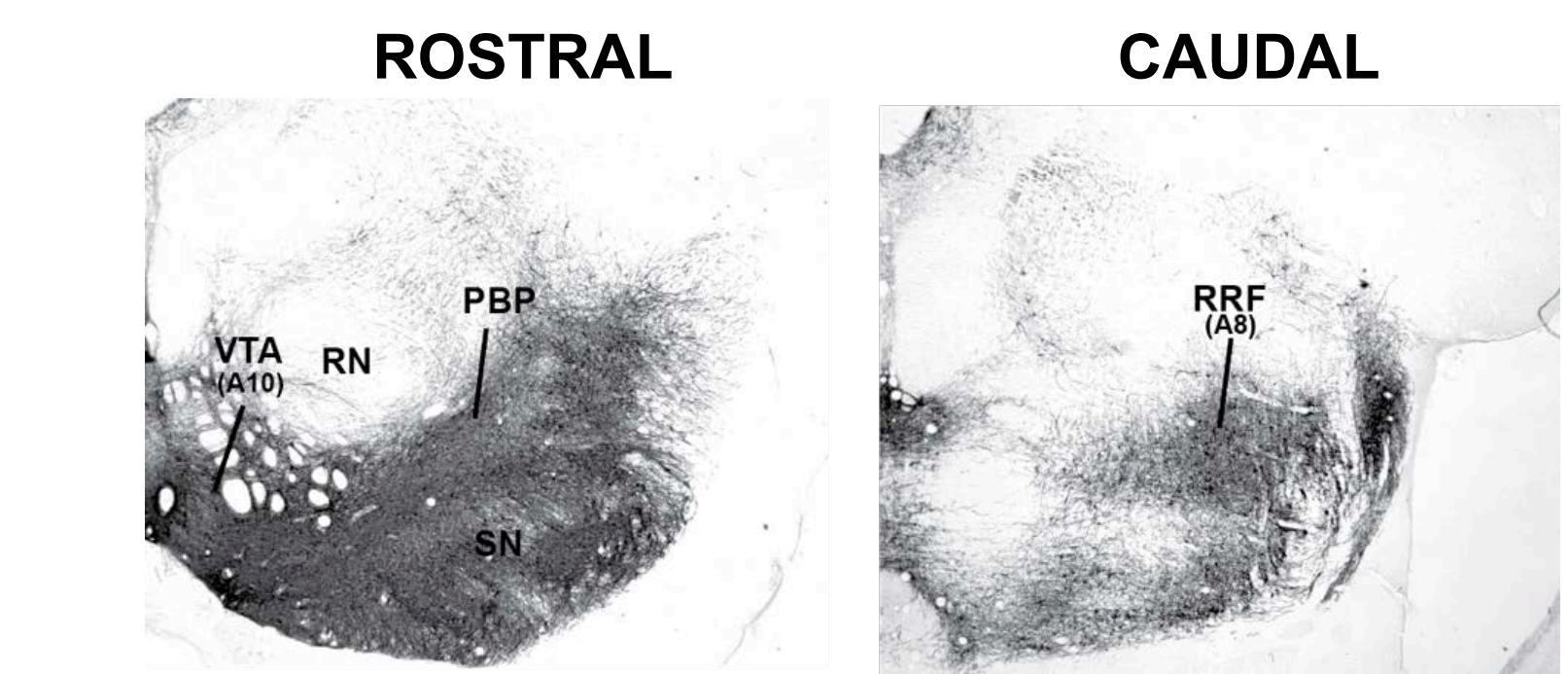


## BACKGROUND

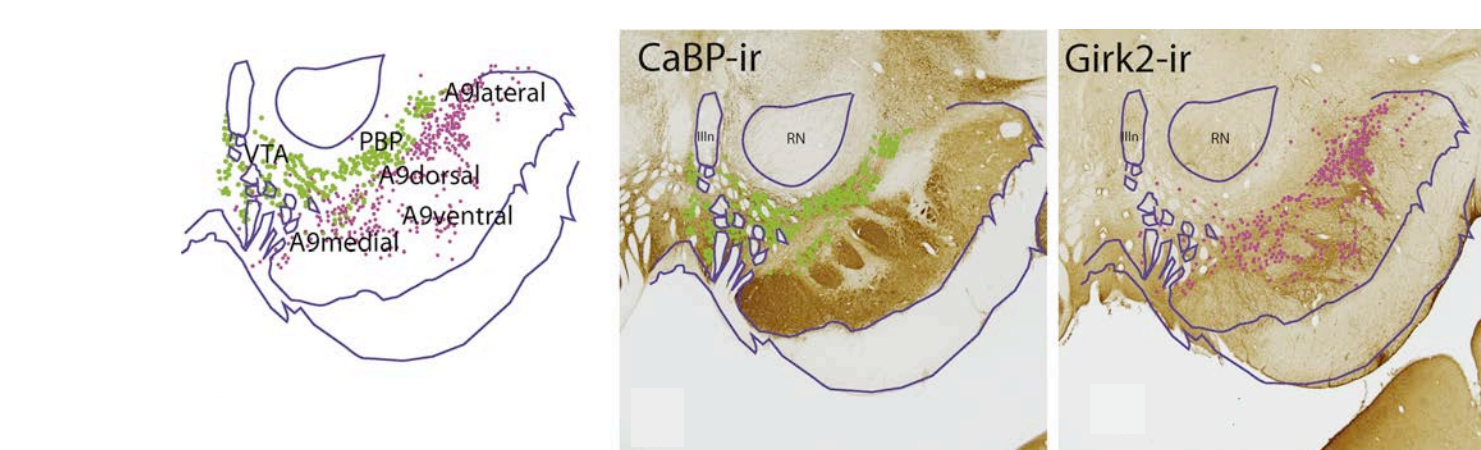
- Dopamine (DA) is important in many fundamental behaviors including positive and negative reinforcement, decision making, working memory, incentive and stimulus salience and purposeful movement.
- This behavioral heterogeneity is due, in part, to the diverse phenotypic characteristics of DA neurons and of the brain structures with which they are connected.
- DA neurons receive excitatory, inhibitory and modulatory input from diverse sources.
- Corticotropin-releasing factor (CRF) is a neuropeptide shown to regulate dopaminergic signaling by modulating its signaling capabilities.
- DA is a key target of CRF in the ventral midbrain. (fig 4)
- Mesolimbic analysis in rodent has primarily focused on the ventral tegmental area (VTA) due to predominant efferent and afferent projections through this subregion (fig 5).
- Evolutionary expansion of the ventral midbrain in primates, results in differential efferent/ afferent patterns (fig 5).

