

Cell Centered Database

University of California, San Diego

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Microscopy Product #:4059 112006dd

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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	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	4059
IMAGE_BASENAME	112006dd
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_4059
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 by 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- α -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 4°C. 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 rinses 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
	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: polyclonal
Raised in animal: rabbit
Antibody type: IgG

Reagent (15714)

Reagent name	anti mGluR5 antibody
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Temperature	
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Chemical	Chemical ID: 15719 Chemical name: anti mGluR5 antibody Vendor: Chemicon Concentration: .25 % Catalog number: AB5675
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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	16793
ATLAS_COORD	, ,
ORGAN	brain
REGION	hippocampus
SYSTEM	central nervous system

Imaging Parameters:

Image Type -	
OPTICAL_SECTION_SERIES	16792
OPTICAL_SECTION_SERIES_D ESC	Only a single optical slice was acquired for each image.

Light Microscopy Product -	
LMPRODUCT_ID	16794
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 (16803)

Confocal image ID 16803

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 (16811)

Confocal image ID 16811

Fluorophor DAPI

Color Blue

Excitation 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 (16797)

Confocal image ID 16797

Fluorophor Alexa 488

Color Green

Excitation wavelength 488 nm

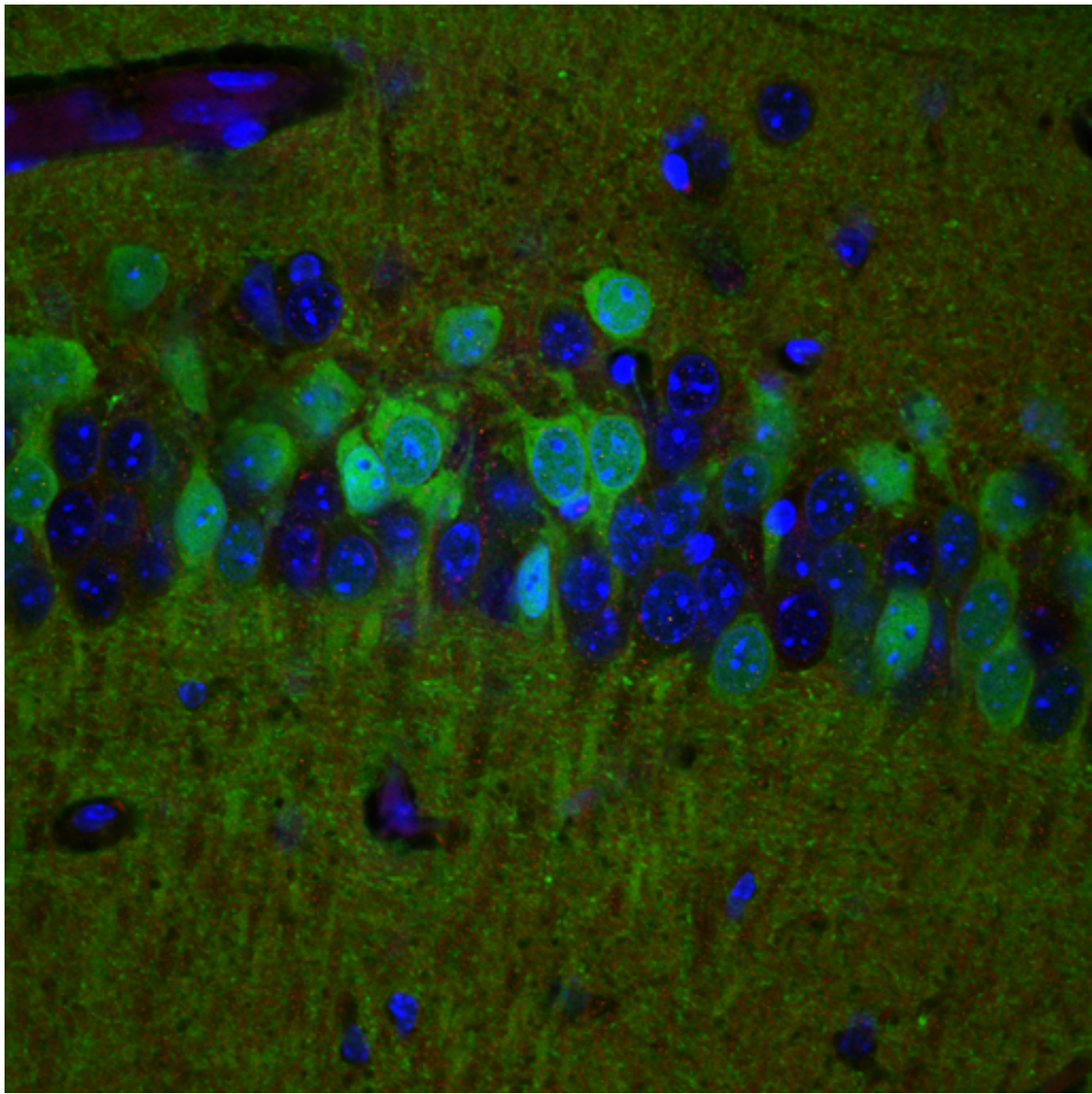
Emission wavelength 520 nm

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

Raw 2D Image

Raw Low Resolution 2D Image -



Raw 2D Image -	
IMAGE2D_ID	16819
IMAGE_DATE	2006-11-20 00:00:00.0
IMAGE_DESC	Zip archive containing the 3 channel image file in tiff format (11200dd_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/112006dd_img.jpg
RAW_DATA_FILE	/telescience/home/CCDB_DATA_USER.portal/P1723/Experiment_3482/Subject_255/Tissue_368/Microscopy_4059/112006dd_img.zip
THUMBNAIL_DESC	Triple labeled confocal image of hippocampal area CA1 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.
THUMBNAIL_FILE	/usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/112006dd_img_thmb.jpg
X_RESOLUTION	.207 um/pixel
Y_RESOLUTION	.207 um/pixel
X_SIZE	1024 pixels
Y_SIZE	1024 pixels

USER AGREEMENT

Data Sharing and Citation Policy: The mission of the CCDB is to promote data sharing among scientists interested in cellular and subcellular anatomy and in developing computer algorithms for 3D reconstruction and modeling of such data. Data sets may be viewed or shared at the discretion of the author of the data. In some cases, the data may be freely viewed and downloaded without contacting the original author while in other cases, permission of the author may have to be obtained prior to downloading the data. In either case, failure to cite or give proper credit to the original authors who collected these data in subsequent published articles or presentations is a material breach of this User Agreement. CCDB requires all researchers re-analyzing these published data via the CCDB access to reference the original published article and the CCDB. An example of an appropriate acknowledgement is provided on the CCDB web site. CCDB is not in a position to police every intended use of these data. The scientific community will self-police the compliance of this contractual obligation.

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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.

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Maryann Martone