Mistake-making: a theoretical framework for generating research questions in biology, with illustrative application to blood clotting

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MISTAKE-MAKING: A THEORETICAL FRAMEWORK FOR GENERATING RESEARCH QUESTIONS IN BIOLOGY, WITH ILLUSTRATIVE APPLICATION TO BLOOD CLOTTING

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ABSTRACT

It is a matter of contention whether or not a general explanatory framework for the biological sciences would be of scientific value, or whether it is even achievable. In this paper we suggest that both are the case, and we outline proposals for a framework capable of generating new scientific questions. Starting with one clear characteristic of biological systems—that they all have the potential to make mistakes—we aim to describe the nature of this potential and the common processes that lie behind it. Given that under most circumstances biological systems function effectively, an examination of different kinds of mistake-making provides pointers to mechanisms that must exist to make failure uncommon. This, in turn, informs a framework for systematic inquiry, which in this paper we apply to the hemostatic system, but we believe could be applied to any system across biology.

Introduction

WHETHER or not an explanatory framework applicable across the biological sciences is possible, and indeed whether the pursuit of such a framework is a worthwhile activity, has been the focus of much discussion (Minelli and Pradeu 2014). Some of the perspectives are philosophical, for example, focused on whether general unifying theories are characteristic of the mature sciences (such as physics) and hence would also be a mark of maturity in the biological sciences. Other debates address the topic in relation to the practices of the biological sciences, posing the question whether generally applicable frameworks are likely to lead to more productive research. In a seminal paper arguing for a general framework to guide investigation, John R. Platt noted an increasing tendency for scientific research to be driven by technological advances and opportunistic experimentation rather than by the systematic generation and testing of competing hypotheses (Platt 1964). He argued for "strong inference," by which he meant the application of the same sequence of hypothesizing and testing, repeated through successive generations of findings, hypotheses, and experiments. Following the dramatic expansion of technological and computing power over the past 50 years, recent authors have voiced similar concerns, arguing for more hypothesis- and theory-driven research (Coveney and Dougherty 2016). There is widespread agreement regarding the diagnosis of the problem (Slack 2013), but very little regarding the solution. Although Platt's argument for a systematic approach to investigation along the lines of "strong inference" seems uncontroversial, the desirability of a general explanatory framework or theory is contested. Sometimes a more piecemeal approach is advocated, which avoids the assumption that the same theoretical features will be found in different areas of the biological sciences (Giere 1999). This question is not only of concern to philosophers of biology. For example, discussions regarding the possibility and desirability of a general and generative theory for cancers are central to current debates regarding the future of cancer research (Sonnenschein and Soto 2020).

Notwithstanding these arguments, there are good reasons to consider the possibility of a generally applicable theoretical framework for biology. Perhaps the most compelling is that we already have a theory of evolutionary processes that is immensely successful. It is strikingly ambitious in its scope, generating hypotheses across the divide between the biological sciences (as they are generally understood) and the behavioral and social sciences (Dunbar and Shultz 2007; Braithwaite et al. 2020). Increasingly, evolutionary concepts such as competition and selection have been applied across the biological sciences, for example, in cancer research (Thomas et al. 2013). Equally, it remains to be seen whether evolutionary hypotheses will make a substantial contribution to emerging theories of carcinogenesis (Sonnenschein and Soto 2020) and more generally whether

evolutionary theory can provide the kind of detailed template that is needed to guide hypothesis generation and experimentation. Therefore, a challenge for any formulation of a general framework is that it should be sufficiently specific to be useful in scientific practice, while at the same time preserving the general applicability found in evolutionary hypotheses. In this paper we make a proposal that aims to contribute both to scientific practice and to general theorizing. We start by pointing out some features of biological systems in general, building on them to identify a set of underlying processes that are common to such systems. We then argue that the details of these processes provide a means of interrogating biological systems in a systematic way—providing, in other words, the basis for a framework for systematic inquiry—and illustrate how the framework can be applied to the hemostatic system (blood clotting) as an example. Although our proposal is in many respects philosophical, it is firmly in line with the view that one should have "two outcomes in mind: philosophical comprehension and scientific benefit. It is insufficient and unhelpful to simply provide an alternative philosophical perspective. A practice-oriented perspective on theory should profit ongoing empirical investigation" (Love 2013:326).

