SUPPLEMENTARY DATA

Extracellular Space Barrier Dysfunction Disrupts Interstitial Fluid Drainage and Is Associated with Memory Heterogeneity in Cognitive Aging

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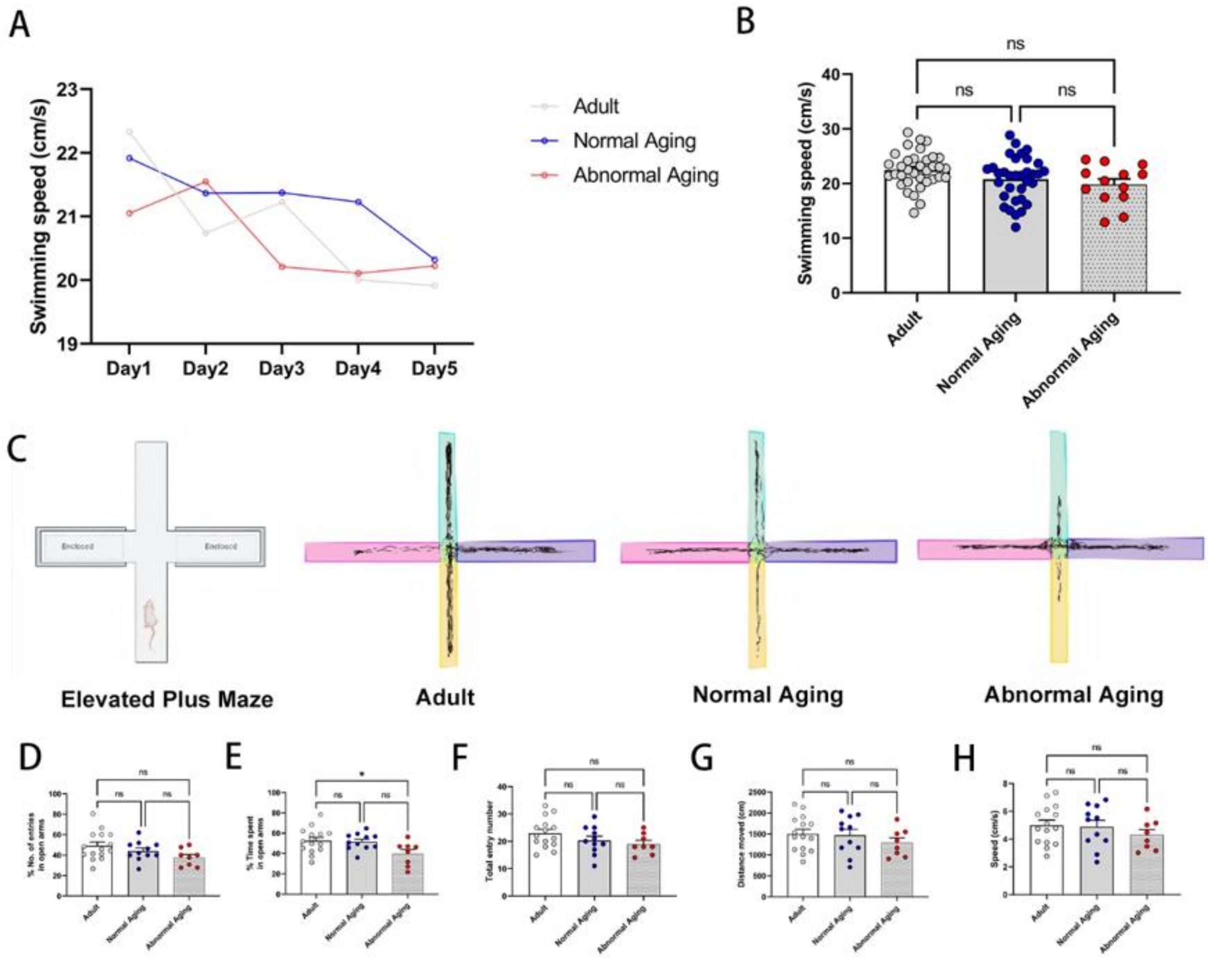
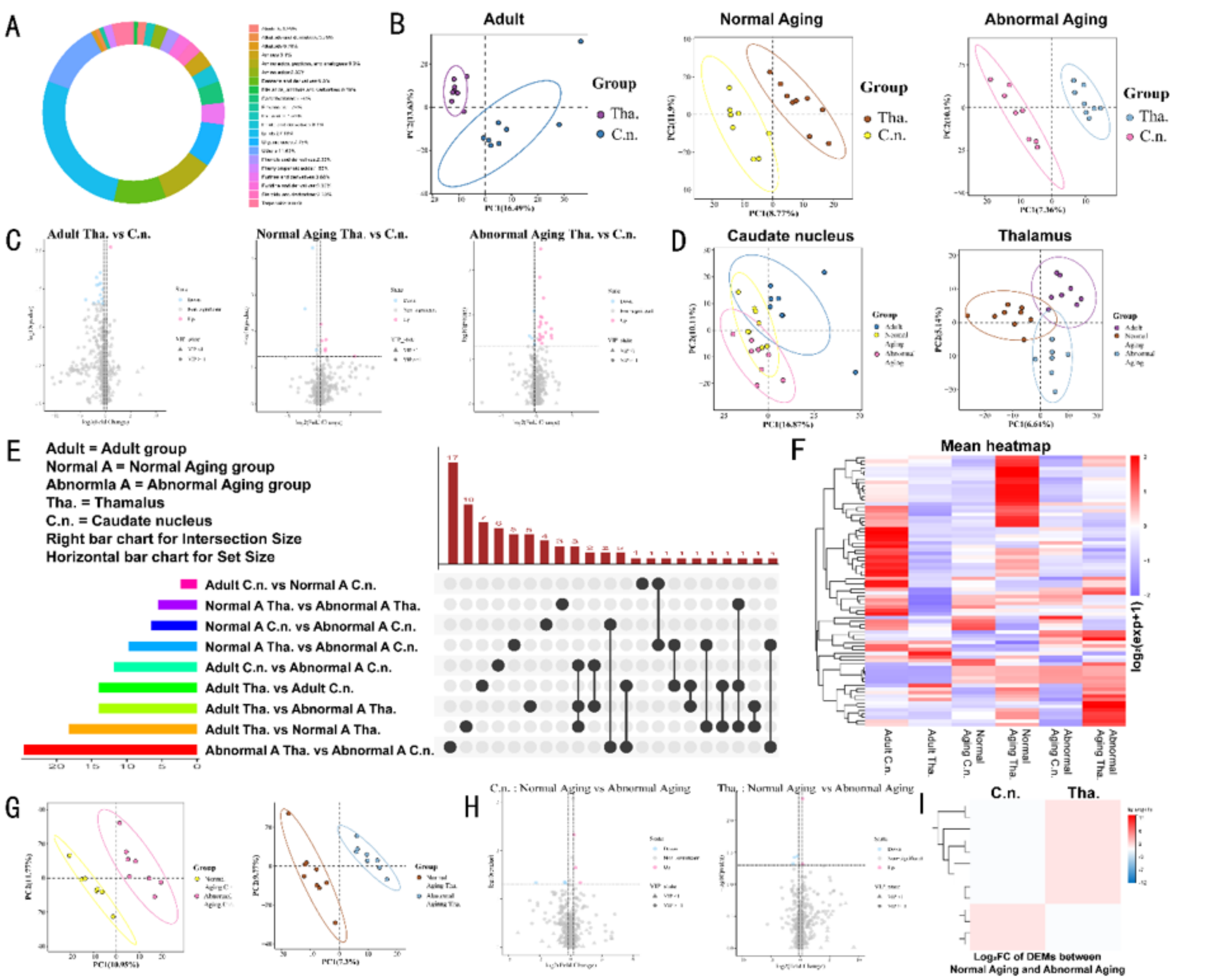


