OPERATIONS MANUAL

NIH/NIA

Ver. 04182025

I. Project Background and Design

A. Project History: Resilience and Cognitive Aging & ADRD

Aging in humans is a diverse process. Many older individuals retain relatively good cognitive function, even at very advanced ages or in the face of other common health conditions. Do these fortunate individuals simply age slowly, retaining their youthful cognitive phenotype until late in life? Growing evidence points to an alternative, that inherent endowment and life experience in some individuals builds reserve and resilience against the negative effects of aging, in some cases despite levels of age-related neuropathology usually indicative of clinical disease. The important implication is that, alongside the search for disease-targeted treatment, effort might be fruitfully directed at identifying mechanisms of reserve and resilience against cognitive aging, AD and related dementias (ADRD). To address this topic, STARRRS, "Successful Trajectories of Aging: Reserve and Resilience in Rats," was conceived as a first-of-its-kind open science resource for the mechanistic study of reserve and resilience.

STARRRS design reflects the efforts of many NIA intra- and extramural contributors. Taking advantage of a widely validated long-established Long-Evans rat model, STARRRS aims to provide extensive longitudinal neuroimaging, phenotypic, and biospecimen data for research on the basis of successful neurocognitive aging, adequately powered to determine the influence of biological sex. A key feature of the model is that hippocampus-dependent memory measured in the Morris water maze at ~2 years of age is normally distributed across a much broader range than in young subjects, from old animals that score on par with the best young adults, to others that exhibit substantial impairment. Impaired and unimpaired aged subgroups in this model show robust test/re-test reliability and provide a valuable framework for linking cognitive outcome with associated neurobiological mechanisms. STARRRS organizational plan, outlined below, balances the logistical challenges of longitudinal neuroimaging, detailed behavioral phenotyping, biospecimen collection and other project activities with the goal of maximizing sample size and specimen yield. By making all data and materials collected openly available to the research community, STARRRS aims to provide a unique resource for the discovery of antecedent predictors of healthy cognitive aging, and ultimately, for the development of effective strategies to prevent or delay age-related cognitive decline and ADRD.

II. Outline

A. Animals

Male and female virgin Long Evans outbred rats from Charles River Laboratories (CRL) Kingston facility enter the NIA colony at 1 month of age. Rats comprising the young control group for the final spatial memory assessment and necropsy samples enter the NIA colony at 3 months of age. Details are found in Section IV.

B. Entry Schedule

A phased-in schedule of animals began in 2022. Each month a cohort of 10 male and 10 female rats 1-month of age enters the longitudinal study. Beginning in the 21st month of the project, 4 male and 4 female 3-month-old rats also began entering the study each month. These rats serve

as young controls at 6 months of age, when their corresponding longitudinal cohort reaches 24 months old.

C. Timeline:

- 1 month of age: arrival intake, body weight, ID transponder implant, and feces collected.
- 2-4 months of age: Rats assigned to battery, receive up to four weeks of task-specific pre-training.
- 5-6 months of age: **EARLY** period behavioral assessments, magnetic resonance neuroimaging, and physiological assessments.
- 15-16 months of age: **MIDDLE** period behavioral assessments, magnetic resonance neuroimaging, and physiological assessments.
- 22-23 months of age: **OLD** period behavioral assessments, magnetic resonance neuroimaging, and physiological assessments.
- 24 months of age: Endpoint spatial memory assessment in the water maze.
- 25 months of age: Necropsy to collect biospecimens.

D. Assessments

1. Physiological Assessments

- Body weight
- Frailty Index (FI)
- Plasma Samples
- Fecal Samples
- Estrus Cytology

2. Magnetic Resonance Imaging

Longitudinal neuroimaging is the backbone of STARRRS and a bidirectional translational bridge to human research on neurocognitive aging and reserve. Structural and functional brain imaging is performed using a dedicated, 9.4T Bruker Biospec 94/20 USR high-field scanner. The following modalities are included in the first wave of STARRRS:

- Structural Imaging
- Diffusion Tensor Imaging
- Resting State Functional Connectivity

3. Phenotypic Assessments

All rats are assessed for anxiety, gross physical function, and endpoint spatial memory.

- Elevated Plus Maze (EPM) - anxiety
- Home Cage Activity (HCS) - physical and circadian activity
- Water maze- Learning Index (WM-LI) - endpoint spatial memory

As described below, individual rats are assigned to one of 4 test batteries, each defined by the unique cognitive assessment they contain.

- Novel Odor Recognition Memory (NOR) – recognition memory

- Water Maze Delayed Match to Place (WM-DMTP) – spatial memory
- Two-Choice Serial Reaction Time Task (2-Choice RT) – attentional and inhibitory control
- Naïve – control group for assessing the potential effects of the multiple physiological, MRI and cognitive assessments administered in the other batteries on endpoint spatial memory.

E. Batteries

Incoming monthly cohorts of rats in STARRRS are assigned to one of four batteries (see Table). Aside from naïve Controls, the three longitudinal batteries are generally similar, but distinguished by the cognitive domain emphasized. The decision to examine different capacities in separate groups partly reflects practical constraints (i.e., aggregate testing burden, personnel requirements), but also on the possibility that extensive handling, cognitive stimulation, and enrichment might itself impact the primary STARRRS endpoint. As foundational data for the resource, it also seemed prudent to first track longitudinal change within cognitive domains, rather than risk task cross-over influences by testing multiple domains within batteries. In each battery, rats are assessed at 3 points in their lifetime, **EARLY** (5-6 months), **MIDDLE** (15-16 months) and **OLD** (22-23 months) before the final spatial memory assessment at 24 months. By this design, because many measures overlap across batteries, STARRRS yields a well-powered database for users to test the possibility that the distinct elements of the batteries influence longitudinal trajectories of cognitive aging.

Although cognitive aging in experimentally naïve Long-Evans rats is well-documented, these studies have almost exclusively used male LE retired male breeders acquired at 9 months of age. The naïve battery used here serves as an important control for assessing the influence of sex and reproductive history on the final spatial memory assessment. Results from rats in the naïve battery will determine whether the same spatial memory variability at 24 months is observed in virgin male and female rats, as well as provide a reference group for documenting the effect of the repeated, longitudinal testing conducted in the three other batteries.

Age (mo.)	Time Point	Test Battery			
		DMTP	2-Choice RT	ORM	Naïve
1		arrival, transponder, fecal collection			
2-3		handling / shaping for battery task			
5	EARLY	EPM, HCS, battery task			
6	EARLY	fecal & plasma, FI, MRI			fecal, FI
15	MIDDLE	EPM, HCS, battery task			

16	**MIDDLE**	fecal & plasma, FI, MRI, estrus cytology	fecal, FI
22	**OLD**	EPM, HCS, battery task	
23	**OLD**	fecal & plasma, FI, MRI, estrus cytology	fecal, FI
24		spatial memory assessment – WM-LI	
24-25		necropsy	

III. Brief methods and available data

Digital data: A founding principle of STARRRS is to provide raw data from longitudinal assessments that would otherwise be difficult if not impossible for individual investigators to acquire themselves. This includes raw videos and tracking data for behavioral assays, microphotographs for estrus cycle assessment and unprocessed MRI images. Summary files containing commonly assessed dependent variables are provided for many of the assays (described below), but no attempt has been made to include or anticipate all measures that individual investigators might find of interest. Rather, the raw data is considered primary and is provided for investigators to export and analyze as appropriate for their experimental design. All summary indices and data files are dynamic and updated as rats complete the study. Users will gain access to STARRRS data via the Aging Research Biobank (ARB) (https://agingresearchbiobank.nia.nih.gov/).

Biospecimens: Plasma and fecal samples are collected at specified ages and a necropsy to collect brain and body samples is conducted at the end of testing. All material is fresh-frozen and duplicate samples are collected when possible. The inventory of biospecimens is dynamic; it is increased when rats complete the study and decreased when investigators are sent samples.

Incomplete records: Data from rats euthanized for health reasons or that die before final spatial memory assessment may be a valuable additional resource. Up to the point data collection has ended, behavioral, imaging and plasma and fecal samples are available along with all health records described in Section III.C.1, including a full pathology report from the NIH Division of Veterinary Resources.

A. Rat Identification

Each STARRRS rat has a unique ID.

For rats enrolled at 1 month of age for longitudinal assessment, the first two characters of the ID are "ST", representing STARRRS, and the 3rd character notes the sex (either M or F). The next four characters are ascending and non-repeating numbers, starting with 0101. For example, the first male rat in the project is STM0101 and the first female is STF0111.

For rats enrolled at 3 months of age to serve as young control rats, the first three characters represent the cohort number they serve as controls for, the fourth character notes the sex (either M or F), and the last 3 characters are ascending and non-repeating numbers, starting with 001. For example, the first female control rat for cohort 1 is C01F001 and the first male control rat for cohort 1 is C01M005.

B. Index of Rats, Available Data and Biospecimens

A subject summary file lists all rats that have completed the study to date. A partial screen shot below shows an example of the first 14 columns. The first 4 columns (A-D) report Rat ID, Sex, Cohort, and Battery respectively and can be sorted/filtered to provide a tailored data output organization. Columns E-I report the rat date of birth, death, reason for exiting the study, any notes and the Learning Index score for the endpoint spatial memory assessment conducted at 24 months. The next 3 columns (J-L) report the number of health-related files created for each subject. Subsequent columns (not shown) report the number of files for specific assays at specific time points for each rat. This index provides an overview of all the rats in the project, when they exited the study and what data and biospecimens were collected. Biospecimen availability is dependent on prior requests and distribution to users. The Aging Research Biobank maintains an up-to-date inventory of available samples.

A	B	C	D	E	F	G	H	I	J	K	L
Rat ID	Sex	Cohort	Battery	Birth Date	Death/Exit Date	Death/Exit Reason	Notes	WM Learning Index Score	Clinical Cases	Health Records	Pathology Reports
STF0187	Female	Cohort 5	Cohort 5 WM-DMTP	04/24/2022	05/31/2024	Necropsy- completed study		149	1	1	0
STF0188	Female	Cohort 5	Cohort 5 WM-DMTP	04/24/2022	05/31/2024	Necropsy- completed study		176	0	1	0
STF0189	Female	Cohort 5	Cohort 5 WM-DMTP	04/24/2022	05/31/2024	Necropsy- completed study		195	0	0	0
STF0190	Female	Cohort 5	Cohort 5 WM-DMTP	04/24/2022	02/21/2024	Euthanized			0	1	2
STM0191	Male	Cohort 5	Cohort 5 WM-DMTP	04/24/2022	04/14/2024	Died (Injury/Illness)			0	0	2

C. Physiological Assessments

1. Health Status

Details on standard clinical care and colony health status are presented in Section IV. Briefly, rats are observed twice a day by NIA animal care staff and intermittently by the research staff. Reported abnormalities are evaluated by a facility veterinarian. Clinical records document health concerns, veterinary diagnoses, and treatments. These records are created by the NIA veterinary staff and are provided so that the health status during each assessment can be considered in association with the animal's behavioral data. The Frailty Index (described in Section III.C.6) is a separate assessment of overall health and physical condition scored by the research staff at each behavioral assessment timepoint (and at necropsy) and should be consulted in making health status determination. It should also be noted that rats with confirmed physical disabilities or diseases that might confound the interpretation of phenotypic assessment were excluded. Accordingly, the sample of STARRRS rats with complete longitudinal datasets and endpoint

cognitive testing is enriched for healthy animals relative to the overall LE population at 2 years of age.

2. Health Reports

A list of number and type of health reports for each rat is provided in the Clinical Records Summary file as shown in the image below for the first 7 female rats from cohort 1.

	A	B	C	D	E
1	Cohort Number	Rat ID	Clinical Cases	Health Records	Pathology Reports
2	1	STF0111	0	0	0
3	1	STF0112	0	1	3
4	1	STF0113	0	1	2
5	1	STF0114	1	1	2
6	1	STF0115	0	0	0
7	1	STF0116	0	0	0
8	1	STF0117	0	0	0

a) Clinical Cases

These files are digital copies of handwritten notes detailing the clinical treatment history provided by NIA animal care staff for an individual rat. Only rats that have undergone treatment will have a clinical case file and rats with an extensive treatment history may have more than one file. The file name includes the rat ID, a date, and end in "Clinical Case.pdf." Example:

STM0140_November2023-ClinicalCase.pdf

Clinical Case Record

(SOP 3402A Revised 6-19-2017)

History:

Animal ID: STM0140

Date: 11/14/23

Investigator: Rapp

DOB: 1/23/22

ASP: 485-LBN-2024

Species: Rat

Strain: Long Evans

Animal Holding Room: 011A

Rack/Side: 1A

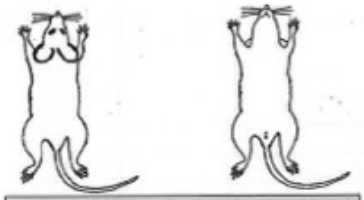
Female ☐ Male ☒

Reported by: Brock

Other Cage Card Info (cage #, active study):

Circle or write in all observed concerns:

Fight Wound	Hunched	Dyspnea	Runting	Eye problem - R L
Dermatitis	Thin	Pale	Abnormal skull shape	Porphyrin staining- R L
Skin Lesion or Ulceration	Rough Hair Coat	Circling	Malocclusion	Ear or pinna problem - R L
Abscess	Dehydration	Head tilt - to Right, to Left	Dystocia	Implant problem
(Lump or Tumor)	Lethargy/Weakness	Ataxia	Vaginal Prolapse	Staple/Suture Present
Abdominal Distension	Listlessness	Seizure Activity	Rectal Prolapse	
Traumatic Injury	Alopecia	Other:	Penile Prolapse	**Pain Score** (0) 1 2 3



Mark on the above figures as appropriate

Mark or write in INITIAL TREATMENT prescribed :

Topicals	Oral/Medicated H2O	Supplements	Monitor	Other	Frequency
TAO	TMS	FOF	(SID)	Clip Lesion	SID X 5
SSD	Acetaminophen (Tylenol)	Soft Chow	BID	Trim Nails	SID X 7
TAO (O)	Enrofloxacin (Baytril)	Soft Chow with STAT	TID	Trim Teeth	BID X 5
DPH	Ibuprofen (Children's Advil)	Trans Gel		Remove Ear Tag	BID X 5 SID X 2 (wknd)
Dermachlor	Meloxicam	NaCl SC		Soft bedding	QW
Sodium Hypochlorite	Other:			Separate Cage	PRN
Green Clay					
Other:					

Date Closed: 1/2/24

Initials: DW

Resolution:

- ☐ Animal Euthanized
- ☐ Investigator Used
- ☒ Clinical Condition Resolved
- ☐ Cage Not Found
- ☐ Animal Found Dead
- ☐ Permanent Defect: _____
- ☐ Sent to Pathology
- ☐ Other: _____

Clinical Case Record (3402A)

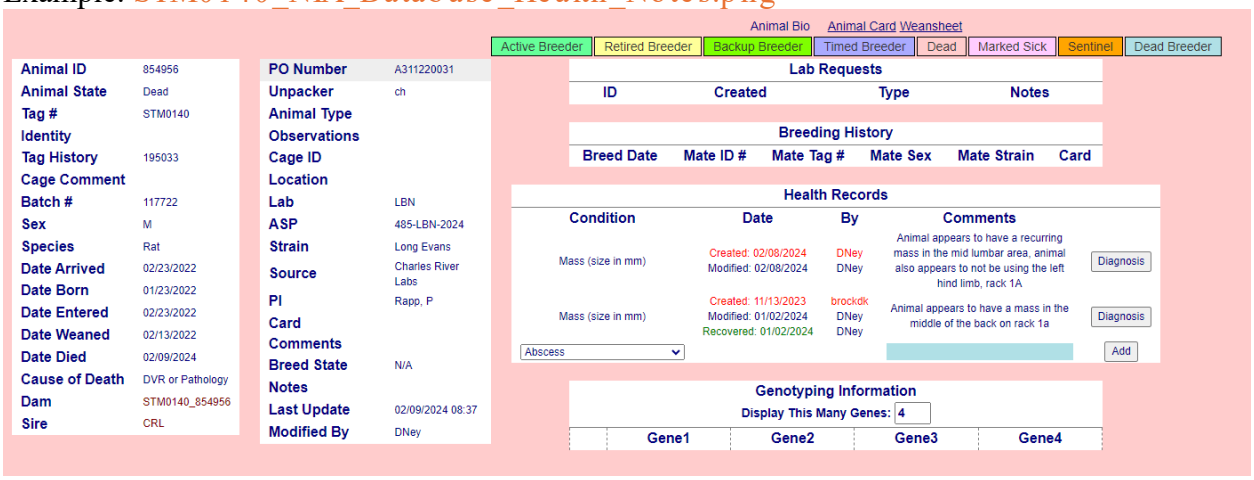
Primary Health Concern:
STM0140 Mass

Date	Time	Follow up Assessment and Updated Treatment Plan	Pain Score	Initials
11/14/23	11:20	Animal appears to have a ~30mm mass		
		in the mid-lumbar area. Monitor SID.		
		Measure weekly.		DW
11/15/23	12:32	BAR		DW
11/16/23	11:32	BAR		DW
11/17/23	2:34	BAR		DW
11.18.23	1218	BAR		RS
11-19-22	11:32	BAR		RD
11-20-23	3:18	S: Animal is BAR. Mass in mid lumbar region		
		A: Mass		
		P: Monitor SID; measure weekly	0-1	
11/20/23	3:23	BAR		DW
11/21/23	2:10	Animal anesthetized w/ isoflurane.		
		Area around mass shaved and cleaned.		
		Mass surgically removed. Incision		
		sutured closed. Recovery was uneventful.		
		0.35ml Meloxicam provided. Sutures		
		removed in 10-14 days. Monitor SID.		DW
11/22/23	1:37	BAR, 0.35ml Meloxicam		DW
11-23-23	11:31	BAR		
11/24/23	9:15	BAR		DW
11-25-23	10:09	BAR		
11-26-23	11:11	BAR		
11-27-23	10:41	S: Animal is BAR. Mass removal surgery		
		A: Healing well. Sutures intact		
		P: Monitor SID	0-1	
11/28/23	10:54	BAR		DW
11/29/23	3:24	BAR		DW
11/30/2023	204	BAR		
12/1/23	1:50	BAR		DW
12/2/23	12:05	BAR		
12/3/23	11:36	BAR		
12.4.23	9:50	BAR		
12/5/23	11:31	BAR, Sutures removed.		DW
12/6/23	9:23	BAR		DW
12/6/23	12:35	not in room	-	
12/7/23	11:57	BAR, Appears healed		DW

b) Health Records

Health concerns noted by the animal care staff are noted in an internal database for subsequent evaluation by NIA veterinary staff. A screen shot of these health notes is available. Only rats with documented health concerns will have a health note file. File names include the rat ID.

Example: STM0140_NIA_Database_Health_Notes.png



c) Pathology Reports

If the NIA veterinary staff determines a rat should be euthanized, the NIH Division of Veterinary Resources provides a postmortem pathology report. A pathology report may also exist for biopsy samples. A pathology report may contain data from several rats, and an individual rat may have multiple pathology reports, e.g., a preliminary and final diagnosis, or findings from other rats listed on the same report. File names will include the rat ID, internal case ID, and either "PathPrelim" or "PathFinal." Example: STF0140_RT2401085-PathFinal.pdf

Pathology Final Report

Division of Veterinary Resources, Office of Research Services, National Institutes of Health

Accession Number: RT2401085

NE / D

Date Submitted: 12/13/2023 **Date received by DVR:** 12/13/2023 **Number of animals:** 2

Species: RAT **Source of animal(s):** Charles River **Date animal(s) received from source:**

Entry #	Animal ID	Sex	Strain	Age	Campus	Building	Room	Cage	Weight	Status	Date & Time of Death	Euthanasia Agent
1	STM0140	M	Long Evans	23 MO	Baltimore NIA	BRC	02C011A			L		
2	STF0123	F	Long Evans	23 MO	Baltimore NIA	BRC	02C011A			L		

Hazards: Infectious agent: **No** Radionuclide: **No** Chemical agent: **Yes** **Import Number:**
10% Buffered Formalin

Remarks / History / Procedures:
STARRRS Project

STM0140 - Mass surgically removed from the mid-lumbar area. No previous health concerns noted.

