Cell Centered Database
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Microscopy Product #:3592 030304a

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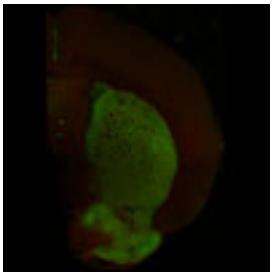
http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=3592

Image2D	Reconstruction	Segmentation
		

Project Information:

PROJECT_ID	P1207
PROJECT_NAME	Correlative microscopic characterization of dendritic spines in a transgenic mouse model of hyperdopaminergia: The dopamine transporter knockout mouse
PROJECT_DESCRIPTION	Multiscale characterization of DAT KO transgenic mouse
LEADER	Diana Price
FUNDING_AGENCY	NIH
PROJECT_START_DATE	2003-01-01 00:00:00.0
PROJECT_END_DATE	
COLLABORATORS	Aki Laakso, Michele Cyr, Maryann Martone, Naoko Yamada, Andrea Thor, Monica Berlanga
PUBLICATION1	
PUBLICATION2	
PUBLICATION3	

Experiment Information -	
PURPOSE	Immunocytochemical localization of VMAT+DARPP-32
TITLE	P1207 Exp 3
EXPERIMENTER	Diana Price
EXPERIMENT_NAME	
EXPERIMENT_DATE	2003-09-17 00:00:00.0

Subject Information -	
GROUP_BY	Genetic manipulation
SUBJECT_NAME	Wildtype control
FIXATION_METHOD_ID	
SCIENTIFIC_NAME	mus musculus
SPECIES	mouse
STRAIN	C57BL6/129SvJ
AGE	days
AGECLASS	adult
ANIMAL_NAME	
LITTER_ID	
SEX	unspecified
VENDOR	obtained from Marc Caron, Duke University
WEIGHT	grams

Tissue -	
ANATOMIC_LOCATION	striatum
MICROTOME	vibratome
ORIENTATION	coronal
THICKNESS	80 um
TISSUE_PROD_STORAGE	Slide box: DAT KO
EXTERNAL_FILE_NAME	
TISSUE_GROUP_TYPE	double labeled

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	3592
IMAGE_BASENAME	030304a
CREATE_DATE	2004-03-03 00:00:00.0
INSTRUMENT	BioRad RTS 2000 Multiphoton
MICROSCOPE_TYPE	MULTIPHOTON
PLANE_COUNT	8772
PRODUCT_TYPE	MOSAIC
PURL	
SESSION_NAME	
TELESCIENCE_SRB	P1207/Experiment_3412/Subject_117/Tissue_135/Microscopy_3592
X_RESOLUTION	.24 um/pixels
Y_RESOLUTION	.24 um/pixels
XSIZE	480
YSIZE	480

Protocol:

P1207: Experiment #3 DAT KO Mouse 9/17/03

Description: Immunolabeling study of VMAT+DARPP-32+Hoescht 33342

Animals: Brains sent from Duke University 9/10/03 (wt 1&2, tg 1&2 = 4 total)

Protocol

1. Perfusion (at Duke U.)
Nembutal; 4% paraformaldehyde + 0.1% gluteraldehyde
Sectioned on Vibratome at NCMIR (80 microns)

2. Wash 3x with PBS 1X (on ice) 3x @ 10min 1 1st 1 2nd 1 3r

3. Make up blocking buffer
PBS w/o NaCl = buffer used
Total amount needed = 33 x 2 mls

Double the following:
Ingredient Amount
0.8 PBS 6.6 ml 5X PBS + 24.2 ml 2x distilled H20
3% NDS (24 , 7/4) 0.96 ml
1% fish gel 3.3 ml
0.3% Triton X-100 0.0996 ml
1% BSA 0.33 g

4. Block slices (2 hr) in blocking buffer
Time started = 12:40 pm 9/17/03
Time ended = 2:50 pm 9/17/03

5. Make up working buffer
" Use blocking buffer to dilute to working buffer

Ingredient 500ml 200ml 150ml 100ml
Blocking buffer 50 ml 20 ml 15 ml 10 ml
0.1% Triton 0.5ml 0.2 ml 0.15 ml 0.1 ml
1X PBS 450 ml 180 ml 135 ml 90 ml