The Universal Phenomenon of Mistake-Making in Biological Systems

We take the making of mistakes as our starting point for three main reasons. First, mistakes are found without exception in biological systems. Every area of biological research of which we are aware is based on the assumption that, in biological systems, accounts of causal mechanisms have to specify how they operate correctly and how they are liable to error (Bolton and Hill 2004). Otherwise, fundamental scientific and medical distinctions between health and disease would become problematic. The universality of mistakes in biological systems addresses one of the challenges that we aim to meet—that a theoretical framework should apply, in a wholly general way, across biology.

Second, in the biological sciences the investigation of different phenomena rests on the assumption that their biological significance can be probed by asking whether they are occurrences of normal behavior and function or whether they are *mistakes*. This is core, and perhaps we should say, axiomatic in biological research. Yet it has received little attention, perhaps as it is so deeply ingrained in current research practice.

Third, the potential of biological systems to make mistakes can be given a generic description (one that applies to all biological systems), which provides an opportunity to work out the nature of this potential and to deduce the common processes that lie behind it. Given that under most circumstances biological systems function effectively, an examination of different kinds of mistake-making provides pointers to mechanisms that must exist to make system failure uncommon. Below we provide this generic description and outline the common processes that we have identified. We then use the details of these processes to create a framework for systematic inquiry—a framework for generating research hypotheses rapidly and systematically—which we apply to the hemostatic system (blood clotting), but we believe could be applied to any system across biology.

Processes Behind Mistake-Making

In biological research, mistakes can be characterized as physical variations that threaten function. Examples include: a change in DNA nucleotide sequence that leads to altered protein function; a change in the structure of a receptor resulting in impaired ligand binding or signal transduction; and elevated potassium levels impairing conduction in the heart leading to life-threatening cardiac arrhythmias. We say "threaten" because mistakes can be corrected or their effects mitigated, in which case functioning may not be impaired. Importantly, physical variations that do not threaten function are not mistakes: a change in DNA sequence with no consequences for protein function; an altered receptor structure that does not affect binding; and variations in heart conduction that have no effect on cardiac rhythm. In medicine they are often referred to as normal variants.

Having observed the link between mistake-making and function, we now need a more precise way of characterizing function and its impairment. Although the definition of function can become less clear in circumstances such as *de novo* gene emergence (Keeling et al. 2019), where the physical structures of interest often display only some of the properties of established genes, we propose that, in general, function can be characterized in terms of the way biological systems generate actions in relation to their environments. In making this proposal we note that there are two concepts of "function" that are of potential relevance here—what we call the "function of" concept, as in the question of the function of the zebra's stripes (see, e.g., Garson 2019), and the other being the "agential" sense of function, which is concerned with action in the environment. Our primary concern is the latter, although of course there will be deep connections between "function of" and "agential function." The function of the zebra's stripes will affect the way the animal functions, agentially, in its environment. In our discussion of agency in relation to the environment, we refer here both to the microenvironments of systems within organisms as well as to the larger external environments of organisms. The actions seen in biological systems are effective where they bring about relevant and well-timed change that preserves, protects, or promotes the welfare of the organism, including survival or reproductive advantage, at the individual or species level. Where an object or event in the biological system's environment poses a threat, effective action removes the threat; where it provides an opportunity, effective action takes advantage of it.

This agential understanding of function, readily recognizable to biologists, and which we have described before in the context of biology and mind (Bolton and Hill 2004), is different from typical philosophical approaches with their focus on intention as the factor differentiating human agency, properly so-called, from mere bodily movement (Woodfield 1976). It might also seem to suggest intent or even consciousness. Yet our theory requires only that action promote the functioning of a system in accordance with its organizing principles. This is consistent with many contemporary accounts of agency in terms of action in the environment that do not require reference to concepts such as intention or conscious activity (e.g., Barandiaran et al. 2009). We eschew the term "teleological" here to avoid irrelevant connotations; that said, philosophy has seen a recent revival of teleological thinking in terms of the metaphysics of powers and what Molnar calls "physical intentionality" (Molnar 2003; Oderberg 2017 on "finality").

From our definition of mistakes as variations that threaten function, and then of function in terms of effective biological action in relation to the system's environment (whether microenvironment or external), it follows that mistakes can arise from physical variations either in the biological system's environment or in the system's structure.

