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The Concept of "Buffering" in Systems and Control Theory: From Metaphor to Math

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The paradigm of "buffering" is used increasingly for the description of diverse "systemic" phenomena encountered in evolutionary genetics, ecology, integrative physiology, and other areas. However, in this new context, the paradigm has not yet matured into a truly quantitative concept inasmuch as it lacks a corresponding quantitative measure of "systems-level buffering strength". Here, I develop such measures on the basis of a formal and general approach to the quantitation of buffering action. "Systems-level buffering" is shown to be synonymous with "disturbance rejection" in feedback-control systems, and can be quantitated by means of dimensionless proportions between partial flows in two-partitioned systems. The units allow either the time-independent, "static" buffering properties or the time-dependent, "dynamic" ones to be measured. Analogous to this "resistance to change", one can define and measure the "conductance to change"; this quantity corresponds to "set-point tracking" in feedback-control systems. Together, these units provide a systematic framework for the quantitation of buffering action in systems biology, and reveal the common principle behind systems-level buffering, classical acid–base buffering, and multiple other manifestations of buffering.

1. Introduction

The paradigm of "buffering" emerged roughly one hundred years ago as biochemists and physiologists were working out the fundamentals of acid–base chemistry. They had observed that certain solutions responded to the addition of acid or base by much smaller changes of acidity than did pure water or saline solution.[1] Moreover, different solutions exhibited this "resistance to change" to different extents. To express that quantitative aspect of buffering action numerically, various "buffering strength units" were proposed, for example, by Henderson, Koppel and Spiro, Van Slyke, and Michaelis.[1–5]

Over the 20th century, the paradigm of buffering gained popularity in many further disciplines, well beyond its original domain of acid–base chemistry. A literature search with "buffering" as the search term will strikingly illustrate that point. It will return numerous relevant articles, including many recent ones, that are related, for instance, to the buffering of electrolytes other than the H^+ ion (e.g. "magnesium buffering", "calcium buffering"[6–17]), of nonelectrolytes (e.g. "oxygen buffering" by hemoglobin and myoglobin[18,19]), or of thermodynamic quantities (e.g. "redox buffering", "thermodynamic buffer enzymes", or "metabolic capacitance"[20–23]).

Importantly, the buffering paradigm is also invoked—with increasing frequency—by "systems biologists". For instance, buffering terminology is applied to classical physiological feedback-control mechanisms. "Blood-pressure buffering" and "autoregulation" stabilize pressure and organ perfusion against disturbances such as fluctuating cardiac output and peripheral resistance.[24–30] Another example is the recent concept of "phenotypic" or "genetic buffering", introduced by evolutionary biologists. Mechanisms such as negative feedback or redundancy are said to minimize or abrogate the effects of genetic mutations on the phenotype, thus decreasing the impact of individual genes on fitness and selection.[31–33] Moreover, ecologists study the "buffering" of animal populations in predator–prey systems, and systemically inclined sociologists and psychologists employ concepts such as "stress buffering", "social buffering", or "cognitive buffering".[34–37]

In addition, the buffering paradigm seems to be in place in the context of further regulatory processes in which it has not been invoked explicitly. For instance, signal transduction at synapses is shaped largely by the mechanisms that decrease the concentration of free transmitters following their triggered exocytosis. Herein, binding to neurotransmitter transporters occurs with much higher speed and efficiency than actual reuptake. The proteins involved in transmitter binding have been fittingly termed "decoy receptors", but their action is still awaiting a quantitative description. Analogously, hormone binding to specific binding proteins represents an important, actively regulated aspect of endocrine signaling through lipophilic hormones. Similarly, intracellular signaling through second messengers such as inositol phosphates, a focus of current research in systems biology, is modulated by binding of these messengers to cytoplasmic factors. Another example is the regulation of oxygen levels in tissues or organisms; herein, oxygen binding to proteins such as hemoglobin or myoglobin contributes to stabilizing free oxygen levels during exercise or diving, at times even to a greater extent than does vasomotion. In all these cases, no suitable framework exists to capture the "buffering effect" of these binding processes.

Apparently, "buffering" is, actually or potentially, an intuitive and useful concept in systems biology, as well as in many other disciplines. However, the increasing popularity of the buffering paradigm also confronts us again, and more press-

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ingly, with the considerable inherent shortcomings of that concept—most importantly, the lack of a single, universally applicable definition of buffering. Consequently, we also lack a single, rigorous, commonly accepted scientific unit for the quantitation of buffering action. For instance, in the field of acid–base buffering, a majority of scientists have adopted Van Slyke's unit $\beta = d[\text{strong base}]/d\text{pH}$ as a quasi-standard;[3] whereas in the field of Ca^{2+} physiology, buffering is more commonly expressed in terms of the "calcium binding ratio" κ, with $\kappa = d[Ca^{2+}]_{bound}/d[Ca^{2+}]_{free}$.[13] For both types of buffering, several further "buffering strength units" exist. The result is a parallel use of multiple units; this is problematic inasmuch as the different units produce qualitatively and quantitatively different conclusions. Another problem causes trouble in different areas of research, namely the use of a buffering paradigm without having any buffering strength unit (e.g. in the field of blood-pressure buffering). The lack of quantitative measures of buffering leads to vague, essentially metaphorical semantics of the term "buffering". Second to none in this respect, contemporary systems biology applies the buffering paradigm lightheartedly although it is equipped neither with a clear concept of "systems-level buffering" nor with an explicitly defined buffering strength unit. It shares this deficit with the more general, mathematical, or engineering varieties of systems and control theory.

