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Supplementary Information for

Seasonal plasticity in the adult somatosensory cortex

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Supplementary Information

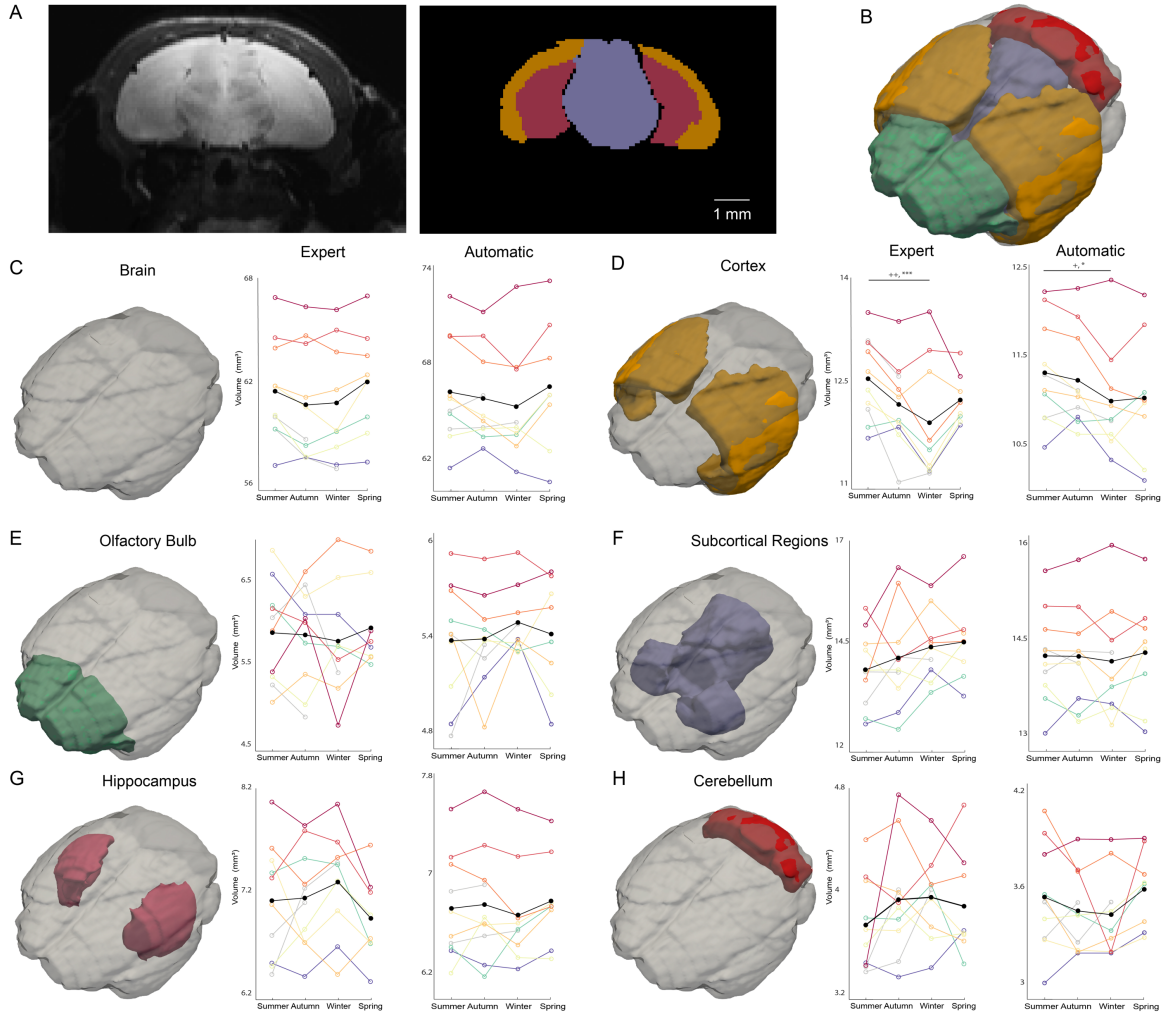


Fig. S1. Seasonal changes in the brains of individual Etruscan shrews determined by manual and automated segmentation of brain regions.

(A) Top, Magnetic resonance imaging was used to obtain T2-weighted images with an in-plane resolution of $75 \times 75 \mu\text{m}$, and $250 \mu\text{m}$ between planes. Bottom, the brain was segmented into cortex (orange), hippocampus (pink), subcortical areas (purple), olfactory bulb (green) and cerebellum (red) and 3 dimensional volumes of these regions and the whole brain (gray) were obtained (B). Measurement of the volumes of the whole brain (C), cortex (D), olfactory bulb (E), subcortical regions (F), hippocampus (G) and cerebellum (H) indicates that only the cortex exhibits consistent shrinkage during winter with no pronounced effect of seasonal variation on the other areas of the brain. The left graph indicates volumes determined by expert-user segmentation, and the right graph indicates volumes determined by automatic segmentation.

Colors in graphs in **C-H** indicate individual animals.
(Black dots indicate means; + indicates Cohen's $d > 0.8$, large effect; ++ indicates Cohen's $d > 1.2$, very large effect; * indicates $p < 0.05$, *** indicates $p < 0.001$, two tailed paired t-test and $p < 0.001$ One-way repeated measure ANOVA).
For statistical information, see table S1.

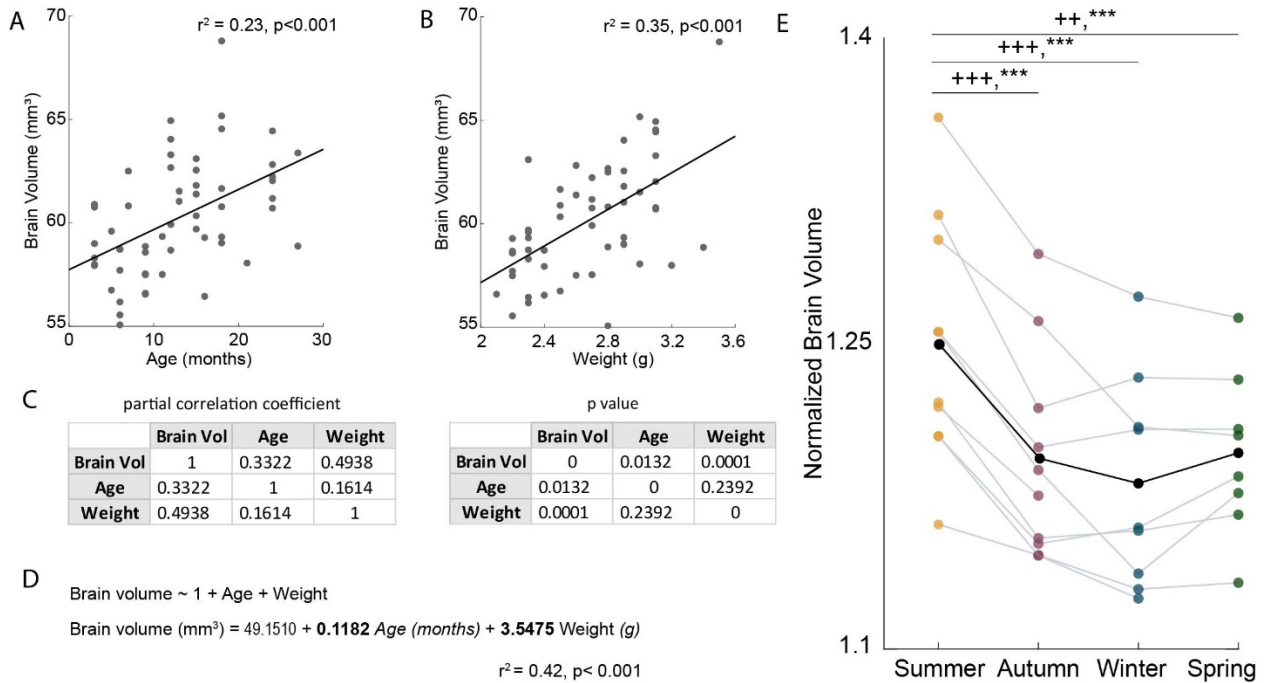


Fig. S2. Shrew brain volume increases with age and weight and shows a more profound decline during winter on normalizing for these parameters.

Brain volume of Etruscan shrews ($n=56$) shows an increase with (A) the age and (B) the weight of a shrew. (C) Partial correlation values indicate a significant relationship between the age and weight of the shrew to its brain volume. (D) Implementing a generalized linear model to correct the brain volume for the age and weight of a shrew, indicates that (E) the normalized brain volumes in Etruscan shrews measured repeatedly over several seasons show a profound decline during autumn and winter.