Supplementary Figure 1. **Abnormal Aging rats exhibit mild emotional alterations. (A)** Quantification of swimming speed during the Morris water maze acquisition phase revealed no significant differences among all groups. **(B)** Quantification of swimming speed during the probe trial also showed no significant group differences. **(C)** Representative movement trajectories in the elevated plus maze test across groups. **(D-H)** Quantitative analysis demonstrated a significant reduction in the percentage of time spent in the open arms (E) in Abnormal Aging rats, with no significant differences in open arm entry frequency (D), total arm entries (F), total moving distance (G), or movement speed (H) across groups. N = 8–14 rats per group for the elevated plus maze test. Each scatter point in the graphs represents data from an individual rat (independent biological replicate), and data are presented as Mean ± SEM. One-way ANOVA with Tukey's post hoc test was applied within three groups comparison. ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$; ns, no significant difference ($P > 0.05$).



Supplementary Figure 2. **Abnormal Aging rats showed a disrupted distribution of extracellular content. (A)** Chemical composition classification of the brain interstitial fluid (ISF) metabolome, showing the distribution of major metabolite classes (including lipids, amino acids, nucleotides, carbohydrates, and bioactive compounds) across all samples. **(B)** Partial Least Squares Discriminant Analysis (PLS-DA) score plot of all ISF metabolome samples, demonstrating a clearer separation of samples by brain region (thalamus, Tha. vs. caudate nucleus, C.n.) across the Adult, compared with Normal Aging, and Abnormal Aging groups. **(C)** Volcano plots highlighting differentially expressed metabolites (DEMs) between the thalamus and caudate nucleus in each group, with screening criteria: $|log_2FC| \geq 1$, $P < 0.05$, variable importance in projection (VIP) ≥ 1. **(D)** PLS-DA score plots of ISF metabolome samples stratified by brain region (thalamus and caudate nucleus), showing distinct separation between the Adult and Abnormal Aging groups. **(E)** UpSet plot illustrating the overlap of differentially expressed metabolite sets across key group comparisons. **(F)** Heatmap of metabolites between different groups and brain regions. **(G)** PLS-DA score plot of ISF metabolome samples comparing the Normal Aging and Abnormal Aging groups, confirming distinct metabolic profiles between the two aging subgroups. **(H)** Volcano plot of differentially expressed metabolites between the Normal Aging and Abnormal Aging groups ($|log_2FC| \geq 1$, $P < 0.05$, VIP ≥ 1). **(I)** Heatmap of regional different metabolites between Normal Aging and Abnormal Aging. N = 8–9 rats per group. Each scatter point in the PLS-DA graphs represents data from an individual rat.

