BIOLOGICAL REVIEWS
Cambridge Philosophical Society
Biol. Rev. (2012), **87**, pp. 275–289.
doi: 10.1111/j.1469-185X.2011.00194.x

Relationships among variables and their equilibrium values: caveats of time-less interpretation

Skirmantas Janušonis*
Department of Psychological and Brain Sciences, University of California, Santa Barbara, California 93106-9660, USA

ABSTRACT

In biology, the researcher often manipulates some variables of a system and observes its other variables, or investigates relationships among naturally occurring variable values. The studied variables are often measured at equilibrium. It is therefore important to know what we can learn from these equilibrium values and how much information they provide about the fundamental properties of the system. We argue that this information is very limited and that statistical relationships that include equilibrium values may lead to grossly incorrect inferences. A number of simple systems are discussed in order to provide examples of such inferential errors and advice is given about how to avoid them in practice. In conclusion, the potential importance of measure theory in biological sciences in considered.

Key words: biological systems, statistical tests, correlation, ANOVA, power analysis, dynamical systems, equilibrium, steady-state, bifurcation, measure theory.

CONTENTS

* Address for correspondence (Tel.: 805-893-6032; Fax: 805-893-4303; E-mail: janusonis@psych.ucsb.edu)

I. INTRODUCTION

Consider a biological system in which a variable X controls a variable Y but has no effect on a variable Z. Such statements are very common in biology, but their exact meaning is rarely given a second thought. Most researchers would take them to mean that (*i*) changing X either decreases or increases Y and that (*ii*) changing X has no effect on Z. These relationships are typically examined with simple statistical techniques [the *t*-test, analysis of variance (ANOVA)] or with more sophisticated approaches that explicitly treat statistical tests as mathematical models and can naturally accommodate the complexities of reality [e.g. the extended family of the generalized linear model (GLM)]. On closer inspection, however, it becomes apparent that unless time is included in the measures of Y and Z, virtually nothing can be said about the theoretical value of these findings. Shockingly, they may have no theoretical or meta-analytical value at all.

If the values of Y and Z are the equilibrium values after the system has settled down, the finding holds true only for the given experimental setup and, generally speaking, may not be replicated in a different experimental setup. This situation may be perfectly acceptable in applied sciences where the same constraints can be imposed repeatedly to achieve the same outcomes (e.g. in the medical or agricultural fields), but it is highly unsatisfactory in fundamental sciences that seek to uncover general properties of systems (e.g. in neuroscience or ecology). In fundamental sciences, the focus on equilibrium states leads to an exponential explosion of possible experiments, each of which can deliver completely new results. By sheer statistics, some of these experiments will produce highly unexpected and therefore ''exciting'' results, even though they may be generated by the same process that has yielded ''negative'' or ''unexciting'' outcomes in other experiments. Importantly, none of these experiments may get us closer to the underlying process, unless one makes a deliberate attempt to find a dynamical system that could reproduce all of the confirmed experimental observations.

Instead of measuring Y and Z after they have settled down, we can measure their movement (rate of change) in time. The difference appears subtle, but it is not. First, the obtained dynamics can be used to predict the equilibrium values of Y and Z. The converse is generally impossible. Second, this information can be combined with the results from other experiments (including those from other research groups) to predict the outcomes of experiments that have never been carried out. By incorporating time in our measures, we break out of empirical constraints and rapidly gain theoretical power.

Most classical (and very successful) physics uses this very approach. Its impressive edifice is built on a nearly trivial observation that all variables can change only in time and that their rate of change therefore represents the simplest universal building block. Natural, complex systems are even more intimately intertwined with time. More precisely, they do not simply exist in time, but time is literally folded into their fundamental properties. While many simple physical (''conservative'') systems are insensitive to the direction of time, biological systems take full advantage of the ''arrow of time'' (Prigogine, 1997) and cannot sustain self-organization when the direction of time is reversed. These systems have been called ''dissipative'' (Nicolis & Prigogine, 1989).

Many approaches in biology use equilibrium values because obtaining time series in biological systems is often impracticable or cost-prohibitive. It is a serious obstacle in some areas of biology (e.g. molecular biology or neuroscience), where the complexity of analysis may not allow multiple sampling of the same subject or cell over time. Multiple sampling may be equally challenging in other areas of biology, for a variety of reasons (e.g. in ecology, it may be the accessibility of a location during an extended period of time). The main argument presented in this review is that the problem lies not with what can or cannot be done in experimental and descriptive research, but rather with the belief that equilibrium values of variables will eventually reveal fundamental properties of biological systems. Here, ''fundamental properties'' are understood to be a small set of the system's properties from which the entire repertoire of the system's behaviour can emerge. It should be stressed that this problem is considerably deeper than knowing how to use statistical tests correctly. While the state of statistical analysis is undoubtedly dismal in some (otherwise highly sophisticated) biology fields (Nakagawa & Cuthill, 2007; Janušonis, 2009; Lazic, 2008, 2010; Nakagawa & Hauber, 2011), the problem discussed in this review is different and will remain unsolved even when statistical illiteracy is vanquished.

One major problem with time-less associations is that they invite intuitive reasoning. Often it is the only kind of reasoning presented in the discussion section of a biological report. However, it is safe to assume that we have little intuition for the dynamics of several interacting elements, even when their interaction is very simple. This lack of intuition is well exemplified by cellular automata (Sigmund, 1993; Wolfram, 2002; also, see www.conwaylife.com). It is worth remembering that the human brain has evolved to perform a specific set of tasks in a specific ecological niche. It can quickly notice and memorize naturally occurring patterns, and then use this knowledge to guide our behaviour efficiently in an inherently unpredictable world. In this regard, the brain is a massive look-up machine of hard-wired (evolutionary) and acquired memory that seeks to minimize the amount of external information needed to operate optimally (Raichle, 2010*a*, *b*). In order to support science, the brain needs to build other, virtual environments—such as axiomatic systems in

mathematics or rigorous experimental designs in natural sciences—and then to learn to navigate them. An average human being requires decades of training to achieve proficiency in these unusual environments. Even then, the brain continues tripping, sometimes mistaking the "obvious" or "elegant" for the truth (Poincaré, 2001).

Despite some entrenched resistance in biological sciences, mathematical modeling presents the only available alternative to intuitive reasoning. As for everything, modeling can be good, bad, and ugly. It has to be based on real data and verified by new experiments. This is often (correctly!) pointed out by experimentalists, some of whom develop generalized aversion to mathematics. Ironically, these same experimentalists often treat *P* values with too much reverence (all statistical tests are in reality mathematical models), but choose to ignore the assumptions of the tests (which are needed to confirm specifically that the model is consistent with the experimental data). In other words, it is an unintentionally pro-modeling stand, with the exception of the deliberately severed link between the model and the reality.

The main goal of this review is to provide a guide for biologists who need to interpret their experimental results based on equilibrium values. Each statement is supported by an example system that exhibits the discussed properties and can be easily simulated computationally. These examples are not meant to be all-inclusive and are deliberately chosen to be as simple as possible. If a property can be demonstrated in a simple system, it can be anticipated in more complex systems. We start by formalizing the general definitions of the time-independent and time-dependent models.

II. TIME-INDEPENDENT AND TIME-DEPENDENT MODELS

(1) The formal structure of the models

We assume that a variable X controls a variable Y. Next, we experimentally change the value of X, allow the system to settle down, and measure Y. Since X controls Y, the value of Y can be expected to change as a function of X. If this actually happens, the relationship (linear or non-linear, with simple or complex measurement errors) is likely to be successfully detected and modeled with the GLM or its extensions [e.g. the generalized additive model (GAM), the generalized linear mixed model (GLMM), and others] (Zuur *et al.*, 2009; Nakagawa & Hauber, 2011). Eventually, one can expect to arrive at a relationship of the form

$$Y = f(X). \tag{1}$$

With some luck, the relationship can be represented simply by

$$Y = aX + b, \tag{2}$$

where a and b are constants. Special cases of this model include the *t*-test (X is binary) and one-way ANOVA (X is discrete).

For example, suppose that a certain gene X is known to control noradrenergic synaptic transmission in the brain. Based on this information, the experimenter may anticipate that knocking down this gene ($X = 0$) will alter noradrenergic synaptic activity. To verify this, she may choose to measure the mRNA or protein levels of adrenergic receptors [e.g. with quantitative reverse-transcription polymerase chain reaction (PCR) or Western blotting] or assess brain noradrenaline levels (e.g. with high-performance liquid chromatography). The experimenter may expect that at least some of these measures (Y) will yield a significant *P* value (i.e. $a \neq 0$).

