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Measures of spatial memory, peripheral inflammation and negative emotional state predict social-cognitive skills in healthy aging cats

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ABSTRACT

Aging is a complex, multidimensional process. Normative aging is typically accompanied by declines in several cognitive domains, including memory and social cognition. As aging progresses, the often-overlooked early manifestations of cognitive decline can advance, leading to an inability to adapt to new situations and environments. Potential mechanisms influencing cognitive decline remain elusive, but, among other mechanisms, inflammaging, an age-related increase in the chronic production of pro-inflammatory mediators, and emotional stress are implicated. Cognitive function in cats is under-researched, with no studies on the link between potential risk factors and cognitive signs in aging subjects. Understanding this link is essential as it may facilitate the early identification of cognitive decline and encourage preventative, individualized interventions. We carried out a citizen science study to investigate, for the first time, the relationship between cognitive functions, stress, and the inflammatory markers interleukin (IL)-1β and IL-10 in 44 healthy, privately owned pet cats (7–14 years of age), who underwent standardized testing in their home environment. A task was employed to test spatial memory, requiring the cat to recall the location of a food-baited container. An unsolvable task was used to assess cats' social cognition and cognitive flexibility by measuring how they used referential communication (i.e., gaze alternation between the apparatus and the caregivers) compared to their persistence in trying to obtain food which was out of reach. Generalized linear regression analysis, conducted while adjusting for demographic factors and stress behaviors, revealed: 1) a predictive positive relationship connecting spatial memory and the use of social referencing with the caregiver (more gaze alternation) in the unsolvable task, highlighting the importance of the interplay between cognitive domains in sustaining complex behaviors, and 2) a reduced engagement in social referencing toward caregivers (less gaze alternation) in the presence of higher serum levels of IL-1β in the unsolvable task, especially as cats get older. A cat's social cognitive performance was also negatively predicted by stress-related behaviors, which is not surprising given that cats are sentient creatures whose everyday behavior is also influenced by emotional states. These findings provide new insights into the crosstalk between different cognitive domains during aging and the potential contributions of emotions and inflammation to cognitive changes in healthy aging cats. Further research using this multidimensional approach will help explore these mechanisms of cognitive aging and potential markers for early detection of cognitive changes in older cats, which is critical for timely interventions.

1. Introduction

According to the most recent estimates, there are over 600 million domestic cats in the world. Of these, approximately 95.6 million reside in households in the US (Megna and Bailie, 2024) and 10.2 million are found in Italian homes ("Statista," 2024). Moreover, a rise has been recorded in the number of cats aged over 11 years, with a lifespan estimated at 15 years, which varies based on geographic location, outdoor access, sex, breed, and other factors (O'Neill et al., 2015; Teng et al., 2018). This increase in life expectancy and demographic shift into

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an older age inevitably raises the question of how to promote healthy aging (HA) in the domestic cat population. A generally accepted standard definition for a 'healthy aged cat' assumes that a cat without any clinically-manifested disease affecting their health and wellbeing is healthy for their age Hajzler et al., (2023)). Among the changes typically observed early during normal aging are those related to various domains of cognition, including memory (e.g., disorientation and confusion) and social interactions (e.g., increased or decreased interaction with people or other pets) (Landsberg et al., 2010; Denenberg et al., 2024). Although memory and social cognition are not the only indicators of a cat's cognitive ability, they provide relevant cues about their capacity to behave flexibly in response to environmental challenges (Azadian and Gunn-Moore, 2022; Vitale Shreve and Udell, 2015). Given that aging is a gradual process, such age-related changes may initially be mild and subtle, and thus may go unnoticed or be considered normal by caregivers (Denenberg et al., 2024). However, their progression may ultimately lead to a pathological state, such as the cognitive dysfunction syndrome (CDS) and/or result in reduced well-being and quality of life (Bellows et al., 2016b). Sometimes, cognition-related behavior changes in elderly animals may also appear before other systemic signs of illness become apparent, and therefore they can be useful early indicators of a decline in general health and welfare. A clear understanding of these changes is invaluable, particularly if efforts are directed toward their early identification, which allows veterinarians and caregivers to counteract the consequences of their progression, facilitate the early recognition of systemic disease, and ultimately support the aging process in elderly cats. This is particularly urgent given the significant prevalence of age-related cognitive changes in cats: approximately 28 % of cats between 11 and 14 years exhibit signs of cognitive dysfunction, and this prevalence increases markedly in cats aged 15 years or older (Landsberg et al., 2010). Unfortunately, cognitive impairment associated with older age remains an underappreciated and understudied condition in pet cats (Klug et al., 2020).

Several cellular and molecular hallmarks have been identified as key drivers of the aging process, including genomic and molecular alterations (e.g., genomic instability, telomere attrition, and epigenetic changes), mitochondrial dysfunction, loss of proteostasis, cellular senescence, stem cell exhaustion, and chronic inflammation (Menassa et al., 2023). Among these, we chose to focus on the inflammatory aspect of aging—specifically persistent, low-grade inflammation without overt infection (Day, 2010; Piotti et al., 2024), or "inflammaging"—because of its documented critical role in cognitive decline, which was the primary target of our investigation, and in the pathogenesis of physical diseases. Inflammaging has, in fact, been implicated in the progressive minor decline of cognitive function, with peripheral cytokine patterns being investigated in humans and animal models for their potential role in mediating the complex interactions between the peripheral and central nervous immune systems across the lifespan (Fard et al., 2022). The range of subclinical yet aging-associated cognitive performance changes in older subjects, before reaching a diagnosis of CDS, represents a critical area of interest in inflammaging research, particularly concerning the relationship between these processes and cognitive function (Fard et al., 2022). Despite its importance, this area remains insufficiently researched (Fard et al., 2022) and, to our knowledge, has yet not been explored in cats.

