University of Nevada, Reno

Harnessing Biological Noise in the Olfactory System

A thesis submitted in partial fulfillment
of the requirements for the degree of

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by

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Glossary

Biological System: A biological system is a network of biological entities. This system can operate on various levels. Examples of biological systems include cells, organ systems, organisms, and populations.

Olfaction: The sense of smell.

Biological Noise: Random variability arising in biological systems.

Stochastic: Involving a random variable. The word stochastic is usually used to describe a system that involves a random component.

Feedback Loop: A system in which an output or product affects the functioning of a system.

Negative Feedback Loop: A system in which an output or product inhibits the functioning of the system.

Positive Feedback Loop: A system in which an output or product enhances the functioning of the system.

Bistable System: A system that has two stable equilibrium states, operating as an on-off switch.

Nongenetic Phenotypic Heterogeneity: Variation in organisms within a population that is not the result of genetics.

Bet-Hedging: A system used by organisms in variable environments where variation exists within populations in order to assure that some subset of the population will survive an environmental fluctuation.

Stochastic Resonance: A phenomena that occurs when a system increases in sensitivity as noise is added to the system.

Abstract

Biological systems are inherently noisy. In this literature based thesis we ask whether biological noise is relevant for function. We suggest that internal noise is an important component of biological systems and that systems have evolved to modulate the levels of this noise to suit function. We describe mechanisms by which biological systems reduce or increase the level of noise in a system. We then describe the benefits to the organism of modulating noise. Finally, we focus our attention on noise generated in sensory systems and search for general principles that might inform how noise plays a role in the functioning of an olfactory system. This research is motivated in part by a desire to learn whether noise plays a part in olfactory system functioning. Overall, this research has implications for revealing intricate details about olfaction that were not known before. These details could be necessary for a complete understanding of neurodegenerative diseases and insect olfaction. Ultimately, translational horizons such as developing solutions for modulating an insect's olfactory abilities as a mode of insect control are likely to be attainable, which could help prevent the spread of insect-borne pathogens as well as insect damage to agriculture.

Acknowledgement

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Introduction

Biological noise refers to the randomness found in biological systems. Generally, the randomness within such systems, particularly the sensory systems, is regarded as undesirable. However, biological noise is a vital component of biological systems. Thus, there is an urgent need to account for noise in biological systems. Not meeting this need is an important problem because, without accounting for noise, there will continue to be an incomplete understanding of various biological processes, especially the senses.

In this thesis, I will identify mechanisms that organisms use to harness and regulate biological noise, and phenomena that result from biological noise. I will use the general principles of noise regulation uncovered through this research to suggest applications and directions for research in olfaction. Olfaction is the least studied of the sensory systems as it relates to noise. This research applies the principles of biological noise discovered in other systems to the olfactory system in order to suggest future research directions.

Biological noise is like static in a signal. It is random variability that is conventionally regarded as damaging. Noise is associated with disruption of signals and disorder in systems. Specifically, noise is often implicated in aging and disease (Kaern, Elston, Blake, & Collins, 2005). Additionally, noise can distort data, and make it more difficult to recognize trends. To make trends more visible, most scientists take averages of their data in order to evaluate overall trends. This can be helpful in recognizing the effects of a variable on a group, but it isn't necessarily a complete representation of the data.

It's easy to think of these problems when we think of people. Although an understanding of the average characteristics of a group of people can be helpful, averages alone are not sufficient in understanding individual people in that group. Almost no human is exactly average.

To deeply understand a system, then, we need to understand variation as well as averages. An understanding of noise could help us to solve many pressing human issues. Noise is a key element of microbial persistence despite drug treatment, a huge medical and agricultural challenge and, as we will see later, noise plays a significant role in sensory systems. I chose to focus on biological noise in the olfactory system because it could have such a large impact on human wellness. Two of the biggest potential applications of research into the mechanisms of olfaction are in insect olfaction as it relates to insect-borne pathogens, and in neurodegenerative diseases.

Understanding olfaction in insects is incredibly important. According to the World Health Organization, several million people die each year as a result of insect borne pathogens. Although repellants and other tools can be helpful in controlling insect populations, a comprehensive understanding of the insect olfactory system would be incredibly useful because it would allow researchers to manipulate the olfactory systems of insects. Additionally, loss of olfaction is one of the first symptoms of degenerative diseases such as Parkinson's and Alzheimer's. Understanding the olfactory system could be key in uncovering the mechanisms of these diseases.

An understanding of biological noise requires an understanding of the mechanisms that regulate biological noise as well as the phenomena that biological noise causes. I will cover two mechanisms of noise management and two effects of noise in biological systems. Specifically, I will cover feedback loops and bistability as mechanisms of modulating biological noise, and nongenetic phenotypic heterogeneity and stochastic resonance as phenomena resulting from biological noise. Feedback loops, bistability, nongenetic phenotypic heterogeneity, and stochastic resonance have all been researched separately as they relate to biological noise.

However, despite their relevance to the topic of biological noise, no research connects all of these topics. This research demonstrates that a complete understanding of biological noise within the olfactory system will require an understanding of all four.

The first mechanism of noise regulation covered in this research is feedback. Feedback loops are one of the most universal regulatory systems of cells. Feedback loops attenuate the outputs of systems, and are found in virtually every biological system. As such, they are one of the best types of systems to study when researching noise. Indeed, a large body of evidence suggests that feedback loops impact the levels of noise produced in the systems they regulate. Negative feedback loops decrease biological noise, while positive feedback loops induce bistability.

A second mechanism of noise regulation is bistability. A bistable system has two stable states and a threshold in between them, preventing the system from sitting between the two states, or moving easily between them. We can think of bistability the way we think about a switch. This kind of system is either ‘on’ or ’off.’ This sort of all or nothing switching can be very sensitive and often leads to increases in biological noise.

Increases in noise are associated with two key beneficial phenomena. The first is phenotypic heterogeneity, or variation within a population. This variation is good for populations because it leads to bet-hedging benefits, and because it allows organisms to adapt to rapidly changing environments. The second is stochastic resonance, whereby organisms can tune their sensitivity to stimuli using noise. Both of these phenomena have been studied as they relate to noise, and the relationship between noise, feedback, and biology has been established. However, no literature links negative or positive feedback directly to nongenetic phenotypic heterogeneity or stochastic resonance. I will argue that feedback is an important mechanism of biological noise,

which is likely to be associated with nongenetic phenotypic heterogeneity and stochastic resonance through bistability.

Biological noise is an important part of biological systems. In some systems biological noise is beneficial and in some deleterious. Biological systems have evolved to regulate the amount of noise in their outputs using feedback loops. This regulation allows systems to tune noise in order to suit system function. Specifically, the olfactory system displays many of the hallmarks of noise utilization and regulation.

Chapter 1: Feedback loops are essential components of regulatory systems

Cells make many decisions to maintain their day to day functioning. They decide when to take up food and when to metabolize it. They decide when and where to move. These decisions are often made using regulatory networks. These are groups of molecules that interact with each other such that the cell can respond to the environment, other cells, or the internal state of the cell. For example, when a cell senses sugar molecules in the environment, it uses a network of molecular components that can respond to the sugar by upregulating the systems needed to pick up and metabolize that sugar. The steps between recognition of the sugar and upregulation of its associated systems are part of a regulatory network.

These regulatory networks rely heavily on feedback loops. Feedback loops are systems in which the output either amplifies or inhibits the function of that system. In the above example, once the sugar is metabolized, the associated systems are once again inactivated. Because these regulatory networks respond to cues, they contain feedback that can *respond* to changes in those cues. Feedback loops are commonly implicated as regulators of noise. This is most likely because feedback loops allow systems to either dull or intensify their responses to stimuli, effectively increasing or decreasing the amount of noise in a system's output. Because feedback networks are so ubiquitous, understanding their role in noise regulation is important to understanding the way noise is utilized in biological systems.

1.A: Positive and negative feedback loops

There are two main types of feedback loops: positive and negative. A negative feedback loop occurs when the product of a system inhibits that system, represented by system A in Figure 1. A

positive feedback occurs when the product of a system amplifies that system, represented by system D in Figure 1.

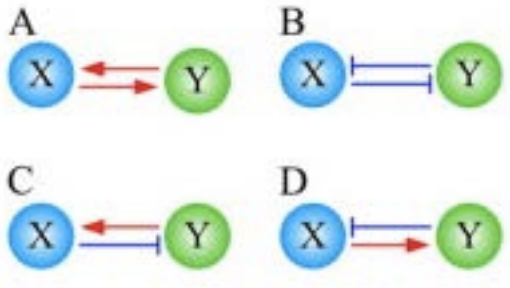

Figure 1. Positive and Negative Feedback Loops*. A) A positive feedback loop. B) A double negative feedback loop. C and D) Negative feedback loops.(Kim, Yoon, & Cho, 2008)*

One example of a biological feedback loop is contractions involved in childbirth. Childbirth is spurred by pressure on the cervix, which releases oxytocin and induces a contraction. Each contraction increases the pressure on the cervix, causing an increase in the release of oxytocin, resulting in another contraction. In this way, contractions are both a product of the release of oxytocin, and a cause of release of oxytocin.

Negative feedback works in the opposite way. A good example is the regulation of blood sugar. A rise in blood sugar prompts the beta cells of the pancreas to secrete insulin, which will lower blood sugar and halt the production of insulin. Insulin is the product of this system, and its presence downregulates the system that produces it.