In both cases the principles are the same. For example, a physical variation in the molecular structure of an antigen (a physical variation in the environment of the system), leads to mistake-making if it threatens effective immune action—if, say, the immune response is activated in the absence of infection. Likewise, a physical variation in an antigen receptor (a physical variation in the biological system's structure) leads to mistake-making if it threatens effective immune action, for example, if the system fails to respond to an infection. We may think of the distinction in terms of either an "uncooperative" environment or a "breakdown" inside the system. If a toad flicks its tongue at a plastic replica of a worm, it makes a mistake due to the unusual and disadvantageous state of the external environment. Disease, however, might cause the toad to regularly ignore perfectly edible prey. Here, an internal failure leads to a specific kind of misidentification.

Our thesis, then, is that all biological systems are mistake-prone in the above sense, and that when studying the biological significance of physical variations, the general definition of mistakes is *physical variations that threaten function*, where "physical variations" can be either in the system's environment or the system's structure and "function" is understood as effective action, meaning action that brings about relevant and well-timed change

that preserves, protects, or promotes the welfare of an organism, including survival or reproductive advantage, at the individual or species level.

Mistake-Making in the Evolutionary Context

The importance of mistake-making to an understanding of biological systems is evident when we consider evolutionary mechanisms. We do this in the context of the distinction made by Ernst Mayr between proximate and ultimate causes in biology (Mayr 1961). Mayr equated proximate causation with immediate factors (for example, physiology) and ultimate causation with evolutionary explanations (for example, natural selection). Using his terminology, our proposal refers to proximate causation. However, and in line with subsequent work that has argued that the distinction is not as clear as originally formulated (Laland et al. 2011), it also is consistent with evolutionary explanations. We suggest that only a molecule capable of making mistakes can be a candidate for creating the scope for novel adaptations required for natural selection. The key property of DNA is that the constraint on nucleotide sequences is provided by the template inherited from previous generations, and not by physicochemical constraints (i.e., not by the constraints of its physical structure). This lack of physicochemical constraints creates the conditions for mistakes, which in DNA are physical variations that threaten protein structure and hence function. This same lack of physicochemical constraint creates the scope for variations that lead to protein structures with novel adaptive potential. Consistent with our definition of function and malfunction as action in relation to the environment, this also creates the conditions whereby the same DNA sequence and protein structure can be either a mistake or an adaptive feature in an environment-dependent way, as illustrated by the sickle cell trait. The same substitution of one amino acid in the hemoglobin molecule leads, in the homozygous individual, to red cells that readily deform when oxygen levels fall and are so liable to block blood vessels, while in the heterozygous case it confers resistance to malaria, and this persists in the human genome (Williams et al. 2005).

Detailing Mistake-Making in Terms of Ineffective Action in Order to Generate Research Questions

We can now start to describe in more detail the types of ineffective action that may occur in biological systems. These include: action in response to the wrong environmental event or object; failure to act when action is required; premature action; action that is too late; action poorly directed in relation to the environmental object or event; disorganized action; action that is too brief or too extended; and action that is too little or too much. With reference to blood clotting, which we consider in detail below for illustration, action of the hemostatic system entails a timely response to injury to generate a clot that limits or terminates blood loss. Ineffective action includes all possible departures from this function, including clotting that is too early or too late, clotting in the absence of injury, failure to initiate clotting in the presence of an injury, and clotting that fails to deal with blood loss.

We propose that each *characteristic of the environment* to which the biological system responds, and each *mechanism required to regulate effective biological action*, is a locus of potentially unidentified mistakes. Thus, research questions can be generated either *forward*, by asking about the potential for mistakes in relation to known environmental characteristics and regulatory mechanisms for biological action, or *backward*, by identifying mistakes and asking whether they provide pointers to hitherto unidentified environmental characteristics or regulatory mechanisms for biological action. Inquiries should not be limited to searching for mistakes that are to be expected but should include the puzzling nonoccurrence of mistakes predicted by the application of the framework. The scope for this line of thinking to generate productive hypotheses is illustrated by J. J. Hopfield's inquiry into why, in view of the similarity between the affinity of correct and incorrect tRNA structures for each mRNA codon, so few errors are made during protein synthesis (Hopfield

1974). His "kinetic proofreading" model predicted the existence of intermediate steps between initial presentation of tRNA and insertion of the amino acid into the elongating protein chain that could increase discrimination between correct and incorrect tRNA.