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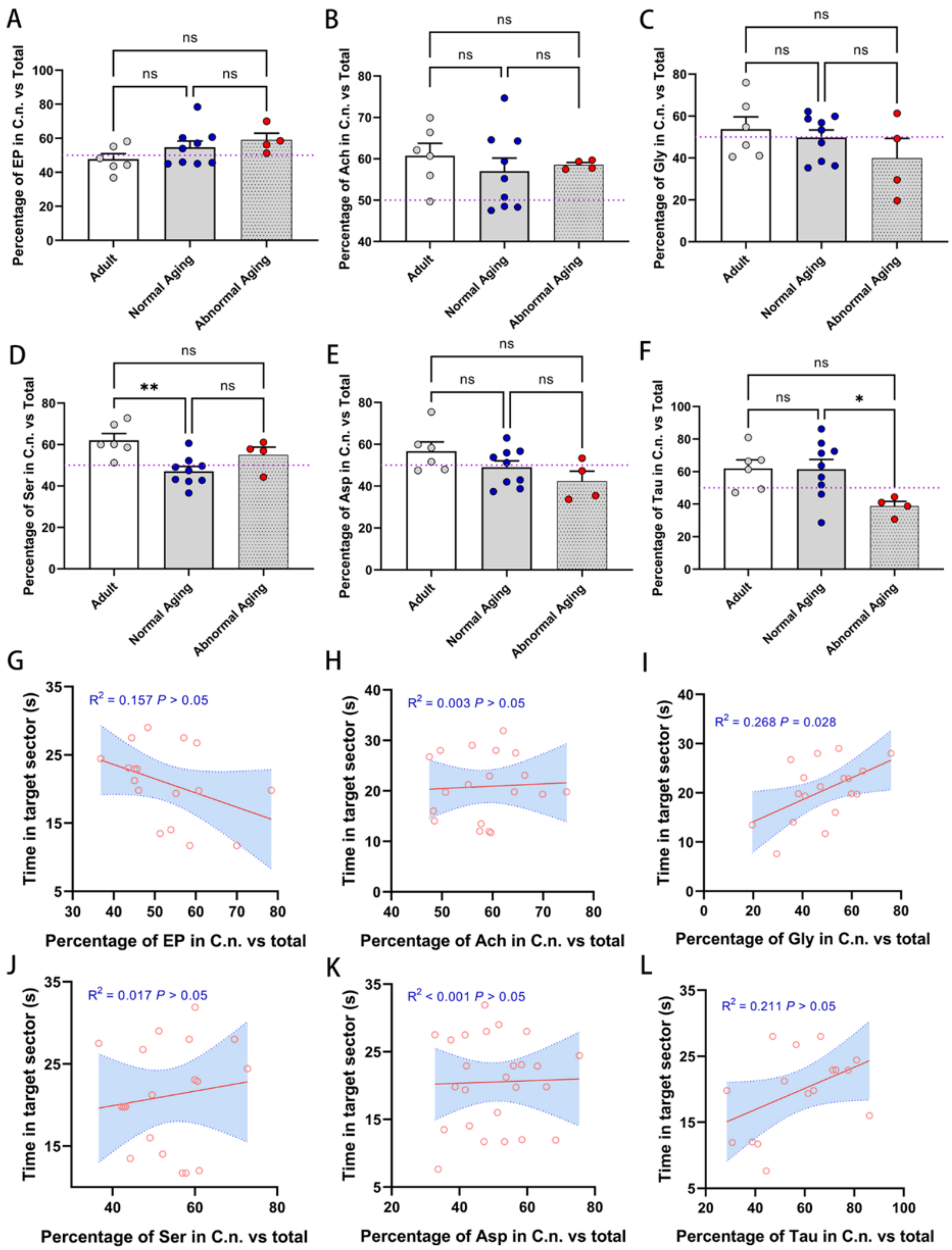
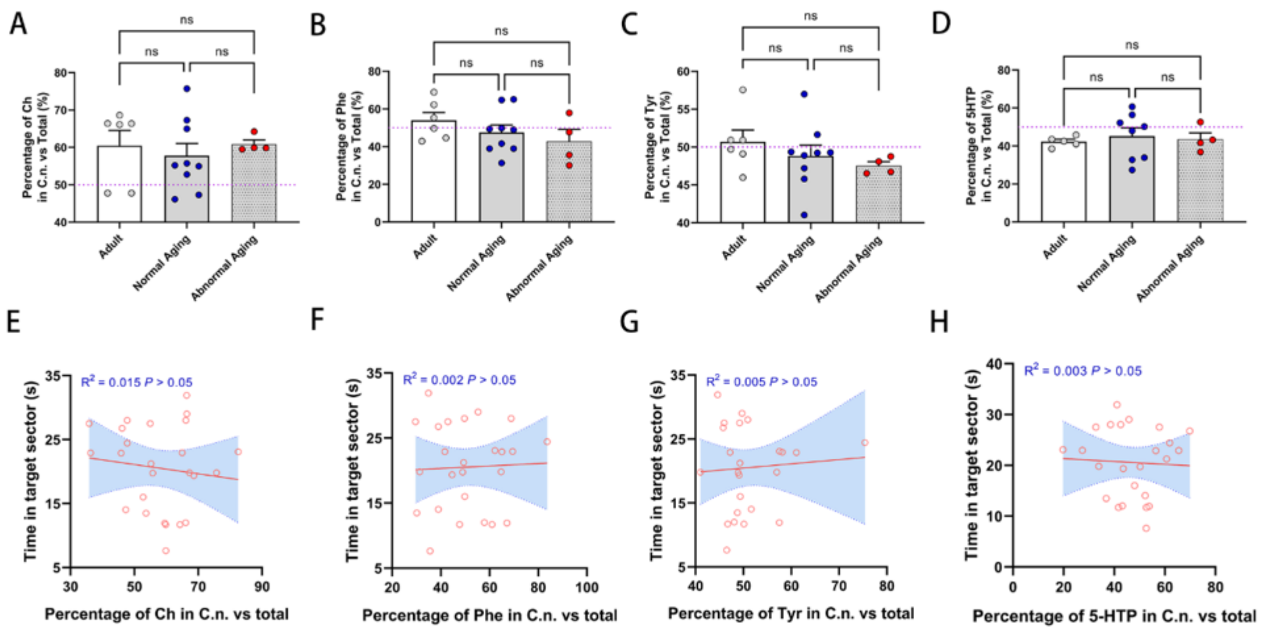


