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Supplementary Figure 1 (magenta-green version of Figure 4 for the assistance of color-blind readers)**:**

Retrogradely labeled neurons in the PV1 nucleus are parvalbumin-positive, demonstrating their projection to the PAG and the LDTg in rats.

A, B: Fluorogold was injected into the tegmental area at Bregma level –9.16. The thick, horizontal arrow in A indicates the location of the needle tip. The same region is depicted in B after illumination with ultraviolet light. Under these illumination conditions, the fluorogold label is seen to have diffused from the needle tip into the laterodorsal tegmental nucleus. The slender arrows in A and B denote cross-sections through two landmark vessels. Scale bar 0.3 mm.

C, D: Three retrogradely-labelled neurons (arrows in C) are visible within the ipsilateral PV1- nucleus of the same animal (Bregma-level –3.30). In D, the same section, incubated with an antiserum against parvalbumin, reveals these three neurons (arrows) to be immunoreactive for this marker protein. Scale bar 20 μm.

E-F: Fluorogold applied in the region ventrolateral to the rat PAG (dashed circle in E), including the region characterized by a high concentration of parvalbumin-positive terminals (circle in F). Scale bars, 0.3 mm. The arrow in E and F points to a large vessel passing through the parvalbumin-rich region..

G-H: Retrogradely fluorogold filled neurons are concentrated in the region of the PV1-nucleus (Fig. G, DAPI-filter) and some of them (arrows) express parvalbumin immunoractivity (H; Cy3-Filter). Both large and small cells are retrogradely filled (G). Notice the presence of two large vessels in the vicinity of the PV1-nucleus (asterisks). Scale bar 75 μm.

Supplementary Figure 2 (magenta-green version of Figure 5 for the assistance of color-blind readers):

Adeno associated viral construct injected in the PV1-nucleus of the mouse.

A-B: Breeding PV-Cre mice with EGFP-floxed mice gave rise to offspring in which all the neurons in the PV1-nucleus are expressing GFP (A; fluorescent microscopy) were also parvalbumin-positive (B; confocal microscopy). Some double labelled cells are indicated by white arrows. Scale bars 30 μm.

C, D: The Cre-dependent adeno-associated-virus-mCherry construct has been taken up by some of the cells (red) in the PV1-nucleus, which are co-labelled with a Cy2-tagged antibody against parvalbumin (green). Nearly all of the red (mCherry labeled) neurons also are green PV1 cells, so the concurrent fluorescence signals appear yellow. However, a few AAV-infected cells emit only a red signal, owing to the poor penetration of the PV antibody through this (80 μm) thick sections. The image in C (experiment 218), is taken at the posterior-level of the PV1-nucleus; that in D at its caudal extremity. The two white arrows in C indicate vessels that can be observed also in figure 1B (small black arrows), cut at a similar level in the rat brain. Scale bars: 30 μm.

E-K: Six consecutive coronal sections at distances of approximately 130 mm from each other through the region of the mouse PV1-nucleus from proximal (E) to distal (L) (injection 188/11). The AAV-cherry positive group of neurons in the PV1 is highlighted with an arrow. The intermediate portion of the PV1-nucleus (F, G) remained unlabelled in this experiment. The small white line in K points to streaming axons. Scale bar for these 6 images: 0.2 mm.

Supplementary Figure 3 (magenta-green version of Figure 6 for the assistance of color-blind readers):

AAV- co-labelling of other structures in the mouse diencephalon

A: In addition to the PV1-nucleus, also neurons in the medial globus pallidus (MGP, also called the entopeduncular nucleus), the subthalamic nucleus (STh) the reticular nucleus of the thalamus (NRTh) and the Zona incerta (in the picture only axons are visible for these two structures; cell bodies are at another level) took up the viral construct (see Table 5 for details). Injection 64/12. Scale bar 0.2 mm.

B: Contralateral projection of the PV-1 neurons. EGFP-positive axons course in the supraoptic decussation (black arrow) and green terminals (white arrows), are close to parvalbumin-positive PV1-neurons (red) of the opposite side.

B1 and B2: are the original images from which the merged image B was generated. The white arrow in B2 indicate axons in the supraoptic decussation and the rectangle in B2 the boundaries of the merged Fig. B. Injection 502/12. Scale bar in B: 30 μm. Scale bars in B1 and B2, 0.2 mm.

