Supplementary Information for

Changes in brain architecture are consistent with altered fear processing in domestic rabbits

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Supplementary Text

Global volumetric measures

First, we evaluated the absolute cerebrum size, being 6.66 ± 0.23 ml (mean ± SEM) for domestic rabbits and 5.73 ± 0.19 ml for wild rabbits. Comparing only the gray matter (GM) volume in the cerebrum also revealed higher average values in domestic rabbits as compared to wild ones: 5.73 ± 0.20 ml vs. 4.92 ± 0.17 ml. Volume estimations of both the entire and gray matter in the cerebrum showed homogeneity of variance according to the Hartley's Fmax test (F_{max} = 1.45 and 1.44, respectively). Differences between domestic and wild rabbits may reflect differences in body size, since domestic as compared to wild rabbits have average hind foot lengths of 82.2±2.56 versus 59.4±0.70 mm and average total body weights of 4.12±0.25 kg and 1.07±0.04 kg, respectively. Using volume-derived estimations of brain weight, the encephalization quotient was 0.217±0.003 in domestic and 0.457±0.017 in wild rabbits ($P = 1.5 \cdot 10^{-9}$; Student's t-test, demonstrating reduced relative brain-to-body size due to domestication.

To reveal differences in brain volume between wild and domestic rabbits, and to minimize inter-group variability, all brain regions of interest (ROIs) were normalized to the total cerebrum volume. Given the limited sample size (eight rabbits per group), we compared the normalized GM volumes between the two groups by using permutation tests (exact tests), which are non-parametric statistical tests that make no assumptions about normal distribution of the samples (1) and thus strengthen the validity of the statistical results. We used a total of 5,000 random permutations for each ROI, a number that is consistent with the one used also for voxel-based morphometry and fractional anisotropy analysis, which represents a good tradeoff between the use of a large number of permutations, strengthening the statistical results, and efficient computational time. The P-values for each of the ROIs, analyzing left

and right hemisphere separately, are shown in SI Appendix (Fig. S2). Regions with statistically significant group differences ($P<0.05$) are presented in SI Appendix (Table S1) along with the statistics.

Voxel-based morphometry (VBM)

P-value maps for decreased and increased gray matter (GM) volume in domestic as compared to wild rabbits outputted by FSL-VBM revealed three clusters with locally larger GM volume in domestic rabbits, and two with larger GM volume in wild rabbits located in both hemispheres. The volume of some of these clusters was intersected by more than one GM region of interest (SI Appendix, Table S2). However, the small clusters 2 and 3, reflecting larger GM volume in domestic rabbits, also largely intersected superficial vessel traces, suggesting that they might not reflect meaningful GM changes.

Portions of both the left and the right amygdala, a structure of particular interest in our study because of its strong contribution to fear, form a major part of both clusters in which the GM volume was significantly smaller in domestic rabbits ($P<0.05$), and extended bilaterally into nearby hippocampi, piriform, and entorhinal cortex. The reductions cover the central-lateral part of the amygdala, corresponding to the lateral, basolateral, and the central nuclei when overlaid on the amygdala rabbit anatomy (2). Increased GM volume was observed in a midline cluster in the frontal cortex, including the medial frontal cortex, that encompassed also temporal and parietal areas.

Fractional anisotropy analysis

All white matter regions contained at least some voxels with a significantly lower fractional anisotropy (FA) in domestic than in wild rabbits. In particular, more than 50% of the voxels of the corona radiata, subcortical white matter and the corpus callosum showed a significantly

lower FA in domestic rabbits. In the corona radiata, the average FA values in the voxels showing such statistically significant difference were equal to 0.35 ± 0.12 (mean ± standard deviation) for domestic and 0.48 ± 0.14 for wild rabbits. In the subcortical white matter, they were equal to 0.30 ± 0.12 and 0.45 ± 0.15 for domestic and wild rabbits, respectively. In the corpus callosum, they were equal to 0.37 ± 0.09 for domestic and 0.46 ± 0.11 for wild rabbits. In contrast, no voxels in any white matter region showed a statistically significant increased FA in domestic rabbits.