Supplementary Figure 3. **Concentration of neurotransmitter and neuromodulators and its correction to cognition. (A–F)** The difference of regional distribution ratio of neurotransmitter and neuromodulator between the Adult, Normal Aging, and Abnormal Aging groups. N = 4–9 rats per group; each scatter point represents an independent biological replicate. Comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. Data are presented as Mean ± SEM. Significance markers: ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *P

< 0.05; ns, no significant difference (*P* > 0.05). **(G–L)** Correlation analyses between the regional distribution ratio of neurotransmitters/neuromodulators and the time spent in the target quadrant during the Morris water maze probe test. Correlations were evaluated using general linear regression, with Pearson or Spearman correlation performed based on data distribution.



Supplementary Figure 4. **Concentration of precursors and its correction to cognition. (A–D)** The difference of regional distribution ratio of precursors between the Adult, Normal Aging, and Abnormal Aging groups. N = 4–9 rats per group; each scatter point represents an independent biological replicate. Comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. Data are presented as Mean ± SEM. Significance markers: ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$; ns, no significant difference ($P > 0.05$). **(E–H)** Correlation analyses between the regional distribution ratio of precursors and the time spent in the target quadrant during the Morris water maze probe test. Correlations were evaluated using general linear regression, with Pearson or Spearman correlation performed based on data distribution.