Characteristics of the Environment and Regulatory Mechanisms for Biological Action

What do we mean by the *characteristics of the environment*? This refers to the kinds of objects (such as antigens, in the case of the immune system) or events (such as injury, in the case of the hemostatic system) in the biological system's environment that are relevant to the system because they require action. Relevant objects or events may be strongly dissimilar to all others encountered in the system's environment, so that the system needs few or no discriminatory capabilities. In this case it will not be prone to making mistakes by responding to the wrong objects or events. By contrast, in an environment where, among similar objects or events, some require action but for others action would be irrelevant or damaging, the system requires discriminatory capabilities and will therefore be prone to mistakes involving incorrect responses. The immune system, for example, is highly prone to discriminatory mistakes. Similarly, in an environment where different kinds of objects or events tend to have the same implications for action, the system may classify otherwise dissimilar objects or events together. For example, blackbirds classify physically dissimilar earthworms, insects, and snails as "edible" and with the same implications for the action of eating (Snow 1988). In relation to responding to characteristics of the environment we refer to discrimination and classification as *operations* performed by the system.

In view of the complexity of so many objects and events, do biological systems respond to them in their entirety? This is unlikely, given that detecting and processing all potential characteristics of complex objects and events would be difficult and time-consuming, thereby threatening timely action. We therefore predict that discrimination and classification are likely to be based on some marker, or even a limited number of features of that marker. In our example of blood clotting, where the key discriminatory task is detecting the presence or absence of an injury, its initiation through platelet activation is not a response to the injury as such, but rather to exposure to collagen from the blood vessel wall, which provides a reliable marker of injury. Furthermore, it is not a response to the entire molecule, but to a subset of repeated amino acid sequences within the structure of collagen (Farndale et al. 2004; Herr and Farndale 2009).

What do we mean by *regulatory mechanisms for biological action*? All of the dimensions of action—onset, termination, duration, amount, organization, direction, and fit with the environment—require regulation. If these dimensions are always the same, or are tightly predetermined (e.g., according to the parameters of the object or event), there will be only limited scope for regulation and hence for mistakes. If, by contrast, they are influenced by feedback from the impact of the action on the object or event, or by internal or external monitoring of the progression of action, we expect them both to make important contributions to fine-tuned regulation and also to create greater scope for mistakes. All of these possible regulatory mechanisms of action, of which discrimination and classification are examples, we also refer to as *operations*.

Throughout biological systems that are responsive to the prevailing environmental conditions, there is a potential tension between operations oriented to the environment and those implicated in timely action. Complex and sophisticated appraisal of the environment may increase accuracy, for example, of discrimination, but at the expense of timeliness. Simpler appraisal by contrast may lead to timely but poorly directed and hence ineffective action. This is beautifully illustrated by the process of kinetic proofreading in T-cell activation. Kinetic proofreading, as we noted earlier, reduces the likelihood of mistake-making by increasing discrimination between physically similar environmental objects, in this case between nonself and self-antigens. It does this by introducing steps between the presentation

of the antigen and T-cell activation in the form of intermediate molecules that are either retained or detached from the activation sequence depending on the duration of binding between the antigen and the T-cell receptor (Hopfield 1974). However, these processes prolong the time between presentation of the antigen and T-cell activation, which could lead to accurate but delayed action. This has led to the search for mechanisms that may speed up kinetic proofreading at an acceptable error rate (Murugan et al. 2012; Yousefi et al. 2019).

A Framework for the Systematic Generation of Hypotheses in Biological Research

Based on the processes described so far, we can now formulate an outline of the way our framework can generate research hypotheses. We do this first, in some detail, in relation to ways in which biological systems respond to their environments (A) and, secondly, more briefly, in relation to the ways they generate effective action (B).

(A) In relation to ways in which biological systems respond to their environments, we start with the fundamental question:

> What are the characteristics of the environment to which the system responds, and what are the corresponding operations found in the system?

This can be separated naturally (but perhaps not exhaustively) into the following subquestions:

I. Do objects or events resemble others in the environment in relevant ways, thereby creating scope for mistakes of discrimination?
II. Do dissimilar objects or events have the same implications for action, and does the system then classify them together, creating scope for mistakes of classification?
III. Does the system respond to markers (or features of markers) of the object or event rather than to the object or event itself? If so, do failures of discrimination or classification arise from this simplification?

