A myopic view of "necessity and sufficiency:" a case of conjunctivitis?

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ABSTRACT

A recent perspective has argued that the phrase "Necessary and Sufficient," long a staple of the genetics literature, has been misapplied in the context of neuroscience, and should be abandoned (Yoshihara and Yoshihara, 2018). Here we rebut this proposal on both logical and semantic grounds. We argue that the claim that "Necessary and Sufficient" is "misapplied" in genetics and neuroscience rests on its narrow meaning in formal logic, in which the phrase is used to define the properties of classes of objects. In genetics, however, this term is used as shorthand to summarize the results of different kinds of experiments. This logical conflict, moreover, applies only to the conjunctive phrase "Necessary *and* Sufficient;" the unlinked use of those words to describe genetic results is simply a matter of semantics.

Introduction

For the last 70 years or so, geneticists have used the terms "necessary" and "sufficient" to refer to the results of experiments in which the function of specific genes is either removed, or added. The use of this terminology to describe loss-of-function (LOF) and gain-of-function (GOF) manipulations has been central to genetic formal logic and scientific rigor. This language has also been adopted, more recently, by systems neuroscientists to describe the results of experiments in which neuronal function, rather than gene function, is either inhibited or increased, for example using optogenetics (Zhang *et al*., 2010).

Recently, it has been argued that the phrase "necessary and sufficient" is routinely misapplied by biologists, i.e., is used in a manner inconsistent with its meaning in formal logic,

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and therefore should be abandoned (Yoshihara and Yoshihara, 2018). The claim is that this is not merely a matter of semantics, but rather one of logical rigor, and that the misapplication of this term is misleading and dangerous. Here the authors (one a geneticist, the other a molecular neuroscientist) respond to this criticism.

A conjunctive confound

Yoshihara and Yoshihara (2018) have argued against the use of the phrase "necessary and sufficient" on both logical and semantic grounds. The logical argument is that in the conjunction "necessary *and* sufficient," the word "and" implies logical equivalence, and that this is not the case in its biological usage. In the illustrative example they provide ((Yoshihara and Yoshihara, 2018); Fig. 1A), the properties of being a polygon with four equal sides and equal angles are necessary *and* sufficient to describe a square. This equivalence implies full interconvertibility of universal affirmatives describing squares: "all squares are polygons with four equal angles and sides" implies that "all polygons with four equal angles and sides are squares." In other words, any set of characteristics that are *sufficient* to exclusively define a class of objects are also *necessary*. In this definitional usage, sufficiency always implies necessity.

In biology, however, this is not always the case. Some genes can be sufficient for a process, but not necessary (if, for example, there are redundant genes controlling the same process; see Fig. 1). If there are cases of sufficiency without necessity, the Yoshiharas argue, it means that "sufficient" does not always imply "necessary," and therefore that the phrase "necessary *and* sufficient" no longer retains its formal logical meaning in biology, and should be abandoned.

The problem with this argument is that the conjunctive phrase "necessary *and* sufficient" is being applied in a *definitional* context (from Set Theory), in which the phrase refers to the *properties* or *characteristics* of a defined *object* or category of objects. In contrast, in genetics the

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term is used as a shorthand to describe *the results of experiments* designed to perturb a biological process: a gene is “necessary” for a biological process *if a loss-of-function (LOF) mutation in the gene prevents the process from occurring*; it is “sufficient” for the process *if a gain-of-function (GOF) mutation causes the process to occur in excess*, or at a time or place when or where it normally does not occur.