### TH+ IMMUNOREACTIVITY OF DA CELLS IN MACAQUE MIDBRAIN



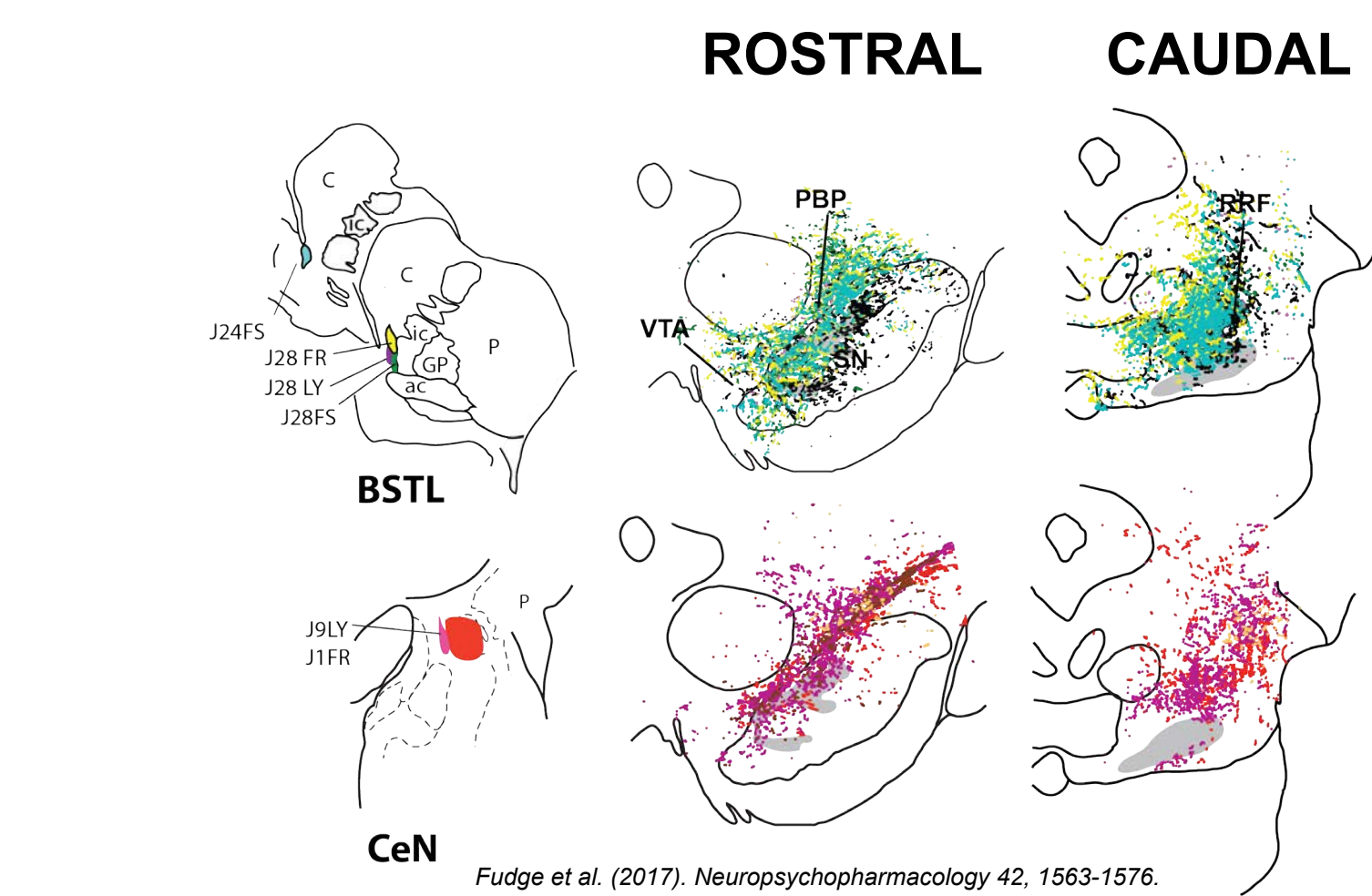
**Fig 1. Tyrosine hydroxylase (TH) immunohistochemistry in macaque midbrain labels DA+ neurons and fibers.** VTA, ventral tegmental area; RN, red nucleus; PBP, parabrachial nucleus; SN, substantia nigra; RRF, retrorubral field.

