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A systemic multidisciplinary approach to study aging in retired sled dogs

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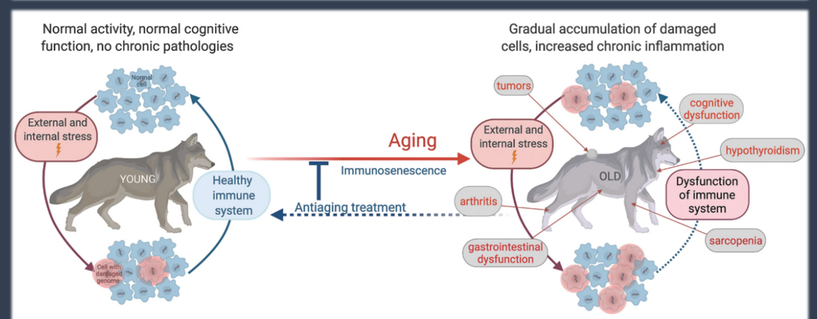
Abstract

Canines represent a valuable model for mammalian aging studies as large animals with short lifespans, allowing longitudinal analyses within a reasonable time frame. Moreover, they develop a spectrum of aging-related diseases resembling that of humans, are exposed to similar environments, and have been reasonably well studied in terms of physiology and genetics. To overcome substantial variables that complicate studies of privately-owned household dogs, we have focused on a more uniform population composed of retired Alaskan sled dogs that shared similar lifestyles, including exposure to natural stresses, and are less prone to breed[1]specific biases than a pure breed population. To reduce variability even further, we have collected a population of 103 retired (8-11 years-old) sled dogs from multiple North American kennels in a specialized research facility named Vaika. Vaika dogs are maintained under standardized conditions with professional veterinary care and participate in a multidisciplinary program to assess the longitudinal dynamics of aging. The established Vaika infrastructure enables periodic gathering of quantitative data reflecting physical, physiological, immunological, neurological, and cognitive decline, as well as monitoring of aging-associated genetic and epigenetic alterations occurring in somatic cells. In addition, we assess the development of age-related diseases such as arthritis and cancer. In-depth data analysis, including artificial intelligence-based approaches, will build a comprehensive, integrated model of canine aging and potentially identify aging biomarkers that will allow use of this model for future testing of antiaging therapies.



Schematic illustration of age-related physiological alterations and their underlying cellular mechanisms.

Table 1. Schedule of assessments performed in aging sled dogs.

Test	Parameters	Timepoints	Participants
	General health		
Physical examination	General appearance, physical palpation, weight, body condition	Every 6 months	
Complete blood counts (CBC)	Standard		
Serum biochemistry	Standard + thyroid hormones		
Morbidity	Diagnosed disease(s)	As needed	All dogs
Therapy	Treatment(s) prescribed		
Whole Body CT scans	Cancer, arthritis		
Sarcopenia assessment	Biomarkers in biopsies, CT body mass index, metabolomics	Annually	
Osteoarthritis, pain assessment	CT scan- and physical exam-based diagnostics		
	Physical fitness		
Treadmill test	Endurance, lactate increase; heart rate resilience; cytokine response to exercising	Every 6 months	60 dogs
Pull test	Average time of pulling 1.5x bodyweight cart		
	Immune system status		
T-cell phenotyping	% of CD4, CD8, CD25, CD28, FoxP3 cells	Annually	60 dogs
T-cell functionality by ELISPOT	IFN-γ, IL-2, IL-4, IL-10, and IL-17 in response to stimulation	Annually	60 dogs
Phagocytosis by peripheral blood cells	Neutrophil and monocyte ability to phagocytose fluorescent beads	Annually	All dogs
Response to vaccination	Antibody titers following leptospirosis vaccine	Annually	All dogs
Steady state of circulating cytokines	GM-CSF, IL-2, IFN-γ, IL-6, IL-8, IL-15, IP-10, IL-10, KC-like, IL-18, MCP1, TNF-alpha	Every 6 months	60 dogs
	Cognitive dysfunction		
Open field tests	Locomotion/activity, interaction with a person, toys and mirror	Every 9 months	
Questionnaire	Behavioral assessment by caretakers and researchers	Every 9 months	All dogs
Neuromarkers in plasma	NfL and β-amyloid variants and glial fibrillary acidic protein	Every 18 months	
Problem solving test	V-test	Every 9 months	
	Somatic cell genome modifications		
Whole genome sequencing	Content of SINEs and LINEs		
DNA methylation	"Methylation clock" assessment	Annually	All dogs
Cellular senescence marker	*p16/Ink4a* RNA level in PBMC		

