The importance of reward-evaluating mechanisms for animal welfare

Johanneke E. van der Harst and Berry M. Spruijt

Animal use and animal welfare

Animals have been exploited by man for all kinds of purposes. The dominion of man over animals implies that we determine how and where they will live. According to many, society should therefore be concerned about the welfare of these animals. Since animals are thought to have less mental capacities than man, it has been questioned whether animal welfare concerned more than physical health alone. However, as early as the 18th century, Bentham addressed this in his classic dictum: 'The question is not, Can they reason? Nor, Can they talk? But, Can they suffer?' [c.f. 25]. Nowadays, it is assumed that similar to man, chronic stress may induce mental suffering in animals, which is not necessarily associated with physical health problems.

Using animals for certain purposes implies keeping them in captivity, which means that we are responsible for their housing conditions. The design of these housing conditions is mainly based on economical and ergonomical factors with little attention for behavioural needs of animals. For laboratory animals, not only economical and ergonomical factors played a role in the design of animal cages, but also standardization requirements. These factors led to more and more impoverished housing conditions that prohibit the performance of a large part of the natural behavioural repertoire. Due to this behavioural deprivation the current housing systems are inadequate in creating an environment that guarantees the welfare of animals, a statement which is often acknowledged in guidelines. As long as the use of animals for research is necessary, society should be concerned about their welfare. In addition, a lack of adequate environmental stimulation is shown to cause a range of brain- and behavioural deficits which do not only jeopardize animal welfare but also the validity of research results. It is stated by others that if welfare is not guaranteed, the validity of laboratory animals as a research model is questionable. Currently, there is an urgent need to be able to assess and improve animal welfare. Several scientific studies have addressed this issue during the past decades and have made substantial progress. However, because of the complexity of the concept of animal welfare, and despite of the extensive amount of relevant research results, animal welfare experts failed to reach a clear consensus on how animal welfare should be defined and measured. Until now, animal welfare has mostly been addressed as inversely related to stress and its related parameters. This is not in line with the scientific delineation of stress and is also different from the approach of human welfare.

An important aspect of animal welfare includes brain processes which are involved in the animal's subjective evaluation of its internal state and its environment. Thus, to be able to determine animal welfare we need to find ways to 'read their minds'. In other words: we need a tool to 'ask' an animal how it 'feels' and be able to understand the 'answer'. It is important that the answer to this question can be scrutinized in the phase before the animal's adaptive mechanisms are chronically challenged and subsequently fail over a longer period of time allowing the early detection and solving of problems. For successfully realizing improvements in animal welfare, we need: 1) to know how these improvements are perceived by the animals and 2) to be able to monitor the effects of these improvements.

The ultimate 'product' of various complex interacting mechanisms in the brain is behaviour. A behavioural response can be described as being the 'answer' of an animal to challenges in its external and internal environment. Part of this review explains a new multidisciplinary approach that has been followed in order to develop a tool to assess the state of animals by means of posing 'questions' to animals and deduce the 'answers' from their behaviour. This tool is based on how an animal evaluates its own situation, adapts its behaviour and optimizes its energy use under natural conditions [23]. Furthermore, new insights and methodologies are proposed to improve animal welfare and assess an animal's appraisal of certain conditions.

Animal welfare: Definitions and indicators

To investigate and discuss animal welfare a comprehensive and practicable definition and analysis of welfare is required. It should not only be defined by how an animal feels at a certain time within a continuum that ranges from suffering to pleasure but also by its ability to cope with environmental changes and challenges over a longer period of time.

Definitions of welfare

One of the first definitions of welfare was put forward in 1965 by the Brambell Committee [c.f. 25] that was constituted after the publication of 'Animal Machines' which described the loss of identity of animals kept in commercial husbandry systems [c.f. 25]. This definition was devised as a checklist of minimal requirements for farm animals but was considered to serve equally well for other captive animals. These minimal standards became to be known as the 'Five freedoms'. The 'Five Freedoms' have evolved somewhat with time and have been revised in 1993 by UK Farm Animal Welfare Council [c.f. 25]: 1) Freedom from thirst, hunger and malnutrition – by ready access to fresh water and diet to maintain full health and vigour. 2) Freedom from discomfort – by providing a suitable environment including shelter and comfortable resting area. 3) Freedom from pain, injury and disease – by prevention or rapid diagnosis and treatment. 4) Freedom to express normal behaviour – by providing sufficient space, proper facilities and company of the animal's own kind. 5) Freedom from fear and distress – by ensuing conditions that avoid mental suffering.

Although these five standards are easily memorized, comprehensible, and will definitely improve quality of life if complied with, the main flaw in the concept of the five freedoms is that it is not necessary to the welfare of an animal (or man) to have absolute freedom from hunger, cold, pain, fear etc.; only that the animal should be able to cope with these problems by taking effective action to avoid suffering. Perhaps a sixth freedom should be added: the freedom to exert control over the quality of life. It was proposed that the welfare of managed animals relates to the degree to which they can adapt without suffering in the environments designated by man. Correspondingly, many others have characterized animal welfare as a state of mental and physical health indicating living in harmony with its environment. Thus, the abovementioned definitions indicate that the features relevant for animal welfare relate to how well the animal is coping, how well it adapts, or whether it is in harmony with its environment. This was biologically translated in: 'welfare is present when an individual can reliably predict or control relevant events by means of species-specific signals or means.' [c.f. 25]. Predictability and controllability are key concepts in this respect. Another somewhat tentative but nevertheless important conclusion drawn is that for optimal welfare some uncertainty (unpredictability and uncontrollability) is of great positive significance [c.f. 25]. This implies that welfare is not fully dependent on complete control, certainty and solely positive experiences, which might even result in a rather insensitive animal. Under natural conditions, animals are exposed to both negative and positive stimuli and 'living in harmony with its environment' probably implies that it must be possible to keep a (positive) balance between these stimuli. An animal can never be 'perfectly' adapted to its environment since the latter is always subject to change but animals develop behavioural strategies to approach 'optimality' and maintain homeostasis. A threatened homeostasis can be defined as the result of the difference between the actual and the expected (or preferred/desired) state and the (stress) responses of animal to minimize this difference can be interpreted as reflecting its efforts to cope. In this sense, 'homeostasis' does not only refer to a physiological state but is also related to the environment and thus to the abovementioned expression 'living in harmony with its environment'. It seems that animal welfare can be regarded as being related to both positive and negative experiences. Measuring welfare by focusing on only one of these categories will probably yield an insufficient picture.

Welfare indicators: classic criteria based on stress responses

It is obvious that animal welfare cannot be simply measured as such; rather, welfare research must focus on variables and criteria that are relevant to animal welfare. Previously, animal welfare has been mainly addressed as inversely related to stress-induced physiological and behavioural changes. The classic criteria utilized in welfare research appear to concern mainly measures of non-welfare that are based on the presence or absence of stress. The acute stress response is generally considered to facilitate the functional adaptation of an organism in order to cope with a challenge. Failure in the attempt to cope is often seen after chronic stress and is considered to indicate that welfare is severely impaired.

Physiological and behavioural parameters

Changes associated with the stress response have been widely used as physiological indicators of (poor) welfare. The increased secretion of hormones from the adrenal cortex (e.g. cortisol) and medulla (e.g. adrenaline) in the initial alarm phase are designed to condition the animal for immediate

action by switching the flow of blood and nutrients from long-term goals like growth towards immediate problems like fight or flight. Since increased secretion of adrenocortical hormones, typically cortisol, is a constant feature of the alarm response, concentrations of these hormones in the blood or saliva of animals are regularly used as an index of stress. This approach, however, can fail to distinguish between alarm as a potential source of suffering, and excitement as a potential source of pleasure. Several other physiological measures, related to increased corticosteroid levels, are traditionally used as indicators of poor welfare as well. For instance: a change in plasma concentrations of glucose, urea or protein, indicating a significant metabolic cost to the animal, and immunosuppression, indicating a potential health risk.

Similar to the classic physiological criteria, most behavioural indicators of welfare also rely on showing evidence of changes that are indicative of (chronic) stress. Particularly, the occurrence of abnormal behaviour has been used as an indicator of stress, or (non)welfare. Stereotypy for instance, is traditionally considered to indicate reduced welfare and are typically observed in situations of conflict or frustration. The performance of stereotypic behaviour may be regarded as a mechanism that helps animals to cope with and adapt to environmental changes. However, it is essential to emphasize that it is generally accepted to represent an animal's response to an inadequate environment. Thus, stereotypy may not be directly indicative of the welfare of the individual animal that performs it (the expression of stereotypy has been argued to be rewarding via a positive feedback effect of sensory stimulation on their underlying control systems) but is certainly indicative for the insufficiency of housing conditions in which they occur. In some cases the presence or absence of certain natural behaviours are used as indicators. For instance, play behaviour may be a reliable indicator of good welfare in mammals since one of the common characteristics of play behaviour is that it is absent under stressful conditions.

