Cell Centered Database
University of California, San Diego
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Microscopy Product #:4067 112006eeee

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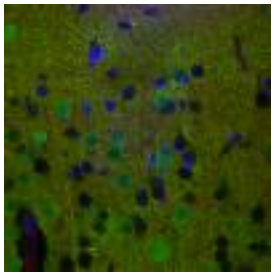
http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=4067

Image2D	Reconstruction	Segmentation
		

Project Information:

PROJECT_ID	P1723
PROJECT_NAME	Localization of Metabotropic Glutamate Receptors in Alpha Synuclein Overexpressing Mouse
PROJECT_DESCRIPTION	Characterization of staining for mGluR5 glutamate receptor in animal model of Parkinsonian disorders
LEADER	Diana Price
FUNDING_AGENCY	Branfman Family Foundation
PROJECT_START_DATE	
PROJECT_END_DATE	
COLLABORATORS	Edward Rockenstein, Eliezer Masliah, Mark Ellisman
PUBLICATION1	
PUBLICATION2	
PUBLICATION3	

Experiment Information -	
PURPOSE	To determine the relationship between mGluR5 and alpha synuclein staining in different lines of alpha synuclein overexpressing mouse
TITLE	Comparison of mGluR5 and synuclein staining
EXPERIMENTER	Diana Price
EXPERIMENT_NAME	
EXPERIMENT_DATE	

Subject Information -	
GROUP_BY	Genetic Modification
SUBJECT_NAME	Thy-1/asyn
FIXATION_METHOD_ID	
SCIENTIFIC_NAME	mus musculus
SPECIES	mouse
STRAIN	C57BL/6-DBA/2
AGE	days
AGECLASS	adult
ANIMAL_NAME	
LITTER_ID	
SEX	male
VENDOR	Eliezer Masliah
WEIGHT	grams

Tissue -	
ANATOMIC_LOCATION	
MICROTOME	vibratome
ORIENTATION	coronal
THICKNESS	80 um
TISSUE_PROD_STORAGE	
EXTERNAL_FILE_NAME	
TISSUE_GROUP_TYPE	triple label

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	4067
IMAGE_BASENAME	112006eeee
CREATE_DATE	2006-11-20 00:00:00.0
INSTRUMENT	Olympus Fluoview 1000
MICROSCOPE_TYPE	LASER SCANNING CONFOCAL
PLANE_COUNT	1
PRODUCT_TYPE	SURVEY
PURL	
SESSION_NAME	Survey of multiple brain areas
TELESCIENCE_SRB	P1723/Experiment_3482/Subject_256/Tissue_369/Microscopy_4067
X_RESOLUTION	.207 um/pixels
Y_RESOLUTION	.207 um/pixels
XSIZE	1024
YSIZE	1024

Protocol:

For more information on transgenic animals, see: Rockenstein et al 2002 E. Rockenstein, M. Mallory, M. Hashimoto, D. Song, C.W. Shults, I. Lang and E. Masliah, Differential neuropathological alterations in transgenic mice expressing alpha-synuclein from the platelet-derived growth factor and Thy-1 promoters, J Neurosci Res 68 (2002), pp. 568 578

Specimen processing: Tissue section acquisition from transgenic animals

Animals were deeply anesthetized with Nembutal" (pentobarbital) and perfused via intracardiac catheterization. Perfusion with oxygenated Ringer's solution containing 250U/ml heparin, 0.2 mg/ml xylocaine and 1% dextrose was followed 4% paraformadehyde in 0.1 M phosphate buffer solution (PBS) (both at 37 degrees Celsius). The brains were carefully removed from the skull and postfixed for 1 hour in the same fixative used in the perfusion. The brain was blocked and cut into 2 mm thick sections using an acrylic brain matrix (David Kopf; Tujunga, CA) to facilitate reproducibility of sections. These thick sections were then sectioned into 80 micron thick coronal sections using a Vibratome (VT1000E, Leica Microsystems, Wetzlar , Germany).

