

SCHOOL of MEDICINE

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Introduction

Excessive alcohol ingestion results in medical disorders affecting the brain and behavior as well as other organs. Detailed research into the neurobiological consequences of alcohol use disorders and alcoholism however, is dependent upon appropriate animal models that parallel the voluntary levels and patterns of alcohol intake observed in human alcoholics. Much of what is known about risk for and the results of heavy alcohol consumption derives from rodent studies or retrospective human studies. We are now funded by NIAAA to supply central nervous system (CNS) and peripheral tissues, as well as, associated bioinformatics to the greater scientific community, in order to conduct a wide array of assays. This resource is unique to the alcohol research field and provides a means for investigators that typically focus on rodent studies to expand their research programs into a nonhuman primate model of ethanol self-administration. The name of this resource is the Monkey Alcohol Tissue Research Resource (MATRR).

Our nonhuman primate model of ethanol self-administration helps to bridge the gap between basic and clinical science research. The basis of this model is a well-characterized nonhuman primate model of ethanol self-administration (Grant et al., 2008) that results in excessive amounts of ethanol(>3.0 g/kg or a 12 drink equivalent/day) over long periods of time (12-30 months) with concomitant pathological changes in endocrine, hepatic and central nervous system (CNS) processes. These longitudinal designs span "stages of drinking" from ethanol-naïve to early alcohol exposure to chronic abuse. To provide access to tissues from this primate model, we have established a Monkey Alcohol Tissue Research Resource (MATRR). From this resource both tissue and associated bioinformatics tools are readily available to the wider alcohol research community. The tissue is derived from a standard protocol of ethanol self-administration in 3 species of monkeys (rhesus, cynomolgus and vervet) that all self-administer ethanol under identical conditions. This resource provides tissues and behavioral data for hypothesis testing relating the risk for and consequences of alcohol consumption. The CNS and peripheral organs from these animals comprise a unique translational resource for mechanistic and genetic studies of ethanol-induced pathologies. The state-of-the-art necropsy protocol provides fresh, fixed and frozen tissues that are appropriate for ex vivo electrophysiology and neurochemical recordings, histological studies and genomic, proteomic and metabolomic approaches, respectively.

The demand for, and quality of, the tissues are high as reflected by numerous peer-reviewed publications, abstracts, and data presentations using tissues supplied by this resource. These tissues also offer the opportunity to collect preliminary data for grant submissions and the animal model that is the basis for this resource has resulted in at least 32 funded projects. Our primary goal is to continue to build the resources of this tissue bank and distribute these tissues and/or associated bioinformatics to the broader alcohol research community to better understand the effects of chronic alcohol abuse. To date, we have interacted with over 40 individual laboratories, resulting in multiple peer-reviewed publications, numerous data presentations and funded projects.

Structural MRI images acquired prior to necropsy are used to identify the precise location of specific brain regions to be harvested.



After the brain is removed from the skull. it is placed in a brain matrix. Our post-mortem interval for brain harvest is typically less than 5 min.





The frontal block is removed and dissected into 12-14 cortical fields. The rest of the brain is sectioned into 4 mm slices. 4 mm slices are positioned onto aluminum plates placed on dry ice. Blocks are numbered sequentially and the sections are digitally archived. After the sections freeze, they are placed into individual plastic bags sized to ▶ minimize air and placed into a freezer at -800C conditions for storage.



Regions of interest (ROI) are identified on digital images. Brain blocks are removed from freezer and ROIs are microdisssected under -35°C conditions.



The hippocampus (hpc), entorhinal cortex (Ent Ctx), ventral tegmental area (VTA), substantia nigra (SN) and lateral geniculate nucleus (LGN) were collected from this block and distributed to recipient investigators.

Monkey Alcohol Tissue Research Resource





Microdissected ROIs are individually stored with proper identification and sent on dry ice to researcher who has an approved tissue request.

Areas that were identified in this block include the dorsal putamen (DP), dorsolateral caudate (DLC), dorsomedial caudate (DMC), central caudate (CC), ventral caudate (VC), Nucleus accumbens shell, Nucleus accumbens core, central putamen (CP) and Ventral putamen (VP).