Disadvantages of stress-related parameters

As mentioned in the previous section, changes associated with the stress response have been widely used as physiological and behavioural indicators of poor welfare. However, the absence of stress does not necessarily indicate good welfare and the presence of stress does not necessarily indicate poor welfare. Mild stress might even improve welfare by optimizing alertness and preventing boredom. Similarly, it was demonstrated that daily mild stressors have protective effects against the effects of isolation. The acute stress responses of an animal are suggested to reflect its efforts to minimize the difference between the actual and the desired state and, thus, attempting to cope with the situation and maintaining homeostasis. Therefore, acute stress responses can also be interpreted as beneficial or having rewarding properties to the individual.

Concentrations of cortisol can be misleading. Namely, corticosteroid release is also seen in the absence of behavioural evidence of aversion and the HPA-axis is also activated by novelty in rats, which can hardly considered to be stressful since rats often show a preference for novel environments. It is argued that this might be related to 'sensation-seeking' which can also be observed in humans and suggests that corticosteroid release may be related to a state of arousal, rather than to psychological stress as defined in terms of aversive behaviour. This is in line with the recent findings which suggested that neuroendocrine responses to competition in young men are associated with cognitive appraisal. Such a relationship is also suggested by the finding that the size of the corticosteroid response to novelty is predictive of the ease with which rats acquire amphetamine self-administration behaviour and is also consistent with notion that acute administration of corticosteroids usually elevates mood in human subjects. This indicates that stress seems hard to define and lacks specificity: the responses can be elicited by neutral or even pleasant events as well. Thus, it appears that animal welfare cannot simply be addressed as being inversely related to stress and the physiological and behavioural responses to it.

Relation to human welfare

It is important to note that animal welfare is traditionally defined on the basis of other criteria and indicators than those used for the definition of human welfare. However, animal welfare research and human welfare research are more closely related than is recognised so far. Animal models are often employed for elucidating human (neuro)psychology or psychopathology based on the homology and analogy between them. An extensive amount of research results point to mutual neural circuits underlying experience and expression of emotions in both man and animal. Thus, it should be self-evident that there should be no discrepancy in the general definition of emotional states of humans and animals. Where animal welfare is mostly approached as being related to the presence or absence of stress, it is acknowledged in humans that the absence of negative symptoms alone is not a guarantee for the presence of welfare. The absence of behavioural expressions of positive

experiences and ‘low mood’ are acknowledged in humans to be an indicator of poor welfare and is probably equally important for the assessment of animal welfare. Thus, not only a general definition for both animal and human welfare might be useful for welfare research in general, but also existing knowledge from either human or animal welfare research might be applicable for the benefit of both. Importantly, animal welfare is related to the quality of animal models that are used to improve human welfare and is thus related to the validity of the data.

A concept of welfare based on the balance between positive and negative experiences

Welfare is related to both positive and negative experiences in the sense that the outcome of the integration of these experiences eventually determines welfare. Impaired welfare does not refer to acute positive or negative experiences, but refers to a chronic imbalance between these experiences reflecting a chronic failure to cope. As long as signs of satisfaction are in balance with signs of stress, the situation is not hopeless. Welfare can thus be conceptualized as the state of the balance between positive and negative experiences [23]. This concept of welfare is based on the hypothesis that rewarding experiences can compensate aversive experiences and that such a compensatory mechanism serves the organization and efficiency of behaviour in all vertebrate species.

Biological background of welfare

Efficiency of behaviour and motivational states

Animals appear to be capable of high efficiency in the use of environmental resources and the avoidance of potentially harmful stimuli and situations. The nervous system of an organism receives information on internal and external events, processes this information, and selects the most efficient behavioural response. Such efficiency of behaviour is based on the relationship between the investment of an organism and the consequences of its actions [22]. This can be translated in terms of costs and benefits that are related to the economy of behaviour. This means that animals adapt their behavioural responses in such a way that a maximum benefit is achieved with a minimum of effort (investment of energy or taking risks). Efficiency requires a continuously changing sensitivity to stimuli dependent on the actual situation. For instance, under stressful circumstances both the sensitivity to rewarding and aversive stimuli increases [8] [25] whereas repeated exposure to rewarding stimuli induces insensitivity to these stimuli in terms of behavioural and neurophysiological reactions [25]. Thus, the variable reward-sensitivity may be part of the adaptive repertoire that allows the animal to reduce reactivity in case of abundantly present rewards or increase its attempts to obtain rewards in their absence. A general theory that exists about behaviour is that its primary goal is to obtain rewards (and avoid punishment) [9] and thus is - from an evolutionary perspective - an instrument to reach ultimate goals (maximal fitness) [23]. The general adaptive significance of the capacity for goal-directed action allows man and animal to control their environment to satisfy their needs. Goal-directed actions are controlled via activation of motivational systems that signal differences between actual and preferred states. Goal-directed behaviour is defined as behaviour controlled by representation of a goal or an understanding of a causal relationship between behaviour and capture of a goal. The induction of expectation (i.e. anticipation) through the acquisition of associations between certain (environmental) stimuli and its consequences is regarded as having high adaptive value since an animal can evaluate and regulate the necessary investment in order to achieve a goal. This means that an animal must be able to evaluate its own state and the significance (rewarding value) of certain stimuli for this state before goal-directed action is performed. This implies that motivation consists of an appetitive phase in which this evaluation takes place and a consummatory phase in which the goal is captured and consumed. The relationship between internal physiological changes on one hand and behavioural changes on the other in relation to the availability of different commodities in living organisms are captured as motivational systems. Motivation is the tendency of an animal to perform certain behaviour, whereby its motivational state is determined by the interaction of internal and external factors. Motivational systems can be defined as feedback mechanisms that are activated by a certain class of stimuli and deactivated following specific events or behavioural patterns. The various motivational systems can be distinguished by the type of responses or events that terminate the activation of a specific motivational system. Every behavioural response that diminishes the difference between the actual and the expected/preferred state, and thus terminates the activation of the motivational system, can be regarded as having rewarding properties.

The analyses and evaluation of external stimuli, which is essential for subsequent the behavioural output, has its counterpart in the organization of the central nervous system. The central nervous

system may be conceptualized as a hierarchically organized series of feedback mechanisms in which stimuli are processed in different levels and in which each level adds its specific component or programming rule to ongoing behaviour [22]. At the highest levels different types of information are evaluated and integrated to be able to elicit the most beneficial response (decision-making mechanism). This processing is not very fast and elicits delayed responses. In case immediate action is required, such as fight or flight, processing of stimuli needs to be direct and fast and thus solely involves the lowest levels.

Evaluation and integration of positive and negative experiences

Efficiency of behaviour implies a continuous evaluation and integration of positive and negative experiences that result in activation of motivational systems to reduce the difference between actual and desired states. Selection of the most efficient (rewarding) response implies that a common 'evaluation' system is available that functions at a higher level than specific motivational systems to be able to compare costs and benefits in a case of conflicting motivations. The largest possible reduction of the signalled difference will then lead to the activation of the motivational system involved. For the process of evaluation, a "common currency" must be available to be able to compare different motivational systems and their respective responses (e.g. hunger versus thirst). Because the reduction between the actual and the expected state can be considered to be rewarding, the type of response (e.g. eating or drinking) that causes the largest reduction between the actual and the desired state is the criterion for the response selection. Therefore, reward may be considered as the common currency of this evaluation system, which is supported by the theory [7] that 'pleasure' is the common currency of the brain. In 1971 Cabanac had already captured the importance of reward in his classic dictum: 'Pleasant is useful' [6]. Nowadays, several animal welfare scientists emphasize the importance of assessment and inducement of positive emotions in animals [5].

