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Philosophical perspectives on neuroendocrine-immune interactions: the building block model and complementary ecological approaches

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Glossary

NEIMS: Neuro-endocrine-immune-microbiota systems, a term used to indicate a recomposition of elements of historically distinct biological systems (which were largely based on anatomical criteria or the mapping of one function to one structure) based on functional criteria at a systemic level.

Structure: Roughly, anything that can be ordered with respect to some criteria can be considered "structured." On the molecular level, primary, secondary, and tertiary structure denote sequences or three-dimensional folding patterns, respectively. On a "higher" level, cellular structures or objects at higher levels of organization are often divided into individual structures (e.g., organelles, organs, specific **systems**).

Function: Usually considered to be closely related to their corresponding **structures**, functions often describe what certain structures (or **systems**) do. Sometimes, researchers have in mind *why* they do

something, i.e., they want to explain why it has been evolutionarily selected for. Philosophers have amply debated these various conceptions of “function” and the relations between them. Biologists frequently use “knock-out” experiments or investigate pathological cases to study what happens when a certain part is removed/dysfunctional and infer its normal function from how the **system** breaks down.

Holism: Holism is a theoretical position, often opposed to **reductionism**, according to which the properties of a system cannot be explained exclusively through its individual components, since the functional sum of the parts is always greater (or in any case different) of the same parts taken individually. For instance, in biology a holistic approach conceives an organism as such, as not reducible to a simple assembly of its constituent parts. Some types of holism are more tangible than others.

Reductionism: In philosophy, the term "reductionism", with respect to any science, holds that the entities, methodologies, or concepts of a research field can be traced back to a more fundamental level, sufficiently to explain the facts of the theory in question. For example, according to reductionism, psychology can be reduced and explained in biological terms. In turn, biology can be reduced and explained in chemical-physical terms. Reductionism, therefore, argues that it is possible to formulate the concepts and language of a scientific theory in terms of another theory considered more fundamental.

System: A collection of things that is either spatially connected, i.e., due to structural reasons, or working together, i.e., functional reasons, is frequently subsumed under the umbrella of being a system. Whether these are divisions that correspond to something in nature or rather arbitrary divisions through the researchers’ perspective is often an issue of debate. Sometimes systems will be characterized in a circular manner, e.g., the immune system is anything of interest to the immunologist.

Eco-Evo-Devo: Ecological evolutionary developmental biology (eco-evo-devo) is an area of biology that focuses on the ecological context of development and evolution. Eco-evo-devo acknowledges that organism-environment interactions, including symbiosis, niche construction, plasticity, etc., are important sources of variation, inheritance, development, and natural selection. Eco-evo-devo is a branch of evo-devo (evolutionary developmental biology), which treats development, not genes, as the fundamental unit of evolution. Evolution from an evo-devo perspective is characterized as changes in the heritable properties of development. This is contrary to the standard definition of evolution as changes in the genetic frequencies of a population.

Abstract

The study of the interactions between the neuroendocrine and immune systems is a highly interdisciplinary research endeavor, in which the boundaries between the systems being studied become blurred. We address a common scientific perspective in dealing with intertwined complex systems, namely the conceptual approach in science that treats each system (e.g., nervous, immune, endocrine systems) as separate units or “building blocks” with unique functions that correspond to specific structures. While there are merits to this way of decomposing complex systems, there are several reasons why such an approach is limited when trying to recompose a physiological system that is engaged in intricate co-functioning and that is the result of, co-development, and co-evolution, not just between these systems, but with the gut microbiota as well. Our suggestion is to take an alternative ecological evolutionary developmental approach to the neuro-endocrine-immune-microbiota system (NEIMS) as a whole, which can serve as complementary to the predominant building block perspective.

> "In trying to analyze the natural world, scientists are seldom aware of the degree to which their ideas are influenced both by their way of perceiving the everyday world and by the constraints that our cognitive development puts on our formulations. At every moment of perception of the world around us, we isolate objects as discrete entities with clear boundaries while we relegate the rest to a background in which the objects exist.
> That tendency [...] is one of the most powerful influences on our scientific understanding. As we change our intent, also we identify anew what is object and what is background."
> — Richard C. Lewontin (1929-2021)[1]

> "Until recently, many scientists viewed immune cells and the central nervous system (CNS) as a deadly mix. [...] Decades of research on this autoimmune disorder [multiple sclerosis] opened a window into how the immune system and the CNS interact, but more recent research efforts have revealed the exceptionally broad scope of communication between the two. We now know that the immune system is very likely a key player in many neurological diseases and, surprisingly, that immune-CNS interactions may not all be bad."
> — (Mueller et al., 2016, p. 760)

1. Introduction

Neuroimmunology is a relatively new and interdisciplinary enterprise. The term 'neuroimmunology' has been around for several years, but the canon of topics that can be categorized as such continues to evolve. Other terms – like 'psychoneuroimmunology' and 'psycho-neuro-endocrino-immunology' have been used as well, indicating both the interconnectedness of these various systems and the disciplines studying them.[2] For reasons of simplicity, 'neuroimmunology' will be employed here as the most inclusive term that refers to the study of interactions between the neuroendocrine and immune systems.

The biological systems in question, however, are anything but simple. From a methodological point of view, in order to deal with complex systems and study them in ways that are appropriate to arrive at empirically meaningful statements, researchers tend to dissect or decompose them into smaller units

[1] "It's Even Less in Your Genes", *The New York Review*, May 26, 2011 issue, https://www.nybooks.com/articles/2011/05/26/its-even-less-your-genes/ [accessed: 28-01-2022]

[2] Historically, various terms have been used reflecting slightly different approaches, from psychoneuroimmunology and neuroimmunomodulation, which, at least initially, were rather top-down in nature, to immunopsychiatry, being more bottom-up (Konsman, 2019; Pariante, 2015). However, in all cases, some interactions between neuroendocrine and immune systems are invoked or assumed. Therefore, we propose to use the term 'neuroimmune' as shorthand for these different approaches while being well aware that neuroimmunology refers to a scientific field.

(Bechtel & Richardson, 2010). The overall organization of the organism has thus been traditionally broken down into the nervous, endocrine, and immune system, studied in isolation as structurally or functionally distinct biological systems. Even though host microbiota are not historically considered a bodily "system", they are nevertheless increasingly thought of as constituting an ecological system within the host body.[3] In this case, the designation of "system" is also driven by structural-functional considerations, as well as the distinct genealogical lineages of macro- versus microorganismal cells.

Another tendency is to assume a natural hierarchy between these decomposed systems. The brain, for instance, as a complex system is supposed to deal with regulating complexities internal and external to the body. Two metaphoric images of the brain have been distinguished, one "as governor and [the other] as transducer" with "the former treat[ing] the brain as the executive control center of the body, [and] the latter as an interface between the organism and reality at large" (Fuller, 2014). In several domains, the "governor vision" of the brain is dominant, for example when it comes to emotions (Colombetti & Zavala, 2019). Furthermore, historically the "brain as governor" vision has proven fruitful regarding the idea of neuroendocrine systems by postulating and establishing an important role for the hypothalamus in controlling pituitary-endocrine gland functional axes (see chapter 1). It is therefore not surprising that initially the fields studying neuroimmune interactions, such as psychoneuroimmunology and neuroimmunomodulation, were largely motivated by the 'top-down' idea that the brain controlled immune responses via neuroendocrine mechanisms.