In order to formulate, test, and interpret quantitative propositions, however, it is clearly vital to have a coherent, unambiguous system of basic and derived scientific units. The epitome of such a system is the "Système International d'Unités", which is at the core of the most mature natural sciences. Lord Kelvin noted: "When you can measure what you are talking about and express it in numbers, you know something about it." Conversely, without a quantitative concept of "systems level buffering", the buffering paradigm in systems biology must remain flimsy in theory and unproductive in practice.

I have presented elsewhere a formal and general concept of "buffering".[38–39] This concept comes with a dimensionless unit of "buffering strength". The first of these articles[38] presents a detailed discussion of the problems that arise from the use of multiple incommensurate measures of buffering action, whereas the second article[39] provides analyses of several classical buffering phenomena and demonstrates the advantage of the unified concept as compared to other approaches.

The formal and general concept is universally applicable and also offers, in principle, a way to get a numerical grasp of systems-level buffering. The present article fleshes out this specific aspect of the general concept, and presents explicitly customized terms and definitions that can turn the general concept into a handy mathematical tool for systems biologists. For the systematic theoretical foundation of the general concept, and for exemplary treatments of various other types of buffering that are of interest to chemists (e.g. H^+ buffering by weak acids or bases, H^+ buffering in pure water, or redox buffering), the reader is referred to other articles.[38–39]

2. Buffering Can Be Quantitated in Terms of Proportions between Partial Flows in a Two-Partitioned System

The key to the formal and general concept of buffering is to view the underlying phenomenon as a partitioning process (specifically, of one given quantity into two complementary compartments), and then describe it in terms of the proportions between the flows into the individual compartments. This approach is illustrated in Figure 1.

For instance, in the case of classic H^+ buffering, the quantity in question is the total concentration of H^+ ions in a solution, and the complementary compartments correspond to the individual concentrations of "free" and "bound" (that is, "buffered") H^+ ions. Let us take total H^+ ion concentration as independent variable x, and the corresponding equilibrium concentrations of free and bound H^+ ion as dependent variables y and z. Transitions between various equilibrium states of such a two-partitioned system involve changes of the independent variable (Δx), and well-defined associated changes of the dependent variables (Δy, Δz). We designate by the term "transfer function" the function $x \rightarrow y(x)$, and by "buffering function" the function $x \rightarrow z(x)$. Accordingly, at a given value of x, the coupling between independent and dependent variables is characterized by two differentials, namely a "transfer coefficient" t given as

$$t = \frac{dy}{dx} = \frac{d(\text{free})}{d(\text{total})}$$

and by a "buffering coefficient" b given as

$$b = \frac{dz}{dx} = \frac{d(\text{bound})}{d(\text{total})}$$

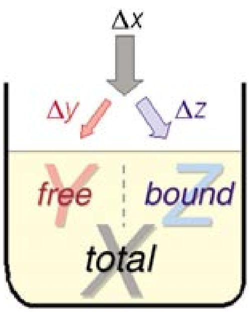

Figure 1. Buffering can be viewed and formalized as a partitioning process. For instance, H^+ ions in an aqueous solution (x) can exist either in a free (y) or bound (z) form. A change of total H^+ ion concentration (Δx) is translated partially into a change of free H^+ ion concentration (Δy), and partially into a change of bound H^+ ion concentration (Δz). The greater the partial change Δz relative to the total change Δx, or relative to the complementary change Δy, the greater the buffering of the variable y in this system. Buffering strength can thus be expressed either by the dimensionless differential ("buffering coefficient") on a scale from 0 to 1, or by the dimensionless differential dz/dy = B ("buffering odds"). Buffering odds B yield an absolute ratio scale, that is, a scale with equal intervals and an absolute zero that does not require any arbitrary proportionality factors.

These coefficients can be thought of as the "fractional flows" into (or out of) the compartments of a two-partitioned system that are observed in response to a net flow into (or out of) the system at a given operating point *x*.

The greater the flow into the second "buffering compartment", the greater the "buffering strength" of the system. One way to express the magnitude of this flow (that is, the magnitude of buffering strength) is the proportion between "a part and the whole"; here, the proportion between partial flow into the "buffering compartment" and total flow into the system. This proportion is given by the buffering coefficient *b*; it reflects buffering strength by a dimensionless number between zero (no buffering) and unity (perfect buffering). For instance, intracellular Mg^{2+} ions are buffered with a buffering coefficient of $b \approx 0.6$. This means that ~60% of Mg^{2+} added to the cytoplasm will be bound by buffer molecules. Thus, the buffering of Mg^{2+} is very weak compared to the cytoplasmic buffering of H^+ ions. Doubling the concentration of Mg^{2+} buffers will increase the buffering coefficient from 0.6 to 0.75.