STF0123 - Mass surgically removed from the left axillary region. Animal previously had a lipoma removed from the left shoulder: RT2304424.

Both masses were placed in 10% buffered formalin for submission. PI would like any information regarding the nature of these masses. Please perform any tests deemed necessary by the pathologist. Please include Wright, Darlene; Knisley, Gabby; Cary, Cindy; Zamora, Bernadette; and Radakovic, Emily on all preliminary and final results.

Preliminary Diagnosis Summary:
Masses are submitted from 2 rats that were removed surgically. From rat STM0140. the mass was removed from the mid lumbar region. This rat is designated as A. From rat STF0123, the mass was removed from the left axillary region. This rat had previously had a lipoma removed from the left shoulder. This rat is designated as B. The findings are as follows:

The mass from Rat A measures 3.8 x 3.0 x 2.0 cm. On cut section, the mass is relatively firm, white to tan and appears finely encapsulated. A section is collected for histopathology.

The mass from Rat B measures 3.5 x 3.0 x 2.0 cm. It is round, tan, relatively firm, white to tan on cut section and appears encapsulated. A section is obtained for histopathology. A separate small mass is present which measures 0.8 x 0.5 x 0.5 cm which appears finely encapsulated and white and semi-firm on cut section.

Final Diagnosis:

A. ID STM0140 Soft tissue mass, neurofibroma,

B. ID STF0123 Mass, large, neurofibroma,

B Mass, small, mammary fibroadenoma,

Final Diagnosis Remarks:
A. ID STM0140: The mass is a mesenchymal neoplasm composed of wavy spindle cells with hyperchromatic nuclei and whirling. Abundant connective tissue stroma is present. Neoplastic cells extend to the surgical margin.

B. ID STF0123:
1) Large mass: This mass is similar to above with small wavy spindle cells in abundant cytoplasm. It invades into surrounding soft tissue ad extends to the surgical margin.

2) Small mass: This mass is a mammary fibroadenoma. Surgical margins are clear of neoplastic cells.

Comment: The neurofibromas are likely to recur.

Diagnostician: Hoffmann, Victoria ***Completion date:*** 12/06/2023

3. Body Weights and Temperature

Body Weight: Rats are weighed within 14 days of receipt and monthly thereafter.

Body Temperature: Temperature is collected via a microchip transponder (detailed in Section IV.C). Temperature information is recorded on arrival, on the first and last day of the WM-LI task and at necropsy.

Data available: Body weights and temperature data are organized by cohort. That is, data for all rats in a cohort are included in a single file. The rat ID is in column A, and the date of assessment in row 2.

4. Fecal collection

Three fecal samples are collected at each of the timepoints (arrival, **EARLY**, **MIDDLE**, **OLD** and necropsy), either defecated directly from the animal or in a clean empty housing cage. Each sample pellet is placed into a 1.5 mL plastic collection tube and stored at -80 ℃.

5. Plasma collection

Scheduled for collection after other assessments, blood is collected into heparinized tubes. At the **EARLY**, **MIDDLE** and **OLD** timepoints, blood is collected from the ventral tail artery and at necropsy, from the left ventricle of the heart during euthanasia. Blood samples are processed for plasma extraction within 4 hrs. of collection. At all timepoints, 3 plasma samples are collected.

6. Frailty Index

The Rat Clinical Frailty Index (FI) assesses health deficit accumulations (Yorke et al., 2017), using a scale (0=absent, 0.5= mild, 1=severe). Assessments are conducted while the rat is in its home cage at each of the behavioral assessment time points (**EARLY**, **MIDDLE**, **OLD**) and at necropsy. The assessment form and evaluation criteria are described in Section V.C.

Data available: Frailty Index summary data file contains data for all rats in the project. Columns A-D list the cohort number, rat ID, time point and the date of the assessment respectively. Columns E-AE contain the scores from the assessment form described in Section V.C.

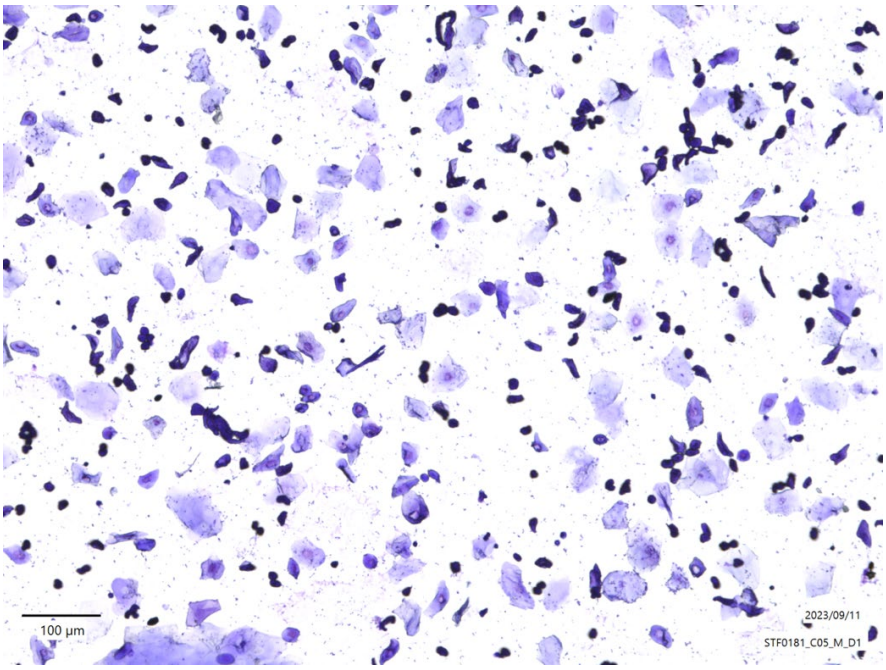
7. Estrus Cycle

Vaginal lavage samples stained with Crystal Violet are obtained for five consecutive days at the **MIDDLE** time point and for two consecutive days at the **OLD** time point. Procedural details are described in Section V.D.

Data available: Digitized photomicrographs of each stained sample are available for user determination of estrus stage. An index of all images available (partial spreadsheet shown below) provides the rat ID and assessment timepoint in columns A and D respectively. Sorting and filtering by these columns allow for a tailored presentation of the data. Columns E-N indicate photomicrographs availability at each time point (Y= yes, an image is available, N= no image is available) and any associated notes. An example sorted by rat ID is shown below.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Rat ID	Sex	Cohort	Timepoint	Day 1 image available	Day 1 Notes	Day 2 image available	Day 2 Notes	Day 3 image available	Day 3 Notes	Day 4 image available	Day 4 Notes	Day 5 image available	Day 5 Notes
2	STF0181	Female	Cohort 5	Middle	Y	Crowded Sample; Two images available	Y		Y		Y		Y	
3	STF0181	Female	Cohort 5	Old	Y		Y							

Each photomicrograph file name contains the rat ID, the time point, the day of assessment and if there is more than one photograph taken that day, an ascending number for each photograph. For example, file STF0181_M_D1_I2.tif represents the 2nd photograph file for that rat on Day 1 of the **MIDDLE** time point. In addition, as shown below, each photomicrograph contains the rat ID, cohort #, time point, the day of assessment, date and a scale bar.



8. Necropsy

At the end of testing, a necropsy is conducted to rapidly collect tissue samples (including plasma and feces) for frozen storage and distribution. The tissues collected and the number of samples collected for each tissue type is shown below.

Tissue	Number of samples
Adrenal	2
Lung	3
Liver	3
Kidney	3
Spleen	3
White Adipose Tissue	3
Testicle or Ovaries	2
Brown Adipose Tissue	3
Gastrocnemius Muscle	3
Skin - pinna	3
Striatum	2
Frontal Cortex	1
Cerebellum	2
Hippocampus	2
Parietal cortex	2
Spinal Cord	2
Eye	2
Heart	1

A standard necropsy form (Section V.A) is available from the ARB for each rat noting the tissue collected and any associated comments.

D. MRI- minimum methods detail, full data description.

1. Animal Prep & Support

Rats are scanned under anesthesia using a combination of isoflurane inhalation (0-2.5% in 30% O2:70% N2) and subcutaneous dexmedetomidine infusion (0.015 mg/kg bolus plus 0.015 mg/kg/hr continuous infusion). Body temperature is maintained with warm air circulation and respiration, rectal temperature, pulse rate, SpO2 and end-tidal CO2 (EtCO2) are continuously monitored and recorded. Isoflurane dose is continuously adjusted to maintain respiration rate between 40-60 breaths per minute.

2. Scanner

MRI data are collected using a Bruker Biospec 9.4T 20 cm scanner equipped with a 12 cm gradient/shim set, 86 mm quadrature transmit coil and four-channel receive-only head array coil.

3. Anatomy

Following a tri-axial localizer scan, two-dimensional multi-slice fast spin echo (RARE) scans are acquired in sagittal, axial and coronal orientations to provide anatomical reference data. A three-dimensional T_2-weighted RARE scan is then performed to generate a data set with 150-micron isotropic voxels, suitable for volumetric measurements.

4. DTI (Diffusion Tensor Imaging)

Using the same slice geometry as the two-dimensional coronal RARE scan, a spin echo diffusion pulse sequence with two-segment EPI readout and respiratory gating is used to acquire 5 control (a0) and 30 diffusion-weighted images with distinct diffusion-sensitizing gradient directions for diffusion tensor (DTI) calculations. These data sets have an in-plane spatial resolution of 375 × 375 μm and a slice thickness of 0.6 mm with 35 slices.

5. EPI (Echo-Planar Imaging) – resting state

Using the same slice geometry and spatial resolution as the DTI scan above, a single-shot T2*-weighted gradient echo pulse sequence with EPI readout is used to acquire two sets of 300 repetitions each with a time resolution of 1.5 s. Two additional sets of 300 repetitions are then acquired with reversed phase encoding to permit EPI distortion correction in post-processing.

Data available: MRI data are presented in four formats: raw and reconstructed Bruker data with parameter files (proprietary, hierarchical format), enhanced DICOM files (https://www.dicomstandard.org/; one file per scan, containing all slices and repetitions), NIFTI files (https://nifti.nimh.nih.gov/) and BIDS (https://bids-specification.readthedocs.io/en/stable/index.html; open source, hierarchical format containing entire study, i.e. all scans for a given rat at a given timepoint).

For each cohort at each time point (**EARLY**, **MIDDLE** or **OLD**), an Excel file lists the scan number corresponding to each MRI modality (2D RARE, 3D RARE, fMRI EPI or DTI) for each rat. For example, the spreadsheet C09_E_MRI_Final.xlsx in the folder C09_MRI_DATA/ shows scan numbers for all Cohort 9 rats at the early timepoint:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	Animal ID	Cohort	DOB	Timepoint	Scan Date	Sex	Weight (g)	Axial 2D RARE	T2W 3D RARE	EPI Reverse	EPI Reverse repeat	EPI Forward	EPI Forward repeat	DTI EPI 30dir	Time under iso before fMRI	Time under dex before fMRI	Total Scan Duration	NOTES					
2	STM0271	C09	8/22/2022	E	3/6/2023	M	725	e4	e14 & 15	e8	e9	e10	e11	e12	1:14	1:02	1:28	Used dataset from December to see if epi scans were be					
3	STF0261	C09	8/22/2022	E	3/7/2023	F	311	e4	e6 & 7	e9	e10	e11	e12	e13	1:16	1:07	1:27	Used newest protocol, messy.					
4	STM0272	C09	8/22/2022	E	3/6/2023	M	660	e4	e14 & 15	e8	e9	e10	e11	e12	1:45	1:33	1:52						
5	STF0262	C09	8/22/2022	E	3/7/2023	F	296	e4	e15 &16	e8	e9	e10	e11	e12	1:13	1:05	1:30	Used 12/14 protocol- melodic reports do appear slightly					
6	STM0273	C09	8/22/2022	E	3/8/2023	M	585	e4	e6 & 7	e9	e10	e11	e12	e13	1:22	1:12	1:39	LabChart's SpO2 section also appears off. Animal appear					
7	STF0263	C09	8/22/2022	E	3/8/2023	F	326	e4	e6 & 18	e9	e10	e11	e12	e13	1:17	1:08	1:32						
8	STM0274	C09	8/22/2022	E	3/9/2023	M	555	e4	e6 & 7	e9	e10	e11	e12	e13	1:16	1:03	1:31						
9	STF0264	C09	8/22/2022	E	3/9/2023	F	349	e4	e17 & 7	e9	e10	e11	e12	e13	1:20	1:13	1:48	Aliasing/ghosting on the T2W scans - doesn't really inter					
10	STM0275	C09	8/22/2022	E	3/10/2023	M	716	e4	e6 & 7	e9	e10	e11	e12	e13	1:22	1:09	1:28						
11	STF0265	C09	8/22/2022	E	3/10/2023	F	342	e4	e6 & 7	e9	e10	e11	e12	e13	1:14	1:06	1:30						
12	STM0276	C09	8/22/2022	E	3/14/2023	M	661	e4	e6 & 7	e9	e10	e11	e12	e13	1:11	1:01	1:31						
13	STF0266	C09	8/22/2022	E	3/14/2023	F	322	e4	e6 & 7	e9	e10	e11	e12	e13	1:22	1:13	1:43	Respiration maintained but very low even at 0.3% iso - u					
14	STM0277	C09	8/22/2022	E	3/14/2023	M	749	e4	e6 & 7	e9	e10	e11	e12	e13	1:11	1:02	1:27	Ran DTI before EPI due to bolus not yet worn off					
15	STF0267	C09	8/22/2022	E	3/14/2023	F	374	e4	e6 & 7	e9	e10	e11	e12	e13	1:13	1:02	1:30						
16	STM0278	C09	8/22/2022	E	3/15/2023	M	607	e4	e6 & 7	e9	e10	e11	e12	e13	1:12	1:01	1:26						
17	STF0268	C09	8/22/2022	E	3/15/2023	F	350	e4	e6 & e7	e9	e10	e11	e12	e13	1:29	1:21	1:38	Body temp lower but stable in the 36 range					
18	STM0279	C09	8/22/2022	E	3/16/2023	M	647	e4	e6 & 7	e9	e10	e11	e12	e13	1:12	1:01	1:24						
19	STF0269	C09	8/22/2022	E	3/16/2023	F	284	e4	e6 &7	e9	e10	e11	e12	e13	1:17	1:08	1:41						
20	STM0280	C09	8/22/2022	E	3/17/2023	M	770	e4	e6 & 7	e9	e10	e11	e12	e13	1:23	1:12	1:37						

For example, at the early timepoint, the scan number for the first fMRI EPI data set for Cohort 9 rat STF0262 is "**e8**". Note that the scan number for a given MRI modality may vary among rats and/or timepoints for a given rat, so the user should refer to this table before retrieving MRI data files. For each rat at each timepoint, four fMRI EPI data sets with 300 repetitions each are acquired: two with reverse ($+k_{max}$ to $-k_{max}$) phase encoding and two with forward ($-k_{max}$ to $+k_{max}$) phase encoding. By combining images acquired with forward and reverse phase encoding, third-party software such as the "topup" routine in FSL (https://fsl.fmrib.ox.ac.uk/fsl/docs/#/diffusion/topup/index) can correct for distortions common in EPI images.

In the same folder as the Excel file, there are subfolders containing MRI data for each rat in a certain cohort acquired at a certain timepoint. For example, the early timepoint data for rat STF0262 in Cohort 9 is stored in the subfolder STF0262_C09_E/. Within each of these folders, there are subfolders containing MRI data in each format plus a FSL MELODIC principal component analysis of each fMRI data set (https://fsl.fmrib.ox.ac.uk/fslcourse/graduate/lectures/practicals/ica/) and a LabChart 8 recording of the rat's vital signs, isoflurane dose and timing marks indicating the duration of each scan.

Raw Bruker Data:

The original Bruker data and parameter files for the MRI study of this rat at this timepoint are stored in a folder with a name beginning as follows:

<scandate>_<scantime>_<ratname>_<ratname>_<cohortnumber>_<timepoint>

where "scandate" and "scantime" refer to the date and time at the beginning of the study and "timepoint" is "E", "M" or "O" for the **EARLY**, **MIDDLE** and **OLD** timepoints, respectively. For example, the folder 20230307_105357_STF0262_STF0262_C09_E_1_2/ contains raw data for rat STF0262 of Cohort 9 acquired at the early timepoint.

Within this folder, there are subfolders corresponding to each of the scan numbers listed for this rat at this timepoint in the Excel file. For example, the first fMRI dataset acquired with reverse phase encoding is in subfolder "8/". In addition, there is a file called "subject" which contains information such as the rat's date of birth and its weight at the time of scanning; these data are transcribed in the Excel spreadsheet.

Within the folder for each scan, there is a file called "rawdata.job0" which contains the complex raw (k-space) binary data recorded for each of four receive channels of the rat head coil. Also present is a text file called "method", which contains a detailed list of parameter values, including many that are not included in the DICOM file headers, such as pulse shapes, durations and power levels.

For each scan, there is a folder called "pdata/", which contains the processed (reconstructed) data corresponding to rawdata.job0. Within pdata/, there is a subfolder "1/", which contains magnitude images reconstructed using a default algorithm. For DTI scans, there is also a subfolder "2/", which contains images calculated from a diffusion tensor reconstruction of the diffusion-weighted images.

Within the "1/" folder, reconstructed images are stored in three formats. The file "2dseq" is a headerless file containing one 16-bit signed binary integer for each pixel, a standard Bruker format. The parameters corresponding to 2dseq are stored in the text file "reco". The reco file describes the details of the reconstruction, including the number of pixels before, during and after Fourier transformation in each dimension and parameters describing intensity rescaling, filtering, etc.

Also in the "1/" folder are subfolders called "dicom/" and "nifti/" containing the reconstructed data in the open-source DICOM and NIFTI formats. For convenience, the DICOM and NIFTI files for all scans for a given rat at a given timepoint are also copied to the "dicom/" and "nifti/" folders at the top level of the file tree, e.g. in the folder STF0262_C09_E.

DICOM data are stored in the "enhanced" DICOM format, with a single file containing all images for a given scan; see "DICOM" section below for file naming conventions.

NIFTI data may be stored with one file containing all images (2D and 3D RARE) or with separate files for each repetition (fMRI scans), slice (diffusion-weighted DTI data) or parameter map (DTI tensor reconstruction). Each of these NIFTI files has a name of the form:

<ratname>_<ratname>_C<cohortnumber>_<timepoint>_<scannumber>_1_<studynumber>.nii.