However, progressively these fields have been enriched by a network or systems approach, in which the immune system can influence brain function as well. The latter even seems to have become the main working hypothesis of the more recent field of immunopsychiatry (Konsman, 2019). Many authors have noticed that some important functional features seem to be shared between the immune and nervous systems, for example memory. This can further contribute to questions, such as, whether the nervous system controls the immune system, or the other way round, what kind of interactions exist between these systems, etc. (for a general and informative discussion of conceptual questions about such relations, see (Pradeu, 2020, pp. 54 ff.)).

It is now widely accepted that the immune system and the neuroendocrine system contribute to an integrated physiology that regulates the homeostasis of the whole organism (beginning with (Ader & Cohen, 1975; Besedovsky et al., 1985; Besedovsky & Rey, 1996; Blalock, 1994), see also (Ader, 2000; Ader & Kelley, 2007) and many more reviewed in (Ashley & Demas, 2017)). We seem to have moved (at

[3] Microorganisms were initially studied (mainly in medicine) in their role as pathogens (consider for instance the studies of Koch and Pasteur). This means that they were studied in isolation without consideration of the structures they usually assume in nature, i.e., biofilms. Indeed, biofilms were first proposed by microbial ecologists and then adapted and adopted in clinical settings. Microorganisms are now seen as systems in virtue of their ecological nature. This is another example of how the way to look at things can "change" the nature of the objects of scientific investigations, including the methods to study those entities.

least partially) beyond the idea that these systems are isolated and separated and only interacting under pathological situations (e.g., the neuroinflammation). When approaching functional aspects of how these systems operate in an organismic context, we are starting to recognize that one cannot neglect the ample interactions and crosstalk between these systems, with important contributions coming from entities and activities that usually are not considered to be part of the same system.

Yet how should we think about the reintegration, or recomposition of the neuroendocrine and immune systems? A bottom-up approach might investigate how neuroimmune circuits and networks cluster and overlap. Such a "connectome," similar to genetic regulatory networks and circuits, can be formed on the basis of a "common language" of molecules and receptors (e.g., cytokines or chemokines, neurotransmitters, hormones) or interconnected feedback loops. Some evidence seems to point towards that direction. The three types of molecules usually/traditionally thought of as unique for each system– cytokines (immune system), neurotransmitters (nervous system), and hormones (endocrine system) seem to share evolutionary and developmental origins (Petrovsky, 2001). Evolution might have picked out pathways that criss-cross all three systems instead of evolving each system separately, up to a certain point, after which they evolved together (Ashley & Demas, 2017; Verburg-van Kemenade et al., 2017).

From this bottom-up perspective, systems biology becomes an attractive methodological approach. The aim of systems biology is to "understand how functional properties and behavior of living organisms are brought about by the interactions of their constituents" (Boogerd et al., 2007, p. 3) with the constituents being mainly molecules such as mRNA, proteins and metabolites, including for neuroendocrinology and particularly immunology (Boonen et al., 2009; Eiden et al., 2020; Gardy et al., 2009; Germain et al., 2011; Gottschalk et al., 2013). Systems biology is often based on high-throughput technologies allowing for the measurement of thousands of transcripts, proteins or metabolites and bioinformatics approaches to generate hypotheses regarding functional behaviors of interest. Therefore, one might think that, focusing on understanding and dissecting the structural patterns detected by these techniques, might be the key to comprehending these systems in a unified solution.

However, it would be naïve to think that these approaches can, by themselves, recompose the various objects of scientific investigation in their systemic nature/features of biological systems. Merely elucidating the structure of networks is not enough to understand their mechanisms and function. Similar problems have frustrated big data -omics approaches in other areas of biology. While trying to understand the genetic regulatory networks that govern developmental processes, molecules and pathways form an intractable "hairball" of nodes and edges that does not lend itself to scientific understanding, explanation, or prediction of the mechanisms involved (Jaeger, 2017). Rather, the connectome approach needs to be supplemented with a framework that elucidates the structure, the function, and the mechanisms and processes. For instance, computational methods and bioinformatics

can provide a complementary look at the **reductionism** of certain compartmentalized and mechanistic investigations (see (Boem, 2016; Ratti, 2016; Leonelli, 2019)) – however, they do not constitute, *per se*, a privileged point of view that is somehow superior to others.[4]

Another widely followed approach lies somewhere in between simplistic reductionism and intangible **holism**. This middle ground keeps the relative autonomy of each system (nervous, endocrine, immune) as largely independent modules but acknowledges the rich and overlapping interactions that form a comprehensive whole. This *building block model* does take structure, function, and process into account, assigning each block a unique structure, function, and types of processes. They are then integrated like Lego blocks, with each block retaining their unique features as they interact to maintain the homeostasis and functioning of the whole organism. However, there are multiple problems with this approach, which we will address below.

We suggest addressing these issues from a different angle, one that does not presuppose different systems as given units or building blocks. When we take a re-integrated neuro-endocrine-immune-microbiota systems (**NEIMS**) approach, we include additional elements of co-development, co-functioning, coevolution, and ecological context.

The "normal" physiological role of neuroimmune interactions beyond disease might not be so "surprising" – as (Mueller et al., 2016) proclaimed in the epigraph – when we approach these systems with a more integrative perspective that takes their interactions, not their distinctiveness, as starting points. By doing so, we "extend" the roles of each system into the other, which in turn could trigger a rethink of what they are and what they do. There are important methodological consequences to this reorientation: when each system is conceived as distinct, one might think that they could be studied separately – as they have been for quite a while. For example, it was widely believed that the brain was "immuno-privileged," in the sense of being outside the immune system's reach, because of the existence of the so-called blood-brain barrier that, in a way, seemed to neatly separate the immune and central nervous systems. Many are now reconsidering the barrier metaphor, with early questioning

[4] It is essential here to recall the recent developments in computational biology and systems biology. The tools used in computational and system biology by these research sectors, allow one to consider and analyze vast amounts of data and to examine and manipulate some properties by abstracting vast amounts of them from the objects of scientific investigation. This practice has led some to believe that this was the main way to deal with the complexity of biological phenomena and, to avoid simplistic reductionism. The discussion of these aspects is too extensive to be fully reported here. However, it is enough for us to point out that, while it is certainly true that, while these approaches have made it possible to build new "privileged observation points" on biological phenomena (not otherwise investigable), it is also important to remember that complexity is not just a question of quantity or computational capacity, but something that (unlike "complication") is inherently irreducible. By providing a complementary look at the reductionism of certain compartmentalized and mechanistic investigations, computational methods will enable researchers to have a more authentic picture of their field of investigation, because even if they employ reductionist methods (the use of which is often a harbinger of discoveries), they will be able to give a broader and more legitimate meaning to their results within the general framework.

starting after the discovery of glial cells as immunocompetent cells in the brain.

This chapter proceeds as follows. In Section 2 we critically examine the "building block model." Section 3 criticizes the view that each block can be investigated independently of each other by examining the immune system; this section also offers an extended view of the immune system and invites us to rethink its structure and function. Section 4 is concerned with how the building block model conceptualizes the integration of multiple systems, illustrated by the mammalian gut. From these examples, we outline a NEIMS perspective driven by the developmental, ecological, and evolutionary relationships between the neuro-endocrine-immune-microbiota systems. In section 5, we conclude with general lessons for dealing with neuro-endocrine-immune-microbiota systems.