### CELLULAR AND REGIONAL MARKERS ASSIST IN SPECIFIC POPULATION ANALYSIS



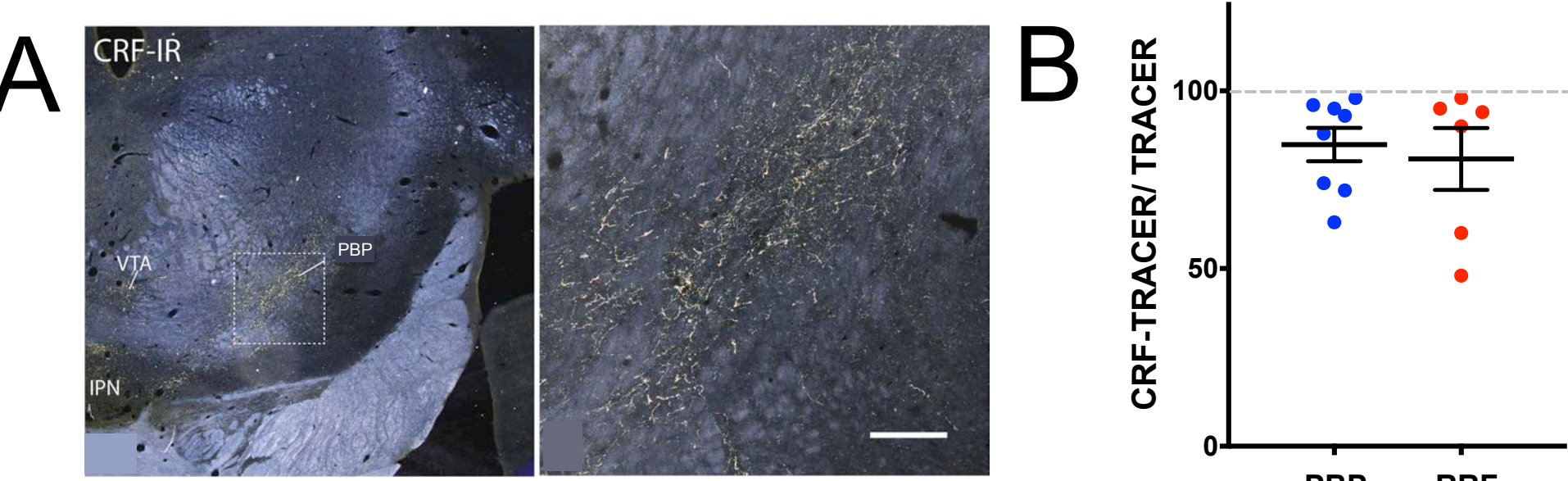
**Fig 2. DA cell subpopulations.** (A) CaBP (green, labels PBP and RRF) and Girk2 (pink, labels the SN) distribution labels distinct DA subpopulations in the macaque midbrain. VTA, ventral tegmental area; RN, red nucleus; PBP, parabrachial nucleus; SN, substantia nigra; RRF, retrorubral field.