Let us now restart the system and again experimentally change X. Will Y immediately change its value to the new value recorded in the previous experiment? Obviously, not! A variable cannot quantum-tunnel to another state in zero time, so Y will start moving towards its new equilibrium value. In the case of noradrenergic activity, this value will depend on time-dependent shifts in mRNA transport and translation (Tholanikunnel *et al.*, 2010), as well as protein internalization and recycling (Luttrell & Gesty-Palmer, 2010; Vayttaden *et al.*, 2010). But then one can argue that what X actually controls is not the equilibrium value of Y, but rather its direction of movement. Strictly speaking, we changed the velocity of Y from zero (Y was stable) to non-zero (it is now moving). We can formally write this as

$$\frac{dY}{dt} = f(X), \tag{3}$$

where t is time. Note that the equation can be rewritten to emphasize that Y starts moving only if X changes from its "normal" value (X_0):

$$\frac{dY}{dt} = f(X - X_0), \tag{4}$$

where we assume $f(0) = 0$. This form is more biologically intuitive, but it is equivalent to the shorter form (Equation 3), which we will use in the following considerations. Again, with luck, we can have a simple relationship:

$$\frac{dY}{dt} = aX + b, \tag{5}$$

where a and b are constants. Note that these equations automatically model the velocity (not just the movement direction) of Y. If the velocity is not known, the equation can still capture the general effect:

$$\frac{dY}{dt} = k \ \operatorname{sgn}(f(X)) \tag{6}$$

where k is a constant and "sgn" is the sign function. The equation now has a built-in degree of uncertainty (the direction is known, but not the velocity), but it is still valuable because the uncertainty has been made explicit. This uncertainty may predict a broad spectrum of what the system can do, or it may turn out to be inconsequential. A striking example of the latter is the wide range of dynamical systems

that approach chaotic behaviour by making stereotypic transitions described by the first Feigenbaum constant (Mitchell, 2009).

In reality, the change of a variable will also depend on the current state of the variable. For instance, most experimentally measurable variables will eventually plateau even if X becomes very large or very small. Some variables (e.g. concentration, length) will never become negative even if X continues to drive them down. Therefore, if X controls Y, it is more realistic to write

$$\frac{dY}{dt} = f(X, Y). \tag{7}$$

To summarize, the same assumption (X controls Y) leads to two different model specifications represented by Equation 1 and Equations 3 & 7. Both of them were derived based on simple logical considerations. The key difference is that Equations 3 & 7 emphasize that Y cannot change instantaneously. However, the transition of a variable to its new state may be very fast. In other cases, this transition may be difficult or nearly impossible to measure because of other reasons. Therefore, the difference between the models appears to be subtle and almost philosophical. We will now show that this conclusion is incorrect and that the two models represent two radically different conceptualizations.

For convenience, we call the first model (Equation 1) the time-independent model and the second model (Equations 3 & 7) the time-dependent model. We assume that, after an initial perturbation, all variables in a time-dependent system approach equilibrium values that show no further change. This represents a typical experimental situation but excludes such important dynamical phenomena as periodic behaviour (e.g. limit cycles) and deterministic chaos (e.g. strange attractors). We define equilibrium values as true steady-state values or quasi-steady-state values, the further change of which can be considered negligible (e.g., for all practical purposes, e^{-t} becomes zero when t grows large). In the following discussion, we focus on potential pitfalls in interpretation of equilibrium values in experimental and descriptive research.

(2) Relationships between dynamics and equilibrium values

(*a*) *An experimental manipulation can control a variable without affecting its equilibrium value*

(*i*) *Example.* An experimenter sets X at each of several different values and records the corresponding values of Y after the system has reached its new respective states. ANOVA shows that the experimental manipulation has no significant effect. The experimenter concludes that X does not control Y. This conclusion may be incorrect.

(*ii*) *Formal demonstration.* Consider a simple time-dependent relationship:

$$\frac{dY}{dt} = X(A - Y), \tag{8}$$

where $A > 0$ is constant. We experimentally vary $X > 0$ and measure the equilibrium values of Y (Fig. 1A). Irrespective of the initial value of Y and the value of X, the equilibrium value of Y will be very close to A. Therefore, time-independent models will detect no relationship between X and Y, even though X directly controls Y.

The experimenter may be more successful if he measures Y in a specific time window (e.g. around $t = 0.5$ in Fig. 1B). In this window, X and Y strongly correlate and small samples would be sufficient to produce a significant P value. However, with time their correlation rapidly decreases and requires increasingly larger samples to be detected. In order to achieve a statistical power of at least 0.8 in the given example, one would need sample sizes of 8, 52, and around 5000 for the time-points $t = 1$, 2, and 4, respectively (the significance level is assumed to be 0.05). If the experimenter has an approximate estimate of how much time the system needs to settle down, he may be better off sampling the time axis rather than blindly increasing the sample size at a pre-determined time-point. In the presented example, the relationship between X and Y can be detected by analyzing five evenly spaced time-points with around 10 cases per time-point. This

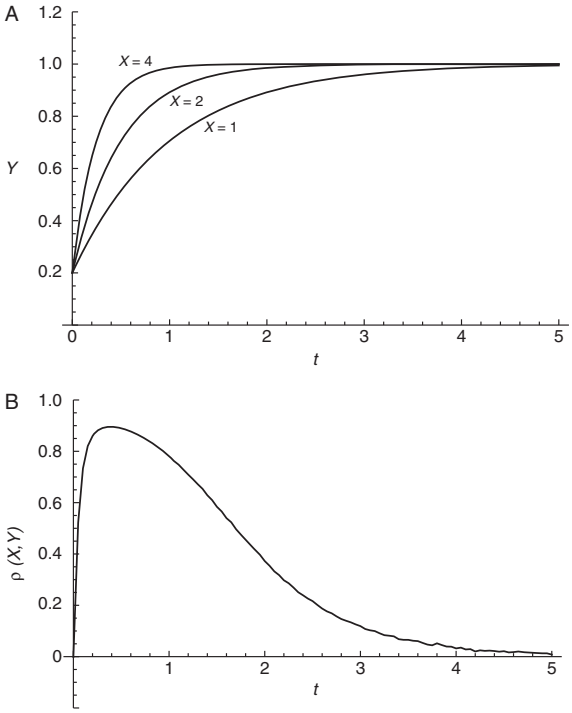

Fig. 1. The dynamics and equilibrium states of the system described by Equation 8 with $A = 1$ and the initial value of Y set at 0.2. (A) The time-evolution of Y as a function of X. (B) The correlation (ρ) between X and Y as a function of time. Note that the correlation is very strong at the time $t = 0.5$, but that it becomes virtually zero when Y approaches equilibrium. At each time-point the correlation was calculated by obtaining a sample of 100,000 uniformly distributed (on [1, 4]) X values and the corresponding values of Y, each of which was assumed to be measured with a normally distributed error (mean = 0, standard deviation = 0.05).

yields 50 cases, which is two orders of magnitude fewer than the unrealistic 5000 cases required for the time-point $t = 4$. This behaviour is relevant to many experimental studies.

Knockout mice that lack a gene known to be highly expressed in the developing and adult brain often appear surprisingly normal in adulthood (Heisler *et al.*, 1998; Chruscinski *et al.*, 1999; Compan *et al.*, 2004). Even disruption of the normal differentiation of most serotonin-producing neurons in the brain has no effect on the gross morphology and cytoarchitecture of the adult forebrain (Hendricks *et al.*, 2003). Careful analysis is often needed to show that knockout mice actually exhibit subtle behavioural and other abnormalities, such as higher anxiety levels in the absence of the serotonin 5-HT_{1A} receptor or *Pet-1* (Heisler *et al.*, 1998; Hendricks *et al.*, 2003), or attenuation of novelty-induced exploratory activity in the absence of the serotonin 5-HT_4 receptor (Compan *et al.*, 2004). These abnormalities suggest micro- or nano-scale perturbations in the brain, but the detection of these changes (e.g. altered synaptic structure) may be exceedingly difficult. The task may become easier if knockout mice are compared with wild-type mice at several developmental time-points.

Suppose that at a certain developmental point the cerebral cortex of a wild-type mouse contains many serotonergic fibres, while the cortex of a mutant mouse contains none. This difference can be detected with simple immnohistochemistry. Next, suppose that by adulthood the densities of cortical serotonergic fibres become indistinguishable in these mice. This scenario is certainly possible if the growth of serotonergic fibres in simply delayed in the mutant mouse. Since precise timing is critical for normal development, the delay is likely to alter brain micro- and nano-architecture and lead to behavioural abnormalities. However, the adult brain will contain little evidence as to what caused them.

The discussed situation is relevant to serious methodological challenges in autism spectrum disorders, where the reported multitude and complexity of brain alterations detected at the time of examination may not give insights into the underlying developmental problem. It is possible that the large number of subtle alterations may be caused by a small number of major, time-dependent perturbations that occur consistently during the early development of the autistic brain. These perturbations may become virtually undetectable by the time the individual is diagnosed to be on the autism spectrum (Janušonis, 2008; Janušonis *et al.*, 2006). This hypothesis is consistent with the well-replicated finding that during early postnatal development autistic brains grow much faster than normally developing brains, but that by adulthood autistic and normal brains no longer differ in size (Courchesne *et al.*, 2001; Courchesne, Campbell & Solso, 2011).

(*b*) *If an experimental manipulation has no effect on a variable in time, it cannot alter its equilibrium value*

This statement appears obvious, but it has interesting implications. If the experimenter has detected an effect of X on the equilibrium value of Y, she can be certain that X acted on Y in time (i.e. it controlled the velocity of Y). This in turn means that if the experimenter moved the sampling backward, she might come across a highly sensitive time window that is more informative and requires smaller samples than the one that is currently being used.

(*c*) *The effect of an experimental manipulation on the equilibrium value of a variable cannot predict how the manipulation affects the variable in time*

We have already demonstrated this phenomenon (Fig. 1). A zero-correlation between an experimentally controlled variable and the equilibrium value of another variable can be obtained when these two variables are truly independent or when the former directly controls the latter. These are radically different alternatives, but they cannot be distinguished by measuring equilibrium values.