Alternatively, the magnitude of this flow can be expressed in terms of the proportion between the two individual parts of a whole; here, the proportion between the partial flows into the "buffering compartment" and the "transfer compartment". This proportion, here referred to as "buffering odds" *B*, is given as a ratio $B = b/t$ or, equivalently, as a derivative $B = dz/dy$. In contrast to the buffering coefficient *b*, the buffering odds *B* reflect buffering by a dimensionless number between zero (no buffering) and positive infinity (perfect buffering). For instance, intracellular Ca^{2+} is buffered with buffering odds of $B = 75$. This means that 75 times more added Ca^{2+} ions will be bound than will remain free, or the proportion bound:free among the added H^+ ions equals 75:1. In other words, it takes 76 additional free Ca^{2+} ions to retain 1 additional free Ca^{2+} ion after reaching the new equilibrium. Doubling the concentration of Ca^{2+} buffers will simply double the buffering odds.

Buffering coefficient and buffering odds carry exactly the same information, but have different mathematical properties. Buffering coefficients behave exactly as the familiar "probabilities" or "relative frequencies". In contrast, buffering odds behave like the SI units (e.g. for length, mass, or time), which all yield ratio scales, the highest possible type of a scientific scale. As additional advantages, the buffering odds are dimensionless and absolute (i.e., the numerical value can be interpreted unambiguously, because it involves no choice between different units such as meters or yards or inches). The meaning of buffering coefficients and odds can be understood in a very straightforward and intuitive way (in terms of fraction or percentage that is buffered, or in terms of the number of additional buffered elements for every additional unbuffered element), without loss of formal correctness. In contrast, a H^+ buffering strength of $\beta = 24$ mM/pH according to Van Slyke's unit relinquishes the more intuitive numbers (such as the number of H^+ ions one needs to add to get one more permanently free H^+ ion) only after spiny mental arithmetic, and the popular visualization stating that 24 mmoles of strong acid will shift the pH of this solution by one unit is patently incorrect.

With these measures of buffering, we are already in a position to describe all classical buffering phenomena, such as the "self-buffering" of H^+ by pure water, and H^+ buffering by a mixture of a weak acid and one of its salts. Example analyses (with some surprising conclusions) have been presented elsewhere in detail,[39] together with further examples showing that these measures allow the buffering paradigm to be applied directly to phenomena that involve quantities other than ion concentrations (e.g., heat energy or redox equivalents). In exactly the same way, one can treat all other nonclassical buffering phenomena that involve the binding of a conserved molecular species, including the examples mentioned above (e.g., the buffering of hormones, transmitters, or oxygen).

3. Buffering in Nonconservative Systems

Importantly, the phenomena considered so far all obeyed a conservation law: the sum of the partial flows into the individual compartments equaled the total flow into the system. Conservation resulted from physical or chemical constraints. From a mathematical point of view, we may drop this constraint with impunity. One the one hand, this generalization allows us to describe systems that are conservative by nature in alternative "parametric" form. For instance, we could express bound and free H^+ ion concentration as a function of "grams" or "milliliters" of a strong acid, instead of "moles" of strong acid. On the other hand, and more importantly, it allows us to deal with functional relationships between completely heterogeneous physical quantities, and to apply the buffering concept to this class of phenomena. This approach is illustrated in Figure 2.

For example, the volume flow $\varphi°$ in a rigid tube is a linear function of the pressure difference ΔP across it; here flow and pressure have different physical dimensions of $length^3 \times time^{-1}$ versus $mass \times length^{-1} \times time^{-2}$, respectively. The response of the system to pressure changes is given by the differential $d\varphi°/d(\Delta P)$and is equal to the hydraulic conductance $L°$ of the tube. If we add another such tube in series, it will draw off half of the available pressure difference, and the response of

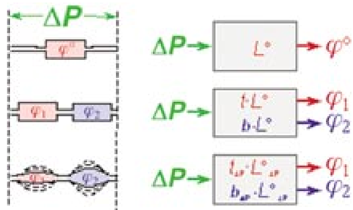

Figure 2. Partitioning in nonconservative two-partitioned systems. Buffering of volume flow (φ) against changes of perfusion pressure (Δp). Upper panel: zero buffering: changes of pressure difference are completely translated into changes of volume flow, with the hydraulic conductivity L° as proportionality factor. Middle panel: a second vessel diminishes the effect of pressure changes on volume flow in the red tube, the latter quantity is now "buffered". The extent of buffering can be expressed again by dimensionless buffering coefficients or buffering odds (see Figure 1 and text). Bottom panel: similarly rigorous quantitation of buffering is possible if hydraulic conductance does not have a constant value L, but a variable value L_P that depends on pressure P. Then, similarly, the buffering coefficient is not constant, but a variable b_p of pressure.

volume flow φ to pressure changes will be smaller than $L°$, namely:

$$L = \frac{d\varphi}{d(\Delta P)} = \frac{L^o}{2}$$

We can say that the hydraulic series resistance contributed by the second tube “buffers” the effect of a given pressure change on volume flow in the first vessel. Our question here is, how can we derive a quantitative measure of buffering in such a context from this formal description? Incidentally, this is already a systems biology question. Organisms with a blood circulation employ this basic strategy to stabilize organ perfusion in the face of fluctuating perfusion pressure. So far, no satisfying unit has been presented for the quantitation of such “blood-pressure buffering”.

