

MARMOSET RESEARCH GROUP OF THE AMERICAS



PROGRAM AND ABSTRACTS

INAUGURAL MEETING – JUNE 13-14, 2004

**UNIVERSITY OF WISCONSIN, MADISON
MEMORIAL UNION**

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Welcome



Welcome to the inaugural meeting of the Marmoset Research Group of the Americas (MaRGA). MaRGA is modeled after the European Marmoset Research Group and like the EMRG, we intend to provide a forum for bringing together people from a wide variety of disciplines and types of institutions to improve the understanding of marmoset biology, and in turn, the quality of research conducted using marmosets. The integration and coordination of efforts by those in both North and South America is necessary to support the expanding use of these primates in research.

We are particularly pleased about the diverse make-up of the registrants for this meeting and have high hopes that this diversity will foster true interdisciplinary exchange. In addition, we are delighted to be able to host two colleagues from Brazil, Drs. Sousa and Yamamoto.

MaRGA gratefully acknowledges the generous support of Harlan, Inc. in sponsoring Drs. Sousa, Yamamoto and Genain.

In addition, the Steering Committee wishes to thank:

- Wisconsin National Primate Research Center at the University of Wisconsin for providing all local arrangements.
- Southwest National Primate Research Center for providing the program/abstract book and travel support for Dr. M. Power.
- New England National Primate Research Center for providing travel support for Dr. Austad.
- University of Nebraska at Omaha, for providing travel support for Dr. Ferris.

We hope you will find the meeting informative and stimulating. Your input will play a critical role in how this young organization develops and matures. We do want MaRGA to be your organization. We have, therefore, left large blocks of time in the meeting for discussion and urge you to participate with your knowledge, questions and concerns.

Thanks to everyone for your interest in MaRGA and have a great meeting.

Suzette D. Tardif for the MaRGA Steering Committee*

*Steering Committee Members:

David Abbott, Ph.D.
Jeffrey French, Ph.D.
Donna Layne, B.S.
Elisabeth Ludlage, D.V.M.
Keith Mansfield, D.V.M.
Rachel Power, Ph.D.
Nancy Schultz-Darken, Ph.D.
Suzette Tardif, Ph.D.

MARMOSET RESEARCH GROUP OF THE AMERICAS (MaRGA)

Inaugural Meeting - 2004
Memorial Union, University of Wisconsin, Madison

SUNDAY, JUNE 13, 2004

- 8:30 a.m. **Introductions, Description of MaRGA and Meeting**
S. Tardif, Southwest National Primate Research Center
- 8:45 a.m. **Marmoset Longevity: Its Place in the Mammalian Scheme**
Speaker: S. Austad
University of Idaho/University of Texas Health Science Center, San Antonio
- 9:45 a.m. Break

SESSION 1: NUTRITION

Chair: N. Schultz-Darken

- 10:00 a.m. **Nutrient Requirements of the Marmoset: Phylogeny, Ecology and Some Hard Data**
M. Power, National Zoo, Smithsonian Institution
- 11:00 a.m. **The Marmoset as a Model of Diet Induced Obesity**
R. Bertram, Glaxo Smith Kline
- 11:20 a.m. **Effects of Energy Restriction on Gestation and Lactation**
S. Tardif, Southwest National Primate Research Center
- 11:40 a.m. Roundtable Discussion – *Nutrition & Related Topics*
- 12:15 p.m. Break for Lunch

SESSION 2: CLINICAL/HUSBANDRY/MANAGEMENT

Chair: R. Power

- 1:30 p.m. **Pathology of Common Marmoset (*Callithrix jacchus*) Diseases**
K. Mansfield, New England National Primate Research Center
- 2:30 p.m. **Development and Use of a Multi Colony Marmoset Demographic Database as a Research Resource**
D. Smucny, Southwest National Primate Research Center
- 2:50 p.m. **A Demographic Review of the Captive Colony of *Callithrix kuhlii* at the University of Nebraska Callitrichid Research Facility**
C. Ross, University of Nebraska
- 3:10 p.m. **The Influence of Litter Size on Inter Birth Interval in Captive *Callithrix kuhlii* at the University of Nebraska at Omaha's Callitrichid Research Center**
H. Jensen, University of Nebraska
- 3:30 p.m. Break
- 3:50 p.m. **Systemic AA Amyloidosis in the Common Marmoset**
E. Ludlage, New England National Primate Research Center
- 4:10 p.m. **Custom Designed Experimental Housing for Marmosets**
D. Layne, Southwest National Primate Research Center
- 4:30 p.m. Roundtable Discussion – *Clinical & Husbandry Issues*
- 5:00 p.m. Discussion of Plans for Next Meeting/Other MaRGA Issues
- 5:30 p.m. Adjourn

MONDAY, JUNE 14

SESSION 3: NEUROSCIENCE/COMMUNICATION/BEHAVIOR

Chair: J. French

- 8:30 a.m. **Functional Magnetic Resonance Imaging in Conscious Marmosets: Methods and Applications in Neuroscience Research**
C. Ferris, University of Massachusetts
- 9:30 a.m. **Common Marmoset as a Model to Study the Neural Basis of Primate Vocal Communication**
X. Wang, Johns Hopkins University
- 9:50 a.m. Break
- 10:10 a.m. **Preliminary Evidence of Early Long Call Acquisition and Usage in a Callitrichid Primate (*Callithrix kuhlii*)**
M. Rukstalis, University of Nebraska
- 10:30 a.m. **Making Sense Out of Scents: Olfaction and Reproduction in Marmosets and Tamarins**
T. Ziegler, National Primate Center, University of Wisconsin
- 10:50 a.m. **Mating Systems in Marmosets: Data from Captive and Wild Groups**
M.E. Yamamoto, Universidade Federal do Rio Grande do Norte, Natal, Brazil
- 11:10 a.m. **Sex Differences in Play Behavior Among Captive Common Marmosets (*Callithrix jacchus*)**
L. Zemba, NICHD, National Institutes of Health
- 11:30 a.m. Roundtable – *Neuroscience & Related Topics*
- 12:00 p.m. Break for Lunch

SESSION 4: PHYSIOLOGY AND IMMUNOLOGY

Chair: E. Ludlage

- 1:00 p.m. **Primate Models of Inflammation with Emphasis on Multiple Sclerosis**
C. Genain, University of California, San Francisco
- 2:00 p.m. **Immunogenicity of GBV-B DNA Vaccines in Common Marmosets**
S. Biswas, University of Massachusetts
- 2:20 p.m. **Androgens and Aging in Male Marmosets**
J. French, University of Nebraska
- 2:40 p.m. **Getting Hormonal Data From Wild Common Marmoset Females: New Insights About Immigration and Mating Systems Mechanisms**
M.B. Sousa, Universidade Federal do Rio Grande do Norte, Natal, Brazil
- 3:00 p.m. Break
- 3:20 p.m. **Bone Mineral Density During Lactation in Common Marmosets**
R. Power, Pennington Biomedical Research Center
- 3:40 p.m. Roundtable – *Physiology & Immunology*
- 4:20 p.m. Adjourn Meeting

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Marmoset Longevity: Its Place in the Mammalian Scheme
S. Austad

Affiliation: University of Texas Health Science Center, San Antonio, TX

Marmoset longevity will be compared to life history data on more than 600 species of mammals in order to evaluate its longevity both in the context of general mammalian life history patterns and primate-specific life histories. Such an analysis reveals that: (1) primates are not the longest-lived mammalian order, either by absolute longevity, longevity corrected for body size, or metabolic expenditure per lifetime; (2) although relative brain size is highly correlated with longevity in primates, this is an aberrant trend for mammals in general, and other body organs account for an even greater amount of variation in longevity; (3) marmosets live about as long as an average primate of their body size; and (4) the limits of marmoset longevity in a captive setting have probably not yet been approached.