(++ indicates Cohen's $d > 1.2$, very large effect; +++ indicates Cohen's $d > 2$, huge effect; *** indicates $p < 0.001$, two tailed paired t-test and $p < 0.001$ One-way repeated measure ANOVA).

For statistical information, see table S1.

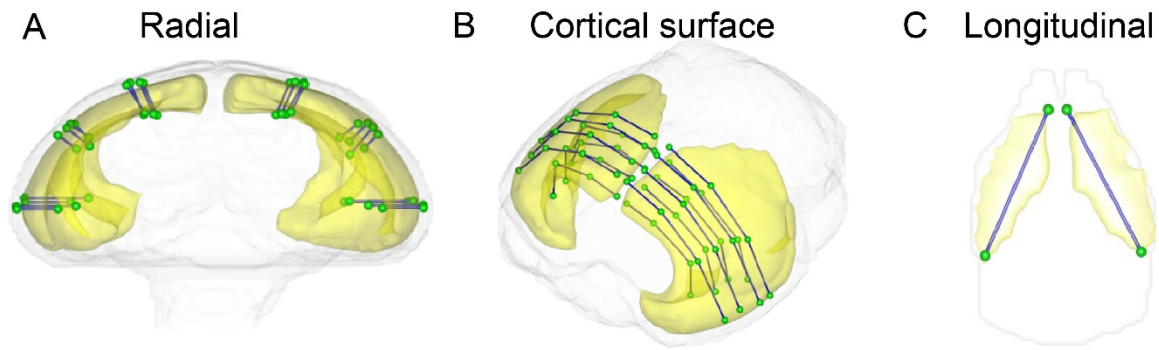


Fig. S3. Determination of changes along different cortical axes.

Measurements (purple lines) were made along radial (A), cortical surface (B) and longitudinal axes (C) of the cortex (yellow) in summer and winter, pairwise in the same animals ($n = 9$ shrews) at 24 distinct locations (coordinates of nodes, marked in green here, in Table S2) to determine how the cortex changed from summer to winter along these axes. For the cortical surface and longitudinal axes, only a subset of measurement points are displayed for visual purposes. Specifically in the longitudinal axes, points were selected at different horizontal planes corresponding to the anterior (and medial) and posterior (and lateral) most points of the cortex in that plane.

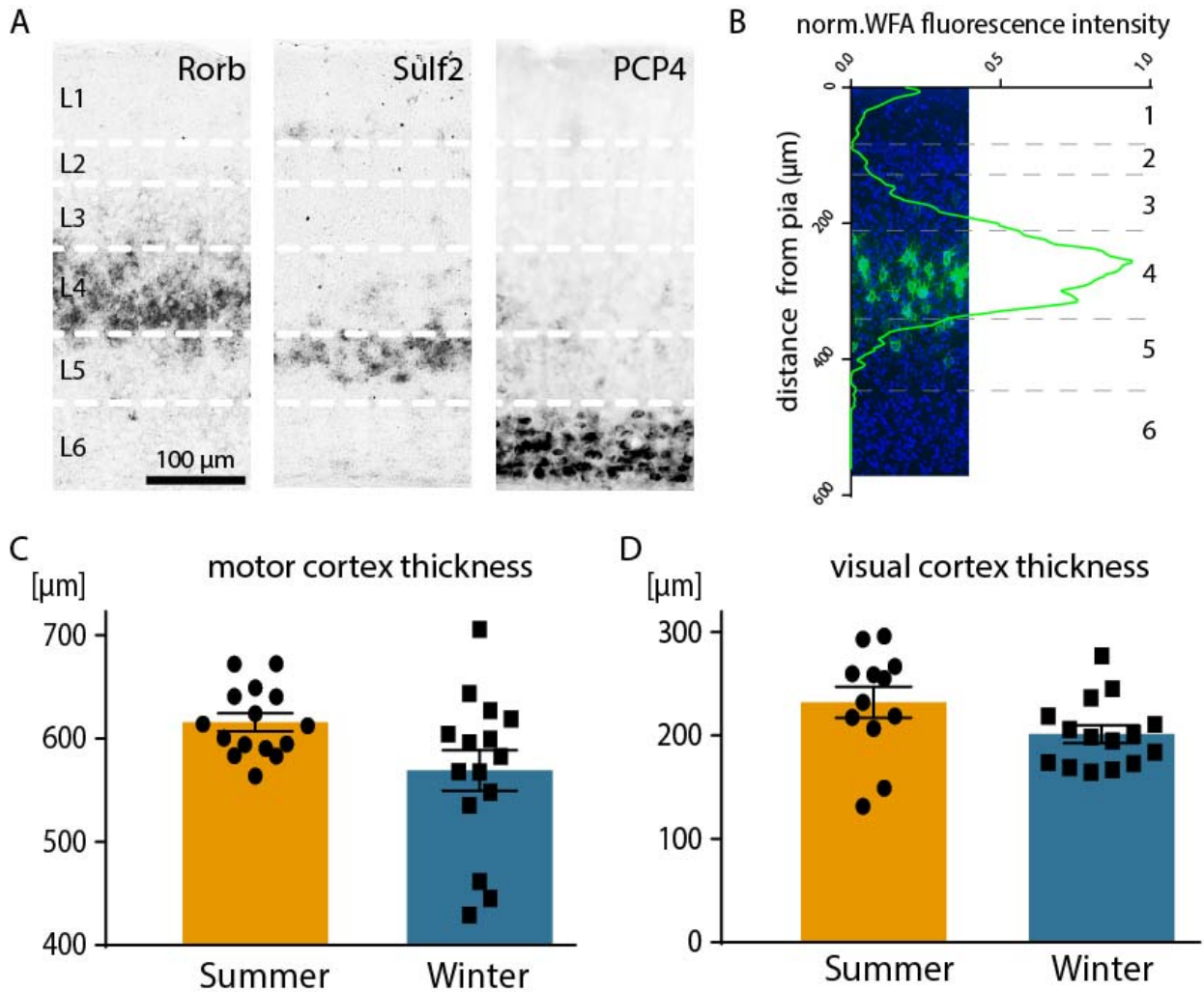


Fig. S4. Cortical layers and cortical thickness in motor and visual cortices.

(A) Gene expression (Rorb, Sulf2), immunohistochemistry (PCP4) and **(B)** histochemistry (WFA) demarcate the cortical layers in the somatosensory cortex with the WFA intensity peaking in layer 4 of the somatosensory cortex **(B)**. **(C)** The motor cortex (n=10 shrews, p=0.0477) and **(D)** visual cortex (n=9 shrews, p=0.0672) show a marginal decline in cortical thickness in the winter but are not statistically significant at the 95-percentile level on correcting for multiple comparisons (Two tailed Mann Whitney U test, Bonferroni corrected $\alpha = 0.017$). Error bars are mean \pm SEM.

For statistical information, see table S1.

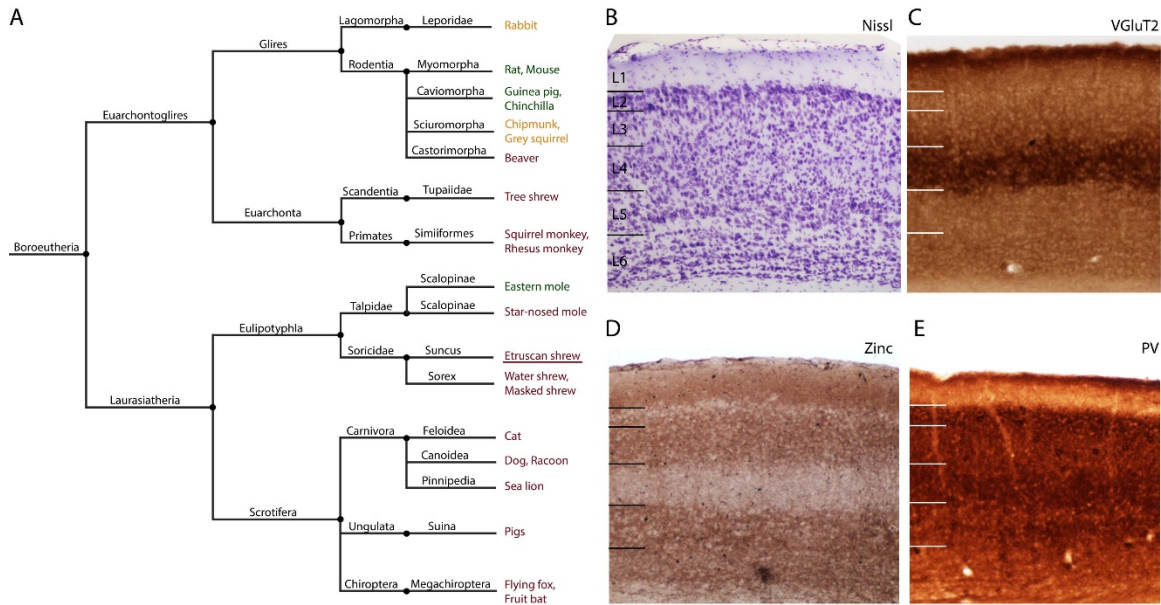


Fig. S5. Etruscan shrews lack anatomically defined barrels for whiskers in layer 4 of the somatosensory cortex.