Biological function of subjective states

Psychological stress and behavioural and mental disorders are features that are likely to be present in both man and animals, at least higher vertebrates [8] [25]. Negative subjective states and experiences such as stress and fear play a role in the survival of an animal. These negative subjective states can be viewed as proximate mechanisms registering the organism's problem and triggering appropriate motivational systems and consequential courses of action to cope with the problem. As mentioned in the previous section, successful reduction of the difference between the actual and the preferred state is considered to be rewarding. On the other hand, a failure in reducing this difference induces a negative/aversive experience (i.e. a state of stress). An animal may use behavioural strategies that alter motivational states to try to counteract adverse environmental conditions [23]. It can be argued that stress may be counteracted by rewarding activities and thus, that stress increases the motivation and subsequent sensitivity for rewards. The fundamental role of reward in the survival and welfare of organisms ranges from the control of autonomous functions to the organization of voluntary, goal-directed behaviour. Rewards have several basic functions: they may induce subjective feelings of pleasure and contribute to positive emotions, they can act as positive reinforcers by increasing the frequency and intensity of behaviour that leads to the acquisition of goals, and they can act as goals in their own right and elicit approach and consummatory behaviour [25]. Via these mechanisms rewards influence behaviour and play an important role in the survival and welfare of animals. This is also indicated by the abovementioned variable sensitivity to reward, which has adaptive value in the sense that it can be utilized to counteract negative experiences. The importance of reward for survival can also be illustrated by the fact that some behaviours seem to be rewarding in itself without necessarily obtaining an immediate goal at that particular moment, such as exploration, sexual behaviour and grooming. These behaviours, also indicated as ethological needs (see **Ethological needs, environmental enrichment, and animal welfare**), are not immediately followed and reinforced by the goal (which is the case for proximate mechanisms) but are essential in the long run (ultimate mechanism) for the animal's survival and that of the species. It may be that the course of evolution has attributed rewarding properties to the display of these behaviours to guarantee their expression.

Interaction between stress and reward systems

It is known that previous experiences such as stress can alter the sensitivity to rewarding and aversive stimuli in both man and animal [8] [19]. Much information has been collected over the years concerning the consequences of acute and chronic stress on reward-sensitivity. For instance, post-weaning isolation rearing causes, besides a range of other behavioural and physiological changes, an increase in the behavioural responses to both primary reinforcers and reward-related stimuli [13]. Acute or short-term stress, such as isolation, foot shock or tail shock, is reported to lead to an increased motivation for rewards [3] [25]. Continuously changing reward-sensitivity is an adaptive

response that allows the organism to fulfil its needs and maintain a balance between negative and positive experiences. For instance, stress urges an animal to react more eagerly, or be very sensitive, in case of the presence of a valuable reward to compensate for the negative state of the balance. In case of deprivation of certain essential stimuli an animal can become very sensitive for non-related or relatively neutral stimuli as well. The rewarding properties of these stimuli will increase due to the deprived condition of the animal. This can for instance be seen in individually housed rats that display an increase in preference for environmental novelty [c.f. 25] or in play-deprived rats that show an enhanced preference for sucrose and food [24]. Under less stressful circumstances the sensitivity to rewards will decrease again. Via this variable reward-sensitivity a balance between positive and negative experiences can be achieved and this way welfare is guarded.

If the attempts to cope fail over a longer period of time – and the balance is too heavily loaded on the negative side – the animal will enter a depression-like stage that is, among other symptoms, reflected by insensitivity to rewarding stimuli. It is known that chronic stress leads to a strong decrease or even a total loss of reward-sensitivity [25] [34]. Chronic stress is applied in animal models of depression since it is known that stress has a precipitating effect on the development of this disorder [36]. Insensitivity to rewards in chronically stressed animals is reminiscent of reward alteration in human depression. This stressor-induced insensitivity to rewards simulates anhedonia (loss of the ability to experience pleasure), which is considered to be a major symptom of human depression. Insensitivity to reward may therefore indicate that welfare is severely impaired. Thus, because of its flexibility that is affected by previous experiences such as stress, reward-sensitivity may be a useful tool to assess the state of an animal in terms of welfare. Furthermore, animal models that are used to study stress-related disorders and their symptoms might be very useful for animal welfare research [32] since they investigate the consequences of previous experiences that are relevant for animal welfare as well.

Underlying substrate of reward and reward-sensitivity

The dopaminergic and opioidergic reward pathways of the brain are critical for survival since they provide the ‘pleasure’ drives for, for instance, food consumption and rewarding activities such as sexual behaviour. Rewarding events are known to be accompanied by activation of the mesolimbic dopamine system and in particular by the release of dopamine from the nucleus accumbens [c.f. 25]. It is often suggested that processing of reward is mediated by both the mesolimbic dopamine system and the opioid system via an interaction between these systems [2] [25]. This idea was based on studies indicating that the opioid-system is involved in the regulation of the reward-system and the fact that opiates are reported to stimulate dopamine turnover and release [c.f. 25].

Opioid systems are involved in the mediation of ‘liking’ (pleasure/palatability) related to food reward whereas dopamine systems are involved in the mediation of ‘wanting’ (appetite/incentive motivation) [2]. In line with this, it has been discovered that dopamine release (in the ventral striatum) is triggered by the expectation of a reward and not by the actual receipt of the reward [21]. Furthermore, it has become apparent that dopamine neurons react to a novel reward but their activation will be transferred from this primary reward to a predictive stimulus during the course of association learning [21]. Also, dopamine neurons are activated when a reward is better than predicted and depressed when a reward is worse than predicted [21] [25]. Thus, the activity of dopamine neurons is related to signalling the difference between the actual and the expected state and is therefore important for the activation of motivational systems that aim to reduce this difference. This is in line with the observation that dopaminergic neurons of the ventral tegmental area (VTA) and substantia nigra send their axons to brain structures involved in reward, motivation and goal-directed behaviours [14] [25] each of which are important factors in the efficiency of behaviour and maintaining homeostasis.

A concept of welfare based on reward-evaluating mechanisms in the brain

As mentioned earlier, welfare can be conceptualized as the transient balance between positive and negative experiences [23]. This concept implies that, to assess welfare, it is important to assess the state of the balance in terms of the outcome of the integration of past en present positive and negative experiences. It is proposed that an animal is continuously monitoring its own welfare state in the sense that when it encounters a challenge/stimulus, an internal evaluation-system determines how to respond depending on its previous (positive and negative) experiences [23]. Thus, by offering a challenge to an animal its state is internally evaluated and this state can be assessed via analysis of its response. In other words, via the presentation of a certain challenge/stimulus an animal can be ‘asked’ to evaluate its own state and the ‘answer’ can be deduced from its response. A way to ‘ask’ an animal about its own welfare state is to present a rewarding stimulus and to investigate the reward-

related behavioural response to this stimulus. In case of a large number of previous negative experiences, an animal will show an increased motivation for (i.e. increased sensitivity to) positive experiences (i.e. rewards) in order to compensate the negative state of the balance. Therefore, quantification of reward-sensitivity is considered as a potentially valuable tool to assess animal welfare. Because this measure of sensitivity reflects the outcome of the integration of positive and negative experiences it may not only detect a long-lasting process of failure to cope (the balance is (too) heavy-loaded on the negative side) but may also detect positive welfare states. In the latter case, an animal will have less need for reward and, thus, will be less motivated to obtain a reward.

Reward-related behaviour as an indicator for the sensitivity of the reward system

Reward-sensitivity can be measured by the behavioural response to expectation (i.e. anticipation) of a reward. In several studies it has been noted that rats display an increase in activity prior to the arrival of a reward. This state of behavioural arousal results from endorphinergic modulation of neural reward systems [23]. Mesolimbic dopaminergic areas are involved in mediating appetitive aspects of motivated behaviour and the activity (sensitivity) of this system is related to quantitative aspects of motivation [c.f. 25]. Thus, the level of activation depends on the incentive value of the reward (appraisal) but, is also dependent on the internal state of the animal which is influenced by its previous (positive and negative) experiences [12] [24] [25] [34]. The relation between sensitivity of the reward-system, the consequent behavioural response to rewards, the state of an animal that is influenced by its history, and the characteristics of a reward is represented in Figure 1. In short, this figure indicates that the sensitivity of the reward system, which determines the level of behavioural activation in anticipation of a reward (i.e. anticipatory behaviour), is related to the welfare state of an animal, which is in its turn determined by previous positive and negative experiences. This indicates that anticipatory behaviour might be a useful tool to assess welfare [23] [25]. Furthermore, the fact that the level of activation is also influenced by the rewarding properties of the reward indicates that anticipatory behaviour might also be used to assess the perception (appraisal) of certain stimuli. The potential utilities of anticipatory behaviour will be further explained later in this review.