This study investigated, in a cross-sectional predictive design, the relationship of short-term spatial memory and peripheral cytokines (IL-1β and IL-10) with social cognition in healthy older cats (7 years and above). Since the reactivity of the hypothalamic-pituitary-adrenal (HPA) axis to emotional stress increases with age (Traustadóttir et al., 2005) and can negatively affect cognitive function in both the short and long term (Scott et al., 2015), the effect of cats' stress-related behaviors on social cognitive abilities was also explored. The cognitive assessments used in this study were standardized tests previously applied in scientific practice to measure cognitive abilities in animals, including cats, in a home environment (Azadian and Gunn-Moore, 2022). In particular, a short-memory task was employed to test spatial memory, requiring the cat to recall the location of a food-baited container (Piotti et al., 2022). Social cognition was tested using the unsolvable task, a tool that assesses both the tendency to engage in social behaviors toward humans and decision-making (Piotti et al., 2021; Scandurra et al., 2023). In this procedure, the animals face a problem they cannot solve on their own (obtaining food that is out of their reach), and react turning towards a human partner (Miklósi et al., 2003) both by gazing toward them and alternating their gaze between the object of their interest and the human face Pongrácz et al., (2019)). In recent years, there has been growing scientific interest in the cognitive and social abilities of cats. Within this framework, gazing at humans has emerged as a relatively recent discovery. It is now well established that cats use gaze to communicate with their owners or caregivers, and in some contexts, such as during unsolvable tasks, they even prefer this behavior over physical contact (Miklósi et al., 2005; Scandurra et al., 2023; Zhang et al., 2021). This gazing behavior is significant because it suggests a level of social cognition that was once thought to be primarily present in other domesticated animals, such as dogs. Particularly, recent findings in canine research suggest that alternative mechanisms, such as 'giving up' behavior, might be involved, while perseverance could also play a role (Lazzaroni et al., 2020; Rao et al., 2018; Hirschi et al., 2022).

Of the two gazing strategies, gaze alternation meets the landmarks for intentional and referential communication (Leavens et al., 2005) as it includes a referential component to the object of desire (Merola et al., 2015; Miklósi et al., 2000), it is affected by the attentional state of the audience (Marshall-Pescini et al., 2013), it can be preceded by attention-seeking behaviors (Gaunet and Deputte, 2011), and it shows persistence and elaboration (Savalli et al., 2014). The present study aimed to answer the following question: are measured IL-1β and IL-10 serum concentrations, along with spatial memory performance and emotional distress, predictive of social cognitive performance as a function of aging (inflammaging) in cats? Based on the literature previously reviewed, we hypothesized that healthy elderly cats with lower serum cytokine concentrations, better performance on short-term memory tests and lower levels of stress would have higher odds of demonstrating enhanced social cognition, as evidenced by increased gaze alternation in the unsolvable task.

2. Methods

This study was conducted as part of a multicentric research project (ethical approval IACUC protocol 807030; Regulations of the University of Milan, decision EC 29 Oct 2012, renewed under protocol No. 02–2016) designed to investigate the effects of age-related chronic inflammation on the health, behavior, and welfare of elderly cats. Recruitment occurred through opportunistic sampling among the patients attending routine preventative care appointments at the Primary Care Service of the University of Pennsylvania School of Veterinary Medicine (PennVet) and the University Veterinary Hospital of Lodi, University of Milan (OVU UNIMI). All caregivers signed written informed consent and agreed to participate in the study.

2.1. Participants

The cats included in this study are part of a larger population of 97 cats enrolled in a research project investigating the role of chronic inflammation on behavior and cognition of aging cats (Morris Animal Foundation D21FE-508). The cats were selected from an initial group of 156 cat owners who expressed interest in participating in the project. Before enrollment, a pre-screening was conducted during preventative care appointments. These included a behavioral and physical examination, guided by a standardized checklist for the health evaluation of elderly cats (adapted from Bellows et al., 2016b, Bellows et al., 2016a). Furthermore, a fecal flotation was conducted to check for intestinal parasites. A blood sample was obtained for routine analysis, including tests for FIV, FeLV, and heartworm (SNAP Feline Triple Test, IDEXX Laboratories, USA), in adherence to the minimum laboratory evaluation standards for mature, senior, and geriatric cats as outlined in the AAHA guidelines for senior pets (Epstein et al., 2005). The recorded results of blood work were assessed based on the reference intervals for healthy aged cats (Bellows et al., 2016a). To be eligible for the study, cats had to be 7 years or older, and free from clinically relevant signs or existing diagnoses of physical or mental disease, such as sensory dysfunction and pain. Behavioral issues not stemming from underlying behavioral pathology (e.g. chronic or excessive anxiety) —for instance, house soiling due to substrate preference—were not considered behavior diseases. Additionally, cats needed to be food-motivated and show interest in new situations and objects, as food and novel objects were involved in the cognitive tests they would undergo if they were enrolled.

