The learnt phenotype: Physiological, behavioural and immunological adaptation to environmental stimuli

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Abstract

Measuring the response of animals to environmental stimuli is a central component of assessing animal welfare. Host responses are usually considered to be governed through three interrelated modalities: physiology, behaviour and immune defence. Understanding how these responses are regulated is necessary before we can interpret what the responses tell us about the animal. The traditional view of regulation of physiological process is encompassed by the concept of homeostasis. This view holds that regulation occurs through feedback loops that drive physiological variables, for example, blood pressure or metabolite levels, towards a constant set point. More recently this model has been superseded by the concept of allostasis, which propses that regulation is a dynamic process that anticipates the predicted load on the host. For instance, the host learns though experience the oxygen demand of tissues associated with a particular pattern of exercise and elevates heart rate at the commencement of exercise before the need actually occurs for increased blood flow to the relevant tissues. Participation of the central nervous system in the processes of learning, prediction and subsequent dynamic regulation is a key component of the concept of physiological regulation through allostasis.

With the realisation that prior experience modifies physiological processes, we can see that “adaptation through learning” is **the** central strategy for host adaptation to environmental stimuli. Modification of behaviour through learning is well documented. In addition, both the adaptive immune system and the innate immune system exhibit characteristics of a system that can be modified by prior experience. From an evolutionary perspective we can consider that the consequence of learning may be to minimise costs to the host by optimizing responses both to noxious and to beneficial environmental inputs.

We can conclude that the stress response phenotype of an animal is a learnt attribute. What are the consequences of the learnt phenotype for measuring animal welfare? When

we measure host responses we need to consider them in the context of the life history of the animal and its community. The magnitude and type of responses to a stimulus at a particular point in time can be influenced both specifically by prior experience, and non-specifically, for example by age, diet, gender, physical conditions of the environment. Responses may be part of the learning experience of the animal and thus the initial experience may diminish or exacerbate the impact of future exposure to the stressor. This viewpoint of “how animals work” should help inform the assessment of animal welfare, as well as lead to better design of studies on animal welfare.

Many examples of physiological, behavioural and immunological adaptation through learning are currently only phenomenological descriptions. An important challenge for the future is to better understand at the molecular level the mechanistic basis that underpins adaptation.

Introduction

When we attempt to assess the welfare of an animal we usually measure a range of variables that provide information about its physiological, behavioural, immunological and emotional status, together with the context within which the animal exhibits these responses. These measurable attributes or traits are components of the phenotype of the animal. The phenotype arises from the interaction of the genotype with the environment ($P \sim G \times E$) (Johannsen 1911). Typically, the traits measured when assessing the welfare of an animal are dynamic and change over a relatively short time frame in response to the environmental inputs the animal receives. Indeed the capacity to change over short time frames (hours, days or weeks) is a characteristic that can make traits informative about the current welfare of the animal. To better understand what the traits tell us about the animal, it is useful to consider how phenotype is regulated.

Regulation of phenotype

Following the rapid growth in recent years of knowledge about genomes, the construction and maintenance of phenotype has (re)emerged as a central puzzle of biology, and in particular how regulation is integrated across the scales of complexity from genes to organs, whole animals and their communities. We can envisage the path from genome to organism to be composed of a series of processes including

1. expression of genes,
2. accumulation and utilisation of resources,
3. transmission of information across boundaries (eg the cell membrane),
4. turnover of structural components such as molecules and cells,
5. replication, and
6. repair.

These processes are influenced by

1. errors,
2. adventitious damage, and
3. senescence

and are regulated by mechanisms including

1. structural changes to molecules that affect their function or their rate of turn over (eg glycosylation, methylation, acetylation, ubiquitination, nitration, nitrosylation)
2. positive and negative feedback pathways, including higher order modulation of the feedback pathways (eg feedback on feedback), and
3. delivery and removal of substrates and products. (eg through vasodilatation)

A fundamental organisational principle of metazoan (multi-cellular) organisms like mammals is that they are composed of communities of cells which individually lack the autonomy to control their own uptake of nutrients and rate of metabolic activity. Rather, these activities are controlled by signals such as hormones delivered to the cell. Indeed, it is this absolute “social” dependence of cells that creates the opportunity for regulation of life processes within the multi-cellular organism. Table 1 provides a brief summary of some structural elements of the life process and some examples of regulatory mechanisms.