Characteristics of anticipatory behaviour

Anticipatory behaviour was as early as 1918 described as a typical arousal with goal-directed activity that occurs in the appetitive phase when the actual reward is not present yet [10]. Thus, anticipation requires the ability to internally represent expectations of a forthcoming reward during the appetitive phase that precedes the actual consumption of the reward (consummatory phase) [22]. In several experiments conducted with rats anticipatory behaviour prior to the arrival of, for instance, food, water or sexual contact has been described as an increase in activity [c.f. 25]. In some studies it was mentioned that rats showed an increase in alertness, grooming, exploration and running [4] [c.f. 25]. A state of behavioural arousal as a part of the anticipatory response is supposed to result from endorphinergic modulation of neural reward systems . In some studies the ‘spontaneous’ behavioural response in anticipation of a reward is shaped by the test situation, for instance, the number of level changes in a bi-level box as a measure of motivation for sexual contact or the performance of an operant task in an instrumental conditioning paradigm. As a consequence of an association between a certain stimulus and a reward rats display enhanced locomotor activity in anticipation of the delivery of the reward, which is defined as being an expression of biologically significant preparatory behaviours [15]. Thus, anticipatory behaviour can also be referred to as ‘preparatory’ behaviour, which is indicative of the function that has been ascribed to this response. It is said to prepare an animal for a forthcoming change and leads to and facilitates consummatory behaviour [4]. Historically, terms such as approach behaviour and goal-directed behaviour have been used as well [12]. Although anticipatory behaviour has been observed on several occasions, in neither of the aforementioned studies the behavioural profile of the spontaneous anticipatory response was further quantified since it never had the prime interest.

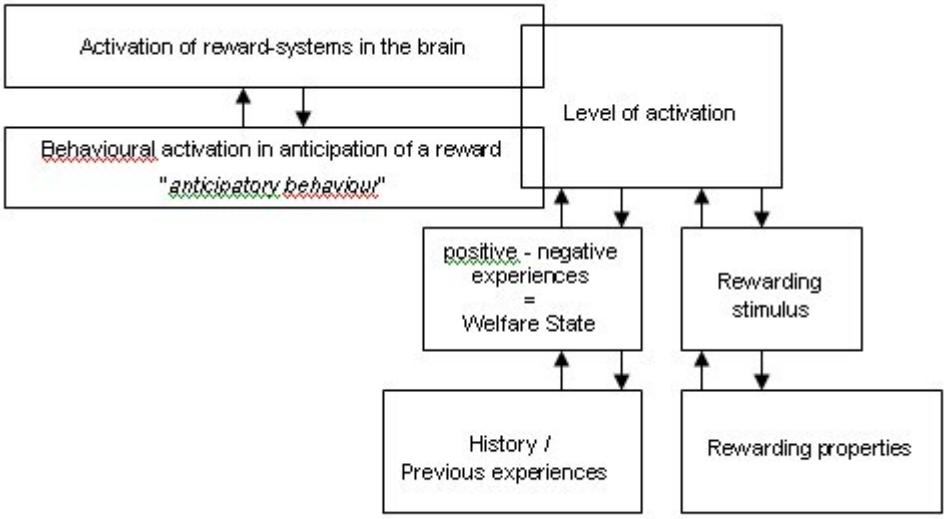

Figure 1. Relation between the sensitivity (and consequent activation) of the reward-system and the consequent behavioural response to rewards (anticipatory behaviour) that are influenced by both the welfare state of an animal and the properties of the reward.

Anticipatory behaviour can be induced in conditioning paradigms. These paradigms involve motivation and reinforcement in relation to rewards that are anticipated via acquisition of associations between certain stimuli (or context) and the reward. In a classical conditioning paradigm, an animal will learn to associate an initially neutral (visual or auditory) stimulus with the delivery of a reward (unconditioned stimulus; US) (Pavlovian conditioning). Via repeated pairing of the stimulus (conditioned stimulus; CS) with the US the initial (unconditioned) appetitive behavioural response to the reward will shift towards the presentation of the CS and thus becomes a conditioned response. This transfer coincides with the activation of dopamine neurons that shifts from just after the time of reward delivery (consummatory phase) to the time of CS-onset (appetitive phase) after repeated pairings of this CS and the reward. The involvement of dopamine in the display of appetitive (anticipatory) behaviour and Pavlovian conditioning has been shown by various authors [18] [21].

Utilities of anticipatory behaviour in relation to animal welfare

As depicted in figure 1, anticipatory behaviour is related to the activation of reward-systems in the brain and it reflects the way an animal perceives stimuli from the outside world, which is in its turn influenced by its history. Therefore, it is hypothesized that anticipatory behaviour can serve several purposes in relation to animal welfare (Table 1):

(**i**) Assessment of welfare

It is argued that anticipatory behaviour reflects the activation of the reward system, and thus reward-sensitivity, and is influenced by previous experiences. Acute or mild stress appears to cause an increased reward-sensitivity whereas chronic or severe stress appears to cause a decrease or even a total absence of reward-sensitivity. It was previously shown that social isolation in rats caused an increase in anticipatory activity for a sucrose reward [24] and that a chronic and more severe stressor caused a total absence of anticipatory activity in rats [34]. Thus, it is argued that anticipatory activity may be indicative of the state of animal in terms of welfare.

(**ii**) Improvement of welfare: counteracting stress

In line with the presently used concept of welfare as being a transient balance between positive and negative experiences and the consequent hypothesis of compensatory mechanisms, it is posed that rewards are able to compensate stress. Because it is known that stress affects the sensitivity of the reward-system and even leads to a total loss of reward sensitivity if this stress is chronic and severe and an animal fails to cope, regular activation of the reward-system may be a tool to counteract these effects, and thus, improve welfare. Because it is known that dopaminergic activation shifts to the expectation phase after several encounters with a certain reward and its predictive stimuli, it is hypothesised that inducing expectation via a Pavlovian conditioning schedule may be of additional value for the therapeutic efficacy of rewards.

(**iii**) Assessment of appraisal of stimuli

Because it is argued that the level op anticipatory behaviour is influenced by the (rewarding) properties

of the forthcoming stimulus/event, the level of anticipation could be indicative for the perception of these stimuli/events in terms of appraisal. In this way, the anticipatory response may be used to investigate the rewarding or aversive value of certain housing conditions and experimental procedures (provided that the welfare state of the animals is constant at that moment).

	Previous experiences (positive/ negative)	Unconditioned stimulus	Utility
	Van der Harst et al. 2003b		
i	A B C	I I I	Assessing the welfare state of animals (as a result of previous positive and negative experiences, (A,B,C) by means of their reward-sensitivity - reflected by the level of anticitipatory behaviour after announcement of a reward (I)
	Van der Harst et al. 2005 / 2003a (Chapter8), Dudink et al. 2006		
ii	D or (E F)	I or (II III)	Improving the state of animals by regularly activating the reward-system via announcements of rewards (I or (II /III), thereby counteracting negative experiences (D (or E/F))
	Van der Harst et al. 2003c		
iii	A A A	I II III	Assessing the perception of different stimuli/events (I, II,III) by the level of anticipatory behaviour, displayed after announcement of these stimuli/events to animals with the same previous experience (A)

Table 1: Several utilities of anticipatory behaviour that can be investigated in a conditioning paradigm in which an initially neutral stimulus is repeatedly paired with the delivery of a reward (unconditioned stimulus). The independent variables (i.e. previous experiences and unconditioned stimuli) vary according to the research question. (**i**) The state of animals with different previous experiences such as housing conditions or stressful events (A,B,C) that influence their welfare can be investigated by announcing a reward (I) and observing anticipatory behaviour in the period before delivery of the reward. (**ii**) Perception of different stimuli or events (I,II,III) can be investigated by announcing these stimuli to animals with the same previous experiences (A). (**iii**) The welfare state of animals can be improved by regularly activating their reward-system by means of reward-announcements, thereby counteracting stress.

A descriptive overview will be presented of the evidence obtained thus far for the proposed uses of reward-related behaviour. But first the background of the setup of these studies, with a special focus on the ethological factors, will be explained in the following sections.

Ethological needs, environmental enrichment, and animal welfare

Another way to maintain the balance between positive and negative experiences may be to increase the biological relevance of a captive environment to allow an animal to satisfy its ethological needs. In the past, standardization efforts led to more and more impoverished housing conditions for laboratory animals that are stimulus-poor and in which the performance of a large part of the natural behavioural repertoire is not possible. The ability to satisfy ethological needs should be an important factor in improving and evaluating housing conditions of animals. Enriching the housing environment of animals by the addition of objects in their cage increases the complexity of this environment and the ability to perform a more extensive repertoire of their natural behaviour including the possibility for activity and

control at a social and a spatial level. If an enriched environment allows the animal to satisfy its ethological needs [20] [25] and thus results into rewarding activities, stress is counteracted continuously. The stimulation of a variety of physiological and behavioural responses provides the animals with an extended adaptive repertoire of responses in case a challenge is encountered. Thus, enrichment may contribute to improved welfare in two ways that both counteract the effects of stress: (**i**) activating the reward-system through the display of natural behaviour and (**ii**) increasing the adaptive capacity of the animals.

