AUTISM TISSUE PROGRAM

Tissue Request Guidelines 2009

I. ATP Tissue Request Information

The Autism Tissue Program (ATP) was established to promote brain donation to support autism neuroscience research. Neuroscience investigators are invited to apply for postmortem brain tissue obtained from persons with autism, relatives of autistic persons and non-affected control donors to advance biomedical research in autism. Applicants submit tissue requests online on the ATP Informatics Portal at www.atpportal.org. Email the ATP at atp@brainbank.org to request a login to the portal for access to donor and tissue information and to submit a request.

Review Process. Proposals submitted to the Autism Tissue Program are reviewed by the ATP Tissue Advisory Board (TAB) quarterly. The TAB has the responsibility to 1) define optimal brain bank collection protocols, including retrieval, sectioning and storage; 2) review applications for tissue and 3) advise on tissue distribution based on both the scientific merit of the proposed research and the potential of the research to contribute to the further understanding of autism in light of past and current projects. You can access a list of TAB-approved research project abstracts, and selected publications, on the ATP Informatics Portal – click on the Research Projects/Data tab on the welcome page at www.atpportal.org.

Collaborative efforts with consortium members or colleagues, who can share tissue, or processed tissue, are recommended to conserve the resource. Researchers receiving tissue, who also have autism tissue or control tissue, are encouraged to inform the ATP of the resource and to make that material available to other investigators using ATP tissue whenever possible.

Tissue selection is an important part of an application. It is the task of the PI to review cases in the portal to see what cases are available that would support the proposed project. Brain region selection is also crucial to a successful application. The Portal has a **brain slice viewer** located on the Project pages. We ask that applicants use these figures, or other diagrams, to show the precise brain regions needed for the proposed study. Note that precise selection of brain regions for study is not only critical for tissue distribution, but must be justified with respect to the hypotheses proposed and/or known associations with autism.

Analysis. Applicants for tissue distribution should provide a power analysis for their studies to assure the ATP that the proposed study is adequately powered to test the hypothesis in question. Many studies fail to find statistically significant differences when in fact true differences exist because the variance in the measure of interest is too high and the sample size in the study is too small. Thus, valuable data is lost, good hypotheses are rejected, and invaluable time, energy and in our case, tissues, are wasted. Statistical power analysis provides a method for estimating how large a sample size is necessary to adequately test a hypothesis.

Blinding Samples. Tissues for distribution to an approved project will be listed on a Project Distribution Table by the brain bank case number. Each tissue sample will be given a code at the time of distribution. The code will be broken when data is returned.

Data-sharing is a requirement of the program. Recipients will provide the ATP results related to tissue cases, by providing a progress report to the ATP Director of Brain Resources and Data for the TAB every six months following receipt of tissue. Pre-publication data will be shared with other investigators only at the discretion of the PI. Recipients of this rare tissue are required to make available to the ATP within 3 months post-publication: data, slides and images or any tissue derivative that could be useful for further scientific exploration to the ATP and other tissue applicants.

II. APPLICATION FOR TISSUE CHECKLIST

Include ALL the following components in your application. For a list of available ATP cases, login to the ATP Informatics Portal at www.atpportal.org. Applications for tissue are submitted online by the deadline indicated on the Welcome page. You will be able to enter the information in A-C and upload documents D-G.

Please follow this checklist in your application.

A. PI Name & Title of Project Proposal.
B. Abstract of project GOALS and HYPOTHESIS(ES) to be tested. This should be limited to no more than 250 words. More detail can be provided in the Tissue Request Proposal.
C. Laboratory address and contact information.
D. Tissue Project Proposal containing the following information. This document should be uploaded to the Research Portal with other required documents. There is no space limit; however, it is important to be concise and precise about what you plan to do.
    1. Describe preliminary studies and alternate ways that you have attempted to solve this problem.
    2. Address the potential clinical benefit of your proposed study.
    3. Research plan.
        a. Investigative techniques and equipment to be used. What will you measure?
        b. State the donor profile (sex, age, PMI, etc.) required for the proposed research.
        c. Rationale with respect to autism and the proposed hypothesis to be tested for specific brain regions requested, a diagram is required.
        d. How much tissue is needed (size of block, sections, etc.). Note that because of the scarcity of tissue resources this question should be addressed with detailed justification.
        e. Indicate method of preservation (fixed/frozen, etc.) required.
        f. If you have an inventory of tissue that you will use, indicate the type of ASD cases and what control tissue you have or require.
        g. If applicable, indicate intention/plan to make slides or unused tissue available to other investigators after your study is complete.
    4. Timeline for work to be completed.
    5. Describe quantitative and statistical analyses, and a numerical analysis to justify the number of samples to be examined in order to produce scientifically valid measures and conclusions.
    6. Indicate if you have funding for this project or if you have an application pending. List personnel; if hiring, please include a position description. *Please note that if you intend to apply to Autism Speaks for funding to use postmortem tissue from the ATP, that your tissue request must be submitted online on the ATP portal at www.atpportal.org to the Autism Tissue Program Tissue Advisory Board. . Recommendations on tissue distribution will be documented and communicated to the Autism Speaks Scientific Advisory Board to ensure the application for funding is complete.*