Table 1. Some mechanisms by which life process of animals is regulated

Structural element	**Regulatory mechanism**
Gene transcription	DNA methylation at CpG dinucleotides Histone acylation Heterochromatin binding Modifiers (eg promotors, repressors) Non-coding RNAs
Expressed gene products	Post-translational modifications eg: ubiquitination, nitration and nitrosylation
Cells and organs	Hormones (eg morphogens, classical hormones, interleukins) Contact dependent feedback from extracellular matrix Contact inhibition

	Modulation of receptor prevalence and sensitivity Modulation of signal transduction pathways Nutrient delivery (blood flow, vascular permeability) Gas tensions pH Tissue temperature
Whole animal	Behaviour Homeostasis Allostasis
Community	Group behaviours Socially transmitted behaviours

Homeostasis and allostasis

Since phenotype arises from the interaction of genotype with environment, it follows that animals, and cells within them, need to sense the environment in which they are present. Information about the environment is gathered through receptors that process signals from the environment. In fact animals have two environments: the internal environment within the body, and the external environment. The importance of stimuli in these two locales is quite different. Whereas the animal attempts to manage the internal environment within certain limits to optimise biological process, the external environment and the stream of stimuli it delivers is to a large degree beyond the control of the animal. The importance of maintenance of a constant internal environment was first recognised by the French physician, Claude Bernard, through a process subsequently called homeostasis by Walter Cannon (1929). The common conception of homeostasis is of a process of feedback that drives physiological variables towards a set point, for instance a constant O_2 tension in blood. The concept of homeostasis was expanded by Sterling and Eyre (1988) to describe the dynamic change of physiological variables that occurs in order to meet anticipated demands of the host; a process they termed allostasis. Like homeostastis, the outcome of allostatis is maintenance of the internal environment, but unlike homeostastis, allostasis identifies the role that change in physiological variables plays in maintaining a stable internal environment. (In this respect, homeorrhesis can be considered to be a subclass of allostasis). A key feature of allostasis is that change in the variable can precede the demand. Sterling uses the example of blood glucose concentration in elite footballers, which can rise to such a high level in the minutes before a match that players exhibit glucosuria.

For a given genotype, it is common for phenotype to vary with environment. Indeed the contrary case, expression of a constant phenotype across a range of environments is sufficiently unusual to attract a label, canalisation (Waddington 1942). Thus, typically, individuals adapt through phenotypic change to environmental stimuli. When stimuli are sufficiently noxious to threaten viability of the individual the host response is often termed a defence reaction. Adaptation to threats from the environment occurs principally through three interrelated modalities: physiology, behaviour and immune defence, although changes in structure and function of organs and tissues can also occur. Together these changes are sometimes termed phenotypic plasticity (Price *et al.* 2003).

Role of learning in phenotypic adaptation

Omitted from the list of processes for regulating phenotype noted above is learning. Prediction of the change in a physiological variable (eg glucose) that is needed to meet an anticipated load (eg physical exertion during a football match) is a learned response; it is a response that is acquired by experience and orchestrated through activity of the central nervous system (Stockhorst 2005). To what extent are other aspects of phenotypic adaptation learned responses? A useful distinction here is the difference between innate responses, where a fixed type and pattern of response occurs on each exposure to a stimulus, and learned responses, where the type or pattern of response is modified by experience. An important aspect of learned responses is prediction or anticipation of recurrence of a stimulus.

A capacity to learn is a phyogenetically ancient trait that is even exhibited by single celled organisms. The slime mould, *Physarum* can learn patterns in environmental stimuli and modify its behaviour in anticipation of recurrence of the environmental stimulus (Saigusa *et al.* 2008). A classical model of learning is Pavlovian conditioning in which a novel conditioning stimulus is associated by the animal with a physiological response that normally occurs as an involuntary or reflex response to an environmental stimulus. Pavlov's famous demonstration of the phenomenon was the association by dogs of the sound of a bell with salivation in anticipation of being fed. A large number of fear, endocrine, physiological and immunological responses can be enlisted as conditioned responses. Perhaps surprisely amongst these is a considerable number of immunological responses, including the acute phase response, fever, cytotoxic T cell activity, NK cell activity and antibody production, all of which can be conditioned (reviewed by (Husband

1993). Once conditioned, these responses do not require a classical immunological stimulus such as endotoxin or antigen for re-enlistment, but can be recalled by exposure to the “non-immunological” conditioning stimulus. Such classical conditioned responses fit the model of homeostatic regulation described as allostasis. They are learned responses overseen by activity in the central nervous system that predict that a specific consequence will follow exposure to the conditioned stimulus. An important question is to what extent do normal life experience and environmental inputs, as opposed to contrived experimental conditions, lead to learned predictive responses being deployed by animals? It may be that, as time passes, the stimuli an animal experiences are constantly undergoing association and disassociation with homeostatic responses, depending on the strength of reinforcement. Other important types of learning include habituation, where responses to a stimulus diminish over time, and sensitization, in which the response is amplified upon repeated exposure to the stimulus. These differ from Pavlovian conditioning in that the associative link between the stimulus and the response remains unchanged. (Pavlovian conditioning is a class of associative learning; operant conditioning in which an animal learns to modify its voluntary behaviour in response to a stimulus is another).

