Astrogliosis and Reduction in Cortical Neuron Density in the SAMP8 Mouse Model of Accelerated Senescence

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#1032

Introduction

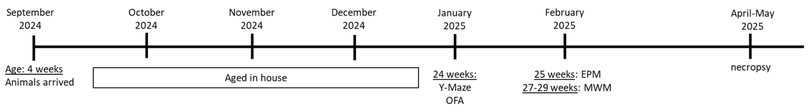
The study of age-related changes in learning, memory, cognition, muscle physiology, and other senescence-related biological factors is an important task in the face of an aging population and rising healthcare costs. Understanding the biological mechanisms underlying deficits in these processes can lead to better diagnostic tests and interventional treatments to improve quality of life and extend lifespan. Translational animal models are an important tool for studying senescence; however, aging animals to an advanced age for study can be costly and may require years of waiting before data are obtained. The senescence-accelerated mouse (SAM) lines were selectively bred from AKR mice that exhibited phenotypes of early-onset aging[1]. The SAM-prone mouse line 8 (SAMP8) has been especially useful in the study of aging-related cognitive decline and sarcopenia[2,3]. SAMP8 mice exhibit deficits in several cognitive and motor behavioral paradigms, which may be explained by neuro inflammation/neurodegeneration in associated brain regions. In this study, we examined behaviors associated with cognition and anxiety, and measured astrogliosis, microgliosis, and neuronal cell counts in the hippocampus and cortex of SAMP8 mice and their wild type controls.

Methods

- Animals: 48 mice from Inotiv's breeding colony were acquired for study: 12M/12F SAM-resistant (SAMR1/Ta) and 12M/12F SAMP8/TaHsd mice. Animals were aged to 24-weeks-old before behavioral assessments took place.

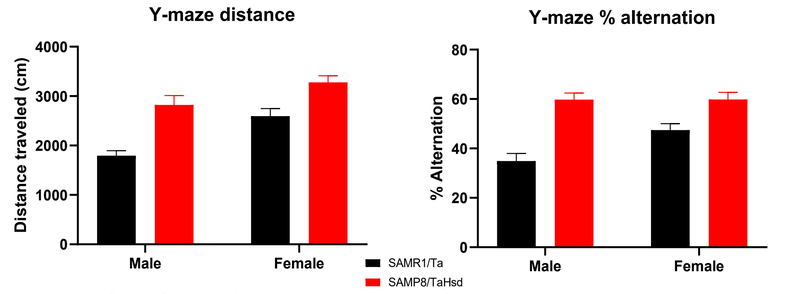
Group	n	Sex	Genotype
1	12	Male	SAMR1
2	12	Female	SAMR1
3	12	Male	SAMP8
4	12	Female	SAMP8

- Study timeline:



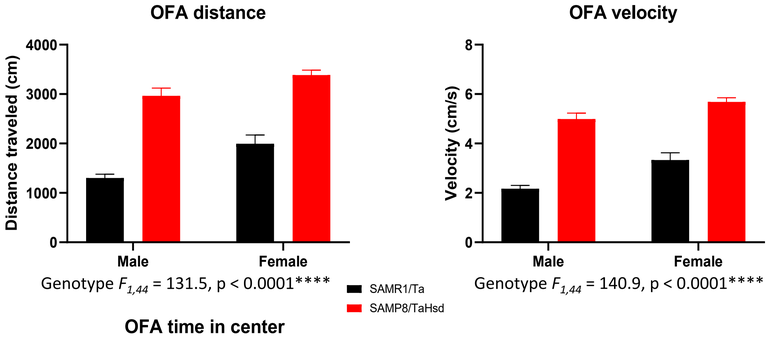
- Behaviors assessed:
  - >**Y-maze**: At 24-weeks-old. 10-minute test of spontaneous alternation %, tracked using Noldus EthoVision software.
  - >**Open field analysis (OFA)**: At 24-weeks-old. 10-minute test to track locomotion and time spent in center of apparatus, tracked using Noldus EthoVision software.
  - >**Morris water maze (MWM)**: Tested in cohorts at 27- to 29-weeks-old. 6 days of training followed by a short-term memory probe 2 hours after the final training session and a long-term memory probe 24 hours after the final training session. Tracked using Noldus EthoVision software.
  - >**Elevated plus maze (EPM)**: At 25-weeks-old. 10-minute test on EPM apparatus, measuring time spent in the open arms. Tracked using Noldus EthoVision software.
- Immunohistochemistry and image analysis
  - >Brains from 9-month-old SAMR1 and SAMP8 mice were fixed and embedded in paraffin, and two 4 µm sections were taken parasagittally +/- 1.5 mm ML from Bregma. Sections were stained with DAPI and immunofluorescent markers for GFAP, Iba-1, and NeuN to image astrocytes, microglia, and neurons, respectively. Images were collected with at 250 µm resolution and analyzed for the percent positive area for GFAP and Iba-1 staining in the hippocampus and cortex, as well as for NeuN-positive cell counts in the hippocampus and cortex using Visiopharm software under pathologist oversight.

