

Anxiety in Relation to Animal Environment and Welfare

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Summary

Negative emotions do not compromise welfare, as long as they do not exceed the individual's adaptive capabilities. Anxiety, though a negative emotion, is highly conserved during evolution, and essential for enabling an individual to both escape from dangerous situations and to avoid them in the future, i.e. to adapt to environmental challenges. However, the interactions between anxiety and environment are highly dynamic and can result in non-adaptive anxiety responses. Non-adaptive anxiety responses not only compromise the animal's welfare, but may be substantially detrimental to experimental results even in non-behavioural studies by dramatically reducing the reliability of the study results obtained. Detailed knowledge about the emotional phenotype of experimental animals used is necessary to reach a balance between reliability of experimental research and the welfare of laboratory animals.

Aversive emotions in welfare

Definitions of welfare vary widely, but scientists and society agree that welfare is about more than just good physical health (*Mills, 2008*). Today it is generally accepted that animals perceive a variety of emotions and, consequently, the individual's emotional state has to be taken into account when welfare is considered. One broadly used welfare concept is the five degrees of freedom by Brambell (*1965*), who states that the absence of aversive states, such as hunger, distress and pain, as well as the possibility to express normal behaviour can be used as safeguards for welfare. This concept has been criticised because it neglects positive emotions as important part of intact welfare. However, it can be questioned whether negative emotions, such as fear and anxiety, necessarily compromise welfare.

Broom states that welfare is "the animal's state in relation to its ability to cope with its environment" (*1986*). This definition allows negative as well as positive emotions – as long as the individual can adequately cope with them. "Thus, the most widely accepted definition of animal welfare is that it comprises the state of the animal's body and mind, and the extent to which its nature (genetic traits manifest in breed and temperament) is satisfied" (*Hewson, 2003*).

Taking into consideration that an individual's needs depend on its biological nature, the absence of aversive experiences/emotions might not be sufficient to guarantee the individual's welfare: the animal may need positive experiences as well. But the experience of aversive experiences/emotions need not be detrimental to the animal's welfare providing the individual is capable of coping with those negative experiences. Based on the example of anxiety, we will outline in the following that negative emotions do not necessarily compromise welfare, as long as they do not exceed adaptive capabilities.

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Homeostasis versus allostasis

When an animal is confronted with environmental challenges, it responds with behavioural and physiological mechanisms to maintain a constant internal milieu, a mechanism called homeostasis (Bernard, 1865; Cannon, 1932). Homeostasis requires that physiological parameters are maintained at a certain level, termed the set point. For distinct physiological systems, such as body temperature and blood pH level, maintenance of homeostasis at a fixed set point is crucial for survival. Consequently, these systems will be modulated when challenged in a time limited manner and turn back to normal as fast as possible. Other systems, such as central nervous receptor systems, in contrast, may show long-lasting changes, i.e. shifts of their set point, due to external challenges. These shifts characterize the adaptation of the organism in a changing environment.

When applying the concept of homeostasis to animal welfare, it means that any environmental challenge will compromise welfare. Another concept, allostasis, which was first introduced by Sterling (Sterling and Eyer, 1988), implies that an organism must be able to vary its internal milieu in order to appropriately adjust to changing environmental demands (Koolhaas et al., 1999; McEwen, 2000; Koob and Le Moal, 2001). Allostasis thus refers to maintaining stability through change, i.e. by adaptive processes, contrasting the idea of a fixed, context-independent homeostatic level (McEwen, 2000; Koob and Le Moal, 2001). The costs to the body for adapting to environmental challenges, called allostatic load, correlates to the magnitude of environmental stimulation (McEwen & Stellar, 1993). Following this concept, animal welfare can be described as the physiological and behavioural ability of an animal to maintain allostasis, i.e. to adapt to environmental challenges (Korte et al., 2007).

The impact of the environment

The individual's adaptive capability depends upon the interaction of internal and external factors.