Having laid this groundwork, we can probe the boundaries of current knowledge. This starts by posing questions going *forward* from known operations to ask about possible mistakes (1), and *backward* from known mistakes to possible operations (2):

1. Based on the known operations that it performs, does the system make all of the mistakes that would be expected?
   a. If not, is this because not all possible (kinds of) mistakes have been explored and tested?
   b. Or, if not, is this because known compensatory or corrective mechanisms limit or prevent dysfunction, making these mistakes difficult to identify?
2. Are there any known mistakes that may provide novel clues to mechanisms of normal functioning?

We then go on to ask about additional possible operations and compensations and the mistakes they might generate:

3. Might there be so far unidentified operations involved in the system's response to its environment, and if so what kinds of mistakes would point to such operations?

These general questions can then be rendered into practical research proposals. We provide some examples below, which still maintain a high level of generality:

i. Given that a possible operation (discrimination, classification) is considered absent in this system, has that been systematically investigated and excluded, or is this more an (unexamined) assumption in the field?
ii. There are structures of this system that have been identified but do not seem essential for known functions. Could it be that they serve hitherto unidentified operations? Is the system really doing more than we have assumed so far?
iii. Can we think of operations that seem useful to us for this system to possess, but which have not yet been identified? What structures or processes would be needed to support such operations? How can we look for them?
iv. Listing the mistakes the system is known to make, and comparing them to the mistakes we predict the system should make given its known operations, we may find them incongruent. Do we need to search for more mistakes, for more operations, for more ways in which operations lead to

mistakes, or for more ways in which compensation and correction limit mistakes?

We can summarize this analytic framework as follows: Questions (I) to (III) explore what is known about the operations and mistakes of the system. Questions (1) to (3) show how we can then generate questions that go beyond what is currently known, providing a basis for novel research ideas. Questions (i) to (iv) help to translate these more generic questions into concrete research projects. Since the analysis in terms of mistake-making is fundamental to biological systems, any incongruence between what is known and what is conceivable translates directly and easily into research proposals of potential value.

(B) In relation to the ways biological systems generate effective action, we need to consider that operations must continuously match appropriate action to the environmental conditions. The progression of action—its organization, direction, and relevance to the object or event—may be modified by feedback about changes in the object or event itself, or by the effect that the object or event has on the system. Furthermore, the overall progression of action and its termination could be regulated by coordination among the units that generate the action, or by top-down monitoring and control. Next, different operations may be required to occur jointly so as to generate effective action, for example, integrating both feedback from the effect of the action on the environment and coordination among responding elements. Finally, since compensatory and corrective mechanisms may prevent mistakes from resulting in failed action and dysfunction, they must be explored with as much thoroughness as the mistakes whose effects they mitigate.

Application of the Framework for Inquiry to the Hemostatic System (Blood Clotting)

responses to the hemostatic system's environment

In relation to ways in which biological systems respond to their environments, we first consider the intravascular environment of the platelet. In answer to Question I (*Do objects or events resemble others in the environment in relevant ways, thereby creating scope for mistakes of discrimination?*), we note that based on current evidence, platelets do not discriminate between vessel wall injuries leading to blood loss and events that resemble them, and hence are not prone to mistakes of discrimination. With reference to Question II (*Do dissimilar objects or events have the same implications for action, and does the system then classify them together, creating scope for mistakes of classification?*), we note that blood clotting does not appear to be an appropriate action in response to events other than vessel wall injuries and hence should not be prone to mistakes of classification. Considering Question III (*Does the system respond to markers (or features of markers) of the object or event rather than to the object or event itself? If so, do failures of discrimination or classification arise from this simplification?*), we note that the system does respond to markers rather than to the object or event itself; that is, following vessel wall injury clotting is initiated by exposure to collagen, however, not to the entire molecule itself but to particular structural features of collagen (Farndale et al. 2004; Herr and Farndale 2009). It will therefore be vulnerable to mistake-making where other environmental events or objects present the same structural markers.