2. Biological systems and their functions: it's a difficult relationship, not just a sum of building blocks

What we call the "building block model" is a way of practicing science and a conceptual model of how the biological world is organized. It acknowledges that systems interact with each other but treats each of them as separable subsystems that can be re-integrated in an additive fashion as Lego blocks snapped together. Researchers each study their own domains but come together only when interactions between systems originate issues that affect diverse systems at the same time.

The underlying assumption of the building block model is a "one system - one function" mapping, where each system corresponds to a unique and main function. This way of thinking is pervasive in the life sciences, like, e.g. the famous one gene – one enzyme hypothesis (Beadle & Tatum, 1941). Similarly, it is all too common to think along the lines of other one-to-one mappings, such as one gene – one disease, one cell-one function, one tissue – one function, one pathogen – one disease, etc.

In traditional neuroimmunology and neuroendocrinology, the building block model is apparent. Historically, the nervous system, the endocrine system, and the immune system were studied largely in isolation from each other, and each attributed a main function. The function of the nervous system would be information processing or cognition, that of the endocrine system, control of body metabolism, that of the immune system, defense, and that of the gut (which we discuss below), digestion. While these might not always be clear-cut cases, the general notion seems to be widespread and inherent in the division of the fields that study these systems. When it comes to integrating the immune system, the nervous system (and many other systems), the expectation was often that it just consisted of adding one system to another, thus ending up with a combination of the two respective main functions of these individual systems. For instance, neuroimmunology would be assumed as the study of the immune defense of the nervous system (which is indeed how the field started) (Yoo &

Mazmanian, 2017; Rankin & Artis, 2018; Fung et al., 2017). Furthermore, many introductory overview articles or textbooks on neuroimmunology emphasize the interactions between systems only in the context of pathologies and disease. For instance, when components of the nervous system are involved in fighting pathogens or, conversely, when immunological components are involved in diseases of the nervous system.

According to the building block model, together with assumptions about independent developmental and evolutionary origins, (causally) linear and additive systems, often as (structurally and functionally) modular sub-systems could be put together like pieces – building blocks (Fig. 1). Each building block is prescribed a unique, main function.

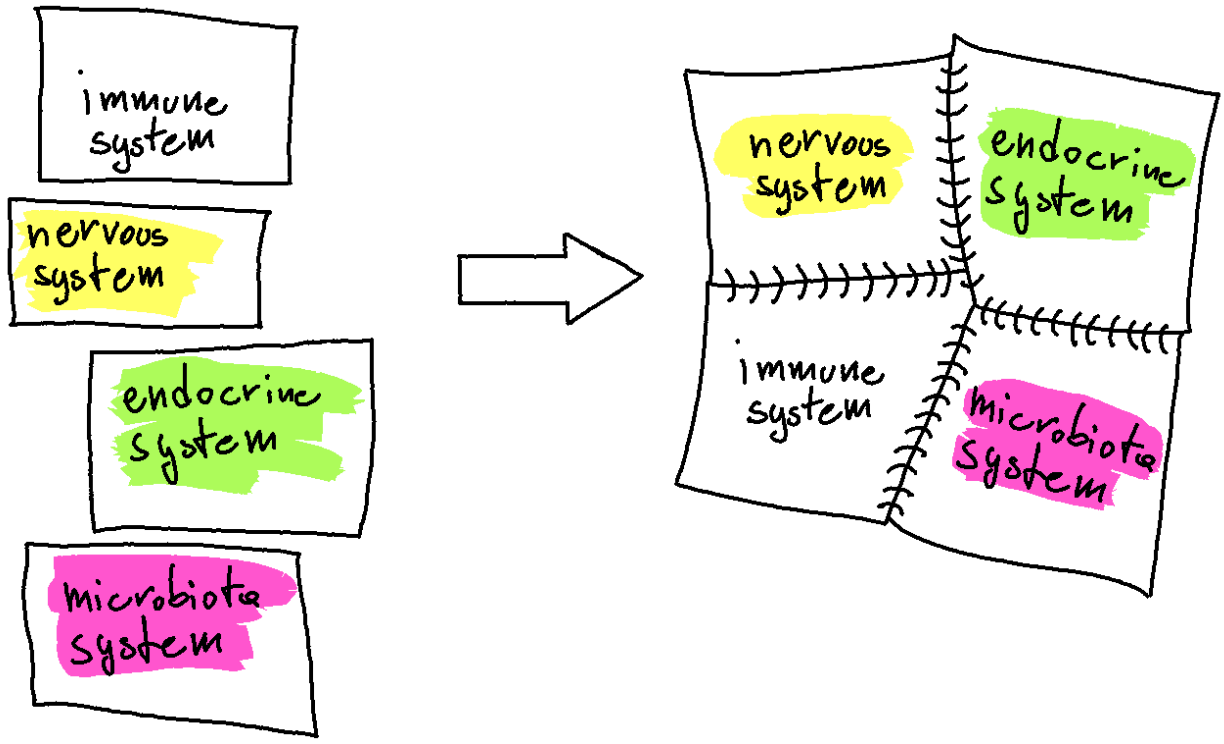

Fig. 1: Illustration of the building block model that systems (and their functions, in color) could be taken as independent units and be stacked together.

Yet it is not sufficient to merely acknowledge the presence of multi-directional interactions between systems. Doing so still attributes specific functions to each system. By focusing primarily (or even exclusively) on the role of the immune system as fighting disease and the nervous system mostly as a

cognitive or 'governing' organ, we may miss out on many of the other important functional roles these systems have as a result of their physiology, development, and evolution.

The building block model does not just make problematic assumptions about the nature and boundaries of systems, but also assumes that we know what systems there are in the first place. How do we demarcate something like a system, and according to which aspects? Decomposing and recomposing according to various functional and evolutionary aspects raise the question of where to localize these functions in the first place. All these questions will play a crucial role in addressing the questions surrounding neuro-endocrine-immune-microbiota systems (NEIMs) and how to integrate them.

To go beyond thinking of various systems being brought together like building blocks, we need new guidelines to take the integrative nature of these systems seriously without lumping them into a holistic, intangible network. One can better understand these systems by putting aside their "traditional functions" to see how their functionalities instead emerge from their interactions, and by examining how their respective structures and functions originate from a process of co-development and ecological interactions. NEIMS, as an ecological, developmental, and evolutionary approach, should serve as a guide for how these systems should be studied and conceptualized, to facilitate a better understanding of these systems, elaborating new hypotheses and experiments to test them, including the potential for novel therapeutic approaches.

The NEIMS approach also has therapeutic consequences. For instance, it has become clear over the years that the properties of the blood-brain interfaces cannot be reduced to the tight junction molecules sealing brain endothelial cells, but are determined by the interplay of astrocytes, pericytes and microglia/macrophages, that is, it includes parts of what is traditionally considered the immune system. As a result, the blood-brain barrier is now being conceived of as one aspect of a blood-brain interface that functions more as a border than a barrier, recognizing among others the importance of the meninges constituting another aspect of this interface (Rustenhoven & Kipnis, 2019). Furthermore, it has recently been proposed that the bona fide macrophages of the brain meninges, choroid plexus and perivascular spaces, which share many cell markers (Faraco et al., 2017) should be referred to as "border-associated macrophages" (Pedragosa et al., 2018; Van Hove et al., 2019). By acknowledging the structural and dynamical properties of the blood-brain interface, it may indeed be more appropriate to use the 'border' metaphor rather than that of a 'barrier' (Badaut et al., Blood-brain borders: a new proposed concept to address limitations of historical blood-brain barrier terminology, *in preparation*).

