CHAPTER 1

General introduction
Summary

The term “preconditioning” has become widely used in various fields. It describes a correlation between the responses to two consecutive stimuli, similar in type but increasing in strength. In this thesis, we will expand the original definition of preconditioning, and distinguish between two sub-types, i.e. progressive preconditioning (PROP) and regressive preconditioning (REP). PROP refers to the standard definition of preconditioning, where an initial, non-lethal stimulus makes cells, tissues or whole organisms tolerant to a subsequent, otherwise lethal stimulus. Examples of PROP are well established and include ischemic preconditioning and the effect of vaccination (Dirnagl et al. 2003; Iliodromitis et al. 2007; Lin et al. 2014; Michael Bell 2002). REP refers to conditions where the initial small stimulus leads to an exaggerated, possibly fatal, response to a second, larger or similar stimulus, which by itself would not be lethal. Examples of REP include allergy and apoptosis progression (Costa et al. 2013; Drouhet et al. 1981; Samarasinghe et al. 2014).

This study uses systems biology approaches to examine the network principles underlying these two types of adaptation. Its next two chapters examine how variations in network design and in early stress exposure affect the response to subsequent stressors. In these chapters, network structures producing either PROP or REP are identified. Chapter four examines the effect of preconditioning on a cell’s decision between apoptosis and mitoptosis. It is disclosed that mitoptosis can lead to an emergence of PROP when the first stimulus cause significant decrease in total mitochondrial volume at the time when the second stimulus is applied.
Introduction

The term preconditioning was introduced by Murry and colleagues when they observed that an initial, low intensity ischemia decreased the intensity of a myocardial infarction in response to a subsequent potentially lethal ischemic injury (Murry et al. 1986). This phenomenon was named “ischemic preconditioning” and via this observation, preconditioning emerged as the definition of any state whereby a stressful non-lethal stimulus sets in motion a cascade of biochemical events that renders cells, tissues or whole organism more tolerant to a subsequent, potentially more harmful, stimulus (Flack et al. 1991; Michael Bell 2002; Murry et al. 1986).

Several studies refer to preconditioning as a protective mechanism that allows cells, tissues or an entire organism to adapt to repetitive external stimuli, without the need for reinforcement between stimuli (Carroll and Yellon 1999; Czibik et al. 2008; Faircloth et al. 2004; Heusch and Schulz 1997; Iliodromitis et al. 2007; Tomai et al. 1999; Ziegelhoffer et al. 1995). The underlying mechanisms that enable this adaptation in favor of tolerance, meeting our definition of PROP, may be both intuitive (e.g. up-regulation of metabolic enzymes to increase clearance of a drug upon second exposure) and unorthodox (e.g. autophagy, apoptosis or inflammation to a cell/tissue to protect the whole organism) (Aban et al. 2005; Cinel et al. 2003; Lin et al. 2013; Maulik et al. 1999; Shi et al. 2013; Yan et al. 2013). It should be noted that adaptation through preconditioning is not always total, leading to amelioration of the effects so as to provide ultimate survival of organism. In this chapter, we will elaborate on these two forms of preconditioning and introduce two new concepts, i.e. progressive and regressive preconditioning.

