Resting state fMRI based identification of brain networks associated with behavioral traits in mice with human APOE2 alleles

Daniel Jin, Ali Mahzarnia, Jacques Stout, Hae Sol Moon, Robert J. Anderson, Jessica Tremblay, Zay Yar Han, Alexandra Badea

Pratt REU for Meeting the Grand Challenges

Abstract

The human APOE alleles have been associated with genetic risk for Alzheimer's disease and cardiovascular disease[1]. APOE2 is considered protective, APOE3 is the control, and APOE4 is associated with increased vulnerability[1]. Here, we study APOE2 targeted replacement mice where the mouse APOE gene has been replaced with the human APOE2 gene. These mouse models have been understudied in comparison to their APOE3 and APOE4 counterparts and may present with increased resilience to environmental stressors such as a high fat diet. In this project, we investigate behavior traits related to learning and memory in APOE2 mice and how they change with sex (male vs female), age (12 months vs 18 months), and diet (control vs high fat diet), as well as which brain regions and networks are involved in these behaviors. We acquired behavior metrics on 15 APOE2 mice of different sexes, ages, and diets via a fear conditioning experiment that tests the learning, memory, and anxiety of the mice. Structural MRI and resting state multi-echo functional MRI images of the mice were acquired on a 7T Bruker 70/20 using a T2* EPI protocol. Images were processed using ANTsX[2,3], Convert3D[4], FSL[5], ITK-SNAP[6,7], tedana[8], and RABIES[9] to obtain functional connectivity matrices (connectomes) from the mice. After obtaining functional connectivity matrices and behavior data, we performed signal search via vertex screening[11] and sparse canonical correlation analysis[12] over the connectomes and behavior metrics to identify several brain regions and networks that are involved in memory function and learning in APOE2 mice.

Methods

15 APOE2 mice that vary in sex and diet were subject to the fear conditioning experiment at either 12 or 18 months of age. Mice on the high fat diet treatment were switched to Research Diets D12451i for 4 months between either 9-12 months or 15-18 months of age.

Fear conditioning experiment methods:

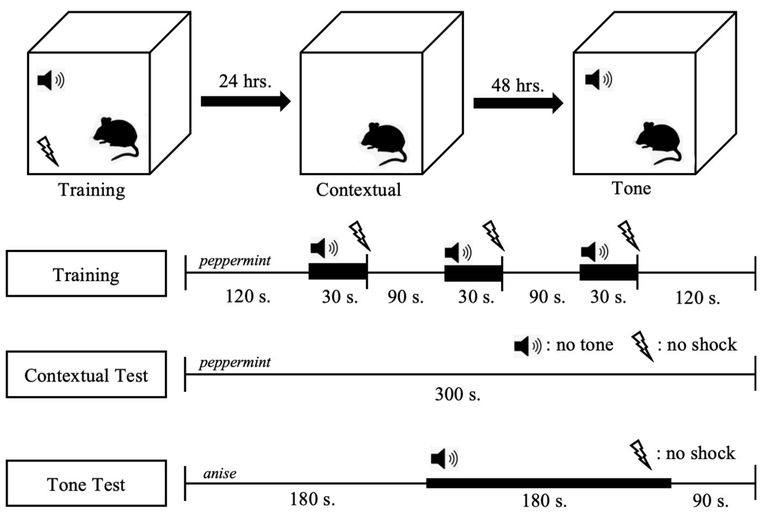
Day 0 (learning): Place the mice in a fear conditioning chamber with a peppermint odor contextual cue (1 peppermint to 10 mineral oil dilution). After 2 min, present a conditioned stimulus (72-dB 12-kHz tone) for 30 sec followed by a 2 sec 0.5 mAmp scrambled foot-shock. After 90 sec, repeat the tone-shock pairing and do this 2 more times. Record the freezing time of the mice (complete lack of movement for >5 sec, with the exception for respiration) when the tone (conditioned stimulus) is played.

→ Day 1 (contextual test): 24 hrs later, return the mice to the conditioning chamber (100 lx light and the peppermint odor contextual cue) for 5 min and record the freezing time of the mice.