Regulatory networks are slightly stochastic by nature: they are driven by semi-random interactions between molecules. The types of feedback loops in a regulatory system determine how an organism will respond to this stochasticity. In general, negative feedback loops decrease noise, combinations of negative and positive feedback loops can result in precise and reliable decision making, and positive feedback loops amplify noise. It is important to note that a double-negative feedback loop is another kind of positive feedback loop, in which a product inhibits its own inhibitor (Ferrell, 2002).

1.B: Negative feedback loops make signals less noisy

Negative feedback loops and coupled negative feedback loops are stabilizing forces within a regulatory system. They are commonly found within systems that regulate homeostasis. Homeostasis is the maintenance of stability within an organism. For example, the body maintains stable temperature, pH, and blood sugar levels. (This is because systems maintaining homeostasis must be responsive, but they cannot be noisy.) Consistency and lack of noise are the strengths of negative feedback loops. Again, negative feedback loops and coupled feedback loops should not be confused with double negative feedback loops, which are functionally identical to positive feedback loops.

A recent mathematical model developed to investigate the roles of single and coupled feedback loops found that negative feedback loops reduce responses to noisy signals, acting as a filter (Kim, Yoon, & Cho, 2008). Based on several such examples, we conclude that negative feedback loops are noise reduction tools within regulatory systems. This is desirable in situations where heterogeneity is not desirable, for example during key stages of animal development or while maintaining homeostasis (Raj, & van Oudenaarden, 2008). It can also help organisms to respond to stimuli selectively and keeps them from wasting energy upregulating systems unnecessarily.

1.B.i: Negative feedback in the galactose uptake system of yeast

The Galactose uptake system in yeast is a popular model system used in studying feedback loops. This system activates the uptake of galactose, a sugar, into the cell by activating a group of genes called GAL genes that aid in the uptake of galactose. The system begins when Gal2p imports galactose into the cell, which binds to Gal3p. Gal3p then binds to Gal80p, preventing it from moving into the nucleus. This allows the transcription factor - Gal4p, which is

normally inhibited by Gal80p, to activate the expression of the downstream GAL genes. In this way, the presence of galactose upregulates the transcription of galactose uptake genes.

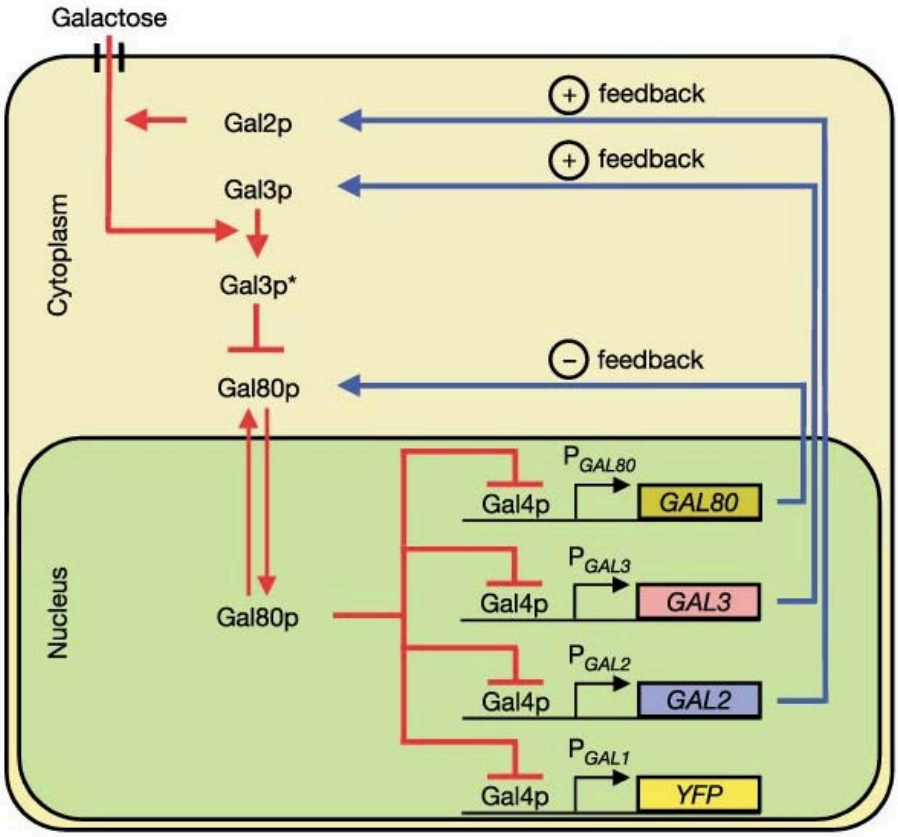

Figure 2. The Galactose Uptake System of Yeast*. Uptake of galactose leads to the activation of Gal3p, which inhibits Gal80p. Gal80p inhibits the transcription of the GAL genes: GAL80, GAL3, GAL2 and YFP. These genes, when activated, reactivate Gal2p and Gal3p, in addition to further inhibiting Gal80p. In this way, a small amount of Galactose can turn on the GAL genes significantly, and keep them on. (Acar, Becskei, & van Oudenaarden, 2005).*

Gal2p and Gal3p are signaling molecules that increase the transcription of GAL genes. They upregulate their own transcription, creating positive feedback loops. Gal80p has the opposite effect, decreasing its own transcription. For this reason, Gal80p forms a negative feedback loop. By increasing Gal80p within the cell, researchers discovered that this single negative feedback loop reduces the fluctuations between cellular states as measured by their uptake of glucose. In other words, when the negative feedback within this system is increased, the output is less noisy and more consistent. This indicates that the presence and strength of

negative feedback within a system decreases the amount of noise in that system's output (Acar, Becskei, & van Oudenaarden, 2005).

1.B.ii: Negative feedback increases stability in E. Coli gene circuits

Forty percent of known E. Coli transcription factors self-regulate with negative feedback loops. It has been hypothesized that this feedback provides stability to the cell and aids in the maintenance of homeostasis. To test the impact of negative feedback specifically, researchers created a simple circuit in E. Coli by creating a mutant that expresses three unusual genes: TetR, EGFP, and firefly luciferase. In one circuit, researchers induced a negative feedback loop. All other aspects of the circuit remain intact. This left researchers with two nearly identical circuits, one containing a negative feedback loop and one unregulated system. Researchers analyzed the stability of each system using a linear stability analysis, which used differential equation models of the gene circuits they designed. This helped to isolate the impacts of the specific system within the organism. Isolating the system was important because there are many other systems impacting the state of the cell. The system that lacked negative feedback and did not autoregulate was significantly less stable than the autoregulatory system. This supports the hypothesis that negative feedback provides the stability and consistency needed for homeostasis by filtering noise out of signals, (Becskei, & Serrano, 2000).

1.B.iii: The strength of negative feedback must be tuned to decrease noise

One potential problem with the use of negative feedback to control or decrease the noise within a regulatory system is that it needs to be tuned to a range of strength to be maximally effective. The experiment detailed above was repeated at a higher sensitivity, where fluorescence activated cell sorting (FACS sorting) replaced microscopy to quantify GFP expression, the measure of output. FACS sorting is capable of more accurately determining the degree to which

the cells in each group fluoresced. It does this by measuring the fluorescence in each live cell and sorting the cells based on those values.

Results supported the conclusion that negative feedback loops significantly reduce noise. However, intermediate concentrations of the feedback molecule produced the largest reduction in noise. When there was excessive negative feedback noise increased again, until it was at levels comparable to the unregulated circuit. This evidence supports a model wherein negative feedback has a U-shaped effect on circuits. In other words, negative feedback decreases noise up to a point, after which noise will increase again. Negative feedback must be tuned to maximally limit biological noise. This should be kept in mind when investigating systems that include particularly strong negative feedback loops, (Dublanche, Michalodimitrakis, Kummerer, Foglierini, & Serrano, 2006).

1.C: Combining negative and positive feedback loops can create oscillations

Coupled negative and positive feedback loops can aid cells in decision making, especially where signals are not particularly noisy to begin with (Kim, Yoon, & Cho, 2008). They have little effect on the amount of noise present in a system, but these arrangements of feedback loops do have the interesting potential to create oscillatory patterns. There are two oscillatory systems whose feedback loop systems have been modeled.

The first is the *Neurospora crassa* oscillator. *Neurospora crassa* is a fungus commonly used to study circadian rhythms. In this system, a protein called FRQ is produced as an output. FRQ inhibits the system by decreasing the productivity of the white color complex (WCC), which in turn inhibits the production of clock controlled genes (ccgs). In the short term, this halts the output of the system. However, FRQ also activates two genes called wc-1 and wc-2. These accumulate in the WCC, and activate the system again. So in the short term FRQ is a part of a

negative feedback loop that halts the system. On a slightly longer time scale FRQ is part of a positive feedback loop that reactivates that system, starting the cycle again (Merrow, Roenneberg, Macino, & Franchi, 2001). This creates an interlocking system of positive and negative feedback, illustrated in Figure 3.