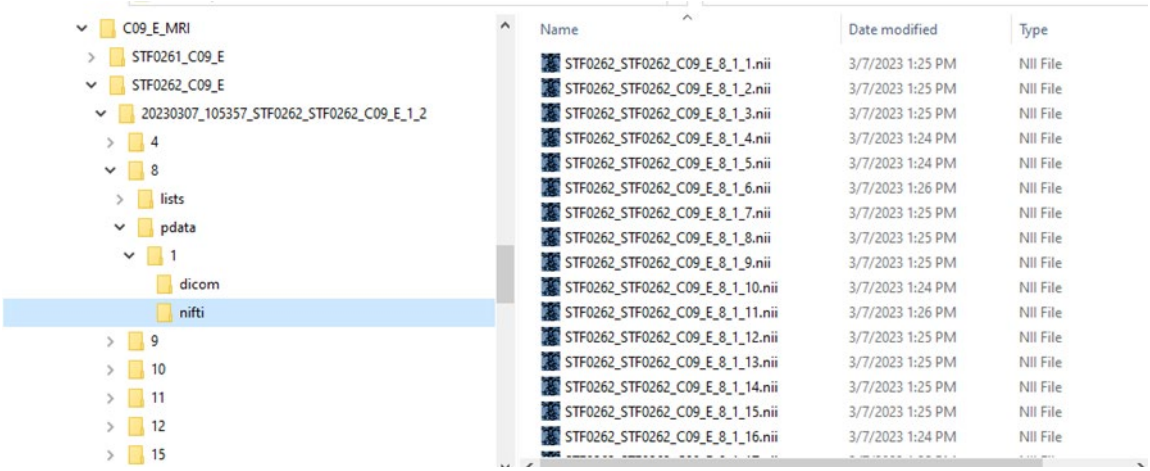
Note that "studynumber" is not simply related to "timepoint" and can be ignored for purposes of data processing.

As an example, there are 300 NIFTI files corresponding to fMRI scan 8 in the folder 20230307_105357_STF0262_STF0262_C09_E_1_2/8/pdata/nifti/. These files have names of the form:

STF0262_STF0262_C09_E_8_1_<repnumber>.nii

where "repnumber" is the repetition number, from 1 to 300. Thus, STF0262_STF0262_C09_E_8_1_7.nii contains images of all 35 slices for repetition 7 of 300 in this fMRI scan.

Here is a summary of the file structure for scan 8 (fMRI EPI with reverse phase encoding, first repetition) of STF0262_C09_E:

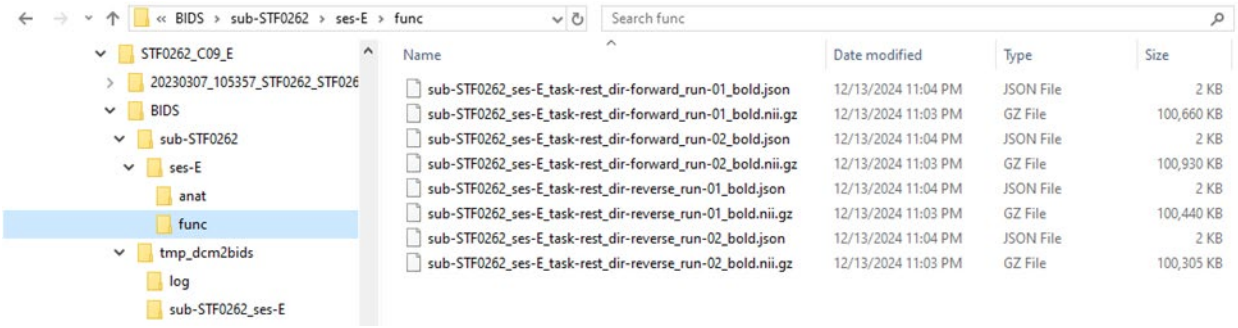


BIDS:

In the folder containing MRI data for each rat at a given timepoint, e.g. STF0262_C09_E/, there is also a folder called "BIDS/". BIDS files are generated using the program dcm2bids (https://doi.org/10.5281/zenodo.8436509), which converts the DICOM files for each study to compressed NIFTI files (*.nii.gz) and reorganizes and renames them according to BIDS specifications. The BIDS folder contains a subfolder with a name of the form "sub-<ratname>/", e.g. sub-STF0262/. Inside this folder is a subfolder with a name of the form "ses-<timepoint>/", e.g. ses-E/ for the early timepoint of this rat. Within this folder,

subfolders named "anat/", "dwi/" and "func/" contain data and parameters for the anatomical scans (2D and 3D RARE), diffusion tensor scans (DTI) and functional scans (fMRI EPI), respectively. Within each of these folders, the image data from each scan are stored as a compressed NIFTI (.nii.gz) file which can be decompressed with the GNU function "gunzip". Each *.nii.gz file in BIDS should decompress to a single NIFTI file, which includes all images in the corresponding scan. Parameters for each scan are stored in a matching text file in the open-source JSON format.

Here is a summary of the BIDS file structure for the fMRI scans in the early timepoint study of Cohort 9 rat STF0262:



This reflects the presence of two forward and two reverse-encoded fMRI scans for each rat at each timepoint.

Here is the BIDS file structure for the anatomical scans in this study:

The "RARE2D" files refer to the two-dimensional multi-slice scan used as an anatomical reference corresponding to the fMRI and DTI scans. The "RARE3D_run-02" files refer to the high-resolution (200 μm^3) three-dimensional scan available for volumetric measurements.

And here is the BIDS file structure for the DTI scans in this study:

For DTI, in addition to the image (*.nii.gz) and general parameter (*.json) files, there are parameter files listing the directions (*.bvec) and strengths (*.bval) of the diffusion-sensitizing gradient.

DICOM:

Although the DICOM files for each scan are available within the pdata/ folders in the Bruker data tree described above, for convenience, they are also collected in the "dicom/" subfolder within the folder containing MRI data for each rat at a given timepoint, e.g. STF0262_C09_E/. For each scan, there is an enhanced DICOM file named:

<ratname>_<ratname>_<ratname>_C<cohortnumber>_<timepoint>_E<scannumber>_P1_EnIm11.dcm

For example, the reconstructed image data of scan 8 acquired on rat STF0262 of cohort 9 at the early timepoint is stored in the file STF0262_STF0262_STF0262_C09_E_E8_P1_EnIm1.dcm.

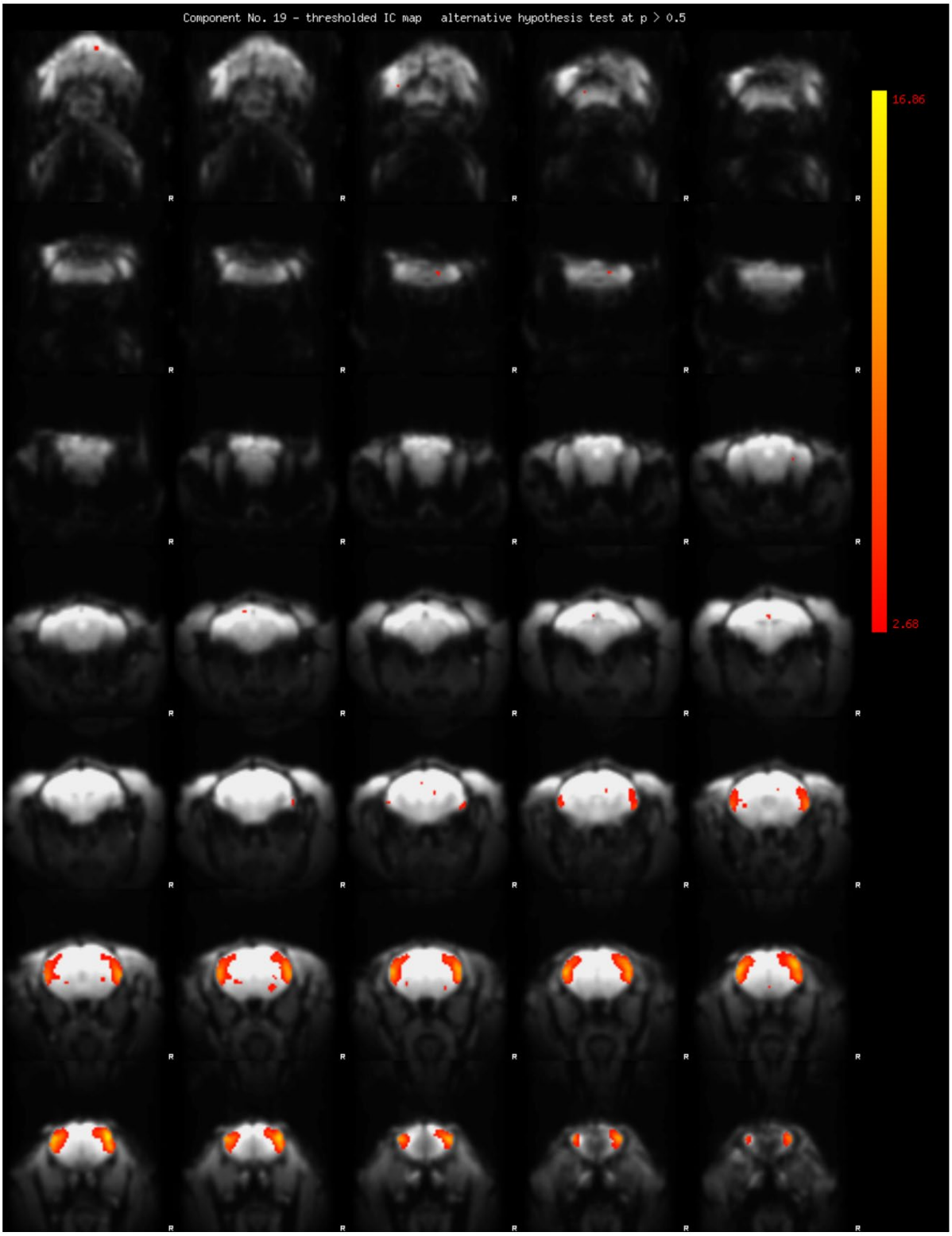
Note that these are "enhanced" DICOM files, containing all images for a given scan in a single file.

MELODIC:

In the folder containing MRI data for each rat at a given timepoint, e.g. STF0262_C09_E/, there is a folder called "melodic/" which contains subfolders for each of the fMRI scans. These folders have names of the form "E<scan number>.ica/". For example, the results of the FSL MELODIC analysis on scan 8 are in the folder "E08.ica/". These analyses were performed immediately after the acquisition of each fMRI scan as a quality control test. The 30 principal components found by MELODIC were reviewed to identify those with physiologically "sensible", bilateral activation patterns (see image below from STF0262_C09_E scan 8). A scan was deemed successful if at least six such components were detected.

MELODIC Component 19

2.42 % of explained variance; 1.74 % of total variance



NIFTI:

Although the NIFTI files for each scan are available within the pdata/ folders in the Bruker data tree described above as well as in the BIDS tree, for convenience, they are also collected in the "nifti/" subfolder within the folder containing MRI data for each rat at a given timepoint, e.g. STF0262_C09_E/.

For each scan, there is a GNU-zipped archive of NIFTI files named:

<ratname>_<timepoint>_e<scannumber>.nii.gz or
<ratname>_<timepoint>_e<scannumber>.tar.gz

For example, the reconstructed image data of scan 8 acquired on rat STF0262 of cohort 9 at the early timepoint is stored in the file STF0262_E_e8.nii.gz.

Note that each *.gz archive may contain one or more individual NIFTI files, depending on the MRI modality (e.g. RARE or fMRI-EPI). This differs from the *.nii.gz files in the BIDS folders, which decompress to a single *.nii file.

LabChart:

Finally, in the folder containing MRI data for each rat at a given timepoint, e.g. STF0262_C09_E/, there is a file containing the physiological data recorded by AD Instruments (ADI) LabChart 8 software during the MRI study. This file has a name of the form:

<ratname>_C<cohortnumber>_<timepoint>.adicht

For example, the physiological data recorded during MRI scanning of rat STF0262 of cohort 9 at the early timepoint is stored in the file STF0262_C09_E.adicht.

These files can be displayed and data extracted and saved using LabChart 8, proprietary software from ADI. AD Instruments also offers a free, display and analysis-only version of LabChart called "LabChart 8 Reader" (https://www.adinstruments.com/products/labchart-reader).

The following physiological and timing data are recorded at a rate of 2000 samples per second (i.e. 0.5 ms per point) throughout each MRI study:

P-resp Effort: Air pressure signal from SA Instruments pneumatic respiration sensor placed below the rat's abdomen. This signal is used to calculate respiration rate and to synchronize MRI data acquisition during DTI scans to avoid motion artifacts.

Resp Rate: Respiration rate calculated from the P-resp Effort signal by SA Instruments' PC-SAM software, in units of breaths per minute.

Pulse Ox Wave: Signal from SA Instruments pulse oximeter sensor placed on the rat's hindpaw.

RF Pulses: TTL logic signal from the MRI scanner marking the beginning of each dynamic in a fMRI scan. This signal permits matching fMRI dynamics to physiological data. This signal is "active-low", i.e. it "floats" at a positive voltage and switches to zero volts at the beginning of each dynamic.

SpO2: Blood oxygenation saturation (in percent) calculated by PC-SAM software from the Pulse Ox Wave signal. This number should be used with caution since it is often unreliable in rats and mice.

Pulse Rate: Pulse rate in beats per minute calculated by PC-SAM software from the Pulse Ox Wave.

FOT1: Rectal temperature in Celsius measured by SA Instruments fiber optic temperature (FOT) sensor.

T1: Temperature in Celsius of warm air blown across rat to maintain normal body temperature. The air heater current is adjusted by the PC-SAM software to stabilize the rectal temperature measured by the FOT sensor at 37-38 °C.

Supply CO2: Percent concentration of carbon dioxide in gas sampled from rat's expired air. This signal could be used to calculate end-tidal CO_2 concentration ($EtCO_2$).

Supply O2: Concentration of oxygen in the isoflurane:O2:N2 anesthesia mixture flowing to the rat's nose cone. This is typically set to 30% by volume.

ERT Gate: Logic signal calculated by PC-SAM to trigger the MRI scanner in DTI experiments. This signal is "active low", i.e. the scanner is triggered only while the ERT Gate singal is at 0 volts. The scanner may or may not actually pulse during this period, depending on the timings of the MRI pulse sequence; the ERT Gate signal merely "allows" the scanner to pulse if it is ready.

Isoflurane: Percent isoflurane in anesthesia mixture, as estimated by a home-built sensor calibrated to the position of the dose adjustment knob on the isoflurane vaporizer. This measurement is accurate to about ± 0.2% over the range of 0-2.5%.

XMTR gate 1: TTL logic signal from the MRI scanner indicating transmitter unblanking prior to a radio-frequency (RF) pulse. Recording this signal permits matching individual slice images to physiological data recorded during the scan. This signal is "active-low", i.e. the scanner's RF transmitter is enabled to transmit a pulse only when this signal is zero. In a fMRI scan, there is a pulse on this channel for each slice in each dynamic, i.e. there are 35 pulses on XMTR gate 1 for each pulse on the "RF Pulses" signal.

For detailed instructions on extracting the physiological data corresponding to each MRI scan, please see Appendix "Extracting Physiological Data for STARRRS fMRI Scans.pdf".

E. Phenotypic Assessments

6. WM-LI

Background: Medial temporal lobe-dependent spatial memory is assessed in a well-established water maze learning index (WM-LI) procedure specifically tuned to detect age-related impairment (Gallagher 1993). In this task, the spatial relationships among distal visual cues provide information regarding the escape platform location. Aged Long Evans rats exhibit marked individual differences in this task, with some performing on par with young rats, and others performing much worse.

Performance in the place version of the water maze critically requires the hippocampus, with severe impairments following lesions involving the hippocampus itself (RGM Morris 1990; RJ Steele 1999) or transection of its input/output pathways (RGM Morris 1990; RJ Steele 1999). Performance is also impaired by lesions of retrosplenial, medial prefrontal, or posterior cingulate cortices, as well as dorsomedial striatum (KT Harker 2004; RJ Sutherland 1988; RJ McDonald 2008).

Brief Summary: Across sparsely spaced training trials rats learn the location of a hidden escape platform submerged in opaque water. Three trials/day are provided for eight consecutive days with the escape platform inaccessible for the first 30 sec. on the last trial every other day (probe trials). Performance on the last three probe trials is used to calculate a learning index (LI) score that serves as the main dependent measure of spatial learning and memory. The LI score includes a correction for differences across trials in distance from the start location to the platform and is relatively unbiased by swim speed. Rats are subsequently tested on a non-hippocampal, visible platform version of the task in a single session to screen for rats with non-mnemonic sensory, motor or motivation impairment.

Data available: A summary file for the final spatial memory assessment for all rats completing the test is available. The screen shot below shows the Rat ID, Sex, Cohort, test date and any notes in columns A-E respectively. The main dependent variable, the learning index score is reported in column F. Data from the hidden platform trials (Acquisition; AQ) are reported in columns G-K. Each AQ block comprises the 5 trials preceding each of the probe tests during training. The search error measure (Gallagher et. al., 1993), reflecting deviation from the optimal path to the goal, implements a correction procedure so that scores are relatively unbiased by differences in distance to the goal from the various start locations and swim speed. Column L reports the mean latency to find the visible platform during the cue training session, where column M flags values in column K over 25 seconds. A mean visible platform latency over 25 seconds is the operational criterion for excluding rats suspected of non-specific performance deficits (e.g., sensorimotor or motivational impairment). Notes for the WM procedure (column

E) typically identify idiosyncratic technical or animal issues, e.g., unreliable data or the existence of raw tracking data from rats that were subsequently dropped from the task for health reasons.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Rat ID	Sex	Cohort	Test Date	Notes	WM Learning Index Score	Search Error Trial 1 (m.s)	AQ, BLock 1 Search Error (m.s)	AQ, BLock 2 Search Error (m.s)	AQ, BLock 3 Search Error (m.s)	AQ, BLock 4 Search Error (m.s)	Cue Mean Latency (sec)	Cue Fail Flag
2	STF0111	Female	Cohort 1	01/16/2024		210	3	14	21	28	32	8	
3	STF0115	Female	Cohort 1	01/16/2024		203	12	7	6	18	6	13	
4	STF0116	Female	Cohort 1	01/16/2024		224	26	23	28	32	30	20	
5	STF0117	Female	Cohort 1	01/16/2024		180	39	22	17	31	22	8	
6	STF0119	Female	Cohort 1	01/16/2024		223	3	3	13	8	13	8	
7	STM0104	Male	Cohort 1	01/16/2024		189	16	23	11	25	43	23	
8	STM0107	Male	Cohort 1	01/16/2024		237	15	30	25	30	46	30	Fail

The raw tracking data and videos data are available for users to conduct additional analyses. The tracking program used to acquire the data, ANY-maze (https://stoeltingco.com/Neuroscience/Anymaze/Any-maze-Video-Tracking), is free to download and can be used for reanalyzing swim paths, exporting other dependent variables and viewing associated videos. Videos can also be exported for use in other analytic pipelines. Rats from the same cohort were tested together and separate experimental files were created for the hidden and visible platform versions of the task. ANY-maze experimental files have the extension .szd and the folders containing the videos follow the same naming as their associated experimental file.

7. EPM

Background: The elevated plus maze (EPM) is used to assess behavior related to anxiety in rodents. Rats explore an elevated plus (+) shaped maze containing two open arms and two closed arms. The test takes advantage of rodents' natural fear of open spaces. Drugs that reduce or increase anxiety in humans induce corresponding shifts in rats' open arm exploration on the EPM (Pellow 1985), conferring predictive validity as a measure of "anxiety-like" behavior. Multiple brain regions are implicated including the amygdala, bed nucleus of the stria terminalis, prefrontal cortex, ventral hippocampus, and lateral septum (reviewed in Calhoon 2015). Exploratory activity in the EPM also reportedly predicts longevity in mice (Fahlstrom 2012).

Although ethopharmacological analysis of EPM data has identified several stereotyped behaviors related to exploration and risk assessment (Cruz 1994; Anseloni 1997), these behaviors are not reliably scored with ANY-maze software and are not quantified in STARRRS. However, video files are available for end-users to explore alternate analytic pipelines.

Brief Summary: Rats are placed in the intersection of an elevated plus (+) shaped maze and allowed to explore freely for 5 minutes.