(A) Phylogenetic tree indicating species lacking whisker barrels in layer 4 of somatosensory cortex (red), those having indistinct barrels (orange) or clearly defined barrels (green). The Etruscan shrew, like most other mammals with whiskers, lacks barrels.

(B) Nissl histochemistry of somatosensory cortex of an Etruscan shrew showing no barrels.

(C) Immunohistochemistry for vesicular glutamate 2 in the somatosensory cortex of an Etruscan shrew showing no barrels.

(D) Histochemistry for zinc in the somatosensory cortex of an Etruscan shrew showing no barrels.

(E) Immunohistochemistry for parvalbumin in the somatosensory cortex of an Etruscan shrew showing no barrels.

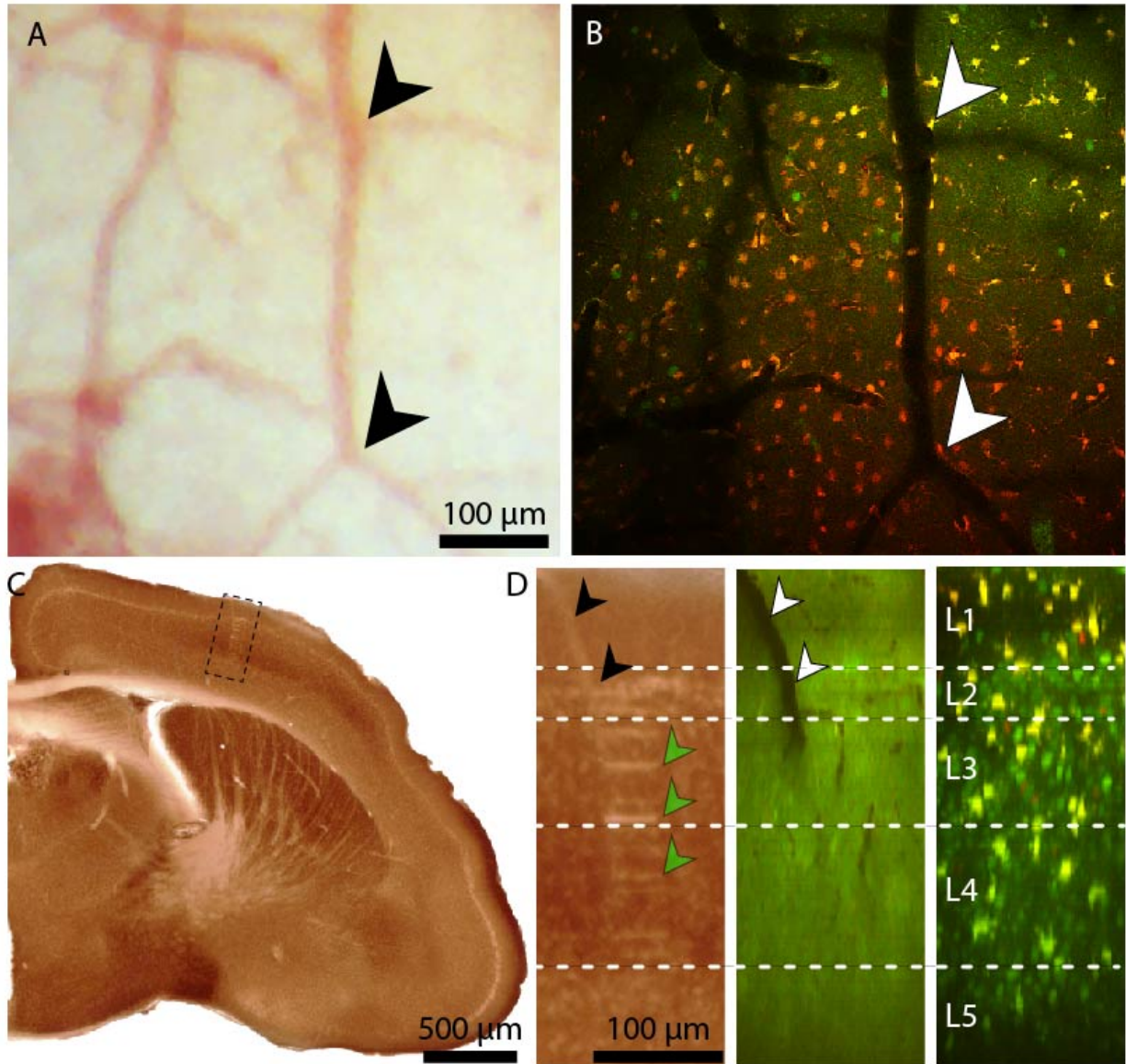


Fig. S6. Regional and laminar alignment of 2-photon imaging and cytochrome oxidase histology.

(A) Surface blood vessel pattern for an imaging region. Black arrowheads indicate blood vessels used for alignment to 2-photon image shown in (B) where corresponding blood vessels are indicating by white arrowheads. (C) Cytochrome oxidase (CO) staining was used to locate 2-photon imaging sites (dashed box) based on aligning blood vessel patterns visible in CO staining (D, left panel, black arrows) and minimum intensity projections of resliced z-stacks (D, middle panel, white arrows). Additionally, faint reductions in cytochrome oxidase activity were visible at imaging sites (D, left panel, green arrows), enabling precise laminar alignment. (D, right panel) shows a maximum intensity projection of the same location.

