

Cell Centered Database

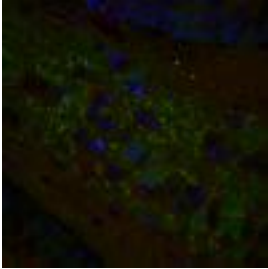
University of California, San Diego

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Microscopy Product #:4058 112006d

For the most updated information, please visit

<http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=4058>

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	Brannman 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	unspecified
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	4058
IMAGE_BASENAME	112006d
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	
TELESCIENCE_SRB	P1723/Experiment_3482/Subject_255/Tissue_368/Microscopy_4058
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% paraformaldehyde 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 mGIR5
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: monoclonal Raised in animal: mouse
Antibody type: IgG

Reagent (15696)

Reagent name	anti alpha synuclein antibody
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Temperature	
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Chemical	Chemical ID: 15695 Chemical name: anti alpha synuclein antibody Vendor: BD Transduction Laboratories Concentration: .25 % Catalog number: AB610787
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Chemical ID: 15704 Chemical name: normal donkey serum Concentration: 1 %
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Chemical ID: 24 Chemical name: phosphate buffer Concentration: .1 M pH: 7.4
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Chemical ID: 31 Chemical name: saline Concentration: .9 % Chemical notes: normal saline
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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: polyclonal
Raised 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 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	16746
ATLAS_COORD	, ,
ORGAN	brain
REGION	dentate gyrus
SYSTEM	central nervous system

Imaging Parameters:

Image Type -	
OPTICAL_SECTION_SERIES	16745
OPTICAL_SECTION_SERIES_DESCRIPTION	Only a single optical section was acquired for each image.

Light Microscopy Product -	
LMPRODUCT_ID	16747
IMMERSION_MEDIUM	oil
LENS	Olympus PlanApo 60X oil
LENS_MAGNIFICATION	60 X
MOUNTING_MEDIUM	Prolong (Molecular Probes)
NUMERICAL_APERTURE	1.42
LM_NOTES	mmartone

Confocal channel (16750)

Confocal image ID

16750

Fluorophor

Alexa 488

Color

Green

Excitation 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

Confocal channel (16756)

Confocal image ID

16756

Fluorophor

Rhodamine Red X

Color

Red

Excitation 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 (16764)	
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Confocal image ID	16764
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Confocal notes	null
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Fluorophor	DAPI
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Color	Blue
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Excitation wavelength	405 nm
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Emission wavelength	461 nm
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Stain (15765)	
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Stain ID	15765
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Stain reagent ID	15760
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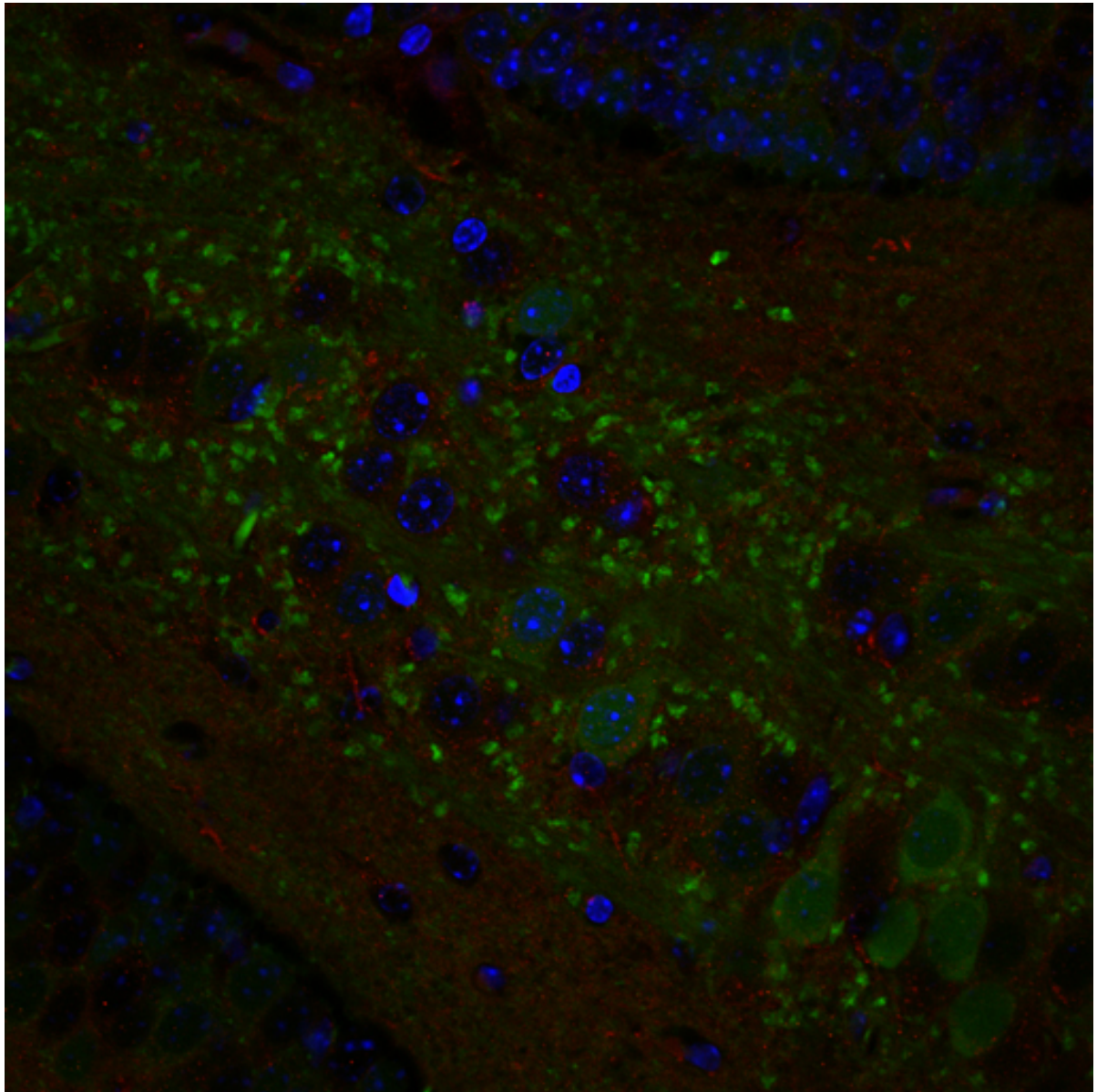
Prepared by	Diana Price
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Temperature	
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Stain notes	DAPI is dissolved in ProLong Mounting medium and applied at time of coverslipping
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Raw 2D Image

Raw Low Resolution 2D Image -



Raw 2D Image -	
IMAGE2D_ID	16784
IMAGE_DATE	2006-11-20 00:00:00.0
IMAGE_DESC	Zip archive containing the 3 channel image file in tiff format (112006d_RGB.tif). Also included is the .oif header file generated by the Olympus Fluoview, which gives additional detail on microscope settings.
IMAGE_FILE_FORMAT	tiff
IMAGE_FILE_NAME	/usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/112006d_img.jpg
RAW_DATA_FILE	/telescience/home/CCDB_DATA_USER.portal/P1723/Experiment_3482/Subject_255/Tissue_368/Microscopy_4058/112006d_img.jpg
THUMBNAIL_DESC	Triple labeled confocal image of the dentate gyrus 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 nuclei.
THUMBNAIL_FILE	/usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/112006d_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 acknowledgement 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