Data available: A summary data file for all rats completing the test is available. The screen shot below shows Cohort number, Rat ID, Sex, Cohort, Time point, Test date and any notes in

columns A-G respectively. The main dependent variable, percent time spent in open arms is reported in column M. Columns H-L report other standard measures (total distance traveled, number of open arm entries, time spent in open arms, number of closed arm entries and time spent in closed arms). Notes associated with this task typically identify trials when technical errors may render the data unreliable.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Cohort Number	Rat ID	Sex	Cohort	Timepoint	Test Date	Notes	Total Distance Traveled (m)	Open Arm Entries	Time Spent in Open Arms (s)	Closed Arms Entries	Time Spent in Closed Arms (s)	Percent Time Spent in Open Arms
2	5	STF0181	Female	Cohort 5	Early	10/31/22		11.305	1	26.6	5	230.9	10.33
3	5	STF0182	Female	Cohort 5	Early	10/31/22		12.778	4	56	7	208.8	21.15
4	5	STF0183	Female	Cohort 5	Early	10/31/22		14.256	7	97.8	8	153.4	38.93
5	5	STF0184	Female	Cohort 5	Early	10/31/22		13.118	6	90.9	7	156.5	36.74

The raw experimental data for each cohort/timepoint are also available. This resource allows users to access the tracking data and videos from the program used to collect the data for analysis in other pipelines.

The tracking program, ANY-maze (https://stoeltingco.com/Neuroscience/Anymaze/Any-maze-Video-Tracking) is free to download and to use for reanalyzing a subject's path and view associated videos. Videos can also be exported to other formats for alternate analytic pipelines. The ANY-maze experimental files have a .szd extension and the folders containing the videos follow the same naming scheme as the associated experimental file.

8. HCS

Background: Home activity patterns, particularly physical activity sedentary behavior, can be significant predictors of geriatric outcomes, including physical performance, cognitive function, and overall health. In rodent models, aging reduces home cage activity, especially in the dark phase and around transitions between light and dark phases (Spangler 1994; Casadesus, 2001).

Brief Summary: Using a custom-designed system (Home Cage Scan, HCS; CleverSys, Reston VA) to document home cage activity in STARRRS, rats are individually housed and video recorded continuously for 48 hours, undisturbed in isolated ventilated cages flanked on three sides by panels emitting both white and infrared light, with the fourth side in line with a camera. Food and water are available *ad libitum,* and the light/dark cycle and cages are the same as the home colony environment.

Data available: Video files appropriate for manual or computer-aided scoring are available. To reduce file size, the recordings are saved as 12, 4-hour video files and 4 camera inputs (one for each rat) are contained in a single video file. The videos are organized by cohort, timepoint, and apparatus cabinet number. An index file identifies the cabinet and box associated with each rat. Since a single video contains simultaneous recording of four rats in a 2×2 array, this index file also identifies the quadrant location for each rat (e.g. upper left, upper right, lower left, lower

right). A partial screen shot of this file is shown below. Section VI.H provides detailed collection methods, video file naming structure and data organization. The notes associated with this task most frequently refer to technical failures resulting in the loss of specific video files.

	A	B	C	D	E	F	G	H	I	J
1	Cohort Number	Rat ID	Sex	Cohort	Timepoint	Test Date	Notes	Cabinet	Box	Video Quadrant
2	1	STM0101	Male	Cohort 1	Middle	4/11/23		1	A	Upper Left
3	1	STM0102	Male	Cohort 1	Middle	4/11/23		1	B	Upper Right
4	1	STF0111	Female	Cohort 1	Middle	4/11/23		1	C	Lower Left
5	1	STF0112	Female	Cohort 1	Middle	4/11/23		1	D	Lower Right

9. WM-DMTP

Background: Prior training in standard 'place' versions of the water maze can profoundly influence subsequent performance when animals age, complicating longitudinal analysis. STARRRS therefore adopted a significantly streamlined alternative for examining life course trajectories of spatial memory, aimed at minimizing the impact of repeated tests on the endpoint outcome at 2 years of age. In this 'delayed match-to-place' variant, rats are trained to swim to a hidden platform location in a single short session, and to learn a new escape location on each of three days of testing.

Brief Summary: At 3 months of age rats were given five days of shaping. This consisted of five acquisition trials/day only; no retention trials were administered. For WM-DMTP testing at the three assessment time points, rats learn a new escape location in each of three, 5-trial test sessions, each including a 6-hour retention test. Swim path to the escape platform is recorded on all trials, and a search error score, representing the deviation from the shortest path to the goal, is computed.

Data available: The summary data file for this task (partial image below) includes the cohort number, Rat ID, sex, cohort, timepoint, test date and any associated notes in columns A-G respectively. The search error measure (cumulative distance from the goal) for both the shaping phase and the DMTP procedure with 6-hour retention test are shown in columns H-AI. Notes associated with this task most often reference trials where technical issues resulted in unreliable data or instances in which partial raw tracking data are available, but an animal was ultimately removed from the experiment for health reasons.

The screen shot below presents two days of shaping data for two rats, and two days of WM-DMTP test data at the **EARLY** timepoint for two others. Note the shaping task does not include a retention trial.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	Cohort Number	Rat ID	Sex	Cohort	Timepoint	Test Date	Notes	Day 1 Trial 1 (Search Error, m.s)	Day 1 Trial 2 (Search Error, m.s)	Day 1 Trial 3 (Search Error, m.s)	Day 1 Trial 4 (Search Error, m.s)	Day 1 Trial 5 (Search Error, m.s)	Day 1 Retention Trial (Search Error, m.s)	Day 2 Trial 1 (Search Error, m.s)	Day 2 Trial 2 (Search Error, m.s)	Day 2 Trial 3 (Search Error, m.s)	Day 2 Trial 4 (Search Error, m.s)	Day 2 Trial 5 (Search Error, m.s)	Day 2 Retention Trial (Search Error, m.s)
2	5	STM0199	Male	Cohort 5	A_Shaping	8/22/22		54.52	37.542	5.66	2.051	16.817		34.815	33.836	0.958	0.076	6.408	
3	5	STM0200	Male	Cohort 5	A_Shaping	8/22/22		41.792	0.699	0.805	0.047	9.347		40.67	33.82	8.261	0.615	12.465	
4	5	STF0181	Female	Cohort 5	Early	10/3/22		54.593	5.84	2.939	3.564	3.257	0.122	3.11	0.115	0.84	9.517	1.394	0.06
5	5	STF0182	Female	Cohort 5	Early	10/3/22		46.191	35.02	5.978	0.412	0.108	4.621	14.691	38.657	29.833	12.585	29.865	12.759

The raw experimental data for each cohort/timepoint are also available. This resource allows users to conduct additional analyses by accessing the tracking data and videos from the program used to collect the data. This tracking software, ANY-maze (https://stoeltingco.com/Neuroscience/Anymaze/Any-maze-Video-Tracking) is free to download for reanalyzing swim paths and view associated videos. Videos are saved in a proprietary ANY-maze .szv file format but can be converted within ANY-maze to other formats. The ANY-maze experimental files have a .szd extension and the folders containing the videos follow the naming scheme of their associated experimental file.

10. ORM

Background: The Odor Recognition Memory (ORM) task takes advantage of rodents' robust olfaction and natural preference to explore novelty to assess long-term recognition memory (Weiler 2021). Recognition memory requires the hippocampal system, and ORM performance in aged LE rats broadly replicates the variability in spatial memory observed in older subjects. In STARRRS, ORM provided an independent assessment for documenting the longitudinal trajectory of hippocampal memory.

Brief Summary: Animals are initially habituated to the test chamber across multiple sessions, in order to increase the relative salience of the odor stimuli used in the formal memory test. The next day, rats are placed in the familiar open field chamber and presented with two identical vials containing the same odorant (sample phase). Twenty-four hours later, the two identical vials are presented in the same locations, one containing the same scent as the day before, and the other containing a novel odorant (test phase). Rats with intact recognition memory spend more time exploring the vial with the novel scent in the test phase. The recognition index score, calculated as the percentage of total odor-directed exploration time spent investigating the novel scent, is the primary dependent measure in ORM.

Data available: The summary file from the ORM task contains data from the different phases of the task. Column H identifies the specific odor pair tested in each subject, and column P identifies the novel odor. Columns I-L report the distance traveled in the empty arena during the habituation phase (comprising open field exploratory activity and habituation). Columns M-N report the time engaged in olfactory exploration for each vial in the sample phase, and column O

reports the total distance traveled during the sample phase. Columns R and S report the time spent exploring the novel and familiar odors respectively in the test phase, column T is the distance traveled in the test phase, and column U reports the recognition index score calculated as the time spent exploring the novel odor divided by the total olfactory exploratory time across both vials. Column Q indicates the position of vials containing the familiar and novel odors in the associated video recordings. This key is required for evaluating performance in videos ported to different software package for analysis. Additional details regarding associated data and video files are presented in Section VI.

A video tracking system (TopScan, CleverSys) was used to record and quantify exploratory behavior in the ORM task. Odor-directed exploration was defined as the nose of the rat being within 1cm of a vial opening. The derived data collected in STARRRS comes directly from the tracking system; although the accuracy of automated scoring relative to a trained observer was validated in initial pilot studies, it is not practical to conduct case-by-case confirmation for the volume data collected in STARRRS. Excel files containing detailed data of each rat's exploratory activity are available along with all files and videos necessary to reanalyze behavior in TopScan or other analytic pipelines.

Summary files note instances in which individual odor exploratory bouts exceeded 6 seconds in column (E), flagging data that may warrant inspection for potential tracking errors. Long exploratory bouts are common, but large values can also indicate tracking or idiosyncratic performance issues. Similarly, "Technical error, data unreliable" entries do not signal that the recorded data should be excluded, but that the quantitative results merit direct verification from the video. For example, rats sometimes knock over a vial during exploration, leading to automated spatial tracking errors in the course of the trial. Users are advised to evaluate the validity of such data and consider potential alternate scoring modifications on a case-by-case basis.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Cohort Number	Rat ID	Sex	Cohort	Timepoint	Test Date	Notes	Odor Pairing	Habituation Day 1-Total Distance (mm)	Habituation Day 2-Total Distance (mm)	Habituation Day 3-Total Distance (mm)	Habituation Day 4-Total Distance (mm)	Sample-Olfactory Exploration at Position 1 (s)	Sample-Olfactory Exploration at Position 2 (s)	Sample-Total Distance (mm)	Novel Odor	Video Quadrant NE/SW	Test-Olfactory Exploration at Novel Odor (s)	Test-Olfactory Exploration Familiar Odor (s)	Test-Total Distance (mm)	Recognition Index Score
2	3	STF0141	Female	Cohort 3	Early	8/29/22	Greater than 6 seconds - Test / 4th habituation trial not conducted	Vanilla/Anise	7735.52	7337.31	7187.8		47.85	42.04	26656.60	Anise	Familiar/Novel	20.15	18.15	14910.23	0.52611
3	3	STF0141	Female	Cohort 3	Middle	6/30/23		Orange/Lime	7480.91	8089.01	8875.81	9362.03	9.84	15.78	20561.16	Lime	Familiar/Novel	3.57	1.77	10635.33	0.668539
4	3	STF0141	Female	Cohort 3	Old	2/2/24		Almond/Banana	8368.11	5577.07	4606.69	4456.58	3.7	4.04	13895.75	Banana	Familiar/Novel	1.53	7.57	8515.95	0.168132

11. 2-Choice RT

Background: Aging and a variety of age-associated neurocognitive disorders prominently affect executive function, i.e., a diverse class of cognitive/behavioral capacities including attention, cognitive flexibility and inhibitory control. Assessment of visuospatial attentional control and behavioral inhibition in STARRRS is performed in the 2-choice serial reaction time task ("2-Choice RT), adapted from a 5-choice version (Reviewed in Fizet J 2016; Voon V 2014; Bari & Robbins 2013). Briefly, rats are trained to fixate attention on a wall of a chamber and respond to brief flashes of light at one of two locations. Successful performance requires control of

visuospatial attention to correctly identify which light port was illuminated, as well the capacity to inhibit responding during inappropriate times.

Attentional control is measured by response accuracy, defined as percentage of responses to the correct port versus total number of correct and incorrect responses. This is impaired by lesion of anterior cingulate cortex (Muir 1992; McGaughy 2002; Lehmann 2003) or blockade of cholinergic receptors in prefrontal cortex (Robbins 1998).

Inhibitory control is measured by (lack of) premature responding, or selecting a response location before a stimulus light was illuminated. Sometimes described as "impulsive action", this behavior is pharmacologically dissociable from "impulsive choice" as measured in a delayed discounting task (Paterson NE 2012). Premature responding is increased following lesions of infralimbic cortex or ventral hippocampus (Chudasama 2003, 2012).

Brief Summary: Rats undergo 45-minute tests in operant chambers, where they fixate attention to one wall and watch for a brief flash of light in one of two apertures. Rats must withhold responding until after the stimulus light is extinguished and then perform a nose-poke at the aperture that was illuminated. Successful trials are rewarded with food pellets; this repeats for 45 minutes.

At three months of age, behavior is shaped on procedural aspects of the task under conditions of food restriction to increase motivation. Shaping is initially conducted under the least demanding test conditions (e.g., long signal light durations), and gradually adjusted in accord with an individual's performance to accommodate the more challenging conditions (Bari 2008).

For assessments at **EARLY**, **MIDDLE**, and **OLD** age, rats are tested under ad libitum feeding conditions to avoid the confounding effects of food restriction on the aging process. Each assessment begins with two days of testing using standard conditions to reorient rats with task procedures. Test parameters are then varied to probe different levels of difficulty for attentional and inhibitory control. This consists of six test days under easy, medium, and hard attentional difficulties using 4, 1, and 0.2 second stimulus light durations, respectively, with two days of testing at each difficulty. Next there are two days probing inhibitory control by varying, within session, the interval before stimulus light presentation from 5-11 seconds. Finally, there is a single day of testing under standard conditions to help reinforce procedural aspects of the task until the next assessment.

Data available: Two different output worksheets are available within the data spreadsheet. The "concise" sheet gives one row per rat at each assessment:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Rat ID	Sex	Cohort	Timepoint	Notes	4.0sec Stimulus % accuracy	1.0sec Stimulus % accuracy	0.2sec Stimulus % accuracy	5sec Interval % premature	7sec Interval % premature	11sec Interval % premature	1.0sec Stimulus % omissions	# Perseverative head entries per correct trial	Stimulus response latency (s)	Food retrieval latency (s)
2	STF0161	Female	Cohort 4	Early		86.364	70.339	59.259	9.6774	20.6349	58.0645	36.500	.02410	1.3850	1.6075

A selection of the most informative data metrics is provided as columns here, with each column representing the cumulative calculation from two consecutive days of testing with the same parameters. Columns F-H contain % accuracy, defined as fraction of timely port nose-pokes that

were to the correct port (50% is random chance). This is reported across three different stimulus durations. Columns I-K contain % premature response rate, defined as fraction of trials in which the rat performs a nose-poke response before any port was illuminated. Also tested parametrically across three different interval durations. Columns L-O represent data calculated from the "standard" task parameters used on days 6-7 of testing; namely, 1.0 second stimulus duration and 5 second intervals. Omissions refer to trials in which the response window expires without a nose-poke response. Perseverative responses are additional, inconsequential nose-pokes to the light aperture that occur after a pellet was successfully earned, but before it was collected from the food receptacle. This metric is normalized to number of correct trials, since by definition they can only occur on trials in which a rat has earned a reward pellet. Stimulus and response latencies are calculated as median times within a session, thus minimizing influence of individual trials with unusually long latencies (especially a problem with food retrieval). The "notes" column reflects any issues that arose at the time of testing, as well as two important flags. "Shaping failure" is presented for a rat across all time points if it failed to graduate past the 2.5 second stimulus duration during initial task shaping. We have empirically found this to be a threshold that separates performance during subsequent testing. We advise excluding all 2-Choice RT data from these animals. "Omission failure" warning is presented if the omission rate exceeded 80% on any of the individual days of testing with stimulus duration set to 1 or 4 seconds (0.2 second stimulus duration trials are difficult by design, and thus expected to substantially increase omission rates). Omissions are especially common in male rats at **MIDDLE** and **OLD** time points.

The "verbose" worksheet presents one row per rat for each day of testing. Raw data is available in the form of lengthy text files, one per rat per day of testing. Trial-by-trial data are provided in several columns. Outcomes shows whether each trial resulted in a correct response, incorrect response, omission, or premature response (C,I,O,P, respectively). Stimulus locations are reported for each trial, left or right (L,R). Stimulus response latency and food retrieval latency are provided for each trial in which a correct response was performed. Interval selections are provided for variable interval trials; these may be 5, 7, or 11 seconds. Finally, the filename of the associated raw text file output is provided, should end users wish to explore data more comprehensively.

IV. Animals and Housing

F. Animals

For longitudinal assessments, male and female virgin Long Evans outbred rats are purchased from Charles River Laboratory's Kingston facility at 1 month of age. Rats used as controls are purchased at 3 months of age (and tested at 6 months as described below). On receipt, rats are assessed for normal alertness, overt posture, gait abnormalities, and gross external anatomical defects prior to housing.

Following a 5–14 day acclimation period, rats are weighed, receive a subcutaneous transponder, feces collected, and body weight and temperature are recorded.

G. Note

The foundation of the cognitive aging model for this longitudinal project comes from over 30 years of experiments using 9-month-old male retired breeder LE rats from Charles River Laboratories (CRL), Raleigh NC facility, room 06 as subjects. Rats from this facility have exhibited consistent mortality and range of cognitive performance over this period.

During the COVID-19 pandemic CRL closed the Raleigh LE facility, and from the start of the STARRRS project the male and female virgin 1-month-old LE rats were shipped from the Charles River Kingston facility, room K72. Rats from this room were originally transferred from the Portage LE facility when it closed in 2014 and analysis by CRL has shown a genetic difference between rats derived from Raleigh and rats derived from rats from Kingston. The subpopulations these rats represent have been separated for decades, so this is not unexpected. Although unexpected at the start of this project, rats from the Kingston facility have exhibited a higher than anticipated mortality rate under 24 months compared to rats from the Raleigh facility. Similarly increased early mortality in LE rats from Kingston was also observed at another animal facility receiving LE rats from Kingston (Johns Hopkins University). When the increased mortality became apparent (over the first 24 months of STARRRS), CRL configured breeding a sufficient supply of rats from the original Raleigh stock for STARRRS (housed in Kingston room K71). Therefore, STARRRS cohorts 1-30 originated from room K72 in the Kingston facility, and rats in cohorts 31 and above are from litters derived from the Raleigh facility (room K71).

Cohort	Room at Kingston CRL
1-30	K72
31+	K71
Control 1-9	K72
Control 10+	K71

Siblings: Although the supplier was asked to minimize the inclusion of siblings in rats provided, the percent of siblings included per cohort could not be confirmed.

H. Transponders

The Bio Medic Data Systems (BMDS) transponders (IPPT-300, IPPT-500, IPPT1000) HTEC transponders (Seaford, DE) are glass encased and battery free. They are placed subcutaneously using a 12-gauge stainless steel, OD 0.071 inches (1.8 millimeters) insertion needle. The transponder is programmed with a code that incorporates a STARRRS ID followed by the NIA Progeny ID number. The transponders can hold 32 characters, including special characters. Per the BMDS website, the temperature range of the device is 20.0-42.0 °C with a resolution or 0.1 °C, and accuracy of 0.2 °C from 34-42 °C, 0.5 °C from 30-34 °C, and 1.0 °C from true at 20-30 °C.

