Supplemental Information

Histamine regulation of microglia: gene-environment interaction in the regulation of central nervous system inflammation

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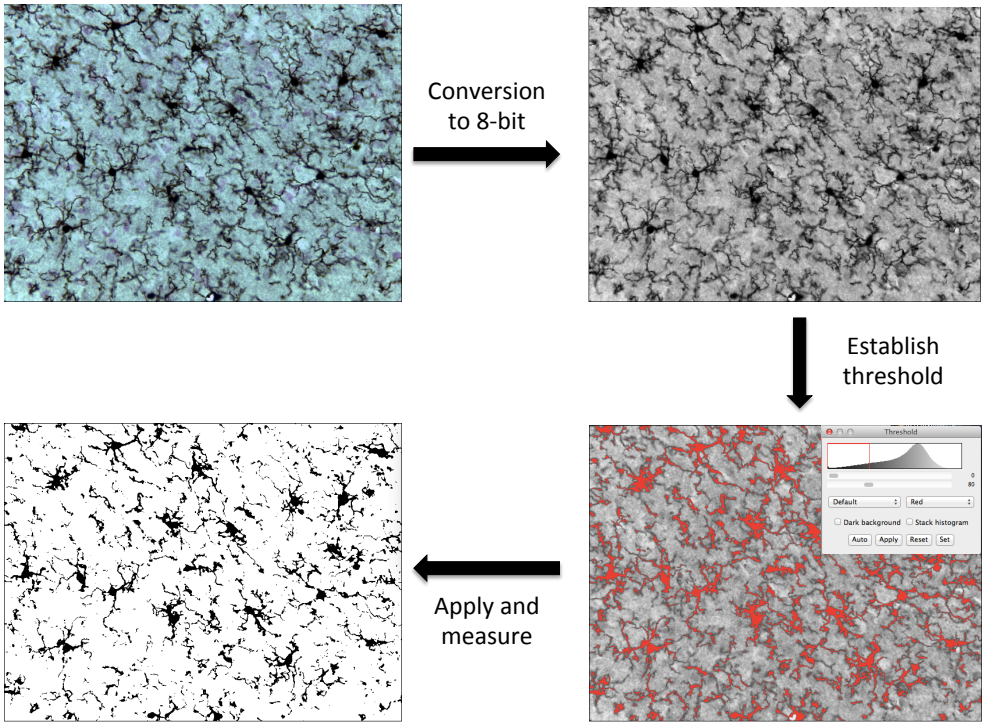
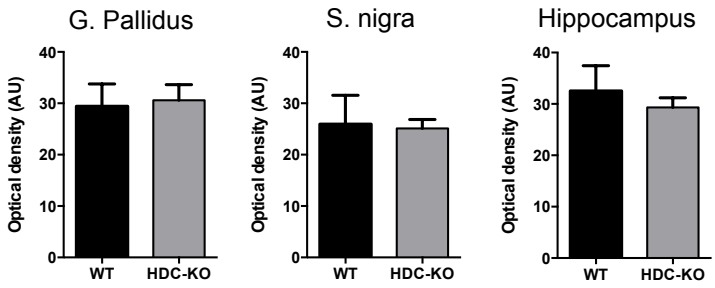
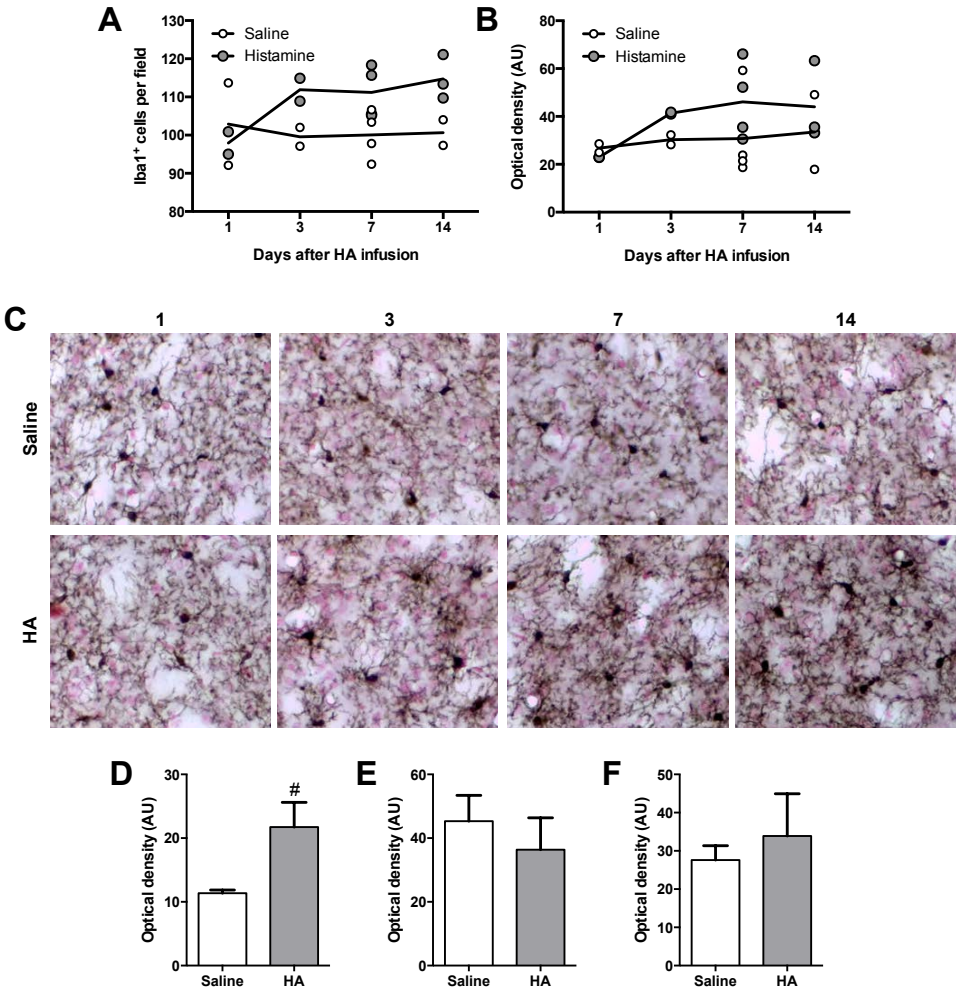