We can now make use of Questions 1 through 3 to further interrogate our understanding of clotting. Is platelet activation known to occur mistakenly in all of the expected ways based on current knowledge? If not, why not? Is that because there are unexplored kinds of mistakes, or because of error-correction mechanisms (Question 1)? Are there mistakes in platelet activation that could be examined as indicators of previously unidentified operations (Question 2)? Are there potential characteristics of the platelet environment beyond those that have already been identified, which if present would open up potential for further (types or instances of) mistakes and hence operations (Question 3)? For example, does the platelet environment include previously unidentified molecules that resemble injury or collagen exposure, for which action would be a mistake, and so requiring more discriminatory capabilities than are currently known? Or, if there are other platelet activators associated with blood vessel injury, could activation be based on the classification

of physically dissimilar trigger molecules, which would open up the scope for further mistakes of activation that it would be informative to explore? We illustrate these questions in Figure 1. Figure 1A summarizes current knowledge regarding platelet activation and Figure 1B illustrates these two further possibilities generated by the framework. In Figure 1B.1, the potential mistake is that platelets incorrectly adhere to the vessel wall through

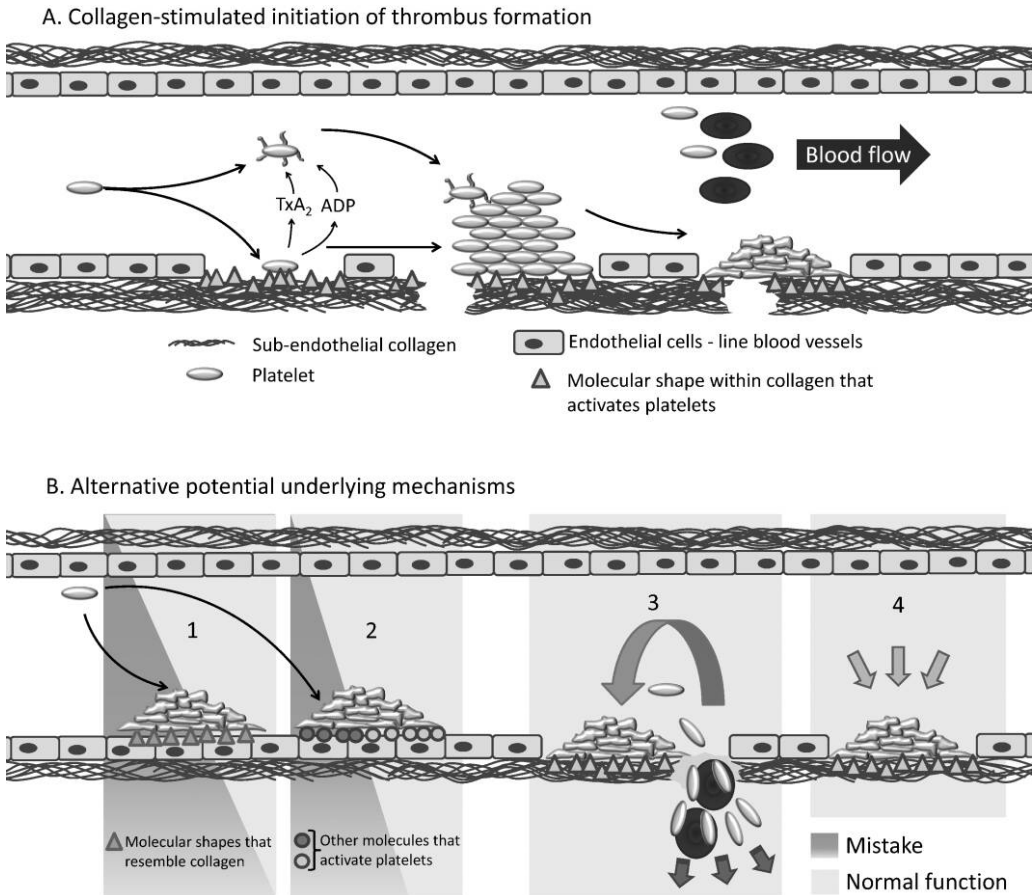

Figure 1. Mechanisms of Platelet Activation and Thrombus Formation

A. Thrombus formation in the damaged arterial circulation. Damage to the endothelial lining of blood vessels, or vessel breaches, result in the exposure of extracellular matrix collagen. Receptor proteins present on the platelet surface enable binding of platelets to specific structures within collagen (triangles) that triggers biochemical pathways within these cells. Resultant platelet activation culminates in the adhesion of platelets to each other and the formation of a thrombus that serves to stem the loss of blood from the vessel. B. Alternative potential explanations for the formation of platelet thrombi. Normal platelet function or normal outcomes (cessation of bleeding) are indicated through light shading. Erroneous platelet activation and thrombus formation (mistakes) are indicated by dark shading. 1. Platelets incorrectly adhere to the vessel wall through binding to molecules with a similar shape to that of collagen (triangles). To prevent this, platelets make more discriminations than currently known. 2. As part of normal function, alternative molecules (light circles) are able, through hitherto unrecognized means, to activate platelets. This expanded classification of normal activators carries risk of mistaken activation (dark circles). In both 1 and 2: at sites of injury these may represent alternative mechanisms to prevent blood loss, or in nondamaged vessels (as depicted) would result in unwanted platelet activation and thrombosis. 3. Platelet thrombus formation stimulated by blood flow/loss-induced local signals or feedback. 4. Currently unrecognized potential "top-down" regulation of thrombus formation, e.g., by the central nervous system or via neurohormonal pathways. See the online edition for a color version of this figure.

binding to molecules with similar shape to that of collagen. If such a mistake were to be identified it would imply that, in order to avoid this, platelets would make more discriminations than currently known. In Figure 1B.2, as part of normal function, alternative molecules are able, through hitherto unrecognized means, to activate platelets. This expanded classification of normal activators carries the risk of mistaken activation. Figures 1B.1 and 1B.2 represent possible alternative mechanisms to prevent blood loss or, in nondamaged vessels, possible routes to unwanted platelet activation and thrombosis.