(*d*) *The effect of an experimental manipulation on the dynamics of a variable predicts the effect of this manipulation on the equilibrium value of the variable*

Suppose the dynamics of Y as a function of X is known. Without sacrificing generality, we can use Equation 7: $\frac{dY}{dt} = f(X, Y)$. If X is experimentally fixed and the system is allowed to settle down, the equilibrium value of Y (Y_{eq}) can be found by simply solving

$$f(X, Y_{eq}) = 0. \tag{9}$$

In addition, the same equation can be used to assess the homeostatic (asymptotic) stability of Y_{eq}. Specifically, Y_{eq} is stable (i.e. it returns to its original value after a slight perturbation) if

$$\frac{\partial f}{\partial Y}(X, Y_{eq}) < 0. \tag{10}$$

For example, consider the system given by Equation 8. Assuming $X > 0$, we solve

$$X(A - Y_{eq}) = 0 \tag{11}$$

and obtain $Y_{eq} = A$, as expected. This equilibrium value is homeostatically stable because

$$\frac{\partial(X(A - Y))}{\partial Y}(X, Y_{eq}) = -X < 0. \tag{12}$$

(*e*) *Experimental manipulations with a focus on equilibrium values can produce contradictory results and meaningless latent variables*

(*i*) *Example.* A researcher is interested in the relationship between a gene polymorphism and a neurological condition. He observes that 50% of individuals who are homozygous for a specific allele develop the condition. By contrast, individuals who are homozygous for a different allele never develop the condition. The researcher concludes that the condition is caused by several genes and that the

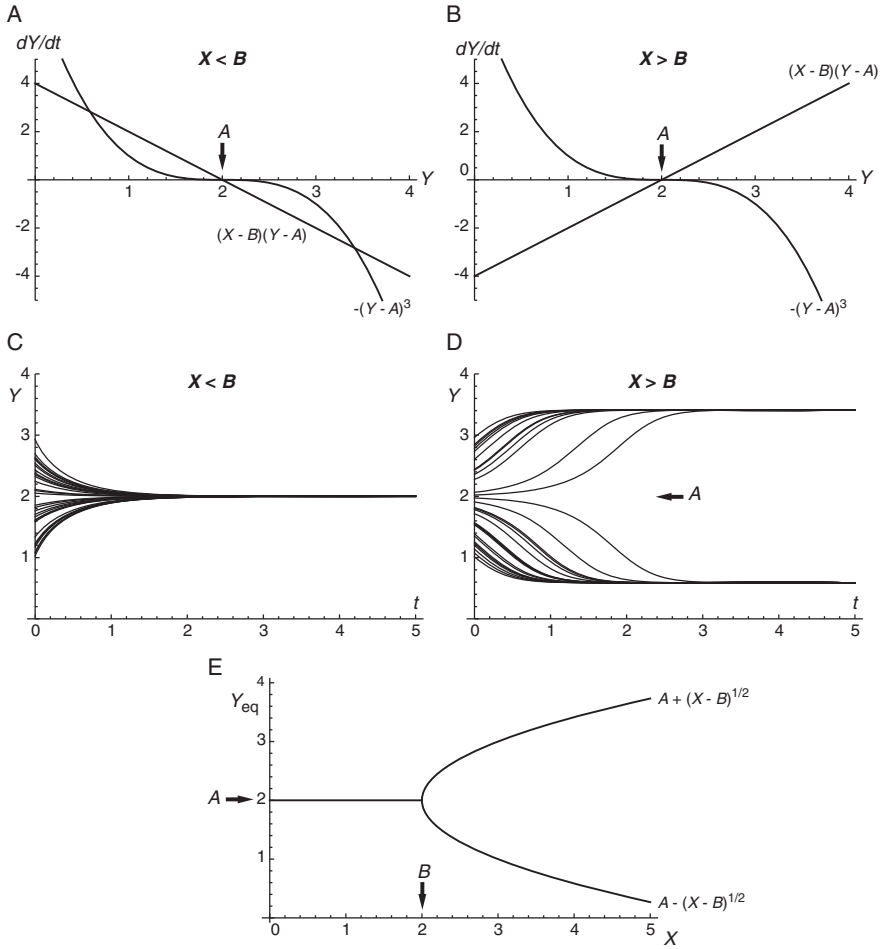

Fig. 2. The dynamics and equilibrium states of the system described by Equation 13 with $A = 2$, $B = 2$, and $X = 0$ (A, C) or $X = 4$ (B, D). (A, B) The two forces controlling the velocity of Y as functions of the current Y value. (C) The dynamics and one stable equilibrium state reached by Y when $X < B$. (D) The dynamics and two stable equilibrium states reached by Y when $X > B$. Note that Y may develop into two different "phenotypes" even when its initial values are close to A and experimentally indistinguishable. (E) When X crosses the critical value (B), the stable equilibrium value of Y (Y_{eq}) "chooses" one of the two possibilities.

studied gene is only one of the risk factors. This conclusion may be incorrect: the condition may be caused solely by the studied gene.

(*ii*) *Formal demonstration.* Consider the following dynamical system (Nicolis & Prigogine, 1989; Strogatz, 2001):

$$\frac{dY}{dt} = (X - B)(Y - A) - (Y - A)^3, \tag{13}$$

where $A > 0$ and B are constant and X is a control variable fixed at an experimentally set value. Despite its somewhat intimidating appearance, the system can formalize various natural biological processes.

If $X < B$, the value of Y increases if $Y < A$ and decreases if $Y > A$ (Fig. 2A). Consider a biological system that, when perturbed, simply returns to its previous homeostatic state (e.g. cardiac output after exercise or the charge of an electric eel after stunning the prey). If the homeostatic state is A, Equation 13 can describe this behaviour (Fig. 2C) and is fundamentally similar to the simpler system in Equation 8.

If $X > B$, the system becomes more interesting and is now controlled by two opposing processes. The first process represents unimpeded exponential growth if Y exceeds a threshold (A) and exponential decay if Y falls below the threshold (Fig. 2B):

$$\frac{dY}{dt} = (X - B)(Y - A). \tag{14}$$

It is easy to find relevant biological examples. In the developing brain, serotonin released by growing serotonergic fibres acts on serotonin 5-HT_{1A} receptors expressed on astroglial cells. In response, astroglial cells release S-100β (a growth factor), which in turn promotes the growth of serotoninergic fibres and leads to higher extracellular serotonin levels (Whitaker-Azmitia, 2001).

In reality, the system cannot grow into positive infinity or decay into negative infinity. Sooner or later, it will encounter an opposing force that may be negligible when

Y is moderate but that will increase rapidly if Y grows too small or too large. This force is modeled by the second half of the equation, where the cubic power is simply a mathematical trick to bend the curve into the needed shape (Fig. 2B):

$$\frac{dY}{dt} = -(Y - A)^3. \quad (15)$$

Now consider the development of Y when $X < B$ and when $X > B$. In the first case ($X < B$), the system reaches a single equilibrium value ($Y = A$) that is independent of X and homeostatically stable (Fig. 2C). In the second case ($X > B$), the system settles into one of two possible equilibrium values ($Y_{eq} = A \pm (X-B)^{1/2}$), both of which are homeostatically stable (Fig. 2D). In each experimental run, the equilibrium value of Y will be determined not by the underlying dynamics (which is always the same), but by the initial value of Y. If the initial value of Y is greater than A, the variable will reach the "upper" equilibrium value. If, however, the initial value of Y is less than A, the variable will reach the "lower" equilibrium value. While at first glance this behaviour appears to be predictable, Y may be so close to A that its actual initial value will always be beyond the precision of experimental instruments. This means that, for all practical purposes, Y will randomly take on one of the two equilibrium values.

To summarize, this simple deterministic dynamics can produce one outcome at some X values and two virtually unpredictable outcomes at some other X values (Fig. 2E). Importantly, this radical change is triggered by a smooth and otherwise inconspicuous transition of X from being less than B to being greater than B (to make it worse, the experimenter may not be aware of the very existence of B). If X is less than B, the equilibrium value of Y is independent of X. Experimental journals are likely to consider such a result "negative" and unworthy of publishing. If X is greater than B, each of the two equilibrium values of Y is determined by the value of X. Experimental journals may consider such a result "exciting," though reviewers may be concerned about a possible heterogeneity of the sample. All of these conclusions would be misguided. In reality, both results are of equal value and complementary. Moreover, there is no heterogeneity in the sample (the initial values of $A - 10^{-10}$ and $A + 10^{-10}$ hardly qualify as being different). Taken together, these studies suggest an underlying simplicity rather than complexity—if only one were willing to consider time.

Let us return to the example at the beginning of this section. Suppose that the genetic polymorphism sets the X value and that the normal neurological state requires that Y be at the level of A or above. Some alleles may set X above B, which will split the population into a normal subset and a subset that will develop the condition (Fig. 2D). Importantly, this stratification will be caused by the inherent dynamics of the system and not by unknown latent variables (e.g. other genes). The proportion of the subsets will be 50:50 if the initial values of Y are symmetrically distributed around A, but it can be anything else if the initial distribution is non-symmetric (as is likely to be the case in practice).

The possibility of this phenomenon should always be considered in the interpretation of experimental studies, including those that have potential clinical significance. Altered serotonin function is thought to play a major role in autism spectrum disorders (Anderson, 2002; Azmitia, Singh & Whitaker-Azmitia, 2011). Mulder *et al.* (2004) have reported that the normally unimodal distribution of blood serotonin levels may become bimodal in individuals diagnosed with autism. One simple explanation is that a typical autistic group contains a subgroup of individuals whose multiple gene variations cumulatively cause abnormally high blood serotonin levels. Another equally simple, but much less intuitive explanation is that the bimodal distribution is caused by altered activity of one gene in all autistic individuals. If the activity of the gene crosses the bifurcation point (again, in all individuals!), two subgroups can emerge spontaneously because of random environmental fluctuations (Fig. 2). Focusing on the subgroups would be misleading, since most of the important information is carried by the bifurcation itself. Paradoxically, it may suggest a single causal factor.