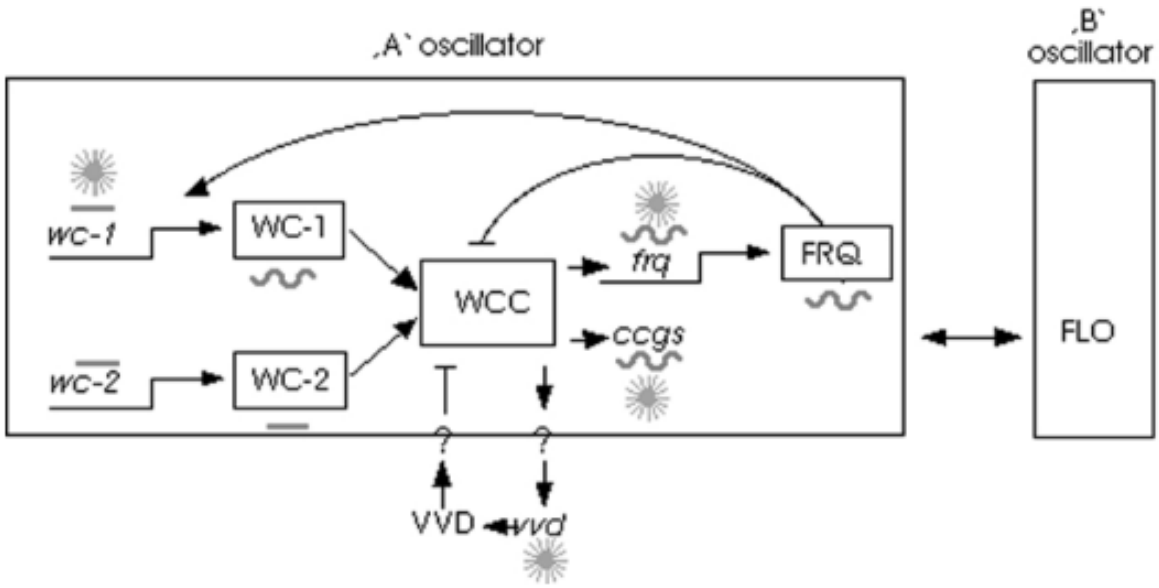

Figure 3. Circadian rhythm system of Neurospora crassa*. FRQ inhibits the system immediately, but provides positive feedback through WC-1 and WC-2. This pattern of inhibition followed by slower effects of positive feedback creates oscillatory patterns.(Merrow, Roenneberg, Macino, & Franchi, 2001).*

A second oscillatory system is the *Drosophila melanogaster* circadian oscillators. This system is the circadian clock of the fruit fly. There are four key proteins that create feedback in this system: Period (PER), Timeless (TIM), dClock (dCLK) and Cycle (CYC). dCLK is the main regulator of this system, activating time dependent genes. PER and TIM, as well as dCLK and CYC, bind together to form complexes. The dCLK and CYC complex activates the production of PER and TIM, but PER and TIM provide negative feedback and reduce this effect. However, they also upregulate dCLK, which provides positive feedback on a slightly longer timescale. (These relationships are illustrated in Figure 4.) In this way, the *Drosophila* circadian rhythm functions similarly to the *Neuroprosa crassa* system, creating both positive and negative

feedback on slightly different timescales to create oscillations. (Bae, Lee, Hardin, & Edery, 2000).

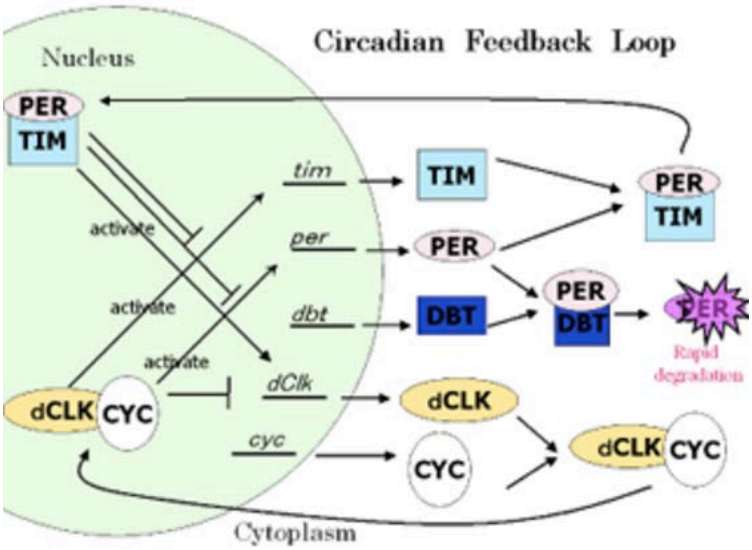

Figure 4. The circadian rythym of drosophila*. PER and TIM, as well as dCLK and CYC, bind together to form complexes. The dCLK and CYC complex activates the production of PER and TIM. PER and TIM provide negative feedback and reduce this effect. PER and TIM also upregulate dCLK, which provides positive feedback on a slightly longer timescale (Circadian rhythms in drosophila, 2009).*

Both of these oscillatory systems contain interlocked positive and negative feedback loops, indicating that oscillatory patterns could be maintained by this system of feedback. However, when modeled, both systems maintained this patterning when positive feedback was removed. The impact that positive feedback loops have on these systems is not well understood and should be investigated further (Smolen, Baxter, & Byrne, 2001).

1.D: Positive feedback creates bistability

Positive feedback, including single and coupled positive feedback loops and double-negative feedback loops, increase the noise in a biological system by introducing bistability (Ferrel, 2002). Bistable systems have two expression states at which they are stable, and operate in an on/off pattern. Either a bistable system is in one stable state or the other, they do not produce graded responses. The features of bistability will be discussed further in the subsequent

chapter. Here we will demonstrate that positive feedback causes bistability by creating stable states that reinforce themselves.

1.D.i: Bistable systems resulting from positive feedback

Many bistable biological systems contain positive feedback loops (Zordan, Galgoczy, & Johnson, 2006, and Maamar, & Dubnau, 2005). Most of these bistable systems are not fully understood, because the mechanisms of regulation in most cells are highly complex and contain many related feedback loops. However, research into specific, well researched systems such as the galactose uptake system of yeast, and the creation of simplified synthetic circuits have demonstrated the strong connection between positive feedback and bistability.

Galactose uptake system of yeast

The galactose uptake system of yeast discussed in Section 1Bi is also a bistable system fueled by positive feedback. This system's two stable states are taking up galactose, or not taking up galactose. We can refer to these as the 'on' and 'off' state of the galactose uptake system. In the off state the transcription of GAL genes is inhibited by Gal80p. Gal3p inactivates Gal80p when the cell picks up a small amount of galactose. This is enough to cause a small increase in the transcription of GAL genes, but this response would decrease quickly in a system without positive feedback. Instead, Gal3p is part of a positive feedback loop. This means that once Gal3- begins repressing Gal80p it allows more Gal3p to be transcribed, resulting in a stable 'on' state that continuously represses the activity of Gal80p and transcribes galactose uptake genes. In this way, the positive feedback loop created by Gal3p creates a second stable state for the galactose uptake system in yeast, making it a bistable system (Acar, Becskei, & van Oudenaarden, 2005).

Genetic toggle switch in E. Coli.

Although the link between bistability and positive feedback can be observed in many organisms, there are many components of bistable systems that could be clouding our understanding. To investigate more thoroughly the impact of positive feedback in situ a synthetic bistable regulatory system was developed in E. Coli. The goal of creating this system was to test a theory of bistability by its predictive capability. Researchers predicted that using two negative feedback loops which are interlocked to form a double negative feedback loop would be the most stable and effective way to induce bistability. They note that one positive feedback loop is theoretically sufficient to create a bistable system. However, they theorize that double negative feedback loops are more likely to be robust and bistable in a larger variety of circumstances. They created five of these synthetic bistable systems, each using double-negative feedback loops of a different strength. If the system was active, it would produce fluorescence. Cells started in an 'off' state, the first steady state in the system. The 'on' state would then be induced over a period of approximately 14 hours. Cells switched abruptly between the 'off' and 'on' state, and became stable as the 'on' state was induced. Each system demonstrated strong bistability. This evidence supports the claim that positive feedback and double negative feedback are sufficient for inducing bistability (Gardner, Cantor, & Collins, 2000).

Genetic toggle switch in Saccharomyces cerevisiae

A similar genetic toggle switch has also been constructed in the eukaryotic *Saccharomyces cerevisiae*. Because prokaryotic organisms like *E. Coli* and eukaryotic organisms like *Saccharomyces cerevisiae* have such different systems for the transcription and translation of genetic material, their responses to positive feedback are not necessarily the same. To investigate possible variations in response, a positive feedback loop was created in *Saccharomyces cerevisiae* in order to construct a similar toggle switch to that in the example

above. As in *E. Coli*, the system was constructed to produce fluorescence when active. The system to which positive feedback was added normally produces graded results. This means that the level of activation of the system does not resemble a switch and instead produces many intermediate values. The positive feedback loops converted this graded system into a binary system, where fluorescence was either not present or strongly present. This evidence supports the claim that positive feedback loops create bistable systems, (Becskei, Seraphin, & Serrano, 2001). Although both *Saccharomyces cerevisiae* and *E. Coli* both contain examples of positive feedback loops leading to bistability, many bistable systems contain more than one feedback loop.