The cats that completed successfully all the cognitive tests and for which we obtained valid cytokine measurements were selected for the study presented here. For example, cats that failed to interact with the food containers used during the testing (see the following section on Experimental Protocol) were excluded. Consequently, 44 cats aged 7–14 years (median age = 8 years), with a median body condition score (BCS) of 6 (minimum = 4, maximum = 8), an equal sex ratio of 1:1, all desexed and from various breeds, were included in this study. The majority were domestic short-haired (n = 39), followed by one each of domestic long-haired, Maine Coon, Persian, Ragdoll, and Siamese. Within a two-week period from their initial clinic visit, the cats underwent cognitive testing. The blood remaining serum after their blood work was used for the measurement of serum cytokine concentrations (IL-1β, IL-10).

2.2. Experimental protocol

2.2.1. Cognitive testing

To avoid stress related to travel, new environments, and unfamiliar people (Bradshaw et al., 1996; Riemer et al., 2021), as well as COVID-19 constraints due to lockdowns (Fukimoto et al., 2023), the animals were tested in their home environment, within a familiar room of approximately 2 by 2 m. In case furniture had to be moved around, this room was prepared by the caregivers 2–3 days before the test, following appropriate written instructions. The evaluations were conducted remotely by the same two researchers, with one at OVU UNIMI and the other at PennVet, using institutional Zoom or Microsoft Teams. The researcher at OVU UNIMI was a resident of the European College of Animal Behaviour and Welfare, while the researcher at PennVet was a resident of the American College of Veterinary Behaviorists. For the video call, the caregiver utilized a computer or tablet to record the test. The researcher had a view of the entire testing area on her device and ensured that the caregiver's face was clearly visible, facilitating the coding of specific cat behaviors (e.g., gaze alternations). The researcher began recording the cat's cognitive testing session for subsequent video-coding. The cats underwent two consecutive short behavioral tests that had been previously standardized and validated. The first test assessed spatial memory (Fig. 1A). During this task, the cat was allowed to search for food that they had previously observed being hidden. Following the researcher's instructions, the caregivers arranged the room by positioning five containers in a semicircle, labeled from A to E from the caregiver's left to right. The containers were equidistant from each other and from the position where the caregiver was seated with the cat on their lap and secured to the floor with double-sided tape. A test trial involved presenting each cat with one baited container out of the five. To obtain the food, the cats were required to recall the location of the baited container after a 30-second interval. Trials were repeated 5 times, changing the baited container according to a pre-determined pseudo-random sequence produced using a dedicate software (www.random.org). Highly favored cat food was used (e.g., cat kibble, cat treats, pieces of cheese or ham, etc.).

Each trial comprised three distinct and sequentially structured phases:

1) Encoding (Fig. 2A): the caregiver slowly baited one container while holding the cat and ensuring that they were observing (calling for the cat to gain their attention, if needed).
2) Distraction (Fig. 2B): immediately after baiting the containers, the caregiver turned around, holding the cat in front of them, so that both faced away from the containers. During this time, the cat was distracted for 30 seconds through cuddling and talking by the caregiver.
3) Recall phase (Fig. 2C): the caregiver turned around and released the cat in front of them. The cat was then given 30 seconds to return to the containers and attempt to retrieve the food from the one it had previously observed being baited. Caregivers were instructed not to talk to the cat during the search period, and to refrain from pointing at the containers at any time. After the 30 seconds, if the cat had not found the food, the caregiver was permitted to approach the baited container, show the food to the cat, and allow them to eat it.

The second test assessed the cats' social cognition and cognitive flexibility using an unsolvable task (Fig. 1B). The procedure (adapted from Piotti et al., 2022 and Scandurra et al., 2023), involved securely attaching a small container to the floor using double-sided tape. The

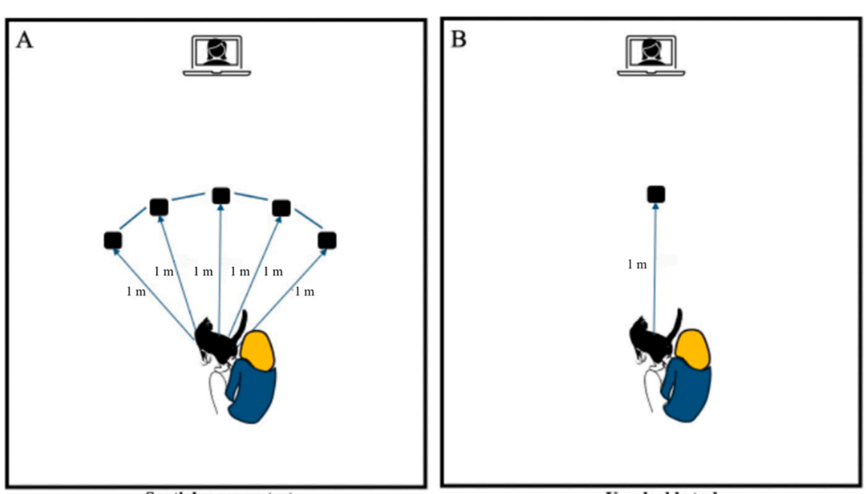

Fig. 1. Schematic drawings of the testing room setup for the spatial memory test (A) and the unsolvable task (B). The caregiver used a computer to allow the researcher a full view of the testing area and record the cat's behavior during the test via video call.

Fig. 2. Cognitive test for short-term spatial memory. A: Encoding - the caregiver baits one container, ensuring the cat is watching. B: Distraction - the cat is moved away and distracted by the caregiver for 30 seconds. C: Recall - the cat has 30 seconds to try and retrieve the food. This procedure was repeated for each of the 5 containers.

container was placed directly in front of the caregiver who held the cat. The test included three solvable trials and one unsolvable trial.