The genetic makeup of the individual provides it with a distinct susceptibility to the impact of any environmental stimulation. Nevertheless, the behavioural expression of the individual's genetic background can be modulated by environmental factors (Paylor et al., 1992). Parameters measuring alterations in brain, behaviour and physiology related to emotions such as anxiety are thought to be highly vulnerable to environmental factors (Pryce et al., 2002), but literature from research in laboratory rodents also shows an inconsistency in the effects of distinct environmental conditions on anxiety-related behaviour. Early aversive experiences can induce long-lasting changes with respect to responses to aversive events in later life (Chorpita & Barlow, 1998; Barros et al., 2006), but it has not been investigated whether this response probability indicates good or poor adaptive abilities. Dimitsantos et al. (2007) found anxiety-related behaviour of Sprague-Dawley rats in an elevated plus maze (EPM) to be affected by pre-weaning litter size. In contrast, increased cage size had no impact on reproductive behaviour in C57BL/6Tac mice or behaviour of offspring (Whitaker et al., 2007) and 2 weeks of enrichment by a nest box revealed no effects on anxiety-indicating EPM behaviour in BALB/c mice (Okva et al., 2007).

Cage enrichment was also found to lead to an increased ability to adapt to novel situations in mice (Tuli et al., 1995; Van de Weerd & Baumans, 1995; Baumans, 1997) and to result in a broader behavioural repertoire as well as less sensitivity to stressful experiences (Van de Weerd et al., 1997). Morphology, physiology and chemistry of the CNS and the psychic abilities of animals are affected by the complexity of housing conditions (Rosenzweig, 1998). Notably, environmental enrichment (interpreted as positive stimulation) can stimulate dendritic growth, cortical thickness, levels of nerve growth factor, increased brain weight (Winoccur, 1998), hippocampal neurogenesis and behavioural and cognitive flexibility (Kemperman et al., 1997). Furthermore, Soffié et al. (1999) found that an enriched environment positively affects plasticity of

e.g. hippocampus and cerebral cortex and facilitates e.g. the performance of complex cognitive functions. Environmental enrichment however, does not *per se* increase the emotional response of animals: several studies found decreased levels of emotional responses due to environmental enrichment (Chamvove, 1989; Chapillon *et al.*, 2002; Phan *et al.*, 1999).

Findings on the influence of an important environmental factor, the social environment, are also inconsistent. In our own studies, we did not find any anxiety-related behavioural differences in male mice from different inbred strains after being housed either singly or in social groups (unpublished observations). Other groups either found no (Rodgers and Cole, 1993), anxiolytic-like (Voikar *et al.*, 2005) or anxiogenic-like (Ferrari *et al.*, 1998) effects of individual housing in mice. For high-anxiety rats, anxiogenic-like effects due to individual housing have been described as well (Ohl *et al.*, 2002).

One possible explanation for the inconsistency of these results might be the within-cage testing order of group-housed animals, as suggested by Lyte *et al.* (2005). They found behavioural differences in the EPM between first- and second-tested CF-1 male mice housed in pairs. First-tested animals made fewer closed-arm entries, an indication of being less anxious. Chesler and colleagues (2002) were able to eliminate within-cage testing order effects on analgesic and nociceptive sensitivity by preventing the exposure of tested male Swiss Webster mice to naïve male conspecifics. Distress, due to the exposure to a novel environment, might thus be communicated, e.g. by ultrasonic calls (Liu *et al.*, 2006) or odours and might even negatively affect welfare of the animals by modulating regulatory adaptive systems. Another factor potentially confounding results, e.g. of tests for anxiety, which has to be taken into consideration at least for male mice, is their territorial behaviour, that can lead to aggressive encounters (e.g. van Loo *et al.*, 2003; Van Oortmerssen, 1971; Bisazza, 1981; Brain *et al.*, 1990). Importantly, it has also been shown

that identical environmental experiences can lead to different responses due to differences in innate anxiety-levels (Ohl *et al.*, 2001).

Systematic research on the nature of environmental factors that are important for animal welfare is incomplete. However, this short selection of scientific results indicates that the effects of environmental conditions are related to the animal's adaptive abilities, and standardized conditions do not necessarily guarantee good animal welfare. Standardised housing conditions still remain a useful tool since i) it is unrealistic to create tailored conditions for every laboratory animal strain and ii) they allow for the systematic investigation of gene-environment interactions. In any case, detailed knowledge about the emotional phenotype of the animals used is essential for the reliable interpretation of experimental results.