→ Day 2 (tone test): 24 hrs later, place the mice in a new environment with a different enclosure, tactile interface (VFC-005A), and odorant (anise). Record the freezing time of the mice mice during 3 min of acclimation (pre-tone component), 3 min during which the conditioned stimulus is played (tone component), and 90 sec of exploration (post-tone component).

→ For day 0, perform a repeated measure multi-factor ANOVA to test for the significance of age, sex, treatment, and time to percent freezing time. For day 1, perform a multi-factor ANOVA to test for the significance of age, sex, and treatment to percent freezing time. For day 2, perform a multi-factor ANOVA to test for the significance of age, sex, and treatment to percent freezing time for the pre-tone, tone, and post-tone components.

→ Determine the learning rate of the mice in day 0 by computing the slope of the line of best-fit for the percent freezing time over time data of the mice. Extract the day 0 learning rates, the day 1 contextual percent freezing times, and the day 2 percent freezing times during the tone as the behavior metrics of the mice.



Methods

fMRI processing methods:

Acquire structural MRI and resting-state multi-echo functional MRI images of the mice on a 7T Bruker 70/20 with a volume RF coil and a 4 channel surface receiver array. Use a T2* EPI protocol with 3 echoes beginning at TE=5 ms and spaced 14.315 ms apart, TR=2252 ms, flip angle=60 degrees, over a field of view of 19.2 x 15 x 9.6 mm, matrix=64 x 50 x 32, and reconstructed at 300 um isotropic resolution. Acquire 650 volumes in 17 minutes.

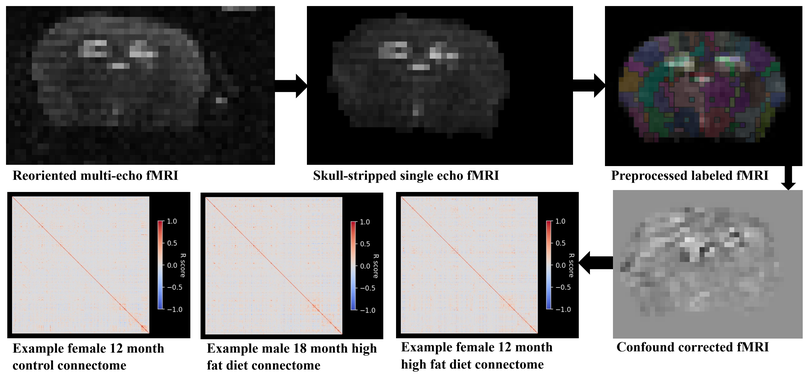
→ Convert the acquired MRI image files from BRUKER to NIfTI and reorient all the images to the RAS coordinate system using ANTsX[2,3], Convert3D[4], and FSL[5].

→ Create masks of the brains in the fMRI images using ITK-SNAP[6,7]. Use tedana[8] to skull strip the brains with the masks and optimally combine the different echoes of the fMRI images.

→ Use RABIES[9] to perform the following preprocessing steps on the fMRI images: inhomogeneity correction of the structural and functional images, head motion correction, registration between the functional and structural images, and registration of the structural images and functional images to the DSURQE mouse template[10].

→ Use RABIES[9] to perform the following confound correction steps: model and remove the confound effects of head motion and the white matter, vascular, and cerebral spinal fluid signals using ordinary least squares (OLS) linear regression and apply a 0.3 mm smoothing filter.

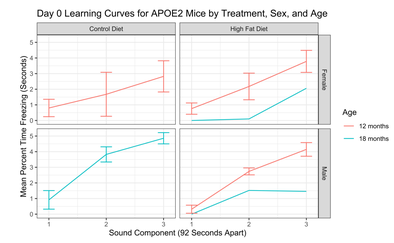
→ Use RABIES[9] to obtain whole brain functional connectivity matrices (functional connectomes) of the mice that provide the cross-correlation (Pearson's r) values between the activation timeseries of brain regions in the mice.