1.D.ii: Coupling positive feedback loops creates a faster and more selective bistable response

If one positive feedback loop is sufficient to create a bistable response, then why are biological circuits general comprised of multiple interlocked and coupled feedback loops? The complexity of these feedback systems actually benefits the regulatory circuit. Linking together slower positive feedback loops with faster positive feedback loops results in more reliable and timely bistable systems. This is because fast positive feedback loops are often volatile, and slow feedback loops, though more reactive, are not timely. Combining the two loops can lead to a response that is more timely than a slow feedback loop, but still reliable.

Budding yeast uses two feedback loops. One involves activity cycling of Cdc42 which provides rapid feedback and the second involves a slower actin mediated transport system for Cdc42. Calcium ion signaling, a common cellular mechanism, often involves two feedback loops. The first is the inositol 1,4,5-triphosphate (IP3) rapid feedback loop, and the second is a slower Calcium ion influx caused by a depletion of Calcium ions within the cell. *Xenopus* oocytes use a fast phosphorylation and dephosphorylation feedback loop and a slow feedback loop using Cdc2 and MAPK.

These two feedback loops regulate each other in order to moderately filter noise from upstream. Rapid feedback loops create extremely noisy outputs. For example, the IP3 feedback loop found in Calcium ion signaling will provide a response even for very brief spikes of Calcium ion. However, the second feedback loop, mediated by a gradual depletion of Calcium ion, requires a longer and more consistent input to result in a response. Combining these two feedback loops creates a system that is sufficiently sensitive to inputs due to the rapid feedback loop but not noisy. (Brandman, Ferrett, Li, & Meyer, 2005).

Conclusions from feedback loops

Feedback loops are a pervasive part of biological systems. They affect the variability of systems' outputs and are therefore good candidates for noise regulation. There are two main types of feedback loops, negative and positive. Negative feedback loops reduce noise within biological systems. Biological systems might want to reduce biological noise in order to maintains steady states like blood pressure or to send signals. Combinations of positive and negative feedback loops create oscillatory systems. These systems are not stable like blood sugar, but they are still resistant to noise from outside the system. Positive feedback loops create a characteristic called bistability.

Understanding the effects of feedback loops upon noise is important for two key reasons. Firstly, the types of feedback within a biological system are an indication of how noise functions within that system. Negative feedback loops are an indication that noise is downregulated in a biological system. Coupled negative and positive feedback loops suggest oscillatory behavior is a possibility. Positive feedback indicates that noise is upregulated through bistability. Secondly, an understanding of positive feedback is vital to an understanding of bistability. Bistability is

inherently noisy, and is therefore important to a comprehensive understanding of biological noise.

Chapter 2: Bistable systems are inherently stochastic

Bistable systems are another mechanism of biological noise. They are closely tied to feedback systems since positive feedback loops induce bistable systems. Bistable systems can be inherently stochastic, introducing noise to a biological system. This could lead to stochastic resonance. Bistable systems also lead to nongenetic phenotypic heterogeneity, a type of noise often found at the population level. The relationship between feedback loops and bistability is well established, as is the relationship between bistability and nongenetic phenotypic heterogeneity. However, the relationship between bistability and stochastic resonance is simply implied by their shared relationship with biological noise and is not discussed in the literature. Bistable systems are systems in which there are two stable equilibrium states. As a result, bistable systems operate like a switch, they are either on or off. Bistable systems are an important component of cellular systems. Many cellular systems contain mechanisms described as switch-like. These systems can be very noisy because they do not contain intermediate states, their responses to stimuli are always extreme.

2.A: Properties of Bistable Systems

A bistable system has two stable states and a threshold of input that must be overcome to move between those states. They contrast with systems where responses are graded, where a small input causes a small change in a system, and a large input causes a large change in a system. Instead of producing a graded response, bistable systems result in all-or-nothing responses, much like the firing of a neuron, which either fires or does not.

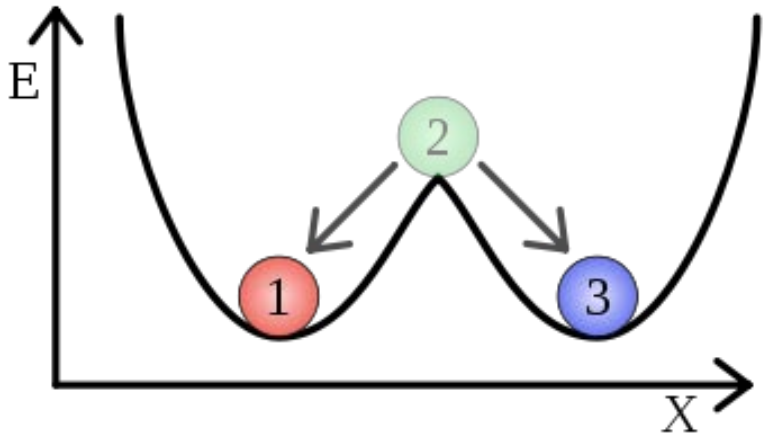

Figure 5. Bistable systems. A bistable system has two stable states, divided by an unstable intermediate state. This intermediate state, often referred to as a barrier, is higher energy than either of the stable states. (Illustration by Georg Wiora)

A bistable system can be imagined as a plane containing two valleys, onto which a ball is placed. Although the ball can be moved from one valley to another a threshold of energy input must be achieved to do so. Weak movement will not be sufficient to move the ball out of one valley and into the other (Bulsara, & Gammaitoni, 1996). In some bistable systems a sufficient amount of input to move between states can be produced by a cell, but some bistable systems are arranged such that once one state has been determined the process is irreversible. In these systems one can imagine that the barrier between states is so high as to be insurmountable by the cell (Ferrell, 2002).

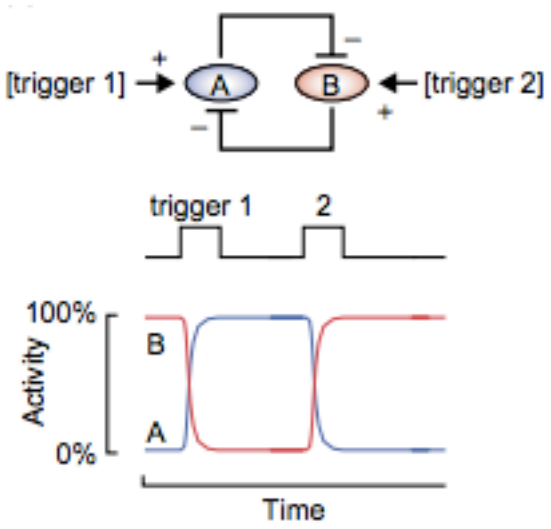

Figure 6. Double negative feedback. In a double negative feedback loop each stable state inhibits the other state, creating a barrier between the two. In these systems a trigger can move the system between states. (Ferrel, 2002).

This barrier between states is in part produced by feedback mechanisms that reinforce each state. This barrier can be created with either positive or negative feedback. A positive feedback loop will reinforce itself, creating stability. In order to harness negative feedback to create two stable states, the cell uses double-negative feedback loops. In a double-negative feedback loop each stable state inhibits the other. This type of feedback loop can be used to regulate transcription or protein activity, giving it diverse applications within the cell.

Bistability also requires these feedback loops to be balanced and sensitive. If one state inhibits the other more strongly, then the system will become monostable instead of bistable. Additional negative feedback can be added to create the same affect. In a double negative feedback loop there are two sources of negative feedback. Any even number of negative feedback sources could potentially result in bistability. However, odd numbers of negative feedback loops do not have the same effects (Ferrell, 2002).

2.B: The bistable switch of the lactose utilization network of E. Coli

One of the best studied bistable systems is the lactose utilization network of E. Coli. This system is regulated by the lac operon, a group of genes relevant to lactose uptake and utilization. This operon contains three relevant genes, termed lacZ, lacY, and lacA. Each of these genes codes for an enzyme which is involved in metabolizing a different substance. LacZ produces an enzyme necessary for conversion of lactose into allolactose. The enzyme coded for in LacY is used in the uptake of lactose and TMG (thio-methylgalactoside). LacA codes for an enzyme used in sugar metabolism.

The lac operon can be activated by the cyclic AMP receptor protein (CRP) and repressed by LacI, both of which regulate transcription. Inhibition by LacI can be reduced by allolactose and TMG, and activation by CRP can be increased by cAMP. The key element of this system is that TMG can upregulate the transcription of the lac operon. The transcription of the lac operon leads to the translation of lacY, which leads to further TMG. This is a positive feedback loop that results in a bistable response to TMG concentrations, illustrated in Figure 4.