Decomposition, a central assumption of the building block model, is often a legitimate research strategy when dealing with complex systems (Bechtel & Richardson, 2010). Many researchers, including philosophers, have helped come up with different criteria for complex systems and how to deal with them (Ladyman & Wiesner, 2020). But there is always an inherent risk involved in dividing a system into components. For instance, there is the possibility of erroneously attributing fixed properties to a

dynamic, complex system[5].

Each partitioning – with respect to such features – comes with certain trade-offs and biases. We cannot treat them as objective demarcations that would allow us to "carve nature at its joints". Most of the time, any decomposition or classification claiming to "carve nature at its joints" results in classifications that can operationally work but are nevertheless relative to the research concern and experimental design. For instance, we tend to classify and conceive of the nervous system (and its cells) as mainly associated with "perception" and "cognition" because those interests are dominant and central in these research fields. Thus, those interests played a crucial role in shaping the way scientific investigations have been pursued.

The NEIMS approach is a much more integrative approach that takes the interwoven components, their interactions, co-development, and co-evolution seriously. It starts with the recognition that there might not be a "main function" associated with each subsystem. Furthermore, the specific function a subsystem plays depends on its relationship with other subsystems and the larger organismal environment.

Combining the nervous and immune systems is not just about putting together the functions of cognition and defense. In the next sections, we argue against the building block model by looking at how the function of the immune system needs to be understood in light of new findings. In addition, we look at what happens when we add other systems, including the endocrine system and microbiota in their ecological context.

3. Rethinking individual building blocks: the case of the immune system

Between the 18th and 19th centuries, the notion of system became progressively employed in anatomy to indicate a combination of bodily tissues or organs having the same characteristics in physiology, like the vascular and nervous systems (Keel, 1982; Moulin, 1991). In the 20th century, the philosopher Joseph Woodger affirmed that "the machine theory of the organism tacitly acknowledges an organization above the chemical level" in the sense that "the organism is analysable into organs systems, organs, tissues, cells and cell-parts" (Woodger, 1929, pp. 292-293). Thus, "according to the 'standard' hierarchical model of physiology, each living organism comprises organ systems" and "[e]ach organ system, in turn, is composed of individual organs" (Ashrafian, 2018). Although the relationships between

[5] This does not necessarily imply that a particular way of dividing subsystems was the result of inattention, the lack of a critical attitude, or methodological negligence, but instead reflects the need to be mindful of the nature and necessity of boundaries. Even if past divisions have successfully worked for certain experimental results, the adopted partition still calls for continuous, additional justification.

functions and organ systems have already been challenged by findings outlined above, indicating that functions attributed to the immune systems involve mediators classically associated with the neuroendocrine system and vice versa, the very notion of systems has also changed in biology. Indeed, different forms of systems biology exist between 20th century systems biology "considering whole living systems, which include their organization [and] the dynamics within systems and the interplay between different levels" and "today's systems biology, which is often a bottom-up approach from molecular dynamics to cellular behavior" (Drack & Wolkenhauer, 2011).

Traditionally, the immune system is conceived as formed by a set of certain cell types, sharing distinctive and similar tasks that are extremely specialized. It is characterized as a system of defense, as a means to protect the organism, the "self," from external threats, that is, the "non-self" (including tumor cells which would become "strangers" to the organism). The lexicon adopted within the sciences of protection and threat already guides us to conceive of immunity as a sort of "defense system". This conceptual, and thus terminological, stance is explicit in the historical development of immunology as a research field (Burnet, 1961).

Argument 1: immune system beyond defense

The first critical problem of the building block model regarding the immune system is its "static" characterization, mainly as a defense system. However, it has progressively been shown that the activities of the immune system go far beyond the defensive functions and are involved, in other various biological processes of the organism, from those related to development, metabolism, tissue repair, to the regulation of the nervous system (e.g., affecting signaling pathways, producing neuroendocrine mediators, facilitating synapsis activity) and the preservation of homeostasis, both locally and organismically (Dantzer, 2018; Rankin & Artis, 2018; Pradeu, 2020). Once the immune system is recognized as having functions that go beyond those of defense, it is easier to understand why it is problematic to use simple self/non-self and internal/external distinctions to define the immune system.

For instance, from a genetic point of view, there are a plethora of exogenous elements which are not perceived as such but rather allowed or tolerated within the boundaries of the extended immune system. First of all, during mammalian reproduction, the fetus constitutes an external entity to the mother, yet it is tolerated by its immune system. Other cases showing the pervasiveness and flexibility of immunological tolerance include, for example, the cases of chimeras, organisms that are composed of cells of different individuals (both of the same and of different species), and those of genetic mosaicism, i.e., the presence, in a multicellular organism, of two different genetic lines derived from a single zygote[6].

[6] In chimerism, each cell population retains its own characteristics, given that they are genetically distinct because they originate from different zygotes. Thus, the resulting organism is a mixture of differently matched regions. On

Argument 2: immune system as an ecological and whole-body system

Differently from other systems of the organism, however, the immune system is not spatially circumscribed, tied to a specific organ, or pertaining only to certain tissues. On the contrary, its structure is "sparse", "diffuse", highly dynamic, and usually it is studied according to its functionality[7]. Dissecting and understanding the functionality and the organization of the immune system is a crucial task for both scientific and clinical investigations. Yet immune cells can differ drastically in functionality despite sharing a common developmental route. It has been shown that functional fluctuations in immune cells activities and specialization can be due to both individual (genetic) variability and epigenetic modifications. Moreover the activity of the immune system presents an impressive range of variability, not only between distinct single individuals, but also within the same person during the different phases of its life (Poon & Farber, 2020). Furthermore, immune functions cannot be fully disjointed or separately considered from their crosstalk with specific tissues and cell types (Farber, 2021). These characteristics showcase the systemic and plastic nature of immune cells, as context and the types of interactions determine their capacities and activities.

More fundamentally, the context-dependency of immune cell functionality corroborates the idea that the immune system presents an ecological character. Subgroups of immune cells that share their origin, mature and functionally develop in different ways, in strict relation to the microenvironment in which they operate (Poon & Farber, 2020). The immune system, due to its widespread and capillary nature, is thought to interact with all the other bodily systems and constitutes a complex functional network, thus resembling in some respects an ecosystem that comprises the organism itself, in its totality (Poon & Farber, 2020).

Herein lies a second clue to the limitations of the building block model. From both a developmental and structural point of view, the immune system is composed of a specific macro-type of cells: leukocytes. However, from a functional perspective, the activities of the immune system, from the defensive ones to the more general and regulatory ones, do not end with the operational possibilities of the leukocytes alone but must instead be understood as the product of the interaction of these cells with other cells of the organism, like epithelial cells, and with symbiotic residents (we will discuss this second aspect in the next section). The interaction of the immune system with the organism concerns at least two levels. On the one hand, there is a local immune activity, determined by the interaction of single compartments of leukocytes linked to specific tissues or areas. On the other hand, there is global crosstalk that involves the whole organism both through the blood flow and also the interaction of the immune system with

the other hand, in genetic mosaicism, different cells, with a diverse genetic heritage, arise from the same zygote.

[7] Even though this structural comprehension of the immune system is possible, and although immune cells share developmental origins (and evolutionary as well), their definition as such relies more upon their capabilities and mutual interactions.

other systems, such as the endocrine and nervous system (Poon & Farber, 2020). Therefore, we can argue that functionally, it is the organism as a whole that is the system of the immune system[8].

