

Supplementary Material

Harnessing axonal transport to map reward circuitry: Differing brain-wide projections from medial forebrain domains

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1 Supplementary Data List

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Supplementary Table S1. Abbreviations used in the column graphs in Fig. 6 and 8, in the order in which the columns appear in the graph. Nomenclature is according to the Allen Institute for Brain Science Mouse Brain Reference Atlas.

Abbreviation	Nominal Label	Abbreviation	Nominal Label
AAA	Anterior amygdalar area	MO	Somatomotor areas
ACA	Anterior cingulate area	MOBgl	Main olfactory bulb glomerular
ACB	Nucleus accumbens (a.k., NAc)	MOBgr	Main olfactory bulb granule layer
aco	Anterior commissure olfactory limb	MOBipl	Main olfactory bulb inner plexiform layer
AOB	Accessory olfactory bulb	MOBmi	Main olfactory bulb mitral layer
AON	Anterior olfactory nucleus	MOBopl	Main olfactory bulb outer plexiform layer
aot	Accessory optic tract	MS	Medial septal nucleus
AV	Anteroventral nucleus of thalamus	MY	Medulla
BLA	Basolateral amygdala nucleus	NDB	Diagonal band nucleus
BST	Bed nuclei of the stria terminalis	NOD	Nodulus X
CA1-CA3	Field ca1 ca2 ca3 pyramidal layer	onl	Nerve layer of main olfactory bulb
CB	Cerebellum	opt	Optic tract
cc	Corpus callosum	ORB	Orbital area
CEA	Central amygdalar nucleus	OT	Olfactory tubercle
CLI	Central linear nucleus raphe	P	Pons
CM	Central medial nucleus of the thalamus	PA	Posterior amygdalar nucleus
COA	Cortical amygdala area	PAG	Periaqueductal gray
CP	Caudoputamen	PB	Parabrachial nucleus
CS	Superior central raphe nucleus	PCG	Pontine central gray
CTX	Cerebral cortex	PF	Parafascicular nucleus
DEC	Declive VI	PG	Pontine gray
DG	Dentate gyrus	PL	Prelimbic area
DP	Dorsal peduncular area	PO	Posterior complex of the thalamus
DR	Dorsal raphe nucleus	PRN	Pontine reticular nucleus
em	External medullary lamina	PT	Parataenial nucleus
EPd	Endopiriform nucleus dorsal part	PTL	Posterior parietal association areas
fi	Fimbria	PVT	Paraventricular nucleus of the thalamus
FOTU	Folium-tuber Vermis VII	PYR	Pyramus VIII
FS	Fundus of striatum	RE	Nucleus of reunions
GPe	Globus pallidus	RN	Red nucleus
GR	Gracile nucleus	RSP	Retrosplenial area
HPF	Hippocampal formation	RT	Reticular nucleus of the thalamus
HY	Hypothalamus	SEZ	Subependymal zone
IAM	Interanteromedial nucleus of thalamus	SI	Substantia innominata
ILA	Infralimbic area	SIM	Simple lobule
IMD	Intermedial dorsal thalamus	sm	Stria medullaris
int	Internal capsule	SNe	Substantia nigra compact part
IPN	Interpeduncular nucleus	SNr	Substantia nigra reticular part
LA	Lateral amygdala nucleus	SPA	Subparafascicular area
LC	Loc coeruleus	SS	Somatosensory areas
LGd	Lateral geniculate complex dorsal part	st	Stria terminalis
lot	Lateral olfactory tract body	TT	Taenia tecta dorsal part
LP	Lateral posterior nucleus of the thalamus	UVU	Uvula IX
LSc	Lateral septal nucleus caudal part	V3	Third ventricle
LSr	Lateral septal nucleus rostral part	VAL	Ventral anterior lateral thalamic complex
MB	Midbrain	vhc	Ventral hippocampal commissure
MD	Mediodorsal nucleus of thalamus	VL	Lateral ventricle
MEA	Medial amygdalar area	VM	Ventral posterolateral thalamus
MEP	Median preoptic nucleus	VPL	Ventral posteromedial nucleus of the thalamus
MG	Medial geniculate complex	VPM	Ventral medial thalamic nucleus
MH	Medial habenula	VTA	Ventral tegmental area
MM	Medial mammillary nucleus	ZI	Zona incerta

Supplementary Table S2. Coordinates for Regions of Interest Analysis relative to Bregma (in mm)