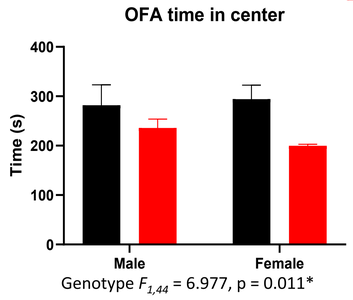
Y-maze: Working memory



SAMP8 mice display increased locomotion compared to SAMR1 controls, as expected. Working memory performance is increased in SAMP8 mice compared to SAMR1 controls, which is the opposite of expected results[5].

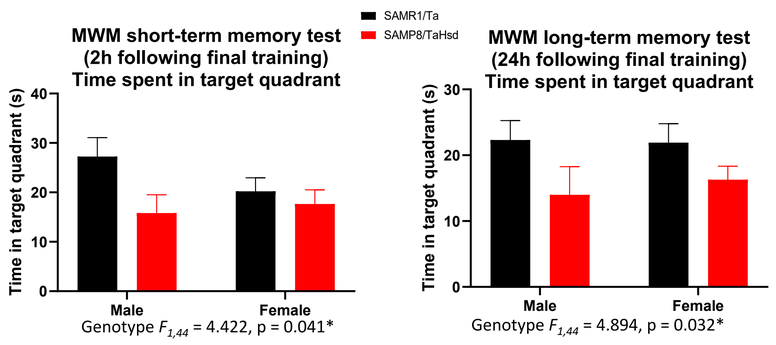
Open field analysis (OFA): Locomotion and anxiety





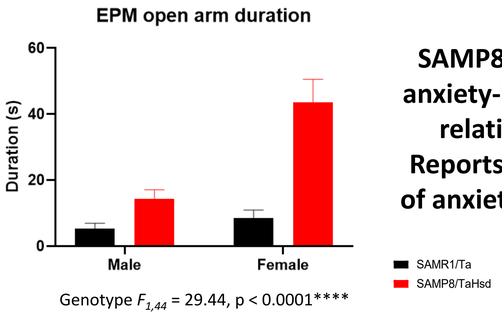
SAMP8 mice display increased locomotion compared to SAMR1 controls, as expected from previous literature[4]. Reduced time in center of OFA indicates an increase in anxiety-like behaviors in these mice.

