The majority of brain mast cells in B10.PL mice is present in the hippocampal formation

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Abstract

In the healthy mammalian CNS, mast cells (MCs) are thought to be located mostly in the thalamus. In this study, we have systematically assessed the presence of MCs in the hippocampal formation (HF) and in the thalamus of normal male and female B10.PL mice. Giemsa+ and Toluidine Blue+ MCs were detected by histomorphometric analyses at perivascular and intraparenchymal sites of both the hippocampus and the entorhinal cortex. We found a mean number of 4.4 MCs in the HF of female and 3.3 MCs in male B10.PL mice. In contrast to the HF, no MCs were present in the thalamus of these mice. Notably, all HF-MCs showed immunoreactivity for Kit, the receptor for the MC growth and maturation factor SCF, as assessed by FITC–avidin/Kit double labelling. We demonstrate that the majority of brain MCs is found in the hippocampus and entorhinal cortex of B10.PL mice, though the total number of MCs is small compared to other mouse strains or rats. The presence of most brain MCs in the HF of B10.PL mice suggests a potential role of MCs in hippocampal physiology and pathology.

Keywords: Hippocampus; Entorhinal cortex; Thalamus; Giemsa; Toluidine Blue

Mast cells (MCs) are abundantly located at host/environment interfaces, i.e. the skin, airways and gut, where they are known to contribute significantly to the induction of inflammation in the context of allergic reactions and innate immune responses to pathogens [8,11]. In contrast, MCs are scarce (or not expressed at all) at anatomical sites that are generally protected from allergens or pathogens, such as the bones or the brain [23]. MCs in the healthy brain of rats are located mostly in the thalamus [4], where they are thought to contribute to sensory processing, blood vessel permeability and local haemodynamics [27]. Brain MCs are increased by stress [5,24] and highly sensitive to reproductive hormones [2,26,29,30].

Mast cell-derived histamine was reported to potentiate synaptically mediated excitotoxicity in hippocampal neurons of mice in vitro [19] and mast cell activation reportedly promotes delayed neurodegeneration in murine mixed neuron–glia hippocampal cultures [20]. Furthermore, genetically MC-deficient mice, i.e. Kit+/Kit+ - d mice, have been shown to be deficient in hippocampal learning [13], which might be associated with MC function.

Because these observations suggest a role of MCs in the physiology and pathology of the murine hippocampal formation (HF), we have carefully investigated normal adult mice of both sexes for the expression of MCs in the hippocampus and entorhinal cortex by quantitative histomorphometry using highly sensitive and specific MC markers. In addition, we have compared MC numbers in the HF and in the thalamus, where most of the brain MCs were expected. We demonstrate that most brain MCs in healthy B10.PL mice are present in the hippocampus and entorhinal cortex, albeit in small numbers, whereas there are no MCs in the thalamus.

Brain sections were prepared from a total of 30 female and 20 male 7-week-old B10.PL-H2u H2-T18a/JNS/Jsn mice obtained from Jackson Laboratories (Boston, MA, USA). The animals were housed separately by sex, four females or four males per cage in a conventional, non-SPF animal facility, and allowed to acclimatize for at least 1 week prior to use.
were allowed free access to food and water, and housed in a room with a constant temperature of 23 °C on a 12-h light:12-h dark cycle. All experiments were performed in compliance with the German guidelines on the use of laboratory animals.

Brain samples were processed for routine histology following standard protocols [16]. In brief, three different fixation protocols were used because MCs may exhibit site-specific sensitivity/resistance to formalin fixation: (1) transcardial perfusion with 4% PFA, 4% PFA fixation overnight and paraffin embedding; (2) PFA-free phosphate buffered saline (PBS) perfusion and Carnoy fixation; (3) PFA-free PBS perfusion and cryo-embedding. Interestingly, MCs were best demonstrated (both, in terms of numbers and morphology) in formalin-fixed brain tissue (PFA > Cryo > Carnoy).

MCs were detected in Giemsa-stained sections. Additionally, slides were stained with 0.5% Toluidine Blue O in 0.5 N hydrochloric acid (Merck, Darmstadt, Germany) and counterstained with eosin [12,14,25]. FITC–avidin labelling and Kit immunoreactivity were detected as described previously [3,15].

For quantitative histomorphometry, serial sections (thickness: 7 µm) of both hemispheres of the entire HF in all mice were analysed at 400× magnification. Serial sections were numbered and every odd-numbered section was Giemsa stained, while every even-numbered section was Toluidine Blue stained. The HF was defined according to Amaral and Witter [1] as a structure including the dentate gyrus, hippocampus proper, subicular complex and entorhinal cortex. A ‘perivascular’ MC was defined as an MC in direct contact with a blood vessel (lumen demarcated by spindle-like endothelial cells); a ‘parenchymal’ MC was defined as an MC embedded within the brain tissue (PFA > Cryo > Carnoy).

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Giemsa-stained MCs were detected by quantitative planimetric histomorphometry in the HF of all mice analysed (Fig. 1). Notably, all MCs exhibited strong metachromatic staining of densely packed cytoplasmatic granules and, thus, resembled connective tissue type MCs. Within the HF, MCs were detected in both the hippocampus and the entorhinal cortex (Fig. 1A–R).

