# Introduction
# The Blood-Brain Barrier: An Integrated Concept

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Writing about the blood-brain barrier (BBB) these days has become a multidisciplinary enterprise. While the classic view formulated by the pioneering work of Ehrlich (1885) and Goldman (1913) was based on evidence that blood-borne substances were excluded from the brain, this concept was systematically reformulated over the past century, cumulating in the compartment concept put forward by Davson (1967) in which he redefined the BBB as the "sum of all bidirectional exchange processes which occur at the morphological blood-brain interfaces". This definition has shifted the more or less endotheliocentric view to a more integrated concept that takes into account not only the bidirectionality of the exchange processes, but also the discovery that besides the endothelium additional components constitute integral parts of the barrier mechanisms. These additional components include the perivascular structural adjuncts such as pericytes, microglia and macrophages and the astroglial interface, interacting in an orchestrated fashion in order to achieve the "functional complex" that allows the transmission of metabolic and homeostatic information between the blood and the brain parenchyma and vice versa. In a former review on the BBB, one of the editors of this handbook described the function of this barrier as a dynamic *homeostat* (Dermietzel and Krause 1991) that regulates the interchange between the body and the brain border. By its nature, this homeostat is not simply constituted by the sum of its parts. The integration of all molecular and structural components and their concerted interplay at the critical locations gives rise to the "more" that we believe is the essence of synergism. Consequently, when conceptualizing this handbook we had to take into account the entire spectrum of disciplines that contribute to our modern understanding of the BBB. The picture we tried to paint cannot, of course, be complete and the reader may find missing a contribution that exactly covers his field of interest. In this case, we must direct the interested scholar to the list of references that are included in each of the articles and which may be well suited as a guideline for further deepening his knowledge.

The collection of articles is thus structured in a way that reflects our understanding of the BBB as a dynamic homeostat. The first part deals with the onto-

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genesis of brain vasculature and begins with a basic article written by *Britta Engelhardt* that not only describes the maturation of the BBB and its structural substrate, but also goes into details regarding molecular aspects of the developmental processes. What we have learned during recent years, especially through the work by the late *Werner Risau* and his coworkers, is that when the BBB is first established it is by no means a static structure, but rather a dynamic construct that requires permanent feeding by growth and differentiation factors in order to maintain and elaborate its complex machinery as an exquisite barrier. As we now know, the microvasculature is not uniformly tight throughout the brain, but is a composite building that contains segments of leakiness integrated into the mass of tight vessels. These leaky segments prevail at sites where a requirement for differential exchange between the blood site and the brain site (or vice versa) exists, i.e. neurohaemal regions which entail neurosecretory and neurosensory areas for controlling specific parameters of the body fluid. An understanding of how the brain manages to keep leaky segments maintained in an embedment of tight vessels will provide a key to the understanding of BBB development. The article by *Jeong Ae Park, Yoon Kyung Choi, Sae-Won Kim* and *Kyu-Won Kim* focuses on some of these factors with emphasis on hypoxia inducible factor (HIF), VEGF and other neuroglia-derived factors. It describes the main sources of these potent humoral factors and their effects during angiogenesis on the developing brain including aspects for barriergenesis.

An extension of the discussion on humoral regulation of brain angiogenesis is provided by *Christina Lilliehook* and *Steven Goldman*. Their focus is on a rather new and exciting field of BBB research which considers the brain microvasculature as a niche that conditions brain stem cells for further lineaging. The observation that hippocampal stem cells before they integrate into the granular cells of the dentate gyrus associate with cerebral capillaries has made this niche a favorable subject for unravelling the conditioning mechanisms taking place at the sites of contact. With the concept of a *vascular niche* a further aspect of the BBB emerges, namely that brain vessels not only provide targets for autocrine and paracrine regulation to maintain their own degree of differentiation, but constitute a putative source of fostering brain stem cells.