Notes:

Nutrient Requirements of the Marmoset: Phylogeny, Ecology and Some Hard Data
M. Power

Affiliation: Nutrition Laboratory, Department of Conservation Biology, Smithsonian’s National Zoological Park, Washington DC

Estimates of nutrient requirements for exotic specie are more often arrived at by intelligent guesswork than by actual hard data. Nutritionists and animal managers tasked with the responsibility of maintaining collections of exotic species frequently must rely on information concerning the phylogeny, anatomy (especially gut morphology), ecology, natural history, and their personal experience with a species in order to devise satisfactory diets. Even for important laboratory animals, such as the common marmoset (*Callithrix jacchus*) which has been kept in laboratory colonies for more than 40 years, studies that contribute data on nutrient requirements are few and far between. I will briefly review what is known and what can be deduced regarding nutrient requirements for common marmosets based on their phylogeny, anatomy, and natural history. For example, the fact that they are primates all but guarantees that they have a requirement for vitamin C. Their small body size and gut morphology imply that they have a limited ability to digest insoluble fiber, although they would appear to be able to digest soluble fiber, such as gum. Although gums and other plant exudates have an important place in their wild diet, there are no data that suggest that these foods provide any required nutrition that cannot be provided by other means in captivity. I will briefly explore this issue, and the possible role that fermentable substrates may or may not play in marmoset nutrition. There are good data relevant to energy and protein requirements, and I will discuss these in more detail. Finally, I will discuss the theoretically fascinating, but practically confusing and aggravating issue of vitamin D and calcium metabolism in marmosets and other New World primates.

Notes:

The Marmoset as a Model of Diet Induced Obesity

Mark Paulik¹, Diane Ignar¹, Mary Lancaster¹, Tula Miliken¹, Wesley Young¹, Christy Britt¹, Lisa Clifton¹, **Rick Bertram**², Carmen McLamb², and Tom Tlusty²

Affiliation: ¹Metabolic Diseases and Virology Center of Excellence in Drug Discovery; and ²Laboratory Animal Science, Glaxo Smith Kline, Research Triangle Park, NC

Obesity has become a major public health concern in the United States. A predictive preclinical model of diet-induced obesity (DIO) could accelerate the development of pharmaceuticals to treat this rising epidemic. A non-human primate could provide such a predictive model. Thus, the common marmoset (*Callithrix jacchus*) was evaluated for its propensity to becoming obese when challenged with high fat diets. Marmosets were placed on either a standard diet (Mazuri 5MI5) (SD), or a SD supplemented with 12% or 24% lard. The marmosets fed the 12% lard diet (HF) for 8 weeks gained the most weight when compared with the 24% lard or the SD groups. Marmosets placed on the HF diet for 3 months had a 27% increase in body weight, a 51% increase in fat mass, and a 25% increase in lean mass. In addition, the marmosets on the HF diet had significant increases in total serum cholesterol, LDL-cholesterol, triglycerides, Hb IAc, glucose, insulin and creatine kinase when compared to marmosets on SD. To determine how marmosets would respond to pharmaceutical intervention, male marmosets were dosed with a CB-1 antagonist (15 and 45 mg/kg) or with Sibutramine (5 and 15 mg/kg) for four weeks. Compared to vehicle treated animals, the Sibutramine groups lost 2.3% and 7.9% weight, respectively. Whereas, the CB-1 antagonist treated marmosets lost 9.5% and 17.4% of their starting weights, respectively. Furthermore, CB-1 antagonist (45 mg/kg) treated marmosets demonstrated significant lowering of serum triglycerides vs. vehicle control animals. After initial evaluation, the common marmoset shows promise as a DIO model.

Notes:

Effects of Energy Restriction on Gestation and Lactation

S. Tardif, D. Layne, and D. Smucny

Affiliation: Southwest National Primate Research Center, San Antonio, TX

We report on the effects of energy restriction during gestation or lactation on maternal condition and reproductive outcomes. For all studies, females were restricted to 75% of expected ad lib energy consumption of a purified diet. Mid gestation restriction (day 66) reliably induced loss of pregnancy prior to term (mean delivery age = 92). Of the late (day 99) restricted pregnancies, 4 of 7 were normal term length while three were preterm deliveries, at 101, 117 and 132 days. Restriction reliably prevented the normal weight gains seen in control pregnancies. In late restricted females, higher daily weight loss was associated with earlier delivery age and females who began pregnancy at lower weights tended to have higher weight losses. Energy restriction during lactation for females of relatively high body weight did not, in and of itself, affect milk composition or infant growth rates – i.e., mothers did not sacrifice infant condition to protect their own reserves. Restricted mothers lost weight during lactation. The percent time spent nursing did not differ between energy restricted and control mothers, however the percent time that mothers carried infants while not nursing was lower for restricted females. Comparisons of maternal outcomes in the nonmanipulated population and in the energy restricted population both point to maternal weight loss during lactation as affecting not only maternal behavior but also milk output. These results suggest that maternal investment in lactation is affected by maternal condition in the most extreme cases (i.e., mothers with low reserves nursing the largest litter size). However, they also suggest that the impact of energy restriction upon reproductive function is strikingly different, dependent upon when in reproduction the restriction is imposed. Specifically, it appears that, as one might expect, the further along the female is in a given reproductive event, the more likely she is to continue to invest.

Notes:

Pathology of Common Marmoset (*Callithrix jacchus*) Diseases
K. Mansfield

Affiliation: New England National Primate Research Center, Harvard Medical School, Southborough, MA

The common marmoset (*Callithrix jacchus*) is a small New World primate that has been used extensively in biomedical research for the past 30 years. Spontaneous pathology and diseases recognized in common marmosets and other Callitrichidae differ from that seen in Old World primate species. These differences result from unique susceptibilities to infectious agents, dietary requirements and species specific degenerative diseases that have arisen due to extensive evolutionary divergence coupled with adaptation to the neotropical environment. The laboratory animal scientist must appreciate these significant differences when utilizing marmosets in pursuit of their research goals. Furthermore these differences may also present unique opportunities in model development not available in more traditional nonhuman primate species. Knowledge of disease entities within a colony is critical in providing well defined research animals and is dependant on proactive disease surveillance. Colony disease surveillance should include routine preventative health care, a rigorous diagnostic approach to clinical illness and gross and histologic examination of all colony animals at necropsy. Common entities observed within the New England Primate Research Center marmoset breeding colony include systemic amyloidosis, inflammatory bowel disease, interstitial nephritis, adenocarcinoma of the small intestine, metabolic bone disease and a variety of enteric pathogens including enteropathogenic *E. coli*, *Giardia* and *Campylobacter jejuni*. Such spontaneous disease entities must be recognized in order to understand their impact on biomedical research programs and colony health.