References

1. Ernst MD (2004) Permutation methods: a basis for exact inference. *Statistical Science* 19:676-685.

2. Równiak M*, et al.* (2007) The morphometric study of the amygdala in the rabbit. *Folia Morphol (Warsz)* 66:44-53.

Table S1. **Brain regions with significant differences in normalized gray matter between wild and domestic rabbits.**

Region of Interest	P-values		Percent change (Domestic vs. Wild)	
	Right	Left	Right	Left
Medial frontal cortex	<0.01	<0.01	+12.1%	+11.1%
Entorhinal cortex	0.03	<0.01	+5.0%	+6.4%
Insular cortex	0.66	0.02	+0.7%	-3.9%
Temporal cortex	0.02	0.41	+3.1%	+1.3%
Claustrum	0.86	0.02	-0.3%	-3.9%
Amygdala	<0.01	<0.01	-10.1%	-8.7%
Hippocampus	<0.01	<0.01	-5.2%	-5.0%

Table S2. **Voxel-based morphometry.**

Regions with smaller gray matter in domestic rabbits					Regions with larger gray matter in domestic rabbits				
Cluster	Minimum P-value	Maximum T-statistic*	Cluster extent	Regions of interest	Cluster	Minimum P-value	Maximum T-statistic*	Cluster extent	Regions of interest
1	0.001	7.58	21229	Piriform cortex left (42%) Amygdala left (22%) Entorhinal cortex left (10%) Hippocampus left (10%)	1	0.002	9.77	80017	Frontal cortex left (25%) Frontal cortex right (12%) Temporal cortex right (8%) Parietal cortex right (5%) Medial frontal cortex left (5%)
2	0.009	6.17	17458	Piriform cortex right (44%) Amygdala right (22%) Entorhinal cortex right (14%) Hippocampus right (5%)	2**	0.04	4.12	1712	Entorhinal cortex left (21%) Temporal cortex left (17%)
					3**	0.03	5.66	1427	Entorhinal cortex right (21%) Temporal cortex right (31%)

* The T-statistic was derived using the Threshold Free Cluster Enhancement method (32).
** This cluster largely intersects also a superficial vessel trace, so it may not reflect meaningful gray matter changes.

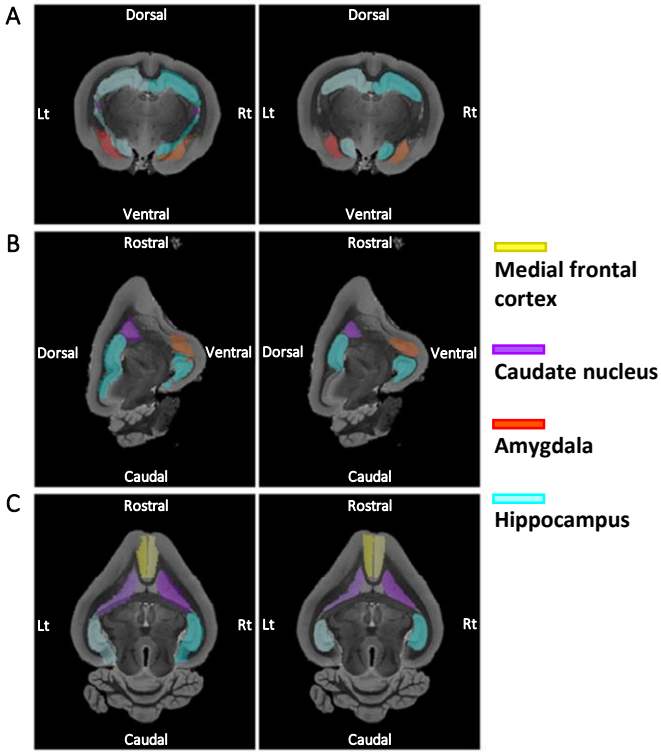

Fig. S1. Illustration of the automatic atlas-based (left column) and manually refined (right column) segmentation of certain structures of interest (medial frontal cortex, caudate nuclei, amygdala and hippocampus) from three different views. *(A)* Coronal view. *(B)* Sagittal view. *(C)* Ventral view.

