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# Animal sentience and suffering: a restriction on scientific research or a challenge?

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## Abstract

The Author treats the involvement of the statement of “animal sentience” as regards to the utilization of different animal models in research activities, by presenting and discussing the actual knowledge on neurofunctional bases of emotions and cognitive abilities in the principal animal models. On these bases, the importance of an ethical use of animals in research and the refinement of conditions of animal handling and management are emphasized.

**Key-Words:** animal models; cognitive abilities; emotions; neurofunctional organization of stress responses

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## Introduction

The statement of "animal sentience" (Lisbon Treaty, December, the 13$^{th}$, 2007) has committed the EU Member States to ensure the protection and welfare of animals in all the practical applications in which animals are involved. The scientific staff and technical operators are requested to assure the compliance with the physiological and ethological needs of different animal species.

In the research activities, the principle of "animal sentience" implies that the possibility of expressing the functional and emotional homeostasis also in situations potentially producing pain, suffering and distress must be guaranteed to experimental animals.

In Italy, the Decree No. 26/2014, implementing Directive 2010/63/EU on the use of animals in research, has adopted the principles of Replacement, Reduction, Refinement and has regulated the assessment of aims and rules of the experimental procedures, defining the prerequisites of experimental areas and their characteristics. It has also introduced specific roles for the technical and scientific evaluation and monitoring of experiments or scientific trials in general.

Actually, in lack of extensive alternative methods, most of the medico-biological research activities may require the use of species with advanced nervous architecture and function as

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animal models. This implies that the veterinary surgeon has the double role of investigator and sponsor subject of animal welfare.

## Animal welfare and emotional homeostasis

Welfare of an animal is currently defined as the result of appropriate integration of the subject with its own means and with the environment. The ability to satisfy the essential needs of an individual, hierarchically organized, to evoke a generalized physiological activation of organism (arousal) to positive or negative stimuli, and to induce behavioral manifestations and biochemical and functional adaptations, aim at maintenance of functional and emotional homeostasis (1).

The capability of an animal to cope with stressors in order to maintain welfare is expressed through dynamic mechanisms of physiological adaptation (coping), with species-specific and individual characteristics. Multiple "collative" factors affect different activation modules of stressful conditions (e.g., degree of novelty, social support, etc.), and make use of specific neurofunctional mechanisms and biomodulators of nervous function and metabolic, functional and immunological processes (2,3).

In the course of experimental trials, in case of non–compliance of adequate treatment, the subjects of different animal species could experience discomfort, distress, pain and suffering .In neurofunctional terms, pain is a physical sensation negatively perceived and emotions are currently defined as mental and physiological states associated with modifications of psychophysiological response to natural or learned stimuli, also expressed through behavior. In humans as well as in different species, emotions trigger mostly unconscious level response in relation to certain stimuli that results in adaptive functions. The coping response, expressed through specific markers (functional, behavioral, endocrine, immune), gives content and shape to an animal's capacity to adapt and maintain a state of physical and mental well-being. Therefore, emotions are transactions with the environment, associated with physiological, experiential changes and behavior, processed by the nervous system according to species' specificity, individual characteristics and previous experiences.

Primary emotions are detectable in all the mammals studied.The primary emotions are often associated to qualities or traits of temperament and since Darwin (4) it is well accepted that all animal species usually express the emotions also through facial or postural typical expressions. The ability to control stress and pain is also evident in all species.

## Neurobiology of emotions

In cognitive neurosciences, the emotions and cognitive abilities of different animal species are studied by observations on patients affected by brain injuries, mainly in human subjects, as well

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as by means of techniques of functional exploration of neurometabolic correlates of nervous activity and behavior. Often with the aid of molecular biology and/or conditioning of experimental animals, electrophysiological registration (EEG,PET) and neurofunctional imaging (RMNf, optometric techniques) allow to identify nervous structures and neurocircuits responsible for the processing of emotional responses. Computational models emulating the information processing mechanism in nervous structures have been also recently proposed (5).

To explain the individual characteristics of emotion in dimensional terms, the neurobiological studies have given a good representation of emotion by means of the "vectorial model", that takes into consideration both the arousal (physiological and individual intensity of the emotional stimulus) and the valence (pleasant/unpleasant, approach/avoidance) of emotional stimulus for the subject (6).