Notes:

Development and Use of a Multi Colony Marmoset Demographic Database as a Research Resource

D. Smucny¹, D.H. Abbott², K.G. Mansfield³, N. Schultz-Darken², M.E. Yamamoto⁴, A.I. Alencar⁴, and S.D. Tardif¹

Affiliation: ¹Southwest National Primate Research Center, San Antonio, TX; ²Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI; ³New England National Primate Research Center, Harvard Medical School, Southborough, MA; and ⁴Universidade Federal do Rio Grande do Norte, Department of Physiology, Natal, Brazil

Common marmosets (*Callithrix jacchus*) represent an important nonhuman primate model in biomedical research. Demographic and reproductive studies of captive marmosets have been limited by relatively small sample sizes, with most studies focused on a single colony. A pooled data source across different marmoset colonies provides a valuable research resource to increase sample sizes to better study topics such as demography, reproduction, aging and heritability. Since 2001, we have compiled demographic and reproductive information for five different common marmoset colonies into a large pooled database. The colonies included in the database are: Universidade Federal do Rio Grande do Norte (Natal, Brazil), New England National Primate Research Center (Southborough, MA), Oak Ridge Associated Universities (ORAU) Marmoset Research Center (Oak Ridge, TN), Southwest National Primate Research Center (San Antonio, TX), and Wisconsin National Primate Research Center (Madison, WI). All of these colonies have at least 8 years of breeding records available, with three of the colonies for > 13 years. As of last update, the multicolony demographic database consisted of records for over 5100 marmoset individuals, including 625 dams and 570 sires with known birthdates. For analyses, the records may be summarized with respect to each litter, for each dam (i.e., each dam’s reproductive history), as well as by colony or year. In future work, the marmoset database may be expanded and refined to include morbidity data, disease incidence and phenotype information. The multicolony database also may be used to explore differences in animal husbandry and colony management practices.

Notes:

A Demographic Review of the Captive Colony of *Callithrix kuhlii* at the University of Nebraska Callitrichid Research Facility

C.N. Ross¹, H. Jensen², J.E.Fite², and J.A. French²

Affiliation: ¹Department of Biological Sciences, University of Nebraska, Lincoln, NE; and ²Department of Psychology, University of Nebraska at Omaha, Omaha, NE

Although reports on colony demographics for a variety of callitrichid species are available in the literature, to date there has not been a detailed examination of Wied’s black tufted-ear marmoset, *Callithrix kuhlii*. The purpose of this study, therefore, is to present colony demographics for *Callithrix kuhlii* from the University of Nebraska’s Callitrichid Research Center from 1990-2003. *C. kuhlii* are currently held at 16 zoological parks and 1 research institution in the U.S. and abroad. The UNO colony represents the only North American breeding colony. Demographic data was collected throughout the 13 years of reproduction in standardized studbook databases including ARKS, MedARKS, and SPARKS. The colony was founded with 12 animals, and grew to a high of approximately 100 animals before breeding was concluded. Analyses that will be presented include temporal analyses of reproductive output, average lifespan, mortality, sex ratios and health issues. Additionally, we will focus upon the impact of management practices on health, as well as mortality and reproductive output during that time. We will use data from colony records to examine the effectiveness of prostaglandin treatment on reproductive suppression, as well as the need for, and health issues related to Vitamin D supplementation.

Notes:

The Influence of Litter Size on Inter Birth Interval in Captive *Callithrix kuhlii* at the University of Nebraska at Omaha’s Callitrichid Research Center

H.A. Jensen, J.E. Fite, and J.A. French

Affiliation: Department of Psychology, University of Nebraska at Omaha, Omaha, NE

Many factors can influence the total reproductive success for captive female marmosets. These can include maternal age, condition and experience, litter size, interbirth interval, and the presence of alloparents. Studies have shown that these factors can work in conjunction with each other to either increase or decrease a dam’s total lifetime infant production. With the important role that captive marmosets play in research and conservation today, it is vital that we understand how these factors interact in order to manage breeding colonies that optimize healthy infant production. This study looks at two of these factors, interbirth interval and litter size in female Wied’s black tufted-ear marmoset (*Callithrix kuhlii*). Information on the reproductive management of kuhlii marmosets is less documented than other, more prevalent species such as *C. jacchus*. The information for this study comes from databases at the University of Nebraska at Omaha’s Callitrichid Research Center. Reproductive data was collected on 16 breeding females over 10 years. Litter number and sizes were collected for each female, and interbirth interval was determined for each birth after the first. In all, 69 litters were examined; 1 quintuplet, 1 quadruplet, 17 triplet, 42 twin, and 8 singleton litters. No significant effect of litter size was found on interbirth interval. Infant survival data was also analyzed and shown to have no effect on interbirth interval. These findings confirm the results of studies on other callitrichid primate species (Snowdon, Sousa, Tardif, Ziegler, and others), and may prove useful in the reproductive management of *C. kuhlii*.

Notes:

Systemic AA Amyloidosis in the Common Marmoset

E. Ludlage¹, C.L. Murphy², S.M. Davern², A. Solomon², D.T. Weiss², D. Glenn-Smith³, S. Dworkin¹, and K.G. Mansfield¹

Affiliation: ¹New England National Primate Research Center, Harvard Medical School, Southborough, MA; ²University of Tennessee Graduate School of Medicine, Knoxville, TN; and ³University of California, Department of Anthropology, Davis, CA

The common marmoset (*Callithrix jacchus*) is a small New World primate native to Brazil that has been used extensively in biomedical research. A retrospective analysis of archived hematoxylin and eosin (H&E)-stained tissue sections and clinical records was conducted at the New England Primate Research Center (NEPRC) on 86 marmosets >1 year of age that were euthanized over the past decade due to morbidity and failure to thrive. Approximately 17% (15/86) were found to have amyloid deposits in one or more organs, including the liver, adrenal glands, kidneys, and intestine. This material was shown by amino acid sequence analysis to be composed of serum amyloid A (SAA)-related protein. This type of amyloidosis, designated AA or “secondary,” typically is associated with an inflammatory process that induces elevated levels of the SAA amyloidogenic precursor molecule. Remarkably, there were no significant differences in SAA concentrations or other distinguishing features in animals with versus those without amyloid, thus suggesting the possible inheritable nature of the disorder. In this respect, the common marmoset provides a unique experimental model for study of the pathogenesis and treatment of AA and other forms of systemic amyloidosis.