Not only are there cases where a gene can be *sufficient* but not necessary for a process to occur (due to redundancy, as mentioned above), but there are also cases where a gene may be *necessary* but not sufficient (e.g., because it is one of several genes that are all required *in combination* for the process to occur). A Venn diagram describing the results of genetic

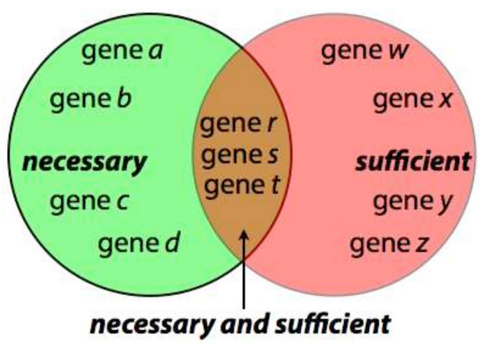

Figure 1. Necessity and sufficiency in genetics. The Venn diagram illustrates three categories of results obtained by manipulating genes involved in a biological process “X” (where X is, e.g., cell division, gastrulation, synaptic transmission), based on LOF or GOF mutations in those genes (see text for LOF/GOF definition). Genes *a-d* are necessary for process X (LOF manipulation blocks X), but not sufficient (GOF manipulation has no effect). Genes *w-z* are sufficient (GOF manipulation causes X), but not necessary (LOF manipulation has no effect). Genes *r-t* are necessary and also sufficient: both LOF and GOF manipulations have effects on X.

experiments of these types is illustrated in Figure 1. Here, the overlap between the circles describes those cases in which a gene is both *necessary* for a process and also *sufficient* for the process to occur. But these two conditions are met in two different experimental conditions. Therefore, they do not describe the characteristic properties of a gene (e.g., made of DNA, double-stranded, specific nucleotide sequence, etc.); rather the results of experimental manipulations.

As pointed out correctly by the Yoshiharas, fully interconvertible universal affirmatives are not implied by cases of genes that behave as “necessary and sufficient:” the fact that gene *a* is sufficient for biological process X does not imply that all genes sufficient for process X are gene *a*; nor does it imply that wherever gene *a* is expressed, process X must occur. Conversely, the fact gene *a* is necessary for process X does not mean that no other genes are necessary for process X other than gene *a*; nor does it imply that process X cannot occur without gene *a* under some other experimental conditions. But the Yoshiharas argue that since fully interconvertible universal affirmatives *are* implied by "necessary and sufficient" (in its definitional sense), the phrase should not be used in biology because it is practically impossible to prove negatives (e.g., one cannot prove that there are no genes other than *a* that are necessary for process X).

In a nutshell, therefore, the Yoshiharas are arguing that when geneticists use the phrase "necessary *and* sufficient" to describe a gene, they are defining the properties of an *object(s)*: the genes are *defined* as "necessary and sufficient" for a given biological process. If that were correct, then their criticism would be valid. However, as mentioned above geneticists do *not* use the phrase in a definitional sense; rather they use it as shorthand to summarize the results of experimental manipulations. Realizing that, the Yoshiharas fall back on the argument that even though geneticists know and agree on what "necessary and sufficient" means in their field, since the phrase might be confusing or misleading to someone from a different field (e.g., formal logic or philosophy), it follows that biologists should stop using that phrase in their talks and publications.

We respectfully disagree. There is no law that states that philosophers or logicians have a monopoly on the usage of natural language that dominates over all fields of science. In our experience, non-biologists either understand the language usage or ask for clarification. While we share with the Yoshiharas an appreciation for precise language, we do not consider the phrase 'necessary and sufficient' imprecise or ambiguous when used in its biological sense. That

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students' use of the phrase is found annoying to the Yoshiharas more likely reflects other aspects of their scholarship, than logical inconsistency.

"Necessary and sufficient" in neuroscience: population vs. unit manipulations

An important issue raised by the Yoshiharas concerns the validity of adopting "necessary and sufficient" terminology in circuit neuroscience. When this phrase is used by geneticists to summarize LOF and GOF data, it refers to experiments performed on the *same unit* of manipulation – i.e., the same gene. That is, to say that a given gene is "necessary and also sufficient" for process X implies that the tests of necessity and sufficiency refer to the *same* biological unit or element. The situation is different when the manipulations are performed on populations of elements (e.g. populations of neurons). There, the use of the conjunctive phrase, even as a description of results, is arguably more ambiguous because the GOF and LOF results may reflect the same or different units within the population.