1.1. Solvable trials (Fig. 3A): the caregiver baited the container while holding the cat, ensuring they were observing the process. Immediately after, the cat was released and given 30 seconds to retrieve the food from that container, which was slightly ajar. During this period, the caregiver could remain attentive to the cat, but was instructed not to make gestures or speak to them. The trial concluded either when the cat consumed the food or after 30 seconds had elapsed. If the cat had not consumed the food within this time frame, the caregiver was then directed to assist the cat in accessing it. This trial was conducted three times, with the container's lid progressively covering more of the opening each time. If the cat did not try to obtain the food on either of the trial, they were excluded from further testing.
1.2. Unsolvable trial (Fig. 3B): the procedure mirrored that of the solvable trials, except that the container was securely sealed with its lid so that the cat could not access the food. After being released, the cat was given one minute to attempt to retrieve the food, which was inaccessible due to the closed lid. Subsequently, the caregiver opened the lid, allowing the cat to access the food.

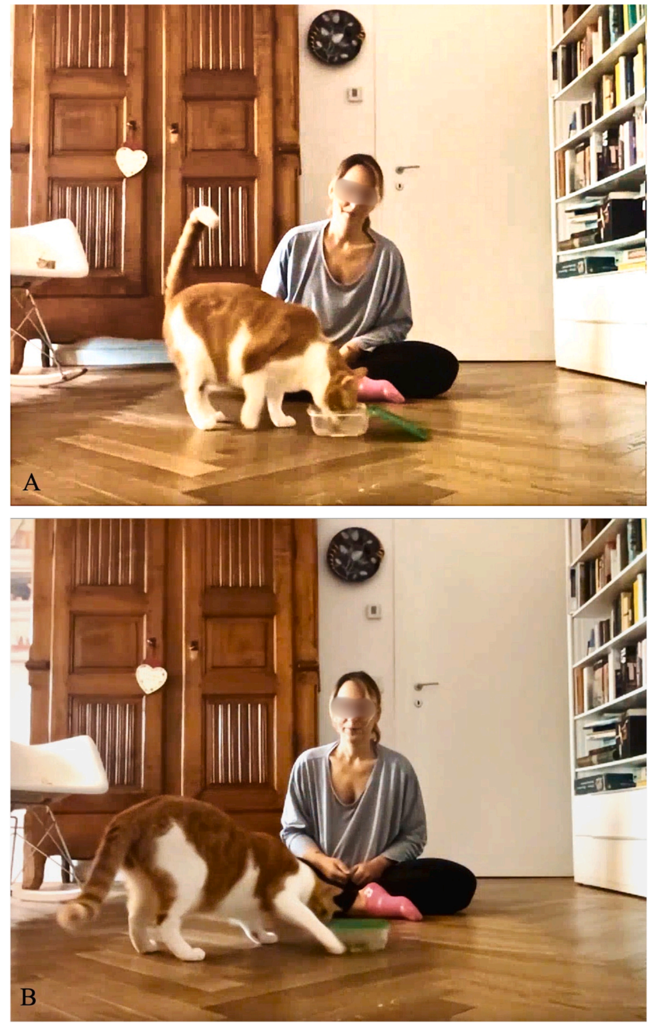

Fig. 3. Unsolvable task procedure. A: Solvable trial - the caregiver baited a Tupperware container, leaving it slightly ajar and ensuring the cat was watching. The cat was then given 30 seconds to retrieve the food from that container in front of the attentive but silent caregiver. This trial was repeated three times, with the container's lid progressively more closed. B: Unsolvable trial - the same procedure as the solvable trials, except that the container was securely sealed with its lid.

If the cat was not inclined to engage in the activity, left the room, began grooming, stopped eating the food, or exhibited high levels of stress or anxiety, the tests were immediately interrupted. Caregivers were provided up to three opportunities to repeat the test on different days unless they opted to withdraw from the study earlier.

2.3. Video coding

The video-recorded tests were analyzed using Solomon Coder, (ELTE TTK, Budapest, Hungary, (András Péter, 2006). The cats' performance in each short-term spatial memory test's trial was characterized by measuring the dependent variable "spatial memory". This binomial measure indicates whether the cat visited firstly the baited container (0 = made mistakes or did not go, 1 = directly approached the correct container). The total spatial memory variable was calculated for each cat by summing the results of each trial, indicating the total number of correct trials in which the cat directly visited the baited container without any errors during the entire task. This yielded a value and

ranging from 0 to 5, where 0 indicated no correct trials and 5 indicated that all trials were completed correctly.

During the unsolvable task, cat behavior was coded throughout the unsolvable trial, from the moment the caregiver released the cat for up to 30 seconds. An ethogram of the coded behaviors, along with each measured parameter (frequency and/or duration), is presented in Table 1. Coding was carried out by a catFACS (Facial Action Coding System) certified coder who was blind to the cat demographics and the aim of the study at the time of coding. CatFACS is a standardized, anatomical and unbiased tool to study facial communication in the domestic cat (Caeiro et al., 2017). Distress behaviors, namely nose licking, yawning, blinking, eye closure, body shaking, panting, and vocalizations, were aggregated into a category labeled "negative valence". Touching and rubbing behaviors were aggregated separately for the object and the caregiver, resulting in the creation of two distinct variables: "physical interaction with the object" and "physical interaction with the caregiver" respectively.