We illustrate further the utility of the questions outlined here by pointing to evidence that platelets are activated not only by blood vessel damage but also by bacteria, viruses, and cancers (Bertling et al. 2012; Assinger 2014; Palacios-Acedo et al. 2019; Darling et al. 2020). If these are mistaken activations leading to damaging clotting, or other damaging consequences of platelet activation, this could lead back to mechanisms involved in known operations. For example, there may be instances where infection or cancer exposes platelets to collagen, or to the same structural features presented following vessel wall injury, which could deceive the system into activation in the absence of blood loss. Alternatively, might these mistakes instead point to previously unidentified operations as illustrated in Figure 1B.1? Could we, for example, be wrong in assuming that molecules implicated in platelet activation do not resemble others in their environment for which activation would be a mistake? In that case activation by infectious agents or cancer cells may arise because platelets make finer discriminations than currently appreciated, and hence are prone to corresponding failures to make those discriminations. This is the case for immune systems that encounter similar molecules that could either be nonself or self-antigens (Tkach and Altan-Bonnet 2013; Voisinne et al. 2015).

GENERATION OF EFFECTIVE HEMOSTATIC ACTION

Considering now the ways biological systems generate effective action, we apply our analysis to action taken by the clotting system within the intravascular environment. Figures 1B.3 and 1B.4 illustrate two possible operations arising from asking Question 3 (*Might there be so far unidentified operations involved in the system's response to its environment, and if so what kinds of mistakes would point to such operations?*): regulation based on feedback from the effect of the clot on blood loss, and top-down regulation from a monitoring and controlling function outside of the clot, respectively. Each could provide regulation of clot progression, organization, direction, or size, in ways that according to current knowledge are not found in clotting. These are both operations found in other biological systems, for example, modulation of arterial wall contraction against changes of blood pressure, and central nervous system (CNS) coordination of muscles in order to generate movement (Ting et al. 2015). As far as we are aware, studies have not been conducted in an attempt to rule out the proposed operations, nor to examine for mistakes that might be seen if they did contribute to clotting.

Regarding the question of possible feedback mechanisms, future studies could examine whether, after accounting for other known influences, altering blood loss from an artery alters clot progression or whether clot progression becomes dysregulated in conditions where information about blood loss is not available. Alternatively, studies could be motivated by a search for structures predicted to be necessary for this operation to be present, such as potential transmitter molecules released as blood flows through a breach in an artery that might provide information on blood loss. Figure 1B.3 shows platelet thrombus formation influenced by currently unrecognized blood flow/loss-induced local signals or feedback.

Similarly, there might be top-down regulation of clot progression, size, and termination. Can we envisage such an operation being implemented via the CNS, perhaps implicating the autonomic nervous system (ANS), with ANS neurotransmitters monitoring and regulating clot progression? Alternatively, might there be a more local mechanism, for example, operating within the blood vessels? Figure 1B.4 shows currently unrecognized

"top-down" regulation of thrombus formation in, for example, the central nervous system or neurohormonal pathways.