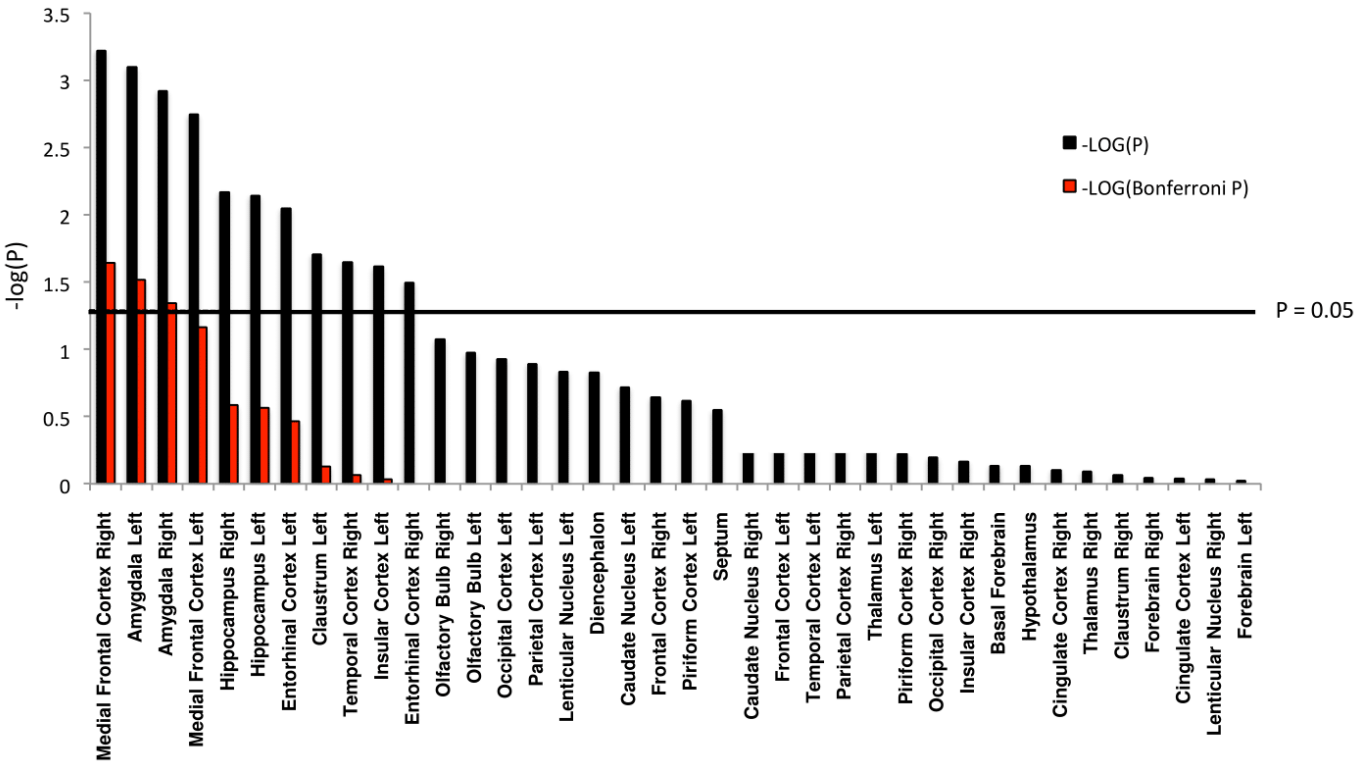

Fig. S2. Volumetric analysis of regions of interest in wild and domestic rabbits. Black bars represent significance levels based on 5,000 permutations for each region of interest and red bars Bonferroni-corrected P-values taking number of regions into account. Here, volumes are analyzed separately for the left and right hemispheres.