Progressive preconditioning (PROP) is consistent with the initial definition of preconditioning (Murry et al. 1986): it is based on a biochemical cascade that is triggered by the initial non-lethal stimulus and constrains the response to the latter stimulus, which may be of similar or larger intensity. The effect of this is to increase the lethal threshold, providing a positive function vis-a-vis survival, which is indicated by its name. Examples of PROP include ischemic preconditioning and vaccination (Lin et al. 2014; Murry et al. 1986). Regressive preconditioning (REP) indicates the alteration in response to two consecutive and non-accumulated stimuli, but this time the result is not an increase in survival probability but rather an increased probability of a fatal outcome. In REP, an endogenous mechanism is activated upon the first stimuli, resulting in a decrease in the lethality threshold (formally defined later). With this decrease in threshold, the probability of any subsequent stimuli being lethal rises even if the stimuli strength drops. This amplifies the probability of death and is thence called “Regressive preconditioning”. The progress from mitoptosis to apoptosis and in allergies both exemplify the REP phenomenon.
The difference between the phenomena of PROP and REP can be illustrated by analogy to the behaviours of two human couples that are facing disagreements in their relationship (Fig. 1.1). PROP is the behaviour of couple “A”, who solve their initial smaller disagreements via communication and are thereby able to handle more substantial subsequent conflicts with faster and better solutions. Effectively, their first response is discussion and the heat in the discussion decreases with time thanks to the trust they have built in their relationship so far. This trust also enhances the threshold preventing a catastrophic response, allowing better handling of larger challenges, which in turn grants a robust and persistent relationship. On the other hand, REP is the behaviour of couple “B”, who ignore small problems within their relationship and pretend everything is fine, instead of taking action to solve the problems. This avoidance could be viewed as a protective mechanism that reduces the risk of potentially damaging arguments, helping the relationship to survive for a long time. However, it also creates a disturbance underneath the surface of the relationship and this lowers the threshold to tolerating subsequent challenges. Hence, when couple B has even a small row, the risk that it will trigger a dangerously heated discussion is increased, and this may eventually end their relationship. The two situations are a bit paradoxical as couple A may have more conflicts than B, yet their relationship persists more than that of couple B.

This social interpretation of PROP and REP demonstrates a fair resemblance with another term widely used in psychology, i.e., the fight-or-flight response. According to Cannon et al., fight-or-flight is a paradoxical primary physiological response of a body that confronts a harmful incident, attack or threat in order to survive (Cannon 1927). This is analogous to the early responses of PROP, whereby couples fight to overcome their problems, and REP, whereby couples flee out of the crisis situations. With these relationships between definitions, we shall be able to make an unconventional connection between fight-or-flight and preconditioning conditions, expand and elaborate on each of their definitions, and even exemplify them with consideration of their role in adaptation.
Computational Systems Biology specializes in the reconstruction of biological realities in mathematical expressions (Westerhoff 2007; Westerhoff et al. 2009). Since we decided to embark on the systems biology approach, we need to reproduce the descriptions above in the forms of mathematical expressions. In order to identify similarities and differences, we shall first introduce a new term, i.e., the preconditioning-response coefficient ($\alpha_R$), which gives the ratio between the maxima of subsequent responses in the presence or absence of the prior stimulus (Eq. 1.1):

$$\alpha_{R21} \equiv \frac{R_{s=2}}{R_{s=1}}$$

(Eq. 1.1)

where $\alpha_R$ is called the preconditioning-response coefficient, and $\frac{R_{s=2}}{R_{s=1}}$ measures the variance in the response coefficients of the latter response in the presence ($R_{s=2}$) or the absence ($R_{s=1}$) of the prior stimulus:

$$R_{s=n} \equiv \frac{\Delta X_{\text{max}}}{X_{t=0}}$$

(Eq. 1.2)

Figure 1.1: Representation of the behaviors of couples A and B, who face problems in their relationship and respond in ways akin to PROP, positive values of preconditioning, and REP, negative values of preconditioning, respectively.
Here \( \Delta X_{\text{max}} \) is the amplitude of the response of the internal variable \( X \) to the stimulus, which is normalized by the level of \( X \) before the application of any stimuli. We consider the case where between stimuli, the stimuli return to its initial value but \( X \) can be any value between its initial value and its maximum value. We also define a lethality threshold (\( T \)) as the level of stimulus that suffices to set in motion a response that leads to catastrophe failure, including death of the cell, tissue or organism, and a lethality probability (\( P \)) as the probability that the system will proceed to this catastrophe. The lethality threshold (\( T(t) \)) is a potentially time dependent variable, calculated after response to the stimuli and based on the change in the response (\( \Delta X_{\text{max}} \)) to the stimuli applied and the type of preconditioning, defined with preconditioning constant (\( \mu \)) in the equation. \( \mu \) is positive for PROP and negative for REP.

\[
T_t \equiv T_{t-1} + \mu \cdot \Delta X_{\text{max}} \text{, if } T_{t-1} + \mu \cdot \Delta X_{\text{max}} < 0 \text{ then } T_t = 0 \quad (\text{Eq. 1.3})
\]