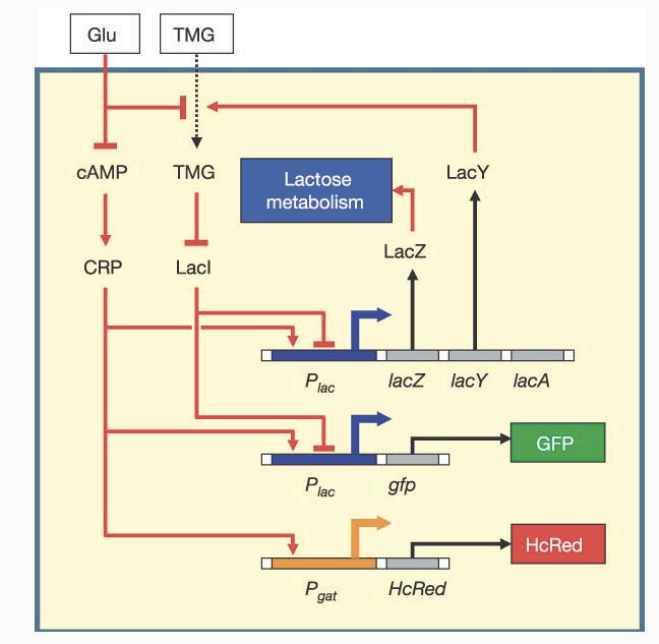

Figure 7. The lac utilization network in E. Coli. *The lac operon is activated by the cyclic AMP receptor protein (CRP) and repressed by LacI. CRP is upregulated by cAMP. LacI is inhibited by TMG. TMG can upregulate the transcription of the lac operon by inhibiting LacI. The transcription of the lac operon leads to the translation of lacY, which leads to further TMG. This is a positive feedback loop (Ozbudak, Thattai, Lim, Shraiman, & van Oudenaarden, 2004).*

Ozbudak et al. found that at TMG concentrations below 3 micromolar, a very low concentration, the lac operon was not induced. At TMG concentrations over 30 micromolar, a relatively high concentration, the lac operon was fully induced. At concentrations in between the lac operon was induced either fully or not at all. The probability that the lac operon would be induced depended on whether that cell had been exposed to high or low levels of TMG in the past and the current concentration of TMG. Ozbudak et al. also established the relationship between feedback systems and bistability by developing a new strain of E. coli where feedback within this system was weaker. As a result of the decreased sensitivity the E. coli in this new strain demonstrated a graded reaction to TMG concentrations instead of the bistable reaction of the wild type strain (Ozbudak, Thattai, Lim, Shraiman, & van Oudenaarden, 2004). This means that bistability is induced by feedback, and that it results in switch-like behavior.

2.C: Bistable systems as producers and users of stochasticity

Bistable systems are inherently noisy. Because they do not produce graded responses to stimuli they result in greater variation between cellular responses. In the lac utilization pathway of E. coli for example, at a moderate concentration of TMG, instead of each cell activating the lac operon moderately, some cells will completely activate the operon and others will completely deactivate it. This produces a much higher standard deviation of expression within the population. In other words, the bistability of the lac utilization system makes its response to TMG concentration noisy. This means that bistability is a potential mechanism for increasing noise within biological systems.

This increase in noise leads to two notable phenomena that provide benefits to the organisms in which they appear. These are stochastic resonance and nongenetic phenotypic heterogeneity. Nongenetic phenotypic heterogeneity uses noise to create different behavior and development in organisms that are genetically identical (Chapter 3). This can confer bet-hedging benefits onto a population, and also help organisms to adapt quickly to volatile environments. Stochastic resonance increases the sensitivity of sensory systems through the addition of noise (Chapter 4). There are specific instances in which the relationship between bistability and nongenetic phenotypic heterogeneity have been established. This demonstrates that bistability is a mechanism for increasing biological noise within a system.

2.C.i: Bistable systems create phenotypic heterogeneity

Often, genotypically identical cells raised in homogenous environments develop different phenotypes. This process is likely a bet-hedging strategy used by the bacterial populations in which it is found, and populations exhibiting this strategy demonstrate increased fitness compared to their homogeneous counterparts.

Sometimes varying phenotypes between genotypically identical cells is a result of cyclical cell cycles. For example, *Caulobacter crescentus* cycles between two cell types throughout its lifespan, but these changes are patterned, not stochastic. However, other examples of varying phenotypes appear to be stochastic and bistable. For example, *Bacillus subtilus*, when in starvation scenarios, will grow in two distinct phenotypes. As starvation advances cells will randomly specialize into one of these two morphologies (Norman, 1999). These phenotypic changes can be reversed during the lifetime of these bacteria or their descendants. This is evidence that the phenotypic heterogeneity is a result of a bistable system (Veening, Smits, & Kuipers, 2008, and Norman, 1999).

2.C.ii: Bistable systems are a key component of stochastic resonance

Stochastic resonance occurs in nonlinear systems when they are exposed to noise and a periodic stimulus (Shulgin, Neiman, & Anishchenko, 1995). Because they produce a binary response that is not graded proportionally with the stimulus, bistable systems are considered nonlinear. This means that bistable systems are a potential source of noise for inducing stochastic resonance. Shulgin, Neiman, and Anishchenko used an overdamped bistable oscillator to simulate a noisy bistable system and a Schmitt trigger as a stimulus. They were able to establish that a bistable system with noise input and periodic excitation (a stimulus) was capable of producing stochastic resonance tuning behavior. Fauve and Heslot conducted a similar experiment also using a Schmitt trigger and noted that they tuned their apparatus's internal noise by adjusting variables corresponding to the size of the barrier between bistable states. Although research has not yet been conducted to link bistable systems to stochastic resonance in biological systems, this suggests that bistability through feedback could be one way of tuning stochasticity in biological systems in order to induce stochastic resonance (Fauve, & Heslot, 1983).

This means that bistable systems are relevant to a comprehensive understanding of noise, not just because they increase the amount of noise within a system, but also because they could lead to stochastic resonance and nongenetic phenotypic heterogeneity. These associations could be useful in understanding the way that the olfactory system utilizes noise. Bistability within the olfactory system indicates that the olfactory system uses noise in some way.

2.D: Bistable systems in the olfactory system

By looking for bistability within the olfactory system we can discover pathways that are likely to utilize noise. There are several key bistable systems specifically associated with the olfactory system. The bistable systems most relevant to our study of harnessing noise in the olfactory system are those that use the increased stochasticity to create variable responses or phenotypes. The Calmodulin Kinase II and Protein Phosphatase I bistable protein network plays a role in long term potentiation and likely affects future behavior as a response to the environment. Olfactory bulb mitral cells are also bistable, which could lead to noisy responses to olfactory signals.

2.D.i: Calmodulin Kinase II and Protein Phosphatase I

The Calmodulin Kinase II (CaMKII) and Protein Phosphatase I bistable protein network is an important example of bistability within the olfactory system. The CaMKII pathway attenuates sensitivity to odors that have been present for a while. Adapting to odors is an important function of the olfactory system. It allows an organism to ignore odors that are present for extended periods of time in order to differentiate new or unusual odors. In vertebrates this process involves a feedback loop mediated by Calcium ions. There are two components to this feedback loop. The first is a cAMP-gated channel, and the other is the reduction of adenylyl cyclase activity by Calmodulin Kinase II. If is the reduction of adenylyl cyclase activity by

Calmodulin Kinase II is disrupted by an inhibitor then adaptation to a sustained stimulus is reduced. This means that the reduction of adenylyl cyclase activity by Calmodulin Kinase II is necessary for the function of the system. The onset rate of adaptation is significantly reduced, as is the recovery rate from adaptation. Adaptation to short stimuli is not affected, which indicates that the function of the CaMKII pathway is highly specific, in that it only responds to long stimuli (Leinders-Zufall, Ma, & Zufall, 1999).

This pathway has bistable outputs. At a standard intracellular calcium ion concentration there are two stable CaMKII phosphorylation levels. This means that in an unaffected cell there are two concentrations of phosphorylated CaMKII that can stably exist. The cell will only display one of these concentrations. These are the two states, or outputs, of the system. The concentration of CaMKII can be changed by a spike in Calcium ion concentration. Therefore Calcium ion concentration represents the force necessary to move between the two states in this bistable system (Graupner, & Brunel, 2007).

Using a bistable system like this in order to induce adaptation to sustained stimulus is advantageous in several ways. Firstly, this system is very energy efficient. Long term potentiation requires a cellular state to change and be maintained for an extended period of time despite ongoing changes within the cell. A bistable system allows the cell to easily maintain a changed state without switching constantly back and forth and does not expend significantly more energy in one state than the other (Lisman, & Zhabotinsky, 2001). Secondly, bistable systems are always stochastic since they turn graded signals into two-state outputs. This means that individuals are likely to respond differently to these extended stimuli. This produces nongenetic heterogeneity, which can provide bet hedging benefits to populations, as well as help individuals adapt to changing environments.

The Calmodulin Kinase II and Protein Phosphatase I pathway is an example of bistability within the olfactory system. It could have several effects on an organisms responses to noise. The pathway could be inducing nongenetic phenotypic heterogeneity by causing different responses to the same stimuli. The pathway also attenuates sensitivity, which means it may be associated with Stochastic Resonance. The presence of bistability within this system indicates that it would be a good source of future research into the function of noise within the olfactory system.

2.D.ii: Olfactory bulb mitral cells

Another source of bistability within the olfactory system is Olfactory bulb mitral cells. The mitral cells of rat main olfactory bulbs are bistable. They alternate between two membrane potentials spontaneously. The first membrane potential is termed an upstate and is about ten millivolts less polarized than the downstate. This is an example of a biological system that increases the noise within the olfactory system as a whole. In one experiment whole cell patch clamp recordings were used to monitor the membrane potentials of mitral cells in response to olfactory nerve stimulation. When mitral cells are in the upstate they are significantly more likely to fire an action potential and respond to a stimulus. This evidence suggests that the randomly switching bistable states of olfactory bulb mitral cells have a significant effect on the output of the olfactory system (Heyward, Ennis, Keller, & Shipley, 2001). There are several possible effects that this stochasticity could have on the system. The increased stochasticity could lead to increased sensitivity through a phenomenon called stochastic resonance (Chapter 4). Alternatively, it could lead to varying responses among a population of individuals such that some individuals respond to a stimulus differently than others, a phenomenon called nongenetic heterogeneity (Chapter 3). In either situation the olfactory system is harnessing noise in order to

increase fitness. This indicates that biological noise can be beneficial to biological systems. In addition, it suggests that olfactory bulb cells are a good candidate for future research in biological noise.