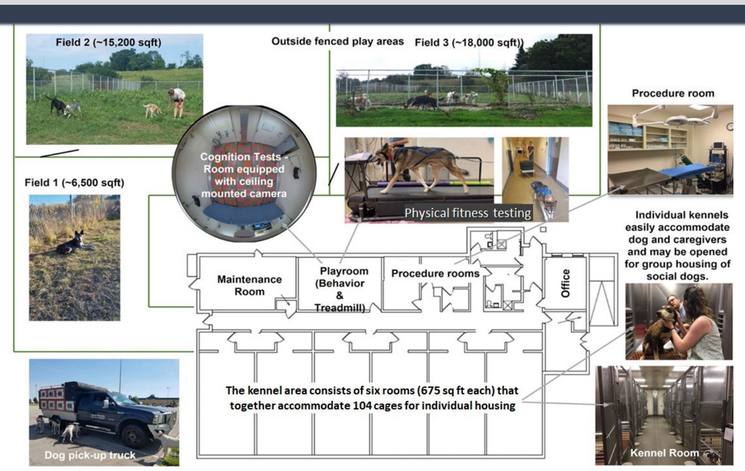
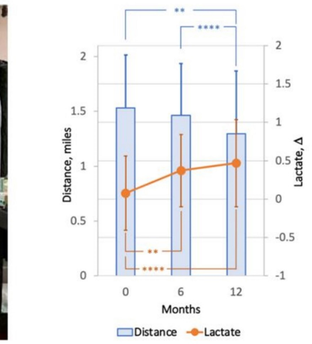
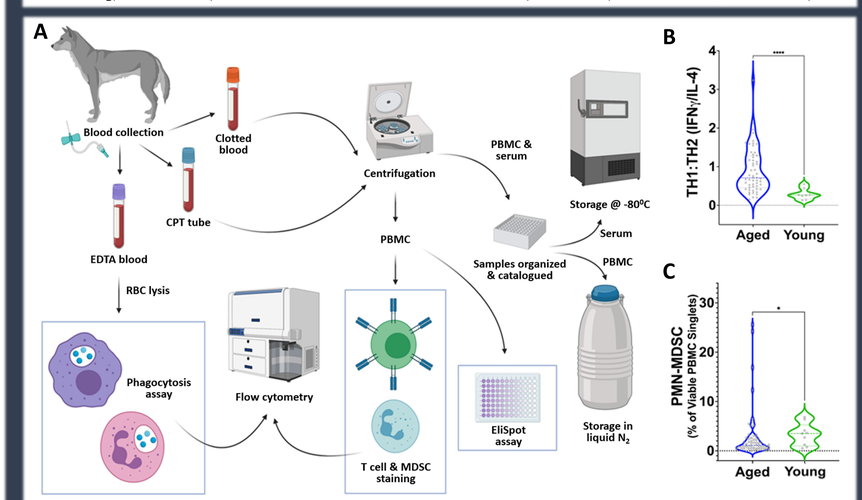


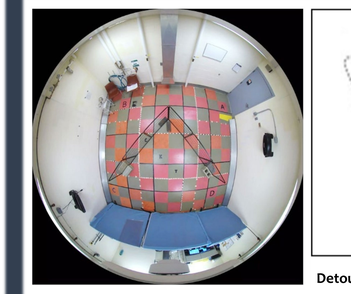
Illustration of the Vaika, Inc. sled dog kennel and research facility at Cornell University, College of Veterinary Medicine.

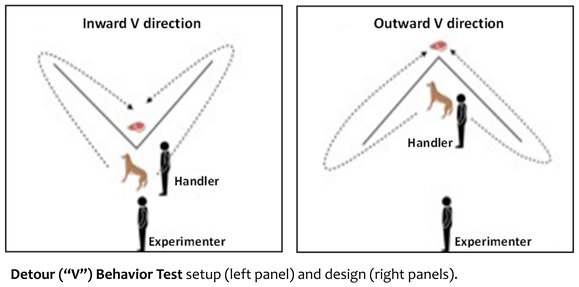


Physical fitness. Use of the treadmill test to assess physical fitness of dogs. Left panel: example of a dog being tested on the treadmill. Two independent parameters measured in the treadmill test show a gradual decline in physical performance of aged sled dogs over 12 months of observation. Mean distance (miles covered by dog running for 20 min at maximum speed adjusted for each dog) and Δ lactate (difference in blood lactate levels before and after performance) are shown. N=65-60 each timepoint.