It should be noted that the discussed system represents a special case of spontaneous "symmetry breaking," which in turn is a special case of highly counterintuitive relationships between determinism and randomness. Deterministic systems can produce quasi-random behaviour (weather dynamics, turbulence, cellular automaton patterns, even the decimal digits of π) (Nicolis & Prigogine, 1989; Wolfram, 2002; Beck, 2009) and, conversely, random behaviour can produce strikingly deterministic outcomes [e.g. see Babloyantz (1977) for spontaneous gradient formation]. These phenomena have been studied by a number of prominent mathematicians and physicists, some of whom have expressed their bewilderment at the counterintuitive results: *"The rule 30 automaton is the most surprising thing I've ever seen in science. [. . .] It took me several years to absorb how important it was."* (Mitchell, 2009; quoting Stephen Wolfram); *"This is counterintuitive, since random variables typically have a built-in fluctuation, and the sharp concentration property in [. . .] is very surprising (to say the least)."* (Beck, 2009).

(*f*) *If two variables are controlled by a third variable, the equilibrium values of the two variables can be independent or correlated*

To see that two variables (X and Y), controlled by the same third variable ($Z > 0$), can produce uncorrelated equilibrium values, consider the following system:

$$\frac{dX}{dt} = Z(A - X), \quad (16)$$

$$\frac{dY}{dt} = Z(B - Y), \quad (17)$$

where A and B are constant. The equilibrium values ($X_{eq} = A$ and $Y_{eq} = B$) are homeostatically stable and uncorrelated if the experimenter varies Z.

To see that two variables (X and $\mathcal{Y}$), controlled by the same third variable ($\mathcal{Z} > 0$), can produce perfectly correlated equilibrium values, consider the following system:

$$\frac{dX}{dt} = A(\mathcal{Z} - kX), \tag{18}$$

$$\frac{d\mathcal{Y}}{dt} = B(\mathcal{Z} - \mathcal{Y}), \tag{19}$$

where $A > 0$, $B > 0$, and $k > 0$ are constant. The equilibrium values ($X_{eq} = \mathcal{Z}/k$ and $\mathcal{Y}_{eq} = \mathcal{Z}$) are homeostatically stable and linearly linked ($\mathcal{Y}_{eq} = kX_{eq}$) if the experimenter varies $\mathcal{Z}$.

Therefore, if manipulating a variable does not produce a significant correlation between the equilibrium values of other variables, one cannot conclude that the manipulated variable does not control the behaviour of both of the observed variables.

(g) *Interaction between two variables controlled by a third variable*

(i) *Example.* In the previous section, the variable $\mathcal{Z}$ controlled the dynamics of X and $\mathcal{Y}$, but X and $\mathcal{Y}$ had no direct effect on each other. Consider the following, more complex situation. An experimenter fixes $\mathcal{Y}$ at four values and measures the corresponding equilibrium values of X. ANOVA reveals no significant effect (Fig. 3A). She next fixes X at four values and measures the equilibrium values of $\mathcal{Y}$. Again, ANOVA reveals no significant effect (Fig. 3B). The experimenter then varies $\mathcal{Z}$ and finds a perfect correlation

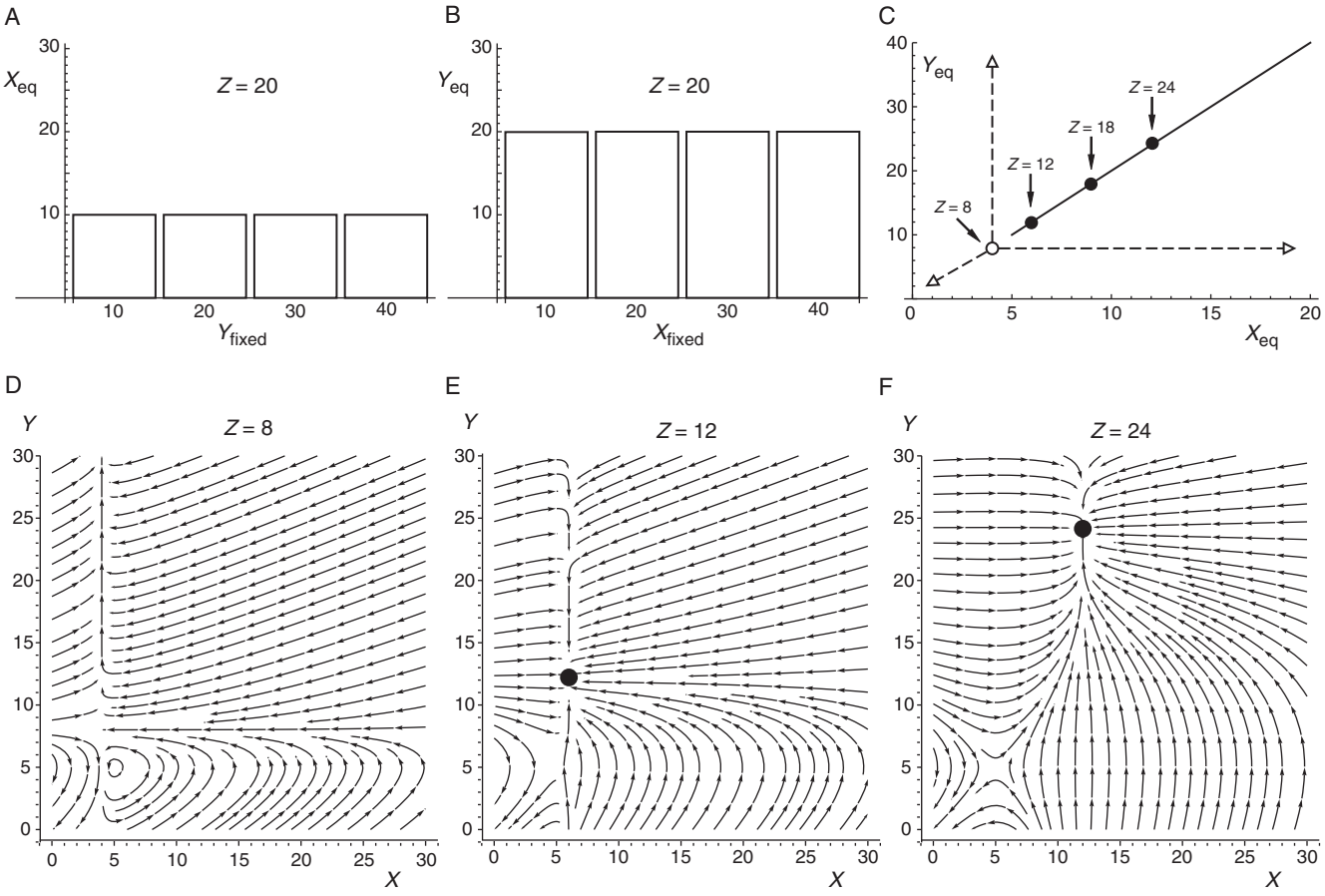

Fig. 3. The dynamics and equilibrium states of the system described by Equations 20 and 21 with $A = 5$, $B = 5$, and $k = 2$. (A) The equilibrium values of X (X_{eq}, measured at the time $t = 1$) with $\mathcal{Z} = 20$ and the values of $\mathcal{Y}$ fixed at 10, 20, 30, and 40. (B) The equilibrium values of $\mathcal{Y}$ ($\mathcal{Y}_{eq}$, measured at $t = 1$) with $\mathcal{Z} = 20$ and the values of X fixed at 10, 20, 30, and 40. In A and B, each bar represents a sample of ten X or $\mathcal{Y}$ values, respectively. These values were obtained from ten initial values uniformly distributed on [5, 25]. The standard errors of the mean are negligible and are not shown. Note that ANOVA or other GLM tests would detect no effect of $\mathcal{Y}$ on X and no effect of X on $\mathcal{Y}$. (C) The linear relationship between the equilibrium values of X and $\mathcal{Y}$ revealed by experimentally fixing $\mathcal{Z}$ at different values. Note that this relationship collapses and becomes practically unpredictable when $\mathcal{Z}$ falls below its critical value [$\max(A, kB) = 10$]. (D) The phase plot of the X and $\mathcal{Y}$ dynamics when $\mathcal{Z}$ is below its critical value. Note the absence of an equilibrium point, the rapidly growing $\mathcal{Y}$ and stable X in the upper left corner of the plot, and the oscillatory, Lotka-Volterra-like dynamics (Lotka, 1925; Berryman, 1992) in the lower left corner of the plot. (E, F) The phase plot of the X and $\mathcal{Y}$ dynamics when $\mathcal{Z}$ is above its critical value. Note that the positions of the X and $\mathcal{Y}$ equilibrium values are linearly related (despite the non-trivial dynamics away from the equilibrium).

between the equilibrium values of X and Y (Fig. 3C). She concludes that both X and Y are controlled by Z, but that there is no direct interaction between X and Y. This conclusion may be incorrect.