For example, in a case where, e.g., optogenetic activation of a genetically identified population of neurons suffices to evoke a behavior, while optogenetic inhibition of the same population inhibits naturally occurring instances of the same behavior (Lee *et al*., 2014), it is formally possible that different subsets of neurons are responsible for the LOF and GOF phenotypes (Yoshihara and Yoshihara, 2018). Therefore in this type of experiment, the phrase "necessary and sufficient" is understood to refer to the manipulated *population*, not to the individual units within the population (Lin *et al*., 2011). Formally, this ambiguity cannot be resolved unless *a single neuron* is being manipulated in both the LOF and GOF experiments. With the exception of *C. elegans* and fly larvae, there are very few cases where this single-cell level of specificity is achieved – including the case of the single pair of interneurons that control feeding in *Drosophila* (Flood et al., 2013): even in that case, one could argue that activation of

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only one of the pair of neurons is "sufficient" to initiate feeding, while inhibition of both neurons is "necessary" to inhibit feeding. Therefore, the same unit (cell) is not certain to be both "necessary and sufficient." While the Yoshiharas propose abandoning the term altogether in such cases (Yoshihara and Yoshihara, 2018), given that the phrase is being used to summarize experimental results, it seems simpler to just indicate that the neurons are "necessary and also sufficient, *as a population*," for behavior X.

"Necessity" and "sufficiency" without conjunctivity

As described above, the logical aspect of the Yoshiharas' criticism boils down to the use of the conjunctive term "necessary and sufficient," because of the formal equivalence of necessity and sufficiency that "and" implies (in a definitional sense). One response to their criticism would be to simply use the words "necessary" and "sufficient" in a non-conjunctive form, i.e., in separate sentences, viz: "Gene *a* is necessary for process X. It is also sufficient for process X." There is no "misapplication" of formal logic in this case. However, the Yoshiharas go a step further and argue that the words "necessary" and "sufficient" are misleading in and of themselves, and should be abandoned by biologists in favor of alternative words such as "indispensable" and "inducing," respectively.

This is, however, a purely semantic argument, and as such it is a weak one. The objection to the word "sufficient" is that a literal interpretation of the word implies that *no other condition* is required in order to achieve the experimental result. Because these words are used by biologists to describe the results of experiments performed in living organisms, it is of course implicitly understood that the organism is required to observe the result. No biologists who states that "gene *a* is sufficient for process X" means that a piece of DNA containing gene *a* floating in a test tube full of water is sufficient for process X. Words and language are used in a *context*. If we restrict

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the usage of words to contexts that are universal across fields of research, then we will greatly impoverish our rich language.

This is not, however, to argue for imprecise language usage. We are all in favor of clarifying or qualifying statements such as "gene *a* is *sufficient* when mis-expressed to cause precocious or ectopic occurrence of process X," or "activation of cell type K is *sufficient* to trigger behavior Y in the absence of other deliberate manipulations or conditions." Of course, it becomes cumbersome to include such extra verbiage in abstracts or short communications. But to argue that substituting "adequate," "causal," "inducing," "activating," "promoting" or any one of dozens of other words is preferable to "sufficient," is not a question of formal logic, it is just to argue in favor of one type of verbal shorthand over another. The same goes for substituting "necessary" with words like "indispensable," "essential," "required," "requisite" or other synonyms. It is a directive to send scientists to waste time poring over their dog-eared copies of Roget's *Thesaurus*, scratching their heads.

Necessity and permissivity

The Yoshiharas go on to argue that the demonstration that a gene or cell is "necessary" for a biological process is not even particularly informative, because it could reflect a relatively trivial and indirect role for a gene in a process. For example, they cite a hypothetical case where the loss of neurons that control leg movements might indirectly lead to a loss of aggressive behavior, by "making the animal less energetic."