As noted above, responses of the animal to environmental stimuli can be innate or learned. The capacity for some behavioural responses to be learned is well recognised. Some examples of the capacity for physiological responses to be learned are described above. The extent to which the traditional senses involve innate and learned pathways is still being discovered. For instance, it was recently reported that olfaction in mice involves both innate and learnt pathways (Kobayakawa *et al.* 2007). Further research to identify the extent to which learnt allostatic responses underlie physiological process is needed. To date, the concept of allostasis has been taken up most strongly by neurobiologists and social scientists to describe the interface of behaviour with physiology that occurs between social stress, diet, disease and substance abuse in humans (Sterling 2004). Recently the concept of allostasis has been proposed as a model for assessing welfare of livestock (Korte *et al.* 2007).

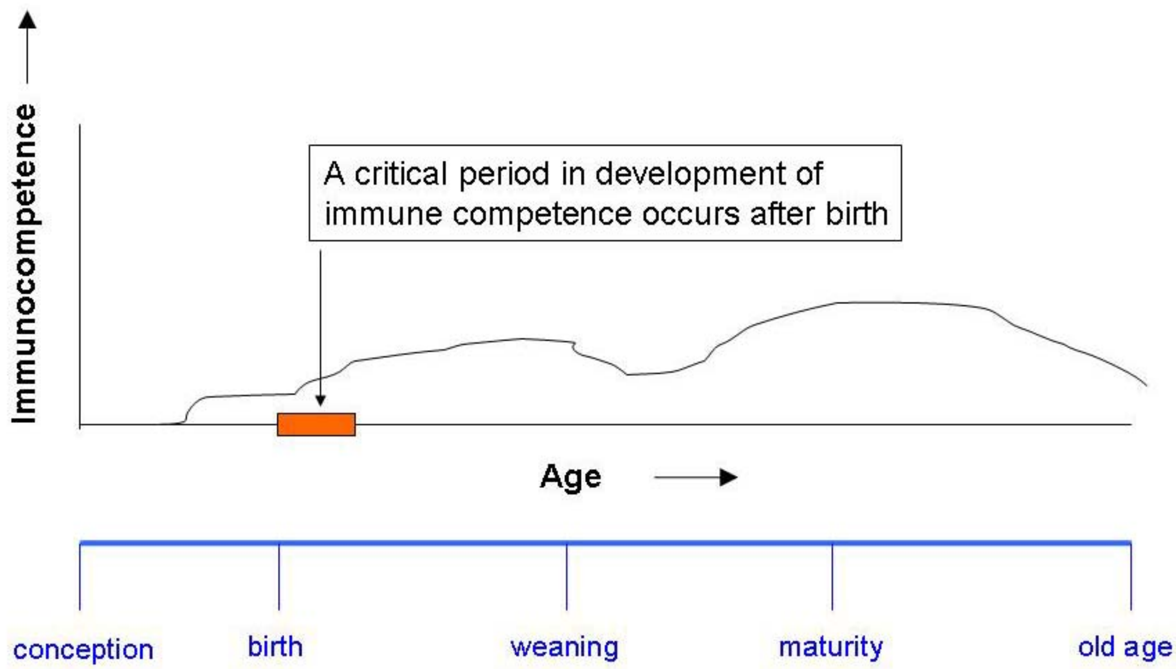
The third arm of host responses to environmental stimuli is the immune system. Separate to the phenomenon of Pavlovian conditioning of immune responses noted above, the capacity for the immune system to exhibit secondary, anamnestic, learned responses is central to our understanding of adaptive immunity. We can see here a common strategy employed by the host for responding to environmental stimuli and in particular to noxious stimuli that may threaten the integrity of the animal. In addition to innate responses, there is the capacity to learn from experience and deploy specialised physiological, behavioural and immunological responses that are specific for the inciting stimulus. As I have discussed elsewhere, in evolutionary terms it seems likely that the specialised response is less costly for host resources than the non-specific defence response (Colditz 2008). Thus the capacity to learn can confer an advantage. To emphasize the role of experience in fine-tuning the specificity of host defence reactions and thereby modifying the phenotype of the animal, I propose the term, the *learnt phenotype*.