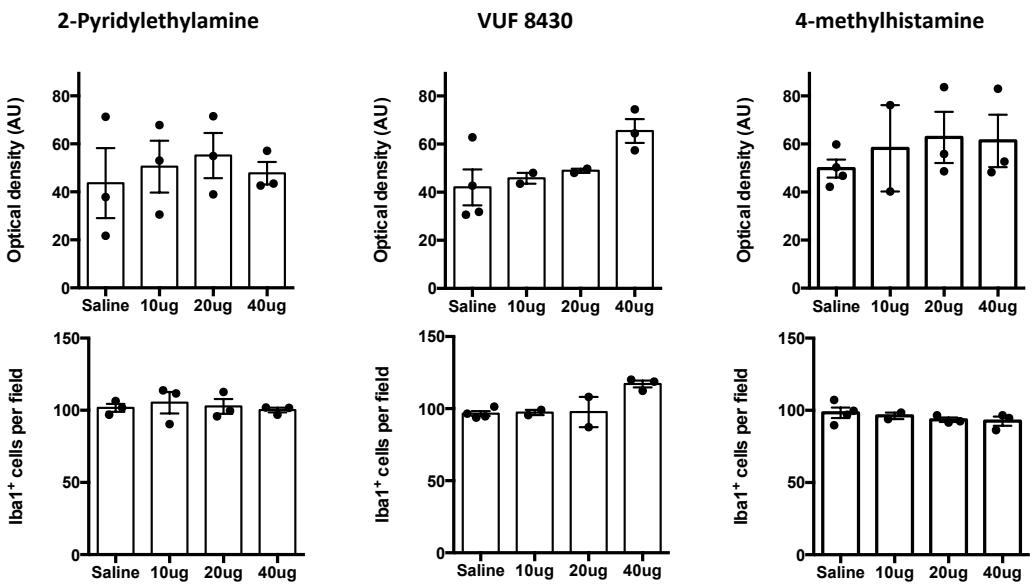
Supplementary Figure 1. Automated quantification of Iba1 staining using ImageJ software. Striatal sections were immunostained with an anti-Iba1 antibody and visualized with diaminobenzidine (DAB). Pictures were taken in a bright field microscope under the same light intensity, aperture, and exposure time for all samples within an experiment to ensure comparability. Then images were converted to 8-bit grayscale resolution. The threshold value was determined to cover the ramifications of the microglial cells and not to detect background, as it can be clearly seen after conversion to final images. The same threshold was applied to all images within each experiment. Then, pixels were quantified with the 'measure' plugin for ImageJ.