E. Signed Investigator Agreement.
F. Biographical sketch (NIH format) for each investigator.

Note: IRB review is not required at the application phase. However, approved projects will not receive tissue distributions until documentation of review and/or exemption by an IRB committee is obtained by the ATP.

Questions: please contact Jane Pickett, Ph.D., ATP Director of Brain Resources and Data, 5119 Alejo Street, San Diego CA 92124, 877-333-0999, Fax: 858-694-0748 and email at: atp@brainbank.org.
Dr. Pickett will notify you about the decision of the TAB

III. Datasharing Agreement

In consideration of receipt of such a limited resource, the ATP has established procedures and expectations of recipients, which are detailed below. In addition, recipients are encouraged to attend meetings of tissue researchers to communicate research results, encourage brain donation to the ATP for the purpose of accelerating research and potentially collaborate with other tissue recipients of ATP cases. Please read the following and indicate your understanding and agreement by signing below.

Procedures and Expectations

Approved PIs will be required to sign Tissue Transfer Agreements (TTA) with each bank that has tissue to distribution for their project. As of August 1, 2002, each principal investigator is responsible for any tissue processing and/or shipping costs.

Approved projects will be listed on the ATP Informatics Portal by Title, Principal Investigator Institution and Abstract.

Please initial each of the items below

_____I will provide the ATP Director of Brain Resources and Data with results related to tissue cases, by providing a written summary of findings as well as electronic raw data from the experiments every six months (or earlier) following receipt of tissue. I understand that brain samples from the ATP will be coded and that the code will be broken when I return data to the ATP.

_____I understand that pre-publication data will be kept confidential by the ATP. Pre publication data, summaries and other "proprietary" research information will remain strictly confidential. Each six-month report shall contain disclosure of any inventions and discoveries related to the tissue made during the reporting period, whether or not such inventions or discoveries are patentable.

_____I will acknowledge the Autism Tissue Program and appropriate brain banks in all publications. *Original brain bank numbers will be used in all publications.* For example; 'We gratefully acknowledge the Harvard Brain Tissue Resource Center, (other brain bank) and the Autism Tissue Program for providing brain tissues for this study.'

_____I will acknowledge the Autism Tissue Program in any and all presentations to the scientific and lay community.

_____I will make available, post-publication, extra tissue sections, slides and images or any tissue derivative that could be useful for further scientific exploration to the ATP and other tissue applicants.

_____I will provide the ATP Director of Brain Resources and Data with abstracts and copie of any scientific publication relating to tissue research. Abstracts and research information for publication will be made available in ATP written materials and on the ATP website.

Signature: _____

Name: _____

Date: _____

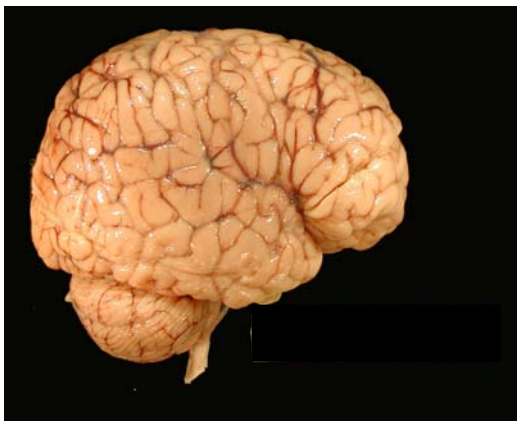
Please upload a scanned copy of the signed agreement to the ATP Autism Informatics Portal (www.atpportal.org). Alternatively, you can FAX a copy to Jane Pickett, Ph.D., 858-694-0748.