Conclusions from bistability

Olfactory bulb mitral cells and the Calmodulin Kinase II and Protein Phosphatase I pathways are both good candidates for future research on bistability and noise within the olfactory system. In researching these pathways it is important to remember that bistable systems are closely tied to feedback systems. Positive feedback loops induce bistable systems. Bistable systems function in a switch like manner because they have two stable states and a threshold that sits between them. Bistable systems can be inherently stochastic, introducing noise in a system. The introduction of noise into a system could result in stochastic resonance (Chapter 4). Bistability is also closely linked to nongenetic phenotypic heterogeneity (Chapter 3). Therefore, an understanding of bistability helps to connect feedback loops, the most basic mechanism of noise regulation, to stochastic resonance and nongenetic phenotypic heterogeneity.

Chapter 3: Nongenetic Phenotypic Heterogeneity increases fitness

Nongenetic phenotypic heterogeneity is a type of noise found in groups of cells or organisms. It is often produced by bistability and feedback. Heterogeneity refers to the variation between organisms. A significant amount of heterogeneity is the result of variations in genetic code across organisms. For example, eye color in humans is a trait that displays genetic heterogeneity. People have differently colored eyes because they have different genes.

However, some of the variation between organisms is not the result of genetic differences. This is why identical twins are never perfectly identical; various environmental and stochastic factors lead to nongenetic heterogeneity between them. Phenotypic heterogeneity simply means that the differences between these organisms are in the expression of their genes, and not in the genes themselves. Differences between genetically identical organisms can be beneficial to organisms and populations. These benefits could be particularly beneficial in the olfactory system, where nongenetic phenotypic heterogeneity could produce differing feeding behavior in populations and confer bet-hedging benefits. Stochastic systems, such as bistable systems, can lead to heterogeneity in the olfactory systems of populations and individuals that increase their fitness.

3.A: Stochastic systems lead to heterogeneity in phenotypes

There are several known ways for organisms to introduce nongenetic phenotypic heterogeneity into their populations, stochasticity is certainly not the only method. Variations in phenotype throughout life cycles, epigenetic modification, and ultraradian rhythms are all valid explanations of some types of nongenetic phenotypic heterogeneity. However, stochasticity has been credited as the source of nongenetic phenotypic heterogeneity in systems such as competence and sporulation individuality, phage lambda decisions, bacterial motility,

chemotaxis, and antibiotic persistence. Stochastic systems can lead to heterogeneity in phenotypes (Avery, 2006). This heterogeneity confers benefits onto a population, and is therefore an example of biological noise being harnessed by a biological system.

3.A.i: Gene expression fluctuation affects variation

One form of biological noise that can lead to nongenetic phenotypic heterogeneity is fluctuation in gene expression. Copies of DNA and RNA, as well as vital cellular components, exist in limited amounts in the cell. Because the concentration of these molecules can be relatively low within the cell, the probability that the necessary proteins will come into contact with any given gene is variable. Based simply on physical circumstance, some cells may transcribe and translate a gene significantly more than others. This effect can be attenuated by positive feedback but is also effective in creating heterogeneity. It is possible to introduce nongenetic phenotypic heterogeneity with noise in gene expression alone (Elowitz, Levine, Siggia, & Swain, 2002, and Raser, & O'Shea, 2005).

Stochastic gene expression is one type of biological noise, and it is usually regarded as a detriment. It has been suggested as a potential source of disease and cellular malfunction. However, there are several benefits to stochastic gene expression. These include cellular differentiation in development, population level heterogeneity, and flexible responses to fluctuations in the environment. (Kaern, elston, Blake, & Collins, 2005).

3.A.ii: Bistable systems and feedback circuitry lead to phenotypic heterogeneity

Through a phenomenon called feedback-based multistability, feedback regulation can lead to a population with multiple subpopulations with varying phenotypes (Smits, Kuipers, & Veening, 2006). This is largely through the creation of bistable systems, which can result in two stable phenotypes within a population. Such systems can become multistable, containing more

than two stable states, simply through the addition of feedback loops that create further stable states (Smits, Kuipers, & Veening, 2006 and Veening, Smits, & Kuipers, 2008)

These bistable systems are responsible for the majority of notable cellular phenotypic heterogeneity that is usually ascribed to stochasticity. Phage lambda decisions, the lactose utilization network of E. Coli, chemotaxis in E. Coli, cellular differentiation, and pathogenic phase variation are all attributable to bistable systems. (Veening, Smits, & Kuipers, 2008). We can conclude that bistable systems lead to nongenetic phenotypic heterogeneity, which is a variation in phenotypes. This variation can be utilized at a population level, but it can also be used to create variation in cell types within an organism.

3.B: Nongenetic phenotypic heterogeneity is necessary for cell differentiation

Within many multicellular organisms, such as most plants and animals, cells have different specializations. This requires permanent nongenetic phenotypic heterogeneity. The variation between genetically identical cells in the same organism needs to be permanently maintained. In these cases, cells enter a stable state within the system and stay in that same state for the duration of their lifespan. This usually occurs when the positive feedback securing the stable state is particularly strong. One of the most common uses of this kind of permanent multistability is cellular differentiation (Veening, Smits, & Kuipers, 2008).

Cellular differentiation is the process by which cells in a multicellular organism specialize. These cells often assume vastly different roles, requiring different cell structure, gene expression, and metabolism. Despite this, they usually share an identical genome. For example, all of the cells in a human body start with the same genetic code. Despite the fact that each of the cells contains the same genes, some become muscle cells, some become neurons, and so on. This is an extreme and permanent example of nongenetic phenotypic heterogeneity.

Bistable systems can also result in cell differentiation in single-celled organisms. Single celled organisms like bacteria often differentiate into specialized cell types that allow them to respond more specifically to environmental conditions. For example, both Bacillus and Clostridia bacteria can produce endospores. Endospores are dormant cells that can survive periods of extreme nutrient depletion. Processes of cellular differentiation like this are often driven by stochasticity resulting from bistable systems (Norman, Lord, Paulsson, & Lossick, 2015). The presence of biological noise in cell differentiation suggests that biological noise is utilized broadly in development. Research in cell differentiation should include an understanding of biological noise and nongenetic phenotypic heterogeneity.

3.C: Heterogeneity confers a benefit to populations

Variation is generally regarded as a positive characteristic of a population. Mutation and sexual recombination are partially successful because of their ability to provide variation within a population (Avery, 2006). This is because random variation in phenotype across a population increases the likelihood that a subset of that population will be able to survive a change in environment (Beaumont, Gallie, Kost, Ferguson, & Rainey, 2009). This is why monocultures in farming are sometimes regarded as dangerous. If all of the organisms being raised are identical, they are more susceptible to being entirely knocked out by a single disease. This is largely a bet hedging effect. Nongenetic phenotypic heterogeneity can serve the same purpose in populations (Avery, 2006).

In environments that are highly variable, it is advantageous for organisms to be able to change their phenotype completely during the course of their lifetimes. Stochastic phenotype switching refers to a case where an environment changes rapidly and often (Rainey et al., 2011).

In these cases, organisms often exhibit dramatic changes in phenotype over a short period of time.

Another way of utilizing heterogeneity in a highly variable environment is bet-hedging. In bet-hedging members of a genetically identical population will have varying phenotypes. Each of these phenotypes will have a different level of fitness depending on the state of the environment at the time. This stabilizes the number of offspring per member of the population over time. Although individual organisms may not benefit from a bet-hedging strategy, all of the individuals within the population are genetically identical, it is ultimately a valid evolutionary strategy, (de Jong, Haccou, & Kuipers, 2011).

However, in more stable environments, or in organisms that may be disbursed over a range of environments, a less extreme version of stochastic phenotype switching exists, called adaptive diversification (Kisdi, 2001). Adaptive diversification demonstrates that although bet hedging and stochastic phenotype switching are pronounced in highly variable environments heterogeneity is advantageous even in more stable environments.

3.C.i: Heterogeneity increases the fitness of stress resistant yeast

One example of heterogeneity conferring advantages is found in the comparison of wild type yeast with a less stress resistant, but more heterogeneous strain. Wild type yeast is relatively stress resistant. The survival of a wild type strain has been compared to the survival of a strain that is less stress resistant, but also more heterogeneous. Generally, the mutant strains would be expected to perform poorly compared to the wild types strains. The mutant strains (cma3, ctr1, sod1) were uncommonly sensitive to doses of metals like nickel and copper, as well as pH changes and some other chemicals. However, the heterogeneity of the mutant strains resulted in

enhanced survival amongst those populations. Heterogeneity increases the fitness of stress resistant yeast (Bishop, Rab, Sumner, & Avery, 2007, and Sumner, & Avery, 2002)

3.C.ii: Stochastic life cycle duration increases mean fitness of chestnut weevils

Another example of heterogeneity increasing fitness is the life cycle duration of chestnut weevils. Chestnut weevils have very variable life cycle lengths. The length of a chestnut weevils diapause, essentially the time it remains dormant as an egg, is determined by how deep it is buried in the soil. Directly, it is impacted by the temperature and size of the egg. These factors do not indicate information useful in determining whether an individual should hatch at a given time to maximize likelihood of survival. Instead, the depth at which an egg is laid is determined randomly by the female laying the eggs.

