Cell Centered Database
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Microscopy Product #:4046 112006aaaa

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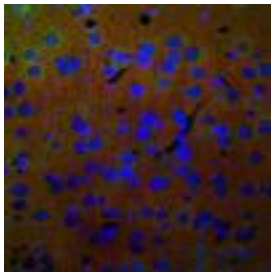
Image2D	Reconstruction	Segmentation
		

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	A53T15+
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	unknown
MICROTOME	Vibratome
ORIENTATION	coronal
THICKNESS	80 um
TISSUE_PROD_STORAGE	
EXTERNAL_FILE_NAME	
TISSUE_GROUP_TYPE	triple label

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	4046
IMAGE_BASENAME	112006aaaa
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_252/Tissue_365/Microscopy_4046
X_RESOLUTION	.207 um/pixels
Y_RESOLUTION	.207 um/pixels
XSIZE	1024
YSIZE	1024

Protocol:

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	15820
ATLAS_COORD	, ,
ORGAN	brain
REGION	cerebral cortex
SYSTEM	central nervous

Imaging Parameters:

Image Type -	
OPTICAL_SECTION_SERIES	15819
OPTICAL_NOTES	Only a single optical section was taken for each image.

Light Microscopy Product -	
LMPRODUCT_ID	15821
IMMERSION_MEDIUM	oil
LENS	Olympus PlanApo 60X oil
LENS_MAGNIFICATION	60 X
MOUNTING_MEDIUM	Prolong (Molecular Probes)
NUMERICAL_APERTURE	1.4
LM_NOTES	DAPI was added to the mounting medium

Confocal channel (15824)

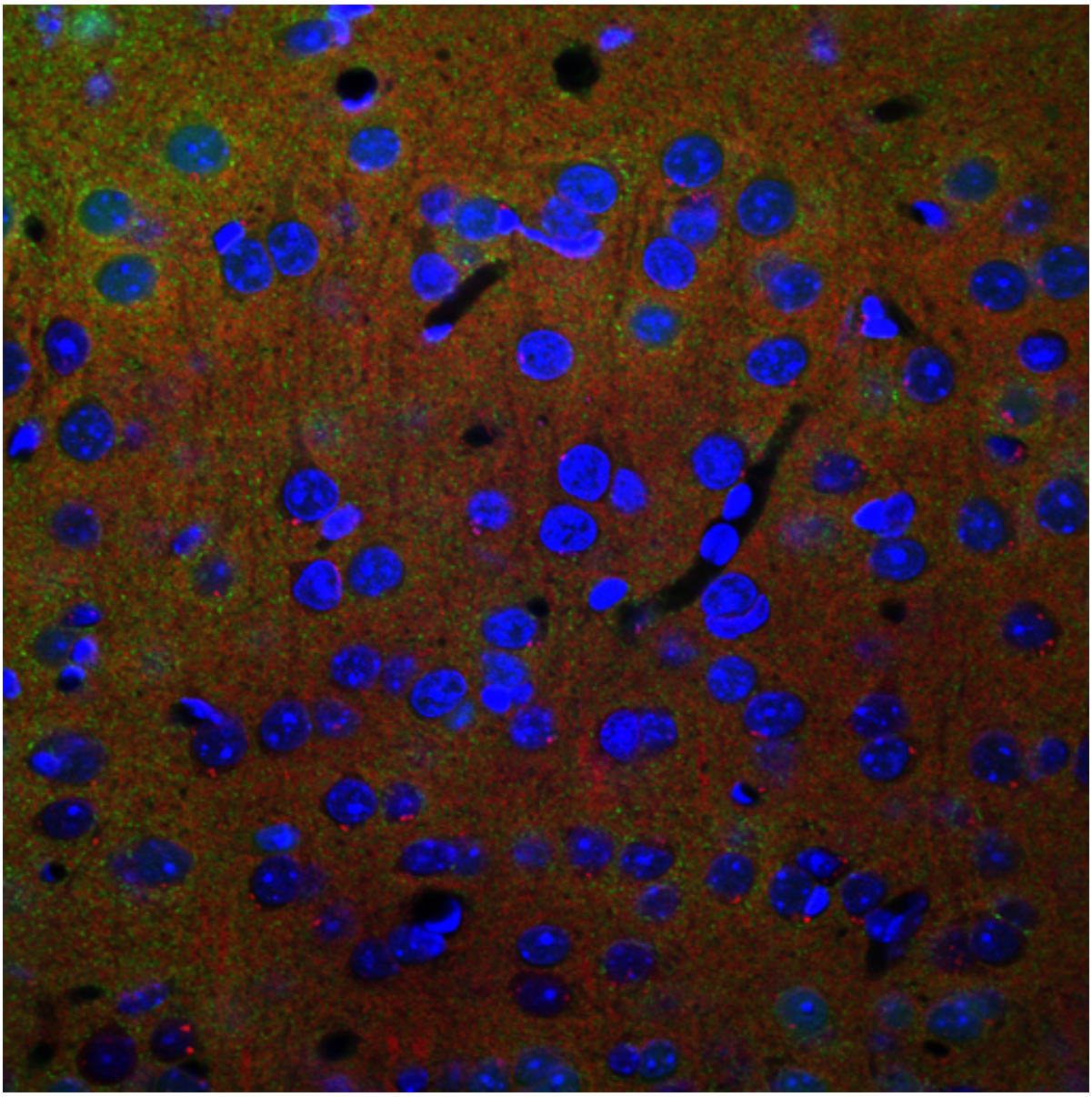
Confocal image ID	15824
Fluorophor	Alexa 488
Color	Green
Exitation wavelength	488 nm
Emission wavelength	520 nm

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	15846
IMAGE_DATE	2006-11-20 00:00:00.0
IMAGE_DESC	Zip archive containing the 3 channel image file in tiff format (112006aaaa_RGB.tiff). Also included are the .oif header file generated by the Olympus Fluoview, which give additional detail on microscope settings.
IMAGE_FILE_FORMAT	tiff
IMAGE_FILE_NAME	/usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/112006aaaa_img.jpg
RAW_DATA_FILE	/telescience/home/CCDB_DATA_USER.portal/P1723/Experiment_3482/Subject_252/Tissue_365/Microscopy_4046/112006aaaa_img.zip
THUMBNAIL_DESC	Triple labeled confocal image of the cerebral cortex of a transgenic mouse engineered to overexpress a mutant form of human alpha synuclein (A53T), 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/112006aaaa_img_thmb.jpg
X_RESOLUTION	.207 um/pixel
Y_RESOLUTION	.207 um/pixel
X_SIZE	1024 pixels
Y_SIZE	1024 pixels
NOTES	Pixel resolution was read from the .oif file submitted with the tiff file but was not verified.

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.

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