IV. Resources from Collaborative Projects (additional biomaterials and tissue available from ATP projects).

The ATP Informatics Portal provides information about donor brain tissue available. Additionally, several projects have generated processed tissue or DNA from donated brains that is also available. There are currently five such projects. To apply, use regular application process described above and Identify the resource.

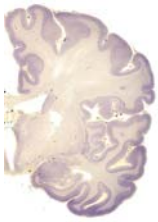
I. **Brain Atlas Celloidin 200u sections**
II. **Brain Atlas PEG 50u sections**
III. **Limbic Block 50u sections**
IV. **Tissue Array I**

I. Brain Atlas Celloidin 200u sections



Description of resource: Hemispheres of 14 matched donors embedded in celloidin and sectioned in 200u. The table of cases is listed below as well as the description of areas being assessed in the Brain Atlas Project. All of these cases have post mortem MRI images. Cresyl violet stained sections are available on loan. An entire third series (every third) of unstained sections of the 28 hemispheres is available.

Stained section. **Unstained sections**



Brain Atlas Project – Celloidin. Stereologic analysis (morphometry and volumetry) are being performed or are planned for the following cortical and subcortical regions:

Cortical Regions
Entire cortical gray matter
Brodmann Area 17
Fusiform gyrus
Inferior temporal gyrus
Middle temporal gyrus
Pars opercularis in the inferior frontal gyrus
Fronto-insular cortex
Anterior cingulate gyrus

Subcortical Regions
Paraventricular nucleus
Supraoptic nucleus (lateral, medial and dorsal)
Superchiasmatic nucleus
Preoptic area
Mammillary nucleus (medialis and lateralis)

Hippocampal Complex and Amygdala:
Entorhinal cortex: Volume of six layers and the number of neurons in five layers;
Subicular complex with the subiculum proper (molecular and pyramidal layer),
presubiculum (molecular, parvopyramidal, and pyramidal layers), and
parasubiculum (molecular and pyramidal layers);
Cornu Ammonis: Volume of the alveus and the strata: oriens, pyramidale, radiatum,
lacunosum/moleculare;
Number of neurons: stratum pyramidale in the CA1, 2, 3 and 4 sectors;
Dentate gyrus: Volume of the molecular and granule cell layers; number of granule cells.
Amygdaloid body. Volume and number of neurons in the
corticomedial group of nuclei (cortical, medial, and central nuclei),
basolateral group of nuclei (basal, lateral, accessory basal)
anterior amygdaloid area.

Cholinergic system - nucleus basalis of Meynert:
CH1 (medial septal nucleus)
CH2 (vertical limb nucleus of the diagonal band of Broca)
CH3 (horizontal limb nucleus of the diagonal band of Broca)
CH4 (anteromedial, anterolateral, intermediate, and posterior part of the nucleus basalis of Meynert)

Motor system:
Substantia nigra - (volumes and number of neurons with and without melanin)
Pars reticulata
Pars compacta: Dorsal pars compacta - dorsal, dorso-medial, dorso-lateral rostral; lateral; Ventral pars compacta - ventro-medial, ventro-lateral part,
Lateral nucleus
Striatum.
Caudate nucleus (volume; number of large and small neurons)
Putamen (volume; number of large and small neurons)
Globus pallidus - internal and external (volume; number of neurons)
Nucleus accumbens (volume; number of neurons);
Cerebellum (volume)
Cortex (volume)
Molecular layer (volume)
Granule cell layer (volume, number of neurons)
Purkinje cells layer (number)
White matter (volume)

Dentate nucleus (volume, number of neurons)

Brainstem:

Nucleus of facial nerve (volume, number of neurons)

Inferior Olive (volume, number of neurons)

principalis (volume, number of neurons)