(*ii*) *Formal demonstration.* Consider the following system:

$$\frac{dX}{dt} = (Y - A)(Z - kX), \tag{20}$$

$$\frac{dY}{dt} = (X - B)(Z - Y), \tag{21}$$

where $A \geq 0$, $B \geq 0$, and $k > 0$ are constant. In order to keep the system stable, we need to place additional constraints. If Z is constant and we experimentally set Y or X, it is assumed that $Y > A$ and $X > B$, respectively. This guarantees global stability of the system. If we experimentally set Z, it is assumed that $Z > \max(A, kB)$. This guarantees local stability of the system around the equilibrium point $X_{eq} = Z/k$, $Y_{eq} = Z$. In practice, these values may correspond to natural, physiologically relevant values, beyond which the system collapses. Note that if $A = B = 0$, all conditions are satisfied if $X, Y, Z > 0$.

If we keep Z constant and experimentally set Y at four values, the equilibrium values of X will be independent of Y ($X_{eq} = Z/k$) (Fig. 3A). Likewise, if we experimentally set X at four values, the equilibrium values of Y will be independent of X ($Y_{eq} = Z$) (Fig. 3B). Next, if we experimentally set Z and allow both X and Y to reach their natural equilibrium values, these values turn out to be linearly related: $Y_{eq} = kX_{eq}$ (Fig. 3C). Despite the impression that X and Y are linked only through Z, they actually directly interact and this interaction is non-linear (Fig. 3D-F).

The non-linear interaction between X and Y is not inconsequential even if one is interested only in equilibrium values. It brings surprises when Z crosses the system's stability threshold: suddenly, the system may show unstable behaviour that is highly sensitive to initial conditions and perturbations (Fig. 3D). One variable may settle down while the other may increase rapidly, or both variables may get trapped in a permanent oscillation. Importantly, the experimenter may not see the dynamics in time and may continue sampling the system, assuming it has settled down as it had done in the past. This may produce unexpected and extreme outliers which may be different in each experimental run. Unfortunately, such revealing results are typically treated as experimental disasters that undermine the consistency of the completed work and are rarely reported.

The discussed dynamics is important for the correct interpretation of large-scale genetic and protein networks (e.g. Hu, Addington & Hyman, 2011). Consider two proteins in such a network. Suppose that an experimental manipulation of one protein does not affect the equilibrium amount of the other protein and that the available connectivity graph indicates that the two proteins are connected only through a third protein (Fig. 4A). If another manipulation changes both of these proteins, the researcher may be compelled to focus on this indirect interaction, even though nothing in the factual data suggests this course of action (the experimental data do not rule out direct interaction and the graph cannot be assumed to be complete; Fig. 4B).

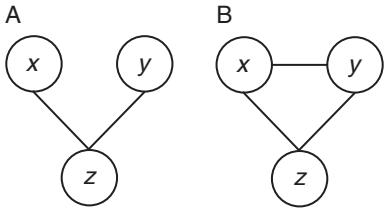

Fig. 4. If experimentally fixing one variable (x) in a system does not affect the equilibrium value of another variable (y), but the equilibrium values of both variables change when a third variable is manipulated (z), it does not imply that x and y interact only indirectly through z or another variable (A). In reality, x and y can still directly interact in time (B). For an example, see Equations 20 & 21 and Fig. 3.

The analysis also shows that well-designed studies may produce drastically different correlation results. It does not mean that any of the studies is flawed or that their results are inconsistent. Instead, attention should shift to the possible dynamics of the system. We give more examples at the end of the next section.

(*h*) *Linear interactions among variables may produce uncorrelated equilibrium values*

We now consider a system in which two variables interact with no experimental manipulation and ask whether their equilibrium values will be correlated. Such systems are important in descriptive studies, but they also guide experimental designs and are used in causal analysis, such as structural equation modeling (Kline, 2005).

Let us restrict our attention to the simplest interaction represented by a system of two coupled linear differential equations. Such linear systems can be used to model accurately real biological phenomena or to approximate the behaviour of non-linear systems around their equilibrium values:

$$\frac{dX}{dt} = m_{11}X + m_{12}Y + q_1, \tag{22}$$

$$\frac{dY}{dt} = m_{21}X + m_{22}Y + q_2, \tag{23}$$

where $m_{ij} \neq 0$ and q_i ($i, j = 1, 2$) are the system's parameters. It should be emphasized that Equations 22 and 23 represent the simplest way of how any two biological variables may interact; therefore, a relationship between two variables immediately implies a dynamic of at least this complexity.

Next, assume that natural environmental factors perturb each of the parameters around their normal values. Will the equilibrium values of X and Y be correlated? The answer turns out to be unexpectedly complex: they may be almost perfectly correlated or nearly uncorrelated, depending on the exact value of the parameters (Fig. 5). Importantly, two

variables that show low or no correlation when their equilibrium values are measured can be tightly linked dynamically (Fig. 5D,E). Since it is the dynamics that determines how these variables will interact with other variables and what equilibrium values they will develop in these extended systems, no useful theoretical or meta-analytical conclusions can be drawn from such correlation analyses. They can, however, play an important practical role in the design or refinement of experiments specifically geared toward theory building.

The underlying dynamics clearly needs to be considered when researchers strongly disagree not only regarding the direction of a correlation, but its very existence. For example, the nature of the relationship between biomass production and species richness is one of the most controversial topics in ecology (Warren, Topping & James, 2009). Since these variables are mutually interdependent in an evolving system, they may correlate in some instances but not others. In comparative mammalian neuroanatomy, the strength of the correlation between the total brain size and the size of the frontal cortex continues to be debated, especially with regard to the human brain (Bush & Allman, 2004; Butler & Hodos, 2005; Kolb, 2007). The frontal cortex has massive connections with the rest of the brain and these parts unavoidably influence each other in evolution. Depending on the initial set of the parameters (of which undoubtedly there are many), different groups of mammals may develop different quantitative relationships between the frontal cortex and the rest of the brain. Recent studies suggest such variability across mammalian orders (Bush & Allman, 2004; Kolb, 2007). Obviously, the dynamical properties discussed in the two last sections are equally relevant to systems that evolve on a more rapid time scale (e.g. hours or seconds).

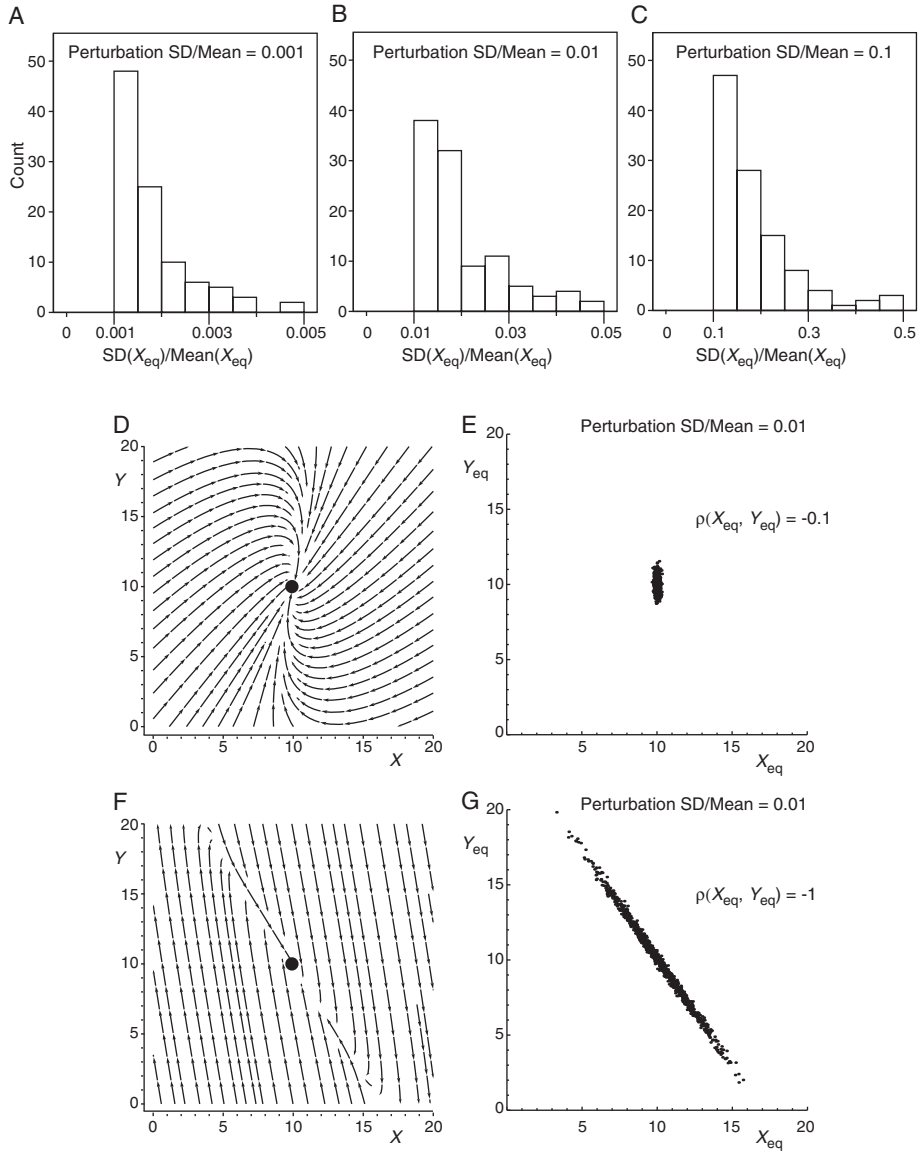

Fig. 5.

(3) Fundamental science of biological systems and measure theory

There is no escape from the conclusion that analyses of relationships among equilibrium values can be grossly misleading, especially if one hopes to build a fundamental science. However, it is equally clear that real-time tracking of multiple variables in a biological system can be extremely difficult or even impossible. This raises an interesting question about variables themselves: what is a variable or, more precisely, what is a measurable variable?

