EMBO reports

Why control an experiment?

From empiricism, via consciousness, toward Implicate Order

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We made a deliberate decision to become scientists and not philosophers, because science offers the opportunity to test ideas using the scientific method. And once we began our formal training as scientists, the greatest challenge beyond formulating a testable or refutable hypothesis was designing appropriate controls for an experiment. In theory, this seems trivial, but in practice, it is often difficult. But where and when did this concept of controlling an experiment start? It is largely attributed to Roger Bacon, who emphasized the use of artificial experiments to provide additional evidence for observations in his *Novum Organum Scientiarum* in 1620. Other philosophers took up the concept of empirical research: in 1877, Charles Peirce redefined the scientific method in *The Fixation of Belief* as the most efficient and reliable way to prove a hypothesis. In the 1930s, Karl Popper emphasized the necessity of refuting hypotheses in *The Logic of Scientific Discoveries*. While these influential works do not explicitly discuss controls as an integral part of experiments, their importance for generating solid and reliable results is nonetheless implicit.

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But the scientific method based on experimentation and observation has come under criticism of late in light of the ever more complex problems faced in physics and biology. Chris Anderson, the editor of *Wired* Magazine, proposed that we should turn to statistical analysis, machine learning, and pattern recognition instead of creating and testing hypotheses, based on the Informatics credo that if you cannot answer the question, you need more data. However, this attitude subsumes that we already have enough data and that we just cannot make sense of it. This assumption is in direct conflict with David Bohm's thesis that there are two "Orders", the Explicate and Implicate [1]. The Explicate Order is the way in which our subjective sensory systems perceive the world [2]. In contrast, Bohm's Implicate Order would represent the objective reality beyond our perception. This view—that we have only a subjective understanding of reality—dates back to Galileo Galilei who, in 1623, criticized the Aristotelian concept of absolute and objective qualities of our sensory perceptions [3] and to Plato's cave allegory that reality is only what our senses allow us to see.

Controlling an experiment

The only way for systematically overcoming the limits of our sensory apparatus and to get a glimpse of the Implicate Order is through the scientific method, through hypothesis-testing, controlled experimentation. Beyond the methodology, controlling an experiment is critically important to ensure that the observed results are not just random events; they help scientists to distinguish between the "signal" and the background "noise" that are inherent in natural and living systems. For example, the detection method for the recent discovery of gravitational waves used four-dimensional reference points to factor out the background noise of the Cosmos. Controls also help to account for errors and variability in the experimental setup and measuring tools: The negative control of an enzyme assay, for instance, tests for any unrelated background signals from the assay or measurement. In short, controls are essential for the unbiased, objective observation and measurement of the dependent variable in response to the experimental setup.

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Nominally, both positive and negative controls are material and procedural; that is, they control for variability of the experimental materials and the procedure itself. But beyond the practical issues to avoid procedural and material artifacts, there is an underlying philosophical question. The need for experimental controls is a subliminal recognition of the relative and subjective nature of the Explicate Order. It requires controls as "reference points" in order to transcend it, and to approximate the Implicate Order.

This is similar to Peter Rowlands' [4] dictum that everything in the Universe adds up to zero, the universal attractor in mathematics. Prior to the introduction of zero, mathematics lacked an absolute reference point similar to a negative or positive control in an experiment. The same is true of biology, where the cell is the reference point owing to its negative entropy: It appears as an attractor for the energy of its environment. Hence, there is a need for

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careful controls in biology: The homeostatic balance that is inherent to life varies during the course of an experiment and therefore must be precisely controlled to distinguish noise from signal and approximate the Implicate Order of life.

P < 0.05 tacitly acknowledges the explicate order

Another example of the "subjectivity" of our perception is the level of accuracy we accept for differences between groups. For example, when we use statistical methods to determine if an observed difference between control and experimental groups is a random occurrence or a specific effect, we conventionally consider a p value of less than or equal to 5% as statistically significant; that is, there is a less than 0.05 probability that the effect is random. The efficacy of this arbitrary convention has been debated for decades; suffice to say that despite questioning the validity of that convention, a *P* value of < 0.05 reflects our acceptance of the subjectivity of our perception of reality.