Morris water maze (MWM): Spatial memory



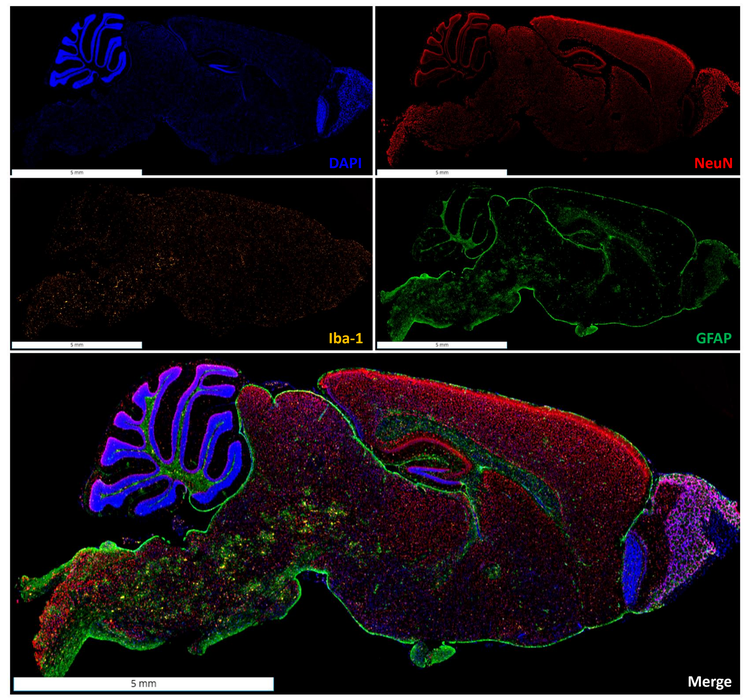
SAMP8 spend less time in the target quadrant during both short- and long-term memory probes with the platform removed than SAMR1 mice, indicating that memory deficits are present in SAMP8 mice as previously reported[5].

Elevated plus maze (EPM): Anxiety

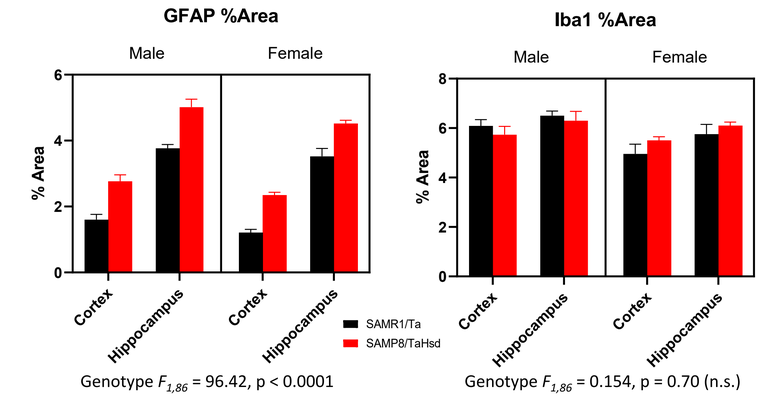


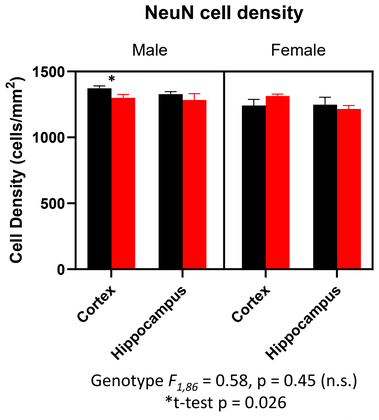
SAMP8 mice display decreased anxiety-like behaviors in the EPM relative to SAMR1 controls. Reports conflict on the direction of anxiety-like behavior in SAMP8 mice.

Immunofluorescent evaluation of astrogliosis, microgliosis, and neuronal cell counts in hippocampus and cortex



Example image of DAPI, NeuN, Iba-1, and GFAP immunofluorescent staining in the brain of a male SAMP8 mouse.





SAMP8 mice display robust astrogliosis, but not microgliosis, in the cortex and hippocampus. SAMP8 male mice had a significant decrease in the number of neurons in the cortex compared to SAMR1 controls.

Summary

SAMP 8 mice display many classically described phenotypes characteristic of the line: increased locomotor activity, altered anxiety, and deficits in cognition. We observed a significant elevation in astrogliosis in SAMP8 mice in both the hippocampus and cortex, which points to increased neuroinflammation that may underlie these behavioral deficits. Male SAMP8 mice also had decreased neuronal counts in the cortex, which could partially account for impaired performance in cognitive assays relative to females.

References

1. Takeda et al., 1981. Mech Ageing Dev 17(2): 183-194.
2. Yagi et al., 1989. J Neuropathol Exp Neurol 48(5): 577-590.
3. Derave et al., 2005. Exp Gerontol 40(7): 562-572.
4. Markowska et al., 1998. Physiol Behav 64(1): 15-26.
5. Pačesová et al., 2022. Aging 14(18): 7300-7327.