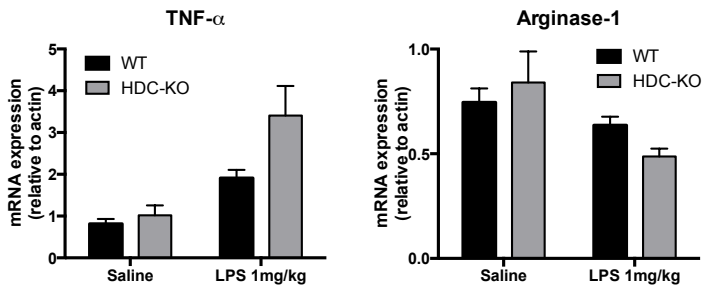
Supplementary Figure 2. Microglial activation in other brain regions in *Hdc*-KO mice. Total area occupied by Iba-1 immunoreactivity was quantified as in **Figure 1** and **Supplementary Figure 1** in different brain regions. n = 3-5 mice per group. For globus pallidus: $t[6] = 0.19$, $p = 0.85$. For substantia nigra: $t[4] = 0.14$, $p = 0.89$. For hippocampus: $t[6] = 0.48$, $p = 0.65$.



Supplementary Figure 3. Time-course of HA-induced microglial activation. HA was infused ICV as described in Figure 1, and microglial activation in the striatum was assayed by Iba1 staining at 1, 3, 7 and 14 days after infusion with the respective saline controls for each time point. **A.** Total cells, 2-way ANOVA: main effect of HA: $F[1,13] = 6.6$, $p = 0.023$; main effect of time: $F[3,13] = 0.85$, $p = 0.49$; Interaction: $F[3,13] = 1.7$, $p = 0.22$. **B.** Optical density, 2-way ANOVA: main effect of HA: $F[1,13] = 1.45$, $p = 0.25$; main effect of time: $F[3,13] = 0.85$, $p = 0.49$; Interaction: $F[3,13] = 0.37$, $p = 0.78$ (n = 2-4 mice per group). **C.** Representative images of time-dependent effects of histamine on striatal microglia. **D.** Regional specificity of HA response: n = 3-4 mice per group. Globus pallidus: $t[3.097] = 2.7$, $p = 0.07$ (Welch's correction). **E.** Substantia nigra: $t[5] = 0.70$, $p > 0.5$. **F.** Hippocampus: $t[6]=0.54$, $p > 0.5$.