Ethological needs

Typically a distinction is made between physiological and ethological (i.e. behavioural) needs of animals. Physiological needs such as nutrition, and climatic influences on the health, productivity, and survival of animals have been recognised for decades. Conversely, ethological needs, although already noted in 1965 (Brambell Report [c.f. 25]; freedom to express normal behaviour), have only been concentrated on since the last decennium. Ethological needs are those activities of which the display is guaranteed by their rewarding properties [23]. Sometimes the term 'psychological needs' is also used which refers to the need to have the possibility, for instance, to hide from conspecifics or frightening external stimuli. In this case, the display of the behaviour (hiding) is not rewarding but the presence of the possibility to do so is important. In this thesis, these needs are considered to be a part of the previously mentioned ethological needs. Concerning the rewarding properties of behaviour, it has been argued that not only those behaviours that lead indirectly to positive feedback (i.e. reinforcement via obtaining (food) reward) but also behaviour that directly causes positive feedback through its mere display rather than through its consequences, are rewarding (e.g grooming) [23]. The latter is especially relevant for animals that are kept under restricted conditions because – in terms of earlier mentioned compensatory mechanisms – it might be that under deprived conditions certain rewarding behaviours may be excessively displayed and develop into stereotypy.

Enriched housing: effects on brain, behaviour and research

In the last decades much research has been devoted to the subject of environmental enrichment. It is well-established that housing rats in a stimulating, enriched environment (e.g. large cages with stimulus objects) compared to housing in a non-stimulating, impoverished environment (e.g. housing in barren cages or isolation) induces a number of neurochemical, neuroanatomical and behavioural alterations. Although the principle of brain alteration due to experience can be traced back to 1928 [c.f. 25], the first study of the consequences of enriched rearing on the behaviour of the rat by means of investigating the effects of problem-solving capacity in aged rats that were reared as pets was published in 1947 [c.f. 25]. Environmental enrichment remained an important experimental manipulation since then, and many studies used enriched housing (of mainly laboratory rats) as an experimental tool to study the facilitation of physiological and physical functions of animals. Brain anatomy, plasticity and functions like learning and memory, development, as well as recovery after brain and spinal cord damage were the centre of interest. Although these studies did not focus on the welfare of experimental animals, their results contribute to the common idea that enrichment of the living-environment improves animal welfare. Because of the effects of environmental enrichment on brain, behaviour and animal welfare, results of scientific studies conducted with animals are also affected. Enriched housed animals are reported to have a larger behavioural repertoire and to be more efficient in assimilating stimuli from the environment [c.f. 25]. Hence, these animals are probably better able to cope with and are less sensitive to stressful experimental situations resulting in more adequate responding. It is therefore expected that enriched housed animals will be more suitable models for many kinds of research questions thereby increasing the scientific validity of the experimental results [c.f. 25].

Enriched housing: assessment of welfare, therapeutic efficacy and appraisal

Because of the above-described potential value of environmental enrichment, the studies that were designed to validate the uses of reward-related behaviour (see Table 1), apply enriched housing both as a method to vary previous experience and as a unconditioned stimulus.

In the following sections, these studies and their results are described. Enriched housing is applied (**i**) to manipulate the previous experience of the animals, and thus be able to assess whether and how this influences their reward-sensitivity as a measure of welfare (**ii**) as a positive stimulus to counteract stress, and thus to investigate the therapeutic efficacy of rewards, (**iii**) as unconditioned stimulus to investigate the anticipatory response for different stimuli as a measure of the appraisal of these stimuli.

Evidence in support of the three proposed utilities of reward-

related behaviour

To come back to the previously proposed uses of reward-related behaviour, now some evidence for each of these uses will be described. The approach of each of these studies is presented in Table 1.

First, we argued that reward-sensitivity is influenced by previous experiences and, thus, may serve as a tool to assess welfare. From addiction-studies it is long known that previous experiences such as stress can alter the sensitivity to rewarding (i.e. addictive) stimuli in humans [19]. Much information has been collected over the years concerning the consequences of acute and chronic stress on reward-sensitivity. Because these lines of research often involve animal models, evidence for this stress-reward interaction is available for animals as well [3] [12] [25]. Based on this information, we hypothesized that animals with different previous experiences would show different levels of reward-sensitivity as measured by their anticipatory behaviour. In other words, more stress will result in a higher level of anticipation for a reward, or the other way around, more positive experiences will result in a lower level of reward-anticipation. To test this, we investigated the level of anticipatory behaviour for a food-reward (sucrose-solution) in groups of laboratory rats with different previous experiences (Table 1-**i**) [26]. One group was housed under 'standard' laboratory housing conditions which are rather stimulus-poor (social housing in cage solely containing saw-dust). The other group was housed in enriched cages that were based on the standard cages with several additions (a shelter/partition-object, gnawing-sticks and a heightened lid) to provide the opportunity to display a more extensive repertoire of their species-specific behaviour (e.g. hiding, climbing, gnawing, rearing). This study revealed that the standard housed rats showed a stronger anticipatory response, as indicated by a stronger increase in activity from baseline to post-training, than the enriched housed animals. Thus, these animals seem to be more sensitive to rewards than the enriched housed animals. This confirms our hypothesis that previous experiences influence reward-sensitivity and thereby the level of anticipatory behaviour. Furthermore, from these results it can be concluded that welfare of standard housed rats is reduced as compared to the enriched housed group. This is likely to be caused by deprivation of the ability to satisfy behavioural needs in these impoverished housing conditions. Further evidence of the relation between previous experiences and reward-sensitivity is provided by other studies of our group [24] [34].

Second, we argued that reward-expectancy may serve to counteract stress and thus, to improve welfare. Stress is part of every day life and may result in depression when it becomes chronic and attempts to cope repeatedly fail – in terms of a 'bank-account' this situation reflects 'bankruptcy': too much has gone out (negative experiences) and too little came in (positive experiences). Depression is an affective disorder of which the symptoms such as insensitivity to rewards can also be observed in animals after chronic stress (the relation of (in)sensitivity to rewards and welfare will be further explained in the last part of this paper). Insensitivity to rewards in animals resembles anhedonia (loss of the ability to experience pleasure) which is considered to be a major symptom of human depression. Several lines of evidence exist for the role of dopamine in the mechanism of action of antidepressant treatments concerning their therapeutic effect on anhedonia and loss of motivation [8] [c.f. 25]. Based on the fact that reward-announcement, thus inducing reward-expectancy, causes activation of mesolimbic reward-systems and subsequent increased dopamine-release [17] [21] we hypothesized that regular activation of the reward system could counteract the effect of chronic stress on reward-sensitivity. For this, it was investigated whether a treatment of regular reward announcements could counteract the development of symptoms of depression (Table 1-**ii**) [27]. For this we used a social stress paradigm in rats, a validated model of depression: after repeated defeats by a larger and more aggressive rat and subsequent individual housing, rats show depressive-like symptoms such as anhedonia that can be reversed by anti-depressant treatment [33] [34] [35].

The first study focussed on preventing the development of anhedonia in socially stressed rats by means of regular announcements of a food-reward during the long-term social isolation period after defeat, which is an important part of the social stress paradigm [28]. This study confirmed the hypothesised therapeutic efficacy of regular activation of the reward-system – the group that received regular reward-announcements did not develop anhedonic symptoms. Interestingly, a subsequent test using an enriched cage as reward, induced a reversal of the depressive-like symptoms in the non-treated social stress group. Therefore, a second study was conducted that investigated whether this reversal was caused by the repeated stay in an enriched cage (short-term, 30 minutes) (thereby providing the ability to display a larger repertoire of species-specific behaviours which is argued to result in rewarding activities) or whether the announcement thereof had an additional effect (by prolonging the activation of the reward-system as compared to providing the mere reward) [25].

MOVIE

Movie 1. Illustration of (hyper-active) behaviour of rats that were transferred to an enriched cage as part of their behavioural therapy.

In this study, besides reward-sensitivity, an additional parameter is measured: synaptic plasticity of the hippocampus, a brain area known to be affected by stress and depression. Socially stressed rats show a severe attenuation of this parameter which can be restored by treatment with an antidepressant indicating the relevance of both the animal model and the parameter to depression [33]. The results showed that both unexpected enriched housing (i.e. the control group was transferred to the enriched cage without a prior announcement) and announced enriched housing restored reward-sensitivity (and thus reversed the anhedonic state) in chronically stressed rats. Furthermore, we found that both unexpected and announced enriched housing caused recovery of synaptic plasticity in the hippocampus. Importantly, the announcement had an additional effect as indicated by a significantly higher level of recovery (almost 50%) as compared to the group without announcement.