Region of Interest	Hemisphere	Coordinates		
		ML	DV	AP
DS	Left	-1.5	0.8	2.7
	Right	1.4	0.8	3.7
GP	Left	-1.6	-0.3	4.7
	Right	1.7	-0.3	4.7
RNT	Left	-1.2	-0.5	4.2
	Right	1.3	-1.0	4.1
NAc/ACB	Left	-1.0	1.0	5.5
	Right	1.2	1.0	5.4
BLA	Left	-3.1	-2.0	5.9
	Right	3.6	-2.0	5.6
SNr	Left	-1.3	-3.1	5.4
	Right	1.5	-3.0	5.4
VTA	Left	-0.8	-3.0	5.2
	Right	0.7	-3.0	5.1
LC	Left	-0.9	-5.7	4.4
	Right	0.8	-5.7	4.6

Bregma locations corresponding to FSL (*fslroi*) defined 3 x 3 x 3 voxel cubes.

Supplementary Table S3. ROI Measurements

See Supplementary Table S3, a separate file available as Excel.

Supplementary Table S4. ROI Statistics (pdf)

Supplementary Table S4. Statistics for ROI within and between group analyses Figures 5 and 7 in the Main text.

Statistics for ACA within group analysis for differences at 6h vs 24h

ROI	t.ratio	p value	Asterisks
DS-L2	3.289	0.0006	*** <0.001
GP_L	-2.861	0.0045	** <0.01
GP_R	-4.304	<0.0001	****
NAC_R2	-1.682	0.0936	* <0.1
RNT_L2	3.212	0.0014	* <0.01
RNT_R2	4.468	<0.0001	****
SNR_L	-2.788	0.0056	** <0.01
SNR_R	-5.578	<0.0001	****
LC_L	3.124	0.0019	** <0.01
LC_R	2.680	0.0077	** <0.01

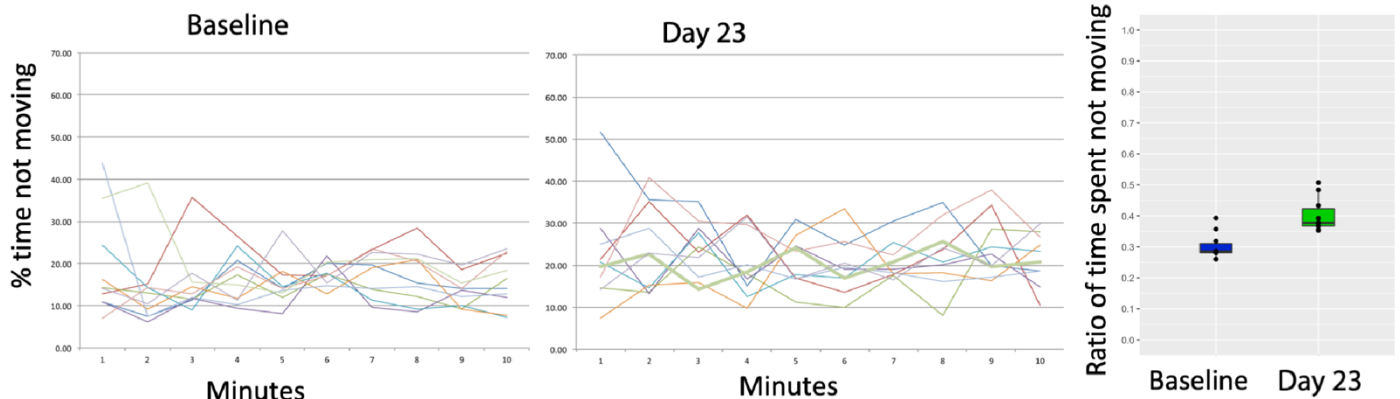
Statistics for between ACA and IL/PL cohorts at 24h post-injection

Region	t.ratio	p value	Asterisks
DS_L2	0.39	0.0642	* <0.1
DS_R2	3.917	0.0009	**** <0.001
RNT_L2	2.1	0.0486	** <0.05
BLA_L	-1.692	0.106	+ <= 0.2
SNr_R	3.624	0.0017	***<0.005

