Inflammation and behavior changes in dogs and cats: a review

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Key Points

- Inflammation is a series of complex response events caused by the host system facing infection or injury.
- Inflammation responses are driven by proinflammatory cytokines that are released peripherally but also act centrally on the brain causing the behavioral symptoms of sickness.

- Sickness behavior results both from peripheral (e.g., infection) and central (e.g., psychological) pathways.
- Proinflammatory cytokines play a major role in inflammaging, that represents a significant risk factor for morbidity and mortality in the elderly animals.

Synopsis

Sickness is a normal response to infections or stress triggered by proinflammatory cytokines, that drive local and systemic inflammatory responses. Proinflammatory cytokines act on the brain causing the so called "sickness behavior", which is thought to improve recovery but can become maladaptive in the long term. Chronic inflammation characterizes many diseases and there is some evidence that dogs and cats experience age-associated increases in inflammation, a condition named "inflammaging". A complex and multifactorial relationship exist between these inflammatory mechanisms, pain and psychological illness that may complicate veterinary diagnosis and affect the outcome.

INTRODUCTION

Proinflammatory cytokines are known to be cell signaling molecules that guide an organism's response to illness, injury, and infection [1]. Therefore, inflammation is traditionally perceived as associated with acute or chronic medical disorders [1]. Nevertheless, it is noticeable that behavior changes are among the first and most important issues related to inflammatory activity. Potent broad-spectrum inflammogens, such as the bacterial endotoxin lipopolysaccharide (LPS), have been shown to induce in rodents depressive-like symptoms, often known as "sickness behaviors" (SB), potentially arisen from an interaction between the immune system and the serotonine (5-

HT) system. [2] Cytokines, such as interferon-alpha, tumor necrosis factor (TNF)-alpha, interleukin (IL)-1 and IL-2, signal to the brain the presence of an infection in the periphery [3] eliciting the sickness behavior through an endocrine mechanism or direct neural transmission. [4] Originally described by Hart, [5] cytokine-induced SB occurs in birds and mammals, including dogs and cats, as part of an adaptive, motivational response to preserve energy and help in recovery from infection. [4]

Inflammation-induced SB refers to both nonspecific clinical and behavioral signs that include fatigue, sleepiness, vomiting, diarrhea, anorexia, or decreased food and/or water intake, fever, decreased general and body-care activities (i.e., grooming), social withdrawal or loss of interest in social activities, and altered cognition. In addition, enhanced pain-like behaviors are often observed, although these can be followed by hypoalgesia during the latest stages of sickness. In humans, proinflammatory cytokines have been reported to induce not only symptoms of sickness, but also true major depressive disorders in vulnerable subjects, even without previous history of mental disorders. [6] All these signs have been shown to be independent of the febrile response, [7] in fact not only infections but also the chronic activation of the stress response system can overtax homeostatic regulatory systems, resulting in SB [8]. In dogs and cats, the hypothalamic stress response system may be activated by external environmental events such as sudden changes, unknown or loud noises, novel and unfamiliar places and objects, and the approach of strangers, or even by psychological stressors. [9,10] Similarly to infection, environmental and psychological stressors may be linked to immune activation and proinflammatory cytokine release [11,12] as well as to changes in mood and pathological pain (Figures. 1-3). [8,13,14]

INFLAMMATION AND THE BRAIN

The brain monitors peripheral innate immune responses by different immune-to-brain communication pathways [15–19] that act in parallel, leading to the production of proinflammatory cytokines by local microglial macrophages. The receptors for these mediators are expressed by both neuronal and non-neuronal brain cells [20]. That said, the brain circuitry that mediates the behavioral effects of cytokines remains mostly unclear. It seems highly probable that different behaviors observed in cytokine-induced SB are controlled by different brain areas: this means that, for example, in case of infection, the social withdrawal likely involves different areas than those involved e.g. by loss of appetite [21] or activation of the hypothalamus–pituitary–adrenal axis. [22]

Many SB-related abnormalities, such as states of anxiety and feeding difficulties, have been reported in family dogs and cats after the exposure to unusual external events, including changes in caretaking and daily routine, or psychological stress, regardless of a disease status. [23] Therefore, veterinary clinicians should consider the possibility of exposure to environmental and psychological stressors in dogs and cats assessed for SB signs.