### EXTENDED AMYGDALA INNERVATES PBP AND RRF



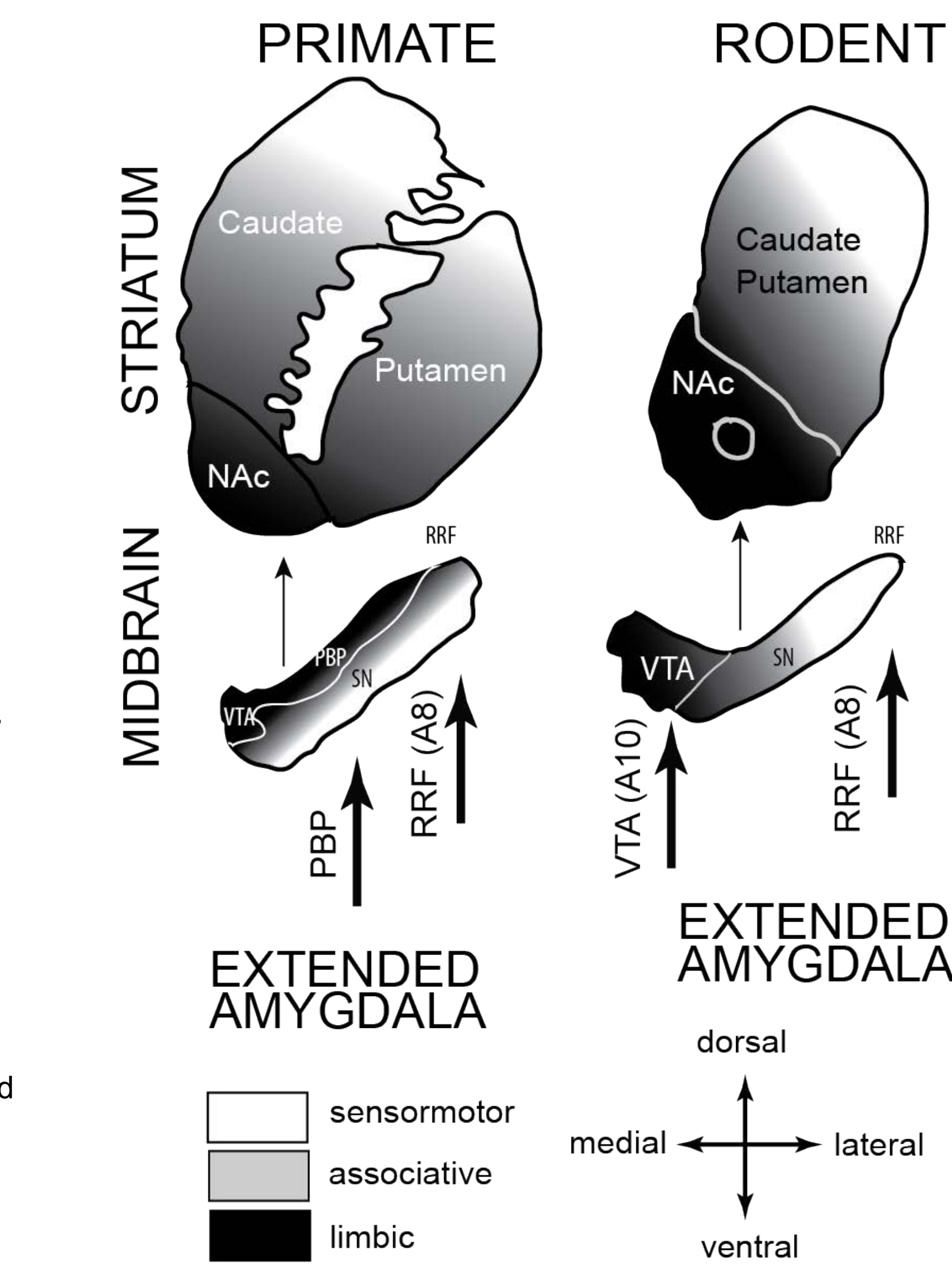
**Fig 3. Extended amygdala sends projections to PBP and RRF.** (A) Anterograde tracer injections into BSTL (top panel) and CeN (bottom panel) results in robust innervation of PBP and RRF. BSTL, bed nucleus of the stria terminalis; CeN, central nucleus; VTA, ventral tegmental area; RN, PBP, parabrachial nucleus; SN, substantia nigra; RRF, retrorubral field.

### EXTENDED AMYGDALA: A CRF SOURCE



**Fig 4. Immunoreactivity of anti-CRF; visualized with darkfield microscopy.** (A) Immunoreactivity of anti-CRF shows robust fiber innervation over the PBP. (B) Retrograde tracer injections in PBP and A8 and subsequent immunolabeling for tracer and CRF revealed a high proportion of CRF+TRACER filled cells projecting to PBP and A8. VTA, ventral tegmental area; PBP, parabrachial nucleus; IPN, interpeduncular nucleus; RRF, retrorubral field. Scale bar= 250 um.