Obvious examples of measurable variables are size, mass, charge, concentration, density etc. All of these variables are equally well suited to describe simple physical systems, such as colliding balls, batteries, or chemical solutions. However, one serious problem with these variables is that one needs a very large number of them to describe even a small biological system. For example, a recent simulation of the internalization and recycling of adrenergic β_2 receptors has used 10 differential equations and nearly 30 parameters (Vayttaden *et al.*, 2010). Another problem is that in biological systems these variables never reach true equilibrium and fluctuate as a function of the circadian cycle, aging, and other natural processes. Even when they do reach "quasi-equilibrium" states (which we call "equilibrium" for convenience), we cannot do much with these values and are forced again to consider the variables in time (as the previous discussion shows). To sum up: on the one hand, equilibrium values produce a precariously shaky foundation; on the other hand, our alternative options appear to be severely limited. Therefore, the main purpose of the previous discussion was not to point out flaws in experimental designs (which may be as good as we can get them today), but rather to call attention to potential inferential errors in the interpretation of experimental results. In particular, seeking a logical connection between the results of two studies that have measured equilibrium values may be a futile exercise, and any perceived consistency or inconsistency between them may be emotionally satisfying but scientifically useless. This situation is obviously unsatisfactory, but seeing it clearly may be a good start towards a solution. Specifically, it may offer insights into why the fundamental structure of biological sciences is still not on a par with exact sciences.

Do we measure what we should measure? If our usual variables should always be considered in time, can we create variables that remain constant in time? If these variables can be created, can they capture the deeper essence of biological

Fig. 5. The effect of perturbation of the parameters in two-variable linear dynamical systems (Equations 22 & 23) on the equilibrium values of the systems. (A-C) The distribution of the standard deviation (SD)/mean ratios of the equilibrium X values (X_{eq}) in around 100 random linear systems, all parameters of which were independently perturbed (each data point represents a sample of around 1000 perturbations applied to the same system). A more precise description follows. Each dynamical system is represented by the equation $d\mathbf{V}/dt = \mathbf{MV} + \mathbf{Q}$, where t is time, $\mathbf{V} = (X, Y)$ is a column-vector containing the two variables, $\mathbf{M} = (m_{ij})$ is a 2x2 matrix, and $\mathbf{Q}$ is a column-vector containing two constants. The matrix $\mathbf{M}$ is a random matrix, the elements of which are uniformly distributed on $[-1, 1]$ and independent. An additional restriction placed on the matrix is that the real parts of both of its eigenvalues should be negative (it guarantees stability of the equilibrium). In order to centre the equilibrium state at $X_{eq} = 10$ and $Y_{eq} = 10$, it was assumed that $\mathbf{Q} = -\mathbf{MV_c}$, where $\mathbf{V_c} = (10, 10)$ is a column-vector. Each perturbation was produced by adding an error term to each of the six parameters (represented by the elements of $\mathbf{M}$ and $\mathbf{Q}$). The error terms were independent and normally distributed with a zero-mean and a standard deviation of $0.001p$, $0.01p$, and $0.1p$, where p is the original value of the parameter. If a system lost its stability after a perturbation, the perturbation was not used (for the standard deviations of $0.001p$ and $0.01p$, less than 1% of systems needed this adjustment). The equilibrium values, represented by the column-vector $\mathbf{V_{eq}} = (X_{eq}, Y_{eq})$, were obtained by $\mathbf{V_{eq}} = -(\mathbf{M}^*)^{-1}\mathbf{Q}^*$, where $\mathbf{M}^*$ and $\mathbf{Q}^*$ contain the perturbed parameters. The results show that, in most systems, the variability of the equilibrium point closely matches the variability of the parameters, even though the link between the two is not linear. However, some "runaway" systems show much larger variability, represented by a long, narrow tail of the histograms (not shown, but see G for an example). (D) The phase plot of the system

$$\frac{dX}{dt} = -0.89X + 0.14Y + 7.50,$$
$$\frac{dY}{dt} = -0.69X - 0.22Y + 9.10.$$

The black circle indicates the position of the stable equilibrium. (E) Low correlation between the X and Y equilibrium values of the system shown in D, each coefficient of which was perturbed around its normal value p with a standard deviation of $0.01p$ (1000 points). Note that even though the equilibrium values show low correlation, X and Y are tightly coupled dynamically. (F) The phase plot of the system

$$\frac{dX}{dt} = 0.12X + 0.09Y - 2.10,$$
$$\frac{dY}{dt} = -0.77X - 0.50Y + 12.70.$$

The black circle indicates the position of the stable equilibrium. (G) High correlation between the X and Y equilibrium values of the system shown in F, each coefficient of which was perturbed around its normal value p with a standard deviation of $0.01p$ (1000 points).

systems? Can we construct the conceptual equivalents of "centre of mass" or "energy" in biological systems?

These questions are not purely philosophical. First, biologists need to consider carefully the very notion of "measure." It is much deeper than the usual measures of length, mass, or concentration, all of which are intuitively obvious and immediately thought of to be located on the real number line. However, it is well known in mathematics and physics that numerical measures can be consistently assigned to more abstract (but perfectly natural) entities. Generally, measures can be consistently assigned to various sets of objects (directly accessed or conceptualized), as long as the sets satisfy certain abstract but intuitively meaningful properties.

In this regard, measure theory (Bogachev, 2007) is underappreciated in biology, perhaps because many biologists find it to be too abstract. It has been used to understand some difficult problems, such as the general properties of perception (Bennet *et al.*, 1996), but such studies are rare. However, biological systems always have a probabilistic (stochastic) component, from molecules to ecological communities (Kurakin, 2005; Pedraza & Paulsson, 2008; Ellwood, Manica & Foster, 2009; Lestas, Vinnicombe & Paulsson, 2010; Thébault & Fontaine, 2010; Huh & Paulsson, 2011), and probability theory itself firmly rests on measure theory (Shiryaev, 1996). Specifically, probability theory successfully deals with a very broad spectrum of events, even though events are not inherently numerical. Probability theory takes advantage of measure theory and maps events and their combinations onto numerical values in a consistent way.

The link between biological systems, probability, and measure theory may be just the tip of a big conceptual iceberg. While in biological systems the usual physical variables (e.g. size, concentration) dynamically fluctuate, some measures of entire sets of objects may remain stable in time. If successful, this approach may radically reduce the need always to consider time, especially if time is already implicitly included in the set (e.g. one object can be replaced with another object without changing the set measure).

To demonstrate the potential power of this approach, consider a neuron in the brain. A typical neuron receives thousands of synapses (in the cerebellum, a single Purkinje cell can receive 100,000 synapses). Functionally, each synapse is composed of a neurotransmitter (e.g. glutamate) released from the presynaptic terminal and a set of postsynaptic neurotransmitter receptors (e.g. glutamate receptors) that detect presynaptic signals and convert them to signals inside the postsynaptic neuron. Traditionally, each neurotransmitter is studied as a separate channel (the glutamate system, the serotonin system, etc.) and each neurotransmitter receptor is assumed to have specific functional roles in the brain (otherwise knocking out neurotransmitter receptors would be meaningless). While this works as a first approximation and provides a pedagogically useful handle, it is not what the postsynaptic neuron sees. Many neurotransmitters act on their receptors and produce signals that feed into a small number of downstream signaling pathways, thus blurring the distinction between which neurotransmitter activated which receptor (Fig. 6). For example, intracellular cyclic AMP (cAMP) levels can be elevated by serotonin acting on 5-HT_4 receptors, or by norepinephrine acting on adrenergic β_2 receptors, or by dopamine acting on dopamine D_1 receptors. A single neuron may have all of these synapses, each of which can be dynamically regulated in time. Unfortunately, this dynamics cannot be directly observed with our current technology.

We can give up, or we can invert the problem and ask what sets of neurotransmitter-receptor pairs can produce the same cAMP signal inside the neuron. We can then think about how to assign these sets (not individual neurotransmitters or receptors!) a meaningful numerical measure. Functionally, it may no longer matter which neurotransmitter acts on which receptor at a given time, as long as the set measure does not change. Therefore, knocking out the dopamine D_1 receptor or the serotonin 5-HT_4 receptor may produce an unexpectedly mild phenotype, as the neuron can maintain its *status quo* by synthesizing additional 5-HT_4 receptors in the first case and additional D_1 receptors in the second case. This implies a considerable degree of redundancy, but this redundancy is different from the typically vague use of this term. First, it leads to a natural, well-defined set that cuts across various neurotransmitter "systems" and instead contains neurotransmitter-receptor pairs. In this regard, neither a single neurotransmitter (e.g. serotonin) nor its full complement of receptors (e.g., 5-HT_{1A}, 5-HT_{1B}, etc.) constitutes a "system," as different receptors of the same neurotransmitter can feed into different signaling cascades inside the postsynaptic neuron. Second, this approach emphasizes the message rather than the messenger, which appears to be a more natural approach. Interestingly, this conceptual reversal ("start with the message rather than the messenger") is somewhat analogous to the transition from the Riemann integral to the more powerful Lebesgue integral in mathematics, which—again—heavily relies on measure theory.

The presented example conveys the gist of the argument, but it is an oversimplification. A neurotransmitter-receptor pair cannot be precisely equivalent to another pair because their signaling may differ in other respects, such as receptor desensitization, internalization, recycling to the membrane, activation of collateral signaling cascades, and others. However, these highly dynamic events again converge inside the neuron (e.g. there is a small number of arrestins that mediate receptor internalization; Luttrell & Gesty-Palmer, 2010) and, therefore, it is meaningful to ask whether a slightly more advanced set would be able to take them into account.