2.4. Cytokine measurements

Serum levels of IL-1β and IL-10 were quantified to assess the degree of inflammation. For cytokine measurement, serum samples were immediately stored at −20°C upon collection, following the manufacturer's instructions, and were stored for up to six months without being thawed. Upon analysis, the samples were thawed at room temperature and assessed by a laboratory technician who was blinded to the hypotheses and the cats' identities, using commercially available enzyme-linked immunosorbent assay (ELISA) kits: the Feline IL-1 beta ELISA Kit and the Feline IL-10 ELISA Kit (Invitrogen, Waltham, Massachusetts), specifically designed for the quantitative determination of IL-1β and IL-10 in cats, respectively. Samples were diluted 1:2 or 1:4 based on pre-experiment results and the manufacturer's recommendations. Each sample was prepared in duplicate, and concentrations were determined using a Micro Read 1000 microplate reader (Global Diagnostic, Geel, Belgium) in accordance with the relevant standard curves, with a range of 16–4000 pg/mL for IL-1β and 0.2–50 ng/mL for IL-10. The average intra-assay coefficient of variation was 3.83 % for IL-1β and 3.06 % for IL-10, while the assay sensibility was 26.02 pg/mL and 0.08 ng/mL, respectively.

2.5. Statistical Analysis

After assessing the normality of continuous variables using the Shapiro–Wilk test and applying decimal logarithm transformations where necessary, the data—both cognitive and cytokine—failed to meet the assumptions required for parametric testing. Consequently, generalized linear models (GzLMs) were employed. For count data, Poisson loglinear models were used, while gamma models with a log link function were applied for scale response variables. Gaze alternation in the unsolvable trial was analyzed as a dependent variable, with separate models run for "gaze alternation frequency" and "gaze alternation duration". Sex, age (years), center (UPENN vs. UNIMI), BCS, total spatial memory, other behaviors in the unsolvable trial (negative valence, physical interaction with the object, physical interaction with the caregiver), and serum cytokines (IL-1b, IL-10) served as independent variables. Moreover, we further refined the models to include specific interactions between age and IL-1b, as well as age and IL-10, to better capture and understand the complex interdependencies among these variables. For each model, we initially implemented a factorial design incorporating all main effects of the factors. Subsequently, we selected the most parsimonious model based on the lowest Akaike Information Criterion (AIC), which involved refining the model by excluding variables that did not contribute significantly. The strength of the associations was expressed as odds ratio (OR) and 95 % confidence interval (95 % CI); $p < 0.05$ were considered significant. Statistical analyses were performed with SPSS 29.0 (IBM SPSS Statistics for MAC, Armonk, NY, USA).

Table 1
Behavioral variables coded during the unsolvable task (modified from Merola et al., 2015; Scandurra et al., 2023; Zhang et al., 2021).

Behaviors	Description	Category	Measured Values (F/D)
Scratching/pawing the object	Paw is within paw distance from the object and the cat is touching it with the paw.	Touch	D/F
Sniffing / nose touch / bite / lick the object	Head is within head distance from object. Associated with the CatFACS AD 40, which refers to the act of smelling, where global movement with varied intensity is observed on the upper lip, nose, and nostrils.	Touch	D
Rubbing the object	Rubbing the head on the object.	Rubbing	D
Rolling over object	Rubbing or rolling the body on the object.	Rubbing	D
Rubbing the caregiver	Rubbing head or body on the caregiver.	Rubbing	D
Jump on the caregiver	Jump or sit on the caregiver.	Rubbing	D
Nose licking	The jaw is lowered, and the tongue is moved beyond the lips and in a dorsal movement, wiping the nose. CatFACS AD137.	Stress	F
Yawning	The mouth is opened by lowering the lower jaw and actively stretching it apart, while the lower teeth, tongue and oral cavity are exposed. CatFACS AU27.	Stress	F
Blink	The eye opens within half a second. CatFACS AU145.	Stress	F
Eye closure	The eyes remain closed for half a second or more while the inactive cat is standing or sitting. CatFACS AU143.	Stress	F
Body shaking	When the body and/or head is twisted, shaking the head and trunk skin, half- rotating the head and/or trunk repeatedly in a craniocaudal axis (e.g., when wet). CatFACS AD160.	Stress	F
Panting	The cat sticks out their tongue, lowers their jaw, and breathes rapidly and noisily through their mouth, while their chest moves rapidly*. CatFACS AD126.	Stress	F
Vocalizations	The cat displays a range of vocalizations, most of which impact the movements of their mouth and nose. CatFACS AD50.	Stress	F
Walk away	The cat exits the testing area and moves out of the camera's view.	Leave	D/F
Gazing object	The cat is looking at the object at least for 0.2 sec. Possibly associated with CatFACS AD57 (the head moves forward, away from the body).	Look	D
Gazing caregiver	The cat is looking at the caregiver at least for 0.2 sec. Possibly associated with CatFACS AD57 (the head moves forward, away from the body).	Look	D
Gaze alternation	Gaze alternation between caregiver and object within 2 sec, featuring two-way sequences between food and	Look	D

(continued on next page)

Table 1 (*continued*)

Behaviors	Description	Category	Measured Values (F/D)
	caregiver (and vice versa). Each look lasts at least 0.2 sec, with gaps no longer than 2 sec between looks.		