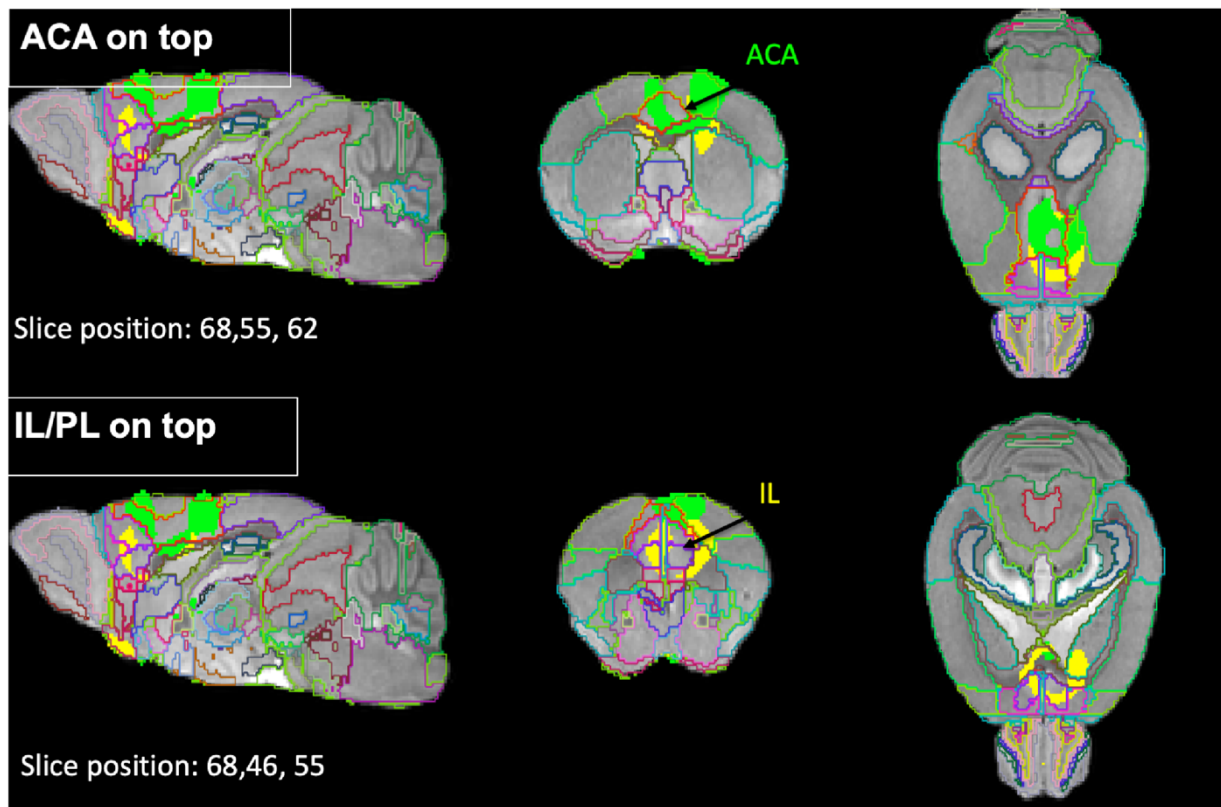
2.2 Supplementary Figures

Supplementary Figure S1: Time spent not moving

Mice were video recorded during the last 10m of 30m time spent in a custom arena at two timepoints before the forebrain injections: At baseline before any handling and at 23 days after handling, imaging and housing. Time spent not-moving within each 1-minute interval was tabulated in Ethovision and results graphed in Excel (Microsoft Office). Statistical comparisons were performed in R by ANOVA between these two time points. A small but statistically significant difference was found between baseline and 23d ($p < 0.01$).



Supplementary Figure S2. Statistical maps of ACA and IL/PL injection sites



The 30m post-injection images for each cohort were compared to pre-injection image by a within-group paired t-test in SPM. Resultant maps of significantly enhanced voxels at a threshold of $p < 0.05$ FDR corrected (T values: IL/PL, $T = 4.75$; ACA, $T = 4.05$) were overlaid on the template image and with *InVivo Atlas* v.10 on the last layer to define position. Slice positions are indicated as voxel positions in the 3D dataset. Note that slice position of the injection halo representing Mn(II) diffusion out of the injection site differs in AP dimension by 9 voxels (0.9mm) and in the DV dimension by 7 voxels (0.7mm), and that there is some overlap in the region between the two sites. Bright pink outline delineates the ACA, and purple the Infralimbic segments as indicated on the coronal slices.