Part II is devoted to the different structural components that define the BBB. Without surprise, this part begins with an account on our recent understanding of the structural and molecular complement of the cerebral endothelium written by *Hartwig Wolburg*. The endothelium plays a crucial role in separating the blood from the two major fluid compartments of the brain: the interstitial cerebral compartment and the cerebrospinal compartment. As mentioned above, this separation entails a constitutive dynamic component which is responsive to the actual metabolic situation on both sites of the barrier. The chapter on the endothelium is not intended to give a complete survey covering all the various carrier and transport mechanisms taking place at the endothelial frontier. Such an encyclopedic detailed overview is outside the scope of this handbook; and we refer here to recent reviews and monographs that deal with these particular

aspects of the BBB (Pardridge 1993; Paulson et al. 1999; Nag 2003). Rather, this chapter presents the major facts and our recent knowledge regarding the two major components of the barrier: its structural and metabolic substrates.

The subsequent articles in this Section concentrate on the cellular adjuncts that surround the vessel wall and should be highlighted together in our conceptual context. Pericytes, presented by *Markus Ramsauer,* brain macrophages by *Frederic Mercier, Sebastien Mambie* and *Glenn Hatton* and microglial cells by *Ingo Bechmann, Angelika Rappert, Joseph Priller* and *Robert Nitsch* are all cellular components which permanently or transiently settle in the perivascular space. Cerebral pericytes have long been neglected by the BBB community although already described in the last third of the 19th century by Rouget. In spite of the fact that brain and retinal capillaries possess the highest density of pericytes per endothelial cell (about 1 : 3), their functional properties and morphological phenotype are still difficult to describe. They seem to be involved in microvascular perfusion by reciprocal contraction as well as in immune responses, a property that has god-fathered them as a "second line of defense" by their ability to enfold macrophagic activity under challenging conditions. Recent discoveries feature pericytes as key players of BBB differentiation, together with astrocytes and regulators of endothelial growth as well as stabilizers of the vascular wall. Their potential to differentiate into other mesenchymal cell types makes them a versatile cellular pool with progenitor features for macrophages and even endothelial cells.

Macrophages, which are of mononuclear origin, reside in the whole CNS vasculature. Together with microglial cells they are responsible for the immune response of the brain and their mostly perivascular association makes them part of the BBB complex. Macrophages are potent producers of extracellular matrix molecules, growth factors and cytokines and are thought to play key roles in regulating glial and neuronal cell function. In contrast to microglial cells which have been detected by recent efforts (Nimmerjahn et al. 2005) to be primarily resident even when BBB injury commences, macrophages seem to be more migratory, patrolling the brain parenchyma and cleansing it from debris after injury or inflammatory attacks. A recent concept pointed out by the paper of *Frederic Mercier, Sebastien Mambie* and *Glenn I. Hatton* calls attention to the presumed function of macrophages as partners in the neurogenic niche, which is supposed to play a crucial role in neural precusor or stem cell priming. Microglial cells are by far the most abundant cellular entity (about 15% of brain cells) involved in the immune response of the CNS. They serve as sensors for pathologic events in the brain tissue and reside in the brain parenchyma and in perivascular and juxtavascular positions. Here, besides their paramount function as executors of the innate and adaptive immune responses of the brain tissue, they seem to function in concert with perivascular macrophages in regulating the tightness of the barrier through chemokine secretion. Both cellular entities, perivascular macrophages and microglial cells, perform fundamental functions in the regulation of the leakiness of the BBB under inflammatory conditions, an issue that is addressed in more detail in part VII of the handbook.