The view of this syndrome as the expression of a motivational state has important implications in terms of homeostasis, particularly as it relates to what is called relative homeostasis. Relative homeostasis considers homeostasis as being featured by various systems, each well-organized at the subjective, behavioral, and physiological levels and adapted to specific physiological conditions. [12] Different motivational states, such as fear, hunger or thirst, or being pathogen-free, have different physiological requirements and their own homeostatic regulatory systems. [3]

From an evolutionary perspective, the behavioral effects of cytokines in response to acute stressors are beneficial for an organism. Seeking rest and care in response to an infection, for example, is advantageous for an individual, in that it allows to shift to a state of increased arousal

and readiness for action when they are confronted with a real or potential threat. Conversely, if sickness is prolonged or exaggerated with respect to the causal factors that have triggered it, the sickness response is no longer adaptive, as it typically occurs during chronic inflammatory disease. In these cases, inappropriate, prolonged activation of proinflammatory cytokines may be involved in brain and systemic disorders, ranging from Alzheimer's' disease to cardiovascular disease, which would explain changes in the mental state and cognition of affected-individuals [3]. Moreover, inflammation is involved in the pathogenesis of many diseases of aging and an increase in age is reported to possibly confer a significant risk for an increase in total number of SBs also in non-human animals, one example is cats with interstitial cystitis. [23] Given the exceptional growth in the worldwide dogs and cats population age, recognizing the effects of inflammation on behavior changes of elderly pets have broad animal health implications.

INFLAMMATION AND AGE (INFLAMMAGING)

Low grade sterile inflammation is a process that is closely related to immunosenescence. [24] Immunosenescence is the dysregulation of the innate immune system that occurs in elderly individuals [25] and predisposes them to increased morbidity and mortality due to infection and age-related pathology [26]. In human medicine, the most consistent findings of recent studies show an age related impairment of the cell-mediated immune function. [27] Moreover, decreased T cell proliferation has been recognized as a peculiarity of immunosenescence in cats [28] dogs, [29] horses [30], and humans[31]. Reduced blood CD4(+) T cells (with imbalance in Th1 *versus* Th2 functional activity), elevation in the CD8(+) subset and reduction in the CD4:CD8 ratio are also reported in senior dogs and cats. Conversely, the dysregulated activity of the innate immune system at brain level leads to an enhanced production of proinflammatory cytokines, such as IL-6, and a

decreased production of anti-inflammatory cytokines, such as IL-10. [32] A large part of the aging phenotype, including immunosenescence, is explained by an imbalance between inflammatory and anti-inflammatory networks, occurring in the absence of overt infection, which results in a status of chronic, low grade inflammation, called inflammaging. [33] Initially defined by Franceschi et al. in 2000, [34] inflammaging became the focus of many subsequent studies, which in the last decade have brought significant progress in knowledge of this condition. [35] Inflammaging is characterized by the following five states: [36] low-grade, controlled, asymptomatic, chronic, and systemic. Normally, inflammatory responses fades away when proinflammatory factors in infection and tissue injuries are eliminated and then turn into a highly active and balanced state known as “resolving inflammation”. [37] Conversely, the inflammation during inflammaging is in an uncontrolled and unbalanced state, named “nonresolving inflammation”. [37] Such imbalance can be a major driving force for frailty and common age-related pathologies, [33] including the nervous and the musculoskeletal system [38]. It ultimately has negative impacts on metabolism, bone density, strength, exercise tolerance, vascular system, cognitive function, and mood. [39] Up to this date, inflammaging is considered a key factor in acceleration of the aging process and lifespan and, in humans, it is highly related to conditions including Alzheimer's disease, [40] Parkinson's disease, acute lateral sclerosis, multiple sclerosis, atherosclerosis, heart disease, age-related macular degeneration, [41] type II diabetes, [42] osteoporosis and insulin resistance, [43] cancer, and other diseases. Overall, inflammaging increases morbidity and mortality, seriously impairing the health and the quality of life of patients. [43] Although this has not been studied systematically yet, empirical observations in veterinary clinics and owners’ reports suggest that this phenomenon also affects the quality of life and welfare of elderly dogs and cats [44,45].

