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Supplemental Data

Synapses with inhibitory neurons differentiate anterior cingulate from dorsolateral prefrontal pathways associated with cognitive control

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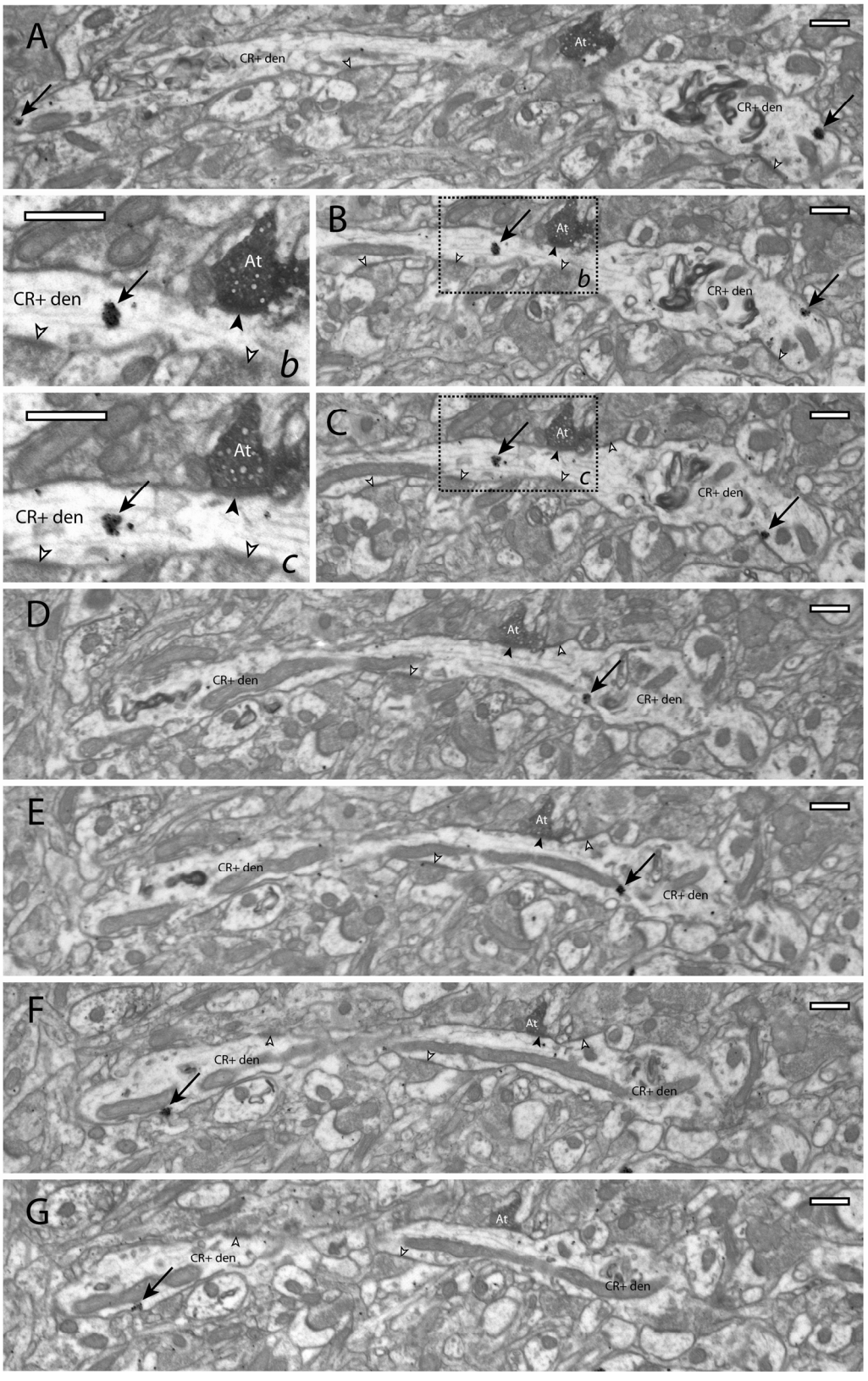
Table S1. Quantitative analysis of synaptic features (mean ± SEM) in pathways from areas 32 and 46 to area 9.