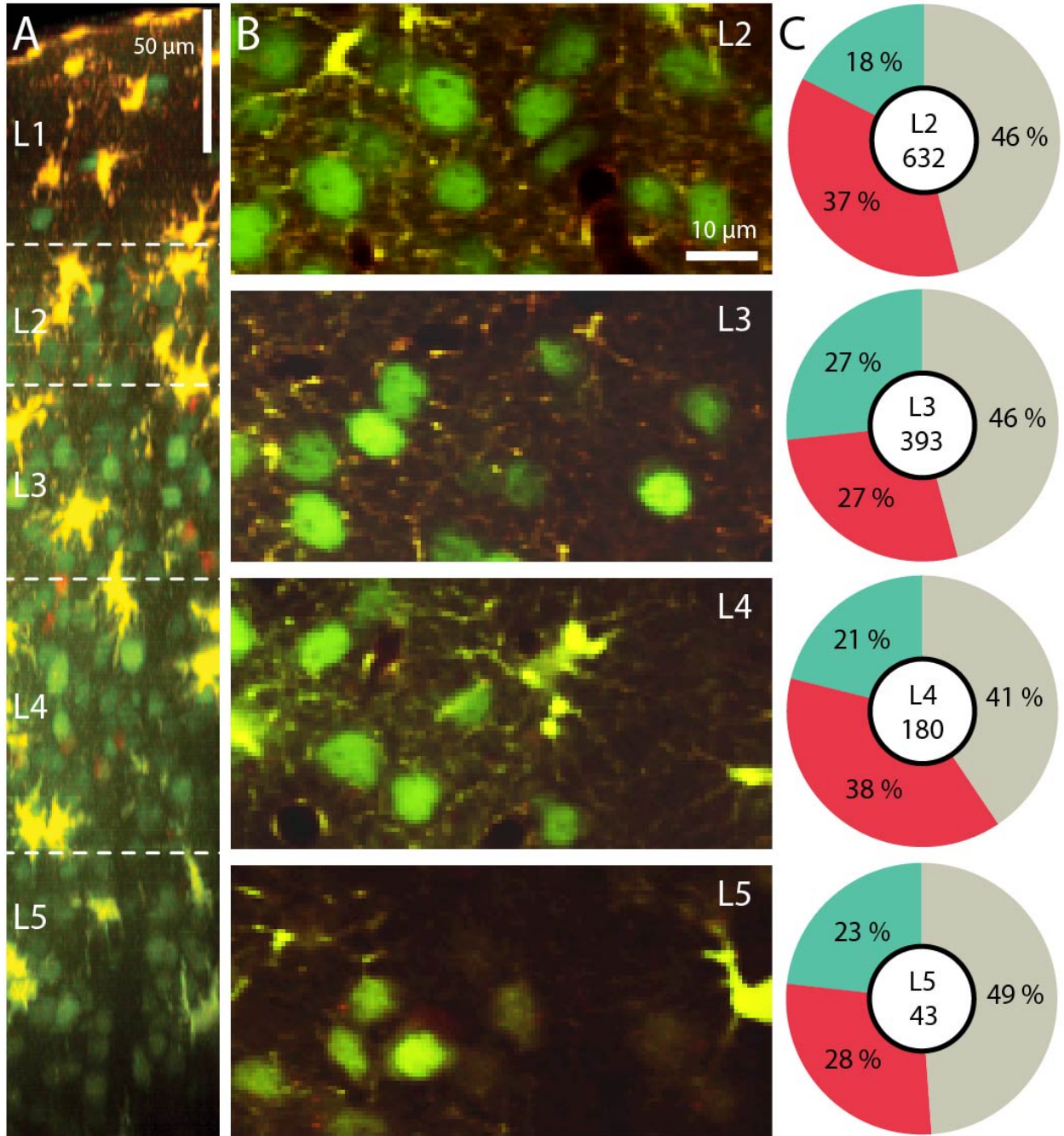


Fig. S7. Two-photon imaging across cortical layers.

(A) Slice through a z-stack of a shrew with two photon imaging performed in layer 2 (B), layer 3 to layer 5 from top to bottom. (C) Cells that were reliably activated (green), suppressed (red) or unmodulated (gray) on touch across each layer. The number in the center of the circle denotes cells recorded in the respective condition.

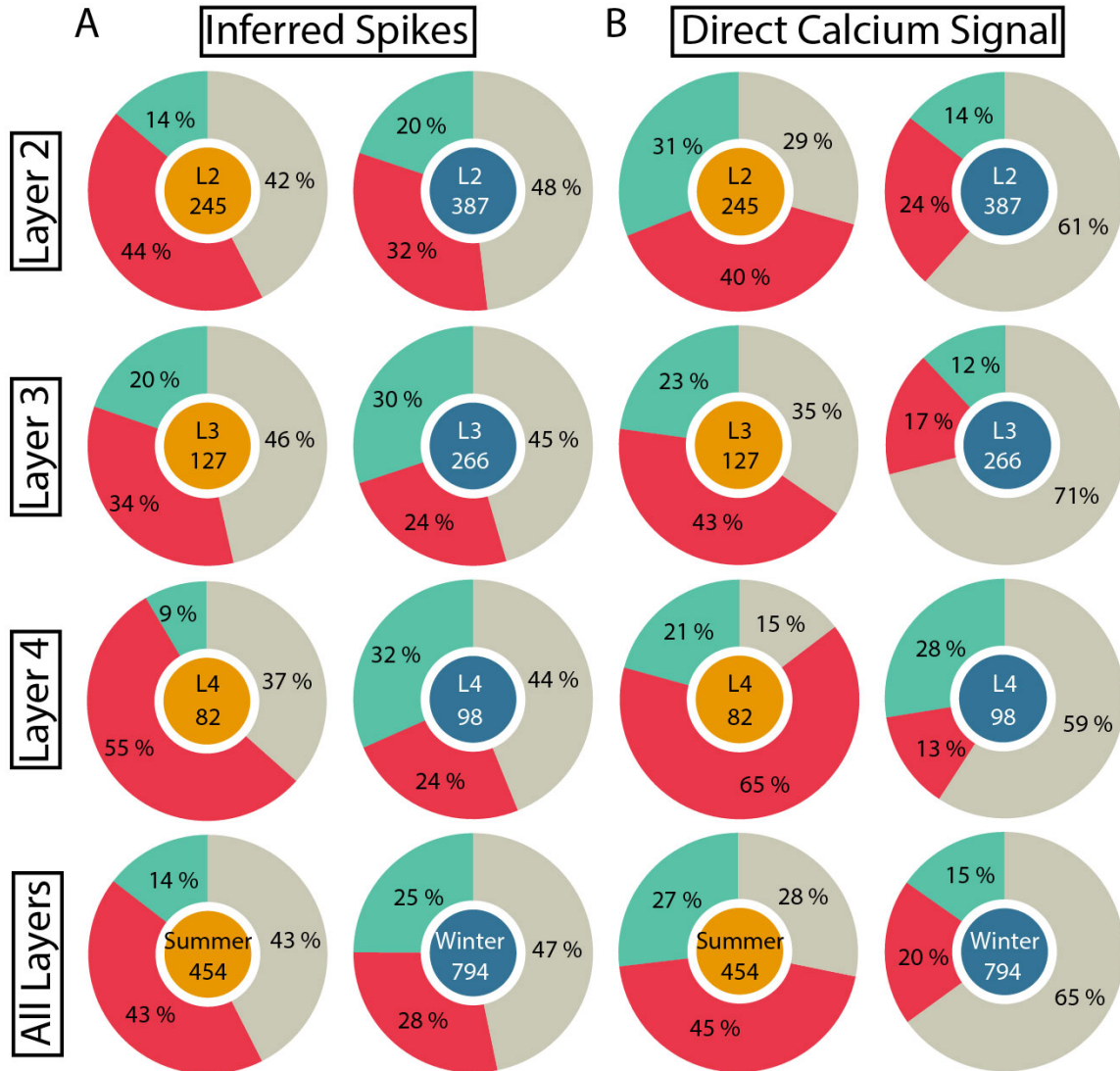


Fig. S8. Two-photon imaging across different seasons with determination of activation and suppression by inferred spikes or directly by fluorescence intensities.

Overall and layer specific responses of cells in the summer and winter based on inferred spikes (A) or directly on fluorescence intensities (B) for layer 2, layer 3, and layer 4 confirms that a lower fraction of cells are suppressed on touch during winter. The number in the center of the circle denotes cells recorded in the respective condition.

For statistical information, see table S1.

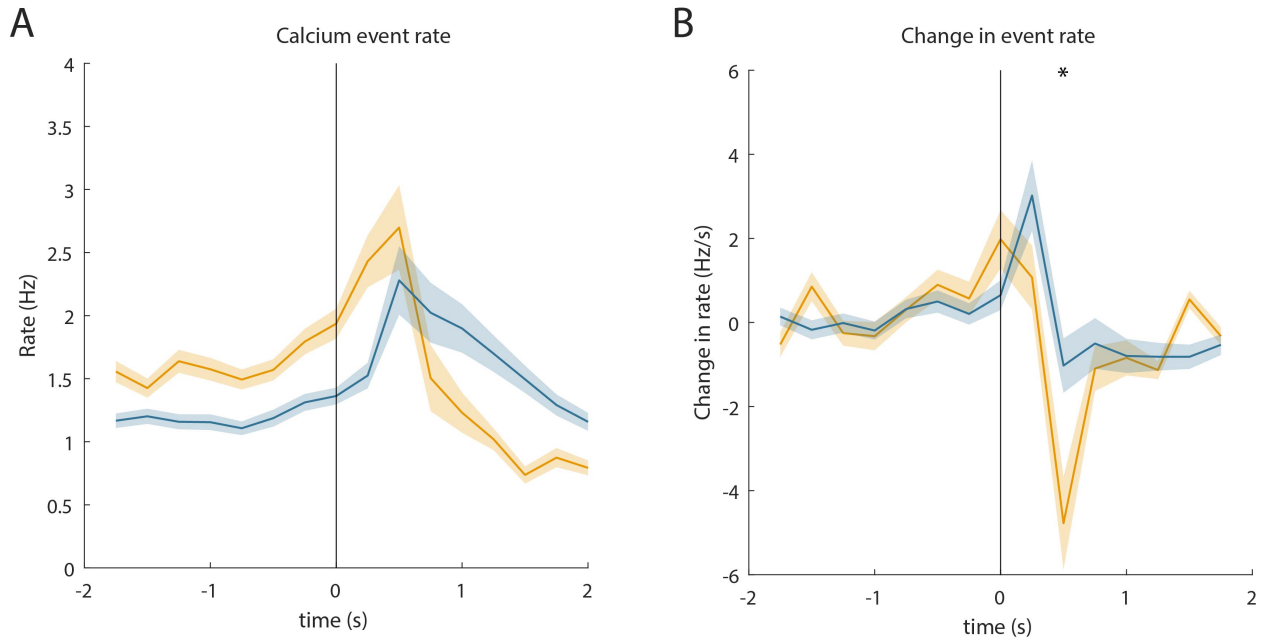


Fig. S9. Responses of cells attenuate faster after touch in summer.