Interestingly, as some recent research suggests, the functionally global interactive nature of the immune system can also be seen as that of a “social” network, layered on different levels (Bergthaler & Menche, 2017). A recent study (based on proteomic analysis of immune cells activities under different stimuli) has shown how the relational and multi-level architecture of the immune system exhibits a particularly high level of intercellular specialization, with divisions and subdivisions resulting in distinct cells performing tailored and context-dependent tasks. Just like any complex system, the overall functionality of the system extends the simple sum of the activities taken individually, exhibiting distinctly emergent properties (Rieckmann et al., 2017).

It is also interesting to note how some studies show that even different cell types, traditionally not associated with the immune system, can become a functional part of it. For example, some cell types, usually classified as structural (such as fibroblasts or epithelial cells), in certain contexts exhibit the ability to communicate with immune cells in order to modulate and coordinate their activity. Indeed, these structural cells can even secrete molecules both attracting and activating immune cells (Gomes & Teichmann, 2020). For instance, studies have shown how epithelial cells in the lungs can detect pathogens’ activities and produce molecules interacting with dendritic cells pushing them towards T-cells activation. Moreover, they can promote fine-grained modulation by recruiting specific subtypes of immune cells (through the production of chemokines) providing a more tailored immune response (Schleimer et al., 2007). Other recent work on airway epithelium (an area obviously exposed to the external environment) has shown how epithelial cells interface and collaborate with specific subpopulations of immune cells, both in coordinating defense activities and in maintaining tissue functionality, including their repair (Hewitt & Lloyd, 2021).

Particularly interesting, still from a systemic perspective, is that in the lungs the "perceptive possibility" of the organism in recognizing the lack of uniformity (such as pathogenic activities) rests on different capacities and "sensory" pathways. This is the case of the neuroimmune response which acts in concert with the pulmonary epithelium. In particular, the lungs are also densely innervated by sensory neurons that are able to activate, differently depending on the stimulus, specific populations of leukocytes (e.g.,

[8] This does not entail a naive (and not very useful) form of reductionism for which every organismic activity is attributable to the immune system. On the contrary, if the immune system extends to the whole body, this means that one cannot ignore the immune system to provide a complete explanation of many activities of the organism itself. It will be the main task, for future science, to clarify the aspects and mechanisms by which this happens.

the T-helper 2 cells), which, in turn, communicate with neuroendocrine cells responsible for the production of mucus. Conversely, cholinergic neurons are able to stimulate a different immune response that entails the production of cytokines by other immune cells (Hewitt & Lloyd, 2021).

This phenomenon is not confined to the lungs alone. The cells of the epithelium of different tissues (from the intestinal tract to the skin) have shown the ability to contribute to the function of the immune system not just activating the "usual" immune compartment but also orchestrating the entire "immune" activity (Larsen et al., 2020). Moreover, recent studies have shown how tissue-related epithelial stem cells preserve an epigenetic memory of their immune activities, potentially altering of further inflammation responses and tissue repair (Naik et al., 2018).

Argument 3: how to distinguish the immune system from other systems

Finally, in the building block model, the functions of regulating and coordinating internal bodily functions and defense against external threats have classically been attributed to the neuroendocrine and immune systems, respectively. The two systems seem to be distinguished mainly by their different cell types, but also by different modes of intercellular signaling. One important way by which cells signal to each other is through direct contact. Another important manner of cell signaling is through the release of molecules in the extracellular space. Depending on the amount released and the expression of receptors for signaling molecules, this may result in signaling to neighboring or distant cells, which have been labeled paracrine and endocrine signaling, respectively (Hartenstein, 2006). The endocrine system has been characterized by hormone-mediated signaling, which is "defined by their ability to send signals to and from different tissues at long distances" (Kodis et al., 2012). Neurons as a cell type, on the other hand, are often associated with synaptic signaling, which involves a hybrid form of close contact and paracrine signaling.

However, over the past decades it has become clear that neuroendocrine responses, for example, activation of the hypothalamus-pituitary adrenal axis, occur in response to detection of microbes or their constituents (Besedovsky & del Rey, 1989; Rivier et al., 1989) and that molecules typically associated with the immune system, such as pro-inflammatory cytokines, can play an important role in regulating body metabolism (Peluso & Palmery, 2016; Wallenius et al., 2002). Moreover, removal of neurons and endocrine cells from Hydra epithelium modifies local bacterial populations (Fraune et al., 2009). Furthermore, mammalian neuropeptides released in endocrine-like manners have antimicrobial properties and can regulate innate immunity against microbes (Aresti Sanz & El Aidy, 2019; Brogden et al., 2005). Finally, the immune system has been proposed to recognize antigenic discontinuity, that is, by the "sudden modification of molecular motifs" (Pradeu et al., 2013), and microbial function in addition to pathogen-associated molecular patterns (Greslehner, 2020), which opens the broader perspective of the immune system being able to detect tissue function.

Furthermore, it is important to note that many neurons also release intercellular signaling molecules, e.g. neuropeptides, outside synaptic clefts (a phenomenon that has been coined volume transmission) into the extracellular space, including into the cerebrospinal fluid or blood and can therefore be labeled neuroendocrine (Hartenstein, 2006). Phylogenetic and ontogenetic findings indicate that the label neuroendocrine cannot only be applied to chains of hormone release regulated by neurons in the hypothalamus, but also to gastrointestinal neuroendocrine cells and nervous fibers targeting such cells (Falkmer, 1993; Hartenstein, 2006; Modlin et al., 2006). Hence, "the gut neuroendocrine system is viewed as a syncytium of neural and endocrine cells" (Modlin et al., 2006). Thus, it seems that neuroendocrine systems, as a whole, can be investigated in different ways based on their mode of intercellular signaling.

Classical examples of ligand-receptor contact-mediated intercellular signaling occur between immune cells, but paracrine and endocrine intercellular signaling is also widespread among immune system components. Consequently, the immune system does not seem to correspond to one particular mode of intercellular signaling either. Although "[t]he classical model of immunity posits that the immune system reacts to pathogens and injury and restores homeostasis," recent models of immunity have proposed that "effector immune responses […] are induced by an antigenic discontinuity; that is, by the sudden modification of molecular motifs" (Pradeu et al., 2013) or that "the healthy immune system is always active and in a state of dynamic equilibrium between antagonistic types of response" (Eberl, 2016). Therefore, it seems that the notion of the immune system refers more to certain stimulus-response modes than a particular intercellular communication mode.

To conclude, an account based on a classical "causal role function", like heart contractions contributing to blood circulation (Brigandt, 2017) no longer allows for a clear characterization of the immune system as a defense system itself nor does it offer a clear distinction between the neuroendocrine and immune. As we have seen, molecular structure does not necessarily determine if a molecule should be considered part of the neuroendocrine or immune system. Furthermore, it is possible to extend the notion of the immune system in its functionality, not only to the compartment of cells traditionally ascribed to it but also to other cells and "to the entire body." It is good to remember that there is no "immune system" as something that is out there in nature, waiting to be discovered. In the real world, there is no clear-cut phenomenon but rather complexity, which comes inherently altogether (Hacking et al., 1983). On the contrary, science investigates some phenomena that it manages to isolate analytically, and to which it attributes properties in order to better understand them. However, it is the progress of research itself that shows that these categories are not fixed and that they can be modified precisely because of new discoveries or new ways of seeing those phenomena.