For conserved quantities, “total change” was equal to the sum of the two “partial changes”. Here, however, we find that dependent versus independent variables are of different physical dimensions; we therefore speak of a “nonconservative” partitioned system. To extend our definition of buffering strength to nonconservative systems, we first introduce the notion of a “sigma function”. The sigma function of a two-partitioned system is the function whose value equals the aggregate value of the two partial functions, or $\sigma(x) = y(x) + z(x)$. Conservative systems are characterized by the equality $x = y(x) + z(x)$, which simplifies the sigma function to $\sigma(x) = x$. In the special case of $z(x) = 0$, equivalent to the complete absence of buffering, the sigma function becomes equal to $y(x)$. Thus, the sigma function tells us how the system would respond if it were not buffered. We can normalize the partial flows with respect to this unbuffered system response, and thus obtain general definitions of t and b that make sure that our measures of buffering are always dimensionless. To this end, we denote the derivatives of the functions with respect to the independent variable x by y', z', and σ', and rewrite the relationship given above in more general form as:

$$t = \frac{y'}{\sigma'}$$

$$b = \frac{z'}{\sigma'}$$

therefore

$$t + b = \frac{y' + z'}{\sigma'} = 1$$

In our hydraulic example, we regarded the situation involving only a single tube as an “unbuffered system”. The corresponding sigma function is:

$$\sigma(x) \leftrightarrow \varphi^o(\Delta P) = L^o \cdot \Delta P$$

The derivative σ' then assumes the specific physical meaning of a hydraulic conductance $L° = d\varphi°/d(\Delta P)$. Nonzero buffering manifests itself as an observed hydraulic conductance L_{obs} that is smaller than $L°$. The sigma function $\sigma{:}\Delta P \rightarrow L°{\cdot}\Delta P$ allows the transfer coefficient to be computed as:

$$t = \frac{y'}{\sigma'} = \frac{L_{obs}}{L^o}$$

This transfer coefficient, in turn, unambiguously determines the other buffering parameters: $b = 1 - t$ and $B = b/t$.

With these buffering parameters at hand, we can now talk meaningfully and unambiguously of “buffering” in the context of pressure-dependent volume flows, and we can rigorously express the appropriate “buffering strengths”, either as buffering coefficient b or buffering odds B. One application of these units in systems biology is the quantification of “blood-pressure buffering” or “autoregulation” of blood flow in the face of variable arterial pressure.

More generally, these parameters express numerically how much an observed change deviates from the “reference” effect seen under conditions of zero buffering. In other words, b and B are the wanted measures of “resistance to change” in an arbitrary two-partitioned system (i.e., of one transfer function and one buffering function, representing the dependence of one state variable, each, in transfer and buffering compartment on a single independent variable of potentially different physical dimension).

By the same token, one can talk meaningfully of “conductance to change” in such a system. Conductance to change, in turn, can be quantitated with similar rigor, either by the transfer coefficient t and by so-called “transfer odds” T, with $T = t/b$.

4. Buffering, Resistance to Change, and Disturbance Rejection in Control Systems

Next, we need to apply our measures of “resistance to change” and “conductance to change” to control systems. This is illustrated by using a simple control system with proportional feedback (Figure 3 A). The system is characterized by a set-point input S, a disturbance input D, and an output Y; a conversion factor K accounts for different physical dimensions or scales of inputs versus outputs. However, the measures of “resistance to change” or “conductance to change” do not depend on the particular system design (proportional–integral–differential feedback, digital vs. analogue, etc.) and are generally applicable to control systems with set-point and disturbance inputs, and one output.

All control systems should satisfy two fundamental requirements. Firstly, they should follow faithfully any changes of the desired set-point; this feature is called “set-point tracking”. Secondly, control system should respond as little as possible to any other parameters, perceived as “disturbances”; this feature is therefore called “disturbance rejection”. Set-point tracking as well as disturbance rejection may be present to a greater or lesser degree, ranging from “perfect” to “completely absent”. To emphasize the quantitative nature of these two fundamental properties of control systems, we here call them “set-point tracking power” and “disturbance rejection power”, by analogy to the familiar concept of “buffering power”.

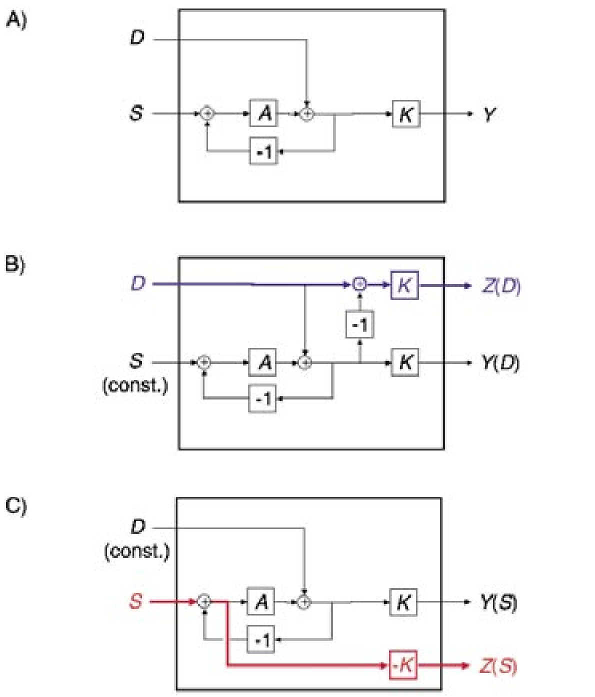

Figure 3. Static disturbance rejection and set-point tracking in feedback-control systems. A) Generic negative-feedback-control system. Dual input–single output system (D, disturbance input; S, set-point input; Y, output) with proportional gain (A, real-valued gain factor). ⊕ symbolizes an element whose output is the sum of its inputs. Square boxes symbolize elements whose output is the product of their single input and the figure shown inside the box. A conversion factor (K, product of a real number and a scientific unit) accounts for scaling and potentially different physical dimensions of inputs versus output. B) Turning a feedback-control system into a two-partitioned system that is suited to measure "disturbance rejection power". C) Turning a feedback-control system into a two-partitioned system suited to measure "set-point tracking power".