Lethality probability is defined as the chance to start of a series of events that will inevitably lead to cell death. Lethality probabilities (\( P_{L,n} \)) indicate the relative relation between the maximal presence of stimuli (\( S_{\text{max}} \)) and the lethality threshold (\( T(t) \)), at the present time. A low \( P_{L,n} \) value indicates a lower probability of death, while any value greater or equal to 1 indicates an inevitable progression to catastrophic failure, and is equalized to the one from then on.

\[
P_{L,t} \equiv 1 - \left( \frac{T_{t-1} - S_{\text{max}}}{T_t} \right), \text{ if } P_{L,t=1} \geq 1 \text{ then } P_{L,t=i} = 1 \text{ where } t \leq i < \infty \quad (\text{Eq. 1.4})
\]

The preconditioning coefficient (\( \alpha_p \)) is defined as the decrease in probability of death after the second stimulus in the presence or absence of the initial stimulus. Hence, the definition equates to the ratio of the lethality probabilities of the second stimulus when the prior stimulus exists (\( P_{L,S=2} \)) over when it is absent (\( P_{L,S=1} \)), such that any value below 1 indicates a reduced probability of death while above and equals to 1 show an increased probability of death:

\[
\alpha_{p21} \equiv \frac{P_{L,S=2}}{P_{L,S=1}} \quad (\text{Eq. 1.5})
\]

Furthermore, we define PROP and REP in terms of \( \alpha_R \) and \( \alpha_p \) for any stimulus that occurs in pulses or prolonged pulses. We here illustrate this for two consecutive stimuli, but the theory would be equally valid for a series of stimuli. PROP is a phenomenon where, even if the stimulus increases somewhat in amplitude, the death probability after the second stimulus is smaller than after the first. In contrast, REP is a phenomenon where the death probability after the second stimulus is higher than after the initial stimulus, even if this second stimulus is smaller in magnitude.
PROP if \( 0 \leq \alpha_R < 1 \)
\[
\begin{align*}
S_1 < S_2 & \text{ where } S_1 < T \\
1 > \alpha_p
\end{align*}
\]
REP if \( 1 \leq \alpha_R \)
\[
\begin{align*}
S_2 < S_1 & \text{ where } S_1 < T \\
\alpha_p > 1
\end{align*}
\] (Eq. 1.6)

Fig. 1.2 describes the two cases of preconditioning, i.e. progressive preconditioning (PROP) in the upper panel and regressive preconditioning (REP) in the lower panel. With progressive preconditioning, the internal variable \( X \) that is being monitored increases after the acute stimulus, returning slowly to its original steady-state level in the absence of further stimuli. If a second stimulus is applied before \( X \) has returned to background, then there is the potential for interaction between the two stimuli responses. In the case of PROP, the emergent behavior is a reduced response to the second stimuli, even if the second stimulus is more intense than the first. In contrast, REP behavior produces an exaggerated response to the second stimuli, even if it is of lower intensity than the first.

In order to exemplify the difference between the two forms of preconditioning, one may confront a system suspected of exhibiting PROP behavior with a set of stimuli of increasing magnitude, and a REP system with stimuli decreasing in magnitude, as illustrated in Fig. 1.2. The key difference between PROP and REP is that with the former, the lethality threshold increases with each new stimulus, whereas with the latter this threshold decreases. As a consequence, for REP, there is an increased probability that a later stimulus will exceed the threshold, therewith increasing the death probability (Fig. 1.2B). In the case of PROP, the threshold increases following each stimulus, moving further and further away from the level elicited by the original stimulus, such that the systems become resistant to much larger stimuli.