### DA SUBPOPULATION OUTPUTS ACROSS SPECIES



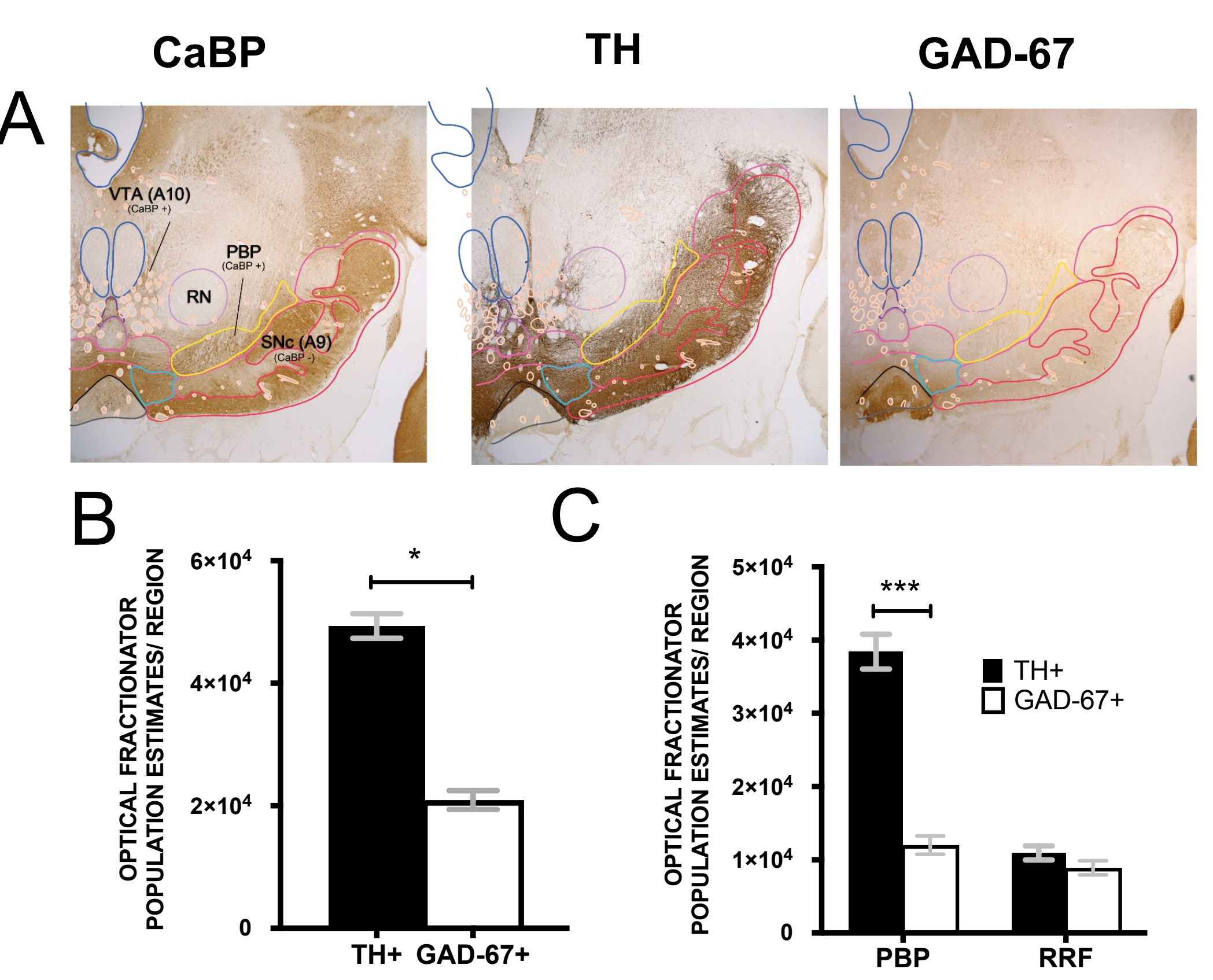
**Fig 5. Functional subdivisions of the dopamine systems across species.** When compared to rodents, there are anatomic positional shifts in the main DA subpopulations (i.e. the PBP of A10 and RRF/A8) resulting in differential innervation profiles across species. Efferents from the extended amygdala show significantly more innervation of VTA in rodents, however in primates, these fibers predominantly target areas OUTSIDE the VTA (PBP and RRF).

## WHAT IS THE SYNAPTIC PROFILE OF CRF CONTACTS ONTO DA/NON-DA CELLS IN PBP/A10 AND RRF/A8 IN MIDBRAIN OF NON-HUMAN PRIMATE?

## MATERIALS AND METHODS

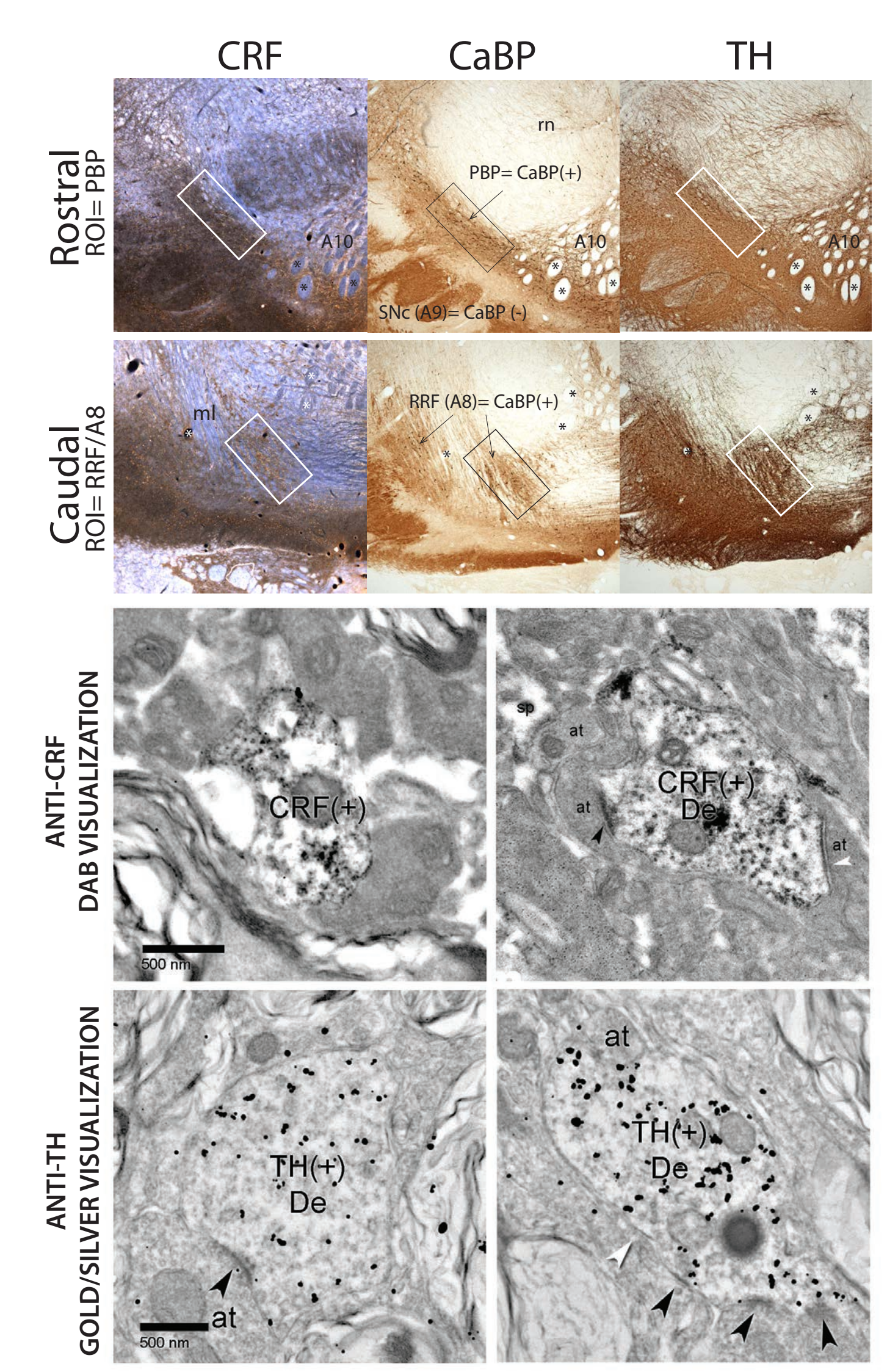
Detailed methods are available upon request