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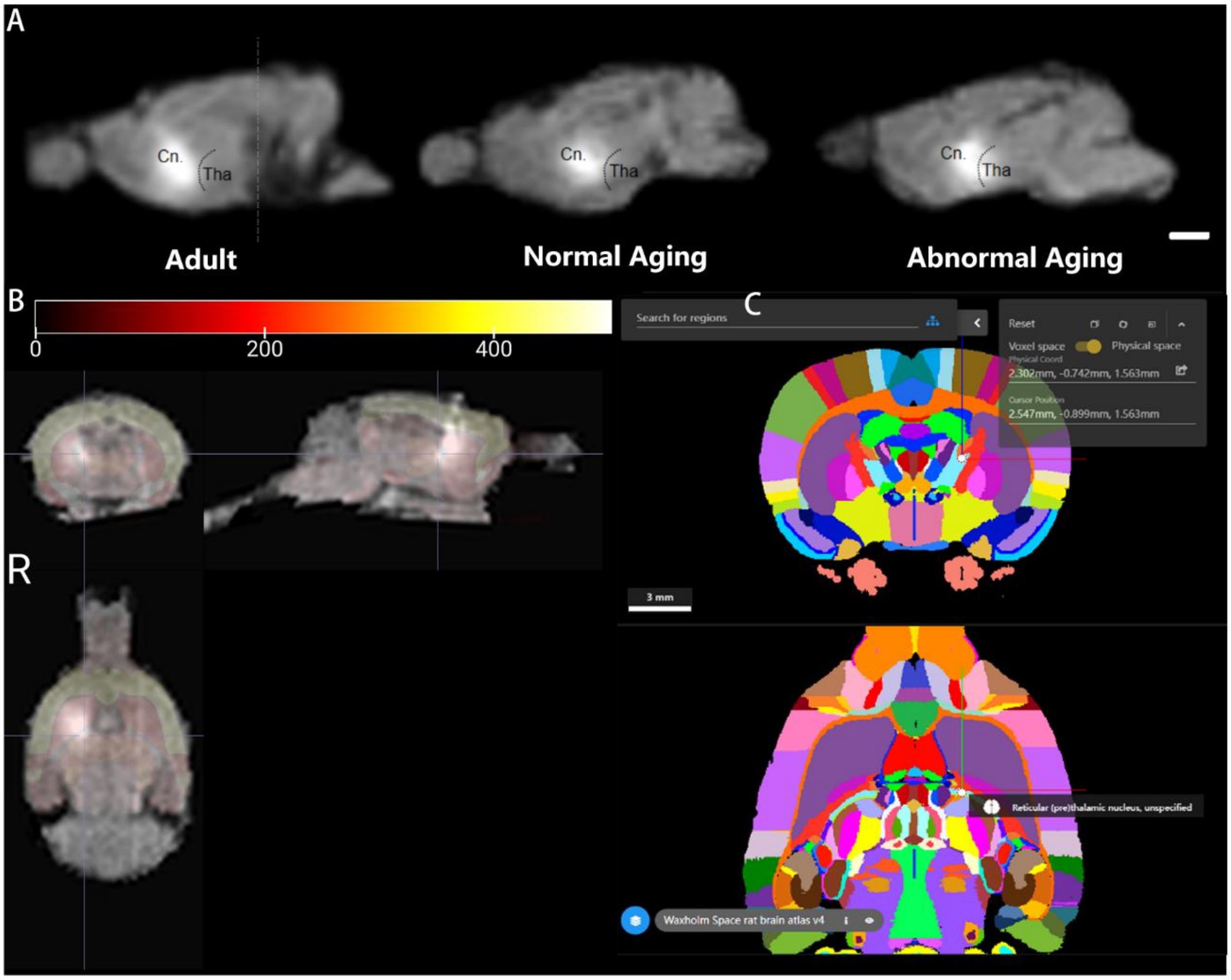

Supplementary Figure 5. **Spontaneous reflux of ISF from C.n. to thalamic reticular nucleus in abnormal aging rats. (A)** Representative image of tracer max diffusion area in axial view of 90-min post-injection revealed a spontaneous reflux of ISF in an abnormal aging rat brain. Scale bar = 5 mm. **(B)** Standardized into the Paxinos and Watson space using DARTEL. **(C)** The edge of diffusion area to thalamic reticular nucleus. Cn., caudate nucleus; i.c., internal capsule; Tha, thalamus.