Specimen processing: Immunocytochemistry

Tissue sections were incubated with monoclonal anti- a-syn (1:250; BD Transduction Laboratories, San Diego, CA, Catalog #AB610787) and rabbit anti-mGluR5 (1:250; Chemicon, Temecula, CA, Catalog #AB5675) followed by incubation with donkey anti-mouse Alexa Fluor 488 (1:100, Molecular Probes, Carlsbad, CA) and donkey anti-rabbit RRX (1:100, Jackson Immunoresearch Laboratories, Inc., West Grove, PA, USA) overnight at 4C. The immunolabeling procedure consisted of the following steps: (1) 6x5 min rinses in 0.1 M PBS; (2) 1 hr blocking step in PBS containing 3% normal donkey (NDS), 0.1% Triton X-100, 1% fish gelatin, and 1% BSA; (3) 48 hr incubation in primary antibodies diluted in working buffer (PBS, 1% NDS) at 20 degrees C; (4) 6 x 5 minute rinsed in working buffer; (5) 24 hr incubation in working buffer containing donkey anti-mouse Alexa Fluor 488 (Molecular Probes, Carlsbad, CA) and donkey anti-rabbit RRX (Jackson Immunoresearch Laboratories, Inc., West Grove, PA, USA). (6) 6 x 10 min rinses in working buffer; (7) 3 x 10 min rinses in PBS; (8) the sections were free floated onto slides and coverslipped using ProLong mounting media (Invitrogen Molecular Probes, Carlsbad, CA) with DAPI nuclear stain. Controls for the mGluR5 antibody experiments included both preabsorption with the control peptide (Chemicon, Catalog #AG374), as well as primary omission studies, which both revealed a lack of non-specific staining. Controls for other antibodies used were performed via omission of primary antibodies, and revealed no non-specific staining. All steps were conducted at 4 degrees C, on wet ice and with ice-cold solutions

Specimen Preparation Information:

Specimen Preparation Information -	
PROTOCOL_ID	15692
PROTOCOL_NAME	Immunolabeling P1723
PROTOCOL_DESCRIPTION	Double labeling immunolabeling of alpha synuclein and mGlR5
Protocol Steps:	1)Molecular Localization(15740) 2)Molecular Localization(15749) 3)Stain(15765) 4)Chemical(15690) 5)Microtomy(15691)

Molecular Localization (15740)

Molecular Target	MOLECULAR TARGET ID: 15741 MOLECULAR LOCALIZATION ID: 15740 MOLECULE: synuclein ISO FROM: alpha MOLECULAR CLASS: protein ABBREVIATION: Snca ENTREZ_ID: 20617
Probe used	PROBE ID: 15742 CONTROLS: omitted primary antibody
	Antibody ID: 15743 Clonality: monoclonalRaised in animal: mouse Antibody type: IgG

Reagent (15696)

Reagent name	anti alpha synuclein antibody
Temperature	
Chemical	Chemical ID: 15695 Chemical name: anti alpha synuclein antibody Vendor: BD Transduction Laboratories Concentration: .25 % Catalog number: AB610787
	Chemical ID: 15704 Chemical name: normal donkey serum Concentration: 1 %
	Chemical ID: 24 Chemical name: phosphate buffer Concentration: .1 M pH: 7.4
	Chemical ID: 31 Chemical name: saline Concentration: .9 % Chemical notes: normal saline

Detection method	Molecule reagent ID: 15709 Molecular type: antibody Chromagen :Alexa 488

Molecular Localization (15749)

Molecular Target	MOLECULAR TARGET ID: 15750 MOLECULAR LOCALIZATION ID: 15749 MOLECULE: metabotropic glutamate receptor ISO FROM: 5 MOLECULAR CLASS: protein ABBREVIATION: GRM5 ENTREZ_ID: 108071
Probe used	PROBE ID: 15751 CONTROLS: omitted primary antibody
	Antibody ID: 15752 Clonality: polyclonalRaised in animal: rabbit Antibody type: IgG

Reagent (15714)

Reagent name	anti mGluR5 antibody
Temperature	
Chemical	Chemical ID: 15719 Chemical name: anti mGluR5 antibody Vendor: Chemicon Concentration: .25 % Catalog number: AB5675

Detection method	Molecule reagent ID: 15721 Molecular type: antibody Chromagen :Rhodamine Red X

Stain (15765)