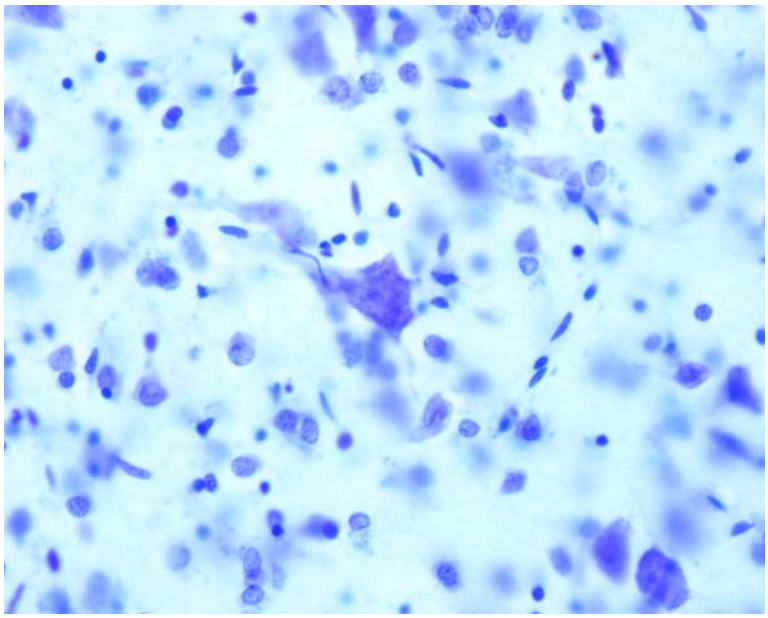
dorsalis (volume, number of neurons)

medialis (volume, number of neurons)

The Brain Atlas Celloidin Project has been assigned 14 autism and 14 control donor brains. Post mortem MRI is part of the every brain examination. A=autism; C=control; S=sex; H-right or left hemisphere; BW=brain weight; Fix-days in formalin fixation prior to processing.

Characteristics of cases and controls used in the Autism Brain Atlas project								
No	Case	Age [y]	S	H	BW [g]	PMI [h]	Fix [d]	Cause of Death
A1	IBR-425-02	4	M	R	1160	30	4560	Drowning
C1	B-6736	4	F	R	1530	17	126	Acute bronchopneumonia after tonsillectomy
A2	UMB-1627	5	F	R	1390	13	1568	Auto trauma
C2	UMB-1499	4	F	R	1222	21	233	Lymphocytic myocarditis
A3	B-6403	7	M	R	1610	25	330	Drowning
C3	UMB-4898	7	M	R	1240	12	130	Drowning
A4	B-5666	8	M	R	1570	22	196	Sarcoma
C4	UMB-1708	8	F	R	1222	20	650	Traumatic multiple injury
A5	B-5535	13	M	L	1470	8	75	Seizure?
C5	BTB-3638	14	M	R	1464	20	1067	Electrocution
A6	B-6115	17	F	L	1158	25	470	Dilated cardiomyopathy
C6	UMB-1843	15	F	R	1250	9	372	Multiple injuries
A7	UMB-1638	21	F	R	1108	50	136	Obstructive pulmonary disease
C7	UMB-1846	20	F	R	1340	9	245	Multiple injuries
A8	IBR-93-01	23	M	R	1610	14	505	Drowning
C8	UMB-1646	23	M	R	1520	6	95	Ruptured spleen
A9	B-5947	32	M	L	1510	23	288	Respiratory failure
C9	UMB-4543	29	M	R	1514	13	89	Traumatic multiple injury
A10	B-6212	36	M	R	1370	24	*	Circulatory failure of cardiac origin; renal failure
C10	UMB-1576	32	M	R	*	24	*	Compressional asphyxia
A11	B-6202	48	M	L	1260	*	875	Oxygen deprivation-chocking on food
C11	BTB-3899	48	M	L	1412	24	215	Atherosclerotic heart disease
A12	BB-1376	52	M	L	1324	11	84	Heart attack
C12	IBR-252-02	51	M	L	1450	18	1819	Myocardial infarct
A13	B-6276	56	M	R	1570	3	692	Arteriosclerotic heart disease
C13	BTB-3983	52	M	R	1430	12	158	atherosclerotic cardiovascular disease
A14	B-6862	66	M	R	1380	13	*	Suspected drowning
C14	B-6874	64	M	R	1250	28	*	Cardiac arrest

II. Brain Atlas - PEG (Polyethylene Glycol) 50u sections



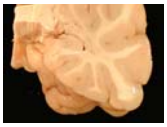
This is a stained image of a section courtesy of William Byne, Mt. Sinai, NY. The processing allows IHC as well. Smithson, MacVicar and Hatton described 'Polyethylene glycol embedding: a technique compatible with immunocytochemistry, enzyme histochemistry, histofluorescence and intracellular staining' in J. Neurosci. Methods. 1983 Jan;7(1):27-41.