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Thus, if we do away with hypothesis-testing science in favor of informatics based on data and statistics—referring to Anderson's suggestion—it reflects our acceptance of the noise in the system. However, mere data analysis without any underlying hypothesis is tantamount to "garbage in-garbage out", in contrast to well-controlled imaginative experiments to separate the wheat from the chaff. Albert Einstein was quoted as saying that imagination was more important than knowledge.

Assessing the implicate order via consciousness

The ultimate purpose of the scientific method is to understand ourselves and our place in Nature. Conventionally, we subscribe to the Anthropic Principle, that we are "in" this Universe, whereas the Endosymbiosis Theory, advocated by Lynn Margulis, stipulates that we are "of" this Universe as a result of the assimilation of the physical environment. According to this theory, the organism endogenizes external factors to make them physiologically "useful", such as iron as the core of the hemoglobin molecule, or ancient bacteria as mitochondria.

> *". . . there is a fundamental difference between knowing via believing and knowing based on empirical research."*

By applying the developmental mechanism of cell–cell communication to phylogeny, we have revealed the interrelationships between cells and explained evolution from its origin as the unicellular state to multicellularity via cell–cell communication. The ultimate outcome of this research is that consciousness is the product of cellular processes and cell–cell communication in order to react to the environment and better anticipate future events [5,6]. Consciousness is an essential prerequisite for transcending the Explicate Order toward the Implicate Order via cellular sensory and cognitive systems that feed an ever-expanding organismal knowledge about both the environment and itself.

The significance of empiricism

It is here where the empirical approach to understanding nature comes in with its emphasis that knowledge comes only from sensual experience rather than innate ideas or traditions. In the context of the cell or higher systems, knowledge about the environment can only be gained by sensing and analyzing the environment. Empiricism is similar to an equation in which the variables and terms form a product, or a chemical reaction, or a biological process where the substrates, aka sensory data, form products, that is, knowledge. However, it requires another step—imagination, according to Albert Einstein—to transcend the Explicate Order in order to gain insight into the Implicate Order. Take for instance, Dmitri Ivanovich Mendeleev's Periodic Table of Elements: his brilliant insight was not just to use Atomic Number to organize it, but also to consider the chemical reactivities of the Elements by sorting them into columns. By introducing chemical reactivity to the Periodic Table, Mendeleev provided something like the "fourth wall" in Drama, which gives the audience an omniscient, god-like perspective on what is happening on stage.

The capacity to transcend the subjective Explicate Order to approximate the objective Implicate Order is not unlike Eastern philosophies like Buddhism or Taoism, which were practiced long before the scientific method. An Indian philosopher once pointed out that the Hindus have known for 30,000 years that the Earth revolves around the sun, while the Europeans only realized this a few hundred years ago based on the work of Copernicus, Brahe, and Galileo. However, there is a fundamental difference between knowing via believing and knowing based on empirical research. A similar example is Aristotle's refusal to test whether a large stone would fall faster than a small one, as he knew the answer already [7]. Galileo eventually performed the experiment from the Leaning Tower in Pisa to demonstrate that the fall time of two objects is independent of their mass—which disproved Aristotle's theory of gravity that stipulated that objects fall at a speed proportional to their mass. Again, it demonstrates the power of empiricism and experimentation as formulated by Francis Bacon, John Locke, and others, over intuition and rationalizing.

> *"Even if our scientific instruments provide us with objective data, we still need to apply our consciousness to evaluate and interpret such data."*

Following the evolution from the unicellular state to multicellular organisms—and reverse-engineering it to a minimal-cell state—reveals that biologic diversity is an artifact of the Explicate Order. Indeed, the unicell seems to be the primary level of selection in the Implicate Order, as it remains proximate to the First Principles of Physiology, namely negative entropy (negentropy), chemiosmosis, and homeostasis. The first two principles are necessary for growth and proliferation, whereas the last reflects Newton's Third Law of Motion that

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every action has an equal and opposite reaction so as to maintain homeostasis.