Notes:

Custom Designed Experimental Housing for Marmosets

D.G. Layne¹, R.A. Power², G. Branson³, K. Pittman⁴, D. Pratt³, D. Bush⁴, and S.D. Tardif¹

Affiliation: ¹Southwest National Primate Research Center, San Antonio, TX; ²Pennington Biomedical Research Center, Baton Rouge, LA; ³Biology Services Facility, University of Tennessee, Knoxville, TN; and ⁴LGL Animal Care Products, Inc., Bryan, TX

With marmosets emerging as one of the biomedical model species in between rodents and the larger, Old World primates, experimental equipment and housing that is commercially available for use with rodents or other primates is often rigged to fit the need. While it is true that marmosets are about the size of rats in terms of body weight (350-500g) their natural posture, locomotion, and social requirements are not the same. This presentation will describe the development and use of two experimental housing units: 1) a cage set and collection system for use in metabolic balance trials (24-72h) by a lactating female with infant(s) and 2) a cage for housing and handling marmosets in bio-safety level 4 conditions.

Notes:

Functional Magnetic Resonance Imaging in Conscious Marmosets: Methods and Applications in Neuroscience Research**C. Ferris**

Affiliation: Center for Comparative Neuroimaging, University of Massachusetts Medical School, Worcester, MA

Functional magnetic resonance imaging (fMRI) at high magnetic field strengths (4.7T – 11.7T) has far superior spatial and temporal resolution than any other non-invasive imaging technique. Functional MRI using the BOLD (blood oxygenation-level-dependent) technique measures changes in blood flow to areas of increased synaptic and neuronal activity. Mobile protons associated with hydrogen atoms in water are the primary source of MR signal. The level of paramagnetic deoxygenated hemoglobin in the blood vessels alters the magnetic-susceptibility of protons aligned in the magnetic field of the spectrometer. Enhanced neuronal activity is accompanied by an increase in metabolism concomitant with changes in cerebral blood flow and volume to the area of activation. The local blood flow to this area exceeds oxygen uptake, lowering the level of deoxygenated hemoglobin and increasing the T2*-relaxation time and MRI signal intensity.

Fully conscious animals are not used routinely in fMRI studies because of technical problems associated with motion artifacts. Any minor head movement distorts the image and may also create a change in signal intensity that can be mistaken for stimulus-associated changes in brain activity. In addition to head movement, motion outside the field of view can also obscure or mimic the signal from neuronal activation. Therefore most fMRI studies use general anesthetics to immobilize the animal. However, anesthetics preclude the study of brain activity involving cognition and emotion. Furthermore, anesthetics depress neuronal activity reducing BOLD signal. To circumvent these problems technology was developed to image fully conscious marmoset monkeys.

Methods

A restrainer was developed consisting of head and body holder with built-in radiofrequency electronics. Prior to imaging animals are habituated to the restraint stress and imaging procedure in a simulated environment. During MR sessions, animals are first lightly sedated with ketamine plus medetomidine (Domitor), and placed in the MR head and body holder. Once securely restrained, anesthesia is reversed with atipamezole (Antiseden). Prior to imaging animals are tested for their sensitivity and recovery to this anesthetic procedure. Animals recovering from anesthesia are judged to be fully conscious when they can both locomote normally and attend to cognitive tasks in their environment. This recovery time from anesthesia is incorporated into the imaging study.

High-resolution anatomical data sets of ca. 12 min in duration are acquired using a multi-slice spin-echo (RARE) pulse sequence at the beginning and end of each imaging session. In plane spatial resolution is ca 120 μm x 120 μm with a slice thickness of 1 mm. Functional images are acquired at 15 sec intervals using a gradient-echo (FLASH) sequence weighted for T2*. BOLD signal changes between control and stimulation periods are analyzed and significant differences presented as activation maps and time-course plots. Subtraction of the first and last anatomical data sets and observation of functional time-series helps detect motion artifact.

Applications in Neuroscience Research

Emotional States

Fear, anger, hunger, and sexual arousal are examples of emotion states and are fertile areas of investigation using fMRI. A library of vocalizations, smells and visual images with proven ethological significance in the animal’s natural habitat and in the semi-natural environment of the laboratory setting can be collected and used to communicate with the animal in the magnet. For example, presentation of the odor of a novel reproductively receptive female marmoset to a male marmoset in the magnet will elicit changes in brain activity related to sexual arousal.

Brain/Environment Interactions in Development

There are myriad examples in animal studies showing early emotional or environmental insult can affect brain development with long-term neurobiological and behavioral consequences. Insights into the etiology of mental illness may be gleaned by longitudinal studies on marmosets examining the interaction between a vulnerable gene pool and a stressful environment at critical times in development. Since fMRI is non-invasive and can be used to study the same animal over the course of its life it is possible to observe developmental changes in neuroanatomy, brain activity and brain chemistry (spectroscopy).

Drugs Effects on Brain Activity

Studying changes in brain activity in response to acute and prolonged exposure to psychotherapeutics and drugs of addiction are two other obvious applications of fMRI. For example, many psychotropic drugs cause a prompt increase in brain levels of neurotransmitters. Nonetheless, patients require weeks of treatment before reporting an improvement in their condition. This would suggest drug efficacy for the treatment of mental illness is due to secondary changes in the neurochemical signals and pathways that are slowly affected by the continuous exposure to the psychotropic agent. Functional MRI would help to resolve this mechanism of action. In the case of drugs of addiction, rhesus monkeys can be trained to self-administer cocaine, withdrawn from the drug and later reinstated in response to conditioned cues. It is feasible to image these different phases of cocaine addiction.

Testing Cognitive Performance

Since animals will readily respond to peripheral stimulation while in the magnet fMRI they may be used in studies of classical conditioning. For example, foot shock can be used as an unconditioned response in associative learning paradigms. When coupled with a conditioned stimulus like scent or light it can be used in learning studies examining discrimination and perception. Operant conditioning would be more difficult because a behavioral action, e.g. bar pressing eliciting rewarding or punishing stimuli, would be necessary. However, recent imaging studies on conscious rhesus monkeys show this is feasible and opens the area of cognitive neuroscience to investigation with fMRI in marmosets.

Acknowledgement: This work was funded by grants from the National Institute of Mental Health MH58700, MH59501

Notes:

Common Marmoset as a Model to Study the Neural Basis of Primate Vocal Communication
X. Wang

Affiliation: Laboratory of Auditory Neurophysiology, Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD

Understanding how the brain processes vocal communication sounds remains one of the most challenging problems in neuroscience. The long-term objective of the research in my laboratory is to reveal physiological mechanisms underlying perception and production of vocal communication sounds in non-human primates. The marmoset is chosen for our studies because it has several important advantages over other non-human primate species for behavioral, neurophysiological and developmental studies in laboratory conditions (namely, highly vocal in captivity, easily bred and a smooth brain). In the past several years, we have established a quantitative database of marmoset vocalizations captured in our breeding colony at Johns Hopkins. Our results showed that marmosets vocalize a rich repertoire of communication sounds that contain exquisite information for discriminating call types and caller identity, similar to some of the fundamental properties demonstrated for human speech. We have also pursued extensive neurophysiological experiments that aim to understand neural encoding mechanisms in auditory cortex of marmosets. Findings from these experiments have showed a close correlation between spectral and temporal characteristics of marmoset vocalizations and response properties of cortical neurons, as well as auditory-vocal interactions in auditory cortex of this non-primate species. Our studies have demonstrated the promising potential of the common marmoset as a neuroethological model to advance the understanding of human speech and language processing in the brain. I will summarize in my presentation a series of studies from my laboratory addressing these issues.