Genomic/proteomic assays of frontal cortical fields (Hemby et al., 2006; Acosta et al., 2010).

> Whole-cell patch clamp ephys and PCR assays – lateral nucleus of the amygdala (Floyd et al., 2004; Anderson et al 2007) and caudate/putamen (Carlson et al., 2009). DA uptake and release assessed with voltammetry in caudate/putamen (Budygin et al. 2003). Dendritic branching and morphology assessed in caudate (Seabold et al., 2010).

Current clamp and voltage clamp recordings done in lateral geniculate nucleus to assess T-type calcium current (Carden et al., 2006; Alexander et al., 2006)

Patch-clamp ephys recordings to assess EtOH effects on GABA, NMDA and AMPA currents in nippocampus (Ariwodola et al., 2003)



Current- and voltage-clamp recordings of primate inferior olivary nucleus Welsh et al, 2011).

Genetic Background

Monkeys in the ethanol self-administration study were genetically characterized to determine their ancestry and/or familial relatedness. Cynomolgus macaques were identified as being from Indonesia and Indochina, using ancestry informative SNP analysis. All of the rhesus macaques were verified to be Indian origin. Rhesus macaques were drawn from a molecularly verified pedigreed population, and were selected to obtain minimal relatedness among subjects (paired kinship < 0.015). Subjects were also genotyped at several loci, including 5-HTTLPR and MAO-LPR, but were not selected based upon genotype.

The vervet monkeys in this resource are part of a 8-generation, pedigreed and partially genotyped colony of vervets. A SNP map for candidate neurobiological genes exists and can be used for association studies. There exists a complete data set on ancestry and relatedness making this colony suitable for identifying quantitative trait loci.

Representative MATRR Publications

- Acosta G, Hasenkamp W, Daunais JB, Friedman DP, Grant KA and Hemby SE. Ethanol self-administration modulation of NMSA receptor subunit and related synaptic protein mRNA expression in prefrontal cortical fields. Brain Res. 2009; [Epub ahead of print]. PMID: 20043891 • Alexander GM, Carden WB, Mu J, Kurukulasuriya NC, McCool BA, Norskog BK, Friedman DP, Daunais JB, Grant KA and Godwin DW, The native T-type calcium current in relay neurons of the primate thalamus. Neuroscience, (2006) 141(1):453-461. PMID: 16690211
- Anderson, N.J., Daunais, J.B., Friedman, D.P., Grant, K.A., and McCool, B.A. (2007) Long-term ethanol self-administration by the non-human primate, Macaca fascicularis, decreases benzodiazepine sensitivity of amygdala GABAA receptors. <u>Alcohol Clin. Exp. Res.</u> (2007) 31:1061-1070. PMID: 17428292 • Ariwodola, O.J., Crowder, T.L., Grant, K.A., Daunais, J.B., Friedman, D.P. and Weiner, J.L. Ethanol modulation of excitatory and inhibitory synaptic transmission in rat
- and monkey dentate granule neurons. Alcohol Clin Exp Res. (2003); 27:1632-1639. PMID: 14574234 • Budygin, E.A., John, C.E., Mateo, Y., Daunais, J.B., Friedman, D.P., Grant, K.A. and Jones, S.R. Chronic ethanol exposure alters presynaptic dopamine function in the striatum of monkeys: a preliminary study. <u>Synapse</u>. (2003); 50:266-268. PMID: 14515345
- Carden WB, Alexander GM, Friedman DP, Daunais JB, Grant KA, Mu J and Godwin DW (2006) Chronic ethanol drinking reduces native T-type calcium current in the thalamus of nonhuman primates. <u>Brain Res</u>. (2006) 1089(1):92-100. PMID: 16631142
- Floyd, D.W., Friedman, D.P., Daunais, J.B., Pierre, P.J., Grant, K.A. and McCool, B.A., Long-term Ethanol Self-administration by Cynomolgus Macaques Alters the Pharmacology and Expression of GABA(A) Receptors Expressed in Basolateral Amygdala. J Pharmacol Exp Ther. (2004) 311:1071-1079. PMID: 15280440
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- PMID: 20644510 • Vivian JA, Green HL, Young JE, Majerksy LS, Thoman BW, Shively CA, Tobin JR, Nader MA, Grant KA. Induction and maintenance of ethanol self-administration in cynomolgus monkeys (Macaca fascicularis): long-term characterization of sex and individual differences. Alcohol Clin. Exp. Res.., 2001; 25:1087-1097. PMID: 11505038
- alcoholic monkeys. Alcohol Clin Exp Res. 2010 Jul;34(7):1171-81. Epub 2010 May 12. PMID: 20477780 • Cuzon Carlson VC, Seabold GK, Helms CM, Garg N, Odagiri M, Rau AR, Daunais J, Alvarez VA, Lovinger DM, Grant KA. Synaptic and morphological neuroadaptations
- Epub 2011 Jul 27. PMID: 21796110
- Freeman WM, Vanguilder HD, Guidone E, Krystal JH, Grant KA, Vrana KE. Plasma proteomic alterations in non-human primates and humans after chronic alcohol selfadministration. Int J Neuropsychopharmacol. 2011 Aug;14(7):899-911. Epub 2011 Feb 8. PMID: 21303580 • Welsh JP, Han VZ, Rossi DJ, Mohr C, Odagiri M, Daunais JB, Grant KA. Bidirectional plasticity in the primate inferior olive induced by chronic ethanol intoxication and sustained abstinence. Proc Natl Acad Sci U S A. 2011 Jun 21;108(25):10314-9. PMID: 21642533