All organisms interact with their surroundings and assimilate their experience as epigenetic marks. Such marks extend to the DNA of germ cells and thus change the phenotypic expression of the offspring. The offspring, in turn, interacts with the environment in response to such epigenetic modifications, giving rise to the concept of the phenotype as an agent that actively and purposefully interacts with its environment in order to adapt and survive. This concept of phenotype based on agency linked to the Explicate Order fundamentally differs from its conventional description as a mere set of biologic characteristics. Organisms' capacities to anticipate future stress situations from past memories are obvious in simple animals such as nematodes, as well as in plants and bacteria [8], suggesting that the subjective Explicate Order controls both organismal behavior and transgenerational evolution.

That perspective offers insight to the nature of consciousness: not as a "mind" that is separate from a "body", but as an endogenization of physical matter, which complies with the Laws of Nature. In other words, consciousness is the physiologic manifestation of endogenized physical surroundings, compartmentalized, and made essential for all organisms by forming the basis for their physiology. Endocytosis and endocytic/synaptic vesicles contribute to endogenization of cellular surroundings, allowing eukaryotic organisms to gain knowledge about the environment. This is true not only for neurons in brains, but also for all eukaryotic cells [5].

Such a view of consciousness offers insight to our awareness of our physical surroundings as the basis for self-referential self-organization. But this is predicated on our capacity to "experiment" with our environment. The burgeoning idea that we are entering the Anthropocene, a man-made world founded on subjective senses instead of Natural Laws, is a dangerous step away from our innate evolutionary arc. Relying on just our senses and emotions, without experimentation and controls to understand the Implicate Order behind reality, is not just an abandonment of the principles of the Enlightenment, but also endangers the planet and its diversity of life.

Bertrand Russell's inductivist turkey and *Homo artefaciens*

Ladislav Kováč discussed the advantages and drawbacks of the inductive method for science and the logic of scientific discoveries [9]. Obviously, technological advances have enabled scientists to expand the borders of knowledge, and informatics allows us to objectively analyze ever larger data-sets. It was the telescope that enabled Tycho Brahe, Johannes Kepler, and Galileo Galilei to make accurate observations and infer the motion of the planets. The microscope provided Robert Koch and Louis Pasteur insights into the microbial world and determines the nature of infectious diseases. Particle colliders now give us a glimpse into the birth of the Universe, while DNA sequencing and bioinformatics have enormously advanced biology's goal to understand the molecular basis of life.

However, Kováč also reminds us that Bayesian inferences and reasoning have serious drawbacks, as documented in the instructive example of Bertrand Russell's "inductivist turkey", which collected large amounts of reproducible data each morning about feeding time. Based on these observations, the turkey correctly predicted the feeding time for the next morning—until Christmas Eve when the turkey's throat was cut [9]. In order to avoid the fate of the "inductivist turkey", mankind should also rely on Popperian deductive science, namely formulating theories, concepts, and hypotheses, which are either confirmed or refuted via stringent experimentation and proper controls. Even if our scientific instruments provide us with objective data, we still need to apply our consciousness to evaluate and interpret such data. Moreover, before we start using our scientific instruments, we need to pose scientific questions. Therefore, as suggested by Albert Szent-Györgyi, we need both Dionysian and Apollonian types of scientists [10]. Unfortunately, as was the case in Szent-Györgyi's times, the Dionysians are still struggling to get proper support.

There have been pleas for reconciling philosophy and science, which parted ways owing to the rise of empiricism. This essay recognizes the centrality experiments and their controls for the advancement of scientific thought, and the attendant advance in philosophy needed to cope with many extant and emerging issues in science and society. We need a common "will" to do so. The rationale is provided herein, if only.

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