Stain ID	15765
Prepared by	Diana Price
Temperature	
Stain notes	DAPI is dissolved in ProLong Mounting medium and applied at time of coverslipping
Reagent	Reagent (15760)

Reagent (15760)

Reagent name	DAPI in ProLong
Temperature	
Chemical	Chemical ID: 15758 Chemical name: DAPI Concentration:
	Chemical ID: 15759 Chemical name: ProLong mounting medium Vendor: Molecular Probes Concentration:

Chemical Fixation (15690)

Time of fixation	
Temperature	37 C
Fixative volume	
Fixation method	perfusion

Microtomy (15691)

Microtome	0
Thickness	80 um
Temperature	
Embedding agent	0
Microtomy notes	Vibratome

Specimen Description -	
ANATOMICAL_DETAIL	17115
ATLAS_COORD	, ,
ORGAN	brain
REGION	cerebral cortex
SYSTEM	central nervous system

Imaging Parameters:

Image Type -	
OPTICAL_SECTION_SERIES	17114
OPTICAL_SECTION_SERIES_DESC	Only a single optical section was acquired for each image.

Light Microscopy Product -	
LMPRODUCT_ID	17116
IMMERSION_MEDIUM	oil
LENS	Olympus PlanApo 60X oil
LENS_MAGNIFICATION	60 X
MOUNTING_MEDIUM	Prolong (Molecular Probes)
NUMERICAL_APERTURE	1.42
LM_NOTES	DAPI was added to the mounting medium.

Confocal channel (17125)

Confocal image ID	17125
Fluorophor	Rhodamine Red X
Color	Red
Exitation wavelength	543 nm
Emission wavelength	591 nm

Molecular Localization (15749)

Molecular Target	MOLECULAR TARGET ID: 15750 MOLECULAR LOCALIZATION ID: 15749 MOLECULE: metabotropic glutamate receptor ISO FROM: 5 MOLECULAR CLASS: protein ABBREVIATION: GRM5 ENTREZ_ID: 108071

Confocal channel (17133)	
Confocal image ID	17133
Fluorophor	DAPI
Color	Blue
Exitation wavelength	405 nm
Emission wavelength	461 nm
Stain (15765)	
Stain ID	15765
Stain reagent ID	15760
Prepared by	Diana Price
Temperature	
Stain notes	DAPI is dissolved in ProLong Mounting medium and applied at time of coverslipping

Confocal channel (17119)