Female chestnut weevils lay eggs randomly at varying depths in varying locations, thereby artificially increasing the heterogeneity of the next generation of chestnut weevils. This strategy increases the mean survival of chestnut weevils, suggesting that it is an adaptation evolved to increase the heterogeneity of the population and confer bet-hedging benefits (Menu, & Desouhant, 2002). This heterogeneity is not necessarily the result of biological noise, but it could potentially be. The process resulting in the laying pattern of chestnut weevils would be a good source of future research on stochastic behavior. Regardless of the mechanisms leading to the chestnut weevils laying behavior, the heterogeneity of their lifespan confers benefits. This heterogeneity should also lead to benefits in other systems, such as olfaction.

3.D: Heterogeneous olfactory behavior resulting from stochasticity

Although no heterogeneous systems in olfaction have been established as advantageous there are components of the olfactory system that display heterogeneous behavior. This heterogeneous behavior that may confer bet-hedging benefits to organisms by providing a variety

of responses to olfactory stimuli. There are several olfactory systems that appear to have heterogeneous outputs. Two of these are the horizontal diagonal band (HDB) and medial preoptic area (MCPO) neurons in the olfactory system (Devore, Pender-Morris, Dean, Smith, & Linster, 2016) and the patterns of variation in responding to olfactory cues in *Drosophila* (Sartorre, Fanara, & Lavagnino, 2014). They both appear to be sources specifically of nongenetic phenotypic heterogeneity, and are both potential sources of research into the benefits of heterogeneity within the olfactory system. If either of these systems introduces nongenetic phenotypic heterogeneity that confers a benefit to an organism or population, that would indicate that biological noise is utilized to introduce nongenetic phenotypic heterogeneity in the olfactory system.

3.D.i: HDB and MCPO neurons in the olfactory system

HDB and MCPO neurons in the olfactory system are associated with differentiating between different odors, in addition to odor-specific learning. HDB and MCPO neurons in rats performing various odor related tasks were directly monitored. These neurons have extremely heterogeneous responses. Each neuron responds differently to the same stimuli and varying times, indicating that other factors are modulating the neural response to stimuli, (Devore, Pender-Morris, Dean, Smith, & Linster, 2016). Although this heterogeneous system is not well studied, it is possible that the heterogeneity in this neural system is the result of stochastic modulating factors. It is also likely that this heterogeneity of response allows the individual to respond in a variety of ways to the same stimulus, an important benefit of nongenetic heterogeneity. If this is the case then HDB and MCPO neurons in the olfactory system could be utilizing biological noise to produce a variety of responses to odors. This is an example of biological noise being used to confer a benefit within the olfactory system.

3.D.ii: Patterns of variation responding to olfactory cues in Drosophila

Another potential example of biological noise being used to produce nongenetic phenotypic heterogeneity is in patterns of variation responding to olfactory cues in *Drosophila*. One of the factors that provides and indicates environmental differences to the fruit fly *Drosophila* is the host fruit on which the fly hatches. Researchers hatched flies on grapes and peaches and then measured their responses to various natural odors. They discovered that flies from each population demonstrated different responses to olfactory stimuli, and even experienced different degrees of plasticity in learning new odors. This indicates that, depending on the environment in which they develop, *Drosophila* display different patterns of variation in response to olfactory cues (Sartorre, Fanara, & Lavagnino, 2014). This phenomena is not yet well studied, but it could be a form of adaptive diversification. If *Drosophila* are utilizing biological noise to produce adaptive diversification, this would be another example of biological noise being utilized to confer benefits through nongenetic phenotypic heterogeneity.

Conclusions from nongenetic phenotypic heterogeneity

Nongenetic phenotypic heterogeneity refers to variation within populations or groups of cells that is not the result of genetic differences. Nongenetic phenotypic heterogeneity is important in a variety of biological contexts. Cell differentiation, the process by which genetically identical cells adopt different roles within an organism, is a form of nongenetic phenotypic heterogeneity. Populations of individuals also use nongenetic phenotypic heterogeneity as a form of bet-hedging. In this way populations can survive highly variable environments, even if they share identical genetics. Understanding nongenetic phenotypic heterogeneity is important to our understanding of biological noise because it is one of the main applications of biological noise at the population level. Nongenetic phenotypic heterogeneity

helps us to understand why two members of one population raised in the same environment with identical genetics can vary. Nongenetic phenotypic heterogeneity is also commonly associated with bistability, and can connect the concepts of bistability and stochasticity.

Nongenetic phenotypic heterogeneity is not the only phenomena caused by biological noise that is advantageous. There is a second phenomenon associated with biological noise called Stochastic resonance that is also advantageous. Nongenetic phenotypic heterogeneity confers benefits to an organism or population through creating variation and diversity. Stochastic resonance confers benefits to an organism by tuning sensitivity of sensory systems.

Chapter 4: Stochastic Resonance in the Olfactory System

Nongenetic phenotypic heterogeneity can lead to benefits in the form of bet-hedging. It does this by creating noisy groups. Stochastic resonance uses noise in cellular signals to confer benefits. There is evidence that sensory systems can be tuned to increase sensitivity by incorporating biological noise; this phenomenon is called stochastic resonance (Wiesenfeld, & Moss, 1995). This is another example of biological noise being harnessed to benefit an organism. Stochastic resonance is particularly relevant to sensory systems such as olfaction, because it tunes the sensitivity of sensory systems specifically. Stochastic resonance can be induced by adding noise into a system or simply studied in a system where it is already present. The mechanisms of stochastic resonance are not well understood. It is possible that feedback and bistability could provide the increase in noise that is needed to produce stochastic resonance.

Stochastic resonance works by increasing the detection of weak information carrying signals by lowering the threshold of action potentials, which allows some subthreshold signals to trigger action potentials. Essentially, some random fluctuations within a system allow weaker stimuli to trigger a neurological response. Because these signals would not normally trigger any neurological response, this incorporation of random fluctuation increases the sensitivity of the sensory system. This is important because it suggests that incorporating biological noise into sensory signals can be beneficial, and may be widely used in sensory systems.

4.A: Adding noise to a neural net varies patterns in action potentials

The relationship between increasing noise in a system and increasing sensitivity has been established by varying the strength of the noise in a system in order to change the patterns of action potential spikes. These action potential spikes are neurological signals that respond to stimuli (Reinoso, Torrent, & Masoller, 2016). Increasing noise, to a certain point, increases the

number of action potentials. This is because, when noise levels are high, there are more opportunities for a low-level stimulus to trigger an action potential. If a sensory system can control the amount of noise incorporated into the signaling process then it can dial up and down the sensitivity of the system.

4.B: Stochastic resonance has been established in most sensory systems

Stochastic resonance has been studied as an inducible phenomenon in most sensory systems. Generally, the phenomenon is induced by introducing Gaussian White noise into the sensory system of an animal, such as a human or a mouse.

One study found that applying subsensory electrical noise through surface electrodes on the ankle can improve tactile perception in the feet. When the stimulation was optimized for the subject being tested their ability to perceive vibration on their feet improved by an average of 16.2% (Breen et al., 2016).

Vibrating insoles were given to human patients with balance problems. Balance problems in these patients were a result of a poor somatosensory function. The vibrating insoles introduced noise into their somatosensory system. The degree of balance problem was quantified by sway data. In other words, the amount that the patients wobbled. The vibrating insoles alone significantly increased the ability of the patience to balance without swaying (Priplata et al., 2006).

In another study the cutaneous mechanoreceptors of rats were stimulated with digitally generated electrical noise, and 11 of the 12 neurons tested showed increased sensitivity at particular levels of noise (Collins, Imhoff, and Grigg, 1996.) Stimulating the auditory nerve or the brainstem with noise can even improve hearing for those with significant hearing damage

(Zeng, Fu, and Morse, 2000). Inducing stochastic resonance is studied primarily as a method of enhancing the senses of elderly or neurologically damaged patients.

4.C: Stochastic resonance in systems similar to olfaction

However, very few articles have been published on stochastic resonance in olfaction. Some comparable systems have been studied. One of the cellular processes most similar to olfaction is chemotaxis, because both involve the sensing of chemicals in order to direct behavior.

The Chemotaxis system of E. Coli has been well studied, and the receptors involved can be sensitized using stochastic resonance. Stochastic resonance plays a role in filtering external noise (Patnaik, 2012).

Additionally, paddlefish use stochastic resonance to detect food using their electroreceptors in a way that is similar to olfaction (Russell, Wilkens, & Moss, 1999). These systems are similar in function and structure to olfactory systems, and support the hypothesis that olfactory systems utilize stochasticity in order to tune sensitivity.