4. Rethinking the recomposition of multiple building blocks: the enteric nervous, immune,

endocrine, and microbiota systems

Discussions around neuroendocrine and immune integration have largely centered on the central nervous system (see, for instance, (Mašek et al., 2003)). The enteric nervous system has received less attention in neuroimmunology, yet its integration with gut immune and endocrine systems presents an important case study to understand neuroimmune-endocrine integration. By taking an ecological evolutionary developmental approach to the gut system, we illustrate the inadequacy of the building-block model of mapping individual functions to individual subsystems and challenge the idea that neuroimmune interactions are relevant only in the context of disease and disorders. With this example, we can illustrate how an integrated NEIMS approach emerges from co-developing, co-functioning, and co-evolving nervous, immune, endocrine, and gut microbiota systems. But first, why focus on the gut?

Proposal 1: the gut complex as a unit of organization

The gut, especially the largely autonomous mid/lower gastrointestinal (GI) system, is a remarkable organ from a neuroimmune perspective. In mammals, for instance, in part due to the enormous surface area of the intestines, the gut is the largest immune and lymphoid organ ("News & Highlights," 2008; Vijay-Kumar et al., 2014), the largest endocrine organ (Ahlman & Nilsson, 2001; Rehfeld, 2004), and contains one of the largest set of intrinsic and interneuron connections amongst the autonomous nervous systems in the body (Kulkarni et al., 2018; Furness & Stebbing, 2018). The enteric mucosal immune system, the enteric endocrine system, and the enteric nervous system are complex systems operating intimately with each other. Altogether, the "gut complex" as a border is exposed to food, toxins, and to the trillions of microorganisms residing in our intestines, it manages immune tolerance and produces localized inflammatory responses to infections.

The operations of these gut subsystems are each relatively autonomous from their respective systemic counterparts. Starting with the gut immune system, the gut mucosal immune system consists of anatomically distinct microcompartments that house gut mucosa-associated lymphoid tissues (GALT) with a unique repertoire of lymphocytes (with IgA as the dominant antibody type) (Janeway et al., 2001; Wershil & Furuta, 2008). GALT compartments and structures, such as Peyer's patches and isolated lymphoid follicles, are sites of local adaptive immune responses that require microbial signals to fully develop (Silva-Sanchez & Randall, 2020).

The enteric nervous system and its extensive local connections makes it also relatively autonomous compared to other nervous systems. In organisms with a central nervous system (CNS), it is well-known that the enteric nervous system (ENS) is a unique neural network distinct but interacting with the CNS. Unlike other peripheral parts of the nervous system, it has interneurons that form a richly interconnected and largely autonomous system with sparse connections to the CNS (Furness & Stebbing, 2018). Evolutionary comparative work of the enteric nervous system across the animal kingdom shows that it is not derived developmentally or evolutionarily from the CNS. Instead, it is evolutionarily conserved across animals with or without the CNS and has been proposed to constitute one of the first nervous systems in evolution, rather than a second or derived brain (Furness & Stebbing,

2018). Even though there are bi-directional pathways linking the gut to the CNS, the complex internal processes of the gut illustrates that gut function is not ultimately driven by the CNS but in large part mediated and controlled by the local regulatory networks and reflexes in the gut. The gut complex is thus an important causal center beyond the inputs from the CNS.

Finally, the gut endocrine system can be considered to correspond to enteroendocrine cells (EEC), which are scattered throughout that gut lining (Gribble & Reimann, 2019) and differentiating into more than 15 subtypes (Posovszky, 2017). EECs form an important bridge between the epithelium and the enteric nervous system through "synapse"-like structures (Kulkarni et al., 2018). As outlined in chapter 1, it has been shown, using anatomical and cytochemical approaches, that enteroendocrine cells share characteristics with pituitary corticotroph cells and pancreatic islet cells, all known to secrete polypeptide hormones. Moreover, at the end of the 1970s, the idea of the gut containing a "diffuse neuroendocrine system" started to emerge (Polak & Bloom, 1979, p. 1400).

Increasingly, the microbiota has started being treated as a "unit". This has a twofold implication. On the one hand, it means that associated bacteria may possess discrete collective boundaries as if they were organs. This can explain why microbial activities are increasingly associated with specific organismic functions (e.g. digestion, immune modulation, etc.). On the other hand, the microbiota can be now considered an object of scientific investigation as a unit (Raman et al., 2019; Cani & Van Hul, 2020).

The functioning of the gut requires all four systems– the gut nervous, immune, endocrine, and microbiota systems– to work together to serve as an interface with the outside world, processing food, expelling toxins, and managing trillions of residential bacteria, viruses, protists, fungi, and sometimes, helminths. In addition, the gut complex has also been proposed to be involved in emotions, sensory processing, cognition, (social) behavior, general motivational state and attitudes, etc. (Mayer, 2011).

Proposal 2: the building block model and ecological models for the gut complex

A building block model of the gut would treat each of these systems as modules with distinct cellular and tissue structure and functioning. Gut motility, for instance, is then thought of as the main function of the enteric nervous system. Gut secretion is the main function of the gut endocrine system while gut defense is the main function of the gut mucosal immune system. Resident commensal microbiota are assigned the roles of probiotics that supplement the body's metabolism and modulate the immune system. Treating the gut as consisting of building blocks implies that we can infer the specific contributions of each system through knock-out and gain-of-function techniques to block or enable parts of the system. A building block model also implies that we can safely study each system in isolation. The control and regulation of the gastrointestinal system would be seen as the "stitched together" combined efforts of these distinct physiological systems. A common "language" might communicate between these systems, for instance, via neurotransmitters, hormones, or cytokines such as interleukin-6, substance P, leptin.

The building block model, however, is inadequate. Ecological developmental biology (**eco-evo-devo**) is a subfield of evolutionary developmental biology (evo-devo) that examines how phenotypes arise from

the processing of environmental signals and cues throughout development, and how such processes in turn affect their evolution (Gilbert & Epel, 2015). Taking an eco-evo-devo angle to the gut complex strongly supports the idea that the gut is not the coming together of multiple independently originated systems, but a *multi-system complex* that has co-developed and co-evolved from the very beginning. These findings compel us to recompose the gut complex as a system of its own with an intermingled NEIMS.

First of all, the subsystems of the gut complex require input from each other to fully develop as a *co-developed unit*. Each physiological system does not differentiate and develop on its own but co-develop as a "gut complex." Signaling molecules from the enteric glial cells of the ENS are implicated in the development of gut immune compartments and immune cells (Yoo & Mazmanian, 2017). Vice versa, the immune system and the microbiota are needed for the full development and maturation of the enteric nervous system (De Vadder et al., 2018; Kabouridis & Pachnis, 2015; Obata & Pachnis, 2016; Vuong et al., 2020) as well as its innervation into gut tissue (Kang et al., 2021). Furthermore, the development and differentiation of enteroendocrine cellular subtypes are highly plastic and dependent on the nutritional and microbiota content of the lumen (Posovszky, 2017).

Secondly, in organisms with nervous, immune, and endocrine systems, the integration between these three with the microbiota is likely ancient and evolutionarily conserved as a co-evolved unit. It is important in this respect to remember that some sort of neuroendocrine system is already present in organisms without immune system elements and that this system regulates bacterial growth (Augustin et al., 2017). Not surprisingly, ever since the appearance of immune cells and mediators during evolution, interactions between elements of endocrine, immune and nervous systems have been documented to occur (Panerai & Ottaviani, 1995; del Rey & Besedovsky, 2017).