Innate anxiety and adaptation

Selective breeding programmes in laboratory animals are generally focused on highly specific characteristics. However, selection may have an (unintended) impact on other characteristics, such as emotional traits, and may lead to reduced adaptive capacities, which can compromise biological functioning and thus may impair welfare and quality of life in animals (Ohl *et al.*, 2007). Anxiety is not a unitary phenomenon: it can be divided into innate (trait) or situation evoked (state) anxiety (Ohl, 2005; Belzung and Griebel, 2001). However, it is extremely difficult to separate the two phenomena as animals with high trait anxiety often show high state anxiety as well. Habituation towards a novel stimulus, on the other hand, might show a discrepancy between state and trait anxiety. As trait anxiety is a durable characteristic of an animal, it depends less on environmental challenges and might show less adaptability over time. High state anxiety evoked by environmental stimuli, will show adaptation over time, when cognitive components gain the upper hand.

Strain comparisons reveal that the same environmental stimulation can elicit varying

behavioural and physiological responses in rodents. Male rats selectively bred for high and low avoidance behaviour (high and low trait anxiety, respectively) in the elevated plus maze show pronounced differences in home cage behaviour when housed socially in groups of 3-5 in non-enriched cages (Henniger *et al.*, 2000), with high anxiety rats being more inactive and affiliative than low anxiety rats. Notably, this contrasting behaviour is not paralleled by differences in stress hormone levels, indicating that both behavioural profiles lead to successful adaptation to the home cage environment. The same rat lines show extremely different behavioural responses to a novel environment, such as the modified hole board test (Ohl *et al.*, 2001). Although in contrast to their low anxiety counterparts, high anxiety rats strongly avoid the unprotected area of the test environment, they still respond with higher stress-hormone responses than low anxiety rats. These results indicate that the more pronounced avoidance behaviour in high anxiety rats is not sufficient to cope with the novel environment. However, repeated exposure of high anxiety rats to the same test environment reveals fast habituation as reflected by a rapid decrease in avoidance behaviour over time (Ohl *et al.*, 2002).

Habituation reflects a waning of a behavioural response elicited over time or after repeated or prolonged exposure to the same environment/stimulus and is a form of non associative learning (Thompson & Spencer, 1966; Leussis and Bolivar, 2006). It allows the animal to differentiate between biological meaningful stimuli to minimize reception and processing of irrelevant sensory input (Eisenstein *et al.*, 1996) The cognitive nature of habituation to a novel environment has been demonstrated for example by effects of pharmacological compounds on learning and memory in rodents (Platel and Porsolt, 1982). Notably, gen-environment interactions might influence habituation processes and may cause a hypersensitive or hyposensitive (i.e. non adaptive) response. Such habituation profiles can be seen in inbred mouse strains often used as contrasting experimental groups in anxiety research.

Both low-anxiety rats and C57BL/6 mice initially show non-anxious behaviour in a novel environment, followed by no further habituation during repeated exposure (Ohl *et al.*, 2002, 2003). High anxiety rats as well as DBA/2 mice, initially being highly anxious, in contrast show rapid habituation during repeated exposure (Ohl *et al.*, 2002, 2003). Notably, initial high anxiety is paralleled by high cognitive performance in both species. In a different study, Thiel *et al.* (1999) found better habituation in rats with higher behavioural reactivity in a novel environment, indicating a close interaction between habituation and anxiety-related behaviour as well. If then anxiety behaviour is cognitively driven in an adaptive manner, it is unlikely to compromise welfare.

In contrast to the adaptive type of anxiety behaviour, the 129P3/J inbred mouse strain, frequently used as background strain for targeted mutagenesis (Simpson *et al.*, 1997; Cook *et al.*, 2002), shows a completely different profile (*unpublished observations*): no habituation of anxiety-related behaviour can be seen over time. Cognitive testing shows that the 129P3/J mice are able to discriminate in a one trial object memory task, suggesting that impaired adaptation after repeated exposure is not caused by general cognitive deficits in this strain (*unpublished own observations*). Further research is certainly needed on these topics, but we hypothesize from our recent findings that high anxiety behaviour may not be a valid indicator for compromised welfare *per se*.