4.D. Stochastic resonance in *Drosophila* olfactory processing

In addition, stochastic resonance appears to be a key factor in the sensitivity of *Drosophila* olfactory processing. There is a circuit in the olfactory system of *Drosophila* where one set of neurons connect to another in a one to one ratio. Although new information is not entering the system and there are the same number of neurons in each set, the second is more broadly tuned to inputs than the first. Specifically, although the synaptic connections between ORNs and PNs are one to one, PNs are more broadly tuned than ORNs. This appears to be due to a stochastic resonance like mechanisms involving lateral excitation, where connectedness within the layers may be introducing internal noise to broaden the sensitivity (Shang, Claridge-Chang,

Sjulson, Pypaert, & Miesenbock, 2007). This research is particularly interesting not only because it focuses on the olfactory system in particular, but also because it suggests a possible mechanism for the incorporation of stochasticity into neurological systems.

The presence of stochastic resonance in sensory systems, particularly in those similar to olfaction and within olfaction, is evidence that stochastic resonance could be an important part of the way our sensory systems function. Although biological noise is not often deeply incorporated into models of olfaction and other sensory systems the existence of stochastic resonance suggests that tools for utilizing biological noise are an important part of sensory function.

4.E: Stochastic resonance could have evolved in biological systems

Several studies suggest that colored noise and non-Gaussian noise can also tune sensitivity. Gaussian noise has a normal distribution of power at any given bandwidth. White noise has an equal power at any bandwidth. Colored noise does not, producing skewed levels of power at different bandwidths. Colored noise has been established in sensory neurons as capable of inducing stochastic resonance. Specifically, rat sensory neurons respond both to white noise and colored noise by enhancing their response to a weak signal (Nozaki, Mar, Grigg, & Collins, 1999).

A separate study induced stochastic resonance using noise that was both colored and non-Gaussian (Fuentes, Toral, & Wio, 2001). In fact, both studies determined that the effects of stochastic resonance were stronger under colored and non-Gaussian noise conditions. This evidence suggests that stochastic resonance is not a product specifically of White Gaussian noise. Biological systems can incorporate several types of noise to receive the benefits of stochastic resonance.

Even given the possibility that colored and non-Gaussian noise are sufficient for inducing Stochastic Resonance, each of the studies above focus on artificially incorporating noise into biological systems. None of them investigate stochastic resonance induced by the organism itself. Even if stochastic resonance is effective at tuning sensitivity, we need evidence that organisms are capable of inducing this phenomenon.

There is evidence that organisms have adapted to use stochastic resonance in vivo. It is possible that neurons do not use this mechanism to increase sensitivity, but it is unlikely. (McDonnell, & Abbott, 2009). Additionally, some of the studies above concern the study of sensory systems without added noise. Examples are Russell, Wilkens, and Moss's research on paddlefish feeding behavior and Shang, Claridge-Chang, Sjulson, Pyaert, and Miesenbock's research on the olfactory circuit of *Drosophila* (Russell, Wilkens, & Moss, 1999 and Shang, Claridge-Chang, Sjulson, Pypaert, & Miesenbock, 2007). This evidence suggests that organisms can use patterns of neurological connections and stochastic chemical fluctuations in order to internally induce stochastic resonance.

Another pitfall lies in the delicate nature of the tuning process. For most simple systems, noise strength needs to be specifically tuned for the stimulus. This means that researchers usually use a specific strength of noise depending on the stimulus they intend to use. This would be impractical for individual organisms, and requires precise and careful use of noise that biological systems may not be capable of. Populations of neurons are more complex than the simple systems often modeled. This gives these populations summation properties. This means that populations of neurons simply need to establish an optimal baseline level of noise to experience benefits from stochastic resonance (Collins, Chow, & Imhoff, 1995).

Finally, although stochastic resonance increases sensitivity to stimulus, the previously mentioned studies do not demonstrate that this sensitivity causes a change in behavior. If the increase in sensitivity is not actionable, there would be no advantage to utilizing stochastic resonance. Nevertheless, evidence suggests that increased sensitivity from stochastic resonance causes adaptive changes in behavior. Paddlefish changed their feeding behavior when stochastic resonance was used to improve their sensitivity. Additionally, humans were also able to optimize their behavioral responses to a visual stimulus using stochastic resonance (Kijato, 2003). This suggests that stochastic resonance is an evolutionary adaptation (Russell, Wilkens, & Moss, 1999).

Conclusions from stochastic resonance

Stochastic Resonance increases sensory systems' sensitivity to weak information carrying signals. It does this by lowering the threshold of action potentials. This allows some subthreshold signals to trigger action potentials. The biological noise, which acts like static, can allow weaker stimuli to trigger a neurological response. In this way, stochastic resonance uses noise in cellular signals to confer benefits. Stochastic resonance has mainly been demonstrated in clinical settings where noise was artificially added to a sensory system. However, it is possible that stochastic resonance has evolved in organisms in order to tune sensitivity.

The mechanisms of stochastic resonance are not well understood. It is possible that feedback and bistability could provide the increase in noise that is needed to produce stochastic resonance. Stochastic resonance is important to an understanding of how noise is used within sensory systems and has many other potential clinical applications. Adding noise to sensory systems to induce stochastic resonance could help patients with decreased sensitivity or help researchers understand the functioning of the senses.

Conclusion

Biological noise is ubiquitous in biological systems. Biological noise can be beneficial, such as in the cases of nongenetic phenotypic heterogeneity and stochastic resonance. It can also be deleterious when it interferes with biological signals. Biological systems have evolved to regulate the amount of noise within them in various ways. One of the key ways that biological systems regulate noise is with feedback. This regulation allows systems to tune noise depending on whether it would be beneficial or deleterious in a given situation. A system can either upregulate (increase) noise, or downregulate (decrease) noise. The reasons why a system might want to downregulate noise are easy to understand; noise can interfere with signals and cause inconsistent responses to stimuli.

The reasons why a system might choose to upregulate noise are less well understood. In this research we have established that there are at least two reasons that a biological system might upregulate noise. One is to upregulate phenotypic heterogeneity. The variation produced this way is good for populations because it leads to bet-hedging benefits and because it allows organisms to adapt to rapidly changing environments. Another is to induce stochastic resonance, which is a mechanism whereby organisms can increase their sensitivity to stimuli by turning up the noise in a sensory system.

There are two mechanisms that biological systems can use to regulate their noise levels. These are feedback loops and bistability. Feedback loops are some of the most universal regulatory systems of cells. Feedback loops change the outputs of systems and, by doing so, increase or decrease the noise in a system’s output. Negative feedback loops decrease biological noise, while positive feedback loops induce bistability, which is the second mechanism by which biological systems can regulate noise.

A bistable system has two stable states and a threshold in between them, preventing the system from sitting between the two states, or moving easily between them. As a result, bistable systems produce all or nothing outputs, which can be noisy. Bistability also allows cells or organisms to develop nongenetic phenotypic heterogeneity through cell differentiation. Because nongenetic phenotypic heterogeneity is a form of biological noise in groups, bistability can be described as increasing the amount of biological noise in systems.

There is no literature connecting all four of these concepts. However, the mechanisms of feedback and bistability, and the phenomena of stochastic resonance and nongenetic phenotypic heterogeneity, are all clearly related to the control of noise within biological systems. Feedback clearly results in bistability. Bistability is connected to nongenetic phenotypic heterogeneity. Feedback is also clearly related to noise regulation, and stochastic resonance is the result of the tuning of biological noise in sensory systems. It follows that each of these topics is necessary for a complete understanding of the functioning of noise within biological systems.

This complete understanding is vitally important to solving many modern day biological problems. Understanding nongenetic phenotypic heterogeneity in olfaction could help us to combat insect borne pathogens by giving researchers a more complete description of insect olfactory behavior. An understanding of stochastic resonance in olfaction could help us to understand the early mechanisms of neurodegenerative diseases. Loss of olfactory sensitivity is one of the first symptoms of degenerative diseases such as Parkinson’s and Alzheimer’s. We now know that sensitivity can be affected by stochastic resonance. It is possible that these early problems in olfaction could be related to a decrease in noise within the olfactory system. Understanding the ways in which the olfactory system uses noise could be key in uncovering the mechanisms of these diseases.

An understanding of biological noise in general may help us to solve many other human problems. Understanding that noise is not a problem in data collection, but rather an aspect of the data collected in biological systems that is important in its own right, could increase our understanding of many biological systems. Phenotypic heterogeneity is an aspect of noise that can lead to bacterial persistence in agriculture, an important modern problem. Noise has also been associated with aging and cancer. Understanding how noise works in biological systems could lead us closer to explanations and solutions for these problems.

In future experiments, researchers should look to feedback loops as indications of how biological noise is regulated within a system. Researchers interested in discovering noisy systems should look for positive feedback and bistability. Researchers seeking to understand bet-hedging and the benefits of noise at the population level should investigate nongenetic phenotypic heterogeneity. Researchers interested in the ways that sensory systems function should investigate the mechanisms of stochastic resonance, and work on finding the source of the biological noise used to create stochastic resonance in sensory systems.

In olfaction researchers can investigate the effects of feedback loops on olfactory systems. Introducing positive and negative feedback into olfactory systems to establish the relationship between positive and negative feedback and biological noise would be a good first step. Researchers in olfaction could also investigate the causes and effects of bistability in olfactory systems such as olfactory bulb mitral cells. Researchers should also investigate stochastic resonance as it relates to olfaction.

Biological noise is an important component of biological systems. An understanding of how biological noise is controlled within a system is important to a complete understanding of

that system as a whole. While biological noise is normally regarded as deleterious, it can also confer certain benefits through stochastic resonance and nongenetic phenotypic heterogeneity.

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