CatFACS AD = Action Descriptor according to the CatFACS. CatFACS AU = Action Unit according to the CatFACS. D = duration; F = frequency. * This behavior is not seen in cats often and is usually associated with a clinical stress response (Kahn, 2010), though it can also be simply a form of thermoregulation (Anrep and Hammouda, 1932).

3. Results

Concentrations of IL-1β were beyond the detectable range of the standard curve in the samples from three cats, as they exceeded the assay's upper detection limit. Consequently, these samples were excluded from the related statistical analysis. The median IL-1β serum concentration in the remaining 41 cats was 685.41 pg/mL (interquartile range [IQR]: 160.39–2944.7 pg/mL). For the entire study sample ($n = 44$), the median IL-10 serum concentration was 1.00 ng/mL (IQR: 0.31–3.09 ng/mL). The median total spatial memory score was 2, with values ranging from a minimum of 0 to a maximum of 5, where 0 indicates the worst performance and 5 the best. Descriptive statistics for gazing, physical interaction with the object and negative valence behaviors during the unsolvable task trial are presented in Table 2.

The "walk away" and "physical interaction with the caregiver" variables, which showed insufficient expression (median frequency and/or duration = 0), were excluded from further analysis. For all the other behaviors coded, intra-rater reliability coding indicated a correlation coefficient between 0.66 and 1.

The results from the GzLM are summarized in Table 3, which reports only statistically significant factors. For the model with gaze duration as the dependent variable: 9 df, $p = 0.001$, AIC = 3.247. For the model with gaze frequency as the dependent variable: 8 df, $p = 0.033$, AIC = 101.193. Notably, shorter physical interaction with the object, less frequent negative valence behaviors, and a higher total spatial memory score significantly predicted prolonged gaze alternation behavior between the object and the caregiver during the unsolvable task. Additionally, older age significantly positively predicted the frequency of gaze alternation, while the interaction between younger age and lower IL-1β serum concentrations significantly negatively predicted both the frequency and duration of gaze alternation.

4. Discussion

In the present study we found a significant relationship of social cognition, measured by gaze alternation during an unsolvable trial task, with spatial memory, stress, and peripheral inflammatory IL-1β in a sample of 44 healthy elderly cats.

Nowadays, domestic cats (*Felis silvestris catus*) are widely recognized as highly socially flexible animals (Finka, 2022), capable of successfully living and communicating with humans, even through social referencing (Zhang et al., 2021). Cats rely on their caregivers for information about unfamiliar stimuli, but they can also flexibly produce communicative cues to influence human behavior. For example, when engaged in an unsolvable task, they may use gaze alternation by consecutively looking at the object and the person, or vice versa (Zhang et al., 2021). In animals, gaze alternation is often considered an indicator of social cognitive abilities as well as of cognitive flexibility (Piotti et al., 2021). Cognitive flexibility generally refers to an animal's ability to switch behavioral responses or strategies according to the context of a situation, thereby adapting to a constantly changing environment (Magnusson and Brim, 2014). In our study, during the unsolvable task, the odds of a cat displaying longer gaze alternation behavior increased by approximately 5 % for every second decrease in manipulation of the food container. Consistently, Marshall-Pescini et al. (2017) and Piotti et al. (2021) found that more object-persistent dogs and wolves—i.e., being more persistent in investigating the object—looked less at humans, regardless of species. Zhang et al. (2021), who investigated cats in a similar unsolvable scenario, argued that animals exhibit social referencing behaviors, like gaze alternation, because they are unsuccessful at accessing the treat and are attempting to attract their caregiver's attention, possibly considering the caregiver's attentional availability. In other words, the less persistent our cats were in interacting with the sealed object using physical

Table 3
Generalized linear model predicting changes in cat gaze alternation.

Predictive factors	B	Std. Error	Sig.	Exp (B)	95 % Wald Confidence Interval for Exp (B)	
					Lower	Upper
Dependent Variable: Duration of gaze alternation in the unsolvable trial.						
Total spatial memory	0.432	0.2165	0.046	1.54	1.008	2.354
Physical interaction with object (duration)	−0.051	0.0173	0.003	0.95	0.918	0.983
Negative valence	−0.301	0.1435	0.036	0.74	0.559	0.981
Age (yrs) * IL−1β (pg/mL)	−0.002	0.0006	0.001	0.998	0.997	0.999
Dependent Variable: Frequency of gaze alternation in the unsolvable trial.						
Age (yrs)	0.196	0.0951	0.039	1.217	1.01	1.466
Age (yrs) * IL−1β (pg/mL)	−0.002	0.0004	0.001	0.998	0.997	0.999

Table 2
Descriptive statistics for behavioral variables in the unsolvable task.

	Gazing at the object		Gazing at the caregiver		Gaze alternation	
	Frequency	Duration	Frequency	Duration	Frequency	Duration
Median	4.5	21.7	2.0	3.1	1.0	0.2
Minimum	1.0	0.4	0.0	0.0	0.0	0.0
Maximum	11.0	57.0	7.0	16.4	5.0	14.0
	Physical interaction with the object		**Negative valence**			
	Frequency	Duration	Frequency			
Median	5.0	16	1.0			
Minimum	0.0	0.0	0.0			
Maximum	31.0	50.2	6.0			

Frequency: number of occurrences in the unsolvable trial. Duration: time spent on the behavior in the unsolvable trial, expressed in seconds.