I. Standard Clinical Care

Rats in the STARRRS project receive the animal facility's standard clinical care. Common treatments include trimming of excessively long toenails, trimming of misaligned teeth, providing soft cage bedding to alleviate pain due to foot sores, and local or oral administration of antibiotics in response to ruptured abscesses or skin sores. Rats that fail to maintain body weight are provided water-softened chow on the cage floor. Rats are euthanized for humane reasons if the attending clinical veterinarian determines a rat is in distress and not responding to treatment. The carcass is then sent to the NIH Division of Veterinary Resources (DVR) Veterinary Pathology Section for analysis.

Long Evans rats are prone to developing subcutaneous tumors and these are excised under specific experimental conditions and veterinary judgement. If the attending veterinarian identifies a tumor as appropriate for surgical removal, and if the rat is not scheduled for a behavior assessment in the upcoming 4 weeks and if the rat is under 22 months of age, then the rat is anesthetized with isoflurane for surgical excision. Rats that have undergone surgery are separated from their cage mate for seven days to allow for wound healing. The mass is sent to the NIH Division of Veterinary Resources (DVR) Veterinary Pathology Section for analysis. For each mass, the full report is included in the rat's pathology report described in Section III.C.2.c. If the tumor regrows or if a tumor is not identified as appropriate for removal and grows to the NIH size exclusion criteria of 4cm, the rat is euthanized and the carcass sent to the NIH Division of Veterinary Resources (DVR) Veterinary Pathology Section for analysis.

J. Housing

Rats are held in two dedicated rooms in the NIA\IRP animal facility in Baltimore MD and are managed under restricted access conditions. Only animal care staff and STARRRS investigators have access to the colony rooms on a regular basis; maintenance, pest control, regulatory oversight, etc., personnel enter as needed. Animal care staff dress in facility dedicated attire. When entering an animal room, all personnel must don a hair bonnet, face mask, sleeve protectors or disposable lab coat, and disposable gloves. There is a defined traffic pattern from the cleanest to the least clean rooms in the facility, which moves from dedicated breeding room to research animal holding rooms to experimental procedure rooms. The animal facility and program have been AAALACi accredited since 1985.

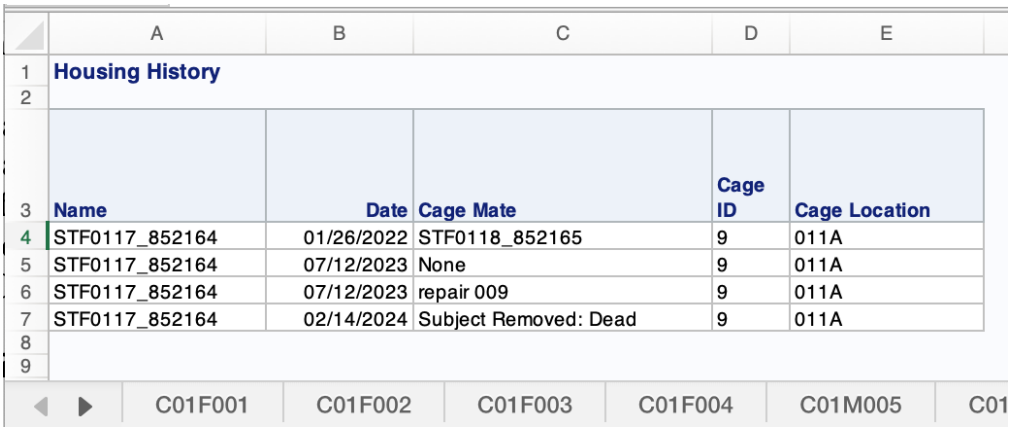
On receipt, same sex rats are housed two per cage (One Cage 2100™ Ventilated Racks & Cages, model 83164AR, Lab Products, Seaford, DE). The racks can hold 64 cages that have a cage floor area of ~210 in^2. The cages are maintained positive to the room air by a high-efficiency

particulate air (HEPA) filtered air supply and exhaust blower units (RAIR Enviro-Gard™, Lab Products) that deliver low velocity HEPA filtered air to each cage; blower units are factory preset to provide the cages with 34 to 75 air changes per hour. The cage positive pressure prevents room air from entering the cages.

Animal rooms are maintained at 72 ± 3 °F (~22 ± 1.5 °C), with relative humidity between 30-70%, and 10 to 15 room air changes per hour. Rats are housed on a 12:12 circadian light cycle; lights go on at 6:30 AM and off at 6:30 PM EST. Daily cycles of light and darkness are timed automatically by a computerized system (Siemens Corporation, Munich, Germany). Room illumination measures 800-1300 lux 3 feet off the floor.

If the cage mate of a rat dies, if possible, same sex and weighted matched rats are provided to serve as a new cage mate. Rats are not repaired once they reach 19 months of age to prevent any stressors/life event impacting final behavioral testing. The replacement rats do not participate in any of the assays. Before housing the rats together, they are placed in a new cage with a clear, perforated divider to allow visual and olfactory exchange, but not physical contact for 4 hrs. Then the divider is removed, and rats are observed for 1-2 hrs. If no fighting occurs the cage is then placed back on the housing rack.

Housing data available: Housing data is organized by cohort. That is, for each cohort housing data is found in a single Excel workbook, and data for each rat in individual tabs. Column A contains the animal ID, column B lists the date a change in housing occurred and column C lists the cage mate of the rat starting at the associated date. Column D identifies the ID of the cage, thus a change in the number represents the subject moving to a different cage. Rats are housed in one of two rooms in the animal colony, and this is identified in column E. An example housing tab for rat STF0117 is shown in the screen shot below. Note that this rat's cage mate (STF0118) died on 7/12/23 (noted as the "none" entry for a cage mate on that date) and on the same day a replacement cage mate (repair 009) was added. The rat's death is recoded as the last entry.



	A	B	C	D	E
1	**Housing History**				
2					
3	**Name**	**Date**	**Cage Mate**	**Cage ID**	**Cage Location**
4	STF0117_852164	01/26/2022	STF0118_852165	9	011A
5	STF0117_852164	07/12/2023	None	9	011A
6	STF0117_852164	07/12/2023	repair 009	9	011A
7	STF0117_852164	02/14/2024	Subject Removed: Dead	9	011A
8					
9					

C01F001 C01F002 C01F003 C01F004 C01M005 C01

K. Husbandry

Rats receive water that is reverse osmosis filtered, then hyper-chlorinated (3-4 ppm), and provided in autoclaved water bottles, ad lib. Rat cages are processed through a tunnel washer and then assembled with 1/8" corncob-based bedding (7092 Envigo, Frederick, MD) and a sterilizable 18% Protein Extruded Rodent Diet (2018SX Global, Envigo, Frederick, MD) in an overhead feed hopper, prior to being autoclaved. Water bottles are placed into the cages at the room level, working within a biological safety cabinet change station. Cages and water bottles are changed weekly.

Cage changes are performed within a biological safety cabinet. Sodium hypochlorite is used to wet gloves and surfaces that the rats may contact. Behavior equipment is sanitized by misting surfaces with disinfectant.

Plastic surfaces of behavioral equipment are misted with Clidox. Disinfectants remain on the equipment for a minimum of 15 minutes contact time. Equipment is wiped dry with a clean paper towel and returned to storage at the end of behavioral assessments.

L. Colony Health Status

STARRRS rats are housed in a larger facility that holds rats, mice, rabbits, and occasionally guinea pigs. Animals are observed twice daily by caretaker staff at the cage level or, during the 48 hr home cage activity assay, by observing them on the CleverSys® external monitors. Colony health surveillance is performed using dirty bedding transfer to sentinel animal cages. Rat sentinels are housed two animals per cage, with one sentinel cage per rack side. Sentinels and/or samples are submitted to the NIH Division of Veterinary Resources (DVR) Veterinary Pathology Section for testing. Sentinels are only tested after a minimum of one month's exposure to dirty bedding. DVR performs gross pathology and histology, ectoparasite and endoparasite examinations, and limited PCR testing. For other PCR and serology tests, DVR submits samples to a commercial laboratory.

The NIA maintains a list of acceptable viral, bacterial, and parasitic organisms (Table 1) as well as a list of excluded organisms. Acceptable organisms are not routinely tested for. Sera from sentinels is submitted quarterly to check for excluded prevalent organisms; annually, a more comprehensive serology panel is performed (Table 2). PCR testing for endo- and ectoparasites is done quarterly on fecal and pelt samples. Annually, sentinels are submitted for a complete necropsy. The complete necropsy includes pelage inspection, gastro-intestinal scrape, direct cecal exam, anal tape test, and fecal float and fecal culture. The pathologists submit any abnormal tissues for histology

Table 1. Acceptable Pathogens List

Organism
Actinobacillus sp.
Bordetella avium (hinzii)
Corynebacterium sp.
Enterococcus spp.

Escherichia coli
Segmented filamentous bacteria
Helicobacter sp.
Klebsiella oxytoca
Klebsiella pneumoniae
Moraxella morganii
*Rodentibacter pneumotropicus (*aka *Pasteurella pneumotropica)*
Proteus mirabilis
Pseudomonas aeruginosa
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus xylosus
Streptococcus sp. Beta (Group B and G) and alpha hemolytic
Trichosporon beigelii
Pneumocystis murina (mice)
Chilomastix sp.
Entamoeba muris
Trichomonas sp.
Tritrichomonas muris
Demodex sp.

Table 2. Screening Panel for Excluded Rat Pathogens

Organism	February, May, August [a b]	October [a]
Rat Serology		
Rat Parvovirus (RPV)	✓	✓
Toolan's H-1 virus (H-1)	✓	✓
Kilham rat virus (KRV)	✓	✓
Rat minute virus (RMV)	✓	✓
Parvovirus NS-1 (NS-1)	✓	✓
Rat coronavirus/ Sialodacryoadenitis virus (RCV/SDAV)	✓	✓
Rat theilovirus (RTV)	✓	✓
Pneumocystis carinii/ Rat respiratory virus (PCAR/RRV)	✓	✓
Sendai virus (SEND)	×	✓
Pneumonia virus of mice (PVM)	×	✓
Reovirus (REO)	×	✓
Mycoplasma pulmonis (MPUL)	×	✓
Lymphocytic choriomeningitis virus (LCMV)	×	✓
Mouse adenovirus (MAV)	×	✓
Rat Polyoma Virus 2 (RPyV2)	✓	✓
Rat Ectoparasites		

Myobia musculi	✓	✓
Mycoptes musculinus	✓	✓
Radfordia affinis	✓	✓
Notoedres muris	✓	✓
Radfordia ensifera	✓	✓
Polyplax serrata	×	✓
Psorergates simplex	×	✓
Polyplax spinulosa	×	✓
Rat Endoparasites		
Flagellates: *Giardia muris, Spironucleus muris, Chilomastix muris,* Trichomonads	×	✓
Entamoeba muris	×	✓
Eimeria spp.	×	✓
Ciliates	×	✓
Helminths: *Syphacia obvelata, Syphacia muris, Aspiculuris tetraptera, Hymenolepis diminuta, Hymenolepis nana*	✓	✓
Rat Pathology (exam)	×	✓

[a] *"✓" indicates that the test is conducted. "×" indicates that the test is not conducted.*
[b] *Serum, fecal and tape test samples are submitted; in October, a live animal is submitted.*

V. Detailed Methods

A. Biospecimen Collection

All biospecimens for distribution are stored in a freezer at -80ºC following collection and during shipment.

1. Fecal Collection

Each rat is placed in a non-bedded clean cage to allow for defecation. If no defecation occurs by 30 minutes, anogenital stimulation is performed to obtain 1 fecal pellet per biospecimen collection tube. Three fecal samples are collected at each of the timepoints (arrival, **EARLY**, **MIDDLE**, **OLD** and necropsy) either as direct deposits from the animal or from a clean empty housing cage. To prevent cross-contamination, either sterile individually wrapped disposable, or stainless-steel forceps replaced between animals are used to collect the feces. Stainless-steel forceps are autoclaved before reuse. One pellet, or approximately 250 mg of feces is placed into a 1.5 mL sterile plastic collection tubes (Fisherbrand™ Snap-Cap™ Flat-Top Graduated Microcentrifuge Tubes), and stored at -80 °C

2. Plasma collection

a) Blood Collection

Blood is collected at the **EARLY**, **MIDDLE**, **OLD** and Necropsy timepoints.

At the **EARLY**, **MIDDLE** and **OLD** timepoints, blood is collected from the ventral tail artery and scheduled after all other assessments. Rats are anesthetized with isoflurane and a 23G, ¾" needle attached to a plastic syringe that has been flushed with heparin, is inserted. The blood volume withdrawn is 1.5-3ml depending on body weight. The needle is removed from the syringe and the blood gently transferred into a 3 ml lithium heparin coated plastic Vacutainer Plus Blood Collection tube and placed on ice until processing.

At necropsy, blood is collected from the left heart ventricle during euthanasia using a 23 G to 21 G, 1" needle. The blood is pulled into a 10-cc plastic syringe previously flushed with heparin. The needle is removed from the syringe, and the blood is gently transferred into a 10 ml lithium heparin coated plastic Vacutainer Plus Blood Collection, 10 mL tube and placed on ice until processing

b) Plasma Extraction

Blood samples are processed within 4 hrs. of collection. The lithium heparin coated tubes of whole blood are centrifuged in a Sorvall™ ST 8R Small Benchtop Centrifuge (Thermo Fisher Scientific, Inc., Waltham, MA) at 1500G for 20 minutes at 4 °C. The plasma is removed via sterile pipette tip and transferred in 200 µL aliquots to new 1.5 mL tubes and the cell fraction discarded. At all timepoints, 3 vials of plasma are collected for each rat.

3. Necropsy

Rats are placed in an induction chamber with 5% isoflurane until recumbent, then kept under anesthesia via a nose cone. Blood is taken from the heart and processed as described in Section V.A. The rat is then decapitated, and necropsies are performed following a set order designed to minimize postmortem tissue degradation. Two technicians work together to dissect tissue from the body, and a third dissects brain subregions and collects tissues located on the head. The tissues and number of samples collected are shown in the table below. To quickly freeze the excised tissues, they are placed on aluminum foil sitting on dry ice. The frozen samples are then placed into cold 1.5mL microcentrifuge tubes and stored at -80 °C.

Tissue	Number of samples
Adrenal	2
Lung	3
Liver	3
Kidney	3
Spleen	3
White Adipose Tissue	3
Testicle or Ovaries	2
Brown Adipose Tissue	3
Gastrocnemius Muscle	3
Skin - pinna	3
Striatum	2
Frontal Cortex	1

Cerebellum	2
Hippocampus	2
Parietal cortex	2
Spinal Cord	2
Eye	2
Heart	1

A standard necropsy form, as provided below, is created for each rat noting the tissue collected and any notes and is available from the ARB. Any additional samples acquired (e.g. pituitary tumors) will be identified in the note section. The file name of the necropsy notes will include the rat ID. Example file name: STF0181_Necropsy.pdf

STARRRS Necropsy Form

STARRRS
Successful Trajectories of Aging: Reserve and Resilience in RatS

Date: _____

Rat ID#: _____

BMDS Body Temperature (oC) prior to euthanasia: _____ Body Weight (gm): _____

Time of euthanasia: _____ Time brain collected: _____ Time necropsy completed: _____

Tissues (**check those collected**)

- ☐ Collect tissues from the left side of the rat. If left side shows abnormalities, take tissues from right side, and note in the comments column. If both left & right sides are abnormal, collect from the left and make a note in the comments column.
- ☐ If any tissues are unable to be collected, make a note in the comments column (sample 3 not collected)
- ☐ Note any general abnormalities in the additional comments section below.

	Blood	
	Adrenal	
	Kidney	
	Spleen	
	White Adipose Tissue	
	Testicle or Ovaries Liver	
	Heart	
	Lung	
	Brown Adipose Tissue	
	Gastrocnemius Muscle	
	Feces	
	Skin - Pinna	
	Eye	
	Spinal Column	
	Brain -Striatum	
	Brain - Basal Forebrain	
	Brain - Frontal Cortex	
	Brain - Hippocampus	
	Brain - Parietal cortex	
	Brain - Cerebellum	

Additional Comments:

B. Biospecimen Storage and Labeling

4. Storage Containers

1) Most biospecimens are placed into 1.5 ml Fisherbrand clear microcentrifuge tubes, Cat. No 02-682-550 prior to freezing. The exception is the heart. It is placed into a 3 mL tube.
2) Storage boxes used are Fisherbrand Cat. No 03-395-464. 5.25 × 5.25 × 2 inches with a 9 × 9 insert (81 cells) or 10 × 10 insert (100 cells).

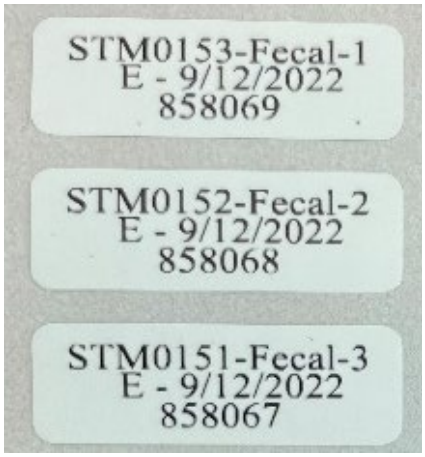
5. Labeling of Biospecimens

Three samples of plasma and feces are obtained per rat per timepoint, placed in collection tubes and stored at -80 °C.

Collection and storage containers are labeled using Polypropylene Autoclave and Liquid Nitrogen Labels with acrylic adhesive that can withstand temperatures from -80 to -70 °C. Labels are printed with a BradyPrinter i5100 600dpi Industrial Label Printer (Brady Corporation, Milwaukee, WI).

1) Top of Tube (Circular Label)
    a) Line 1 – STARRRS prefix (STM/STF)
    b) Line 2 – STARRRS Unique ID number (####)
    c) Line 3 – Sample Type & Number of repeated sampling e.g., F1/2/3 or P 1/2/3

2) Side of Tube (Rectangular Label)
    a) Line 1 – STARRRS Unique ID and Sample Type & Number of repeated sampling e.g., STX####-Fecal/Plasma-#



b) Line 2 – Time Point (A = Arrival, E = Early, M = Middle, O = Old, T = Terminal) Date – M/D/YYYY
c) Line 3 – Internal NIA Animal ID Number

Labeling for samples taken at necropsy adhere to the same format at for plasma and feces. The label abbreviations for each tissue type are listed below.

Tissue	Label abbreviation
Adrenal	ADN
Lung	LUN
Liver	LVR
Kidney	KDN
Spleen	SPL
White Adipose Tissue	WAT
Testicle or Ovaries	TE or OV
Brown Adipose Tissue	BAT
Gastrocnemius Muscle	GM
Skin - pinna	SKN
Brain - Striatum	S
Brain - Frontal Cortex	FC
Brain - Cerebellum	C
Brain - Hippocampus	HC
Brain – Parietal cortex	PR
Spinal Cord	SPC
Eye	EYE
Heart	HRT

C. Frailty Index

Assessments are conducted while the rat is in its home cage at each of the behavioral assessment time points (**EARLY**, **MIDDLE**, **OLD**) and at necropsy using a computer-based version of the scoring sheet below. Details of the scoring methods are included below. The body weight, temperature, and food intake scores are not included with in FI score calculation. Inter-rater reliability is repeatedly validated.