While at this time we cannot do the inventory of all neurotransmitter receptors on the membrane of a single neuron (much less monitor them in time), recent studies have shown that neurotransmitters "cross-talk" much more than has been previously thought. Some neurotransmitter receptors form functional complexes (heteromers), thus effectively creating a multi-neurotransmitter "microchip" in the neuronal membrane (Albizu *et al.*, 2010; Vilardaga *et al.*, 2010). Such complexes have been demonstrated between glutamate and serotonin receptors, glutamate and adrenergic receptors, serotonin and adrenergic receptors, and other neurotransmitter

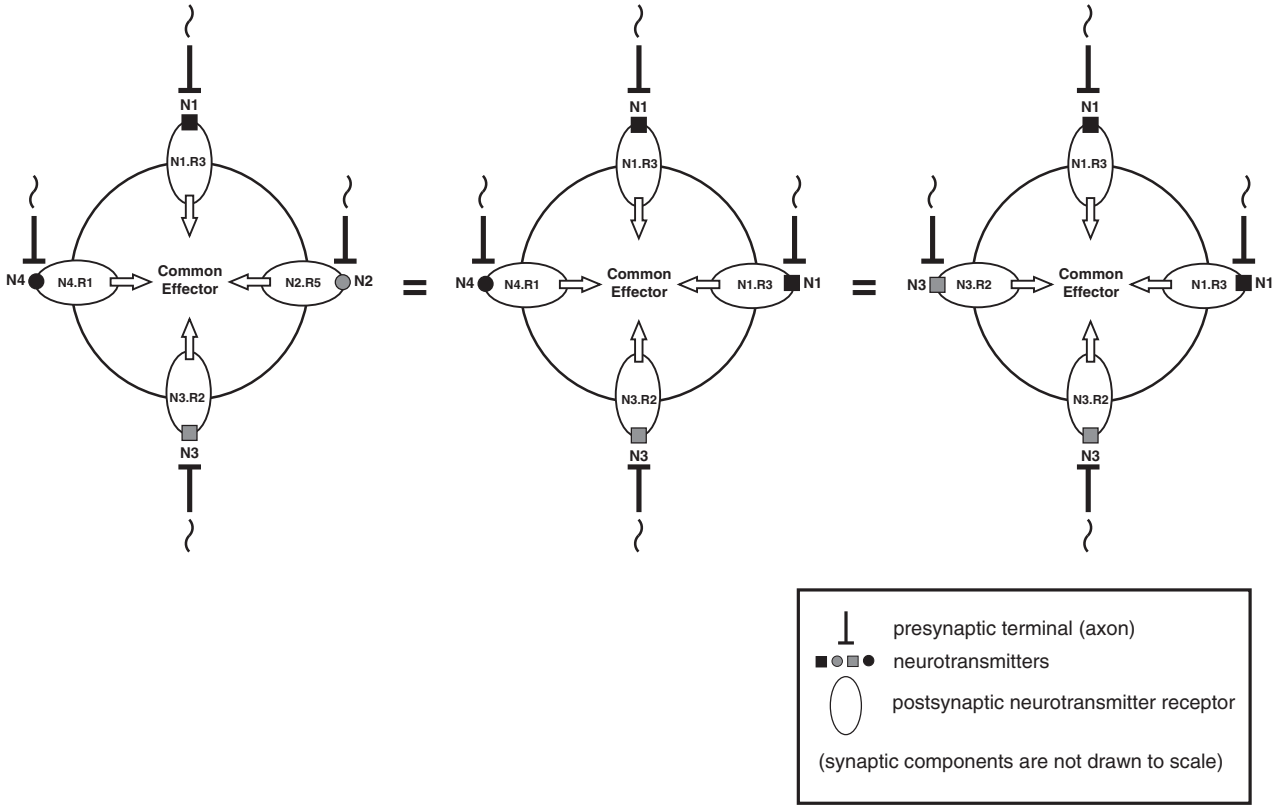

Fig. 6. A typical neuron in the brain makes a great number of synapses, each of which is dynamically regulated in time (wavy lines). Each presynaptic terminal (axon) releases a neurotramitter (N*n*) which acts on some of its postsynaptic receptors (N*n*.R*m*, where *n* and *m* numerically denote a specific neurotransmitter and one of its receptors). The downstream signaling from several neurotransmitter-receptor pairs may converge onto the same effector pathway (e.g. an increase in cyclic AMP or Ca^{2+} levels). Therefore, the internal state of the neuron may be immune to some changes in the synaptic set, as well as to the highly dynamic modulation of each of the individual synapses. Consequently, tracking each of the synapses in time may not be needed. Instead, it may be useful to focus on the properties of the entire synaptic set, which can be assigned a numerical measure. The presented picture is an oversimplification, but it helps to illustrate the proposed approach that is not limited to neural systems.

receptors (Berthouze *et al.*, 2005, 2007; González-Maeso & Sealfon, 2009; Albizu *et al.*, 2010; Joiner *et al.*, 2010). Also, the absence of one neurotransmitter receptor can be functionally compensated for by hyperfunction of another neurotransmitter receptor, even if the two receptors are activated by different neurotransmitters (Segu *et al.*, 2010).

It should be noted in conclusion that it matters greatly what we measure, especially in fundamental sciences. Understanding every minutiae of a system may seem to be a truly fundamental approach: *"[. . .] it would seem that both points of view are valid, although the latter one, leaving out no details, might seem to be the more* fundamental *one [. . .], while the former, being a highly compressed simplification in which vast amounts of information are thrown away, might seem to be the more* useful *one [. . .].* (Hofstadter, 2007)

However, the complexity and degeneracy of biological systems (Whitacre, 2010) forces us to make wise choices. As Henri Poincaré has put it, *"There is no disputing the fact that a selection must be made: however great our activity, facts outstrip us, and we can never overtake them; while the scientist is discovering one fact, millions and millions are produced in every cubic inch of his body. Trying to make science contain nature is like trying to make the part contain the whole. But scientists believe that there is a hierarchy of facts, and that a judicious selection can be made. They are right, for otherwise there would be no science, and science does exist."* (Poincaré, 2001).

III. CONCLUSIONS

(1) Statistical analysis of relationships among equilibrium values has little theoretical or meta-analytical value. The problem lies with the conceptual approach but not with statistical methods that can easily incorporate time as another variable.

(2) Great caution should be exercised in using equilibrium values to understand fundamental properties of biological systems. Generally, negative results are as valuable as positive results; together they may give insight into the underlying dynamical relationships that often cannot be observed directly. Because of the underlying dynamics, correlations in which at least one of the variables is measured at equilibrium may be unstable and inconsistent. This applies to both

experimental studies (where some variables may be fixed by the experimenter) and descriptive research (where both variables may be measured at equilibrium).

(3) Understanding the dynamics of a system may considerably increase the statistical power of small samples, which is currently a serious problem in some areas of biological research.

(4) Experimenters should appreciate the hierarchy of empirical facts. Specifically, some relationships among variables are inherently more valuable than others (at least from the theoretical point of view). Measure theory may be a useful tool in formalizing the fundamental structure of biological systems.

IV. ACKNOWLEDGEMENTS

This work was supported by Hellman Family Faculty Fellowships (2009 & 2010) and UCSB Academic Senate grants.

V. REFERENCES

Albizu, L., Moreno, J. L., González-Maeso, J. & Sealfon, S. C. (2010). Heterodimerization of G protein-coupled receptors: Relevance to neurological disorders and neurotherapeutics. *CNS & Neurological Disorders—Drug Targets* **9**, 636–650.

Anderson, G. M. (2002). Genetics of childhood disorders: XLV. Autism, part 4: serotonin in autism. *Journal of the American Academy of Child & Adolescent Psychiatry* **41**, 1513–1516.

Azmitia, E. C., Singh, J. S. & Whitaker-Azmitia, P. M. (2011). Increased serotonin axons (immunoreactive to 5-HT transporter) in postmortem brains from young autism donors. *Neuropharmacology* **60**, 1347–1354.

Babloyantz, A. (1977). Self-organization phenomena resulting from cell-cell contact. *Journal of Theoretical Biology* **68**, 551–561.

Beck, J. (2009). *Inevitable Randomness in Discrete Mathematics*. American Mathematical Society, Providence, Rhode Island.

Bennett, B. M., Hoffman, D. D., Prakash, C. & Richman, S. N. (1996). Observer theory, Bayes theory, and psychophysics. In *Perception as Bayesian Inference*. (eds D. C. Knill and W. Richards), pp. 163–212. Cambridge University Press, Cambridge.

Berryman, A. A. (1992). The origins and evolution of predator-prey theory. *Ecology* **73**, 1530–1535.

Berthouze, M., Ayoub M., Russo, O., Rivail, L., Sicsic, S., Fischmeister, R., Berque-Bestel, I., Jockers, R. & Lezoualc'h, F. (2005). Constitutive dimerization of human serotonin 5-HT$_4$ receptors in living cells. *FEBS Letters* **579**, 2973–2980.

Berthouze, M., Rivail, L., Lucas, A., Ayoub, M. A., Russo, O., Sicsic, S., Fischmeister, R., Berque-Bestel, I., Jockers, R. & Lezoualc'h, F. (2007). Two transmembrane Cys residues are involved in 5-HT$_4$ receptor dimerization. *Biochemical and Biophysical Research Communications* **356**, 642–647.