## Stereological analysis of TH/GAD-67 cells in PBP/A10 and RRF/A8



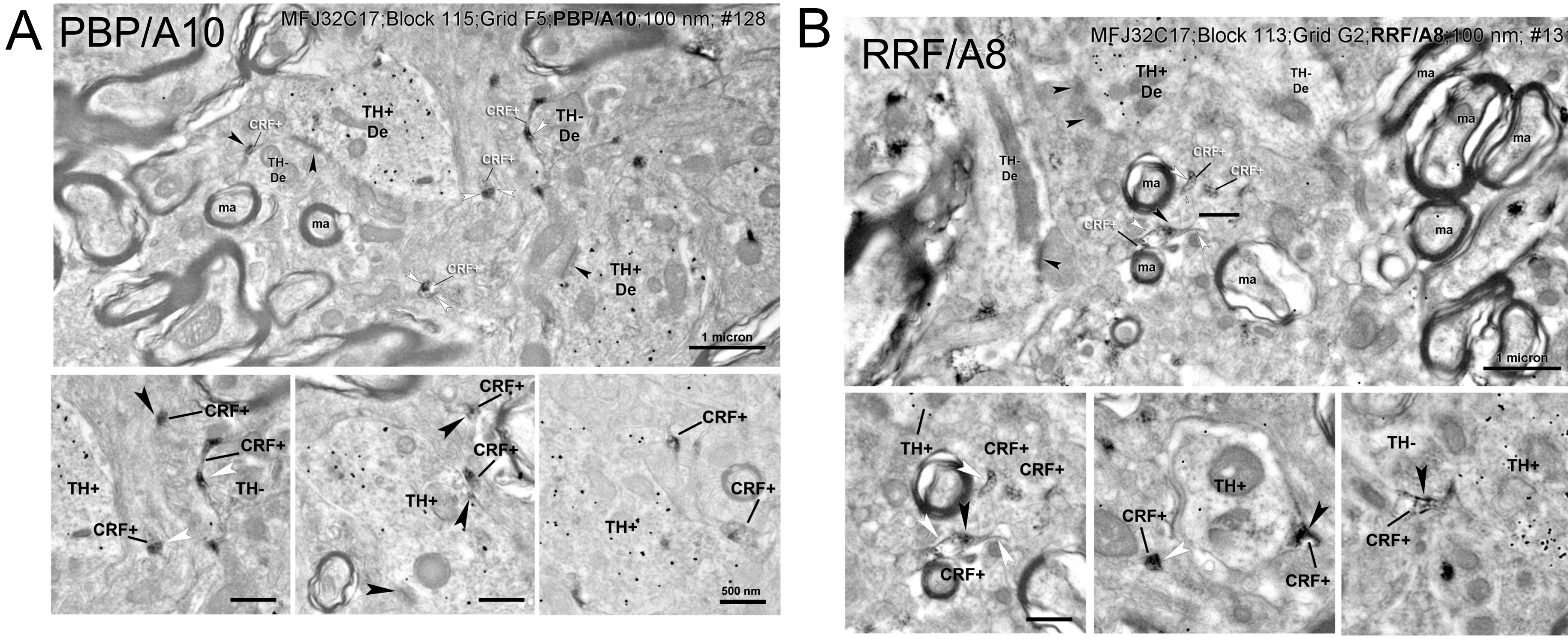
**Fig 6. Unbiased stereology of DA+ and non-DA+ cells shows significantly more DA+ cells in the PBP.** (A) Neighboring sections were immunoreacted for CaBP, TH, and GAD-67. Subregion boundaries were determined using CaBP and overlaid onto TH and GAD-67 sections. (B) Quantitative analysis of DA+ (TH) vs non-DA+ (GAD-67, GABAergic) cells in the PBP and RRF subregions shows 2.5 fold more TH+ cells. (C) TH+ vs GAD-67+ across region shows significantly more TH+ cell in the PBP. GAD-67 cells were not significantly different across region.