Finally, abundant research has focused on how the proper functioning of each system relies on the others. As the functioning of each subsystem is context-dependent on the state of the other systems as well as the situation of the body as a whole, they constitute a *co-functioning unit*. An example is the EECs. They are considered hormone-secreting endocrine cells. We now know that they influence other physiological systems (Posovszky, 2017). On the luminal-facing end, some types of enteroendocrine cells are in direct contact with gut content, detecting and integrating signals from food, toxins, the immune system, and the gut microbiota, etc., with their microvilli. They can secrete peptide mediators into the lumen that regulate gut motility and metabolism but also immune responses and defense (Wikoff et al., 2009; O'Mahony et al., 2015; Kuwahara et al., 2020; Cani & Knauf, 2016; Gribble & Reimann, 2016; Psichas et al., 2015). On the other end, they can regulate gut function and communicate with the CNS by secreting hormones into the bloodstream (the classic hormones include stomach gastrin and the small intestinal cholecystokinin and secretin) or by acting on local sensory nerves or by synaptically communicating with enteric glia via neuropod structures (Kaelberer et al., 2020). Neuropods are associated with local nerve terminals, suggesting that these cells are part of a local neuroendocrine system (Sharkey et al., 2018). The gut endocrine system is thus a part of the gut nervous and immune circuitries, bridging them with luminal microbiota. The neural system of the gut extends beyond just the

enteric neurons to also include enteric glia cells as well as enteroendocrine cells (Bohórquez & Liddle, 2015).

Co-functioning is especially prominent when we consider the gut microbiota. Consider, for instance, that microorganisms are involved in the regulation of peristalsis of food. The short-chain fatty acids secreted by gut microbes stimulate the release of serotonin (De Vadder et al., 2018). Gut microbiota is implicated in the development and functioning of the intestine (Hooper, 2004) and its immune (Belkaid & Hand, 2014; Rhee et al., 2004), endocrine (Wikoff et al., 2009; O'Mahony et al., 2015; Watnick & Jugder, 2020), and enteric nervous systems (De Vadder et al., 2018; Hyland & Cryan, 2016).

The co-development, co-evolution, and co-functioning of the gut nervous, immune, endocrine, and microbiota systems support the idea that together, they form a "gut complex." From the NEIMS perspective, the functions usually assigned to each physiological system emerge from their intermingling– they do not possess their typical functions prior to these interactions. Furthermore, the microbiota is now gradually recognized as an intrinsic yet also "ecological" factor modulating the physiological responses of the human organism, with most of the literature focused on the immune system (Belkaid & Hand, 2014; Boem et al., 2020; Chiu et al., 2017). This intrinsicity, therefore, leads to consider the activity of the microbiota close to an endogenous regulator: i.e. the microbiota can be seen as a functional part of the immune system itself (Amedei & Boem, 2018; Belkaid & Hand, 2014; Fung et al., 2017; Zheng et al., 2020). The eco-evo-devo perspective of NEIMS provides a new way to think about the four systems and their relationships.

So far, we've shown how new insights from recent literature challenge the neuroimmunology building block model. New findings push us to rethink the "one structure-one function" assumption. In the previous section, with the immune system as a key example, we argued that the immune system is not just a defense system. Here, with the gut complex as an illustrative example, we argued that the nervous, immune, and endocrine systems do not come together like Lego pieces, with each system exhibiting their main functions prior to their interactions. Instead, from an eco-evo-devo perspective, the respective functionality of enteric nervous, immune, endocrine, and microbiota systems emerges from their interactions.

Proposal 3: a NEIMS approach

A NEIMS approach invites us to rethink the functionality of the entire neuro-endocrine-immune-microbiota system complex. Once we look at NEIMS as a whole, there are many potential ways in which one can recompose the gut complex and to assign function to NEIMS as a whole. One way is to look at other structural systems (e.g. nervous system) and their preferred associated function (e.g. cognition) and propose that the gut complex as a whole is also capable of it. There are indeed previously underappreciated - sometimes not even considered - potential functions of the gut complex that suggest that the gut is not the mere sum of distinct building blocks "dedicated" to digestion. One possibility is to think of the gut complex as a proto-cognitive organ. It is an example of how using different criteria for systems can lead to the proposal that the gut complex as a unified entity that involves all four categories of host physiological and microbial systems.

The gut has a wide diversity of sensory functions. Instead of analyzing them separately under their respective historical systems (e.g., the gut endocrine system, the gut nervous system, the gut immune and tissue defense system), Furness and colleagues have long argued that we should treat gut detection as a function of an integrated gut sensory organ (Furness et al., 1999, 2013; Furness & Cottrell, 2017). These detection features can be interpreted as an example of integration between the enteric nervous system, the gut mucosal immune system, the enteroendocrine system, and the gut microbiota cells.

The gut has an enormous capacity to sense and distinguish between nutrients, irritants and micro-organisms in its internalized milieu and to differentially respond to those (Mayer, 2011; Breer et al., 2012; Collins et al., 2012; Latorre et al., 2016). It is important to point out first that the gut can exhibit at least six stereotypical patterns of motor and secretion behaviors that can be observed in different physiological and pathological conditions and that can be modulated by numerous signals (Wood, 2004; Schemann et al., 2020). While most of the studies establishing these patterns and their modulation have been done in vertebrates that possess central nervous systems capable of influencing the gut through the peripheral nervous thus making it sometimes hard to determine the part played by the gut, it is important to consider *Cinaderia*, like hydras, box jellies, jellyfish, corals, and sea anemones, which can display several gut motor patterns despite possessing only nerve nets with the most important being situated around the central body cavity.

Signal integration is "[t]he capacity to combine information from multiple sources," and valence, "[t]he capacity of a [biological system] to assign a value to the summary of information about its surroundings at a given moment, relative to its own current state" (Lyon, 2015, p. 4). It should be noted that sensing of gut luminal contents seem to be categorized as beneficial or threatening based on gut signals and responses and can be integrated with systemic hormonal signals at the level of gut endocrine cells and nerve fibers (Dockray, 2003; Wood, 2004; Holzer & Holzer-Petsche, 2009; Mayer, 2011; Brookes et al., 2013; Sclafani, 2013; Neunlist & Schemann, 2014; Maniscalco & Rinaman, 2018; Sharkey et al., 2018; Han et al., 2018; Lu et al., 2021). Behavior can be considered as "[t]he capacity of a a [biological system] to adapt via changing its spatial, structural or functional relation to its external or internal milieu" (Lyon, 2015, p. 4). Accordingly, it can be argued that the gut's modification of motility and secretion in response to nutrients, irritants and infectious microorganisms constitute local behavioral responses, but also that gut responses can give rise to behavioral modifications at the level of the whole organism (Wood, 1999; Stephen, 2001; Khan & Collins, 2006; Chen et al., 2009; Mikkelsen, 2010; Akiho et al., 2011; Brookes et al., 2013; Skibicka & Dickson, 2013; Latorre et al., 2016; Furness, 2016; Yang & Chiu, 2017; Serna-Duque & Esteban, 2020). Thus, the gut is clearly capable of discriminating, integrating, evaluating, and responding to different kinds of stimuli.