III. Limbic Block 50u sections

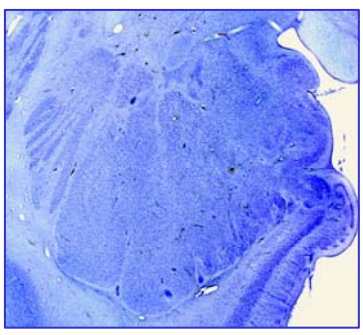
David Amaral and Cynthia Schumann, The M.I.N.D. Institute, UC Davis, CA. Amygdala or larger 'limbic' block is cut from formalin fixed hemisphere, frozen and cut into 6 series of 50µm and 2 series of 100µm. 100µm sections through the amygdala are Nissl stained for stereological analysis: CM Schumann and DG Amaral. 2006. Stereological analysis of amygdala neuron number in autism J. Neuroscience 26: 7674-7679.

a. One series (#8) of 50u **unstained** sections of 19 donors is available
b. Stained sections may be viewed at the MIND Institute.
c. MRI dicom data available on the cases.

4 cm for amygdala block; 6.5 cm includes hippocampus



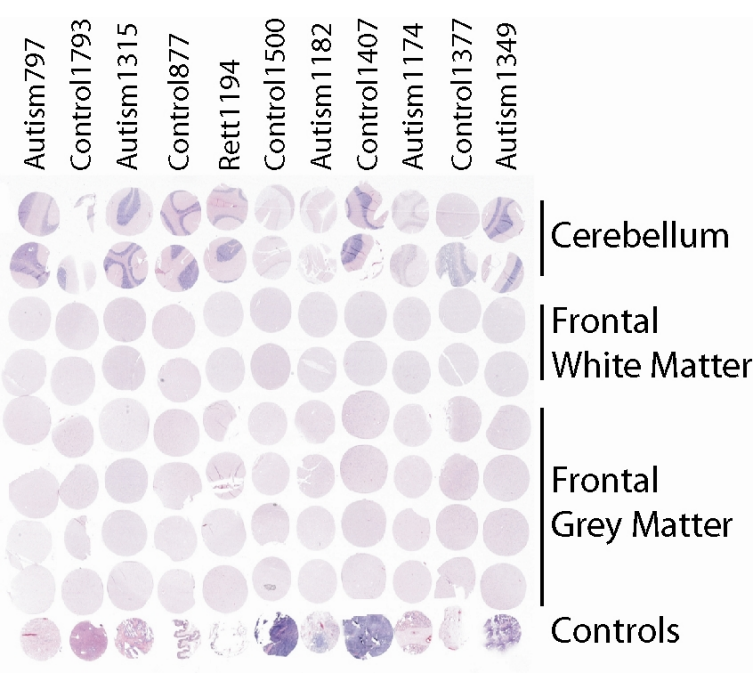
b. Stained section



c. 3-D from image data

IV. Tissue Array I

Charles Eberhart, Johns Hopkins. Tissue Array made using U of Maryland NICHD brain bank autism and Rett cases. Distribution Plan: ATP accepts PI requests for slides for pilot projects and ensures that proper tissue transfer agreements are submitted to Dr. Ron Zielke, University of Maryland brain bank.



Control tissues

1 2 3 4 5 6 7 8 9 10 11

1-kidney, 2-liver, 3-prostate, 4-skin, 5-lung, 6-tonsil, 7-bladder, 8-thymus, 9-placenta, 10-gall bladder, 11-salivary gland

The array was made at the Johns Hopkins Pathology Department Tissue Microarray Core Facility for use in screening for genes and proteins potentially affected in autism using in situ hybridization and/or immunohistochemistry. Case numbers, along with basic clinical, autopsy and demographic data are available on request atp@brainbank.org. Two cores were taken from each region of five autism, one Rett and control brains in order to sample more than one location. Thus, on the array, which is organized in columns, there are two superficial frontal cortex cores, two deep cortex cores, two white matter cores, two cerebellum cores from each case and a single control tissue core. Control cases alternate with affected cases in columns. The tenth slide cut from the block was stained with H&E, and a table is available that describes the cores in more detail. Additional H&E stained slides will be generated from every 10th section.

Eberhart, C, Copeland, J & T Abel. 2006. Brief Report: S6 Ribosomal Protein Phosphorylation in Autistic Frontal Cortex and Cerebellum: a Tissue Array Analysis. J Autism Dev Disord. 36:1131-1136.

If you have questions, please contact Jane Pickett, Ph.D.,
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