(A) The calcium event rate (mean \pm SEM) of cells in summer (orange, n=454 cells, 5 shrews) and winter (blue, n=794 cells, 7 shrews) aligned to whisker stimulation. (B) The change in the event rate (mean \pm SEM) of cells in summer and winter aligned to whisker stimulation indicates significantly faster response attenuation during summer. ($p=0.0017$, Two tailed unpaired t-test, Bonferroni corrected $\alpha = 0.0031$). For statistical information, see table S1.

Table S1. Experiments and respective statistical analyses.

Figure	Experiment	Number of measurements	Number of animals	Mean \pm SE	Effect Size (Cohen's d)	Significance (p-value)	Statistical test
1C (summer-autumn)	Brain Volume (% change)	10	10	-0.93 \pm 0.32	0.91	0.019	Two tailed Paired t-test
1C (summer-winter)	Brain Volume (% change)	9	9	-1.47 \pm 0.38	1.29	0.004	Two tailed Paired t-test
1C (summer-spring)	Brain Volume (% change)	8	8	-0.03 \pm 0.38	0.03	0.93	Two tailed Paired t-test
1C	Brain Volume (% change)	7	7	-1.06 \pm 0.45 (summer-autumn); -1.50 \pm 0.49 (summer-winter); -0.11 \pm 0.42 (summer-spring)	-	0.002	One-way repeated measure ANOVA
1D (summer-autumn)	Cortex Volume (% change)	10	10	-1.72 \pm 0.55	0.97	0.013	Two tailed Paired t-test
1D (summer-winter)	Cortex Volume (% change)	9	9	-3.76 \pm 0.95	1.32	0.004	Two tailed Paired t-test
1D (summer-spring)	Cortex Volume (% change)	8	8	-2.67 \pm 0.77	1.23	0.010	Two tailed Paired t-test
1D	Cortex Volume (% change)	7	7	-1.34 \pm 0.73 (summer-autumn); -3.80 \pm 1.24 (summer-winter); -2.81 \pm 0.88 (summer-spring)	-	<0.001	One-way repeated measure ANOVA
1D	Body Weight (g)	7	7	2.27 \pm 0.08 (summer); 2.61 \pm 0.13 (autumn); 2.69 \pm 0.16 (winter); 2.69 \pm 0.18 (spring)	-	<0.001	One-way repeated measure ANOVA
1F	Cortical axes changes	648	9	-	-	0.234 (radial-longitudinal); <0.00001 (radial-cortical surface); <0.00001 (longitudinal-cortical surface)	Two tailed Fisher's exact test (Bonferroni corrected $\alpha = 0.017$)
2B	Cortex Thickness (μ m)	152	15	544.32 \pm 4.58 (summer); 495.68 \pm 6.36 (winter)	1.03	<0.00001	Two tailed Mann Whitney U test (Bonferroni corrected $\alpha = 0.017$)

2D (L1)	Cortex Thickness (μm)	97	15	71.88 ± 2.98 (summer 1); 74.56 ± 2.32 (winter); 88.25 ± 2.60 (summer 2)	0.17 (summer 1 – winter); 0.99 (summer 2 – winter)	0.2983 (summer 1 – winter); 0.0001 (summer 2 – winter)	Two tailed Mann Whitney U test (Bonferroni corrected $\alpha = 0.004$)
2D (L2)	Cortex Thickness (μm)	97	15	41.86 ± 0.95 (summer 1); 40.75 ± 1.66 (winter); 43.06 ± 2.16 (summer 2)	0.13 (summer 1 – winter); 0.22 (summer 2 – winter)	0.5552 (summer 1 – winter); 0.6031 (summer 2 – winter)	Two tailed Mann Whitney U test (Bonferroni corrected $\alpha = 0.004$)
2D (L3)	Cortex Thickness (μm)	97	15	86.30 ± 2.65 (summer 1); 99.27 ± 5.09 (winter); 97.39 ± 4.91 (summer 2)	0.54 (summer 1 – winter); 0.07 (summer 2 – winter)	0.0455 (summer 1 – winter); 0.6745 (summer 2 – winter)	Two tailed Mann Whitney U test (Bonferroni corrected $\alpha = 0.004$)
2D (L4)	Cortex Thickness (μm)	97	15	165.07 ± 2.84 (summer 1); 119.33 ± 4.88 (winter); 153.18 ± 4.45 (summer 2)	1.94 (summer 1 – winter); 1.27 (summer 2 – winter)	<0.00001 (summer 1 – winter); <0.00001 (summer 2 – winter)	Two tailed Mann Whitney U test (Bonferroni corrected $\alpha = 0.004$)
2D (L5)	Cortex Thickness (μm)	97	15	84.44 ± 3.43 (summer 1); 98.62 ± 5.55 (winter); 104.60 ± 3.52 (summer 2)	0.52 (summer 1 – winter); 0.22 (summer 2 – winter)	0.1285 (summer 1 – winter); 0.1236 (summer 2 – winter)	Two tailed Mann Whitney U test (Bonferroni corrected $\alpha = 0.004$)
2D (L6)	Cortex Thickness (μm)	96	15	70.41 ± 2.41 (summer 1); 66.92 ± 3.25 (winter); 73.22 ± 2.81 (summer 2)	0.21 (summer 1 – winter); 0.36 (summer 2 – winter)	0.2757 (summer 1 – winter); 0.1362 (summer 2 – winter)	Two tailed Mann Whitney U test (Bonferroni corrected $\alpha = 0.004$)
2F	Neuron Density (per mm ²)	3864	10	25933 ± 759 (summer); 18214 ± 789 (winter)	2.62	<0.00001	Two tailed Mann Whitney U test
2F	Neuron Density (per mm ³)	3864	10	169954 ± 5675 (summer); 148120 ± 8454 (winter)	0.84	0.0751	Two tailed Mann Whitney U test
3F (suppressed)	Cell responses	420	12	43% (summer); 28% (winter)	-	<0.0001	z-test
3G (L2)	Cell responses	342	12	-	-	0.006	Chi-square test (Bonferroni corrected $\alpha = 0.017$)
3G (L3)	Cell responses	213	12	-	-	0.012	Chi-square test (Bonferroni corrected $\alpha = 0.017$)

3G (L4)	Cell responses	107	12	-	-	<0.0001	Chi-square test (Bonferroni corrected $\alpha = 0.017$)
4C	Neuron Density (per mm ²)	509	10	3133 ± 137 (summer); 2522 ± 180 (winter)	0.98	0.0151	Two tailed Mann Whitney U test
4E	Brain volume (% change)	7	7	-1.93 ± 0.29	2.48	<0.001	Two tailed Paired t-test
4E	Brain Volume (% change)	7	7	-1.93 ± 0.29 (summer-summer limited food)	-	0.002	One-way repeated measure ANOVA
4E	Cortex volume (% change)	7	7	-6.72 ± 1.50	1.69	0.004	Two tailed Paired t-test
4E	Cortex Volume (% change)	7	7	-6.72 ± 1.50 (summer-summer limited food)	-	<0.001	One-way repeated measure ANOVA
4E	Olfactory bulb volume (% change)	7	7	0.22 ± 2.73	0.03	0.940	Two tailed Paired t-test
4E	Olfactory bulb volume (% change)	7	7	0.22 ± 2.73 (summer-summer limited food)	-	0.994	One-way repeated measure ANOVA
4E	Subcortical regions volume (% change)	7	7	-0.06 ± 1.14	0.02	0.957	Two tailed Paired t-test
4E	Subcortical regions volume (% change)	7	7	-0.06 ± 1.14 (summer-summer limited food)	-	0.105	One-way repeated measure ANOVA
4E	Hippocampus volume (% change)	7	7	-3.00 ± 2.32	0.49	0.24	Two tailed Paired t-test
4E	Hippocampus volume (% change)	7	7	-3.00 ± 2.32 (summer-summer limited food)	-	0.392	One-way repeated measure ANOVA
4E	Cerebellum volume (% change)	7	7	5.42 ± 2.29	0.89	0.056	Two tailed Paired t-test
4E	Cerebellum volume (% change)	7	7	5.42 ± 2.29 (summer-summer limited food)	-	0.068	One-way repeated measure ANOVA
S1C (summer-autumn; expert)	Brain volume (mm ³)	10	10	61.42 ± 0.94 (summer); 60.63 ± 1.06 (autumn)	0.83	0.027	Two tailed Paired t-test
S1C (summer-winter; expert)	Brain volume (mm ³)	9	9	61.59 ± 1.03 (summer); 60.74 ± 1.16 (winter)	0.6	0.053	Two tailed Paired t-test
S1C (summer-spring; expert)	Brain volume (mm ³)	8	8	61.89 ± 1.12 (summer); 61.94 ± 1.11 (spring)	0.89	0.814	Two tailed Paired t-test
S1C (expert)	Brain volume (mm ³)	7	7	61.51 ± 1.22 (summer); 60.84 ± 1.32 (autumn); 60.69 ± 1.24 (winter); 61.57 ± 1.21 (spring)	-	<0.001	One-way repeated measure ANOVA