PATHOPHYSIOLOGY OF INFLAMMAGING

As already anticipated, inflammaging implies elevated levels of circulating proinflammatory cytokines, [46] including IL-6, IL-1β, IL-15, IL-18, TNF-α mRNA, and TNF-α protein, [47] which, through different biochemical reactions and pathways, [48] induce the production of senescent cells. These cells must be effectively removed and replaced, otherwise their accumulation may contribute to the manifestations of aging. Moreover, senescent cells secrete proinflammatory cytokines and other compounds [49], developing a senescence-associated secretory phenotype (SASP) which contributes to the age-associated chronic low-grade inflammatory condition.

As mentioned above, chronic inflammation is a core-aging mechanism that appear to be relevant in the pathophysiology of tissues and organ systems, including brain tissue. There has been extensive investigation of age-associated neurodegenerative disease in the dog. Chronic inflammation is involved in the loss of brain mass and function. Aberrant production of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, by microglia and astrocytes supports a neurotoxic milieu that contributes to neurodegeneration. [50,51] Signs of senescence have been detected in mammalian brains, highlighting their potential role in brain aging, [52] although information is still negligible in the aged canine brain. Conversely, some evidence of increased inflammatory activity and gene expression patterns in aging canine brain tissue is available. [53]

Medicine is working towards the identification of suitable clinical markers which may help understand whether an organism's proinflammatory and anti-inflammatory status is in balance. Unfortunately, biological markers specific of the aging process have not yet been fully identified, which does not help in evaluating the degree of inflammaging. Based on the above-mentioned underlying mechanisms, the most promising markers of inflammaging include immune cell markers (e.g., CD8+ T cells, a decrease in CD4+ T cells and CD19+ B cells), serum cytokine

markers (such as IL-1 and IL-10, [54] which have been identified in dogs and cats [27]) and microRNAs, [35] a class of molecules involved in the regulation of gene expression and biological pathways associated with inflammation, cellular senescence, and age-related diseases. [55]

THE CONCEPT OF ANTINFLAMMAGING

While inflammation could be beneficial to the organism by neutralizing the cytokines early in life, it becomes detrimental in later years as inflammaging. [56] Inflammaging can be counter-acted by anti-inflammaging. [33] One of the endogenous counter-regulators recognized is cortisol. [57] However, besides being the main specific response and counterbalance to inflammaging, the activation of HPA [58] becomes with time the cause of the decline of immunological functions, leading from robustness to frailty. [39] The frailty phenotype is an objectively measured indicator of advanced-stage aging that is characterized by organism-level dysfunction, while the altered inflammation markers level signifies an earlier stage between cellular abnormalities and systems dysfunction: results from human observational studies and randomized controlled trials indicate that these measures facilitate classification of older patients with chronic conditions into groups that vary in disease incidence, prognosis and therapeutic response/toxicity. [59] In veterinary medicine there's been given increasing attention to frailty in the assessment of aging dogs, [60–62] which is defined as a decline in an organism's physiological reserves resulting in increased vulnerability to stressors and a frailty index, which is directly related to survival and can be measured through a validated scale. [62]

To further complicate the picture, coexistence of immunosenescence and inflammaging [25] makes it difficult to establish whether the inflammation-related diseases are caused by one or the other

process. Unfortunately, there is still need for integrated biological and clinical research before a causal relationship may be said to exist between inflammaging and diseases. [35]

While it is difficult to assess the degree of inflammaging in an individual, both human and veterinary medicine have focused on protective and preventative interventions. Several factors probably contribute to the increased inflammatory response in the elderly. Recent attention in geroscience has focused on alterations in cytokine receptor signaling, [63] the imbalance of redox factors, [64] changes in genotypes, [65] increased body fat, [66] and life-long antigenic exposure. [46] These are all likely candidates responsible for chronic immune system activation and inflammation associated to age, and acting against these factors would, therefore, help counter-act inflammaging. In humans, there is some evidence that antiaging interventions, such as exercise and dietary restriction, may mitigate inflammaging-related changes. [27,67] In the study of Alzheimer's disease, it has been observed that physical activity can improve neurogenesis and mitigate the age-related loss of brain mass both in the hippocampus and globally in the brain. [68] Similarly, veterinary medicine has started to recognize aging as a life-long process and healthy-aging as a goal that needs to be addressed early in life. [69,70] Aging research in veterinary medicine has brought some evidence that environmental enrichments and physical activity might help preserve and ameliorate cognitive function in aging dogs, [71,72] while the effect of exercise on feline brain aging is still unexplored. [61]