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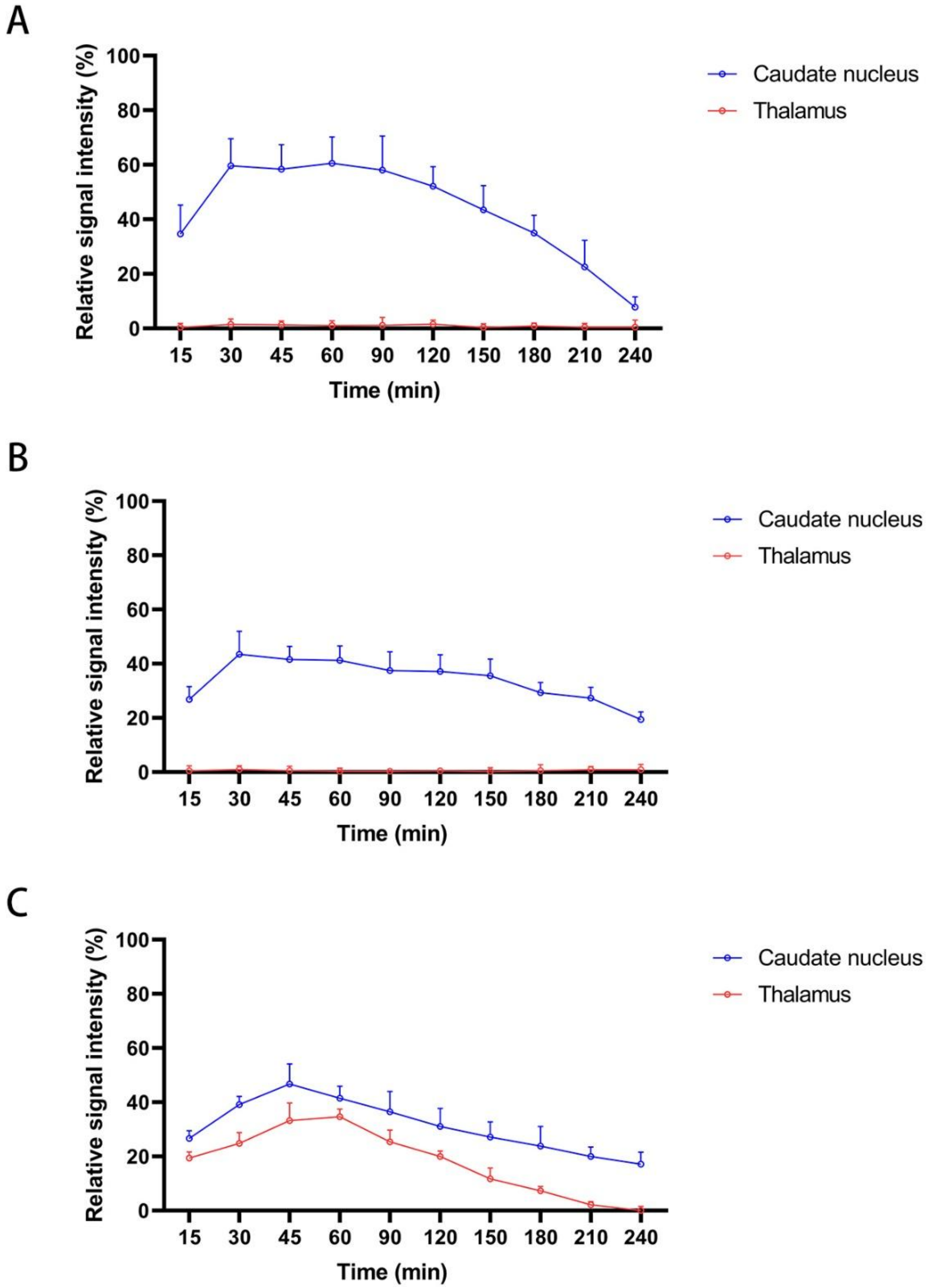

Supplementary Figure 6. **Time-course changes of tracer signal intensity. (A-C)** Tracer signal dynamics in the caudate nucleusand thalamus across Adult (A), Normal Aging (B), and Abnormal Aging (C) rats over a 240-minute observation period. Data are presented as Mean ± SEM.

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Supplementary Tables

Supplementary Table 1. Differential Neuro-metabolites in ISF of Distinct Brain Regions (Tha vs. Cn).