Control can be poor or very efficient. However, "control quality" has multiple aspects, and cannot be characterized comprehensively by a single figure. Nonetheless, set-point tracking and disturbance rejection reflect the most important aspects of control quality. In a first approach to the quantitation of control quality, we focus on the time-independent steady states, ignoring for now the different speed with which systems can respond to changes of set-point or disturbance inputs. To characterize these "static" control properties, we thus need measures of "static set-point tracking power" and of "static disturbance rejection power".

To apply our general definitions of the buffering coefficient *b* and the buffering odds *B* to the analysis of feedback-control systems and obtain a measure of "static disturbance rejection power" in this context, one starts ideally from the explicit mathematical description of the system. For this simple system (Figure 3 A), an analytical solution is available for the complete state space; namely, the steady-state output *Y* is determined by both inputs according to:

$$Y(S,D) = K\left[S\,\frac{A}{A+1} + D\,\frac{1}{A+1}\right]$$

From this equation, the sensitivity $Y_D' = [\partial Y(S,D)]/\partial D$ of the output *Y* to a change of the disturbance input *D* can be calculated as:

$$Y_D' = K\,\frac{1}{(A+1)}$$

Thus, with small feedback gain *A*, a change of the disturbance input *D* will translate almost completely into an change of the output *Y*. In the limiting case of $A=0$, the sensitivity of the system to disturbances assumes a maximum value of $Y_D' = K$. The same sensitivity is obtained by cutting open the feedback loop. In contrast, when feedback gain *A* is high, the output *Y* responds to a changed disturbance input *D* with only a fraction of its maximum sensitivity. In the limiting case of $A \to \infty$, the output *Y* becomes completely insensitive to disturbances.

Thus, the negative-feedback mechanism conveys to the system a "resistance to change" in the face of an external disturbance *D*. Synonymously, control theory talks of "disturbance rejection", in this case, of "static disturbance rejection". Equivalently, we may say that the negative feedback "buffers" the effect of the disturbance input *D* on the output *Y*.

Knowing the state space of a system and the sensitivity Y_D' of the output to a disturbance input is a necessary step toward a quantitative expression of "disturbance rejection". However, sensitivity Y_D' does not constitute of itself a direct measure of disturbance rejection. Firstly, its value varies *inversely* with disturbance rejection. Secondly, its scaling and physical dimensions are contingent on the particular control system (given by the conversion factor *K*). To obtain a better measure of this quantity (namely, a direct, general, and dimensionless one), we need to derive a "buffered system" from the above mathematical representation. In other words, we need to define a transfer function, a buffering function, and a sigma function in this control system.

We say the system has "zero *disturbance* buffering" when a disturbance *D* is not rejected at all, but impacts fully on the output *Y*; in the system shown in Figure 3, this was the case if $A=0$ or with the feedback loop cut open, and was associated with an open-loop sensitivity $\mathring{Y}_D' = K$. This unbuffered response is now described, as in the examples above, by the *sigma function*, and written in general form as $\sigma_D:(S,D) \to Y^{*D}(S,D)$. Here, we obtain $\sigma_D(S,D) = K \cdot D$.

The *transfer function* is then the function that describes the relation between inputs *S*,*D* and output *Y* for any actual value of $A \in \mathbb{R}$, written as $\tau_D:(S,D) \to Y(S,D)$. Here, we found:

$$\tau_D(S,D) = K\left[S\,\frac{A}{A+1} + D\,\frac{1}{A+1}\right]$$

and

$$\tau_D' = Y_D' = K\,\frac{A}{A+1}$$

What is missing is the *buffering function*. Its value cannot be read out anywhere from the system, but we can compute its value from the equation $\sigma(x) = y(x) + z(x)$, which characterizes all two-partitioned systems. Thus, we obtain:

$$\beta_D(S,D) = \sigma_D(S,D) - \tau_D(S,D) = K\left[-S\frac{A}{A+1} + D\frac{A}{A+1}\right]$$

and

$$\beta'_D = Z'_D = K\frac{A}{A+1}$$

Here, Z_D is a "virtual" second output that can be read out from the system as shown in Figure 3B, and Z_D' is the sensitivity of that output to a disturbance D.