Besides genetically based innate (trait) anxiety, environmental conditions can have major influence on an animal's ability to adapt and can finally determine an individual's level of trait anxiety in later life. For example, chronic, inescapable or uncontrolled stress may lead to the impairment of normal regulatory adaptive systems and may result in high anxiety phenotypes. Early postnatally stressed mice are more susceptible to chronic stress during adulthood compared to non stressed control mice (Chung *et al.*, 2005). Additionally, chronic stress during adolescence, created by forming an unstable social environment for a prolonged period

of time (Schmidt *et al.*, 2003) or exposing animals to variable stress (Marin *et al.*, 2007), can result in a permanent alteration of centrally regulated stress systems and increased anxiety as well.

It is of note that chronic stress can have different effects in individuals from the same strain in that some individuals are more vulnerable to chronic stress exposure whilst others seem largely unaffected. This within-strain effect has also been described for responses in other behavioural paradigms. For example Piazza *et al.* (1990) reported that outbred Sprague-Dawley rats can be separated in high and low responders in an amphetamine administration paradigm based on their locomotor response to novelty. Similarly, Homberg *et al.* (2004) reported individual differences in various aspects of cocaine self-administration in outbred Wistar rats following selection based on grooming behaviour or response to amphetamine. These results illustrate the importance of the individual plasticity within the brain's emotional circuits in actively maintaining an emotional homeostasis: while the individual's innate level of anxiety determines its response to environmental stimulation, its emotional level will be modulated by the same stimulation as well. Thus the interaction between anxiety and environment is highly dynamic, following the concept of allostasis.

Anxiety and welfare

Anxiety is an essential emotion, which is highly conserved during evolution. In principle, anxiety is an adaptive reaction when an animal is confronted with danger or threat. Thus, anxiety enables an individual to escape from dangerous situations and to avoid them in the future, i.e. to adapt to environmental challenges. If, however, anxiety-responses are inappropriate, the individual's ability to adapt to external factors can be substantially compromised. In case of inappropriately low anxiety, the individual may be insufficiently prepared to avoid potentially dangerous situations. In case of inappropriately high anxiety, the individual may not be able to adapt to changing environmental

stimuli, resulting in chronic stress and compromised welfare. As outlined in the above, negative emotions do, however, not necessarily compromise welfare, as long as they do not exceed adaptive capabilities (fig. 1).

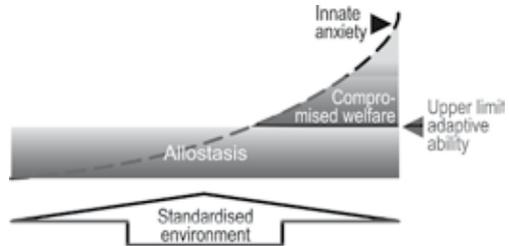


Figure 1. An increased level of innate anxiety does not necessarily result in compromised welfare. Based on the interaction between genetic susceptibility and environmental stimulation, each individual is characterised by its ability to adapt to challenges within a distinct range (allostasis). If the upper limit of this range is reached and adaptive abilities are exceeded, the individual's welfare will be compromised.

Non-adaptive anxiety responses not only compromise the animal's welfare, but also may also fundamentally affect physiological functioning. In laboratory animals this may substantially affect experimental results even in non-behavioural studies. Such effects are likely to dramatically reduce the reliability of the study results obtained, ultimately leading to an undesirable increase in the number of experimental animals to be used.

Given the individual differences in anxiety discussed above, the questions arise whether 1) standardised housing conditions can guarantee good welfare for all strains of one species, and whether 2) specific demands in terms of housing and treatment can be hypothesized for animals differing in anxiety. Even if distinct housing conditions do not exceed the adaptive capabilities of anxious strains, adaptation will result in a specific behavioural profile, profoundly differing from less anxious strains. While the anxiety-phenotype may on the one hand be a

confounding factor for experimental results, on the other hand it offers the opportunity to systematically investigate gene-environment interactions. However, researchers should be aware of the potential conflict between the need of standardized environmental and experimental conditions, the demanded reliability of experimental results and the welfare of experimental animals. Detailed knowledge about the emotional phenotype of the animals used is necessary to reach a balance between reliable experimental research and the welfare of laboratory animals.

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