The following two articles by *Joan Abbott* and the group of *Antonio Frigeri* (*Grazia P. Nicchia, Batrice Nico, Laura M.A. Camassa, Maria G. Mola, Domenica Ribatti, David C. Spray, Alejandra Bosco, Maria Svelto* and *Antonio Frigeri*) concentrate on the astroglial surrounding of the blood-brain interface. Since the work by Goldstein and Betz (1986) and later by Janzer and Raff (1987), the astroglia and in particular the perivascular endfeet have been considered to be crucial elements for maintaining the BBB complex. Astrocytes not only participate in regulating transport through the endothelium and provide trophic support for the tightness of the endothelium, but they also provide the essential link for vascular-neuronal signaling. Because of their ideal strategical position and their molecular and structural polarization, the astroglia are destined to link the neuronal site with the vascular bed of the brain parenchyma and serve as a pathway for metabolic and ionic transfer. Most importantly, astrocytes seem to represent the most dynamic part of the BBB complex, since they are able to respond to neuronal activity and transmit signals to the blood front to regulate local perfusion. Thus, the astroglia are an essential part of an integrated concept of the BBB, not only in terms of their morphogenetic capabilities, but also as transformers of neuronal activation into BBB receptive signals.

Part III is devoted to hormonal and enzymatic control of the brain vessels. The initial article by *Bernhard Reuss* centers on the role of fibroblasts growth factors (FGFs) in regard to their influence on growth and differentiation of brain microvascular endothelium, and thus their involvement in establishing, maintaining and restoring the BBB. In particular, FGF-2 has come into focus as an important cofactor together with secretory products of astrocytes (TGF-$\beta_1$, GDNF) involved in the induction of certain specific barrier properties of brain microvessels. Also, the effect of FGF-2 to influence the synthesis and phosphorylation of the intermediate filament protein GFAP of astrocytes, which has been proven to be important for inducing the BBB phenotype, is covered here, to provide insight of the importance of the FGF family for the stabilization and preservation of BBB features.

This issue of factors that influence the functional properties of the BBB by humoral and enzymatic inputs is further considered by four articles. First, the contribution of *Weihing Pan, Shulin Xiang, Hong Tu* and *Abba Kastin* presents a general overview on the interaction of cytokines with the BBB. In particular, the mechanism of cytokine transport across the endothelial cells is described, as is their action on these cells, which results in altered endothelial function, cytotoxicity or cell proliferation. This article is followed by a description of insulin transport through the barrier and the effect of insulin on the BBB in concert with proinflammatory cytokines, authored by *William Banks* and *Wee Shion Lim*. It also addresses the important clinical issue of insulin resistance and its impact on the inflammatory susceptibility of the brain vasculature, an issue that is of profound socio-economic importance in view of the increasing number of obese people. The reviews on humoral effects on the BBB is completed by *Jean Bernard Dietrich*, who gives a detailed account of the interaction of glucocorticoid hormones and estrogens on the endothelium of the BBB. The focus of this

contribution lies on the influence of glucocorticoids and estrogens with regard to their expression of endothelial adhesion molecules that are pivotal for the transendothelial migration of inflammatory cells through the BBB. In this context, their use as therapeutic tools for the treatment of autoimmune diseases is discussed. Part III on hormone and enzyme interaction ends with the chapter of *Dorothee Krause* and *Christina Lohmann* on metalloproteinases and the brain microvasculature. Besides the structural sealing of the BBB endothelium through tight junctions and its complement of specific transporters and carriers, the vascular wall of brain vessels is endowed with a battery of enzymes constituting a kind of "enzymatic barrier" to the passage of peptides across the BBB. These enzymes are proteinases and have been detected in variable amounts at the BBB. Among them are the metalloproteinases, which are involved in the cleavage of peripheral peptides as well as centrally released peptides, in remodelling the extracellular matrix during angiogenesis and in facilitating perivascular penetration of emigrating blood-borne cells, including tumor cells. The complexity of the metalloproteinases (MPs), and in particular the matrix bound subgroup (MMPs), for the function of the BBB are thoroughly reviewed in this paper.