## Pre-embedding dual-immunoperoxidase reactivity labels CRF+ axons and TH+ dendrites in the macaque midbrain

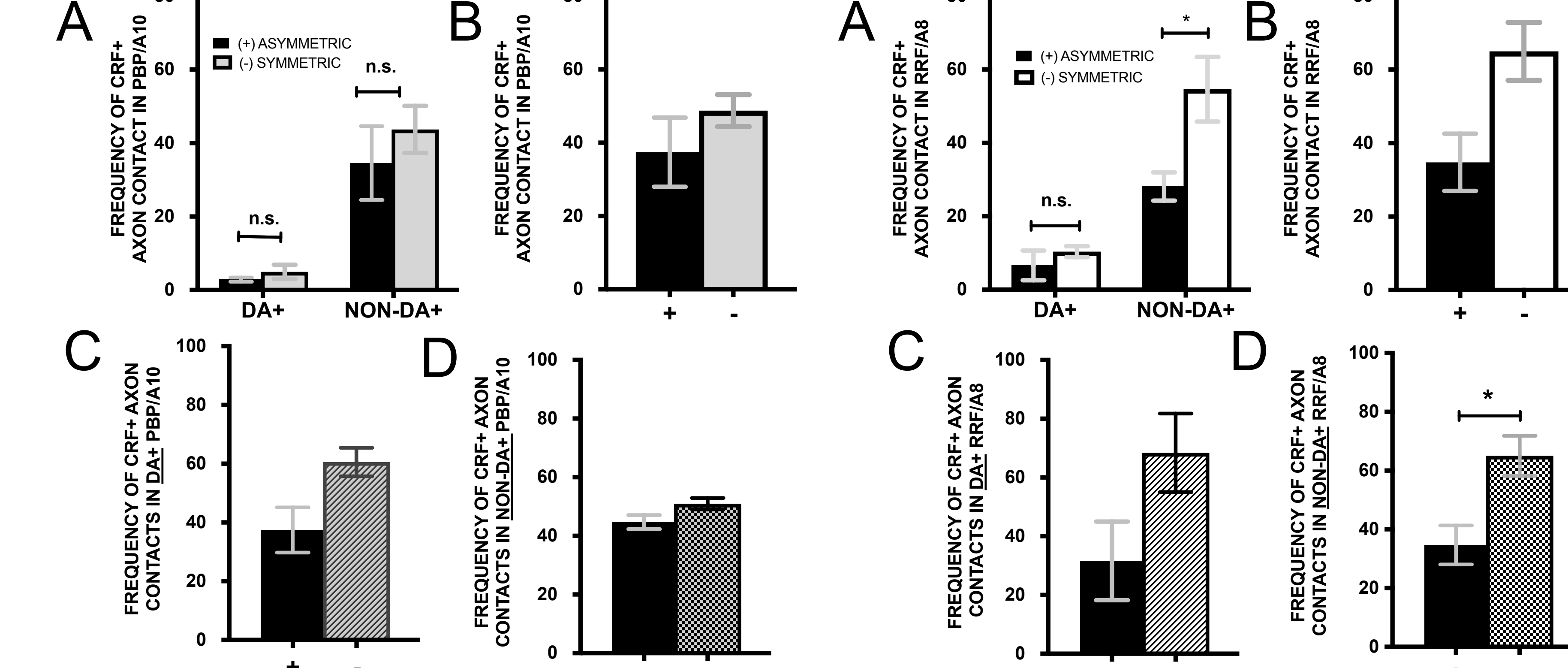


**Fig 7. Electron Microscopy Sample Preparation.** (A) Adjacent tissue sections were immuno-reacted for anti-Corticotropin Releasing Factor (CRF), anti-Calbindin (CaBP), and anti-Tyrosine Hydroxylase (TH). Using staining patterns and blood vessel location across samples, ROI's were selected for EM analysis. (B) Tissue sections were processed for pre-embedding immunoperoxidase reactivity for anti-CRF (DAB visualized) and anti-TH (Gold Silver visualized). (C) Successful processing yielded DAB particulate-filled structures (top panels) and gold particle-filled structures (bottom panels) that did not obstruct pre/postsynaptic visualization of synaptic properties.

## CRF+ fibers predominantly make symmetric contacts on NON-DA+ cells in PBP/A10 and RRF/A8 subpopulations in the macaque midbrain



**Fig 8. CRF+ synaptic profile in the PBP/A10.** Representative examples of pre-embedding immunoreactivity EM in (A) PBP and (B) RRF/A8 shows DAB-filled CRF+ axons and Gold-labeled TH+ dendrites. Excitatory synapses (asymmetric, black arrow heads), inhibitory synapses (symmetric, white arrow heads)



**Fig 9. Quantitative analysis of excitatory (+, asymmetric) vs inhibitory (-, symmetric) synapses in the PBP/A10.** (A) CRF+ axons predominantly form symmetric (-) contacts on non-DA+ cells (B) Total synapse analysis showed no difference between synapse type. (C) No synapse differences were seen in DA+ populations. (D) Within non-DA+ populations only, CRF made significantly more symmetric (-) contacts.