touches (Lazzaroni et al., 2019; Rao et al., 2018) to solve the task by themselves, the more likely they were to gaze at humans referentially. After all, while focused engagement with the task may be potentially beneficial for problem-solving, it may reduce the likelihood of looking away to seek external cues or assistance, which would instead increase their chance to solve the problem when faced with an unsolvable task, given that the food is out of reach. In several studies, referential looking behavior, specifically the gaze alternation described in our paper, has been interpreted as a communicative signal, possibly indicating a request for help in unsolvable tasks (Carballo et al., 2020; Marshall-Pescini et al., 2017; Piotti et al., 2017; Piotti et al., 2021). However, we cannot entirely rule out the possibility that this behavior may simply reflect other forms of communication, such as seeking attention, or even a loss of interest in the task after giving up, although this latter motivation has generally been associated with looking back at the owner, rather than alternating gaze between the owner and the object (Lazzaroni et al., 2020). In any case, the increase in gaze alternations associated with lower persistence in task orientation in this sample of cats reflected a potential shift from a task-oriented to a more likely successful social strategy, once the cats failed to reach their goal to obtain the food. This highlights that the cats showing more gaze alternation were good performers, a condition linked to being more cognitively flexible and better able to adapt to new environmental conditions (Cañas et al., 2003).

Spatial memory, that reflects the capacity to encode and retrieve spatial information about a novel location, is another condition essential for an animal's adaptation to their environment (Goulet et al., 1994). Research in humans has shown that problem-solving abilities are closely linked to spatial memory, as they benefit significantly from the analysis of spatial processes. As such, individuals who are poor problem-solvers often struggle specifically with tasks requiring spatial working memory (Passolunghi and Mammarella, 2010). Spatial memory shares multiple points of convergence with social cognition, and the interaction between these two diverse domains is believed to be a fundamental component of daily life activities. Moreover, both spatial memory and social cognition are markers of age-related cognitive decline (Streuber et al., 2011). Evaluating these two cognitive processes jointly is thus crucial, as it may help understand cognitive abilities and provide a promising way to detect the early stages of cognitive decline. Despite this, they have primarily been examined separately (Spreng, 2013) and their complex relationship is far from being fully understood (Streuber et al., 2011). This is the first study investigating the relationship between spatial memory and social cognition in pet cats. We found a statistically significant positive relationship between spatial memory performance and the duration of gaze alternation in cats. Specifically, the cats performing better on the spatial memory task (i.e., finding the food more often at their first attempt), which required the use of spatial memory to find food, were significantly more likely to engage more in gaze alternation. This finding underscores the potential role of spatial memory cognitive abilities in influencing the tendency of pet cats to flexibly adapt their behavioral strategy in problem-solving tasks, engaging in referential signaling toward humans when this approach is likely to be the most successful. Evaluating both spatial memory and social cognition might therefore be a useful method to monitor healthy cognitive aging in cats, providing broader insights into their cognitive health and helping detect early signs of cognitive decline. The ability to test these cognitive functions remotely, in the animal's home environment and in the presence of their caregivers, while still obtaining reliable results, facilitates the clinical use of these evaluations, particularly with notoriously difficult patients like cats.

Our study demonstrated that cats showed an increased frequency of gaze alternation with increasing age during the unsolvable test. This observation aligns well with results from Passalacqua et al., (2011), who observed that adult dogs engaged in more frequent gaze alternation between a person and a container compared to younger dogs during a similar task. The authors suggested that the improvement in the production of human-directed gazing behavior with age might result from a learning process influenced by positively rewarded human interactions throughout a pet's life. This pattern could have been maintained in our healthy aging cats. However, the dynamics changed when considering the physiological impact of age combined with inflammation. In fact, the interaction term between age and serum IL-1β had a small but significant ($p = 0.001$) negative effect on gaze alternation. Specifically, each unit increase in the interaction of age and IL-1β increased the likelihood of a cat showing less gaze alternation behavior by 0.2 %. These findings indicate that elevated peripheral levels of IL-1β, a marker of inflammation, in combination with the cat's increasing age, may adversely affect the cognitive flexibility or attentional engagement necessary for effective gaze alternation and, more in general, a cats' ability to engage in social cognition strategies when these would be desirable. Research in humans and animal models have revealed that, as age increases, a shift occurs towards a proinflammatory environment in healthy individuals (Uciechowski et al., 2008). In short, aging is related to chronic inflammation. The resultant increase in serum concentrations of cytokines may possibly act as a mediator, partially explaining the relationship between age and cognitive impairment (Lin et al., 2018). Deeper knowledge of the relationships between chronic inflammation and neurocognitive functioning as cats age is needed, as it offers significant insights into the pathophysiological mechanisms underpinning normal and pathological aging processes. To the best of our knowledge, this is the first study analyzing social cognitive performance against IL-1β serum concentrations in heathy aging cats during an unsolvable task. The use of cognitive tasks designed to measure specific cognitive domains has been praised recently, as they may offer greater precision than cognitive screening tests in assessing the relationship between cognition and peripheral inflammation markers (Fard et al., 2022). Beyond all this, it is worth noting that, due to its complexity, inflammation may not be accurately reflected by single specific markers, including the one we tested here. Although IL-1β is prominent mediator linking chronic inflammation and the risk of deficits in neurocognitive functions during aging (Cheng et al., 2022), there is still a quantity of other markers that should be addressed in future analyses, particularly tumor necrosis factor-α (TNF–α) as a key regulator of the inflammatory response. Further research in this field may help identify (a) signatures of accelerated aging that are both informative of the complexity of the organism's response and predictive of cognitive outcomes, and (b) key molecular and behavioral mechanisms that can be targeted for intervention to promote healthy responses against silent inflammaging and mitigate age-related neurocognitive decline. Further consideration is warranted regarding the processing protocols employed in this study and their implications for future research. Many pre-analytical factors, including sample collection, handling, storage, and preparation, are known to influence the outcomes of serum-based studies. Although strong evidence exists on the impact of freeze-thaw cycles on cytokine stability (Kordulewska et al., 2021) and the effects of extended thawing at 4°C (Goodman et al., 2021), there is surprisingly limited data comparing storage temperatures (−20°C vs. −80°C) for serum samples, particularly independent of freeze-thaw cycles (Valo et al., 2022). Importantly, specific data on feline IL-1β and IL-10 cytokines remain unavailable. In general, aliquoting samples to avoid repeated freeze-thaw cycles and storing them at −80°C are considered good practices for long-term preservation. However, certain analytes, including cytokines, have been shown to remain stable when stored at −20°C for up to one year, suggesting that it is feasible to use samples stored at this temperature for biomarker studies. At the same time, some samples may deteriorate over extended storage, even at −80°C (Maki et al., 2021), and there is inconsistency in defining what constitutes "extended" storage in the literature. In this study, serum samples were stored according to the manufacturer's guidelines, confirmed through both the kit manual and direct written communication with the manufacturer's representative. These guidelines recommended storing samples at −20°C or lower, aliquoted into 0.5 mL portions for up to one year without significant loss of stability, provided freeze-thaw cycles were avoided. While we adhered to the manufacturer's protocols, we cannot completely rule out the possibility that the relatively weak cytokine results in our study were partially influenced by molecular degradation due to storage at −20°C, compared to −80°C. Future studies will aim to mitigate this potential limitation by using −80°C storage and/or reducing storage duration, though these adjustments may present practical challenges. The selected protocols in this study helped minimize variance in time and temperature between participants, in alignment with the practical constraints of our study parameters, facilities, and staffing. It is important to note that opportunistically recruiting elderly cats for research on healthy aging may take considerable time due to challenges in finding animals that meet strict health criteria, as well as securing willing owners. This may ultimately delay the rapid collection of a sufficient number of samples, limiting the feasibility of shorter storage durations.