Bogachev, V. I. (2007). *Measure Theory* (vols. 1-2). Springer, New York.

Bush, E. C. & Allman, J. M. (2004). The scaling of frontal cortex in primates and carnivores. *The Proceedings of the National Academy of Sciences of the United States of America* **101**, 3962–3966.

Butler, A. B. & Hodos, W. (2005). *Comparative Vertebrate Neuroanatomy: Evolution and Adaptation* (2nd ed.). Wiley-Interscience, Hoboken, NJ.

Chruscinski, A. J., Rohrer, D. K., Schauble, E., Desai, K. H., Bernstein, D. & Kobilka, B. K. (1999). Targeted disruption of the β_2 adrenergic receptor gene. *The Journal of Biological Chemistry* **274**, 16694–16700.

Compan, V., Zhou, M., Grailhe, R., Gazzara, R. A., Martin, R., Gingrich, J., Dumuis, A., Brunner, D., Bockaert, J. & Hen, R. (2004). Attenuated response to stress and novelty and hypersensitivity to seizures in 5-HT$_4$ receptor knock-out mice. *The Journal of Neuroscience* **24**, 412–419.

Courchesne, E., Campbell, K. & Solso, S. (2011). Brain growth across the life span in autism: Age-specific changes in anatomical pathology. *Brain Research* **1380**, 138–145.

Courchesne, E., Karns, C. M., Davis, H. R., Ziccardi, R., Carper, R. A., Tigue, Z. D., Chisum, H. J., Moses, P., Pierce, K., Lord, C., Lincoln, A. J., Pizzo, S., Schreibman, L., Haas, R. H., Akshoomoff, N. A. & Courchesne, R. Y. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology* **57**, 245–254.

Ellwood, M. D. F., Manica, A. & Foster, W. A. (2009). Stochastic and deterministic processes jointly structure tropical arthropod communities. *Ecology Letters* **12**, 277–284.

González-Maeso, J. & Sealfon, S. C. (2009). Psychedelics and schizophrenia. *Trends in Neurosciences* **32**, 225–232.

Heisler, L. K., Chu, H.-M., Brennan, T. J., Danao, J. A., Bajwa, P., Parsons, L. H. & Tecott, L. H. (1998). Elevated anxiety and anti-depressant-like responses in serotonin 5-HT$_{1A}$ receptor mutant mice. *The Proceedings of the National Academy of Sciences of the United States of America* **95**, 15049–15054.

Hendricks, T. J., Fyodorov, D. V., Wegman, L. J., Lelutiu, N. B., Pehek, E. A., Yamamoto, B., Silver, J., Weeber, E. J., Sweatt, J. D. & Deneris, E. S. (2003). *Pet-1* ETS gene plays a critical role in 5-HT neuron development and is required for normal anxiety-like and aggressive behaviors. *Neuron* **37**, 233–247.

Hofstadter, D. (2007). *I Am a Strange Loop*. Basic Books, New York.

Hu, V. W., Addington, A., & Hyman, A. (2011) Novel autism subtype-dependent genetic variants are revealed by quantitative trait and subphenotype association analyses of published GWAS data. *PLoS ONE* **6**, e19067.

Huh, D. & Paulsson, J. (2011). Non-genetic heterogeneity from stochastic partitioning at cell division. *Nature Genetics* **43**, 95–100.

Janušonis, S. (2008). Origin of the blood hyperserotonemia of autism. *Theoretical Biology & Medical Modelling* **5**, 10.

Janušonis, S. (2009). Comparing two small samples with an unstable, treatment-independent baseline. *Journal of Neuroscience Methods* **179**, 173–178.

Janušonis, S., Anderson, G. M., Shifrovich, I. & Rakic, P. (2006). Ontogeny of brain and blood serotonin levels in 5-HT$_{1A}$ receptor knockout mice: Potential relevance to the neurobiology of autism. *Journal of Neurochemistry* **99**, 1019–1031.

Joiner, M. L., Lisé, M. F., Yuen, E. Y., Kam, A. Y., Zhang, M., Hall, D. D., Malik, Z. A., Qian, H., Chen, Y., Ulrich, J. D., Burette, A. C., Weinberg, R. J., Law, P. Y., El-Husseini, A., Yan, Z. & Hell, J. W. (2010). Assembly of a β_2-adrenergic receptor–GluR1 signalling complex for localized cAMP signaling. *The EMBO Journal* **29**, 482–495.

Kline, R. B. (2005). *Principles and Practice of Structural Equation Modeling* (2nd ed). The Guilford Press, New York.

Kolb, B. (2007). Do all mammals have a prefrontal cortex? In *Evolution of Nervous Systems: A Comprehensive Reference* (vol. 3). (eds J. H. Kaas and L. A. Krubitzer), pp. 443–450.

Kurakin, A. (2005). Self-organization versus Watchmaker: stochastic dynamics of cellular organization. *Biological Chemistry* **386**, 247–254.

Lazic, S. E. (2008). Why we should use simpler models if the data allow this: relevance for ANOVA designs in experimental biology. *BMC Physiology* **8**, 16.

Lazic, S. E. (2010). The problem of pseudoreplication in neuroscientific studies: is it affecting your analysis? *BMC Neuroscience* **11**, 5.

Lestas, I., Vinnicombe, G. & Paulsson, J. (2010). Fundamental limits on the suppression of molecular fluctuations. *Nature* **467**, 174–178.

Lotka, A. J. (1925). *Elements of Physical Biology*. Williams and Wilkins Company, Baltimore.

Luttrell, L. M. & Gesty-Palmer, D. (2010). Beyond desensitization: Physiological relevance of arrestin-dependent signaling. *Pharmacological Reviews* **62**, 305–330.

Mitchell, M. (2009). *Complexity: A Guided Tour*. Oxford University Press, New York.

Mulder, E. J., Anderson, G. M., Kema, I. P., de Bildt, A., van Lang, N. D. J., den Boer, J. & Minderaa, R. B. (2004). Platelet serotonin levels in pervasive developmental disorders and mental retardation: Diagnostic group differences, within-group distribution, and behavioral correlates. *Journal of the American Academy of Child & Adolescent Psychiatry* **43**, 4.

Nakagawa, S. & Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews* **82**, 591–605.

Nakagawa, S. & Hauber, M. E. (2011). Great challenges with few subjects: Statistical strategies for neuroscientists. *Neuroscience & Biobehavioral Reviews* **35**, 462–473.

Nicolis, G. & Prigogine, I. (1989). *Exploring Complexity*. W.H. Freeman and Company, New York.

Pedraza, J. M. & Paulsson, J. (2008). Effects of molecular memory and bursting on fluctuations in gene expression. *Science* **319**, 339–343.

Poincaré, H. (2001). *The Value of Science: Essential Writings of Henri Poincaré*. The Modern Library, New York.

Prigogine, I. (1997). *The End of Certainty: Time, Chaos, and the New Laws of Nature*. The Free Press, New York.

Raichle, M. E. (2010*a*). The brain's dark energy. *Scientific American* **302**, 44–49.

Raichle, M. E. (2010*b*). Two views of brain function. *Trends in Cognitive Sciences* **14**, 180–190.

Segu, L., Lecomte, M.-J., Wolff, M., Santamaria, J., Hen, R., Dumuis, A., Berrard, S., Bockaert, J., Buhot, M.-C. & Compan, V. (2010). Hyperfunction of muscarinic receptor maintains long-term memory in 5-HT$_4$ receptor knock-out mice. *PLoS ONE* **5**, e9529.

Shiryaev, A. N. (1996). *Probability* (2nd ed.). Springer, New York.

Sigmund, K. (1993). *Games of Life: Explorations in Ecology, Evolution and Behaviour*. Penguin Books, London.
Strogatz, S. H. (2001). *Nonlinear Dynamics and Chaos: With Applications to Physics, Biology, Chemistry, and Engineering*. Westview Press, New York.
Thébault, E. & Fontaine, C. (2010). Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science* **329**, 853–856.
Tholanikunnel, B. G., Joseph, K., Kandasamy, K., Baldys, A., Raymond, J. R., Luttrell, L. M., McDermott, P. J. & Fernandez, D. J. (2010). Novel mechanisms in the regulation of G protein-coupled receptor trafficking to the plasma membrane. *The Journal of Biological Chemistry* **285**, 33816–33825.
Vayttaden, S. J., Friedman, J., Tran, T. M., Rich, T. C., Dessauer, C. W. & Clark, R. B. (2010). Quantitative modeling of GRK-mediated β_2AR regulation. *PLoS Computational Biology* **6**, e1000647.
Vilardaga, J.-P., Agnati, L. F., Fuxe, K. & Ciruela, F. (2010). *Journal of Cell Science* **123** (Pt. 24), 4215–4220.
Warren, J., Topping, C. J. & James, P. (2009). A unifying evolutionary theory for the biomass-diversity-fertility relationship. *Theoretical Ecology* **2**, 119–126.
Whitacre, J. M. (2010). Degeneracy: a link between evolvability, robustness and complexity in biological systems. *Theoretical Biology & Medical Modelling* **7**, 6.
Whitaker-Azmitia, P. M. (2001). Serotonin and brain development: Role in human developmental diseases. *Brain Research Bulletin* **56**, 479–485.
Wolfram, S. (2002). *A New Kind of Science*. Wolfram Media, Champaign, IL.
Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. & Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York.

(*Received 28 March 2011; revised 2 July 2011; accepted 8 July 2011; published online 31 July 2011*)