Implications of the learnt phenotype for assessing animal welfare

If the physiological, behavioural and immunological responses of an animal change with experience, there are important consequences for our interpretation of the variables we measure when attempting to assess the welfare of an animal. Typically we are assessing the impact of a husbandry practice, a production environment, a management practice or a disease on the welfare of individuals or of a group of animals at a point in time. Gathering contextual information is part of the assessment process. The capacity of the response profile of the animal to be modified by experience indicates that in addition to contextual information about the environment we should also assess the previous life history of the animal and the probable future experience of the animal. Thus previous experience may diminish or accentuate a current response, while the current experience under assessment may be playing a formative or disruptive role in preparing the animal to cope better with future experiences.

In addition to the place of the current experience in the experiential life-history of the animal, the position of the animal within its own developmental history is also important. Sensory systems of the animal undergo both prenatal and post-natal development and maturation, a sequence known as ontogeny. Developmental histories are displayed not only by the traditional senses but also by the immune system and cell surface receptor systems for sensing molecular events in the extracellular environment. Traits classically measured when applying quantitative genetic methodologies to animal breeding also typically exhibit a developmental history. Importantly, sensory systems require on-going stimulation for normal development and function, and many go through critical periods when there can be modification of the functionality of the system that persists for the life of the animal. The theoretical change in the capacity of the immune system to respond to a stimulus is presented in Figure 1.

Figure 1. The capacity of the immune sensory system to respond to molecular threats changes during the life history of the animal



Many other host and environmental factors can also influence the response variables we measure to assess welfare. These include:

1. reproductive status
2. body condition
3. age
4. concurrent infection
5. diet
6. concurrent stressors
7. social environment

and so forth. While it is well accepted that such contextual information is important to the assessment of animal welfare, an understanding of the influence of prior experience and ontogeny on the learnt phenotype increases our awareness of the importance of also considering the life history of the host when assess animal welfare.

Conclusions

Governance by the central nervous system of physiological, behavioural and immunological responses of the animal to environmental stimuli creates the capacity for many responses to be modified by experience. The allostatic regulation of host defence responses that arises from experience involves prediction of the anticipated load of a stressor on the host and modification of physiological functions in preparation for meeting the anticipated load. Further research is required to better characterise the impact of experience on host defence responses so that we can more accurately interpret the responses being assayed during a welfare assessment within the prior and possible future life story of the animal. An important consequence of the capacity of physiological, behavioural and immunological responses to be modified by experience is that there may be considerable untapped scope to better prepare animals for life experiences through appropriate handling and training, especially during critical developmental periods.

References

Cannon WB (1929) Organization for physiological regulation. *Physiological Reviews* **9,** 399-431.

Colditz IG (2008) The costs of immune responses. In 'Resource Allocation Theory Applied to Farm Animal Production'. (Ed. WM Rauw) in press. (CABI Publishing: Wallingford, UK)

Husband AJ (1993) Role of central nervous system and behaviour in the immune response. *Vaccine* **11,** 805-816.

Johannsen W (1911) The genotype conception of heredity. *American Naturalist* **45,** 129-159.

Kobayakawa K, Kobayakawa R, Matsumoto H, Oka Y, Imai T, Ikawa M, Okabe M, Ikeda T, Itohara S, Kikusui T, Mori K, Sakano H (2007) Innate versus learned odour processing in the mouse olfactory bulb. *Nature* **450,** 503-5U5.

Korte SM, Olivier B, Koolhass JM (2007) A new concept of animal welfare based on allostasis. *Physiology & Behavior* **92,** 422-428.

Price TD, Qvarnström A, Irwin DE (2003) The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B* **270,** 1433-1440.

Saigusa T, Tero A, Nakagaki T, Kuramoto Y (2008) Amoeba anticipate periodic events. *Physical Review Letters* **100,** 018101-1-018101-4.

Sterling P (2004) Principles of allostasis: optimal design, predictive regulation, pathophysiology and rational therapeutics. In 'Allostasis, Homeostasis, and the Costs of Adaptation'. (Ed. J Schulkin) pp. 17-64. (Cambridge University Press: Cambridge)

Sterling P, Eyer J (1988) Allostasis: a new paradigm to explain arousal pathology. In 'Handbook of Life Stress, Cognition and Health'. (Eds S Fisher and J Reason) pp. 629-649. (J. Wiley and Sons: New York)

Stockhorst U (2005) Classical conditioning of endocrine effects. *Current Opinion in Psychiatry* **18,** 181-187.

Waddington CH (1942) Canalization of development and the inheritance of acquired characters. *Nature* **150,** 563-565.