With τ_D, β_D, and σ_D, we are in a position to compute the buffering parameters in the usual way as:

$$b_D = \frac{\beta'_D}{\sigma'_D} = \frac{Z'_D}{Y'_D + Z'_D}$$

and

$$B_D = \frac{\beta'_D}{\tau'_D} = \frac{Z'_D}{Y'_D}$$

If one takes a black-box approach to the system, limiting oneself to the externally accessible parameters (inputs D and S, and output Y) and assuming knowledge of the conversion factor K, but not of the "virtual" output Z, one can express the two buffering parameters alternatively as:

$$b_D = \frac{K - Y'_D}{K}$$

and

$$B_D = \frac{K - Y'_D}{Y'_D}$$

In the context of control systems, buffering coefficient and odds can be called more intuitively the "static disturbance rejection coefficient" and "static disturbance rejection odds". These two measures are denoted b_D and B_D in order to be unambiguous about the independent variable. The static disturbance rejection coefficient b_D represents a normalized sensitivity and thus described by a dimensionless number between 0 and 1 the *fraction* of an imposed change that was diverted or rejected from a specific compartment. In this particular feedback-control system, b_D has the specific value of $b_D = A/(A+1)$. Analogously, the disturbance rejection odds reflect a dimensionless number, the proportion between "transmitted change" and "rejected change", and yield an absolute ratio scale with $B_D \in \mathbb{R}^+$ for $A > 0$. In this particular system, the static disturbance rejection odds are $B_D = A$.

Either one of these two parameters provides a rigorous quantitative measure of "static disturbance rejection". The point is that static disturbance rejection represents a specific and meaningful interpretation of the term "systems-level buffering", and is applicable to any system in which a disturbance is related to an output in a defined way. For instance, the mammalian genome contains multiple genes that encode various membrane transporters for organic cations. Spontaneous or experimental knock-out of one such transporter produces only a partial reduction of the specific transport capacities and a "mild" phenotypic effect. The redundancies in the system provide the organism with "buffering" against such mutations, or, synonymously, "disturbance rejection" (taking the mutations as "disturbance"). For other proteins (e.g. the enzymes biotinidase or 11-β-hydroxylase), no such redundancies exist, and single mutations can result in a complete loss of the associated biological function and severe genetic disease. Provided that the respective phenotype can be quantitated (e.g. by a specific biological function such as transport or enzyme activity), one may use our measures of "static disturbance rejection" to express numerically the extent of such "genetic" or "phenotypic buffering".

5. Buffering, "Conductance to Change", and Static Set-Point Tracking Power in Control Systems

Next, we develop a measure of "static set-point tracking power" in this feedback-control system (Figure 3C). To compare systems with respect to this ability, we look at how the system responds to changes in the set-point input S. This response is again given by a sensitivity of the output $Y(S,D)$, but this time as the partial derivative with respect to the variable S, that is, as:

$$Y'_S = \frac{\partial Y(S,D)}{\partial S}$$

In this particular control system, this sensitivity has the value $K[A/(A+1)]$. Thus, with a small value of the proportional feedback gain A, the set-point S has little or no effect on the output Y. For large values of A, the impact of the set-point S on the output Y will be greater and approach a maximum value of K for $A \rightarrow \infty$.

For the analysis of set-point tracking, we took as sigma function σ_S the function that describes the relation between inputs S and D expected in the absence of *set-point* buffering, that is, the condition under which the set-point S translates perfectly into corresponding changes of output Y, and where the sensitivity $Y_S' = K$. In this particular system, this is the case when feedback gain $A \rightarrow \infty$. In other systems, the "ideal" response to a set-point change may obey different rules. In any case, it is crucial to have an explicit expression for that response (which may in principle be a nonlinear function of S and D). Importantly, if one does not know that expected unbuffered response, it is not possible to quantify buffering action. In general, for the analysis of set-point tracking, we write the sigma function as $\sigma_S:(S,D) \rightarrow Y^{*S}(S,D)$. Here, we find that $\sigma_S(S,D) = K \cdot S$.

The *transfer function* τ_S is the function that describes the relation between inputs S and D and output Y for any given level of set-point buffering and for any $A \in \mathbb{R}$. Here, we find that:

$$\tau_S = K\left[S\frac{A}{A+1} + D\frac{1}{A+1}\right]$$

the sensitivity Y_S' of the output Y to set-point changes follows as:

$$Y_S' = K\frac{A}{A+1}$$

The *buffering function* β_S follows again from the equation $\sigma(x) = y(x) + z(x)$ as:

$$\beta_S = K\frac{S}{A+1}$$

In this particular control system, a read-out of its value can be constructed as shown in Figure 3C. The sensitivity of this output to set-point changes is given by:

$$Z_S' = K\frac{1}{A+1}$$

With τ_S and β_S and σ_S, it is straightforward to derive the parameters t and T as:

$$t_S = \frac{\tau_S'}{\sigma_S'} = \frac{Y_S'}{Y_S' + Z_S'}$$

and

$$T_S = \frac{\tau_S'}{\sigma_S'} = \frac{Y_S'}{Z_S'}$$

In a black-box approach, one may again determine these parameters alternatively as $t_S = Y_S'/K$ and $T_S = Y_S'/(K - Y_S')$. The parameters t_S and T_S represent the wanted measures of "static set-point tracking power", or, synonymously, of "conductance to change" on the systems level. In the control system shown in Figure 3, we find that $t_S = A/(A+1)$ and $T_S = A$.