The neurofunctional domain of emotions has been experimentally and extensively studied by J. Panksepp (7). In primitive mammals as well as in humans, the existence of seven principal neurocircuits or emotional systems (seeking, rage, fear, cure, panic/grief, play, sex/ lust) has been demonstrated, with the involvement of different neurotransmitters and neuromodulators.

In the organization of stress response, amygdala, hippocampus and prefrontal cortex (PFC) are recognized to play a significant role. In these structures, stressors produce changes in the concentrations of different neurotransmitters leading to activation and modulation of stress coping processes. The noradrenergic, dopaminergic and serotoninergic pathways are the most significant systems underlying the response to stress, fear and punishment or reward, so influencing the behavior. Tonic immobility (freezing) is a sign of fear and explorative behaviors and it is a well-established test to evaluate the fear response in a wide range of vertebrates and invertebrates (8).

The amygdala is the central mediator of the emotions, and, from the point of view of functional mechanisms, it plays a role equivalent in the different species. It presents a sensory interface (to olfactory, visual, auditory, somato-sensorial stimuli, etc.) and an interface for control of emotional responses, for which it relies on the specific subsystems for different emotions.

The thalamus-amygdala system, common to humans and animals where the neocortex has not completely developed, is responsible for the processing of emotional stimuli and immediate responses, giving emotional significance to the sensation experienced.

In the same time, the hippocampus processes the inputs from the environment, building a configurational representation of the contextual situation on structured networks of mutual associations among multiple stimuli, developing effective adaptation responses and avoidance of anxiety-provoking stimuli. The hippocampus-amygdala realises, therefore, mutual influence between the emotional

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evaluation and cognitive processing, peculiar in the different animal species and in the different individuals. According to the different type of emotion, the neural processes involve many central nervous structures (prefrontal and orbital cortex, anteriore cingulate, insula, nucleus accumbens, ventral tegmental area, periacqueductal grey).

The adaptive mechanisms also rely on executive structures (hypothalamus, basal nuclei of the prosencephalon, mesencephalic tegment), and numerous central and peripheral biomodulators (dopamine, serotonin, cathecolamines, cytokines, endorphins, prostaglandins) are involved. These biomodulators provide functional and behavioral adaptations to adequate levels of physical and emotional stress tested.

Then, the balance between excitatory signals in the amygdala and hippocampal inhibitory signals sent to the paraventricular nucleus (PVN) determines the actual stress condition, so influencing the hypothalamic-pituitary-adrenal (HPA) axis activity and the amount of corticoliberin (CRH), adrenocorticotropic hormone (ACTH) and cortisol finally released.

## Cognitive control of emotion

Conditioning studies showed that the emotional learning experience can be addressed in a positive or negative way. It is known that the activation of the seeking system and reward, with specific appetitive activation mechanisms (sniff, touch, lick, explore, play) and subsequent consummatory events, promotes positive learning mechanisms of experience (positive emotion), with the mediation of the dopamine - endorphins system; on the contrary, negative emotions, generated by subsystems of fear and anxiety, alter the balance between the "fight" and "flight" responses.

The role of ventromedial prefrontal cortex (vmPFC) in the emotional learning experience has been enlightened by the studies of Ledoux (9). In the fear conditioning test, the vmPFC plays a significant role in the emotional regulation impacted by stress, controlling the neurocircuits of emotional memory and extinction learning processes modulated by serotonin. On the other hand, it has been demonstrated that the hippocampus and prefrontal cortex are rich in glucocorticoids and mineralcorticoid receptors and are particularly vulnerable to glucocorticoid excess (10).

In laboratory animals, very recent studies (11), using optokinetic techniques, discovered a new neural pathway connecting the cerebellum to the ventral tegmental area, that delivers dopamine to the vmPFC, so influencing reward responses and behavior.

The significance of right and left hemispheres in cognitive processing of emotional stimuli

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and emotional control has been also studied in different species (12), according to their species-specificity cognitive abilities and expressed behavior.