24-Item Index to Assess Frailty in Long Evans Rats

Rat #:_____

Date of Birth:_____

Sex: F M Rating: 0 = absent 0.5 = mild 1 = severe

				Comments:
Integument				
1. Alopecia	0	0.5	1	
2. Dermatitis	0	0.5	1	
3. Coat condition	0	0.5	1	
Physical/Musculoskeletal				
4. Tumors	0	0.5	1	
5. Distended abdomen	0	0.5	1	
6. Hunched posture	0	0.5	1	
7. Body condition score	0	0.5	1	
8. Gait disorder	0	0.5	1	
9. Tremor	0	0.5	1	
Vestibulocochlear/Auditory				
10. Hearing loss	0	0.5	1	
Ocular/Nasal				
11. Cataracts	0	0.5	1	
12. Chromodacryorrhea/porphyrin	0	0.5	1	
13. Exophthalmos	0	0.5	1	
14. Microphthalmos	0	0.5	1	
15. Corneal opacity	0	0.5	1	
Neurological				
16. Head tilt	0	0.5	1	
Digestive/Urogenital				
17. Malocclusion	0	0.5	1	
18. Diarrhea	0	0.5	1	
19. Jaundice	0	0.5	1	
20. Penile/vaginal prolapse	0	0.5	1	
21. Rectal prolapse	0	0.5	1	
Respiratory				
22. Breathing rate/depth	0	0.5	1	
Pain/Discomfort				
23. Piloerection	0	0.5	1	
24. Unusual sounds	0	0.5	1	

TOTAL SCORE = _____ TOTAL SCORE/MAX SCORE = _____

Adapted from *Journals of Gerontology: BIOLOGICAL SCIENCES,* 2017, Vol. 72, No. 7. © Susan E. Howlett, 2016.

Frailty Index Scoring Guide

These descriptions are taken from the 2019 McGill SOP in the Literature folder. However, the body condition score is based on an adaptation from the study *Use of a body condition score technique to assess health status in a rat model of polycystic kidney disease* by York *et al*. (Figure 1).

Frailty Item:	**Clinical Description**	**0 (Absent)**	**0.5 (Mild/Present)**	**1 (Severe)**
Alopecia	Hair loss due to age-related balding and/or barbering	normal fur density	<25% fur loss	>25% fur loss
Dermatitis	Inflammation, overgrooming etc causing skin erosion/ulceration	absent	focal lesions	widespread or multifocal lesions
Coat Condition	Ruffled/matted fur, ungroomed appearance	smooth, shiny coat	coat slightly ruffled	unkempt, ungroomed, matted coat
Tumors	Development of visible or palpable tumors/masses	absent	<1cc	≥1cc
Distended Abdomen	Enlarged abdomen	absent	slight bulge	abdomen clearly distended
Hunched Posture	Exaggerated outward curvature of the lower cervical column	absent	mild	clearly hunched posture
Body Condition Score	Visual signs of muscle wasting or obesity, using the BCS system	BCS of 3 (well-conditioned) or 4 (over conditioned)	BCS of 2 (under conditioned) or 5 (obese)	BCS <2 (emaciated)
Gait Disorder	Lack of coordination in movement	no abnormality	abnormal gait but animal can walk	impaired ability to move
Tremor	Involuntary shaking at rest or during movement	no tremor	slight tremor	marked tremor, animal cannot climb
Hearing Loss	Failure to respond to sudden sound (ex. clicker)	always reacts, 3/3 times	reacts 1/3 or 2/3 times	unresponsive, 0/3 times
Cataracts	Clouding of the lens of the eye, opaque spot in center of eye	no cataract	small opaque spot	opaque lens
Chromodacryorrhea/porphyrin	Porphyrin staining around eyes/nose	no staining	minimal staining	marked staining
Exophthalmos	Abnormal protrusion of the eye	normal	slight bulging	marked bulging
Microphthalmos	Abnormally small eye, sunken in appearance	normal	one/both eyes slightly small or sunken	one/both eyes very small or sunken

Corneal opacity	Cornea appears white or clouded	normal	minimal changes in cornea	marked clouding/spotting of cornea
Head tilt	Abnormal head position associated with CNS disturbance	absent	mild head tilt, slight spin	severe disequilibrium
Malocclusion	Abnormal occlusion due to uneven or overgrown incisors	mandibular longer than maxillary incisors	teeth slightly uneven	teeth very uneven and overgrown
Diarrhea	Increased frequency and decreased consistency of bowel movements, fecal smearing	normal stools	some feces or bedding near rectum	marked soft or bloody stools
Jaundice	Yellowing of the feet, nose, ears, and tail	normal	mild yellowing	marked yellowing
Penile/Vaginal Prolapse	Penis cannot reenter penile sheath/vagina or uterus protrudes through vagina and vulva	no prolapse	mild prolapse	marked prolapse
Rectal Prolapse	Protrusion of the rectum just below the tail	no prolapse	mild prolapse	marked prolapse
Breathing Rate/Depth	Difficulty breathing, pulmonary congestion, and/or rapid breathing	normal	slight change in breathing rate/depth	marked changes in breathing rate/depth
Piloerection	Involuntary bristling of the fur, particularly on back of neck	no piloerection	piloerection at base of neck only	generalized piloerection
Unusual Sounds	Acute vocalization in response to touch	no vocalizations	mild vocalizations	marked vocalizations

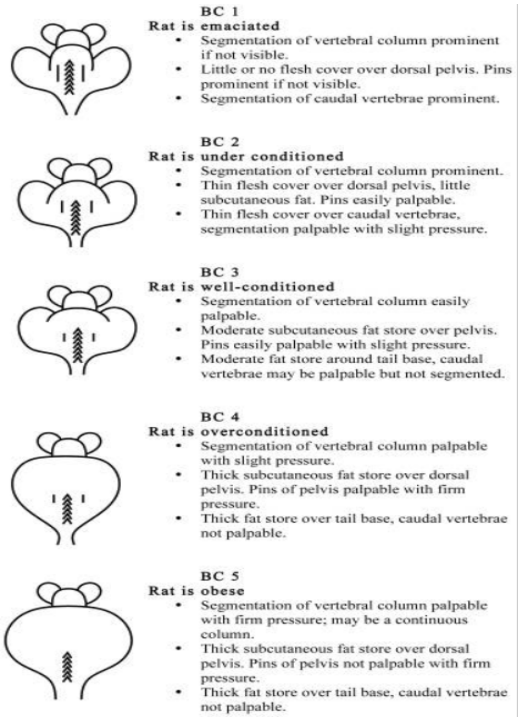


Figure 1. Body Condition Scoring for Rats (Yorke 2017)

D. Estrus Cycle

Vaginal lavage samples are obtained either between 9:30 – 11:00 AM or 12-2 PM for five consecutive days at the **MIDDLE** time point and for two consecutive days at the **OLD** time point. While the vaginal lavage procedure is only performed on females, males undergo similar handling without sampling.

Samples are collected by flushing 0.2 – 0.4 mL of 0.9% saline via disposable transfer pipette and placing a single drop onto a glass slide. Air dried slides are subsequently stained with 0.1% Crystal Violet (Certified Biological Stain, Fisher Chemical™) for 1 minute, and washed in deionized water for 1 minute twice. Slides are then viewed and photographed under a BZ-X810 All-in-One Fluorescence Microscope (Keyence, Itasca, IL) with a 10x objective and saved in a .tif format. After digital archiving, slides are discarded.

E. WM-LI

Apparatus: A white, 1.83 m diameter circular tank was filled with water (24 ± 1 °C) made opaque with white tempera paint and surrounded by a curtain affixed with large black and white geometric patterns to provide distal spatial cues. A circular array of lights directed to the ceiling provide reflected Illumination. The escape platform was 10 cm in diameter and located 1-2 cm below the surface of the water. The swim path was acquired with video tracking system software (ANY-maze; https://stoeltingco.com/Neuroscience/Anymaze/Any-maze-Video-Tracking) connected to a camera and computer.

Test Procedure:

Hidden platform trials: Rats received three training trials per day with a 40-second intertrial interval, over eight consecutive days. On each trial, rats were placed into the water facing the wall of the maze at one of four equally spaced start positions (north, south, east, or west). The start positions were varied in a pseudorandom fashion, but all rats are started from each of the locations approximately the same number of times. Rats were allowed to search until they found the submerged platform or until 90 seconds elapsed, at which time rats are guided to the platform by the experimenter. Rats remained on the platform for 20 seconds and then were placed in a holding chamber for a 20-second intertrial interval. Every sixth trial was a probe trial in which the platform was lowered to the bottom of the maze for the first 30 seconds of the trial, after which it was raised to the standard position of 1-2 cm below the water level. A learning index score was calculated for each rat based on their average proximity to the hidden platform across the last three probe trials. Lower learning index scores indicates better task performance.

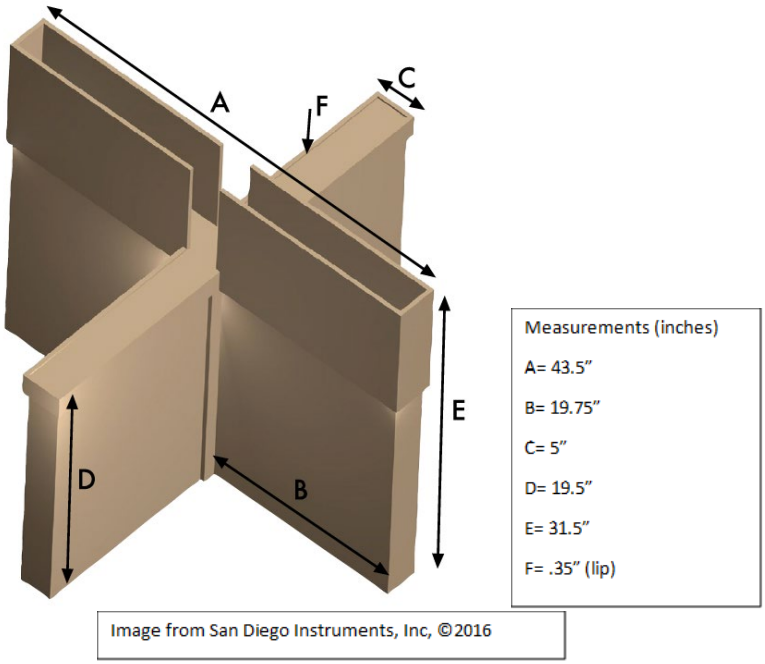
Dependent measure A Search Error measure that reflects how efficiently the animal moved from the start position to the platform was calculated. Lower scores are better. This measure (Gallagher et. al., 1993) implements a correction procedure so that trial performance is relatively unbiased by differences in distance to the goal from the various start locations and swim speed. Briefly, for each rat, the average swimming speed for each trial (path length /latency) is first calculated. Then the amount of time required to swim to the goal at that speed from the start location used on the trial was removed from the record prior to computing trial performance. Then, for every position of the rat, the distance from the platform multiplied by the time the rat stayed at that position is calculated. The Search Error measure for the hidden platform trials is the sum of all these values. The Search Error measure for the probe trials is the average of these values for the first 30 seconds of the probe test. The Learning Index score is the sum of weighted search error values for the last 3 probe trials. The units are meter·seconds.

Visible platform trials: On Day 9, after spatial memory training is complete, rats were tested for six trials on non-hippocampal dependent cue training to assess sensorimotor abilities and motivation to escape. For cue training, rats were trained to swim to a visible platform (painted black and protruding 2 cm above the water's surface). Both the start position and platform location were varied across trials, making the extra-maze cues explicitly irrelevant to the platform location. Trials were 30 s with a 20-s intertrial interval. The mean of all six trials in under 25 sec was the criterion to pass this version of the test.

F. EPM

Apparatus: A beige elevated plus maze made of ABS plastic was obtained from San Diego Instruments, Inc. (https://sandiegoinstruments.com/product/elevated-plus-maze/). The maze arm widths were modified from the manufacturer's standard maze specifications to accommodate the larger size of an aged male Long Evans rat. The maze sits on a 10.5" (height) pedestal made in-house so the arms the rat explored are approximately 30" above the floor. The testing area is surrounded by white curtains. A camera connected to a computer is affixed above the maze to

track rat exploration which is acquired with video tracking system software (ANY-maze). The room is lit with overhead fluorescent lighting (approximately 1,000 lux).



Test Procedure:

Rats are assessed at an **EARLY**, **MIDDLE**, and **OLD** timepoint. During the **EARLY** timepoint, EPM is the first assessment to ensure that rats are tested with minimal previous experience. Rats are brought into the testing room in their cage with their cage mate at least 30 minutes before testing begins. The cage is covered until testing and is not visible from the maze apparatus. At testing, rats are placed in the center of the maze facing one of the closed arms. The experimenter exits the curtained area and starts the tracking session remotely. The rats are allowed to explore the maze for five minutes while the tester is observing the computer tracking in an adjacent room. After the trial ends, the rat is returned to their cage and the maze is cleaned and air-dried for 2 minutes before the next rat is tested.

Dependent Measures:

- Total distance traveled (m): total movement by the center point of the rat
- Open arm entries: number of entries into both open arms. An entry was considered to occur once 80% percent of a rat's visibly tracked body (excluding tail) was inside an open arm. It was then considered to have remained in an arm as long as at least 20% percent of its visible body remained.
- Time spent in open arms (s)
- Closed arm entries
- Time spent in closed arms (s)
- Percent time spent in open arms: (time in open arms) / (time in open arms + time in closed arms). This measurement excludes time the animal was not considered to be in any of the four arms (i.e., in the center square).

Note that detailed ethopharmacological analysis has identified several stereotyped behaviors of rats in the plus maze related to exploration and risk assessment (Cruz 1994; Anseloni 1997). These behaviors were not reliably scored with ANY-maze and are not quantified in STARRRS. However, video files are available to end-users who wish to perform such analysis.

G. HCS

General Outline

To assess home cage activity, rats were housed individually for 48 hrs. in isolated ventilated cages (https://cleversysinc.com/CleverSysInc/csi_products/ivc-rackscan/) flanked on 3 sides by panels emitting both white and infrared light, with the 4th side in line with a camera and video recorded. Rats had ad lib access to food and water and the light/dark cycle and cages matched that of the colony room. To reduce file size, the recordings were saved as 12, 4-hour video files (i.e., 12, 6.5 GB files instead of 1, 80 GB file), and 4 camera inputs (one for each rat) are contained in a single video file. The hardware used to collect the video consisted of 4 identical cabinets, each housing 4 'boxes', with 1 rat cage and 1 camera per box.

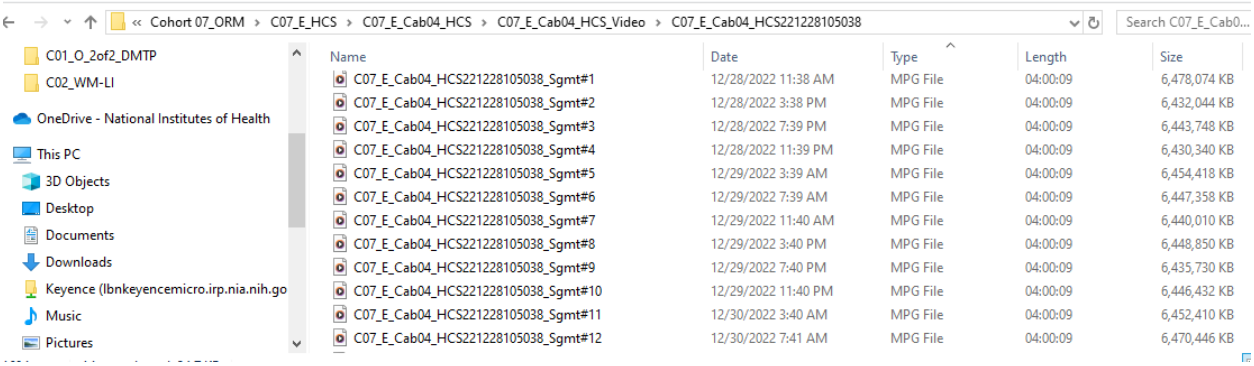
The video files are the primary data and are appropriate for manual or computer-based scoring. Although the video was collected via a system designed to automatically classify home cage behaviors (HomeCageScan 3.0, Clever Sys, Inc.; https://cleversysinc.com), we were not able to define parameters for high-throughput testing that could be held constant and yield acceptable behavioral classification accuracy across videos. Accuracy was greatly improved by individualizing image contrast and other setting adjustments, but this was not practical for large-scale application in STARRRS. However, all the associated files necessary for analyzing videos with the HomeCageScan system are available and investigators interested in using HomeCageScan should contact Clever Sys, Inc. directly. The videos are provided as .MPG files suitable for exploration in a wide variety of analytic pipelines.

Data Organization: The file hcs_summary.xlsx identifies the rat /cabinet/box relationship at each assessment. Since a single video contains simultaneous recording of 4 subjects in a 2 × 2 array, the file identifies the subject in each of the quadrants (e.g. upper left, upper right, lower left, lower right).

	A	B	C	D	E	F	G	H	I	J
1	Cohort Number	Rat ID	Sex	Cohort	Timepoint	Test Date	Notes	Cabinet	Box	Video Quadrant
2	1	STM0101	Male	Cohort 1	Middle	4/11/23		1	A	Upper Left
3	1	STM0102	Male	Cohort 1	Middle	4/11/23		1	B	Upper Right
4	1	STF0111	Female	Cohort 1	Middle	4/11/23		1	C	Lower Left
5	1	STF0112	Female	Cohort 1	Middle	4/11/23		1	D	Lower Right

Video Files: Since a single video file includes images from 4 rats, the video file name includes the cohort number, the time point, and the cabinet number. And since the 48 hrs of recording

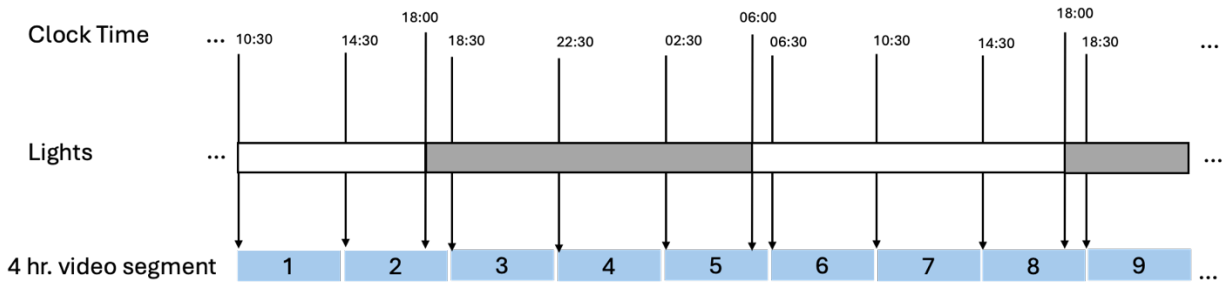
were captured in 12 separate 4hr videos, the last part of the file name includes the segment number. As shown in the screen shot below, for Cohort 7, **EARLY** timepoint, cabinet 4, the video file name also includes alphanumeric characters between the cabinet number and the segment (Sgmt) number. This is an automatically generated date number and unless using the HomeCageScan program to visualize the videos can be ignored.

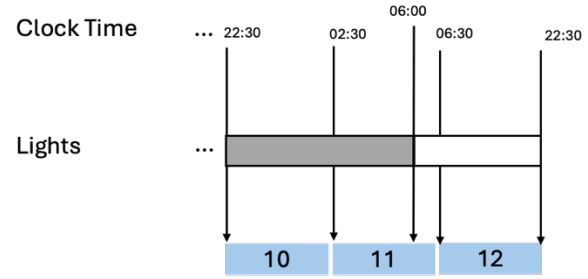


Note: File names for Cohorts 1 and 3 at the **EARLY** time period do not follow this format. The file names for these videos are recorded in the Notes column in the video key file.

Recordings started and ended around 10am and the light/dark cycle matched that in the colony, i.e., lights on at 6am and off at 6pm. Thus the 48 hrs of video contains 2 full dark cycles and one full light cycle in addition to partial light cycles the day rats entered and were removed from the recording chambers. Because recording did not start at the exact time for each test session, the most reliable method for syncing the video segment time with the time of day (clock time), is to locate in the video when the lights turn off. This will identify 6pm on the video, and start of the first 12hr dark cycle.