Part IV centers on different approaches that have been taken to establish *in vitro* systems for culturing the BBB complex. It further includes a strategy that has recently been developed aimed to predict BBB properties by *in silico* approaches. *In vitro* models are of paramount importance to test the transport of drugs through the BBB. The use of alternate tissue culture models are helpful in some ways, but can only be regarded as approximative approaches. Thus, considerable effort has been channeled to develop reliable culture systems to mimic the BBB *in vitro*. The chapter by *Romeo Cecchelli, Caroline Coisne, Lucie Dehouck, Florence Miller, Marie-Pierre Dehouck, Valérie Buée-Scherrer* and *Bénédicte Dehouck* gives an overview on our present knowledge of *in vitro* BBB models that at least fulfil some of the essential criteria that are required for a well differentiated BBB endothelium. In extension of this article, *Alla Zozulya, Christian Weidenfeller* and *Hans-Joachim Galla* describe their specific approach to utilize a coculture system treated with hydrocortisone to achieve monolayers with high endothelial resistance, the key feature of a tight barrier. Both articles provide a comprehensive source of information for those who are interested in model systems of the BBB. The chapter by *Luca Cucullo, Emily Oby, Kerri Hallene, Barbara Aumayr, Ed Rapp* and *Damir Janigro* introduces advanced models that take into account the three-dimensional structure of the vascular tube including shear-factors applied on the endothelium by fluid flow. This dynamic *in vitro* model of BBB (DIV-BBB) and its newly designed model (NDIV-BBB) display the most advanced *ex vivo* approach to the *in vivo* BBB situation with the advantage of large upscaling, an obligatory requirement for industrial application. *Gerhard Eckert* and *Christian Noe* introduce the readers to the new world of *in silico* screening of drugs (pharmacoinformatics) with respect to their ability to penetrate the BBB. These *in silico* methods gain increasing interest in order to economize the process of standard high-throughput screening. The applica-

bility of computational methods on the BBB is a novel promising strategy that may allow discovery of target families and presumably novel bioactive molecules. The fact that the permeation of the BBB is a multifactorial process necessitates advanced computational methods for modelling approaches and opens new perspectives in BBB drug research.

Part V completes consideration of pharmacological aspects of the BBB, and gives access to some fundamental issues of BBB pharmacology. *Sandra Turcotte, Michel Demeule, Anthony Régina, Chantal Fournier, Julie Jodoin, Albert Moghrabi* and *Richard Béliveau* introduce one of the most relevant systems that prevents significant accumulation of many hydrophobic molecules and drugs in the brain: the multidrug resistance transporter P-glycoprotein (P-gp). This efflux transporter is a member of the ATP-binding cassette group of transporters (ABC), which represent the largest family of transmembrane proteins. The existence of the P-gp at the BBB is one of the main causes of failure in chemotherapy, because of its ability to translocate xenobiotics against a concentration gradient across the plasma membrane. Thus, the P-gp plays an important role in brain protection at the BBB site, but by its nature provides a considerable hindrance for successful treatment of a variety of brain diseases. *Nicolas Bodor* and *Peter Buchwald* provide a survey on general aspects of targeting neuropharmacologicals by chemical delivery systems (CDSs). CDSs, as the authors state, represent a rational drug design approach that exploits sequential metabolism not only to deliver, but also to specifically target drugs to their site of action. The authors present a spectrum of approaches intended to deliver drugs, particularly bioactive peptides, through CDSs to the brain tissue. The chapter by *Pieter J. Gaillard, Corine C. Visser* and *Albertus G. de Boer* is centered on certain delivery systems, which include the transferrin system, the insulin receptor, the low-density lipoproteins I and II (LRP1, LRP2) and the diphtheria toxin receptor. The authors present the enormous opportunity that these systems offer for the successful delivery of drugs to the brain, but also enumerate the pitfalls that these systems face and their still limited applicability in clinical therapeutics.