In conclusion, announced short-term enriched housing has a high and long lasting counteracting efficacy on stress-induced alterations of both reward-sensitivity and brain plasticity. This information is important for counteracting the consequences of chronic stress in captive animals and may therefore have therapeutic relevance in case of welfare problems.

MOVIE

Movie 2. Illustration of the effect of anticipation-therapy: regular announcements of a (food) reward to rats housed under impoverished conditions, results in less anxious behaviour and increased exploration. Further evidence for this behavioural therapy is provided by a study that we conducted in pigs [11].

MOVIE

Movie 3. Illustration of behaviour of piglets that were released at a corridor with straw as part of their behavioural therapy. This study showed that announcing the arrival of enrichment increases play behaviour and reduces weaning-stress-induced behaviours of piglets directly after weaning.

Third, we argued that anticipatory behaviour is influenced by the (rewarding) properties of a forthcoming stimulus/event, and thus, may serve as a tool to assess animal perception of this stimulus/event. The behavioural response of animals to a certain stimulus (or event) is likely to be representative for the perception of this stimulus since the neuronal substrates of behavioural activation and the perception of reward are remarkably similar [22]. In line with this, we have argued that behavioural activation in anticipation of the arrival of a reward represents the activation of reward centres in the brain [23]. The level of activation depends on the incentive value of the reward [25]. Therefore, a behavioural parameter based on this response could be very useful for the assessment of the perception (i.e. appraisal) of stimuli and events.

Based on this information, we hypothesized that the level of anticipatory behaviour is influenced by the type and rewarding properties of a stimulus. To test this, the intensity of the behavioural activation occurring in the time-window between the announcement and the arrival of a reward or other event is investigated in rats that expected different types of stimuli (Table 1-**iii**) [27]. The animals all had the same history in terms of housing and management to prevent interference of different previous experiences on their anticipatory response. A secondary aim of this study was to investigate whether rats would perceive a (relatively simple) enriched cage as rewarding. Therefore, transfer to an enriched cage was used as one of the stimuli. Sexual contact was used as a stimulus for the positive reference group since sexual behaviour belongs to a class of naturally occurring behaviours that are generally considered to be highly rewarding. The other groups were either transferred to a standard cage (neutral stimulus) or a cylinder that was filled with water (negative stimulus). The results showed different levels of anticipatory behaviour for the different expected events, thereby confirming the hypothesis.

It is important to note that we used several control groups that received types of stimuli that are known to be either rewarding or stressful to validate that the level of anticipatory activity is related to the rewarding properties of the stimulus (as was hypothesised based on literature about activation of reward centres in the brain) and to exclude the possibility that an increase in activity is also related to the anticipation of a negative event. Furthermore, the strong increase in activity in anticipation of both an enriched cage and sexual contact and the similar response concerning the separate behavioural elements indicate that the appraisal of access to an enriched cage shares a common denominator with the perception of sexual contact. Since the latter is generally accepted to have highly rewarding properties to rats it is concluded that the enriched cage is highly rewarding as well. The anticipatory response for sexual contact or an enriched cage was obviously different from the anticipation for access to a standard cage or a forced swim session indicating that this response is related to the positive nature of the stimulus. Further evidence for anticipatory behaviour as a tool to assess animal

perception is provided by a recent study [1] using anticipatory behaviour to investigate the significance of positive and negative events to laboratory-housed common Marmosets (Callithrix jacchus).

Reward and animal welfare: Anticipatory behaviour: characteristics and utility for animal welfare and scientific research

General characteristics of anticipatory behaviour

MOVIE

Movie 4. Reward-related behaviour in expectation of a reward is generally characterized in rats by an anticipatory increase in activity appearing in the appetitive phase (i.e. before actual receipt of the reward which is referred to as the consummatory phase).

The increase in several specific behavioural elements appeared to be dependent on the characteristics of the reward and are therefore less useful as a general parameter for anticipation. The anticipatory response for a forthcoming reward to reflect 'wanting' and is related to the 'need' for (i.e. sensitivity to) the reward [23]. However, in case of an aversive forthcoming event the anticipatory response was expected to be different and not related to 'wanting' but rather to be related to 'fear' or 'avoidance' (for instance exemplified by freezing behaviour as is seen in conditioning experiments using foot shocks). Thus, to be able to characterise 'positive' anticipation (i.e. anticipation for a reward) and use it as a measure for reward-sensitivity it was important that a distinction would be present between 'positive' and 'negative' anticipation (i.e. anticipation for a 'punishment') in the applied experimental set-up (Pavlovian conditioning). Although the expected freezing behaviour was not seen, negative anticipation was clearly different from positive anticipation concerning the activity after announcement of the negative and positive stimulus.

In the current stage of research we have also been able to evoke and investigate anticipatory behaviour in several other species, including mink [31], pigs [11], cats [c.f. 25], and farmed silverfoxes [16]. Recently, marmosets could be added to this list since our concept and approach to investigate the significance of positive and negative events of these animals was applied [1].

MOVIE

Movie 5. Anticipatory behaviour in pigs [10].

MOVIE

Movie 6. Also, in a research project on overtraining in horses in which brief social contact was used as a behavioural test, anticipatory behaviour was observed [29].

Utilities of anticipatory behaviour for animal welfare research

Concerning the assessment of welfare, a difference in reward-sensitivity was detected between standard and enriched housed rats when the anticipatory response of these animals was investigated. Thus, previous experiences in terms of housing conditions appear to have an effect on anticipatory behaviour. It was confirmed that chronic stressful experiences cause an impaired anticipatory response reflecting anhedonia (reward-insensitivity). Importantly, anticipatory behaviour appeared to be a more sensitive measure than sucrose-consumption (which is widely used as a measure of anhedonia) ; considering the fact that it was shown to be present under different conditions it is a very robust measure. Overall, this parameter may be a useful and robust indicator of the state of the animal in terms of welfare.

A hypothetical relationship between anticipatory activity and the balance between positive and negative experiences – that influences reward-sensitivity and is proposed as a concept of welfare – is depicted in Figure 2: an increasing level of negative experiences that are not compensated for by positive experiences will result in an increased sensitivity to rewards until a 'cut-off' point is reached when the stress becomes chronic. The exact shape of the curve is currently being investigated (UFAW Hume Research Fellowship J.E. van der Harst).

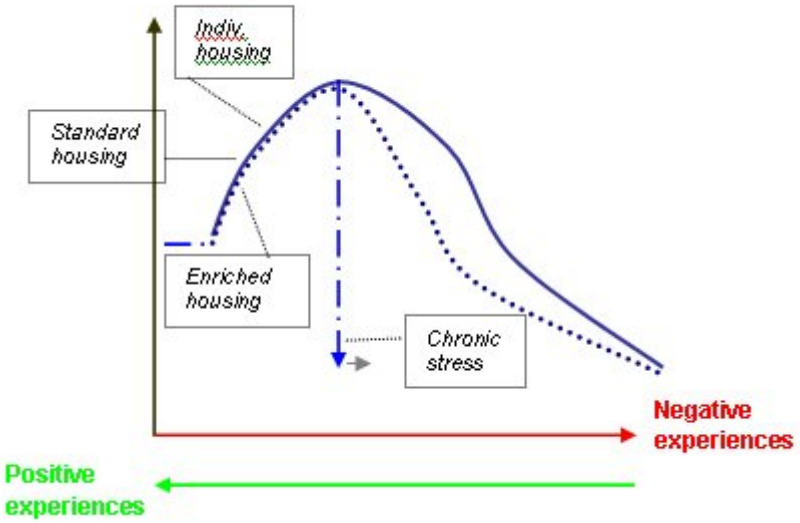

Figure 2. Hypothetical relationships between reward-sensitivity - as reflected by anticipatory activity after announcement of a reward (Y-axis) - and welfare - as reflected by the balance between positive and negative experiences (X-axis).

Implications for scientific research

Animal welfare is important for the validity of the results of scientific research that use these animals as models to improve human welfare. It is therefore not only important to assess animal welfare but also to improve it once it has been assessed to be poor. Counteracting stress via the announcements of rewards may be a useful tool to improve welfare of laboratory animals and the consequential validity of research results. Inducing anticipation via announcement of rewards has proven to be successful in both preventing the development of chronic-stress effects and reversing these effects in rats. It must be mentioned that the latter should not be necessary if the development of these effects is prevented from the start.