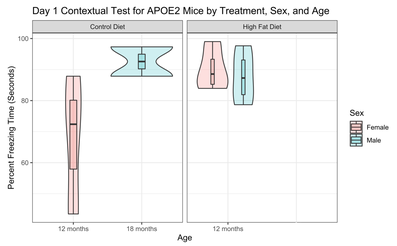
Signal search via vertex screening[11]: Find the distance correlations between X^i, the connectivity of one brain region to all other brain regions in the mice, and Y^i, the behavior metrics of the mice, for each of j brain regions. This results in one distance correlation value per brain region. Rank the brain regions by distance correlation values and remove the bottom 5%. Repeat until you can no longer remove 5% of the remaining regions. For each of the removals, store the maximum correlation value and the associated brain regions. Find the maximum of the maximum correlation values acquired in the previous step and look at the set of regions associated with that maximum. These regions are the most correlated with the behavior metrics.

Sparse canonical correlation analysis[12]: Vectorize the lower triangle of the connectomes and vectorize the behavior metrics of the mice to get X and Y, which are the matrices of the connectome vectors and behavior metric vectors respectively. Find the sparse canonical variables u and v such that the correlation between the linear projections of the connectome vectors and the behavior metric vectors, Cor(Xu, Yv), is maximized. The canonical variables indicate which connectivities (networks) on average are important to the behavioral metrics.

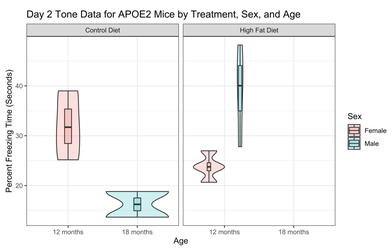
Results



RMANOVA showed that all animals can learn as there is an effect of time ($F(1,24)=87.58$, $p<0.001$) and an effect of the interaction of age, treatment, and time ($F(1,24)=6.14$, $p=0.02$) on day 0 learning.



ANOVA showed that there is an effect of the interaction of age and sex ($F(1,9)=6.19$, $p=0.03$) on freezing during the day 1 contextual test.



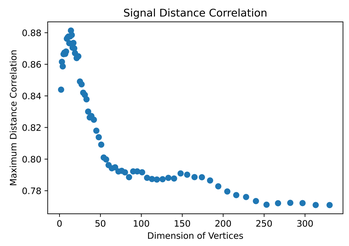
ANOVA showed that there is an effect of age ($F(1,9)=15.61$, $p=0.003$) and an effect of sex ($F(1,9)=6.64$, $p=0.03$) on freezing during the day 2 tone.

Mice behavioral metrics

Sex	Diet	Age (Months)	Day 0 Learning Rate	Day 1 Contextual Percent Freezing	Day 2 Tone Percent Freezing
female	HFD	1.48E+01	1.445	91.42	2.70E+01
female	HFD	14.8	1.33	85.69	23.77
female	HFD	14.8	1.195	83.93	23.73
female	HFD	12.2666667	2.075	99.02	20.66
male	HFD	14.8	2.375	97.66	42.7
male	HFD	14.4	1.65	91.56	37.37
male	HFD	14.4	1.975	78.67	48.2
male	HFD	14.4	1.655	82.99	27.79
male	Control	21.2333333	2.45	97.32	18.81
male	Control	21.2333333	1.495	87.86	13.66
male	HFD	20.2333333	0.73	88.77	18.25
female	HFD	20.2333333	1.03	53.77	17.49
female	Control	13.4	0.735	43.46	25.17
female	Control	13.1666667	1.47	87.86	31.71
female	Control	13.1666667	0.83	72.38	39

Results

Signal search via vertex screening: regions correlated with behavioral metrics

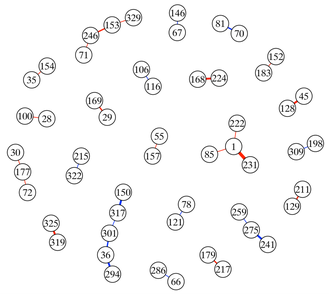


Region Name	Distance Correlation
Cingulate cortex: area 29a left	0.881358609
colliculus: superior right	0.829737455
Cingulate cortex: area 29b left	0.820826213
simple lobule (lobule 6) right	0.819575001
corpus callosum left	0.818370753
CA1Or left	0.798654973
CA1Rad left	0.795103581
colliculus: inferior right	0.784503547
Cingulate cortex: area 32 right	0.781748811
colliculus: superior left	0.775121739
Intermediate nucleus of the endopiriform claustrum right	0.773323128
lobules 4-5: culmen (ventral and dorsal) left	0.771928347
Frontal association cortex left	0.759752133
Lateral orbital cortex right	0.740518829