Both hippocampal and entorhinal cortex MCs were located primarily in the direct vicinity of blood vessels (Fig. 1A–F, J–L). In some mice, the sequential sections revealed the presence of MCs in the parenchyma of the entorhinal cortex, i.e. at non-perivascular sites (Fig. 1G–I). No MCs could be detected in the thalamus of any mouse (Table 1). Virtually identical patterns of MC distribution were found using HF sections stained with Toluidine Blue, a classical marker which is highly specific and sensitive marker for MCs (Fig. 1M–R). Interestingly, all perivascular and intraparenchymal MCs detected in the hippocampus and the entorhinal cortex by FITC–avidin labelling were found to express Kit, as assessed by double immunofluorescence labelling with FITC–avidin and anti-Kit antibody (Fig. 1S–U).

To analyse whether MC presence in the HF or in the thalamus is dependent on the sex of the mice, we have compared perivascular and parenchymal MCs in the hippocampus and entorhinal cortex of age-matched female and male mice (Table 1). The mean number of parenchymal MCs in both hippocampi of female mice was 1.3 and 1 MC in both entorhinal cortices. The mean number of perivascular MCs in both hippocampi was 2.5 and in the entorhinal cortices 0.4 (Table 1). Male mice displayed a mean number of 3.6 perivascular MCs in both hippocampi and one in the entorhinal cortices. Male mice did not express any parenchymal MCs. No MCs were present in the thalamus of any female or male mouse (Table 1).

The presence of MCs in the brain was first reported by Ehrlich [7] more than a century ago. In healthy mammalian brains, MCs occur only in small numbers compared to other organs such as the skin or the gastrointestinal tract. Mammalian brain MCs have been reported to be mainly concentrated in the leptomeninges, the dura mater, the choroid plexus and the parenchyma of the thalamic–hypothalamic region, where they are generally found along the blood vessels (reviewed in [28]). In this study we could demonstrate that MCs are consistently expressed within the hippocampus and entorhinal cortex but not in the thalamus of normal adult B10.PL mice. In the present study we found no MCs in the thalamus of female or male mice. However, we found a mean number of 4.4 entorhinal–hippocampal MCs in female and 3.3 MCs in male B10.PL mice. Thus, brain MC numbers are low in B10.PL mice and they are found in the HF and not in the thalamus. This is in striking contrast to the findings in rats where MC numbers in the HF are much higher [6,10] and 98% of all brain MCs are located in the thalamus [4,27]. This is also in sharp contrast to Swiss Webster mice, which display high numbers of MCs in the thalamus (approximately 50–100 MCs per thalamus in untreated animals) [22]. The presence of the majority of brain MCs in the HF of B10.PL mice suggests a potential role of MCs in strain-dependent hippocampal physiology and pathology.

Interestingly, all intraparenchymal MCs in the HF displayed c-kit immunoreactivity. This is in contrast to the findings of

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<td></td>
<td>Hippocampal MCs</td>
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<td>Female parenchymal</td>
<td>3.3 ± 0.25</td>
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<td>Male parenchymal</td>
<td>0</td>
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<tr>
<td>Female perivascular</td>
<td>2.5 ± 0.55</td>
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<tr>
<td>Male perivascular</td>
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The number of metachromatic parenchymal and perivascular mast cells in the hippocampus, entorhinal cortex and thalamus of age-matched, female and male B10.PL mice was investigated in Giemsa-stained sections of 4% paraformaldehyde (PFA)-fixed, paraffin-embedded brains. MC numbers are indicated as mean ± S.E.M.
Fig. 1. Mast cells in the hippocampal formation. Giemsa staining (A–L), Toluidine Blue with eosin counterstaining (M–R), and double labelling with FITC–avidin and anti-kit antibody (P–R) in sections of murine hippocampus and entorhinal cortex. Boxes indicate where the adjacent picture has been photographed at a higher magnification. (A–C) Perivascular MCs were detected by Giemsa staining in the hippocampus (A–F) and entorhinal cortex (J–K). Intraparenchymal MCs were found in the entorhinal cortex (G–I). Toluidine Blue revealed similar distribution patterns of intraparenchymal and perivascular MCs in the hippocampus (M–O) and entorhinal cortex (P–R). Double labelling with FITC–avidin (S) and for kit (T) revealed a co-localisation of these two markers in all perivascular and intraparenchymal MCs in the hippocampal formation (R). Bars = 40 μm.

Other authors, who have demonstrated intraparenchymal MCs of mice in the inferior colliculus, medial and lateral geniculate, and dorsal and ventral thalamic nuclei to be devoid of c-kit immunoreactivity [18]. However, the total MC numbers are low in the brain of B10.PL mice and it is not excluded that single KIT-negative MCs have escaped notice.

The hippocampus is highly sensitive to the neurotoxic effects of hypoxia [9] or stress [17]. Hippocampal MCs might be involved in hypoxia- or stress-induced hippocampal neurodegeneration, as MCs have been shown to degranulate during hypoxia and stress, and blockade of hypoxia-induced degranulation with a mast cell stabilizer attenuates the hypoxic
inflammatory response [21]. It is, therefore, a promising aim to identify those factors that influence the number of MCs in the hippocampus and entorhinal cortex and to characterize their potential role in hippocampal physiology and pathology.

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