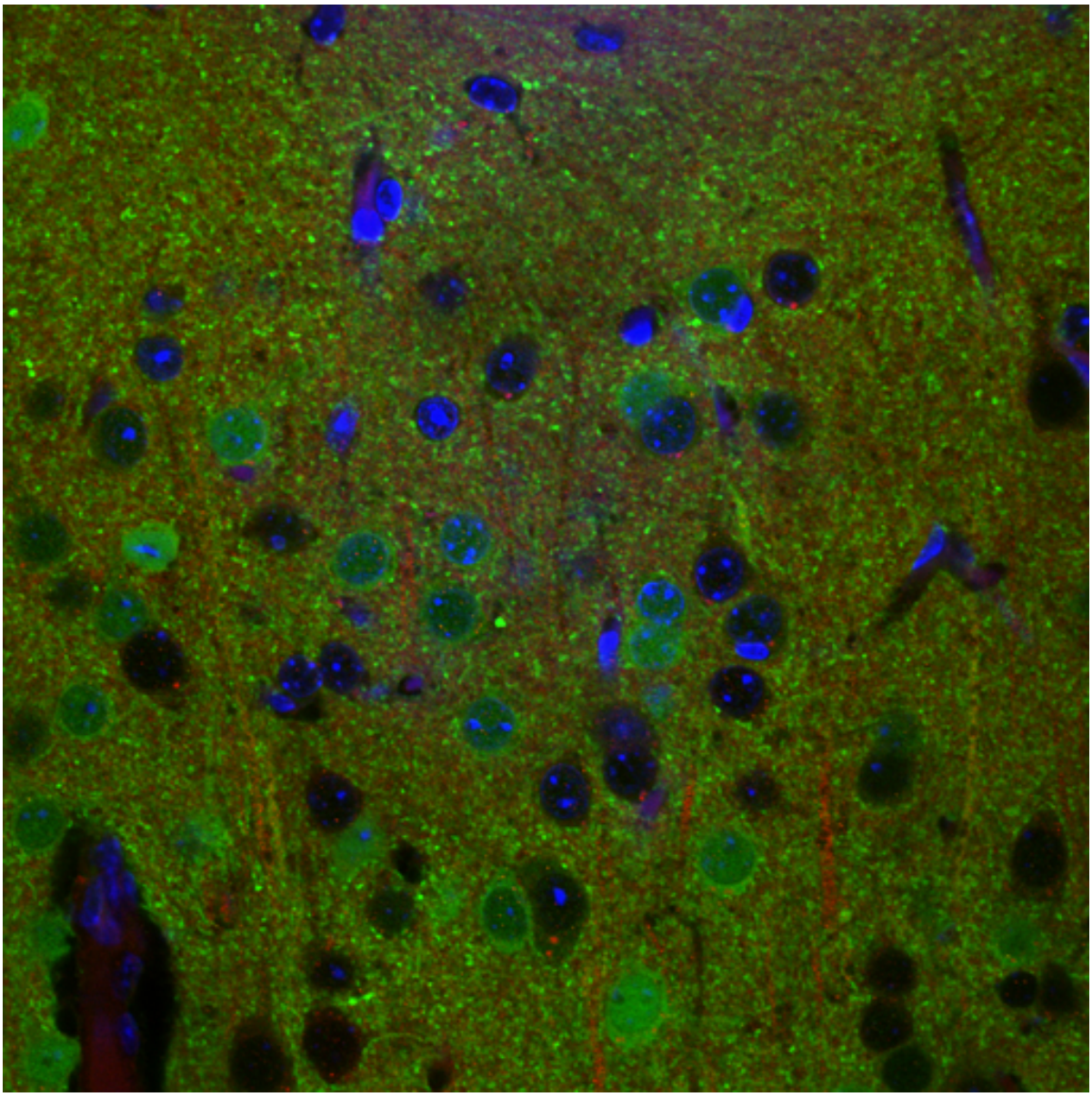
Confocal image ID	17119
Fluorophor	Alexa 488
Color	Green
Exitation wavelength	488 nm
Emission wavelength	520 nm

Molecular Localization (15740)

Molecular Target	MOLECULAR TARGET ID: 15741 MOLECULAR LOCALIZATION ID: 15740 MOLECULE: synuclein ISO FROM: alpha MOLECULAR CLASS: protein ABBREVIATION: Snca ENTREZ_ID: 20617

Raw 2D Image

Raw Low Resolution 2D Image -



Raw 2D Image -	
IMAGE2D_ID	17141
IMAGE_DATE	2006-11-20 00:00:00.0
IMAGE_DESC	Zip archive containing the 3 channel image file in tiff format (112006eeee_RGB.tif). Also included is the .oif header file generated by the Olympus Fluoview, which gives additional detail on microscope settings. Also included is a control section in which the primary antibody was omitted from the immunolabeling reaction (112006h.tif). Although the control was from the same experiment, it was not able to be determined whether it was from the same animal.
IMAGE_FILE_FORMAT	tiff
IMAGE_FILE_NAME	/usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/112006eeee_img.jpg
RAW_DATA_FILE	/telescience/home/CCDB_DATA_USER.portal/P1723/Experiment_3482/Subject_256/Tissue_369/Microscopy_4067/112006eeee_img.zip
THUMBNAIL_DESC	Triple labeled confocal image of the cerebral cortex of a transgenic mouse engineered to overexpress alpha synuclein under the Thy-1 promotor, immunolabeled for mGluR5 (red), alpha synuclein (green) and counterstained with DAPI (blue) to reveal cell nuclei. An image of a no primary control is available for download; some light non-specific labeling was visible in the red and green channels.
THUMBNAIL_FILE	/usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/112006eeee_img_thmb.jpg
X_RESOLUTION	.207 um/pixel
Y_RESOLUTION	.207 um/pixel
X_SIZE	1024 pixels
Y_SIZE	1024 pixels

USER AGREEMENT

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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