Anticipatory behaviour: function, underlying substrate, relation to abnormal behaviours

Now that the results have supported the hypothesized utilities of anticipatory behaviour it is desirable to shed some light on its natural role, underlying substrate and potential relation to other mechanisms. This way, the important factors for the additional validation of this parameter for welfare research will become clear.

Natural role of anticipatory behaviour

As mentioned, reward-related behaviour in anticipation of a reward was as early as 1918 described [10] as typical arousal with goal-directed activity. Anticipatory behaviour might be considered as goal-directed behaviour since goal-directed behaviour is defined as behaviour controlled by the representation of a goal or an understanding of a causal relationship between behaviour and capture of a goal. An anticipating animal can behave efficiently since it can estimate costs and benefits beforehand and adapt its behaviour accordingly [21] [22]. The theory of economy of behaviour implies that an animal will try to behave as efficiently as possible by means of investing a minimum amount of energy to get a maximum profit [22]. The question is why an animal spends energy on increasing its activity in anticipation of a reward when this reward is delivered anyway, thus without necessary responding. This might be explained if the display of the behaviour has become rewarding and activates reward centres in the brain. Furthermore, since anticipatory behaviour is also defined as preparatory behaviour and as a maintained state of attention it might be essential that the animal is prepared to collect the reward once it is delivered. This preparation and attention might be an essential part of its natural response that leads to and facilitates consummatory behaviour. The role and function of reward centres and mechanisms underlying attention are phylogenetically old and seem to be present in all vertebrates [22] indicating a functional role for survival.

Involvement of dopamine and opioids

Several studies have reported the involvement of dopamine in the mediation of anticipatory behaviour [18] [21] [23]. Dopamine is said to be involved in ‘wanting’ [2] and its release thus precedes the

consummatory phase. Opioids, on the other hand, seem to be involved in the direct appraisal of stimuli ('liking'), thereby indirectly affecting 'wanting'. It is as if one system (opioid system) mediates the rewarding component and induces or activates the other system (dopaminergic system) to form and maintain a specific behavioural strategy to obtain a reward [23]. It was argued that opioid systems seem to act more in 'the here and now' when the animal is facing an environmental challenge to evaluate ongoing behaviour whereas mesolimbic dopaminergic systems are more involved in future behaviour [23]. Thus, similar to dopamine, opioids are also involved in anticipation and may even have a key function since opioids are argued to stimulate dopamine turnover and release. As mentioned above, activation of opioid systems, and the subsequent effect on dopamine release, probably occurs prior to the consumption of a reward and thus during the appetitive phase. This is confirmed by studies that indicate that the release of opioids may be conditioned as shown in self-administration models and place preference studies. Because opioids are involved in the release of dopamine and their release may be conditioned, it is plausible that the conditioned anticipatory activity seen in the studies described in this review may be related to opioid activation. A relation between anticipatory activity and the opioid system has been confirmed [30] and showed that an opiate antagonist (naloxone) inhibited the anticipatory level changing in male rats that expected access to a sexually receptive female. Because a co-localisation of dopamine neurons and opiate receptors has been found [c.f. 25], a possible mechanism could be that increased opioid release induces an activation of the mu-receptor in the VTA and that the subsequent activation of an GABA-interneuron disinhibits the dopamine cellbodies which results in a dopamine release in the nucleus accumbens [c.f. 25]. This elevation in dopamine release could result in the increased anticipatory activity as seen in the described studies.

Anticipatory behaviour: relation to abnormal reward-induced behaviours

Although anticipation seems to have a natural function, an attempt to fully unravel the mechanism of reward-related behavioural response in anticipation of a reward may yield considerations of a relation to reward-induced behaviours that have been specified as being abnormal. Some characteristics and definitions of abnormal reward-induced behaviours, such as stereotypy, adjunctive behaviour, and superstitious behaviour have certain similarities with anticipatory behaviour which will be briefly discussed here. The abnormality of the aforementioned behaviours can concern any characteristic of the behaviour such as the form, the intensity or the time of performance. Abnormal behaviours are mainly observed under captive or restricted conditions, suggesting that frustration and deprivation might be determinant in the development of those behaviours.

Methodological considerations

Pavlovian conditioning

Via Pavlovian conditioning a 'spontaneous' behavioural response is evoked. Thus, an animal does not have to learn a certain unnatural response that might interfere with the results in the sense that not each animal might be able to learn it. That is, during an operant task the animals have to perform activities such as pushing or lifting a weight or pressing a lever which are not related to their natural behavioural repertoire. For these reasons an animal may not always be able to learn an operant response. It is important that they associate the required activities with the goals to be reached, and this might be easier if the behavioural response required for expressing the preferences is reasonably natural for the type of reward.

Anhedonia: behavioural parameter versus consumption

The insensitivity to rewards is mostly measured in rats by a decrease in consumption of a sucrose solution. However, the validity and reliability of sucrose consumption as a hedonic measure is questionable. It has been demonstrated that conditioned place preference (appetitive phase) for a sucrose solution was decreased in stressed rats whereas the sucrose consumption (consummatory phase) during the conditioning trials was unchanged [c.f. 25]. In line with this, it has been argued [34] [35] that the absence of reward-related (appetitive) behaviour is a more consistent consequence of chronic stress and representative of anhedonia. This is in accordance with the recent finding that dopamine release is triggered by the expectation of a reward and not by the actual receipt [17] [21]. It was shown that consumption of sucrose was not altered by chronic social stress whereas reward-related behaviour was affected by this stressful previous experience [33] [35]. This is in line with the finding that reward-related behaviour was altered in rats that had experienced brief periods of early maternal separation whereas no alteration in consummatory behaviour and preference for sucrose was found [15].

Approach: positive welfare indicator

Both positive and negative indicators used in the past focus on only one side of the balance which might yield insufficient results for an unequivocal interpretation in terms of the welfare state of an animal. Since welfare is conceptualized here as the balance between positive and negative experiences, a tool to measure the outcome of this weighing as described in this review and earlier [25], might be more sufficient to assess welfare. It was argued that the state of this balance could be reflected by sensitivity to (aversive as well as to rewarding) stimuli. Emphasis was put on rewarding stimuli and reward-related behaviour since it is intuitively more appropriate to approach welfare research by means of a positive method. Importantly, our method can be used to detect welfare problems in an early stage and not solely post-hoc. Considering the positive characteristics of the reward-related parameter described in this review, the fact that it can also indicate good welfare, and is measured in a non-invasive way (i.e. behavioural observation), this parameter may be defined as a 'positive' welfare indicator.

Anticipatory behaviour: improving welfare during assessment of welfare?

The multi-functionality of anticipatory behaviour, in the sense that it can be used for both welfare assessment and improvement, might raise some concerns. One could argue that while the animals are subjected to the anticipation test that takes several days their welfare could be improved during the course of action by the regular reward-announcements. This may indeed be a problem, but can be solved by detailed observation of the anticipatory response over time. Via baseline observations (behavioural response before association training starts) and following the behavioural response over the course of the training sessions creates the possibility to compare the development of this behavioural response. Since animals with relatively good welfare will be less sensitive to a potential therapeutic effect, the response over time should be able to distinguish between animals with good and poor welfare. Furthermore, it was shown that in case of chronic stress, anticipation for sucrose did not have a therapeutic effect. Since the therapeutic efficacy of anticipation was only shown in combination with an enriched cage as stimulus, it seems that a relative mild reward cannot reverse chronic stress. Thus, by using a relatively mild reward and detailed monitoring of the development of the anticipatory response over time it should be possible to distinguish between groups with different previous experiences and detect the occurrence of a potential therapeutic effect.

Environmental enrichment and animal welfare

Importance of environmental enrichment

Although it is common sense that environmental enrichment improves the life of captive animals it seems to be necessary to prove it. It has been stated by others that it is odd that welfare specialists are frequently urged to prove that changes are beneficial to small laboratory animals by the same individuals who accept empirical enrichment for captive carnivores and primates [c.f. 25]. Off course, scientific research of environmental enrichment is necessary to validate the effects, but it seems that regardless of the amount of data it is never sufficient enough to actually proceed to worldwide implementation of enrichment. Nowadays, the use of environmental enrichment for laboratory rodents is promoted widely and is incorporated in European legislation. Furthermore, in 1998, expert working groups were constituted by the Council of Europe to make a proposal for the revision of Appendix A of the Convention (accommodation and care of laboratory animals). In these future principles it is stated that gregarious species such as rats, should be housed in groups whenever possible. Furthermore, it is advocated that cage enrichment should be provided unless there is a justification on experimental or welfare grounds against doing so. However, actual implementation of enrichment for laboratory rats is still not frequently realized in laboratories. Although much research has been conducted on the subject of environmental enrichment, scientists seem to remain cautious since the methods and results seem to vary and do not yield one clear answer. Furthermore, researchers are concerned about the comparability of previous scientific results obtained by using standard housed animals. On one hand, it is preferred that environmental enrichment for experimental animals has no effect on experimental outcomes, but on the other hand, welfare researchers are enquired to show effects to convince them to be beneficial.