This research was supported by NIH Grants DC03180, DC05808; Publications available at <http://www.bme.jhu.edu/~xwang/papers.html>

Notes:

Preliminary Evidence of Early Long Call Acquisition and Usage in a Callitrichid Primate (*Callithrix kuhlii*)

M. Rukstalis¹ and J.A. French²

Affiliation: Callitrichid Primate Research Center, ¹Departments of Biology and ²Psychology, University of Nebraska at Omaha, Omaha, NE

The acquisition and appropriate usage of adult vocalizations among infant primates is a topic which has generated much controversy. Recent research has suggested that primate infants may experience more complex periods of vocal ontogeny than previously believed. However, this study presents preliminary evidence of a limited ontogeny in the acquisition and appropriate usage of one vocalization, the long call, in Wied's black tufted-ear marmosets (*Callithrix kuhlii*). We assessed the morphology and usage of phee calls (i.e., long calls) in isolated marmoset infants from one to seven weeks of age. Infant marmosets removed from their natal cage vocalized frequently, participating in antiphonal calling bouts with parents and siblings. When compared to adults, the structure of marmoset infant long calls was similar even as early as eight days of age (mean duration of call 2.73s vs. 2.87 s, respectively). While infant calls were similar, some structural differences were evident. In general eight day-old infant marmosets had higher maximum and minimum call frequencies than adults (11.98 kHz and 7.93 kHz vs. 7.99 kHz and 5.74 kHz respectively), used more syllables per call (5.2 vs. 3.7), and had shorter average syllable length (348.2 ms vs. 595.5 ms). These differences were reduced by seven weeks of age and may reflect maturational changes (i.e. increasing vocal tract length and body mass) rather than development of a functional call. While additional calls in the marmoset's repertoire may undergo more complex periods of development, the preliminary evidence presented here suggest that long calls may experience a limited ontogeny.

Notes:

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Making Sense Out of Scents: Olfaction and Reproduction in Marmosets and Tamarins
T. Ziegler*

Affiliation: National Primate Center, University of Wisconsin, Madison, WI

Marmosets and tamarins have a well-developed chemosensory system used for reproductive communication. They make use of both the main olfactory system (MOS) and the accessory olfactory system (AOS). Chemical signaling is received in a synergistic manner through both systems with the detection of the volatile substances by the MOS and the non-volatile substances through the vomeronasal of the AOS. Chemical communication enables marmosets and tamarins to adjust both mating effort and parental effort according to their social surroundings. Functional studies will be presented that demonstrate the responses elicited, both hormonal and behavioral, in male marmoset and tamarins to chemical signals.

Notes:

Mating Systems in Marmosets: Data from Captive and Wild Groups

M.E. Yamamoto, A. Araújo, F.S. Albuquerque, M.B. Sousa, A.I. Alencar and M.F. Arruda

Affiliation: Universidade Federal do Rio Grande do Norte, Natal, Brazil

The mating system of callitrichids has been suggested to be monogamous, polygynous and polyandrous. In *Callithrix jacchus*, two breeding female, as well as one breeding female groups have been reported. Reproduction in female common marmosets is usually related to dominance status, which is reflected in agonistic behavior and ovarian function. Socially dominant females have been reported to receive submissive behavior from subordinates, while exhibiting normal ovulatory function, intra-group copulatory behavior, and term births resulting in surviving offspring. Subordinate females, however, receive aggressive behavior from dominants, while exhibiting reduced or absent ovulatory function, and little or no intra-group copulatory behavior, with rare occurrences of term births. The long term monitoring of wild *Callithrix jacchus* groups in Nizia Floresta, RN, Brazil, suggests that wild groups may have one or two breeding females, but some of the two-breeding female groups may be, in fact, monogamous. Subordinate females in these two groups differed regarding the permanence in the group after breeding, the number of surviving offspring and the mating partner. Data from our colony indicated that subordinate females may or may not fight for the dominant rank in their groups, and this reflects in their behavioral and hormonal profiles. We suggest that such variation in female-female social dominance relationships and the associated variation in the degree and reliability of fertility suppression may explain why free-living groups of common marmosets can exhibit either one or two breeding females. We also suggest that subordinate female marmosets can explore reproductive alternatives other than remaining infertile in their natal group while waiting for a breeding vacancy in a neighboring group or migrating.

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Notes:

Sex Differences in Play Behavior Among Captive Common Marmosets (*Callithrix jacchus*)

H. Guard, K.T. Jenkins, **L.A. Zemba**, and R.L. Roberts

Affiliation: Laboratory of Comparative Ethology, NICHD, National Institutes of Health, Poolesville, MD

Social play behavior may be an expression of affiliation that impacts subsequent social development and may function as a means of acquiring knowledge and abilities that provide animals with the optimal chance for survival as social adults. Differences in play behavior between male and female primates have been recorded in various studies, but few studies have examined play in common marmosets, a monogamous New World primate that exhibits relatively little behavioral sexual dimorphism or sexual division of labor. In our study, play behaviors, along with other social and non-social behaviors were recorded in eleven captive common marmoset (*Callithrix jacchus*) family groups. When initiating play, both male infants (1-6 months old) and juveniles (over 7 months old) played for a longer average duration than females, particularly when initiating rough and tumble play. Our results indicate that play behavior differs between males and females in ways that mirror differences in the behavior of adults, and this sex difference is present at the time that play emerges. Individual differences in the composition of litters and family groups might influence the expression of play behavior in ways that inform competing hypotheses about the adaptive function of play behavior.

Notes:

Primate Models of Inflammation with Emphasis on Multiple Sclerosis
C. Genain

Affiliation: Department of Neurology, University of California, San Francisco, CA

Models that adequately reflect the complexity of human multiple sclerosis (MS) are needed, especially for pre-clinical testing of immunomodulatory drugs. A unique experimental system to study MS has been created in the New World common marmoset *Callithrix jacchus jacchus* (*C. jacchus*). Following immunization with myelin, these monkeys develop a chronic, relapsing-remitting form of experimental allergic encephalomyelitis (EAE), which pathologically, recapitulates the hallmark features of lesions of human MS. The MS-like lesion in *C. jacchus* results from a complex immune response against myelin antigens, and requires both T cells and pathogenic antibodies. Studies of *C. jacchus* EAE have permitted the identification of a major target for pathogenic autoantibodies in MS, the myelin/oligodendrocyte glycoprotein. Other advantages of the model include a natural bone marrow chimerism, which permits T cell adoptive transfers between siblings, and the possibility of using different antigens to produce either inflammatory or demyelinating phenotypes of EAE. Despite their small size, sequential *in vivo* imaging and immunological studies are possible in these monkeys, and have been used to monitor efficacy in pre-clinical trials. In addition and similar to tamarins, *C. jacchus* marmosets have a large deletion in the major histocompatibility class I gene region which perhaps favors susceptibility to spontaneous autoimmune diseases such as thyroiditis and inflammatory bowel disease. Due to close phylogeny and high homology of immune and nervous system genes with humans, this model has fast-tracked the development of novel therapeutics for MS such as B cell targeted therapy.