Limitations

In making this proposal, we are aware that the topics we cover touch on many areas in the philosophy and theory of biology for which adequate discussion in this paper is not possible. For example, based on the way we describe the processes underlying mistake-making it can be concluded that, in biological systems, the significance of physical differences depends on their functional implications. Function and malfunction are defined in terms of action in relation to the environment, and not solely in terms of physical differences. This is a proposal that departs from the idea that explanations of biological systems can be reduced to physical laws. However, it is not within the scope of this paper to discuss the case for or against reductionism more broadly (cf. Nicholson and Dupré 2018). In addition, within the context of evolutionary hypotheses for biology, our concept of function (as effective action in the environment) is different from several other ways the term is commonly used (e.g., Wouters 2003), although more in line with some others (e.g., Bock and von Wahlert 1965). Additional discussion would be required to do justice to this extensive literature.

Furthermore, our account here does not address possible challenges from the philosophy of science. For example, it may appear to be based on the assumption that the sciences can plot an assured course via hypothesis and testing toward better approximations to an understanding of the phenomena under consideration. There is a long history of challenge to this notion (see Kuhn 1962) including a more recent argument that commonly, and perhaps invariably, even the most persuasive and empirically supported hypotheses in the sciences face the problem of unconceived alternatives that will fit the evidence equally well (Stanford 2006). Perhaps our proposal is no exception. That said, our framework makes a virtue of open-ended investigation into the possible existence of currently unknown operations of the kind described in Figure 1. This may keep the problem of unconceived alternatives at arm's length—a worry for philosophers but of less practical relevance to working biologists.

A strength of our proposal is that it proceeds from first principles to make the case that, given mistake-making, other phenomena must be present in biological systems. In this way we aim to describe the "logic" of biological systems. This strength is, however, also a potential weakness. Our proposal may fail either by being incompatible with some evidence or, in spite of being compatible, prove unproductive in some contexts. In order to test this, it will be essential to apply it to examples likely to be demanding, such as evolutionary mechanisms. Will it prove productive in providing additional perspectives on current questions in this field, such as what might be the causes of developmental constraints on phenotypes in evolution (Maynard Smith et al. 1985; Brakefield 2006)? Application of our framework to the organization of action across phenotypes in evolution might suggest that there is a limited number of ways in which effective biological action can be organized, and this may point to a source of constraints beyond those so far identified. Given that, in spite of the constraints, there is enormous diversity in life, and assuming the number of possible operations is limited, does this point to their generality across very different phenotypes? Might this be an implication of, for example, the remarkable findings that the same gene, PAX6, has a role in the development of the eye across a wide range of species with vastly different eye structures (Pichaud and Desplan 2002)? At this stage we can only provide preliminary indications of how our framework might be applied; testing across diverse examples will be required before we are able to make strong claims for it.

Conclusion

As we noted earlier, a major challenge in attempting to formulate generally applicable theoretical frameworks for biology is how to provide on the one hand sufficient generality, covering the vast diversity of biological systems, and on the other enough specificity

to mark out phenomena susceptible to testing by working biologists. As we have argued, mistake-making provides a starting point with the required generality, and the pervasive use of the language of mistake-making in biological research suggests that specificity may be achievable. Building on these foundations, our aim has been to show how, by examining the details of mistake-making, we arrive at a general description of the requirements for a biological system to act effectively in its environment. Crucially, we believe, this description is capable of generating specific research questions while at the same time retaining its generality.

The illustrative application to blood clotting has arisen from a detailed discussion between the authors who come from more theoretical and philosophical perspectives and the author who is actively researching hemostasis. The tests of whether our proposal meets the challenge of generality with specificity will be both philosophical and scientific. For example, the generality that we claim for mistake-making requires scrutiny of whether the concept applies in the same way across biology. The specificity that we describe needs to be examined for its potential to generate novel scientific questions over a range of biological systems.

Summarizing the outlook of the French philosopher of science, Georges Canguilhem, philosopher Michel Foucault said: "In the extreme, life is what is capable of error" (Foucault 1991:22). We agree with the thought but deny that this capability for error lies in the extreme. Rather, we see it as fundamental to life itself.

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