This is of course a valid possibility, but it confuses two distinct concepts in biology: necessary vs. sufficient; and *permissive* vs. *instructive*. A gene is said to be "permissive," when it is required for a process to occur, but when increasing its activity does not accelerate or enhance the process. By analogy, gasoline is permissive for automobile function: in its absence, a car will not move;

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however a car with a full tank does not go any faster than one with half a tank. In contrast, the accelerator pedal is "instructive," since pressing harder on it makes the car go faster. Similarly, shifting the transmission into a different gear changes power output, and is also instructive, but in a stepwise (discontinuous) rather than a continuous manner. Both the gas pedal and the transmission, like fuel, have similar LOF phenotypes – the car doesn't run without them. GOF manipulations are necessary to distinguish "instructive" from "permissive" functions. Nevertheless, the fact that a gene may prove to be "permissive" does not mean that LOF experiments are uninformative. The real question is whether a requirement for a given component in a biological process reflects a direct, or indirect, role for that component. Further experiments – not abandonment of certain types of experiments -- are required to make that important distinction.

The Yoshiharas further argue that the inability to demonstrate necessity – i.e., a negative result in a LOF experiment – is not evidence that a gene or cell type is unimportant, because it could reflect redundancy or compensation by other genes. We couldn't agree more. Negative results in genetic LOF experiments are difficult to interpret, because they could reflect biological or technical factors. We would argue the same for negative results in systems neuroscience experiments, such as lesions. In particular, the relatively long time necessary for an animal to recover from a surgical lesion or genetic ablation (days or weeks) can allow many compensating mechanisms to engage (Hong *et al*., 2018). Several comparative studies have shown that rapidly reversible LOF manipulations (e.g, optogenetic inhibition) can yield phenotypes in cases where lesions of the same structure do not (Goshen *et al*., 2011; Otchy *et al*., 2015). Therefore, we would argue that it is dangerous to conclude that a lesion of a brain structure that has no effect on a function of interest implies that that structure does not contribute to that function, only that it

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does not play an "essential" role (meaning one that is not redundant, or which cannot be compensated by other structures or circuits).

Necessity, sufficiency and causality

In genetics, experimental tests of necessity and sufficiency (N&S) are essential to distinguish correlation from causation. The Yoshiharas seem to argue that these tests are not all that informative: "we have never seen a single case where the use of N&S has helped our understanding of biology" (Yoshihara and Yoshihara, 2018). Another recent perspective essay argued that demonstrations of necessity and sufficiency in neural circuit research (and by extension, other areas of biology such as genetics) produce little in the way of "understanding" (Krakauer *et al*., 2017).

Here we run into the epistemological question of what constitutes "understanding" in neuroscience, and whether causality as established using genetic manipulations is an essential part of such understanding. It is beyond the scope of this essay to delve into this important question, about which reasonable people may disagree. Certainly there are fields of science where "understanding" is achieved without causal perturbations: the laws of planetary motion were established without experiments designed to add a planet, delete a planet, change the mass of a planet or alter its distance from the sun, because such experiments are not possible. The question at the center of the current epistemological debate in neuroscience is whether such perturbation experiments, when they are in principle enabled by technology, are potentially useful, or do more harm than good because they alter the system in unphysiological ways (Jazayeri and Afraz, 2017). Only time will tell.

In closing, we would like to point out that in contrast to most physical systems, biological systems such as those that control development, cancer or behavior are highly complex, and

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involve many interacting components that can produce evidence of correlated activity when interrogated using sensitive methods. Such activity measurements (whether based on gene expression, spiking rates or phosphorylation) can produce a wealth of phenomenological, correlative data. However in the absence of causal data, it is extremely difficult to decide which of these phenomena to study further, and therefore the risk of unproductively chasing an observation that ultimately proves to be an epiphenomenon is high. Cancer researchers spent decades searching fruitlessly for the mechanisms of oncogenic transformation, in cellular-level phenomena such as changes in membrane fluidity or cytoskeletal organization, before genetic tests were developed to identify oncogenes functionally (Weinberg, 1984). Similarly, researchers in the circadian rhythm field spent years studying oscillating genes and proteins that were simply readouts of the biological clock, before Konopka and Benzer discovered the *per* genes that are central to the circadian oscillator (Konopka and Benzer, 1971). Experimental tests of necessity and sufficiency are a critical first step, not a solution. As Jim Watson once said about the famous Hershey-Chase experiment (Hershey and Chase, 1952), which showed that hereditary information in bacteriophage is carried by nucleic acids and not by proteins, such experiments may not tell you what the answer is, but they show you where to look.

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