Name	logP	log2FC	VIP	State
Adult				
Ethylbenzene	2.049	1.049	1.761	Up
Methylenediurea;	1.315	-2.302	1.396	Down
2-Mercaptoethanol	1.422	-0.991	1.696	Down
Methylbutylnitrosamine	1.558	-1.294	1.365	Down
Diethylcarbamazine	1.359	-3.907	1.110	Down
N-isopropyl-N'-(2-oxoazepan-3-yl)urea	1.551	-1.688	1.533	Down
Phenethyl isocyanate	1.333	-1.353	1.211	Down
Ropivacaine	1.713	-0.993	1.665	Down
N-butylphenylnitrone	1.650	-1.731	1.148	Down
Ulinastatin	1.575	-0.938	1.428	Down
Aldicarb	1.371	-0.661	1.385	Down
2H-Benzo(a)quinolizin-2-ol	1.494	-0.555	1.497	Down
NP-008563	1.312	-0.529	1.463	Down
Normal Aging				
4-Aminobenzoic acid	1.510	1.019	1.966	Up
N'1-2,2-dimethylpropanohydrazide	2.186	0.288	2.625	Up
2,4-Quinolinediol	1.305	5.625	1.156	Up
4-Hydroxynonanal	1.762	1.093	1.475	Up
10-hydroxydecanoate	1.459	0.997	1.393	Up
4-(Dodecylamino)Phenol	1.733	0.409	2.309	Up
Amipaque	1.480	-0.430	2.400	Down
Pentylenetetrazole	2.613	-2.211	2.514	Down
Ropivacaine	4.290	-1.003	3.332	Down
Abnormal Aging				
Carbamoyl cholesterol	1.564	0.457	2.122	Up
Calcium salicylate;	1.816	1.389	2.166	Up
Triethylamine N-oxide	1.510	0.570	2.144	Up
1-Hydroxyvitamin D5	1.535	2.097	1.742	Up
Arachidoyl Ethanolamide	1.784	0.607	2.385	Up
Melamine	2.374	1.000	2.494	Up
N,N-Diisopropylethylamine (DIPEA)	1.723	0.414	2.436	Up
10-hydroxydecanoate	1.684	2.100	1.972	Up
Tris(2-chloroethyl) phosphate	1.500	0.933	2.013	Up
N-Methylolpentamethylmelamine	1.601	1.313	2.161	Up
p-Menthan-3-ol	1.841	0.578	2.481	Up

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Supplementary Table 1. (Continued) Differential Neuro-metabolites in ISF of Distinct Brain Regions (Tha vs. Cn).

Name	logP	log2FC	VIP	State
Ethylbenzene	1.936	0.801	2.323	Up
N-(3-oxo-hexadecanoyl)-homoserine lactone	1.356	0.769	1.555	Up
3,4-Dihydroxyphenylpropionic acid	2.836	0.480	2.966	Up
Chem A*	1.957	0.615	2.439	Up
Octyl 4-methoxycinnamic acid	3.470	0.607	3.110	Up
Chem B*	1.431	0.344	2.174	Up
trihydroxybutyrophenone	1.444	0.554	2.301	Up
Epoxypheophorbide a	1.473	2.162	1.575	Up
Methyl alpha-D-galactopyranoside	1.471	-0.620	2.026	Down
3,3'-Diaminobenzidine	1.524	-0.965	2.293	Down
Ulinastatin	1.341	-0.272	2.007	Down
LMPR0103030001	2.082	-0.293	2.494	Down

Note: Chem A* stands for (8aR,12S,12aR)-12-hydroxy-4-methyl-4,5,6,7,8,8a,12,12a-octahydro-2H-3-benzoxecine-2,9(1H)-dione; Chem B* stands for ethyl 3-amino-1-(6-(4-methylpiperidino)pyridazin-3-yl)-1H-pyrazole-4-carboxylate.

Supplementary Table 2. Differential Neuro-metabolites in Cerebral ISF Under Distinct Cognitive Aging Statuses (Normal vs. Abnormal)

Name	logP	log2FC	VIP	State
Caudate nucleus				
Carbamoyl cholesterol	1.650	0.459	2.135	Up
Octyl 4-methoxycinnamic acid	2.332	0.307	2.631	Up
Tetradecylamine	1.340	0.909	2.345	Up
1-Hydroxypyrrole-2,5-diol	1.329	-0.515	1.954	Down
2-Aminooxybenzoic acid	1.352	-0.576	2.040	Down
5-Phenyl-3-tetrahydro-1H-pyrrol-1-ylisothiazole-4-carbonitrile	1.341	-3.338	1.241	Down
Thalamus				
NP-018711	1.320	0.370	1.882	Up
Aldicarb	2.342	0.367	2.639	Up
Dimethyl sulfone	1.423	-1.031	1.688	Down
Methylimidazoleacetic acid	1.325	-1.266	1.581	Down
Arachidoyl Ethanolamide	1.445	-0.480	1.901	Down