6. Wash 1X5 minutes with working buffer: 1

7. Add 1o Abs diluted in working buffer

anti-VMAT-2; Host = guinea pig; 1:500 (Oncogene, catalog # 503-01-50)
anti-DARPP-32; Host = mouse; 1:500 (BD Transduction Laboratories, catalog #611520)

8. Place on shaker in cold room labeled & covered with aluminum foil overnight
Time started = 4:30 pm 9/17/03
Time ended = 10:30 am 9/18/03

9. Wash 3x with working buffer 3x @ 10min 1 1st 1 2nd 1 3rdh

10. Prepare 2o Abs : all 1:100

donkey ¿ mouse AF488 (Molecular Probes, Cat #A21202)
donkey ¿ guinea pig RRX (Jackson Immunoresearch Laboratories, Inc)

11. Let sit on shaker covered with foil for 2 hrs at RT
Time started = 12:35pm 9/18/03
Time ended = 2:50pm 9/18/03

12. Wash 3x with 1X PBS 0.8 3x @ 5min 1 1st 1 2nd 1 3rd

13. Prepare nuclear stain (Hoescht 1:1000 for 15-30 minutes)

14. Wash 3x with 1X PBS 0.8 3x @ 10min1 1st 1 2nd 1 3rd

15. Mount sections on slides and coverslip using gelvatol

16. Dry flat in fridge 24-48 hours and seal with nail polish

Image Type -	
MOSAIC_ID	6020
X_POSITION	45 tiles
Y_POSITION	65 tiles
MOSAIC_DESC	10% overlap between tiles
OPTICAL_SECTION_SERIES	6025
OPTICAL_Z_RESOLUTION	3 um

Specimen Description -	
ANATOMICAL_DETAIL	6091
ATLAS_COORD	, ,
ORGAN	brain
REGION	neostriatum
SYSTEM	central nervous system

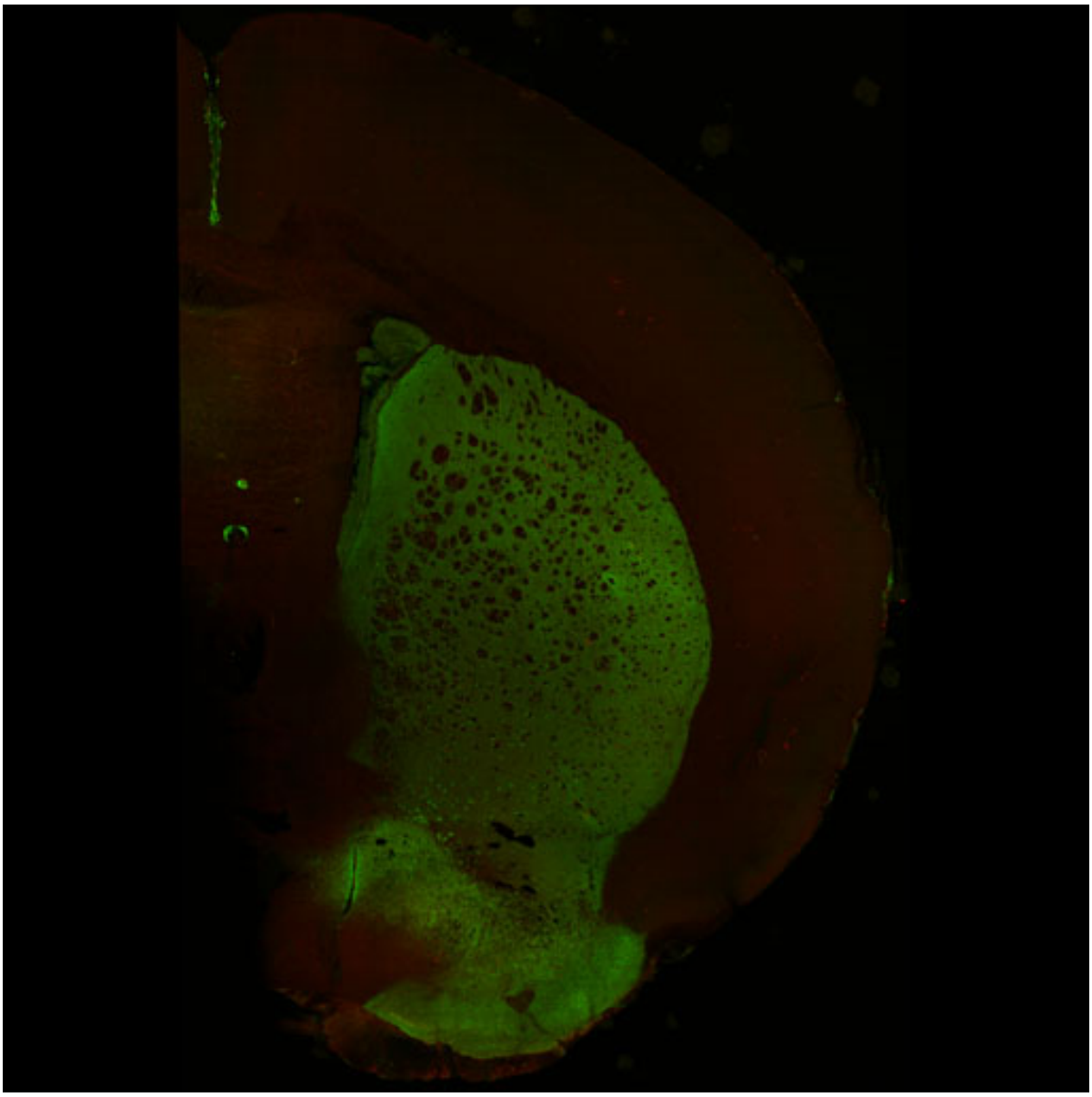
Light Microscopy Product -	
LMPRODUCT_ID	6044
COVER_SLIP_THICKNESS	1 #
IMMERSION_MEDIUM	oil
LENS	Nikon Plan Apo
MOUNTING_MEDIUM	gelvatol
NUMERICAL_APERTURE	1.45