C: Some endings visible in the ipsilateral lateral habenular nucleus (experiment 237/12). In experiments with inadvertent labelling of the medial globus pallidus, the density of terminals is much higher (inset, injection 238/12). Scale bar 0.2 mm

D: Axons and probably terminals deriving perhaps from the PV1 in the ipsilateral region around the retroflex fascicle. The axons then converge medially and are visible as a compact bundle adjacent to the EW, some sections later (6E). Scale bar 0.2 mm

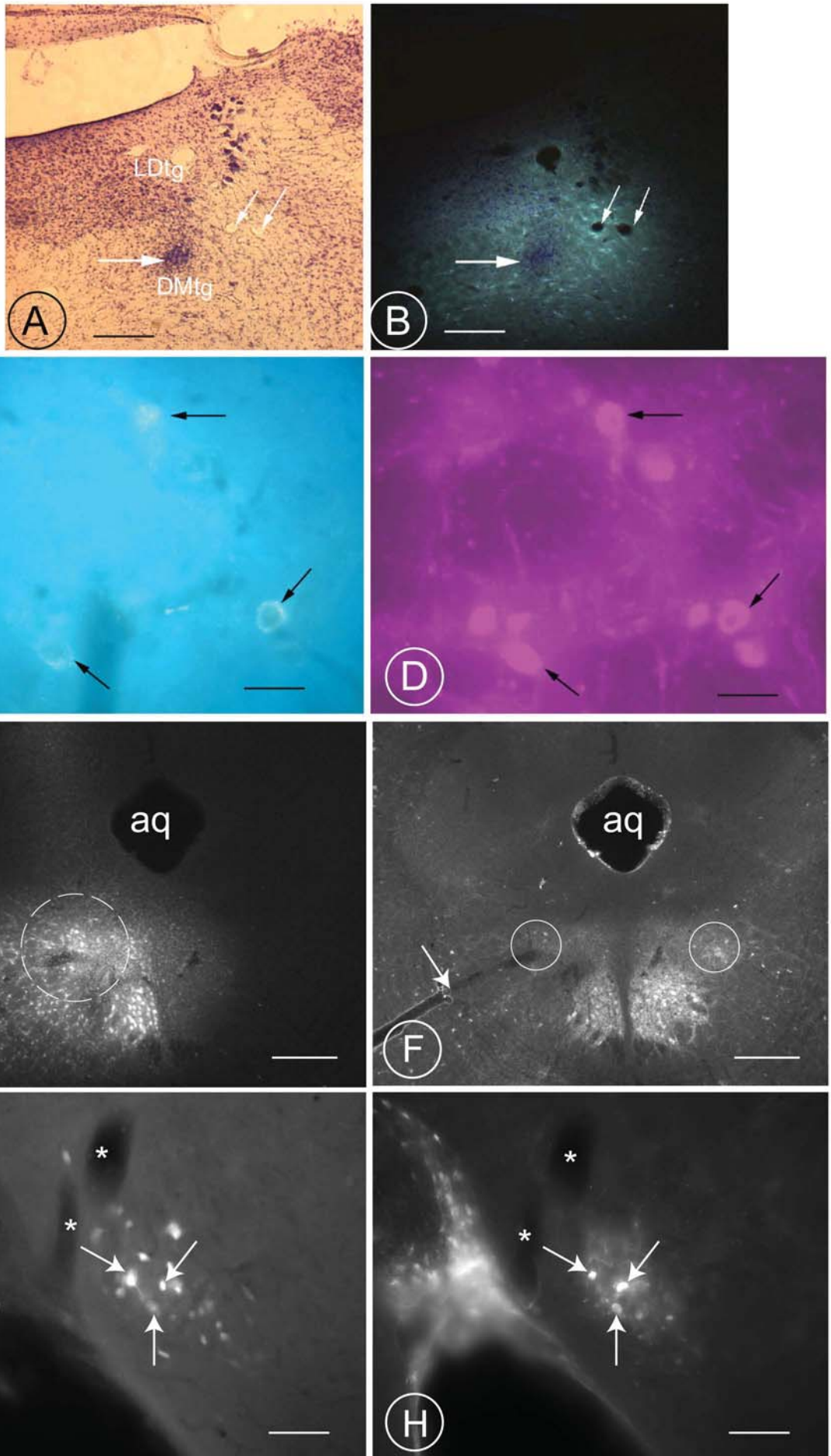
E: A compact bundle of axons in the region of the EW (white arrow. The section is counterstained with an antibody to CART, a marker of the cortically projecting part of the EW-nucleus (EWcp, thin arrows). Terminals are located slightly more laterally (arrow). The vertical dashed line represents the midline. Injection 64/12. Scale bar 50 μm.