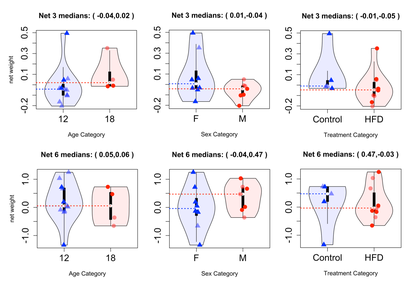
Sparse canonical correlation analysis: networks associated with behavioral metrics

Network Number	Region Numbers in RABIES	Region Names	Total Weight
1	85, 222, 231, 1	crus 2: ansiform lobule (lobule 7) left, lobule 9: uvula left, crus 2 white matter left, nucleus interpositus left	-1.281
2	100, 28	colliculus: superior left, lobule 3 white matter left	-0.1286
3	169, 29	Cingulate cortex: area 29b left, lobules 4-5: culmen (ventral and dorsal) left	-0.4321
4	177, 30, 72	simple lobule (lobule 6) right, Cingulate cortex: area 29b right, Lateral parietal association cortex right	-0.1856
5	154, 35	Ventral tenia tecta right, lobule 10: nodulus left	-0.0614
6	294, 301, 317, 36, 150	CA2Rad left, CA3Rad left, Olfactory bulb: mitral cell layer right, Cingulate cortex: area 32 right, Ventral nucleus of the endopiriform claustrum right	1.43638
7	128, 45	Primary visual cortex: binocular area right, Caudomedial entorhinal cortex right	-0.5548
8	157, 55	cuneate nucleus left, Cortex-amygdala transition zones right	-0.031
9	286, 66	LMol left, Frontal association cortex right	0.08236
10	146, 67	ventral tegmental decussation, Intermediate nucleus of the endopiriform claustrum right	0.12104
11	81, 70	Posteromedial cortical amygdaloid area right, Insular region: not subdivided right	0.22985
12	246, 329, 71, 153	Frontal association cortex left, Accessory olfactory bulb: granule cell layer left, Lateral orbital cortex right, Ventral orbital cortex right	-0.5875
13	121, 78	pre-para subiculum left, Medial parietal association cortex right	0.0501
14	116, 106	fasciculus retroflexus left, anterior commissure: pars anterior right	0.07331
15	211, 129	Caudomedial entorhinal cortex left, lobule 9 white matter left	-0.245
16	183, 152	flocculus (FL) left, Ventral intermediate entorhinal cortex right	-0.0808
17	224, 168	simple lobule white matter right, Cingulate cortex: area 29a left	-0.469
18	217, 179	lobules 4-5 white matter left, crus 2: ansiform lobule (lobule 7) right	-0.2911
19	309, 198	PoDG left, superior olivary complex left	0.19424
20	322, 215	Anterior olfactory nucleus right, lobule 1-2 white matter left	0.2089
21	275, 241, 259	Primary visual cortex: monocular area left, Dorsolateral entorhinal cortex left, Perirhinal cortex left	0.67072
22	325, 319	Olfactory bulb: mitral cell layer left, Olfactory bulb: granule cell layer right	-0.6441

Networks associated with behavioral metrics



Connectivity of networks 3 and 6 based on age, sex, and diet



Conclusions

- From our fear conditioning analysis, we have shown that APOE2 mice of all sexes, ages, and diets are able to learn (associate stimulus with response), and that groups of mice based on sex, age, and diet are not significantly different in this aspect. After learning, we can see that the females are more vulnerable to the high fat diet than the males during the tone test (cued fear conditioning task) but not during the contextual test (contextual fear conditioning task).
- We have established protocols for acquiring BOLD MRI data in 12-20 months old mice.
- We have established protocols for processing resting state fMRI images and acquiring functional connectomes from mice.
- We have used two methods to identify differences in the connectivity between mice of different ages, sexes, and diets based on behaviors related to memory and learning using vertex screening and sparse canonical correlation.
- Future efforts will concentrate on a quantitative comparison and validation of the above connectome analysis methods using predictive modeling approaches. We also plan to expand the number of mice in our samples and including other APOE alleles to better understand their role in aging and Alzheimer's disease.

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