SIC (summer-autumn; automated)	Brain volume (mm ³)	10	10	66.17 ± 1.06 (summer); 65.77 ± 0.92 (autumn)	0.83	0.280	Two tailed Paired t-test
SIC (summer-winter; automated)	Brain volume (mm ³)	9	9	66.30 ± 1.76 (summer); 65.26 ± 1.18 (winter)	0.6	0.057	Two tailed Paired t-test
SIC (summer-spring; automated)	Brain volume (mm ³)	8	8	66.61 ± 1.29 (summer); 66.52 ± 1.45 (spring)	0.89	0.815	Two tailed Paired t-test
SIC (automated)	Brain volume (mm ³)	7	7	66.17 ± 1.40 (summer); 65.45 ± 1.17 (autumn); 65.07 ± 1.50 (winter); 65.97 ± 1.54 (spring)	-	0.160	One-way repeated measure ANOVA
S1D (summer-autumn; expert)	Cortex Volume (mm ³)	10	10	12.54 ± 0.19 (summer); 12.16 ± 0.20 (autumn)	1.03	0.01	Two tailed Paired t-test
S1D (summer-winter; expert)	Cortex Volume (mm ³)	9	9	12.47 ± 0.20 (summer); 11.90 ± 0.30 (winter)	1.26	0.009	Two tailed Paired t-test
S1D (summer-spring; expert)	Cortex Volume (mm ³)	8	8	12.52 ± 0.22 (summer); 12.23 ± 0.13 (spring)	0.78	0.074	Two tailed Paired t-test
S1D (expert)	Cortex Volume (mm ³)	7	7	12.45 ± 0.24 (summer); 12.20 ± 0.22 (autumn); 11.85 ± 0.34 (winter); 12.12 ± 0.10 (spring)	-	<0.001	One-way repeated measure ANOVA
S1D (summer-autumn; automated)	Cortex Volume (mm ³)	10	10	11.30 ± 0.18 (summer); 11.21 ± 0.17 (autumn)	0.42	0.217	Two tailed Paired t-test
S1D (summer-winter; automated)	Cortex Volume (mm ³)	9	9	11.36 ± 0.20 (summer); 10.98 ± 0.20 (winter)	0.95	0.021	Two tailed Paired t-test
S1D (summer-spring; automated)	Cortex Volume (mm ³)	8	8	11.37 ± 0.22 (summer); 11.02 ± 0.25 (spring)	1.04	0.008	Two tailed Paired t-test
S1D (automated)	Cortex Volume (mm ³)	7	7	11.26 ± 0.23 (summer); 11.17 ± 0.22 (autumn); 10.95 ± 0.25 (winter); 10.90 ± 0.18 (spring)	-	0.004	One-way repeated measure ANOVA
S1E (summer-autumn; expert)	Olfactory bulb volume (mm ³)	10	10	5.87 ± 0.19 (summer); 5.84 ± 0.19 (autumn)	0.06	0.854	Two tailed Paired t-test
S1E (summer-winter; expert)	Olfactory bulb volume (mm ³)	9	9	5.94 ± 0.20 (summer); 5.77 ± 0.23 (winter)	0.17	0.301	Two tailed Paired t-test
S1E (summer-spring; expert)	Olfactory bulb volume (mm ³)	8	8	5.93 ± 0.23 (summer); 5.93 ± 0.18 (spring)	0.09	0.996	Two tailed Paired t-test
S1E (expert)	Olfactory bulb volume (mm ³)	7	7	5.90 ± 0.26 (summer); 5.88 ± 0.22 (autumn);	-	0.606	One-way repeated

				5.85 ± 0.29 (winter); 5.95 ± 0.21 (spring)			measure ANOVA
S1E (summer- autumn; automated)	Olfactory bulb volume (mm ³)	10	10	5.36 ± 0.12 (summer); 5.37 ± 0.09 (autumn)	0.03	0.913	Two tailed Paired t-test
S1E (summer- winter; automated)	Olfactory bulb volume (mm ³)	9	9	5.36 ± 0.13 (summer); 5.48 ± 0.07 (winter)	0.52	0.521	Two tailed Paired t-test
S1E (summer- spring; automated)	Olfactory bulb volume (mm ³)	8	8	5.43 ± 0.09 (summer); 5.41 ± 0.13 (spring)	0.26	0.634	Two tailed Paired t-test
S1E (automated)	Olfactory bulb volume (mm ³)	7	7	5.37 ± 0.12 (summer); 5.33 ± 0.10 (autumn); 5.42 ± 0.06 (winter); 5.35 ± 0.13 (spring)	-	0.092	One-way repeated measure ANOVA
S1F (summer- autumn; expert)	Subcortical regions volume (mm ³)	10	10	13.85 ± 0.30 (summer); 14.12 ± 0.39 (autumn)	0.25	0.442	Two tailed Paired t-test
S1F (summer- winter; expert)	Subcortical regions volume (mm ³)	9	9	13.85 ± 0.33 (summer); 14.40 ± 0.29 (winter)	0.78	0.049	Two tailed Paired t-test
S1F (summer- spring; expert)	Subcortical regions volume (mm ³)	8	8	13.96 ± 0.36 (summer); 14.52 ± 0.36 (spring)	0.93	0.063	Two tailed Paired t-test
S1F (expert)	Subcortical regions volume (mm ³)	7	7	13.76 ± 0.34 (summer); 14.17 ± 0.57 (autumn); 14.42 ± 0.37 (winter); 14.47 ± 0.41 (spring)	-	0.078	One-way repeated measure ANOVA
S1F (summer- autumn; automated)	Subcortical regions volume (mm ³)	10	10	14.21 ± 0.23 (summer); 14.21 ± 0.25 (autumn)	0.02	0.943	Two tailed Paired t-test
S1F (summer- winter; automated)	Subcortical regions volume (mm ³)	9	9	14.24 ± 0.26 (summer); 14.13 ± 0.30 (winter)	0.17	0.678	Two tailed Paired t-test
S1F (summer- spring; automated)	Subcortical regions volume (mm ³)	8	8	14.23 ± 0.29 (summer); 14.26 ± 0.31 (spring)	0.16	0.781	Two tailed Paired t-test
S1F (automated)	Subcortical regions volume (mm ³)	7	7	14.12 ± 0.31 (summer); 14.10 ± 0.34 (autumn); 14.06 ± 0.38 (winter); 14.18 ± 0.35 (spring)	-	0.706	One-way repeated measure ANOVA
S1G (summer- autumn; expert)	Hippocampus volume (mm ³)	10	10	7.10 ± 0.18 (summer); 7.12 ± 0.15 (autumn)	0.05	0.88	Two tailed Paired t-test
S1G (summer- winter; expert)	Hippocampus volume (mm ³)	9	9	7.14 ± 0.20 (summer); 7.27 ± 0.17 (winter)	0.31	0.49	Two tailed Paired t-test
S1G (summer- spring; expert)	Hippocampus volume (mm ³)	8	8	7.23 ± 0.19 (summer); 6.93 ± 0.15 (spring)	0.38	0.102	Two tailed Paired t-test