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Notes:

Immunogenicity of GBV-B DNA Vaccines in Common Marmosets

S. Biswas¹, Keith Mansfield², James Jacob³, and Shan Lu¹

Affiliation: ¹Department of Medicine, University of Massachusetts Medical School, Worcester, MA; ²New England Regional Primate Research Center, Harvard, Medical School, Southborough, MA; and ³College of Veterinary Medicine, Cornell University, Ithaca, NY

The common marmoset, *Callithrix jacchus*, an important animal model potentially represents an alternative species to more traditional nonhuman primates. A major impediment in the study of Hepatitis C virus (HCV) is the lack of affordable yet relevant animal models. GB virus-B (GBV-B), which infects and causes hepatitis in marmosets, has been described to be phylogenetically most closely related to HCV. We have developed the common marmosets as a relevant animal model for HCV vaccine development studies and also to generate and identify useful immunological tools and reagents that will be used for further research involving this animal model.

The envelope proteins of HCV are likely vaccine candidates and their counterparts in GBV-B, E1 and E2, were examined for their immunogenicity in marmosets. The genes coding for GBV-B envelope proteins were cloned in a mammalian expression vector and used to immunize marmosets via gene gun methodology. It was evident that DNA immunization with the DNA vaccine expressing only GBV-B E2 antigen and not where E1 was expressed in cis, generated high levels of E2-specific antibodies as demonstrated by ELISA and Western blot analysis of the marmoset sera, using anti-human antibodies as the secondary antibody. The effect of DNA priming was further demonstrated when the immunized animals showed enhanced anti-E2 antibody responses (anamnestic) after being challenged with GBV-B while there was no detectable anti-E2 antibody in naïve animals shortly after viral challenge. Thus, we describe for the first time the generation of a specific immune response mediated by DNA immunization in the common marmosets. We confirm the cross-reactivity of anti-human IgG to marmoset antibodies as observed in other studies and our research further opens the door for studies of many such reagents in the future.

Notes:

Androgens and Aging in Male Marmosets

J.A. French, K.J. Patera, S. Boykins, and A.M. Puffer

Affiliation: Callitrichid Research Center, University of Nebraska at Omaha, Omaha, NE

Aging is associated with dramatic changes in gonadal steroid production in men and women, including increases in hormones in the early stages of life (e.g., peripubertally) and decreases in hormone titers in later years. Similar patterns are apparent in a wide range of mammals, suggesting a ubiquitous underlying process. Callitrichid primates may represent an exception to this rule, at least for females. In tamarins, while females do experience a reduction in ovulatory incidence as they age, ovarian luteal tissue does not appear to lose its steroidogenic properties in older females (Tardif & Ziegler, Biol. Reprod., 1992). We explored patterns of changes in steroid hormone production in male marmosets whether male marmosets exhibit changes in androgens as they age. We measured concentrations of urinary testosterone (uT), urinary estradiol (uE), and urinary cortisol (uC) in male marmosets (*Callithrix kuhlii*) via enzyme immunoassays. Concentrations of uT were high in infants less than three months of age, and levels were lowest in males 5-6 months of age. AT 12 months of age (the age of earliest documented conceptive mating in males), mean levels of uT exceeded 1,000 ng/mg Cr, suggesting this value as a diagnostic for reproductive maturation. Levels of uT and uC were not different in sons residing in family groups and while paired with females, but were elevated in males housed in same-sex groups. In contrast, uE became elevated in males after pairing with adult females. Across the lifespan, uT levels were characterized by a weak inverted U-shaped function, with peak levels at 6-8 years of age and delining levels thereafter. There was a weak but significant correlation between age and uT from 6-15 years of age ($r = 0.25, p < 0.01$). The results of GnRH challenges revealed that gonadal tissue not only retained its steroidogenic properties in older male marmosets, but may be more sensitive to GnRH challenge than in younger males. Change in plasma T and E concentrations were highly and positively correlated with age (r 's = 0.85 and 0.86, $p < 0.01$, respectively). These results suggest that alterations in hypothalamic-pituitary-gonadal activity associated with age in male marmosets are similar to those of other mammals, but unlike other primates (and like female callitrichids), steroidogenic cells in the gonads appear to retain function well into adult aging. The results point to the utility of the male marmoset as a model for the effects of aging on androgen-mediated traits. Supported by NSF (00-91030) and NIH (HD 42882).

Notes:

Getting Hormonal Data From Wild Common Marmoset Females: New Insights About Immigration and Mating Systems Mechanisms

M.B. Sousa, A.C.S.R. Albuquerque, and H.M. Santos

Affiliation: Universidade Federal do Rio Grande do Norte, Natal, Brazil

The use of hormonal analysis in feces is a useful tool to investigate reproductive performance in wild primates. A two-step method to measure fecal progesterone was recently validated for captive common marmosets. These small primates live in secondary forests in the Northeastern region of Brazil and the major aspects related to their reproductive strategies have been clarified by the association between behavioral and hormonal studies. Despite the large hormonal information obtained from captive common marmosets no studies were developed until now for natural groups. Therefore the goals of this study were to determine the methodological concerns related to fecal collection under natural conditions and to establish the reproductive functioning of subordinate females in both situations: 1) before emigration from the natal group and, 2) when they were living in either monogamous and polygynous groups. We found that the chance to collect fecal samples is a 100% when it is done shortly after the animals wake up when they start their motor activities around their sleeping trees. Factors such as temperature, rainfall, pregnancy and parturition do not disrupt the sampling but retards slightly the time of the first defecation. Subordinate females emigrated from natal groups showing different reproductive conditions such as not ovulating, ovulating or even pregnancy and in one occasion we recorded emigration and return to natal group by one subordinate female when a reproductive vacancy appeared. Subordinate females were able to reproduce in both monogamous and polygynous groups but most in occasions they lost their infants. These data indicate that subordinate females when living in natural groups express different strategies trying to obtain their reproductive success.

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Notes:

Bone Mineral Density During Lactation in Common Marmosets

R.A. Power¹, D.G. Layne², D. Smucny², and S.D. Tardif²

Affiliation: ¹Pennington Biomedical Research Center, Baton Rouge, LA, and ²Southwest National Primate Research Center, San Antonio, TX

A significant decline in bone mineral density (BMD) has been observed during lactation in women and Old World monkeys; calcium supplementation does not alter this effect in women. Our study examines the effects of lactation on BMD in common marmosets fed a calcium replete (1.2%; CCL) or deplete (0.3%; CRL) diet. DEXA is performed at baseline (non-pregnant; NP), 22 days post-partum (d22pp), 60 days post-partum (d60pp), and 94 days post-partum (d94pp). Data for 8 subjects have been collected (4 CCL, 4CRL). A significant effect of time, with a significant decrease in BMD at d60pp compared to NP, was observed for the combined 8 subjects. A difference in BMD/NPBMD between CCL and CRL subjects was not observed at any lactation time point. However, only 1 CRL subject, compared to 3 CCL subjects, nursed twins, and within a repeated measures ANOVA model, a significant effect of litter size was observed. In addition, NP females (n=13) were compared to males (n=11). A slight trend toward higher BMD in females compared to males was observed, with no difference in age between the groups.