**Fig 10. Quantitative analysis of excitatory (+, asymmetric) vs inhibitory (-, symmetric) synapses in the RRF/A8.** (A) CRF+ axons predominantly form symmetric (-) contacts on non-DA+ cells (B) Total synapse analysis showed no difference between synapse type. (C) No synapse differences were seen in DA+ populations. (D) Within non-DA+ populations only, CRF made significantly more symmetric (-) contacts.

## CONCLUSIONS

- Dopaminergic (TH+) vs GABAergic (GAD-67+) cell comparisons show significantly more TH+ cells in the parabrachial nucleus of the macaque midbrain. GAD-67+ were equally distributed across regions.
- CRF+ axons predominantly make symmetric (inhibitory) contacts on NON-DA cells in both PBP and RRF.
- CRF is largely expressed in putative GABAergic contacts in the PBP and RRF. Although the extended amygdala is only one source of CRF to these regions, our data suggest the hypothesis that extended amygdala inputs are largely inhibitory and indirectly modulate dopamine firing (fig 11).

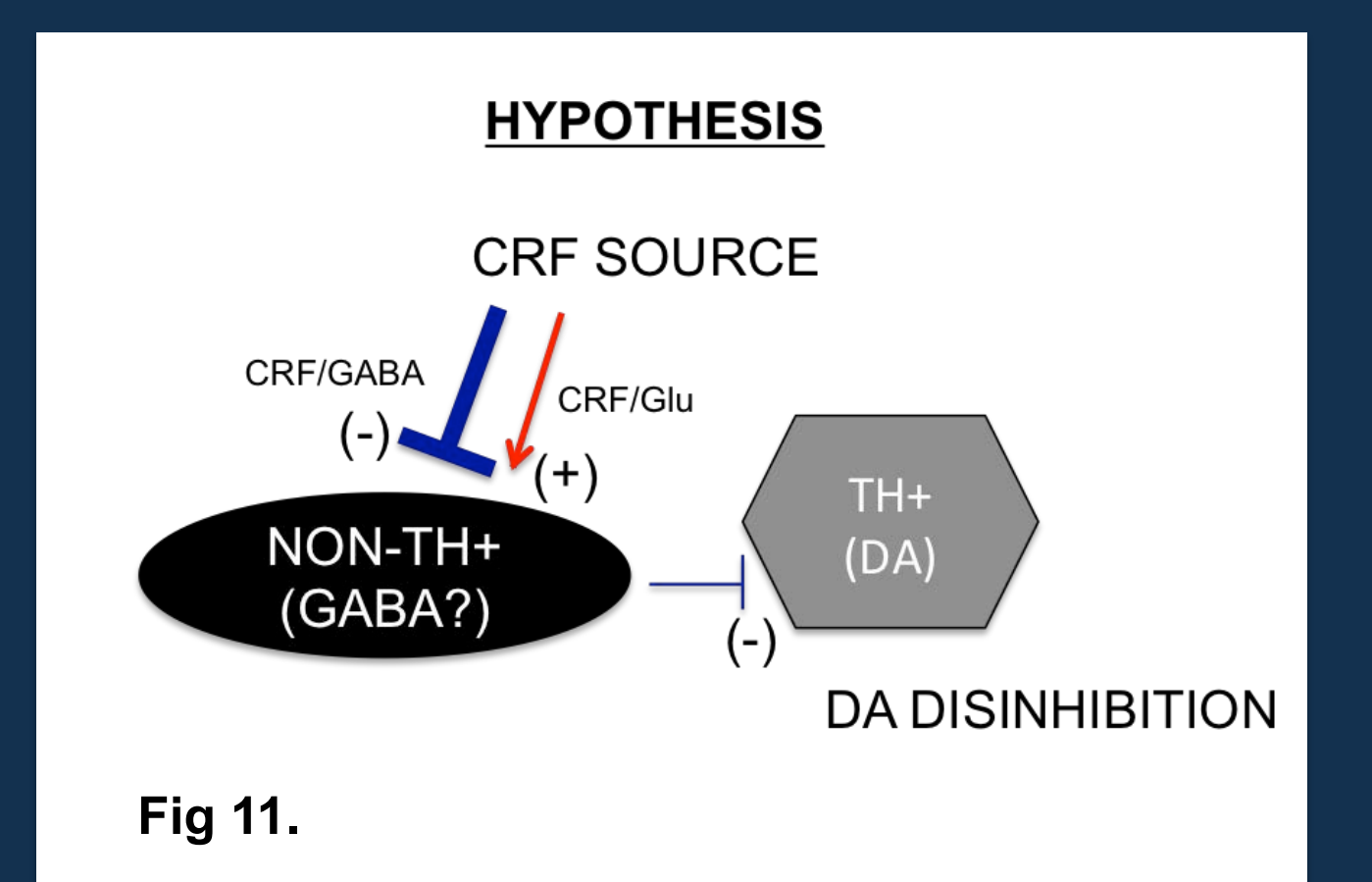


Fig 11.

## REFERENCES

- Fudge et al. (2017). Beyond the classic VTA: extended amygdala projections to the DA-Striatal Paths in the Primate. *Neuropsychopharmacology* 42, 1563-1576.
- Kelly & Fudge (2018). The neuroanatomic complexity of the CRF and DA systems and their interface: What we still don't know. *Neuroscience and Biobehavioral Reviews* 90, 247-259.

This work is supported by the National Institute of Health (NIMH), #RO1MH115016.