PROP and REP are likely to involve different network mechanisms, but at this moment it is not clear what these network topologies are, or how they interact to elicit these two contrasting behaviors. In this thesis, we aim to discover network principles underlying adaptation through these two forms of preconditioning in varied systems and conditions, using systems biology approaches. The following two chapters: chapter 2 and chapter 3, examine variations in network designs and the response to stress exposure. They identify network structures producing either PROP or REP, by including various aspects of biological realities, one at a time. Chapter 4 analyzes the relationship between oxidative stress, mitoptosis, apoptosis and necrosis with regard to cell and tissue survival.
Figure 1.2: Graphical representation of (A) progressive preconditioning (PROP) and (B) regressive preconditioning (REP) as alternative response patterns to the similarly repeated stimuli. The black line represents the intensity of the input signal, sudden peaks of which correspond to the stimuli. The red, orange, blue and dark blue lines represent the response of a measured internal property to the stimuli in the presence (red and blue) and in the absence (orange and dark blue) of the prior stimulus. The dashed grey line represents the lethality threshold: if the stimulus exceeds this threshold, death processes are set in motion and the probability of irreversible transition to the death state. Wider dashed line is in the presence of prior stimulus. The death probability is calculated for the presence (green line) or the absence (yellow line) of the prior stimulus.
Chapter 2: 'The History of a Stress Episode' is built on the analysis of small signal regulatory network of two nuclear receptors, GR and PXR, and their corresponding ligand: cortisol. With a system modelling approach, we examine the design principles underlying the biological response to stress. We show that this network is able to regulate both the magnitude and duration of the physiological response to a stress episode, which we believe is an important design feature to protect against the increased morbidity associated with chronic overstimulation. Then, we demonstrate that the differential kinetics of species within the network produce a 'biological memory' that can lessen the response to subsequent stress episodes, exemplifying PROP. In addition, we demonstrate that under certain biological conditions (i.e. reduced intensity of the initial stress episode, and reduced delays between stress episodes) REP behavior can also be observed. This shift in behavior emphasizes that the way stress episodes are presented are as important as the magnitude of the stress episode in determining the emergent biological response.

Chapter 3: 'Stress, Circadian Rhythms and Preconditioning' uses an extended version of the small signal regulatory network presented in chapter 2, with the addition of another regulatory network that reproduces the circadian rhythm of cortisol, plus a genome-scale reconstruction of human metabolism. This multi-level model enables us to examine genotype-phenotype relationships in human tissues, specifically liver, through mechanistic in silico methodologies. In addition to the possibility to examine molecular mechanisms underlying the emergence of stress desynchrony related complex pathologies, this chapter expands the knowledge acquired in chapter 2. The stress-response relationship, exemplified by TAT concentration, is tested by further examining the influence of cortisol circadian rhythm on preconditioning. The results reveal that the presence of a previous stress episode might diminish (PROP) or boost (REP) the response to a subsequent stress episode, with the emergent behavior dependent on the nature of the episodes; i.e. if they are induced briefly after each other or their magnitudes were large or small. The circadian rhythm, however, does not seem to influence the emergent behavior, possibly due to the fact that our system has a stress response that is a ratiometric scaler instead of an absolute level response.

Chapter 4: 'The tale of a mitochondrion: hero or villain?' analyzes the relationship between mitoptosis, mitophagy, apoptosis and necrosis from a molecular perspective, using dynamic in silico modeling techniques. Examination of the developed multi-level network enhances the understanding of mitoptosis, its role and limitations in protecting a cell from apoptosis and even the role in promoting mitonecrosis. We observe that mitoptosis, under specific circumstances can cause PROP: specifically, when a biological memory of the first stimulus is achieved through a significant decrease in total mitochondrial volume (a.k.a. mitochondrial number) at the time when the second stimuli is applied. Similar to the results obtained in chapters 2 and 3, a shift from PROP
to REP behavior can be obtained through changes in the dynamic nature of stimuli; namely, the intensity or the delay between consecutive stimuli.

The general discussion chapter summarizes the existence and role of preconditioning in different fields from biology to sociology, ecology and even politics. With this chapter, we develop a more generic appreciation of the occurrence and generality of the preconditioning phenomenon by provoking analogies with various topics in other fields of science. Through these analogies, and the simulation results obtained in the previous chapters, we demonstrate the potential benefits of a mathematical formulation of preconditioning concepts: PROP and REP, which enhances our understanding of preconditioning phenomenon in the context of diseases such as metabolic disorder, depression, and cancer. We conclude that the preconditioning is another aspect of biological complexity; an aspect ripe for implementation in systems medicine.