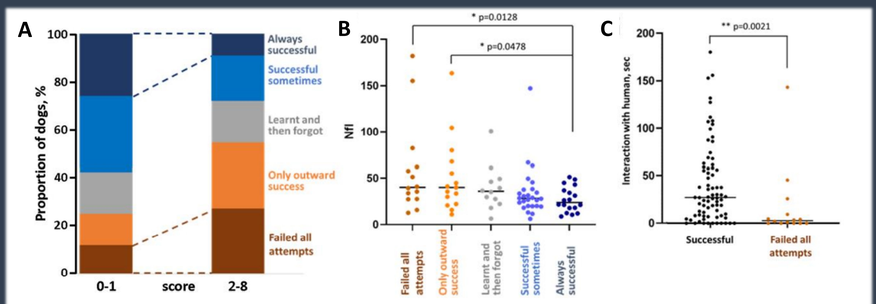


Overview of workflow for immunologic assays. **A**. Blood is collected from dogs into the appropriate tubes (EDTA, CPT) for corresponding downstream assays. Some assays, such as phagocytosis, T cell, MDSC staining, and EliSpot, are conducted immediately. Others, such as determination of cytokine levels and antibody titers elicited by leptospirosis vaccination, use serum and PBMC stored until analysis. **B**. Age-dependence of T cell composition. The ratio of T cells with TH1 vs. TH2 phenotypes was determined by IFNγ/IL-4 EliSpot assays in groups of aged (8-12 years old; N=60) and young (2–4-year-old; N=10) sled dogs. Groups were compared by Mann-Whitney test. **C**. Age-dependence of PMN-MDSC levels in peripheral blood. The percentage of PBMC expressing MDSC markers (CADO48A+CD14+) was determined by flow cytometry for the same groups of dogs as in C. Groups were compared by Mann-Whitney test.

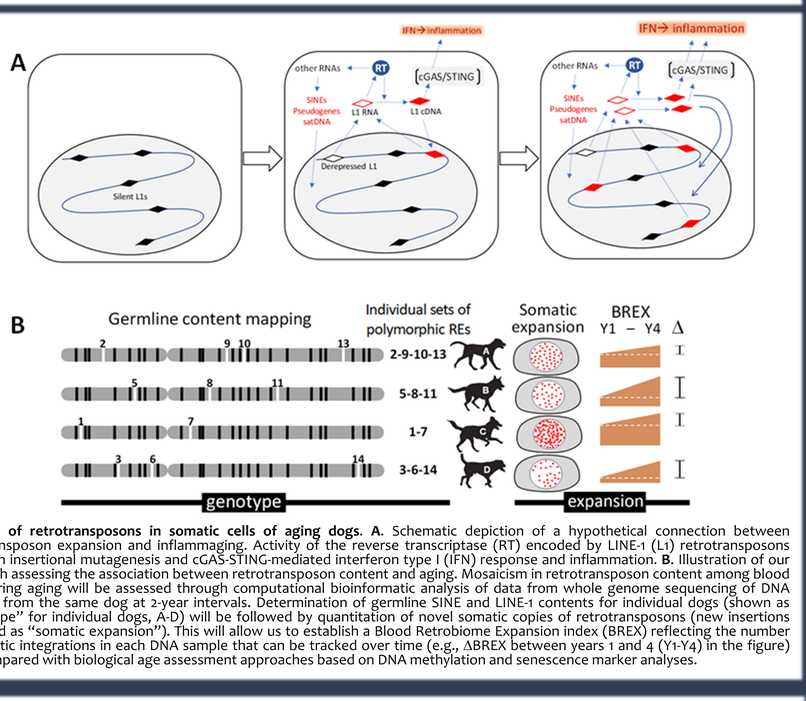




Detour ("V") Behavior Test setup (left panel) and design (right panels).



Cognitive and neural marker tests (cross-validation of results). N=91 **A**. Dogs' performance in problem-solving V-tests correlates with CDS scores obtained from questionnaires completed by handlers. V-test performance is indicated by color as shown on the right y-axis; questionnaire scores reflecting the number of signs of cognitive dysfunction are shown on the x-axis. **B**. Dogs that fail the V-test tend to have higher plasma levels of NfL (pg/ml). **C**. Dogs that fail the V-test tend to interact less with the human in the "novel person" open field test. The "successful" group here includes dogs who were successful at least one attempt. For all panels, *- p<0.05, **- p<0.01, ****- p<0.0001.



Analysis of retrotransposons in somatic cells of aging dogs. **A**. Schematic depiction of a hypothetical connection between retrotransposon expansion and inflammaging. Activity of the reverse transcriptase (RT) encoded by LINE-1 (L1) retrotransposons results in insertional mutagenesis and cGAS-STING-mediated interferon type I (IFN) response and inflammation. **B**. Illustration of our approach assessing the association between retrotransposon content and aging. Mosaicism in retrotransposon content among blood cells during aging will be assessed through computational bioinformatic analysis of data from whole genome sequencing of DNA isolated from the same dog at 2-year intervals. Determination of germline SINE and LINE-1 contents for individual dogs (shown as "genotype" for individual dogs, A-D) will be followed by quantitation of novel somatic copies of retrotransposons (new insertions indicated as "somatic expansion"). This will allow us to establish a Blood Retrobiome Expansion index (BREX) reflecting the number of somatic integrations in each DNA sample that can be tracked over time (e.g., ΔBREX between years 1 and 4 (Y1-Y4) in the figure) and compared with biological age assessment approaches based on DNA methylation and senescence marker analyses.

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