**Cognitive ability of animal models**

Then, cognitive processes allow animals to acquire, process, store and act on information from the environment. Awareness has been defined as the state in which complex brain analysis is used to process sensory stimuli or constructs based on memory (13).

Therefore, the individual and emotional response to stress is the function of individual "allostatic load ", i.e. the individual capacity of perception of stress and its control. Most species used as animal models exhibit cognitive capacities, but with varying degrees of awareness and emotion. The emotional responses, depending on the degree of species-specific and individual awareness, can vary in terms of valence and intensity, creating a conscious emotion or not (feeling), such as fear or pain, that can change the behavior or act as reinforcement in learning processes.

A pivotal review by Broom (14) has extensively discussed the cognitive ability and awareness of principal animal models. In animal species, he proposed four levels of awareness: (i) perceptual awareness, that results in an automatic response, that the individual may or may not be capable of modifying voluntarily; (ii) cognitive awareness, that induces brain processing of a flexible response to sensory inputs or mental constructs; (iii)assessment awareness, that is shown by the individual ability to assess and deduce the significance of a situation in relation to itself; (iv) executive awareness, shown by the individual in assessing, deducing, and planning the behavior.

The differentiated ability of animal species to learn was studied by Kilgour (15), using modified Hebb-Williams mazes for animals of different sizes. These mazes start with a decision point where there are two or more possible directions to take, one being towards a concealed target reached after two further turn. Therefore, it was demonstrated that cows, sheep, goats and pigs performed less well than 5-year-old children, but better than dogs, cats, rats, horses and several other mammals and birds.

Many animal species are able to recognize and discriminate among conspecifics, between calm or nervous individual state, between conspecifics and humans. They are also able to make discrimination and to show self- or not self -recognition by using mirror or iconic images.

In different species, self-recognition relies in different modalities – visual, olfactory, auditory- and the principal sensory modality is an expression of an underlying self-

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processing system (16).

The olfactory self-recognition has been demonstrated in many species and the visual self-recognition has been presented and discussed on the basis of species' peculiarities (17).

The ability of recognition and discrimination using olfaction, for example to get a food reward, has been shown in pigs (18,19), dogs (20, 21), and cattle (22). Many results suggest that sheep can use facial cues to discriminate between different species, breeds and male and female members of the same breed. Discriminatory performance is influenced by orientation, the presence of the eyes and vocal cues (23). Moreover, it has been demonstrated that sheep can learn to distinguish between individual adult sheep faces but that the breed and social familiarity influence the level of performance (24). The ability to discriminate individual sheep and humans has been also demonstrated, and, in association to behavioral discrimination, fired neuronsin the medial temporal and prefrontal lobes of the cerebralcortex were identified (23,25-28). In this species, unlike sheep face stimuli, that are processed in the right hemisphere, the human faces are processed symmetrically in the sheep brain, with a preference in the left hemisphere (29).

In monkeys, in order to study the neural mechanism underlying the ability to discriminate speech and conspecific vocalizations, many electrophysiological studies have investigated the neural responses of the auditory cortex to conspecific vocalizations. The data suggested that vocalizations may be hierarchically processed along an anterior/ventral stream from the primary auditory cortex (A1) to the vmPFC. In the cat, the neural response patterns in posterior auditory field (PAF ) were more complex and had longer latency than those in anterior auditory field (AAF). The selectivity for different vocalizations based on the mean firing rate was low in both AAF and PAF, and not significantly different between them; however, more vocalization information was transmitted when the temporal response profiles were considered. Discrimination accuracy based on the activities of an ensemble of PAF neurons was also better than that of AAF neurons. The data also suggested that AAF and PAF are similar with regard to which vocalizations they represent, but differ in the way they represent these vocalizations, and there may be a complex processing stream between them (30).

Horses are capable of a visual discrimination between conspecifics and not-conspecifics' images (31) and they seem also capable of mirror self-recognition (32).

Many studies also demonstrated that many animal species (dog, pig, parrot, chicken, fishes, not only Primates) have a concept of an object in absence of the object, have the concept of a symbol or of a location and they use these symbols to carry out an action or to have a food reward.

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To give some examples, they are able to find a hidden food, to respond to given commands, to use visual or auditory symbols for object and to use photographs of the objects to carry out an action (33-37).

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