In the example diagramed below, recording started at 10:30. The first 4 hr. video segment ended at 14:30, and the second video segment started. From the video, the lights turned off 3.5 hrs. into the second video segment, marking 18:00 clock time. The lights turned back on 3.5hr. into the 5th video segment, marking 06:00 clock time the next day. Since different experiments will have slightly different recording clock start times, the time within the video segments where the lights change will be different than in this example.





H. WM-DMTP

Apparatus: A white, 1.83 m diameter circular tank was filled with water (24 ± 1 °C) made opaque with white tempera paint and open to room cues except for a curtain encompassing approximately ¼ of the tank that blocked the view of the experimenter and computer from the rat. A large black and white geometric cue was place on the curtain. A circular array of lights directed to the ceiling provide reflected Illumination. The swim path was acquired with video tracking system software (ANY-maze; https://stoeltingco.com/Neuroscience/Anymaze/Any-maze-Video-Tracking) connected to a camera and computer.

Test Procedure:

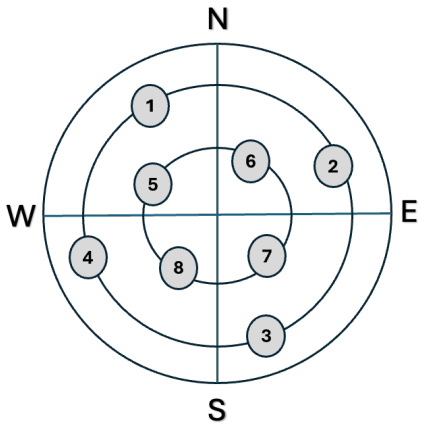
Shaping: At 2-3 months of age, rats were trained to find an escape platform submerged 1-2 cm below the water's surface in one of the eight possible locations for a total of five test days. Each training day consisted of five acquisition trials. To start a trial, rats were placed into the water facing the wall of the maze at one of four equally spaced start positions (north, south, east, or west), and the start positions are varied in a pseudorandom fashion to prevent the use of a response strategy. Rats were allowed to search until they found the platform or until 60 seconds elapsed. If the rat did not locate to platform it was guided to it by the experimenter. Rats

remained on the platform for 20 seconds and were then placed in a holding chamber for a 40-second intertrial interval. The platform and start location positions are detailed below.

Assessments: Testing was identical to that of the shaping phase, except three tests sessions were given every other day and after the fifth acquisition trial, the rat was returned to its home cage for a six-hour delay before a single retention trial was conducted. The platform and start location positions are detailed below.

Dependent measure A Search Error measure that reflects how directly the animal navigated from the start position to the platform was calculated. Lower scores are better. This measure (Gallagher et. al., 1993) implements a correction procedure so that trial performance is relatively unbiased by differences in distance to the goal from the various start locations and swim speed. Briefly, for each rat, the average swimming speed for each trial (path length / latency) is first calculated. Then the amount of time required to swim to the goal at that speed from the start location used on the trial was removed from the record prior to computing trial performance. Then, for every position of the rat, the distance from the platform multiplied by the time the rat stayed at that position is calculated. The Search Error measure is the sum of all these values. The units are meter·seconds.

Platform and Start locations:



Shaping

Day 1:
platform in 1.
Start Locations: S, N, E, W, S

Day 2
platform in 7.
Start Locations: E, W, N, S, E

Day 3

platform in 4.
Start Locations: W, S, E, N, W

Day 4
platform in 6.
Start Locations: N, W, S, E, W

Day 5
platform in 3.
Start Locations: E, W, N, S, E

Testing

Early Timepoint
Day 1: Platform 1
Acquisition Start Locations: S, N, E, W, S
Retention Trail Start Location: E

Day 2: Platform 7
Acquisition Start Locations: E, W, N, S, E
Retention Trail Start Location: N

Day 3: Platform 4
Acquisition Start Locations: W, S, E, N, W
Retention Trail Start Location: E

Middle Timepoint

Day 1: Platform 6
Acquisition Start Locations: N, W, S, E, W
Retention Trail Start Location: S

Day 2: Platform 3
Acquisition Start Locations: E, W, N, S, E
Retention Trail Start Location: N

Day 3: Platform 5
Acquisition Start Locations: W, S, E, N, W
Retention Trail Start Location: E

Old Timepoint

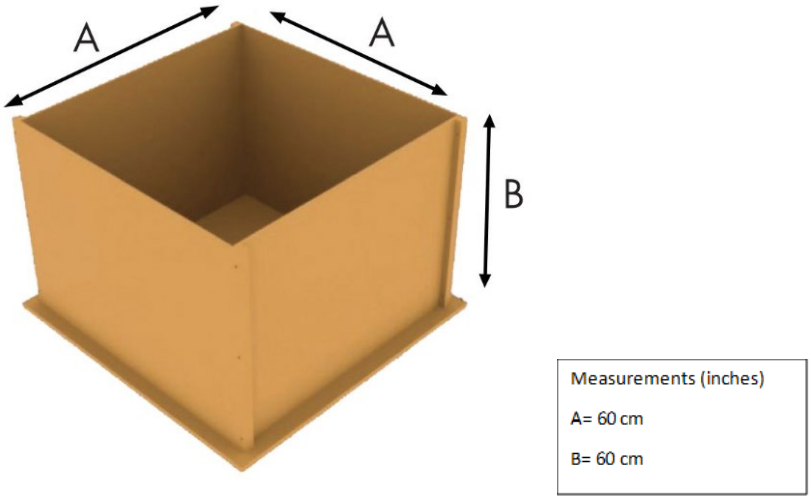
Day 1: Platform 2
Acquisition Start Locations: E, S, W, S, N
Retention Trail Start Location: W

Day 2: Platform 8
Acquisition Start Locations: W, S, N, E, S
Retention Trail Start Location: N

Day 3: Platform 1
Acquisition Start Locations: N, E, W, S, N
Retention Trail Start Location: S

I. ORM

Apparatus: The test is conducted in a custom-made arena (60 cm × 60 cm × 60 cm) constructed of beige ABS plastic (SD Instruments, Inc., San Diego, CA). A rectangular placard with vertical alternating black and white lines to facilitate spatial orientation is positioned, and the floor is lined with corncob bedding. The arena is placed in a dimly lit room with overhead lights off and red lamps on. A digital video camera (Panasonic WV-CP314) is mounted above the arena to record the animal's movements. Animal activity recorded during test sessions is digitized with TopScan Software (Version 3.00; CleverSys Inc., Reston, VA). The arena is cleaned with 70% ethanol and bedding is changed between test animals.



Test Procedure:

Rats are assessed at an **EARLY**, **MIDDLE** and **OLD** timepoint. Male rats are tested one week, and females are tested the next week at the same time of day. Males and females are tested in different arenas. All rats to be tested that day are brought into the holding area in their home cages on a cart. Cages are covered while in the holding area. Rats are placed in a holding cage prior to habituation, sample, and test sessions to ensure that their cage mates are not exposed to any odorants before their sessions.

Habituation
Habituation is three minutes per rat and is assessed across four. Signs of distress are noted, if present. The data acquired during this phase of the assessment is equivalent to open field test data.

Sample Phase
In the sample phase, odorants are presented in two identical amber glass vials (2.5 cm in diameter and 5 cm in height), fixed to the arena floor with Velcro (10 cm from the wall at the back corners). The position of the vials is constant, and both contain a small cotton plug infused with a suprathreshold concentration of the same odorant (counter-balanced across animals within groups) These two odorants were chosen based on background data that demonstrated no systematic preference across animals for one over the other during initial exposure. Different odor vials, thoroughly cleaned with 70% ethanol, are used for each rat tested. Rats are placed in the arena facing the wall opposite the vials and allowed to explore freely for 10 minutes.

Test Phase
Long-term memory is tested 24 hours later. During the memory test, one vial contains the odorant used in the sample phase, and the other holds a novel odorant (with the left/right position of the novel stimulus counter-balanced). Rats explore freely for five minutes during the memory test.

A camera connected to a computer is affixed above the arena to track rat exploration which is acquired with video tracking system software (TopScan, Version 3.00; CleverSys Inc., Reston, VA). Odor exploration is defined as a rat orienting its snout within 1 cm of one of the two stimulus vial openings.

Rats with cumulative odor exploration totaling less than 2 seconds during the memory test, or that failed to explore both odorants during the sample phase, are excluded from the long-term memory test as well as analysis.

Note: If the time spent sniffing the object exceeds 6 seconds, the tracking may be off, and the rat may not be directly sniffing the scent. Video recording is available for researchers to confirm and cross-validate automated scoring of odor-directed exploration.

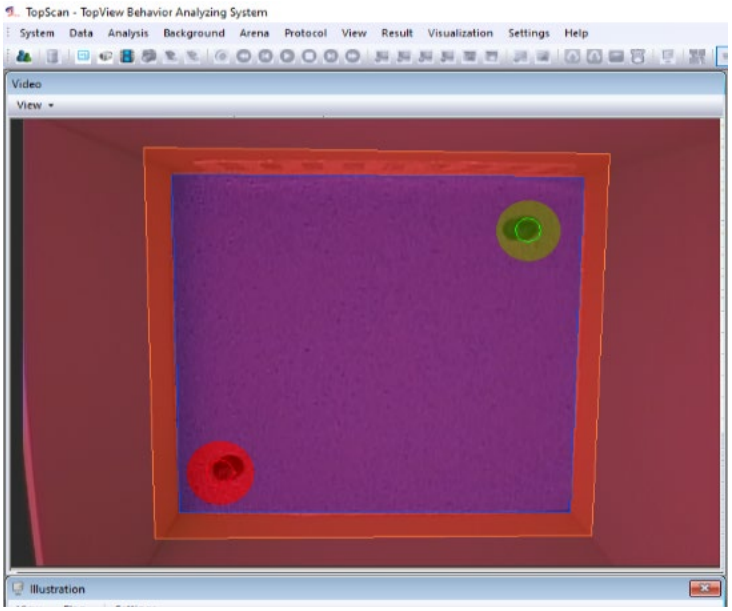
Dependent variable:
The Recognition Index Score is a measure of the time spent at the novel odor divided by the total odor exploration time (sample plus novel odor exploration). A Recognition Index Score of 0.5 indicates no odor preference. A Recognition score greater than 0.5 indicates a preference for the novel odor.

Odor Manufacturing and Dilution Information:
All extracts are manufactured by McCormick (Hunt Valley, MD) and used in the following concentrations and volumes:

1. Vanilla and Anise – 200 µL of odor, undiluted per vial
2. Orange and Lime – 400 µL of odor diluted in 2mL of deionized water, then 200 µL of this dilution per vial
3. Banana and Almond – 2mL of odor diluted in 1mL of deionized water, then 200 µL of this dilution per vial.

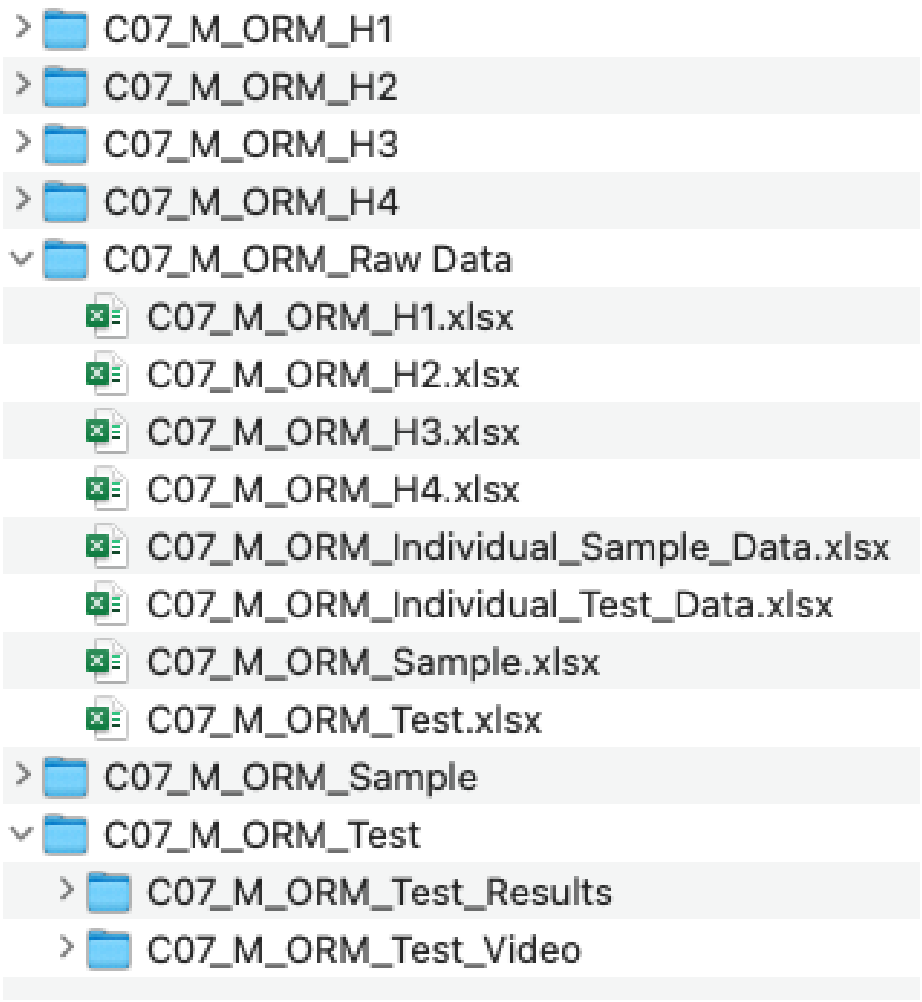
Odor Pair Information

Cohort	Early Odor Pair	Middle Odor Pair	Aged Odor Pair
3	vanilla/anise	orange/lime	banana/almond
7	orange/lime	banana/almond	vanilla/anise
11	banana/almond	vanilla/anise	orange/lime
15	vanilla/anise	banana/almond	orange/lime
19	orange/lime	vanilla/anise	banana/almond
23	banana/almond	orange/lime	vanilla/anise
27	vanilla/anise	orange/lime	banana/almond
31	orange/lime	banana/almond	vanilla/anise
35	banana/almond	vanilla/anise	orange/lime
39	vanilla/anise	banana/almond	orange/lime
41	orange/lime	vanilla/anise	banana/almond
45	banana/almond	orange/lime	vanilla/anise



Data Organization

The raw data are organized in separate folders by Cohort, per timepoint. This is useful for users wishing to conduct additional analyses by accessing the tracking data and videos from the program used to collect the data. The folder organization for the middle timepoint for cohort 7 is shown below.



The 4 habituation days (folders H1, H2, H3, H4) and the sample day and test day folders, all contain two sub folders. The Video folder contains the .mpg files for each rat and these can be used to reanalyze trials. The Results folder contains various files associated with the tracking system used to collect and analyze the data (TopScan, Clever Sys. https://cleversysinc.com/CleverSysInc/automated-behavior-analysis-systems/software/topscan/). Investigators interested in using TopScan should contact the company directly.

The Raw Data folder contains 8 separate Excel workbooks. Six for each experimental phase (H1, H2, H3, H4, Sample, Test) contain the total exploratory times during the trial. Data from these files are compiled to create the ORM summary file. The two other workbooks report individual exploratory bouts during the trial for the sample and test phase. The data for each rat is in a separate tab in the workbook. Examination of these files along with the video may be required to evaluate the consequence of an event listed as a note in the summary file.

J. 2-Choice RT

Apparatus: Testing is conducted in a 30cm wide × 32cm deep × 29cm tall operant chamber (MED-Associates part no. ENV-009A) with metal bars for floor and Plexiglas/metal walls. The chamber is located inside a 60 × 41 × 38cm sound-attenuating MDF cubicle (ENV-022MD) with fan running to circulate air and provide background white noise. The chamber contains a pellet dispenser on one wall, which delivers 45mg chocolate-flavored reward pellets high in sugar and fat (TestDiet 5TUL). On opposite wall are two illuminated nose ports located 6 cm above floor, with centers spaced 8 cm apart. The chamber is constantly lit with a dim bulb pointed towards the ceiling, which is only extinguished during "time out" periods.

Test Procedure:

Shaping: Shaping procedures are performed at three months of age under conditions of food restriction to increase food motivation and engagement in the task. Prior to shaping, rats are restricted to a target body weight of 90% of free-fed, and returned to free feeding as soon as shaping ends. A first acclimation stage consists of 20 minutes acclimation to chambers with ten food pellets pre-loaded into the food receptacle. Rats must eat all ten pellets to graduate to magazine training, in which pellets are delivered individually with random 30-90 second intervals. Each pellet delivery, here and throughout testing, is accompanied by two 50-ms clicks of white noise, separated by 50 ms and delivered through a speaker mounted inside the chamber. To graduate from magazine training rats must retrieve pellets with average latency less than 20 seconds, and accumulate at least 100 head entries into the food receptacle.

Rats then begin the 2-choice shaping task with test conditions that progressively get harder as performance improves. Each daily session is initiated by rat retrieving a free pellet present in the food receptacle. Beginning five seconds after the rat removes its head from food receptacle, one of two stimulus lights is illuminated. To complete a correct response, the rat must respond with a nose-poke to the illuminated aperture while the light is on or during a "limited hold" period after the light is extinguished. Duration of stimulus light and limited hold periods begin with easy conditions and get more challenging each day, pursuant to rat performance. See table below, modified from Bari et al 2008. A "time out" period is initiated after failure to perform a response by the end of the limited hold period ("omission"), response to the aperture that was not illuminated on that trial ("incorrect"), or responding at an aperture before the light is illuminated ("premature.") The time-out period consists of 5 seconds of darkness and constant white noise,

after which the rat must perform a head entry into the (empty) food receptacle to begin the next trial. During shaping, each session continues for 45 minutes.

Performance is calculated at the end of each session using both the full number of trials performed and the first 100 trials. If either calculation allows the rat to advance to the next stage of training (per table below), parameters are adjusted accordingly for the next day. A rat that fails to advance four days in a row is moved back to the previous stage of training. Once a rat achieves criteria at the final stage, its shaping is completed. Accuracy is defined as the ratio of correct responses to the combined total of correct and incorrect responses.

Stimulus (sec)	**Inter-trial interval (sec)**	**Limited hold (sec)**	**Criteria to graduate**
30	2	2	≥ 30 correct
20	2	2	"
10	5	5	≥ 50 correct
5	5	5	≥ 50 correct > 79.5% accuracy
2.5	5	5	≥ 50 correct >79.5% accuracy <20.5% omissions
1.75	5	5	"
1.25	5	5	"
1.0	5	5	"

Assessments: For assessments at **EARLY**, **MIDDLE**, and **OLD** age, rats are tested under *ad libitum* feeding conditions to avoid confounding effects of food restriction on the aging process. In accordance with published findings (Harrison 1997; Grottick 2002), STARRRS pilot testing found that food restriction had no effect on measures of attentional control, although it does increase omission rate.

Each day of testing consists of 100 trials or 45 minutes, whichever comes first. Testing is similar to shaping, but each day follows a specific set of parameters (table below). The first two days are "reminder" sessions with standard parameters to reorient rats to procedural demands of the task. Next rats are tested in six days of testing with progressively increasing attentional demands (via shortening stimulus durations). Next are variable interval test days, in which the interval for each trial is pseudo-randomized to either 5, 7, or 11 seconds in blocks of 9 trials. Finally, rats have a single day of "debriefing" with testing at standard conditions.