INFLAMMATION AND BEHAVIOR FROM THE VETERINARY CLINICIAN'S PERSPECTIVE

Recently, several studies have suggested a causal link between ongoing inflammation and impaired mental health in humans. [6] Inflammation is considered a risk factor for depression and

its correlation with behavior changes has been widely highlighted. [74] Dantzer and colleagues have reported that proinflammatory stimuli, such LPS or IL-1β, increase activity in the tryptophan metabolizing enzyme indoleamine 2,3-dioxygenase, resulting in lowered tryptophan availability and therefore decreased 5-HT synthesis. [6] Although it has been questioned more recently, [75] a link between lowered serotonin and depression has been suggested since the 1960s [76–78] and has been the basis for the use of antidepressants. [79]

Psychological illness, including depression and anxiety disorders, and stress induce the same inflammation-related mechanisms and behavioral changes of sickness caused by diseases and infections [80] and might be responsible for the presence of an animal's SB. [5] For instance, SB has been shown to increase in cats with interstitial cystitis, for which a relationship to psychological stress has been well described. [81] The same has been observed in healthy cats during changes in their environment, including husbandry changes, unfamiliar caretakers, feeding delays, and withdrawal of playtime; decreased food intake and increase of house soiling are the most commonly observed behavioral changes. [23]

Inflammation may also be linked to pain, by cyclooxygenase (COX) enzymes, mostly COX 2, which help synthesize prostaglandins (PGs) found in elevated concentration at the inflammatory site. [78] Once released, PGs either stimulate pain receptors or sensitize them to the effect of other pain producing substances (such as histamine, 5-HT, and bradykinin) which induce neurons to send pain signals to the brain. [78] There is evidence that cytokines (e.g., IL-1β, TNF-α) are involved in the initiation as well as the maintenance of pain by directly activating nociceptive sensory neurons [83]. Pain-induced responses lead to several physiological changes including a decreased serotonin activity in the brain, [84] that is also negatively influenced by the reduction of physical activity caused by pain. [85] In the experience of pain, the emotional brain is strongly

involved as pain is a highly subjective psychophysical experience mediated by the immune system and by the stress response system. Pain-induced alteration in motivation and emotional states results in a wide range of potential changes in behavior, that are the most commons sign of pain in itself [86] (Fig. 4, Video 1). Indeed, the modern approach to pain no longer focuses on measuring the intensity of pain but on subjective feelings [87–89] which, in non-human animals, should be based on the indirect evaluation through behavior changes and response to pain medication. The most common signs of pain are those related to a reduction of previously expressed behaviors and to the development of previously not expressed behaviors (Table 1). [90] For example, pain can lead to aggressive behaviors that are often described as a defensive reaction to avoid physical contact which the animal has learnt may cause further injury. [91] In terms of emotional component, pain can also induce fear, acting as an unconditional stimulus. [92,93] When exposed to a painful stimulus, an individual will try to predict similar situations in the future by creating associations between the stimulus that causes pain and other neutral stimuli. [94] It may be the case for a cat suffering from urinary tract disease or osteoarthritis, who can experience pain while urinating, which in turn may induce her make an association between pain and the litter tray, thus developing periuria. [95] These changes are usually biological adaptive reactions although they are frequently perceived by the owner as problematic. [96]

The nature of the relationship between pain and problem behaviors may be complex and heterogeneous and had been classified as: [97]

1) Presenting complaint as a direct manifestation of pain;
2) Unidentified pain underpinning secondary concerns, within the initial behavior problem;
3) Exacerbation of one or more signs of problem behavior as a result of pain;
4) Adjunctive behavioral signs to the primary complaint, associated with pain.