This analysis leads to an interesting nontrivial statement about control systems that employ proportional feedback. In such a system, the two aspects of control quality, namely static set-point tracking and static disturbance rejection, are always of identical magnitude and depend in exactly the same way on the feedback gain A. In contrast, when the feedback loop is cut open, the resulting open-loop control is characterized by zero disturbance rejection ($\partial Y(S,D)/\partial D = K$) and "amplifying" set-point tracking ($\partial Y(S,D)/\partial S = A \cdot K$).

A biologically relevant control task that can be analyzed in these terms is the adaptive increase of muscle blood flow in response to exercise. Here, we may take as the ideal response that blood flow that exactly matches oxygen supply to oxygen consumption. In this sense, the actual response may be indistinguishable from the ideal one up to relatively high levels of oxygen consumption, but will inevitably deviate from it as the ability to increase muscle perfusion saturates. Our measures of "set-point tracking" allow us to express numerically how well the organism can track the shifting set-point (i.e., muscle perfusion). Such numbers may serve to compare control quality at various operating points (i.e., exercise levels), between trained and untrained individuals, etc.

6. Time-Dependent Buffering: "Dynamic Disturbance Rejection" and "Dynamic Set-Point Tracking"

Quite often, "good" control requires not only accuracy, but speed as well. For instance, a voltage-clamp device must respond quickly to a voltage step command in order to resolve the fast currents produced by voltage-gated ion channels. In animals, conditions such as acidosis, elevated blood pressure, or hypoglycemia need to be counteracted within short time in order to avoid seizures, organ damage, and—ultimately—death. Speedy control is similarly important in social systems plagued by high numbers of unemployed citizens or of criminals on the loose. Speed pertains to both aspects of control: tracking a shifting set-point (e.g., a change of command potential by a voltage-clamp device) and rejection of fluctuating disturbances (e.g., an acid load, a sugar deficit, excess criminality, etc.). The respective aspects of control quality are termed here "dynamic set-point tracking power" and "dynamic disturbance rejection power". Again what we need are ways to rigorously express the quantitative aspect of these features.

First, we derive a measure of "dynamic disturbance rejection power". Reconsider the negative-feedback control system with a fixed set-point S (Figure 3B). We impose a step change ΔD of a disturbance input D, and observe the time course of the output Y (shown schematically in Figure 4). We now ignore the absolute magnitude of Y, and rather consider the deviation $e(t)$ of Y from its value $Y_{baseline}$ before the step change, given as

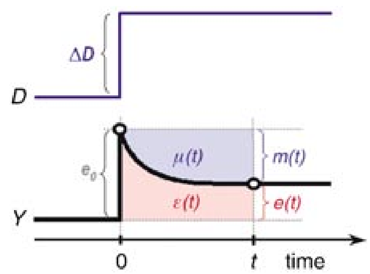

Figure 4. *Dynamic disturbance rejection in feedback-control systems. Upper panel: With the set-point* S *fixed, the disturbance input of a system similar to the one shown in Figure 3B is instantaneously stepped to a new constant value. Lower panel: The step change* ΔD *shifts the output* Y *instantaneously by a specific amount* e_0*, then negative feedback kicks in and pushes* Y *back towards the set-point* S*. The duration and extent of the error* e(t) *are measured by the integral* ε(t)*, whereas duration and extent of the "error reduction"* m(t) *are measured by the integral* μ(t)*. "Dynamic disturbance rejection power" can be expressed by a time-dependent buffering coefficient* $b_D = \mu(t)/[\varepsilon(t) + \mu(t)]$ *or buffering odds* $B_D = \mu(t)/\varepsilon(t)$*. Analogously, one can find measures that express the system's ability to follow shifts of the set-point* S*.*

$e(t) = Y(t) - Y_{baseline}$. The step change ΔD produces an initial error e_0, given as $e_0 = \Delta D \cdot K$. Without control, this error will persist forever: $e(t) = \text{const.} = e_0$. In contrast, negative-feedback control will tend to reduce the error $e(t)$ over time. Note that in the control system shown in Figure 3, the analytical solution would yield instantaneous control; in real systems, however, control requires finite time because of finite time constants, time lags, and less-than-infinite feedback gain. The corresponding analytical solutions are complicated and vary greatly between systems; the following statements are therefore made in a qualitative, generally valid way.

The faster and the more complete the error $e(t)$ decreases, the better the control quality. A good aggregate measure of error magnitude and error duration is the integral $\varepsilon(e_0,t) = \int_0^t e(t)\mathrm{d}t$, a function of initial error and time.

There is another, equivalent way to look at the same process. Rather than in terms of the "error" $e(t)$, we can describe the control process by following the "error reduction" $m(t)$. Herein, we define error reduction as $m(t) = e_0 - e(t)$, that is, the deviation of $Y(t)$ from its value Y_0 immediately *after* the step change ΔD, before any compensation kicks in. An aggregate measure of both magnitude and duration of this error reduction is the integral $\mu(e_0,t) = \int_0^t m(t)\mathrm{d}t$, again a function of initial error and time.