Enteroendocrine cells can respond to gut luminal contents through multiple receptors, including through pattern recognition receptors that recognize particular microbial molecules (Mayer, 2011; Sharkey et al., 2018). It is also important to keep in mind that signaling molecules in the gut are not necessarily specific for one single cell type as enteroendocrine cells, in addition to mast cells, can also release serotonin (Sharkey et al., 2018). In terms of anticipation, it is clear that changes in feeding patterns are met with responses both at the level of the enteric nervous system and gut

neuroendocrine-immune interactions (Schemann et al., 2020). While it is clear that local mast cells play an important role in Pavlovian conditioning of vertebrate gut responses (Wood, 2004), it is still an open question as to whether gut components like the enteric nervous system alone are solely capable of this kind of associative learning or whether it always involves the CNS (Schemann et al., 2020). In this respect, it is interesting to consider that classical conditioning occurs in animals without a brain like sea anemones. Moreover, these *Cinderia* have also been shown to display habituation and sensitization (Cheng, 2021). Finally, it is beyond doubt though that gastrointestinal infections and inflammation can give rise to long-lasting enteric neuroplasticity in vertebrates (Schemann et al., 2020). Taken together, these findings indicate that the gut can be considered as capable of minimal cognition or having proto-cognitive capacities.

Instead of focusing on the nervous and immune systems as historical cognitive systems, the possibility that interactions between neuroendocrine and immune components confer cognitive capacities to the gut should be considered. This gives rise to the questions of the precise contributions of the gut immune and neuroendocrine cells and how the gut engages and interacts with full-blown cognitive systems in animals with a CNS? Indeed, the enteric nervous system and the gut immune and neuroendocrine system have co-developed, co-constructed, co-evolved as part of an integrated biological unit that may exhibit scaffolding and niche construction that also need to be considered regarding gut microbiota.[9]

5. Conclusion and future outlook

After this conceptual tour through a philosophical perspective on the interactions between the neuroendocrine and immune system and how to deal with NEIMS – instead of a building block model –, let us conclude with a couple of take-home messages which we would like the reader to integrate into their thinking (see also Box 1). We hope they will be helpful not just as another building block piece of knowledge, but as conceptual tools for thinking about these systems and approaching them experimentally.

First, the microbiota is a necessary ecological component of our physiology. The microbiota is involved in the constitution of the whole organism during development and functioning[10]. It is known that the

[9] Finally, although not addressed here, the organization of the gut between the endocrine, immune and nervous components may also play a role in the emergence of proto-awareness in animals – a topic to be discussed in the future.

[10] From a systemic point of view, however, it is important to remember that the microbiota is not only involved in the modulation of the local or tissue-specific response. Indeed, treatment with antibiotics has in fact shown how the decrease of the microbiota (both in terms of its number and composition/diversity) has a global impact on the activities of the immune system at the level of the whole organism. This occurs both in situations of inflammatory and defensive response and concerning the general and regulatory functions of the immune system in its broadest sense. There are now countless studies concerning the potential contribution of the microbiota (with either protective or promoting effects) with conditions such as chronic, metabolic, autoimmune, neurodegenerative

microbiota can affect (either positively or negatively) the predisposition to the onset of pathologies (such as autoimmune diseases), moreover, it is now proven that it is fundamental for the maturation of secondary lymphoid structures, in the strengthening of the intestinal epithelium and in the homeostasis of tissues of interest. Other known functions concern the modulation of the immune system, such as the regulation of the inflammatory response and the activation of immune cells related to specific tissues. Next, it carries out an action (both directly and indirectly) against other microorganisms with pathogenic potential. For instance, not only is it now widely recognized that microbiota plays a role in the modulation and the development of the immune system (Belkaid & Hand, 2014) but it can directly interact with other bacterial species determining their capability of interacting with the host. Thus, by preventing some species from inhabiting the host, the so-called "colonization resistance" explains, in an ecological way, how commensal microorganisms constitute a functional extension of the immune system itself (Amedei & Boem, 2018; Ronai et al., 2020).

Taking the microbiota into account may show, to quote from (Nicholson & Wilson, 2003, p. 669), that "systems biology could ultimately turn out to be more like an ecological problem than one of molecular biology." The gut is an outward-facing organ that serves as an interface between the organism and the environment. The involvement of the gut microbiota in the gut neuro-immuno-endocrine system thus introduces an ecological perspective to internal physiology. The diverse ecosystems of microbiota are engaged with the gut in ecological relationships (Costello et al., 2012; Coyte et al., 2015; Dethlefsen et al., 2007), sometimes in ways that integrate with host physiological systems (see (Chiu et al., 2017) for a co-immunity between host immune systems and the microbiota). The gut complex constitutes an extended developmental system that includes environmental cues and stimuli (Griffiths & Gray, 2004; Griffiths et al., 1994).

The symbiotic bond from a functional but also an evolutionary point of view implies that the microbiota of *Homo sapiens* has co-evolved with our species making the individual referred to as the single "human" a functional, and evolutionary unit, composed of the network of interactions formed by the various actors of the symbiotic association.

This means that the concept of symbiosis itself has been updated. Individual beings such as animals or plants, in their purely genetic dimension, can no longer be considered complete and autonomous. Accordingly, singular organisms should now be seen as functional wholes, resulting from this interactive network. Indeed, the developmental biologist Scott Gilbert described this fact by saying that "we have never been individuals" (Gilbert et al., 2012). Because of that, one now cannot now ignore the fact that what was thought to be single and well defined is actually the product of a multitude of different agents, in an ecological and dynamic relationship: i.e. the holobiont (Bordenstein & Theis, 2015). Leaving aside the purely theoretical debates on the nature of the holobiont, the fact remains that it is not only the object of experimental but also therapeutic investigations.

The result is that the "holobiont" (the macro-organism and its microorganisms) can be considered as a new unit of biological organization which is epistemically privileged, from the point of view of functions

diseases, and cancer.

and criteria regarding individuality. This means, given the eco-systemic nature of the holobiont, that the relations between these physiological systems and its extensions can also be uniquely analyzed from an ecological point of view and with methodologies taken from ecology (Martin et al., 2011; Chiu et al., 2017; Ronai et al., 2020; Schneider, 2021).

Ideally, readers will appreciate the eco-evo-devo perspective we propose might change their perspective of how to look at experimental data – especially data inconsistent with the building block model, which would be hard to interpret otherwise. By adopting a different epistemic culture – instead of the building block model – the study of neuroendocrine-immune system interactions could become a "block-buster".

Box 1: Philosophical key points
● Conventional dismantling of systems in terms of structure and function is often biased by conceptual and methodological limitations. ● Division of systems and division of labor are often necessary and helpful, but we also need the other direction of re-integration – for which the building blocks approach often will not do. ● Do not shy away from considering functions classically associated with one system for another and propose new functions by observing systems as a whole. ● Do not take the systems as a given, having a clear "main task". These systems cannot be combined like building blocks in an additive way, resulting in the mere sum of these structures and functions. Nor are functions determined once and for all. ● Do not ask: who is controlling whom? Which system regulates the other? Many of these questions are ill-posed when framed through the lens of the building block model. ● Do not just focus on disease. Lip-service is often being paid through the phrase "in health and disease". However, it is far from clear what "health" is and putting them as polar opposites might not be the most helpful way for understanding how these systems inter- and co-act. ● There is not just bidirectional communication or regulation between different systems. Proper re-integration takes more than just putting them together. ● Thus, multidisciplinary approaches are needed. One cannot just treat the systems nor the disciplines studying them as building blocks. ● Re-integration might be difficult and uncomfortable at times, forcing one to question what seems to be established common knowledge within fields. ● Still, such an approach is more promising than a reductionistic enterprise, in which one tries to find the "most fundamental units". ● This should be kept in mind for training and educating future scientists in the respective fields, including relevant lessons from philosophy of science.

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