F: Terminals are sometimes present in the mediodorsal tegmental nucleus (MdTG). Their number increases drastically when the lateral mamillary nucleus is inadvertently co-stained (inset, white arrow). Injection 238/11. Scale bar 0.2 mm.

Supplementary Figure 4 (magenta-green version of Figure 8 for the assistance of color-blind readers):

Distribution of terminal fields in the caudal PAG of mice

A-B: At the fourth and last level of the PAG (approx. level -4.6 in (Paxinos and Franklin, 2001)), the PV1-CTF flares out and becomes oval (white arrows in A), expands in the reticular formation, and impinges upon a nucleus that is composed of parvalbumin-immunoreactive neurons (B). We have named this uncharted nucleus the PV2 nucleus (injection 503-12, bilaterally in the PV1 with the AAV2/9.CAG.FLEX.EGFP.WPRE.bGH constructs). Scale bar 0.3 mm. C-D: At higher magnification, the density of terminals in the ventrolateral cylinder of terminals can be better appreciated (N, injection 237/11). The intimate relationship between the endings from the PV1-nucleus (red) and the parvalbumin-positive neurons in the PV2-nucleus (green) suggest possible synaptic contacts. C: image taken with the fluorescence microscope (injection 237/11). D: stack of laser scanning confocal images to visualize a similar level to C in injection 218/11. Scale bar in C and D: 20 μm.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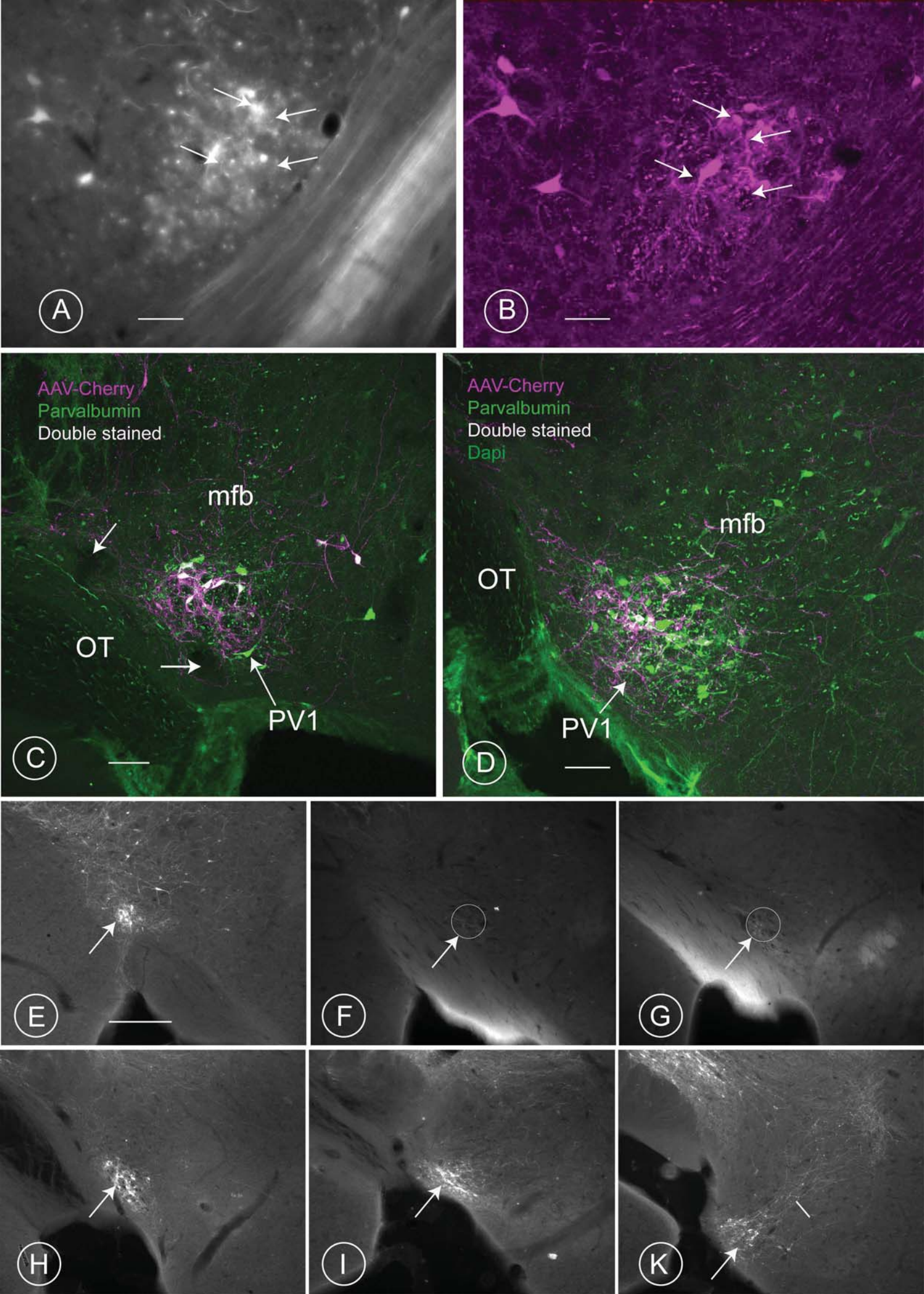
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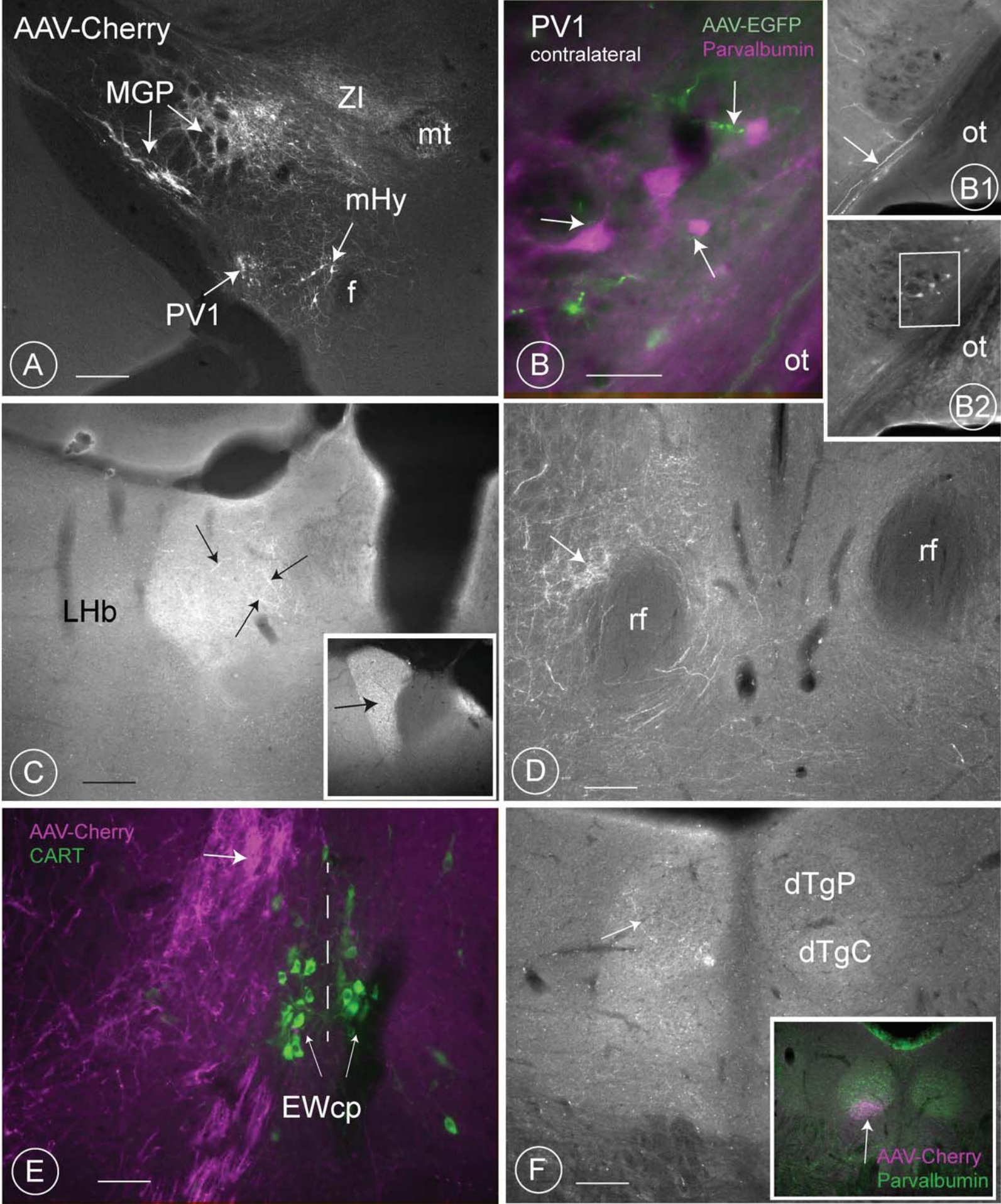
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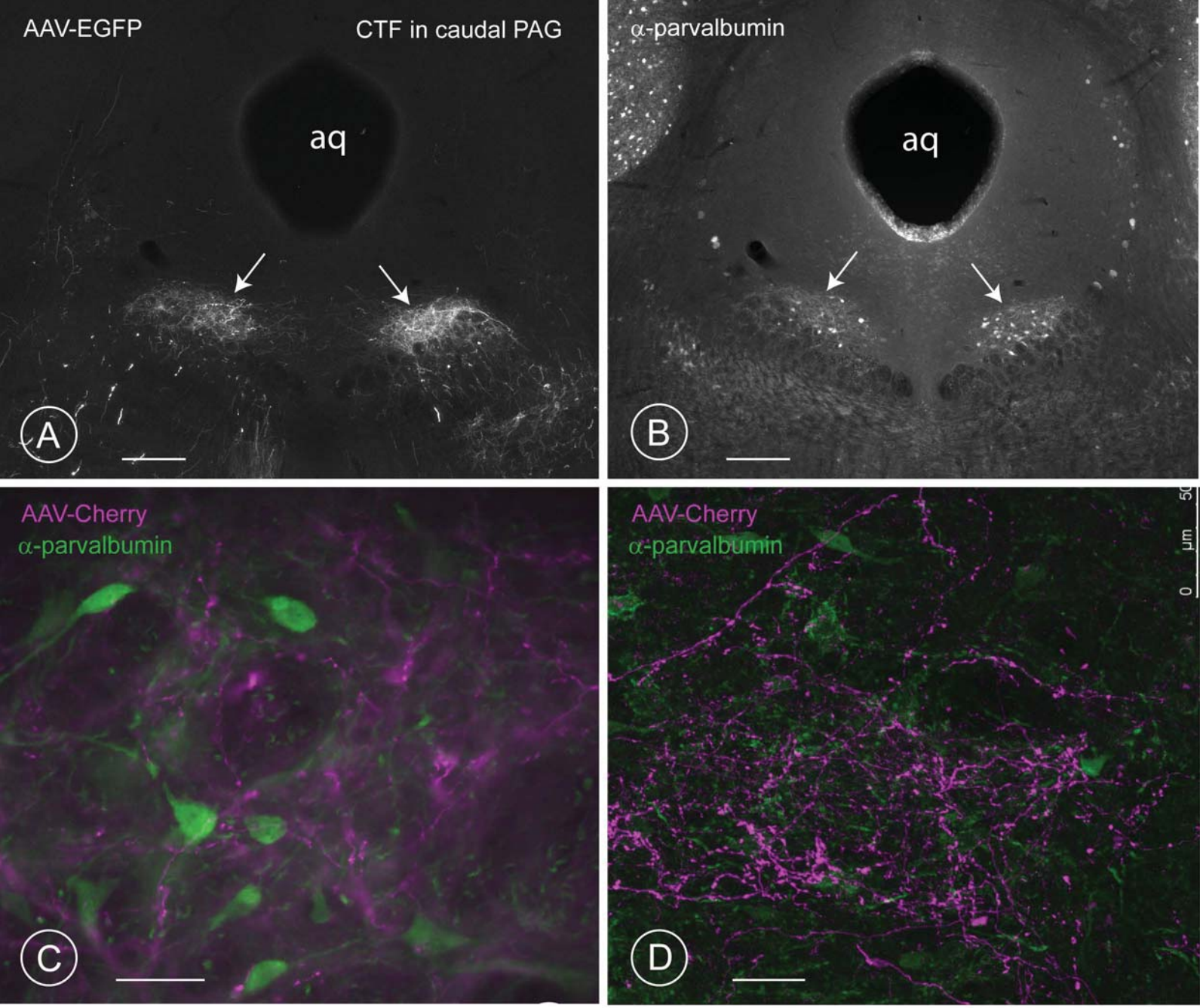
AAV-EGFP
CTF in caudal PAG
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