Raw 2D Image

Raw Low Resolution 2D Image -

Reconstruction

Reconstruction Image -



Reconstruction -	
RECONSTRUCTION3D_ID	6070
CROPPING_COORDINATE1	,
CROPPING_COORDINATE2	,
RECON_DATE	2004-03-02 00:00:00.0
RECON_DESC	Tiff image of the Z projection of the processed mosaic (~1.7 Gb)
RECON_PROGRAM	Custom montaging software using Image J plug ins
RECON_TYPE	Processed mosiac
VOLUME_DIMENSION	20325, 29989, 1
VOLUME_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_3412/Subject_118/Tissue_136/Microscopy_3593/030204A_tg.tif
VOXEL_SCALE	.24, .24, 9
RECONSTRUCTION_IMAGES_ID	6070
NEUROINFORMATICA_URL	http://ccdb-aims.ucsd.edu:8880/slide.jsp?fn=10md5:dcb28b284db5f025fc3a1c3dbb07cfcc&mag=1
RECON_IMAGE_DESC	Large scale brain mosaic showing the distribution of DARRP-32 (green) and VMAT-2 (red) in a hemisection through the anterior neostriatum. To explore the full resolution dataset, click on the "N" in the thumbnail window. This will open up the virtual microscope image explorer.
RECON_FILE_NAME	P1207/Experiment_3412/Subject_117/Tissue_135/Microscopy_3592/030304a_vol.jpg
VOLUME_THUMBNAIL	P1207/030304a_vol_thmb.jpg

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USER NOTIFICATION

For large size image data, it will take several minutes to download, please be patient. Thanks!

ACKNOWLEDGEMENT

Data used from the CCDB should be appropriately referenced, including both the author of the data and the CCDB. If the data were from a published study, the reference is included in the database record. The following reference should be cited for the CCDB:

Martone, M. E., Gupta, A., Wong, M., Qian, X., Sosinsky, G., Ludaescher, B., and Ellisman, M. H. A cell centered database for electron tomographic data. J. Struct. Biology 138: 145-155, 2002.

In addition, the support for the Cell Centered Database should be included in the acknolwedgement section of any publication:

The Cell Centered Database is supported by NIH grants from NCRR RR04050, RR RR08605 and the Human Brain Project DA016602 from the National Institute on Drug Abuse, the National Institute of Biomedical Imaging and Bioengineering and the National Institute of Mental Health, and NSF grants supporting the National Partnership for Advanced Computational Infrastructure NSF-ASC 97-5249 and MCB-9728338.

Maryann Martone