These results suggest a decrease in BMD during lactation in common marmosets, with no apparent effect of dietary calcium content. However, litter size appears to have an effect, and the greater number of twin litters within the CCL group compared to the CRL group should be considered. In addition to these densitometry data, bone turnover data by histomorphometry are currently being collected and will be discussed.

Notes:

List of Attendees – Contact Information



Abbott, David (Organizer)

Wisconsin National Primate Research Center
University of Wisconsin-Madison
Department of Ob/Gyn
1223 Capitol Court
Madison, WI 53715
E-mail: abbott@primate.wisc.edu

Almond, Roz, Ph.D.

University of Wisconsin-Madison
Department of Psychology
1020 West Johnson St.
Madison, WI 53706
Tel: 608-263-5072
E-mail: reaa2@cam.ac.uk

Austad, Steve

University of Idaho and
Univ. of Texas Health Science Center, SA
E-mail: austad@uidaho.edu

Bales, Karen L.

University of Illinois at Chicago
Department of Psychiatry
1601 W. Taylor St.
Chicago, IL 60612
Tel: 312-413-7521
Fax: 312-966-7658
E-mail: baleskaren@aol.com

Benton, Charles E., Ph.D.

Harlan Teklad
2826 Latham Drive
Madison, WI 53716
Tel: 608-277-2016
Fax: 608-277-2066
E-mail: cbenton@harlan.com

Bertram, Rick, D.V.M.

Glaxo Smith Kline
Five Moore Dr.
P.O. Box 13398
Room G-3064-2C
Research Triangle, Park, NC 27709
Tel: 919-483-6082
Fax: 919-483-6070
E-mail: fb48878@gsk.com

Biswas, Subhabrata, Ph.D.

University of Massachusetts
Medical School
Lazare Research Bldg, Lu Lab, 340C
364 Plantation Street
Worcester, MA 06105
Tel: 508-856-1186
Fax: 508-856-6751
E-mail: subhabrata.biswas@umassmed.edu

Boe, Carla

University of Wisconsin-Madison
Zoology Dept. c/o Dept. Psychology
1202 W. Johnson St.
Madison, WI 53706
Tel: 608-263-5072
E-mail: cyboe@wisc.edu

Bolton, Iris, D.V.M.

Pfizer, Inc.
0216-300-233
7000 Portage Road
Kalamazoo, MI 49001
Tel: 269-833-3184
E-mail: iris.d.bolton@pfizer.com

Brackee, Gordon, D.V.M.

University of Texas Southwestern
5323 Harry Hines
Dallas, TX 75235
Tel: 214-648-4100
Fax: 214-648-4104
E-mail: gordon.brackee@utsouthwestern.edu

Burdett, Eric, D.V.M.

University of Utah
50 N 2030 E
Salt Lake City, UT 84112
Tel: 801-585-0915
Fax: 801-581-7005
E-mail: eburdett@arc.utah.edu

Burkholder, Tanya H., D.V.M.

National Institutes of Health
Division of Veterinary Resources
9000 Rockville Pike, Bldg. 28 Rm. 104
Bethesda, MD 20892
Tel: 301-496-9201
Fax: 301-402-0377
E-mail: burkholt@mail.nih.gov

List of Attendees – Contact Information



Butler, Ray

Novartis Pharmaceuticals
One Health Plaza
Bldg. 406/150L
East Hanover, NJ 07936
E-mail: ray.butler@pharma.novartis.com

Cronin, Katherine

Lincoln Park Zoo
2001 N. Clark St.
Chicago, IL 60614
Tel: 312-742-7250
Fax: 312-742-7220
E-mail: kcronin@lpzoo.org

Curlee, Joseph F., Jr., D.V.M.

Harlan-Sprague Dawley, Inc.
P.O. Box 44220
Madison, WI 53744-4420
Tel: 608-277-2000
Fax: 608-437-4327
E-mail: bcurlee@harlan.com

Emerson, Carol, D.V.M.

Lovelace Respiratory Research Institute
2425 Ridgecrest Drive SE
Albuquerque, NM 87108
Tel: 505-348-9611
Fax: 505-348-4980
E-mail: cemerson@lrri.org

Estrada, Alejandro, Ph.D.

University of Mexico
Estación de Biología Los Tuxtlas
Instituto de Biología, UNAM Apdo. Pos. 176
San Andrés Tuxtla, Veracruz, Mexico 95700
Tel: 294-942-4668
Fax: 294-942-4668
E-mail: aestrada@primatesmx.com

Ferrell, Thomas L., D.V.M.

Manager, LAR
Calvert Laboratories, Inc.
Scott Technology Park
100 Discovery Drive
Olyphant, PA 18447
Tel: 570-585-2322
Fax: 570-586-3450
E-mail: thomas.ferrell@calvertlabs.com

Ferris, Craig

University of Massachusetts Medical School
Center for Comparative Neuroimaging
55 Lake Avenue North
Worcester, MA 01655
E-mail: craig.ferris@umassmed.edu

French, Jeff (Organizer)

University of Nebraska at Omaha
Department of Psychology and Nebraska
Behavioral Biology Group
Omaha, NE 68182-0274
Tel: 402-554-2558
Fax: 402-554-2556
E-mail: jfrench@unomaha.edu

Genain, Claude

University of California, San Francisco
Department of Neurology
San Francisco, CA 94143-0435
E-mail: claudeg@itsa.ucsf.edu

Ginther, Anita J., Ph.D.

University of Wisconsin
Psychology Dept.
1202 W. Johnson St.
Madison, WI 53706
Tel: 608-836-6991
E-mail: ajg@itpnet.com

Hadzic, Zezira, V.M.

Wisconsin National Primate Research Center
University of Wisconsin-Madison
1220 Capitol Court
Madison, WI 53715-1299
Tel: 608-265-6247
Fax: 608-265-2067
E-mail: vhadzic@primate.wisc.edu

Humle, Tatyana

University of Wisconsin-Madison
Psychology Dept.
1202 W. Johnson St.
Madison, WI 53706
Tel: 608-262-7550
E-mail: tatyanahumle@eml.cc

List of Attendees – Contact Information



Jaume, Juan C., M.D.

