole-genome expression arrays, enables small-volume analysis characterization of volume-limited brain regions [18,71,93]. In future studies, this may allow for the examination of the sequelae of MC activation and stabilization under varied developmental and disease states, as well as adoptive transfer of MCs that lack one or another mediator into MC-deficient mice to study changes in behavioral and brain functioning.

Cpa3^{Cre/+} MC-deficient mice [78–80]. Some of these discrepancies may be due to differences in environmental factors, genetic background, and associated complex hematopoietic alterations of each MC-deficient mouse strain or the method of EAE induction. Future work based on converging evidence from a variety of protocols and newly developed transgenic mice (Box 1) will help delineate the exact role of MCs in EAE pathophysiology.

Migraine and neuropathic pain

Migraine is persistent and throbbing pain believed to result at least in part from inflammation of the intracranial cerebral meninges and the resultant vasodilation and ongoing activation of primary afferent nociceptive neurons. There is good evidence to suggest a role of MCs in migraine pathophysiology. MCs localize within the dural layer of the

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meninges in association with blood vessels and the terminals of meningeal nociceptors [81]. Humans with mastocytosis or MC activation syndrome are more prone to suffer from headaches as well as visceral pain [82]. Furthermore, injection of c48/80 into the cranial circulation induces migraine, which can be prevented by cromolyn administration. Several MC products, such as histamine, TNF- α , IL-6, endothelin-1, and leukotrienes, can also promote migraine (reviewed in [81]). Thus, it has been hypothesized that degranulation of MCs in the meninges induced by exogenous and endogenous triggers, such as stress, hormonal imbalance, or various neuropeptides, may be a contributing factor to migraine pathophysiology [17]. Congruent with this hypothesis, degranulation of MCs can prolong activation of meningeal nociceptor afferents, which can be blocked by cromolyn treatment [83].

MCs may also function to modulate peripheral pain sensitivity. MCs lie in close proximity to nociceptors but increase in number in response to nerve injury, releasing mediators such as histamine and NGF that sensitize these nociceptors, ultimately leading to increased pain hypersensitivity (hyperalgesia) as well as activating trigemino-cervical and lumbosacral pain pathways [84,85]. Similarly, MC degranulation by c48/80 induced increased hyperalgesia in mice, a response that was abrogated in *Wsh/Wsh* mice but not if these mice had their MCs restored to wild type levels by plantar MC reconstitution. This particular MC degranulation initiation of peripheral inflammatory pain appears to be dependent upon localized neutrophil influx and histamine release [86]. The degranulation of MCs following a thermal pain exposure requires direct contact between this immune cell and peripheral nerve terminals, which is mediated by the calcium-dependent cell adhesion molecule N-cadherin (N-cad). Mice that lack the matrix metalloproteinase MT5-MMP, which is expressed by peripheral nerves and cleaves N-cad, expressed higher levels of N-cad, had much closer MC-nerve interactions, enhanced MC degranulation, and elevated thermal pain sensitivity [87]. Congruent to these findings, MC stabilizers can inhibit hyperalgesia resulting from nerve injury and postoperative pain [85,88].

Cerebral ischemia

Well documented and amply reviewed in both the adult and the immature brain is the participation of MCs in ischemic brain injury [19]. In brief, the inflammatory response triggered by stroke is associated with the influx of many blood-borne cells, including leukocytes, neutrophils, and macrophages [89]. MCs differ from other hematopoietic cells in that they are resident in the brain and meninges and are first responders, even before microglia [90]. They can act rapidly on the cerebral vessels and other CNS compartments during the very earliest phase of acute cerebral ischemia and hemorrhage by releasing their preformed cytoplasmic granules containing a host of readily available vasoactive and neuroactive mediators. MCs act on the basal membrane, promoting BBB damage, brain edema, prolonged extravasation, and hemorrhage. Indeed, MC stabilization inhibits hypoxic ischemic-induced brain damage [91]. Furthermore, following even acute ischemic brain injury, cerebral MCs amplify and prolong the endothelial expression of adhesion

molecules and the continued breakdown of the BBB, thereby enabling the infiltration of other blood-borne cells and signals [19,92].

Concluding remarks

In sum, there are similarities between the role of MCs in allergy and asthma and in their regulation of disease progression and response to injury in the CNS. In particular, MCs function as first responders at sites of injury or infection, are rapidly activated to degranulate and release preformed and newly synthesized mediators. Intriguingly, the recruitment of TNF- α and neutrophils to these sites appears to be a common mechanism through which MCs promote continued inflammation and disease exacerbation. However, it remains a challenge to elucidate the degree of involvement of brain MCs in these processes, especially because of their ubiquitous distribution, mobility, and multifunctional nature.

MCs, similar to many cells of the immune system, are multifunctional and have extensive crosstalk with the nervous system. In evolution, old systems are usurped for new roles, often without diminishing older functions. The MC is an ancient cell that derived from ancestral leukocyte cells at least 500 million years ago; throughout evolution, MCs have acquired novel molecular phenotypes in fulfilling specific functional roles. MCs have been considered a first line of defense against pathogens, allergens, toxins, and tissue injury, but with the further accumulation of various membrane-bound receptors and granule-contained mediators, MCs have diversified into regulating many novel normal physiological functions within the CNS.

This is an exciting time for researchers studying brain MCs. Despite their considerably smaller number in comparison to other immune cells of the brain, there is accumulating evidence from many vertebrate species that MCs and their mediators are key catalysts, integrators, and amplifiers of chemical signaling among blood vessels, neurons, astrocytes, and glia, as well as being modifiers of behavior under both physiological and pathophysiological conditions. (Table 1 summarizes some of the known functional roles of these MC mediators in the CNS and peripheral nervous system described in this review). Nevertheless, much remains to be determined regarding the exact scope and nature of their involvement, including whether they contribute to disease states over the long term. Among the challenges to better understanding brain MCs are some that are common to exploring any neuroimmune relation. In particular, we stress the critical need for converging evidence from a variety of paradigms and sources (*in vitro* work, *in vivo* pharmacological studies, and joint explorations of metabolomics and genomics [93] in established and new generations of transgenic mice) to appreciate fully the tremendous importance of these unique immune cells in the brain during development and adulthood.

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