Day(s)	**Stimulus duration (sec)**	**Limited hold (sec)**	**Inter-trial interval (sec)**
1,2	1	5	5

3,4	4	2	5
5,6	1	5	5
7,8	0.2	5.8	5
9,10	1	5	5, 7, 11
11	1	5	5

Dependent variable:

Two different output worksheets are available within the data spreadsheet. The "concise" sheet gives one row per rat at each assessment. A selection of the most informative data metrics are provided as columns here, with each column representing the cumulative calculation from two consecutive days of testing with the same parameters. The "verbose" worksheet format presents one row per rat for each day of testing. Raw data is available in the form of lengthy text files, one per rat per day of testing, outputting all session variables and event timestamps.

"Concise" worksheet:

- % accuracy: (# correct responses) / (# correct responses + # incorrect responses). A correct response is a nose-poke into the aperture that was illuminated on that trial. Note that this metric is tested across three different stimulus durations.

- % premature responses: (# premature responses) / (total # trials). A premature response is a nose-poke into any aperture during the inter-trial interval, before onset of a stimulus light. These responses are never reinforced with food pellets. Note that this is tested across three different inter-trial interval durations.

- % omissions: (# omission trials) / (total # trials). An omission trial is one in which a stimulus light is illuminated, but the rat does not perform a nose-poke response into either the correct or incorrect aperture within a 6-second response window starting at the onset of the light. The value reported here comes from the 1.0 second stimulus duration test days, which are moderately challenging.

- perseveration rate: (# perseverative responses) / (total # trials) * 100. A perseverative response is defined as nose-poke responses into the correct aperture after the rat had already performed a correct response and earned a food pellet reward, which it had not yet collected. The value reported here comes from the 1.0 second stimulus duration test days.

- stimulus response latency: The time from onset of stimulus light until a nose-poke response is detected into the aperture. Data is only collected for correct response

trials. The median response latency for each day is calculated, and these values are then averaged across all days of testing at 1.0 second stimulus duration.

- food retrieval latency: The time from delivery of food pellet into the receptacle until the animal performs a head-entry into the receptacle. The median latency is calculated for each day, and these values are then averaged across all days of testing at 1.0 second stimulus duration.

"Verbose" worksheet:

- Number of trials with correct, incorrect, premature, and omission responses
- Number of correct responses on trials with left and right apertures illuminated
- Median food retrieval and stimulus response latencies on trials with left and right apertures illuminated.
- (For variable interval test days): The length of each interval used. For each of the three interval types: total number of trials performed and number of trials with correct, incorrect, premature, and omission responses.
- Number of head entries into the food receptacle.
- Trial-by-trial data on several metrics, with each trial separated by a semicolon:
    - Outcome of each trial. C=correct, I=incorrect, P=premature, O=omission.
    - Location of stimulus light. L=left aperture, R=right aperture
    - Stimulus response latencies. A value of 0 is reported for trials with premature or omission responses.
    - Food retrieval latencies. A value of 0 is reported for trials in which a food pellet reward was not delivered.

Data organization: Raw data files are .txt dumps of all variable arrays at the end of each test session per rat. Arrays were defined in the custom-written MPC script file executing the behavioral task. Array and variable definitions may be found in the file "Appendix - script variable definitions.txt"

Notes to end-users: Some rats show poor engagement with the task at assessments, as assessed by high rates of omission trials. This is particularly prominent in male rats and worsens with age. Investigators are advised to use caution in these trials and filter data as they see fit (for example, excluding data from rats with >90% omission rate).

During the early-life shaping sessions, many rats do not learn the task to full criterion. We have not found training performance to be a significant predictor of future performance, provided animals make it past 5.0 second stimulus duration criterion. Rats that failed to make it to this checkpoint during early life shaping are flagged in the "Notes" column of the "concise" worksheet.

VI. MRI Details

Longitudinal neuroimaging studies include 3D T_2-weighted high resolution whole brain imaging for volumetric analysis, diffusion tensor imaging (DTI) to evaluate white matter tract integrity, and functional MR imaging (fMRI) appropriate for functional connectivity analysis.

MRI studies are performed on anesthetized rats using a Bruker BioSpec 94/20 USR scanner equipped with a Bruker 9.4T/20 cm magnet, a 12 cm actively shielded gradient/shim coil, an 86 mm diameter quadrature radiofrequency (RF) transmit body coil and a four-channel rat head RF receive-only array coil (Bruker BioSpec Inc., Billerica, MA). MRI data are acquired using Bruker ParaVision 360 software.

A. Initial Anesthesia and Preparation Outside Magnet Room

After weighing, to induce anesthesia, rats are placed into a box supplied with a mixture of isoflurane in oxygen. The isoflurane vaporizer is set at 3% for induction and adjusted to 1.5 – 2.0% as soon as the rat loses consciousness. Administration of isoflurane anesthesia is continued by nose cone as a small patch is shaved on the upper back and disinfected with 70% isopropanol. A PE20 polyethylene catheter (Stoelting Co., Wood Dale, IL) loaded with the alpha-2 adrenergic agonist dexmedetomidine (pharmaceutical grade) diluted in normal saline is inserted subcutaneously through a tunnel created with a 20-gauge needle. Rats are given a bolus dose of 0.015 mg/kg and the time of this injection is noted. The catheter is secured to the skin with surgical tape, the rat is transferred to the magnet room and the dexmedetomidine syringe is mounted into a remote-controlled MRI-compatible syringe pump (Harvard Apparatus, Holliston, MA) set to deliver a dose of 0.015 mg/kg/hr. This dose is maintained continuously throughout the remainder of the MRI study until fMRI scans are complete, regardless of changes in isoflurane concentration.

B. Magnet Room Setup

The rat is placed on an animal bed equipped with a nose cone, ear bars and incisor bar for head fixation (RAPID MRI International, Columbus, OH) and gas anesthesia delivery is resumed with isoflurane in a 30% O_2:70% N_2 gas mixture. The bed is fitted with a pneumatic respiration sensor connected to a MRI-compatible physiological monitoring system (SA Instruments, Stony Brook, NY) and the rat's respiratory effort is continuously monitored. A sterile ocular lubricant (Puralube, Valley Vet Supply, Marysville, KS) is applied to the rat's eyes. The incisor bar and nose cone are adjusted and ear bars are inserted to immobilize the head. The head array coil is centered at the level of the ear canal and fixed to the animal bed. A fiber optic temperature sensor (SA Instruments) is inserted into a disposable, pre-lubricated sheath and the sheathed sensor is inserted into the rat's rectum and taped to its tail. Finally, a MRI-compatible pulse oximetry sensor (SA Instruments) is attached to the rat's hind paw. The animal's core temperature, pulse signal, pulse rate and SpO_2 are continuously monitored and recorded together with the respiratory effort signal and respiration rate at the MRI operator's station. Air from a remote blower passes through a MRI-compatible heater (SA Instruments) and blows over the rat to maintain a core temperature of 37.0° C with feedback control from the rectal temperature

sensor. During the above preparations, the isoflurane dose is maintained at 2% or as needed to maintain a respiration rate of 40 breaths per minute. The bed bearing the rat is inserted into the MRI magnet and the body transmit coil is tuned to the nominal proton frequency of 400.1 MHz. A tri-axial localizer scan is acquired to ensure that the receive array coil is centered on the brain in the rostral-caudal direction and that the brain and coil are positioned at the center of the magnet. If the array coil needs to be repositioned relative to the brain, the animal bed is pulled out of the magnet and adjustments are made. If the rat and head coil need to be centered in the magnet, the operator slides the bed into or out of the magnet by a few millimeters as needed. Should the array coil be repositioned or the animal bed position adjusted more than 1 cm, then tuning is re-optimized and the localizer scan is repeated with all pre-scan auto-adjustments.

C. Fast Spin Echo (RARE) Anatomical Imaging

Once a final position has been set, the isoflurane dose is adjusted to maintain a respiration rate of 60 breaths per minute. In all subsequent scans, radio frequency pulse timings, isoflurane dose and all vital signs are recorded from the physiological monitoring system and scanner console using a PowerLab data acquisition system (AD Instruments, Colorado Springs, CO). Two-dimensional fat-suppressed multi-slice fast spin echo (RARE) images are acquired in the sagittal, axial, and coronal planes to precisely identify the orientation of the brain in the magnet. Scan parameters include field of view (FOV) 30 × 30 mm, matrix size (MTX) 256 × 256, in-plane resolution 117 × 117 microns, echo spacing 9 ms, echo train length (RARE factor) 8, effective echo time TE_{eff} = 36 ms, repetition time TR = 4 s, acquisition bandwidth 50 kHz and one signal average. For sagittal, coronal (i.e., body-coronal or brain-axial), and axial (i.e. body axial or brain-coronal) scans, 35, 14 and 35 slices are acquired with thickness 0.7 mm, 0.7 mm and 0.6 mm, respectively. Each of these three scans requires 2 minutes 8 seconds to acquire. In sagittal and coronal scans, the readout direction is set to rostral-caudal while it is set to left-right in the axial scan. Slice orientation and position for the sagittal scan is set by referring to the most recent triaxial pilot scan. After the sagittal scan is complete, if pronounced ghosting is observed in the anterior-posterior (phase-encoded) direction, then the animal may be removed from the magnet and the tooth and ear bars adjusted for better restraint. Otherwise, the sagittal images are used to set the orientation and position of the coronal slices, lining up the most ventral coronal slice with the ventral surface of the brain. After the sagittal and coronal scans are complete, these images are used to set the orientation and position of the axial slices, lining up these slices to be perpendicular to the ventral surface of the brain and to the midline, as observed in the sagittal and coronal views, respectively. The axial slice package is adjusted in the rostral-caudal direction so that the anterior commissure, as shown in the midline sagittal image, is contained in one slice.

D. Whole-brain Shimming

Based on the 2D RARE images, a single-voxel PRESS (point-resolved spectroscopy) scan without water suppression is set up with a rectangular voxel (ca. 14 × 10 × 19 mm) inscribed within the brain and an ellipsoidal shim volume enclosing the whole brain excluding the

olfactory lobes and cerebellum. Other parameters for the PRESS scan include TE = 20 ms, TR = 2.5 s, acquisition bandwidth 7937 Hz and number of sampled points 2048. A B_0 field map scan is set up with reference to the most recent tri-axial pilot scan using parameters FOV 40 × 40 × 40 mm, MTX 64 × 64 × 64, coronal rostral-caudal slice orientation, first echo time 1.644 ms, echo spacing 3.568 ms, acquisition bandwidth 119 kHz, flip angle 30° and TR = 20 ms. Acquisition of the B_0 field map scan requires 1 minute 22 seconds. The whole-brain PRESS scan is then acquired with automatic map-based shimming (MAPSHIM) over the ellipsoidal shim volume based on the B_0 field map, followed by localized iterative correction of X, Y and Z shims. The full width at half height (FWHM) of the water peak in the resulting whole-brain spectrum is measured and recorded for quality control; a typical value is 30 Hz. If the measured FWHM greatly exceeds this value, then a new B_0 field map and PRESS scan are acquired, adjusting the shim volume and MAPSHIM parameters as needed to achieve the smallest linewidth possible.

E. Structural T_2-weighted 3D Imaging of the Whole Brain

A 3D T_2-weighted scan is performed to facilitate precise measurement of regional and ventricular volumes. This scan utilizes the same slice angulation and offsets as the 2D axial RARE scan acquired previously. Data are acquired with a fat-suppressed 3D RARE pulse sequence with a field of view of 30 × 30 × 30 mm and parameters TR = 1 s, echo spacing 4.971 ms, RARE factor 25, effective echo time TE_{eff} = 64.62 ms, acquisition bandwidth 100 kHz and a single signal average. To suppress alias artifacts arising from out-of-volume signal, coronal and axial saturation slices are applied ventral and dorsal or rostral and caudal to the brain, respectively. The orientation and thickness of these saturation slices are set by the operator in reference to the tri-axial pilot scan and 2D RARE scans described above. A quick check for image quality, especially the absence of ghost artifacts overlapping the brain, is performed by acquiring a scan with a matrix size of 100 × 100 × 100, resulting in a 300 μm^3 isotropic resolution. This scan takes 6 minutes 40 seconds. If ghosting is observed in the ventral-dorsal direction, then the scan is re-acquired with the read direction changed from left-right to ventral-dorsal. Once adequate image quality is obtained, data are acquired with a matrix size of 150 × 150 × 150 pixels, resulting in an isotropic resolution of 200 μm^3. The duration for this high-resolution 3D RARE scan is 15 minutes.

F. Resting-state Functional Imaging (fMRI) Scans

After a minimum of one hour since the initial dexmedetomidine bolus injection, acquisition of fMRI data may begin. By this time, the isoflurane dose will typically have been reduced to 0.5-0.6% to maintain a respiration rate of 60 breaths per minute. Single-shot gradient echo EPI scans are acquired with FOV 30 × 30 mm, MTX 80 × 80, in-plane pixel size 375 × 375 μm, slice thickness 0.6 mm, 35 slices, TE = 20 ms, TR = 1.5 s and acquisition bandwidth 200 kHz. A gradient echo EPI scan is acquired with forward k-space encoding order to check for correct slice positioning and the absence of major artifacts. Once adequate image quality has been confirmed, two EPI scans with reverse k-space encoding order and 300 repetitions each are acquired to obtain fMRI data. These are followed by two scans with forward phase encoding. Each scan

requires 7 minutes 30 seconds. The images resulting from each scan are immediately analyzed using a custom script in FSL software (FMRIB Analysis Group, Oxford, UK) to check for the presence of bilateral principal component pixel clusters. If these are not observed, then the fMRI scan is repeated, and the images are analyzed until the desired principal components are observed.

G. Diffusion Tensor Imaging (DTI) of the Whole Brain

DTI data are acquired for mapping white matter tracts using a fat-suppressed, respiratory-gated, diffusion-weighted spin-echo EPI sequence with geometry copied from the axial RARE scan acquired at the beginning of the study. Diffusion parameters include gradient duration $\delta = 2.5$ ms, gradient separation $\Delta = 8.5$ ms, diffusion weighting $b = 650$ s/mm^2 and 30 non-colinear gradient directions plus five scans acquired with minimum diffusion weighting (b_0 images). As in the 2D axial RARE scan, the slice thickness is set to 0.6 mm. In-plane geometry parameters include FOV 30 × 30 mm, MTX 80 × 80 and pixel size 375 × 375 μm. Other acquisition parameters include TR = 3 s, TE = 20.5 ms, acquisition bandwidth 341 kHz and one signal average. For the EPI readout, k-space is sampled in two segments. Scan duration for DTI imaging is 3 min 30 s. Following acquisition, Bruker ParaVision software automatically calculates maps of fractional anisotropy, mean diffusivity and other DTI parameters, which are checked for the presence of major white matter tracts (e.g., corpus callosum) in the expected anatomical locations.

H. Recovery

After MRI scanning, the animal bed is withdrawn from the magnet and the pulse oximetry and fiber optic temperature sensors are removed. The head coil is removed, and the rat is released from the ear bars, incisor bar and nose cone. The dexmedetomidine catheter is removed, the rat is immediately moved to a preheated incubator supplied with humidified oxygen and a reversal agent (0.1-1.0 mg/kg Antisedan) is injected subcutaneously to accelerate recovery. The rat is monitored visually and is kept in the incubator until it moves spontaneously and is steady on its feet.

If the rat's vital signs indicate distress during a scan (irregular or excessively slow breathing, abnormal pulse rate, low SpO_2 or abnormally low body temperature), the animal is immediately removed from the magnet, the reversal agent is injected, and the rat is allowed to recover as described above. A rescan is attempted 1 to 2 weeks later if this occurred before the halfway point of the study.

I. Data Export

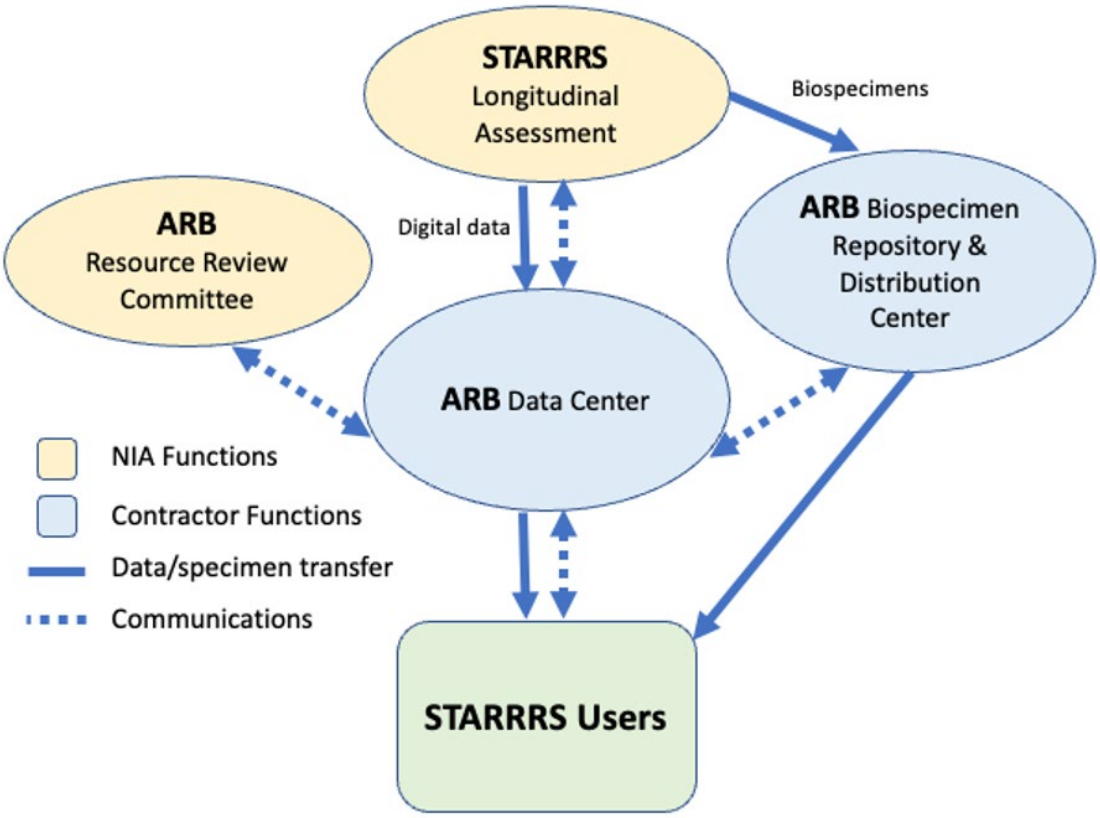
Following scanning, data from the axial 2D RARE, 3D RARE, fMRI and DTI scans are exported to enhanced DICOM files (one file per scan) and NIFTI files (one file per image) using ParaVision. In addition, for each scan, data are presented as a header-less 32-bit signed integer "rawdata.job0" raw data file and 16-bit signed integer "2dseq" reconstructed image file. Finally, data are also presented as a file hierarchy in the BIDS format containing images in NIFTI format.

Physiological data recorded during each MRI study (respiratory effort and rate, pulse oximeter waveform, pulse rate, SpO2, rectal temperature and EtCO2) are stored in an AD Instruments LabChart proprietary format (.adicht file) which may be read by LabChart 8 (licensed software) or LabChart Reader (free, but no save or export capability). Also included in the .adicht file is a synchronous record of isoflurane dose, actual oxygen concentration in the anesthesia gas and MRI pulse sequence markers. These markers can be used to identify portions of the physiological record that correspond to each MRI scan, e.g. a fMRI experiment.

VII. Data and Biospecimen inventory and request.

Biospecimens and data are generated by the NIA STARRRS project investigative staff. Users gain access to these biospecimens and data via the Aging Research Biobank (ARB) (https://agingresearchbiobank.nia.nih.gov/). The ARB:

- Receives, stores and distributes biospecimens and data from the STARRRS project.
- Maintains a website for researcher interaction.
- Receives, routes and tracks requests from investigators through the website.
- Send biospecimen requests to the ARB Resource Review Committee.



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