S1G (expert)	Hippocampus volume (mm ³)	7	7	7.22 ± 0.22 (summer); 7.02 ± 0.20 (autumn); 7.19 ± 0.21 (winter); 6.89 ± 0.16 (spring)	-	0.165	One-way repeated measure ANOVA
S1G (summer-autumn; automated)	Hippocampus volume (mm ³)	10	10	6.72 ± 0.13 (summer); 6.75 ± 0.14 (autumn)	0.16	0.621	Two tailed Paired t-test
S1G (summer-winter; automated)	Hippocampus volume (mm ³)	9	9	6.75 ± 0.14 (summer); 6.66 ± 0.14 (winter)	0.32	0.511	Two tailed Paired t-test
S1G (summer-spring; automated)	Hippocampus volume (mm ³)	8	8	6.74 ± 0.16 (summer); 6.78 ± 0.13 (spring)	0.30	0.576	Two tailed Paired t-test
S1G (automated)	Hippocampus volume (mm ³)	7	7	6.68 ± 0.18 (summer); 6.70 ± 0.19 (autumn); 6.61 ± 0.16 (winter); 6.72 ± 0.14 (spring)	-	0.746	One-way repeated measure ANOVA
S1H (summer-autumn; expert)	Cerebellum volume (mm ³)	10	10	3.72 ± 0.11 (summer); 3.92 ± 0.14 (autumn)	0.44	0.359	Two tailed Paired t-test
S1H (summer-winter; expert)	Cerebellum volume (mm ³)	9	9	3.76 ± 0.12 (summer); 3.94 ± 0.11 (winter)	0.48	0.273	Two tailed Paired t-test
S1H (summer-spring; expert)	Cerebellum volume (mm ³)	8	8	3.81 ± 0.12 (summer); 3.87 ± 0.15 (spring)	0.34	0.679	Two tailed Paired t-test
S1H (expert)	Cerebellum volume (mm ³)	7	7	3.76 ± 0.14 (summer); 3.98 ± 0.19 (autumn); 3.90 ± 0.14 (winter); 3.76 ± 0.11 (spring)	-	0.309	One-way repeated measure ANOVA
S1H (summer-autumn; automated)	Cerebellum volume (mm ³)	10	10	3.53 ± 0.10 (summer); 3.44 ± 0.08 (autumn)	0.40	0.239	Two tailed Paired t-test
S1H (summer-winter; automated)	Cerebellum volume (mm ³)	9	9	3.53 ± 0.12 (summer); 3.42 ± 0.09 (winter)	0.39	0.176	Two tailed Paired t-test
S1H (summer-spring; automated)	Cerebellum volume (mm ³)	8	8	3.57 ± 0.13 (summer); 3.58 ± 0.09 (spring)	0.23	0.850	Two tailed Paired t-test
S1H (automated)	Cerebellum volume (mm ³)	7	7	3.51 ± 0.13 (summer); 3.42 ± 0.11 (autumn); 3.44 ± 0.11 (winter); 3.54 ± 0.09 (spring)	-	0.0504	One-way repeated measure ANOVA
S2E (summer-autumn)	Normalized Brain volume	10	10	1.25 ± 0.02 (summer); 1.19 ± 1.02 (autumn)	2.68	<0.001	Two tailed Paired t-test
S2E (summer-winter)	Normalized Brain volume	9	9	1.25 ± 0.02 (summer); 1.18 ± 0.02 (winter)	2.51	<0.001	Two tailed Paired t-test

S2E (summer- spring)	Normalized Brain volume	8	8	1.26 ± 0.06 (summer); 1.20 ± 0.04 (spring)	1.79	<0.001	Two tailed Paired t-test
S2E	Normalized Brain Volume	8	8	1.26 ± 0.06 (summer); 1.20 ± 0.05 (autumn); 1.19 ± 0.05 (winter); 1.20 ± 0.04 (spring)	-	<0.001	One-way repeated measure ANOVA
S3C	Cortex Thickness (µm)	30	10	615.8 ± 8.57 (summer); 569 ± 19.65 (winter)	0.80	0.0477	Two tailed Mann Whitney U test (Bonferroni corrected α = 0.017)
S3D	Cortex Thickness (µm)	27	9	232.2 ± 14.85 (summer); 201.4 ± 8.4 (winter)	0.73	0.0672	Two tailed Mann Whitney U test (Bonferroni corrected α = 0.017)
S8A (L2, suppressed)	Cell responses	231	12	44% (summer); 32% (winter)	-	0.0023	z-test (Bonferroni corrected α = 0.017)
S8A (L3, suppressed)	Cell responses	108	12	34% (summer); 24% (winter)	-	0.038	z-test (Bonferroni corrected α = 0.017)
S8A (L4, suppressed)	Cell responses	69	12	55% (summer); 24% (winter)	-	<0.0001	z-test (Bonferroni corrected α = 0.017)
S8A (All, suppressed)	Cell responses	420	12	43% (summer); 28% (winter)	-	<0.0001	z-test
S8B (L2, suppressed)	Cell responses	190	12	40% (summer); 34% (winter)	-	<0.0001	z-test (Bonferroni corrected α = 0.017)
S8B (L3, suppressed)	Cell responses	99	12	43% (summer); 17% (winter)	-	<0.0001	z-test (Bonferroni corrected α = 0.017)
S8B (L4, suppressed)	Cell responses	66	12	65% (summer); 13% (winter)	-	<0.0001	z-test (Bonferroni corrected α = 0.017)
S8B (All, suppressed)	Cell responses	435	12	45% (summer); 20% (winter)	-	<0.0001	z-test
S9B	Response rate change (s ²)	1248	12	-1.19 ± 0.27 (summer); -0.26 ± 0.16 (winter)	0.19	0.0017	Two tailed Unpaired t- test (Bonferroni corrected α = 0.0031)

Table S2. Location of nodes used to determine changes along the cortical axes in standard shrew space coordinates.

<u>Axes</u>	<u>LocationID</u>	<u>Node 1</u>	<u>Node 2</u>	<u>Node 3</u>	<u>Node 4</u>
Radial	R1	(-2.47000,-0.72000,-1.77000)	(-3.10000,-0.72000,-1.77000)		
	R2	(-2.19000,-0.72000,-0.86000)	(-2.47000,-0.72000,-0.65000)		
	R3	(-1.14000,-0.72000,-0.30000)	(-1.42000,-0.72000,0.12000)		
	R4	(1.10000,-0.72000,-0.30000)	(1.31000,-0.72000,0.12000)		
	R5	(2.22000,-0.72000,-0.86000)	(2.43000,-0.72000,-0.65000)		
	R6	(2.50000,-0.72000,-1.77000)	(3.13000,-0.72000,-1.77000)		
	R7	(-2.19000,-0.44000,-1.77000)	(-3.10000,-0.44000,-1.77000)		
	R8	(-1.98000,-0.44000,-0.86000)	(-2.33000,-0.44000,-0.58000)		
	R9	(-1.00000,-0.44000,-0.37000)	(-1.21000,-0.44000,0.12000)		
	R10	(0.96000,-0.44000,-0.37000)	(1.17000,-0.44000,0.12000)		
	R11	(2.01000,-0.44000,-1.00000)	(2.36000,-0.44000,-0.58000)		
	R12	(2.29000,-0.44000,-1.77000)	(3.13000,-0.44000,-1.77000)		
	R13	(-2.26000,-0.02000,-1.77000)	(-2.96000,-0.02000,-1.77000)		
	R14	(-1.98000,-0.02000,-0.79000)	(-2.26000,-0.02000,-0.58000)		
	R15	(-1.14000,-0.02000,-0.44000)	(-1.35000,-0.02000,0.05000)		
	R16	(1.03000,-0.02000,-0.44000)	(1.31000,-0.02000,0.05000)		
	R17	(2.01000,-0.02000,-0.86000)	(2.29000,-0.02000,-0.58000)		
	R18	(2.01000,-0.02000,-1.77000)	(2.99000,-0.02000,-1.77000)		
	R19	(-1.98000,0.40000,-1.77000)	(-2.96000,0.40000,-1.77000)		
	R20	(-1.98000,0.40000,-0.86000)	(-2.19000,0.40000,-0.65000)		
	R21	(-0.93000,0.40000,-0.51000)	(-1.14000,0.40000,-0.02000)		
	R22	(0.89000,0.40000,-0.51000)	(1.10000,0.40000,-0.02000)		
	R23	(1.87000,0.40000,-0.86000)	(2.15000,0.40000,-0.65000)		
	R24	(1.94000,0.40000,-1.84000)	(2.92000,0.40000,-1.84000)		
Longitudinal	L1	(-2.34400,-2.19000,-0.66400)	(-1.77000,0.82000,-0.65000)		
	L2	(2.30400,-2.19000,-0.66400)	(1.73000,0.89000,-0.65000)		
	L3	(-2.27400,-2.19000,-0.59400)	(-1.49000,0.82000,-0.58000)		
	L4	(2.22000,-2.19000,-0.59400)	(1.10000,0.75000,-0.58000)		
	L5	(-2.19000,-2.19000,-0.51000)	(-0.23000,0.82000,-0.52400)		
	L6	(2.22000,-2.19000,-0.51000)	(0.20400,0.82000,-0.52400)		