Amygdala



GABA and glutamate slice ephys recordings conducted in BNST

Neurosteroid levels assayed in hippocampal punches

• Cheng HJ, Grant KA, Han QH, Daunais JB, Friedman DP, Masutani S, Little WC, Cheng CP. Up-regulation and functional effect of cardiac β3-adrenoreceptors in in the putamen associated with long-term, relapsing alcohol drinking in primates. Neuropsychopharmacology. 2011 Nov;36(12):2513-28. doi: 10.1038/npp.2011.140.

Data Resources Core

Our **Data Resources Core**, currently available at <u>www.matrr.com</u> (see Screen Shots), acts as a centralized location for specific tissue requests, tissue distributions, advanced tissue filtering, navigation and data mining. Once visitors register, they are able to request very specific tissues from archived or upcoming necropsies. The review process prioritizes requests and manages tissue disbursement. Prioritization is based, in part, on scientific significance, amount of tissue requested and past success. Differing levels of site permissions will allow certain users the ability to identify tissues based on a variety of empirically-derived and cohort- or animal-centric data such as: **Daily behavioral data**; In vivo and in vitro neuroimaging and neurobiological data; In vitro and ex vivo histological, genomic, proteomic and biochemical data; Functional electrophysiology (ephys) and neurochemical data (single cell, slice and bundled electrophysiology; voltammetry, microdialysis) in living tissue

The standard user will be able to access general information about animal cohort drinking habits and protocol timelines. Users with advanced permissions may select tissues associated with monkeys identified through data mining and statistical analysis of the stored population.

Navigating from the (A) MATRR homepage, users can survey available cohorts based, in part (B) on experimental data. From there, users can (C) select tissues of interest and (D) formally request them for use in individual experimental protocols.



Summary and Conclusions

- voltammetry in living tissue as well as an array of assays including genomic and proteomic methods.
- the brain disssections.
- All peripheral tissues are collected for various assays.
- agreements.
- States.
- collaborations using this animal model.
- pending.

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• Our necropsy design results in viable tissue for *ex vivo* slice recording and

• Discrete brain regions of interest can be obtained with 100% accuracy using structural MRI to guide

• Bioinformatics information entails the monkey database, tissue requests and material transfer

• Bioinformatics will also integrate analysis of genomic, genetic and phenotypic information. • We currently collaborate with over 40 principle investigators from 17 universities across the United

• Over 45 peer-reviewed manuscripts and 65 abstracts have resulted from cross-institutional

• Over 30 grants have been funded as a result of this tissue resource and multiple grants are

• This tissue resource has proven to be a critical resource with a very high return on investment.