Two major chapters herald part VI, both of which deal with vascular perfusion. *Albert Gjedde* introduces the field of blood-brain transfer and metabolism of oxygen, a field that sets the basics for functional imaging covered by the article of *Gerald A. Dienel*. The rationale to include these chapters within this handbook on the BBB is multi-faceted. As quoted by *A. Gjedde*, delivery of oxygen to brain tissue differs in major respects from the delivery of oxygen to other tissues. This is not a direct consequence of the properties of the BBB in its narrow sense, but reflects the specific demands that the vascular system of the brain must fulfil in order to satisfy the energetic requirements of the working brain. "The absent recruitment of capillaries in states of activation of neurons as well as the general principle of topographic arrangement of the vessels" account for the differences of brain with regard to other tissues. This statement by *A. Gjedde* pin-points the morphological and physiological specificity of the brain microvasculature, a key feature in the context of an integrated concept of the BBB. This is followed directly by the chapter of *Gerald Dienel*, which covers the

entire spectrum of modern brain imaging and provides a thorough overview of the basic physiological features on which modern brain imaging is founded. The "take-home message" of this chapter is that blood flow, metabolism and cellular function are inseparable aspects of brain activities and the responsiveness of the BBB to neuronal activity represents a hallmark in coupling both sites of the active brain: blood flow and brain work.

The last and most extensive part VII includes a collection of articles on clinical afflictions of the BBB. It starts with a chapter on the impact of inflammation on the BBB by *Pedro Faustmann's* and *Claus Haase's* contribution on inflammatory responses of the blood-brain interface. The brain has long been considered to be an immunoprivileged part of the body. Under pathological conditions such as inflammation, trauma and neurodegeneration blood-borne cells immigrate into the CNS and changes of the permeability of the BBB occur. The mechanisms how these cells enter the brain and which proteins in the cerebrospinal fluid are disease-related are key issues, which represent central questions currently being addressed in neurology, neuropathology and neuroimmunology. The sections on inflammatory attacks on the BBB is followed by *Marilyn J. Cipolla's* chapter on stroke that summarizes the pathophysiological sequelae to the event of a stroke, i.e. development of brain edema, cellular regulation of cerebrovascular permeability, effects of stroke on the cytoskeleton of brain endothelium and reperfusion injury, just to mention a few highlights of this article.

A further important clinical issue is covered by the article of *Arshag D. Mooradian*, which is centered on diabetes and its consequences for the BBB. "The CNS complications of diabetes have not widely appreciated, because of the most overt complication, namely stroke", states *A. Mooradian*, pointing to the more subtle effects on the CNS that extends beyond clinically appreciated cerebrovascular accidents. These include: alterations in cerebral microvessels with poor autoregulation and blood distribution, altered BBB function, neurochemical changes, alterations in neurotransmitter receptor activity and contributing factors such as hypoglycemic reactions. All these changes in the CNS are discussed from the perspective of BBB function.

The chapter by a group of parasitologists contributing to the chapter by *Danny A. Milner* (*Mahalia S. Desruisseaux, Louis M. Weiss, Herbert B. Tanowitz* and *Adam Moss*) focuses on a group of infections with global impact: human parasitic diseases. The involvement of the BBB during parasitic infections has been elaborated in this conceptual context for the first time in this handbook. The chapter by *Milner et al.* offers a taxonomic guideline for those readers who are interested in parasitic infections which affect, disrupt and/or destroy the BBB. From a clinical point of view, these infections are of great relevance since they are more commonly fatal than parasitic diseases which do not destroy the BBB.

The final article by *Tailoi Chang-Ling* introduces the current understanding of the blood-retina interfaces. It describes both the features the retinal interface shares in common with the CNS blood vessels as well as its specific structural features. The unique morphology of the retina as an exposed part of the brain

and the transparency of the ocular media has made it an exceptionally accessible target not only for *in vivo* detection of the vascular physiology but also for monitoring cardiovascular pathology, including changes to arterioles and venular walls due to arteriosclerosis and diabetic retinopathy. We feel that this is an appropriate last chapter, insofar as the article recapitulates many of the concepts regarding both healthy and diseased BBB that are encountered during the excursion through this handbook.

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