Supplementary Figure 4. Effect of H1 and H4 agonists on microglia. In a pilot dose-finding study, drugs were infused ICV over a range of doses and microglial activation in the striatum was evaluated by Iba1 staining 3 days later. The H4 receptor agonist VUF8430 increased Iba1 immunostaining and cell number (n = 2-4 mice per group; Univariate analysis of variance for optical density: $F[10] = 10.1$, $p = 0.01$; for cell number: $F[10] = 15.3$, $p = 0.004$). Another H4 agonist, 4-methylhistamine, produced nonsignificant increases of total microglial density (n = 2-4 mice per group; Iba1 staining density: univariate analysis of variance for optical density: $F[11] = 2.56$, $p = 0.14$ For cell number: $F[11] = 1.02$, $p = 0.34$. The H1 agonist 2-pyridylethylamine did not affect microglia (n = 3 mice per group; Univariate analysis of variance for optical density: $F[11] = 0.06$, $p = 0.8$; for total cells: $F[11] = 0.2$, $p = 0.66$). In a second dexperiment the highest dose of each drug was infused in a larger number of mice; these data confirmed effects of both H4 agonists, as shown in Figure 3.



Supplementary Figure 5. Expression of M1 and M2 markers of microglia in the striatum after LPS challenge. Hdc-KO and WT mice were injected with LPS and mRNA expression of IL-1β, TNF-α and Arginase-1 were evaluated 4 h later. Data for IL-1β are shown in Figure 5. Higher TNF-α mRNA mRNA was observed in *Hdc*-KO mice, with a differential increase after LPS treatment, at trend level (n = 7,7; two-way ANOVA: main effect of LPS: $F[1,24] = 20.01$, $p = 0.0002$; main effect of genotype: $F[1,24] = 4.8$, $p = 0.039$; genotype X LPS interaction: $F[1,24] = 2.8$, $p = 0.11$). In contrast, a trend towards a differential reduction in mRNA for arginase-1, a marker of neuroprotective microglia, was observed in KO mice after LPS challenge (n = 7,7; two-way ANOVA: main effect of LPS: $F[1,24] = 7.247$, $p = 0.013$; main effect of genotype: $F[1,24] = 0.10$, $p = 0.75$; genotype X LPS interaction: $F[1,24] = 2.02$, $p = 0.17$).

Rt-PCR primers

Gene	Primer Forward	Primer Reverse
IL-1β	GAG CAC CTT CTT TTC CTT CAT CTT	CAC ACA CCA GCA GGT TAT CAT CA
IL-6	TTC CAT CCA GTT GCC TTC TTG	GTT GGG AGT GGT ATC CTC TGT GA
TNF-α	GCA CCA CCA TCA AGG ACT CAA	TTG CAG AAC TCA GGA ATG GAC A
iNOS	GGC AGC CTG TGA GAC CTT TG	TGC ATT GGA AGT GAA GCG TTT
IGF1	TTC TAC CTG GCG CTC TGC TT	AGC TCA GCC CCG CAA AG
Actin	CAA CTT GAT GTA TGA AGG CTT TGG T	ACT TTT ATT GGT CTC AAG TCA GTG TAC AG
H1R	TCC ACC AGG GCA CTC TCA CT	TGT CAA ATG ATC CCA GGA ACC T
H4R	TGG GCT CCA TAC TGT CTG TTC A	AAT GCT GTA CCA CAC CGA TTT G

***In situ* hybridization primers** (T7 sequences underlined)

Gene	Primer Forward	Primer Reverse
H1-antisense	GAG CAC CTT CTT TTC CTT CAT CTT	CCA AGC CTT CTA ATA CGA CTC ACT ATA GGG AGA AGC TGA AGC ACG GGT CTT GG
H1-sense	CCA AGC CTT CTA ATA CGA CTC ACT ATA GGG AGA GGT CAC AGT GGG CCT CAA CC	AGC TGA AGC ACG GGT CTT GG
H4-antisense	GAG CCT GTG GAA GCG TAG GG	CCA AGC CTT CTA ATA CGA CTC ACT ATA GGG AGA TTC CGA TCG CCA GAA GGA AC
H4-sense	CCA AGC CTT CTA ATA CGA CTC ACT ATA GGG AGA GAG CCT GTG GAA GCG TAG GG	TTC CGA TCG CCA GAA GGA AC

Supplementary Table 1. Primer sequences for rtPCR and in situ hybridization. Primers were designed using the full transcripts available on Unigene for Mus musculus with the software Primer Express (Applied Biosystems).