Fundamentally, behavioral and medical diagnoses often coexist, as animal behavior reflects the individual physiological state. [98] The traditional clinical approach to behavioral problems has been based on analyzing whether the behavioral changes were "purely behavioral" or secondary to physical conditions, excluded through comprehensive differential diagnoses. The potential influence of medical factors on behavior problems were viewed as diagnoses of exclusion. [99] More modern approaches consider behavior as an output of the brain and the body, in other words, one of the methods by which the individual seeks to establish an equilibrium between internals factors, such as health, and the environment .[96] Any illness or treatment will have an effect on behavior, which should be kept into account, as behavioral and physical health are both components of an integrated system that should be managed and treated as a whole [96]. In parallel with human psychiatry and the multi-axis approach, veterinary clinicians are today encouraged to make a complete and exhaustive assessment of a case by collecting and organizing information about all the factors that, together, may contribute to behavioral and mental health problems in companion animals. [96] In veterinary behavioral medicine, efforts are made for referrals in different specialties in order to have a complete assessment of cases, to a more holistic collection of information regarding the management of the case, and to a synergetic vision regarding the therapy (Fig. 5). In addition, factors such as personality traits and social and physical environment have an influence on whether a particular individual will show a problem behavior [100] and even develop or display signs of illness. [10,45] In other words, it is essential to understand how behavioral medicine and other specialties such as neurology, dermatology, gastroenterology, etc. are linked together to improve diagnosis and therapy process. [97,101–104]

Summary

SB is evoked by proinflammatory cytokines released by macrophages, dendritic cells, and mast cells, which can trigger behavioral changes via the activation of sensory neurons or the secretion of immune signaling molecules from the microglia into the brain. [1–4] These pathways may be activated in response to infectious pathogens or stress, [8] particularly in the aged animals. [106] SB is therefore relevant in various contexts besides fighting infections. Behavioral changes include increased body temperature, sleep, loss of appetite as well as metabolism alterations causing weight loss. Sickness behavior and inflammation are possible factors to consider in the onset and maintenance of pain and should be viewed from a bio-psycho-social perspective.

Clinics Care Points

- Sickness Behavior is a cytokine-mediated motivational adaptive response linked to infection and inflammation.
- Elderly animals present changes that are the result of the balance between and anti-inflammatory activity in the body (senescence and inflammaging). There are no specific markers, therefore the behavior and the physical health of the animal need to be assessed.
- Physical health should be assessed through objective scales (e.g., frailty scales)
- Physical and mental health are closely connected: behavioral and medical diagnoses often coexist.
- Veterinary clinicians should collect information about all the factors that may contribute to behavioral and mental health problems.
- Other factors such as personality traits and social and physical environment have an influence on whether a particular individual will show a problem behavior.

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562

563 **Figures, Tables, Boxes, and Videos:**

564 • Figure Legends

565 **Fig. 1.** Sickness behavior in a 17-year-old dog with acute pyelonephritis. The dog's
566 reactivity, feeding and social behavior are reduced, resting and apathy increases. (Courtesy
567 of Patrizia Piotti DVM, MSc, PhD, MRCVS. Milan, Italy).

568 **Fig. 2.** Cowering behavior and painful posture in a poodle brought to the behavior consult
569 for fear and anxiety. The history and further exams revealed and old fracture to the right
570 foreleg and dysplasia of the left knee (Courtesy of Ludovica Pierantoni DVM,
571 ECAWBM - Behavioural Medicine. Naples, Italy).

572 **Fig. 3.** Left: A 16-year-old cat with hyperthyroidism and CKD IRIS 3 manifested as
573 increased activity levels and decreased appetite (behavioral signs) associated with weight
574 loss, moderate to severe sarcopenia and dehydration (physical signs). Right: the same cat
575 at 17.5 years. An antalgic posture is evident (Courtesy of Patrizia Piotti DVM, MSc,
576 PhD, MRCVS. Milan, Italy).

Fig. 4. The inflammation, depression, and pain cycle.

Fig 5. Extended examinations and tests required by during a behavioral assessment.

- Tables

Table 1: pain induced behavior changes.

- Videos Legends

Video 1 - In the video a dog showing pain postures (cowering, low tail) and attention seeking behavior, later diagnosed with a painful condition (i.e., knew dysplasia). (Courtesy of Ludovica Pierantoni DVM, ECAWBM - Behavioural Medicine. Naples, Italy).