Via the increased stimulation and ability to display a more extensive repertoire of natural behaviour the animals will be better able to cope with and adapt to environmental changes (such as the novelty of an experimental task). Enriched housing enables animals to develop flexible physical and emotional responses to unexpected events in which they experience a sudden loss of control. This increased coping and adaptive capacity will lead to less stressful situations/experiences and consequential more adequate responding and will therefore improve both animal welfare and scientific validity of experiments conducted with enriched housed animals.

Environmental enrichment: preferences and rewarding properties

Preference for environmental features

Concerning mice, a clear preference seems to exist for nesting material and this form of enrichment is frequently implemented. Concerning laboratory rats, several types of cage modifications appeared to be successful in improving their welfare, including social contact, shelters, soft materials, gnawing objects, increased cage size. Division of space also seems to be preferred, either by partitions, platforms or boxes/shelters. Rats however, do not seem to show a particular preference for any specific feature. This is probably caused by the fact that rats need complexity, but also by the fact that preference studies are difficult to interpret since measuring time spent with objects provides only limited information and is dependent upon the choices that are offered. It might be wise to use other methods as well in addition to preference testing to obtain a more complete view of the essential environmental features for rats. As described in this paper, reward-related behaviour in anticipation of a transfer to different housing conditions might be a good candidate. However, there is no exclusive feature that can improve the welfare of rats (and mice) because it is the complexity of the environment allowing them to display a more extensive repertoire of natural behaviour that is important. Thus, when designing housing conditions for animals one should take into account the natural behavioural repertoire of the particular species and provide a certain level of complexity without actually focussing on one central feature.

Rewarding properties of environmental features

Whether extensions of the currently impoverished housing environment of captive animals have rewarding properties can be investigated by their reward-related behaviour in anticipation of a transfer to such an improved housing system. It has been shown that a relatively simple enriched cage for laboratory rats as used in the above-described studies evoked an anticipatory response that was equal to that for sexual contact. This means that even relatively simple adjustments are highly rewarding to rats. The increased ability to display a more extensive behavioural repertoire (and increased social control via the ability to hide and avoid cage mates) can therefore be considered as highly rewarding. The importance of environmental enrichment also became clear by the effect of the repeated stay in an enriched cage on chronically stressed rats: it appeared to cause a reversal of the chronic stress-induced depressive-like state in rats as reflected by their appetitive behavioural response and hippocampal synaptic plasticity. Considering these strong effects, environmental enrichment must be of great significance to these animals and should therefore be implemented at short notice as one of the first steps to improve welfare of laboratory rats and other captive animals.

Environmental enrichment: Implications for scientific research

Validity and variability of results

Animal models can be defined as representing experimental procedures that are developed in one species for the purpose of studying phenomena occurring in other species; the latter mainly concerns humans. Lack of adequate environmental stimulation causes behavioural and neuro-anatomical and neuro-chemical deficits [c.f. 25]. Thus, the scientific validity of experimental results obtained with animals that are standard housed in a stimulus-poor environment may be questionable, at least for studies on brain-behaviour relationships.

Implementation and implications

A variation in aversiveness of the test conditions to differently housed animals is also likely to affect the results and subsequent interpretation of these results. Many effects of environmental enrichment on behaviour have been found over the years and it is obvious that enriched housed animals respond differently to certain conditions than standard housed rats. Enriched housed rats are known to habituate faster to the open field and are overall less anxious/stressed during experimental procedures. It is also possible that the initial response of an enriched housed animal to experimental procedures that involve open areas might appear to be more fearful/anxious because of the large difference between the cage were the animal has the ability to hide and the open area were there is no such possibility. Therefore, when evaluating the effects of housing conditions it is important that responses over time are investigated as well. If these consequences and differences are evaluated and the possible effects of certain subtle differences in the type of environmental enrichment are established, environmental enrichment can be implemented successfully. This implementation may, however, imply that certain standard experimental tests and parameters or analysis-methods need to be adapted.

Animal welfare, reward and emotion

Animal welfare research is strongly related to the attribution of mental states to animals. The study of emotion is related to the current need to 'read the minds' of animals to be able to assess and improve their welfare. In humans, verbal language aids to assess emotional experiences but in animals only behavioural and physiological parameters and their interpretation can aid to detect emotions. The existence of emotions in animals remains a controversial issue. Darwin's publication 'The expression of emotion in man and animals' (1872) can probably be regarded as the corner stone of modern emotion research. Although an extensive amount of studies is conducted that investigate the process of emotion, it appears to be a rather difficult issue to capture because of its subjective characteristics. In behavioural neuroscience the study of emotion is mostly addressed by means of defining emotion by the response of a subject to an emotionally arousing situation. In this way, the emotional reaction is measured by behavioural, neurochemical and neuroendocrine parameters without requiring any preconceived theory about what emotions really are. This may be favourable for scientific research but, is it possible to study emotion without knowing about emotions [c.f. 25]? Several other authors have expressed their concerns about the reduction of a causal explanation to one process or discipline [c.f. 25]. These authors argue that a multidisciplinary approach that acknowledges emotion as involving multiple levels of control and complex interactions should be applied. The different disciplines and consequent approaches of the study of emotion is probably the cause of the lack of consensus in the literature on a definition of emotion: 92 different definitions of emotions are listed.

Among this long list is: 'emotion is any mental experience with high intensity and high hedonic content pleasure/displeasure', which is in line with the earlier explained concept that. pleasure is the common currency of the brain that underlies the economy of behaviour and the maintenance of the balance between positive and negative experiences [23]. From an adaptation perspective the ability to perceive its own emotions enables an individual to detect and assess a discrepancy between its requirements and environmental conditions (actual and expected state) and subsequent take action to regain homeostasis. Since animals appear to have high adaptive capacities in the sense of maintaining homeostasis, it would be logical that (at least part) of this capacity is derived from the ability to 'experience' emotions. This is in line with an extensive amount of research results that point to mutual neural circuits underlying experience and expression of emotions in both man and animal [22].

Main conclusions

Knowledge of the economy and consequential efficiency of behaviour that is related to the variable sensitivity of neuronal structures is very useful for the study of animal welfare. The results described support the three hypothesised utilities of anticipatory behaviour as a tool to measure and improve animal welfare. Therefore, the concept of welfare that was applied and was the basis of their formulation appears to be useful. Because the biological background of the proposed tool can probably be generalized to all (vertebrate) species, the obtained information is not only applicable to laboratory rats but also to other animals (e.g. husbandry animals).

It became apparent that:

(I) anticipatory behaviour as a parameter for reward-sensitivity, may be a useful tool to elucidate the status of the animal's bank account (i.e. welfare in terms of the transient balance between positive and negative experiences with reward as a common currency). This is an approach of welfare from the perspective of the animal in the sense that it measures its response at the moment that it has to evaluate its own state in order to select the appropriate response. Furthermore, this parameter seems to cover the whole range from good (positive balance) to poor (negative balance) welfare. For these reasons, anticipatory behaviour may be a very useful tool to objectively assess the state of animals in terms of welfare.

(II) anticipatory behaviour might be useful as a behavioural therapy to counteract the consequences of stress. Furthermore, induction of anticipation via the announcement of certain events or procedures might also be a way to reduce the stressfulness of events or procedures to which animals are subjected since it is known that a predictable stressor is perceived as less stressful.

(III) anticipatory behaviour as a measure for the rewarding value of different stimuli might be a useful tool to get more insight into the perception of animals in terms of appraisal of certain conditions or events. With such a tool it is possible to investigate the appraisal of housing conditions or other events and procedures to which the animals are subjected.

It also became apparent that even a relatively simple type of environmental enrichment has highly rewarding properties for rats and can be used as a behavioural therapy to counteract the

consequences of (chronic) stress. The fact that the enriched cage used in this study could even reverse depressive-like symptoms indicates that it is highly beneficial for the animals. This is not only important for the welfare of animals but also for scientific research that is conducted with these animals since this would increase the validity of the results. The presented results should once again stress the importance of the worldwide implementation of environmental enrichment and/or other opportunities to engage in rewarding activities for laboratory rats and other captive animals.

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