	L7	(-2.13400,-2.26000,-0.45400)	(-0.23000,0.83400,-0.45400)		
	L8	(2.06600,-2.26000,-0.45400)	(0.12000,0.83400,-0.45400)		
	L9	(-2.06400,-2.26000,-0.38400)	(-0.24400,0.75000,-0.38400)		
	L10	(2.16400,-2.26000,-0.38400)	(0.12000,0.75000,-0.38400)		
	L11	(-1.98000,-2.27400,-0.31400)	(-0.14600,0.75000,-0.31400)		
	L12	(2.08000,-2.26000,-0.30000)	(0.12000,0.75000,-0.31400)		
	L13	(-1.98000,-2.19000,-0.23000)	(-0.23000,0.75000,-0.24400)		
	L14	(2.02400,-2.19000,-0.24400)	(0.10600,0.75000,-0.24400)		
	L15	(-1.77000,-2.26000,-0.16000)	(-0.23000,0.76400,-0.17400)		
	L16	(1.94000,-2.19000,-0.16000)	(0.19000,0.76400,-0.17400)		
	L17	(-1.70000,-2.19000,-0.09000)	(-0.23000,0.68000,-0.10400)		
	L18	(1.80000,-2.19000,-0.09000)	(0.20400,0.68000,-0.10400)		
	L19	(-1.50400,-2.12000,-0.03400)	(-0.23000,0.69400,-0.03400)		
	L20	(1.73000,-2.05000,-0.02000)	(0.12000,0.69400,-0.03400)		
	L21	(-1.42000,-2.12000,0.05000)	(-0.23000,0.62400,0.03600)		
	L22	(1.53400,-2.05000,0.03600)	(0.12000,0.61000,0.05000)		
	L23	(-1.35000,-2.05000,0.12000)	(-0.23000,0.54000,0.12000)		
	L24	(1.45000,-2.05000,0.12000)	(0.19000,0.54000,0.12000)		
Cortical Surface	C1	(-2.47000,-0.72000,-1.77000)	(-2.19000,-0.72000,-0.86000)	(-1.14000,-0.72000,-0.30000)	(-0.23000,-0.72000,-0.09000)
	C2	(-2.78500,-0.72000,-1.77000)	(-2.33000,-0.72000,-0.75500)	(-1.28000,-0.72000,-0.09000)	(-0.23000,-0.72000,0.12000)
	C3	(-3.10000,-0.72000,-1.77000)	(-2.47000,-0.72000,-0.65000)	(-1.42000,-0.72000,0.12000)	(-0.23000,-0.72000,0.33000)
	C4	(0.19000,-0.72000,-0.09000)	(1.10000,-0.72000,-0.30000)	(2.22000,-0.72000,-0.86000)	(2.50000,-0.72000,-1.77000)
	C5	(0.19000,-0.72000,0.12000)	(1.20500,-0.72000,-0.09000)	(2.32500,-0.72000,-0.75500)	(2.81500,-0.72000,-1.77000)
	C6	(0.19000,-0.72000,0.33000)	(1.31000,-0.72000,0.12000)	(2.43000,-0.72000,-0.65000)	(3.13000,-0.72000,-1.77000)
	C7	(-2.19000,-0.44000,-1.77000)	(-1.98000,-0.44000,-0.86000)	(-1.00000,-0.44000,-0.37000)	(-0.16000,-0.44000,-0.37000)
	C8	(-2.64500,-0.44000,-1.77000)	(-2.15500,-0.44000,-0.72000)	(-1.10500,-0.44000,-0.12500)	(-0.16000,-0.44000,-0.05500)
	C9	(-3.10000,-0.44000,-1.77000)	(-2.33000,-0.44000,-0.58000)	(-1.21000,-0.44000,0.12000)	(-0.16000,-0.44000,0.26000)
	C10	(0.19000,-0.44000,-0.37000)	(0.96000,-0.44000,-0.37000)	(2.01000,-0.44000,-1.00000)	(2.29000,-0.44000,-1.77000)
	C11	(0.19000,-0.44000,-0.05500)	(1.06500,-0.44000,-0.12500)	(2.18500,-0.44000,-0.79000)	(2.71000,-0.44000,-1.77000)
	C12	(0.19000,-0.44000,0.26000)	(1.17000,-0.44000,0.12000)	(2.36000,-0.44000,-0.58000)	(3.13000,-0.44000,-1.77000)
	C13	(-2.26000,-0.02000,-1.77000)	(-1.98000,-0.02000,-0.79000)	(-1.14000,-0.02000,-0.44000)	(-0.23000,-0.02000,-0.30000)
	C14	(-2.61000,-0.02000,-1.77000)	(-2.12000,-0.02000,-0.68500)	(-1.24500,-0.02000,-0.19500)	(-0.23000,-0.02000,-0.05500)
	C15	(-2.96000,-0.02000,-1.77000)	(-2.26000,-0.02000,-0.58000)	(-1.35000,-0.02000,0.05000)	(-0.23000,-0.02000,0.19000)

C16	(0.12000,-0.02000,- 0.30000)	(1.03000,-0.02000,- 0.44000)	(2.01000,-0.02000,- 0.86000)	(2.01000,-0.02000,- 1.77000)
C17	(0.12000,-0.02000,- 0.05500)	(1.17000,-0.02000,- 0.19500)	(2.15000,-0.02000,- 0.72000)	(2.50000,-0.02000,- 1.77000)
C18	(0.12000,- 0.02000,0.19000)	(1.31000,- 0.02000,0.05000)	(2.29000,-0.02000,- 0.58000)	(2.99000,-0.02000,- 1.77000)
C19	(-1.98000,0.40000,- 1.77000)	(-1.98000,0.40000,- 0.86000)	(-0.93000,0.40000,- 0.51000)	(-0.16000,0.40000,- 0.51000)
C20	(-2.47000,0.40000,- 1.77000)	(-2.08500,0.40000,- 0.75500)	(-1.03500,0.40000,- 0.26500)	(-0.16000,0.40000,- 0.19500)
C21	(-2.96000,0.40000,- 1.77000)	(-2.19000,0.40000,- 0.65000)	(-1.14000,0.40000,- 0.02000)	(- 0.16000,0.40000,0.12000)
C22	(0.12000,0.40000,- 0.51000)	(0.89000,0.40000,- 0.51000)	(1.87000,0.40000,- 0.86000)	(1.94000,0.40000,- 1.84000)
C23	(0.12000,0.40000,- 0.19500)	(0.99500,0.40000,- 0.26500)	(2.01000,0.40000,- 0.75500)	(2.43000,0.40000,- 1.84000)
C24	(0.12000,0.40000,0.12 000)	(1.10000,0.40000,- 0.02000)	(2.15000,0.40000,- 0.65000)	(2.92000,0.40000,- 1.84000)

Table S3. Primary antibody list.

Antigen	Host	Dilution	Source	Catalog number
Foxp2	Rabbit	1:500	SIGMA	HPA000382
Biotinylated Wisteria Floribunda Lectin	-	1:1000	VECTOR	B-1355
NeuN	Rabbit	1:1000	Servicebio	GB11138
Parvalbumin	Mouse monoclonal	1:5000	Swant	PV235
PCP4	Rabbit	1:500	Sigma	HPA005792

Table S4. Primers used to amplify DNA fragments.

Gene name	Species	Primer set	template
Er81	Suncus Etruscus	Fw-TACAGGAAACATGGCTTGCAGAAGCTCAGGTACC Re-TTCGTTGATGTGACGTTCCATGTCTGTCTTCAGTAGTGG	cDNA
Rorb	Suncus Etruscus	Fw-AGCAGCAGCATCAGTAATGGGCTAAGCAACC Re-AGACGGTGGCACAGTCAGGATTAAGAGCTCC	cDNA
Sulf2	Suncus Etruscus	Fw-ATGTGCCACCCGGCTGGAAGG Re-TTGGAGAGTGCCTGGACCCAGGG	cDNA