For a given initial error e_0 and a specified time t, overall "disturbance rejection" correlates with the integral $\mu(e_0,t)$. Note that this integral has particular physical dimensions, contingent on the physical dimension of the output Y. However, it does not make sense to quantitate "disturbance rejection" by means of a unit that has the dimension of "charge" in one case, of "volume" in another, etc. Rather, we want a single universal, dimensionless measure. Such a measure can be obtained by normalization. Here, we normalize the integrated actually achieved error reduction $\mu(t)$ with respect to the theoretically achievable maximum error reduction; this maximum is equivalent to the error $\int_0^t e_0(t)\mathrm{d}t = e_0 \cdot t$ that would have been observed in the absence of any control. The resulting "proportion between a part and the whole" is given as:

$$b_D(t) = \frac{\mu(e_0,t)}{(e_0 \cdot t)}$$

This measure $b_D(t)$ represents the fraction of the disturbance that was, on average during the chosen time window, "rejected" or "buffered"; it is termed here the "dynamic disturbance rejection coefficient" or "dynamic disturbance buffering coefficient" $b_D(e_0,t)$.

An alternative, dimensionless measure of dynamic disturbance rejection is the proportion between error reduction integral $\mu(e_0,t)$ and error integral $\varepsilon(e_0,t)$; this corresponds to the proportion between the two parts of a whole. This proportion is termed "dynamic disturbance rejection odds" or "dynamic disturbance buffering odds" $B_D(t)$, and is given as:

$$B_D(t) = \frac{\mu(e_0,t)}{\varepsilon(e_0,t)}$$

The dynamic disturbance rejection odds reflect dynamic disturbance rejection by a dimensionless number on an absolute ratio scale.

The dynamic variety of *set-point tracking* can be assessed and compared if the disturbance input D is fixed at a constant value, and the set-point input S is varied systematically, in a similar manner to the arrangement shown in Figure 3C. A step change ΔS causes the output Y to travel over time to a new value. Without any disturbance input ($D = 0$), the deviation $e(t)$ of Y from its initial value will approach a characteristic value $e_0 = S \cdot K$. This "error" is now a desired property of the control system. In the presence of a fixed, non-zero disturbance D, the error $e(t)$ takes a different course, usually approaching e_0 more slowly and less completely. As compared to a given unbuffered response e_0 and at a given time t, the system response ideally translates into an integral $\varepsilon(e_0,t) = \int_0^t e(t)\mathrm{d}t = e_0 \cdot t$; in other words, set-point tracking is perfect. Less than perfect set-point tracking is reflected by an integral $\varepsilon(e_0,t)$ that is smaller than the product $e_0 \cdot t$. Then, we also find that the time integral $\mu(e_0,t) = \int_0^t m(t)\mathrm{d}t$ of the error reduction $m(t) = e_0 - e(t)$ has a non-zero value. The greater the error integral $\varepsilon(e_0,t)$, the greater is the "dynamic set-point tracking power" of the system. We can express this quantity either as a proportion between one part and the whole, namely in terms of the "dynamic set-point tracking coefficient"

$$t_S(t) = \frac{\varepsilon(e_0,t)}{(e_0 \cdot t)}$$

or as a proportion between the two parts of a whole, namely in terms of the "dynamic set-point tracking odds":

$$T_S(t) = \frac{\varepsilon(e_0,t)}{\mu(e_0,t)}$$

Interestingly, there is a strong formal and conceptual link between static and dynamic measures of systems-level buffering. The static measures of disturbance rejection or set-point tracking are contained as special cases in the corresponding dynamic quantities. Namely, the dynamic, time-*dependent* measures will become identical to the static, time-*independent* measures when we let integration time approach infinity and, simultaneously, let the size of the initial step change of disturbance or set-point input approach zero.

7. Conclusion

In this article, we have derived a concept of "systems-level buffering", and ways to quantitate its various aspects, starting from a seemingly abstract definition of "buffering". Specifically, buffering understood as "resistance to change" of a system can be expressed quantitatively by means of the static or dynamic "disturbance rejection odds". A corresponding "conductance to change" or compliance of a system can be measured by static or dynamic "set-point tracking odds".

In our examples, buffering was manifested as a system response that was *smaller* than the unbuffered response. Although not worked out here explicitly, these units handle systems with responses that are *greater* than expected equally well. We propose to call any deviation of the actual system response from the unbuffered response "buffering", irrespective of its direction, and specify buffering further as "moderation" (response smaller than expected) or "amplification" (response greater than expected).

Systems biology as a quantitative discipline relies on the formal language of mathematics, and this basis is completely shared with, or borrowed from, general systems and control theory. This means that our formal measures of static or dynamic "disturbance rejection" and "set-point tracking" are not restricted to biological systems, but applicable to any type of control systems. Albeit there is no shortage of measures of control quality, the measures outlined here are unique and superior inasmuch they provide scales of the highest possible type for the measurement of disturbance rejection or set-point tracking. With these dimensionless ratio scales, our concept of buffering provides a single, universal and coherent framework for the treatment of phenomena that were previously considered separate and unrelated. This unified approach to the quantitation of buffering action exposes the common pattern behind the different manifestations of buffering, linking phenomena involving different physical quantities, or moderation versus amplification, or static versus dynamic aspects of control. To say that the paradigms of "homeostasis", "control", and "buffering" are inherently universal is not an airy commonplace. Our universal measures of buffering action substantiate such a claim, and provide a framework that allows different homeostatic mechanisms to be compared directly with respect to their efficiency in the steady-state or during the transient phase.

Keywords: buffering · control · scientific units · systems biology · theoretical chemistry

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