Area 32 to 9					Area 46 to 9				
Post-synaptic target:	Spine	Shaft	Multiple sites		Post-synaptic target:	Spine	Shaft	Multiple sites	
			>1 Spine	Spine + Shaft				>1 Spine	Spine + Shaft
Analysis of labeled synapses									
Proportion of labeled boutons with distinct postsynaptic sites (%)									
n = 345 total	*n = 235*	*n = 67*	*n = 43*		*n = 325 total*	*n = 261*	*n = 39*	*n = 25*	
	69 ± 2%	18 ± 2%	6 ± 1%	7 ± 1%		80 ± 2%	13 ± 1%	5 ± 2%	3 ± 1%
3D EM analysis of reconstructed labeled synapses									
Bouton volume (µm^3)					**Bouton volume (µm^3)**				
n = 91 total	*n = 62*	*n = 14*	*n = 15*		*n = 90 total*	*n = 65*	*n = 13*	*n = 12*	
0.23 ±0.02	0.18 ±0.01	0.26 ±0.03	0.44 ±0.1	0.39 ±0.06	0.15 ±0.01	0.15 ±0.01	0.1 ±0.01	0.2 ±0.07	0.2 ±0.03
Postsynaptic density (PSD) area (µm^2)					**Postsynaptic density (PSD) area (µm^2)**				
0.13 ±0.01	0.13 ±0.02	0.14 ±0.02	0.1 ±0.01 *total PSD:* 0.22 ±0.04	0.13 ±0.03 *total PSD:* 0.29 ±0.06	0.1 ±0.01	0.11 ±0.01	0.06 ±0.01	0.06 ±0.01 *total PSD:* 0.2 ±0.06	0.1 ±0.04 *total PSD:* 0.12 ±0.02
Spine volume (µm^3)					**Spine volume (µm^3)**				
	0.07 ±0.01		0.09 ±0.03	0.07 ±0.02		0.07 ±0.01		0.06 ±0.01	0.08 ±0.03
2D EM analysis of size of labeled synapses									
Bouton major diameter (µm)					**Bouton major diameter (µm)**				
n = 102 total	*n = 61*	*n = 25*	*n = 16*		*n = 95 total*	*n = 73*	*n = 13*	*n = 9*	
0.93 ±0.04	0.9 ±0.04	0.98 ±0.07	0.91 ±0.16	0.9 ±0.05	0.8 ±0.03	0.8 ±0.03	0.7 ±0.06	0.8 ±0.12	0.8 ±0.07
Analysis of unlabeled asymmetric synapses in the surrounding neuropil									
Proportion of unlabeled boutons with distinct postsynaptic sites (%)									
n = 716 total	*n = 519*	*n = 165*	*n = 29*		*n = 888 total*	*n = 712*	*n = 147*	*n = 25*	
	73 ± 1%	22 ± 1%	4 ± 0.1%	2 ± 0.9%		81 ± 2%	15 ± 3%	2 ± 0.4%	0.8 ± 0.3%
Volume of unlabeled boutons (µm^3)					**Volume of unlabeled boutons (µm^3)**				
n = 99 total	*n = 62*	*n = 21*	*n = 16*		*n = 73 total*	*n = 58*	*n = 10*	*n = 5*	
0.12 ±0.01	0.1 ±0.01	0.11 ±0.02	0.25 ±0.1	0.15 ±0.05	0.13 ±0.02	0.13 ±0.01	0.13 ±0.02	0.21 ±0.03	0.17


A
CR+ den
At
CR+ den
B
At
b
CR+ den
CR+ den
At
b
C
At
c
CR+ den
CR+ den
At
c
D
At
CR+ den
CR+ den
E
At
CR+ den
CR+ den
F
At
CR+ den
CR+ den
G
At
CR+ den
CR+ den

Supplemental Figure 1. EM photomicrographs of serial sections **(A-G)** of a bouton (At) from area 32 labeled with FE tracer forming a synapse (black arrowheads in B-F) with an aspiny dendritic shaft positive for calretinin (CR+ den), labeled with silver-enhanced gold (arrows). The CR+ dendrite is cut longitudinally and gold label appears in clumps, sparsely scattered within the dendrite and evident throughout the series of photomicrographs. Note the specificity of the clumps of gold label to the dendrite and the low level of background. The CR+ dendrite is also identified morphologically, based on the prevalence of shaft synapses (mainly asymmetric) from unlabeled boutons (silhouette arrowheads), and lack of spines. Insets in B and C show synapse of labeled bouton (At) at higher magnification. Scale bars = 0.5 μm.