Supporting Information for:

Chemical Gradients within Brain Extracellular Space Measured using Low Flow Push-Pull Perfusion Sampling *in Vivo*

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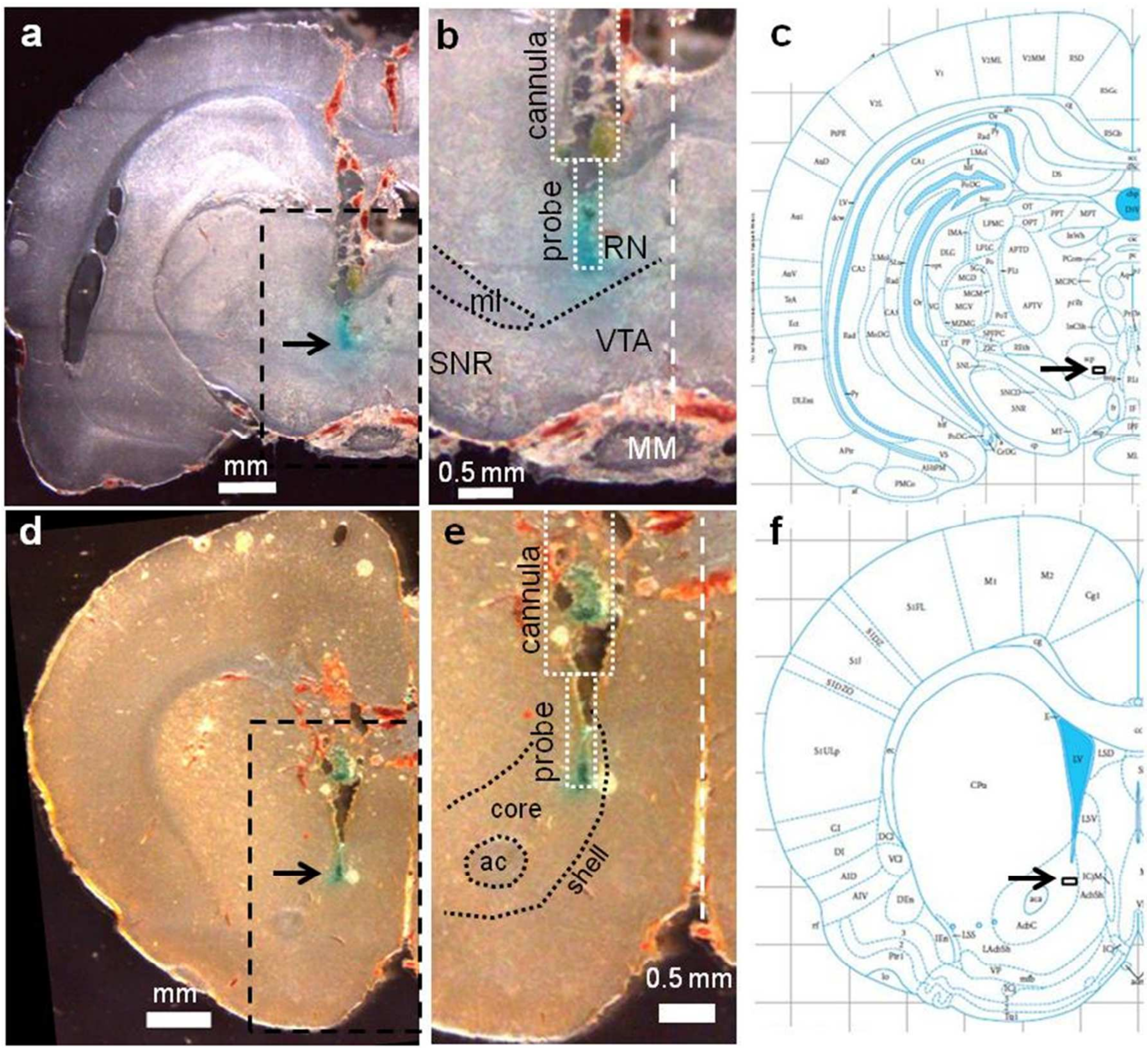

Figure 1. Examples of histology for probe tracking. Coronal brain slices were mounted to microscope slides (**a**, **d**). Fast Green FCF stained the probe track a blue color, whereas the cannula track was inherently visible by displaced tissue (**a**, **b**, **d**, **e**). Anatomical features including white matter, ventricles, and regional boundaries provided references for identifying placements (**b**, **e**). Probe tip placements were then mapped on diagrams of coronal slices(*1*) (**c**, **f**). Panels **b** and **e** include overlays of the probe and cannula locations (drawn to scale), the medial line (white), and visible features. Arrows indicate sampling sites (**a**, **c**, **d**, **e**). Abbreviations: *SNR* – substantia nigra reticulata; *ml* – medial lemniscus; *MM* – medial mammillary nucleus; *ac* – anterior commissure; *RN* – red nucleus; *VTA* – ventral tegmental area.

Region	Neurotransmitter	"No-Net-Flux" Concentration	Species	Push-Pull Concentration
VTA	DA	5.0 ± 0.5 nM(*2*)	♀ Indiana "P"	4.8 ± 1.5 nM
	Glu	3.3 ± 0.8 µM, 4.1 ± 0.5 µM(*3*)	♀ Wistar	8.5 ± 2.7 µM
Accumbens	5-HT	0.7 nM(*4*)	♂ SD	1.3 ± 0.6 nM (core)
		0.7 ± 0.1 nM(*5*) 0.6 ± 0.1 nM(*6*)	♂ Wistar ♂ Wistar	0.6 ± 0.2 nM (shell)
	DA	4.7 ± 0.7 nM(*7*) 5.6 ± 0.4 nM(*5*) 8.3 ± 1.2 nM(*6*)	♂ Holtzman ♂ Wistar ♂ Wistar	7.2 ± 1.2 nM (core) 11 ± 4 nM (shell)
	GABA	32.7 ± 4.0 nM(*8*)	♂ SD	150 ± 110 nM (core) 92 ± 48 nM (shell)
	Glu	1.8 ± 0.4 µM, 2.4 ± 0.5 µM(*9*)	♂ SD	2.4 ± 0.9 µM (core)
		5.6 ± 1.0 µM(*10*)	♂ SD	0.93 ± 0.34 µM (shell)
Striatum (anesthetized)	DA	2.5 ± 0.5 nM(*11*) 6.5 ± 1.1 nM(*12*)	♂ SD ♂ SD	1.7 ± 0.2 nM
	Glu	3.0 ± 0.6 µM(*13*)	♂ SD	1.1 ± 0.2 µM

Table 1. Comparison of concentrations measured by microdialysis calibrated by "no-net-flux", and low-flow push-pull perfusion (this work). Male Sprague-Dawley (SD) rats were utilized for push-pull, whereas animals in referenced microdialysis studies varied, as shown above.

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