Finally, emotional states are likely to influence problem-solving (Trezise and Reeve, 2014). While a certain amount of stress may enhance performance, negative emotions such as distress or anxiety tend to hinder successful problem-solving (Hong et al., 2023). In line with this, results from our study revealed a significant negative relationship between stress-related behaviors and gaze alternation. Specifically, each unit decrease in the expression of behaviors associated with negative emotional valence resulted in a 26 % increase in the odds of observing prolonged gaze alternation in aging cats during the unsolvable trial. Thus, the low level of stress in our cats may have contributed to their good cognitive performances. This finding provides further insight into the link between emotional well-being and cognitive behaviors, emphasizing the importance of minimizing stress and negative emotions to support cognitive health in pet cats.

However, in general, in our study, the cats exhibited very few stress-related behaviors, with a median of just one occurrence per trial. Some factors, either individually or collectively, might have contributed to the reduced stress responses observed during cognitive testing. Firstly, the low frequency of stress behaviors could be attributed to our exclusion criteria, which prevented cats displaying signs of severe distress or anxiety from participating. The minimal level of observed stress might also suggest that the home testing environment, as expected, was perceived as less stressful by the cats compared to a laboratory setting. This observation strengthens the rationale for using citizen science methods in studies on cat cognition. The procedures themselves also may have been non-stressful, although this was not a given. Indeed, Amsel & Roussel (1958) proposed that the unsolvable trial could be viewed as an extinction trial of a previously reinforced response (i.e., interacting with the apparatus to obtain food), potentially leading to a state of frustration. It is therefore important to evaluate stress-related behaviors during the test, such as body shaking, yawning, panting, or nose-licking. To our knowledge, only two other studies have included stress signals in the analysis of cats involved in unsolvable tasks. Scandurra et al. (2023) tested cats with different living styles (e.g., indoor only and indoor/outdoor) and found that indoor/outdoor cats exhibited stress behaviors sooner than indoor cats. Meanwhile, Forman and Leavens (2024) reported that only few cats in their study, which aimed to assess the effects of transparency on task engagement and social behaviors, were excluded for stress-related reasons. Both studies utilized a citizen science methodology, which further highlights the considerable advantages of in-home testing protocols.

5. Conclusions

Findings from this study suggest that social cognitive aging is more than a simple consequence of broader age-related cognitive decline. They underscore how sociocognitive abilities in healthy cats are diversely influenced by the complex interplay with other cognitive domains and factors such as emotions, and the interaction between inflammation and age. Understanding these dynamics is crucial for: a) interpreting and supporting the behavior of older cats, particularly in contexts that demand cognitive flexibility, and b) designing effective prevention and intervention strategies that may mitigate the effects of psychophysiological changes on cognitive performance while improving resilience to cope with these changes through a multidimensional approach rooted in the holistic perspective of contemporary geroscience.

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CRediT authorship contribution statement

federica pirrone: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Eleonora Biffi:** Writing – review & editing, Investigation, Formal analysis. **Patrizia Piotti:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Holly Memoli:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Filipe Soares:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Paola Scarpa:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Irit Grader:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Carlo Siracusa:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mariangela Albertini:** Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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