University of Wisconsin-Madison
Department of Medicine
600 Highland Ave
Madison, WI 53792
Tel: 608-265-7000 ext. 4024
Fax: 608-280-7294
E-mail: jcj@medicine.wisc.edu

Jensen, Heather

University of Nebraska at Omaha
6001 Dodge St.
Omaha, NE 68182
Tel: 402-545-3205
E-mail: hajensen@mail.unomaha.edu

Jordan, (Elaine) Kay

ENS, LORR, CC, National Institutes of Health
Bldg. 10, Rm. BIN256
10 Center Dr. - MSC1074
Bethesda, MD 20892-1074
Tel: 301-435-5253
Fax: 301-402-3216
E-mail: ekj@helix.nih.gov

Kurian, Aimee

University of Wisconsin-Madison
Psychology Dept.
1202 W. Johnson St.
Madison, WI 53706
Tel: 608-262-9654
Fax: 608-262-4029

Layne, Donna G. (Organizer)

Southwest National Primate Research Center
P.O. Box 760549
San Antonio, TX 78245-0549
Tel: 210-258-9866
Fax: 210-258-9883
E-mail: dlayne@icarus.sfbr.org

Ludlage, Elizabeth (Organizer)

New England National Primate Research
Center, Harvard Medical School
P.O. Box 9102
Southborough, MA 01772-9012
E-mail: elizabeth_ludlage@hms.harvard.edu

MacGill, Tracy, D.V.M.

Center for Drug Evaluation & Research, FDA
Division of Counter-Terrorism, HFD-970
7520 Stanish Place
Rockville, MD 20855
Tel: 301-827-7324
Fax: 301-827-7722
E-mail: macgillt@cderr.fda.gov

Mansfield, Keith (Organizer)

New England National Primate Research
Center, Harvard Medical School
P.O. Box 9102
Southborough, MA 01772-9012
E-mail: keith_mansfield@hms.harvard.edu

Maxwell, Heather R.

University of Nebraska at Omaha
6001 Dodge St.
418 Alwine Hall
Omaha, NE 68182
Tel: 402-554-3094
Fax: 402-554-3121
E-mail: hmaxwell@mail.unomaha.edu

Moloo, Badru, D.V.M.

Animal Resources Centre
University Health Network
Toronto, Ontario
Canada M5G 1X6
Tel: 416-946-2250
Fax: 416-946-2340
E-mail: bmoloo@uhnres.utoronto.edu

Moscovice, Liza

University of Wisconsin-Madison
Psychology Dept.
1202 W. Johnson St.
Madison, WI 53706
Tel: 608-262-7550
E-mail: Linzarmos@fastmail.fm

Nadon, Nancy, Ph.D.

National Institute on Aging
Office of Biological Resources and Resource
Development
7201 Wisconsin Ave., GW2C231
Bethesda, MD 20892
Tel: 301-402-7744
Fax: 301-402-5997
E-mail: nadonn@nia.nih.gov

List of Attendees – Contact Information



Newman, John, Ph.D.

NICHD, National Institutes of Health
Laboratory of Comparative Ethology
Box 529
Poolesville, MD 20837
Tel: 301-496-0835
Fax: 301-496-0066
E-mail: jdnewman@helix.nih.gov

Neilsen, Ronald, D.V.M.

University of Utah
50 N 2030 E
Salt Lake City, UT 84112
Tel: 801-585-6870
Fax: 801-581-7005
E-mail: rnielsen@arc.utah.edu

Power, Michael

National Zoo
Smithsonian Institution
Nutrition Laboratory
Washington, DC 20008
E-mail: mpower@acog.org

Power, Rachel (Organizer)

Transgenics
Pennington Biomedical Research Center
6400 Perkins Road
Baton Rouge, LA 70808
Tel: 225-933-7642
Fax: 225-763-0273
E-mail: powerra@pbrc.edu

Reeb, Jamie

Harlan-Sprague Dawley, Inc.
2826 Latham Dr.
Madison, WI 53713
Tel: 608-230-2116
Fax: 608-230-2106
E-mail: bcurlee@harlan.com

Reilly, Sheila M.

Novartis Pharmaceuticals
One Health Plaza
Bldg. 406/150L
East Hanover, NJ 07936
Tel: 862-778-6950
E-mail: sheila.reilly@pharma.novartis.com

Roberts, Lucille, Ph.D.

National Institutes of Health
Laboratory of Comparative Ethology
16701 Elmer School Rd., Bldg. 112, Rm. 205
Dickerson, MD 20842
Tel: 301-435-0962
Fax: 301-435-0962
E-mail: RoberLu@csr.nih.gov

Rolf, Lester L., Jr., D.V.M.

University of Pennsylvania/ULAR
Medical School Mail Room/MC 1604
Philadelphia, PA 19104
Tel: 215-898-2351
Fax: 215-572-4447
E-mail: penrolf@pobox.upenn.edu

Ross, Corinna

University of Nebraska-Lincoln
318 Manter Hall
Lincoln, NE 68588-0118
Tel: 402-472-3999
E-mail: cross@bigred.unl.edu

Rukstalis, Michael

University of Nebraska at Omaha
Dept. of Biology
60th and Dodge Streets
Omaha, NE 68154
Tel: 402-554-3094
Fax: 402-558-2556
E-mail: mrukstalis@mail.unomaha.edu

Schultz-Darken, Nancy (Organizer)

Wisconsin National Primate Research Center
University of Wisconsin-Madison
1220 Capitol Court
Madison, WI 53715-1299
Tel: 608-263-3563
Fax: 608-265-0567
E-mail: darken@primate.wisc.edu

Scott, Jill

Wisconsin National Primate Research Center
University of Wisconsin-Madison
1220 Capitol Court
Madison, WI 53715-1299
Tel: 608-263-3519
E-mail: jscott@primate.wisc.edu

List of Attendees – Contact Information



Siani, Jennifer

University of Maryland
Department of Biology
College Park, MD 20742
Tel: 301-405-0583
Fax: 301-314-9358
E-mail: jmsiani@umd.edu

Smucny, Darlene A., Ph.D.

Southwest National Primate Research Center
P.O. Box 760549
San Antonio, TX 78245-0549
Tel: 210-614-7863
Fax: 210-258-9883
E-mail: smucnydar@hotmail.com

Snowdon, Charles T., Ph.D.

University of Wisconsin-Madison
Psychology Dept.
1202 W. Johnson St.
Madison, WI 53706
Tel: 608-262-3974
Fax: 608-262-4029
E-mail: snowdon@wisc.edu

Sousa, Maria Bernardete

Universidade Federal do Rio Grande do Norte,
Natal, Brazil
E-mail: mdesousa@cb.ufrn.br

Sramek, Mary, D.V.M.

Abbott Laboratories
Dept. R403, Bldg/F1 AP1 3/1
100 Abbott Park Road
Abbott Park, IL 60064-6105
Tel: 847-938-8697
Fax: 847-938-6524
E-mail: mary.sramek@abbott.com

Tardif, Suzette, Ph.D. (Organizer)

Southwest National Primate Research Center
P.O. Box 760549
San Antonio, TX 78245-0549
Tel: 210-258-9885
Fax: 210-258-9883
E-mail: stardif@icarus.sfbr.org

Thorsen, Peter, D.V.M.

University of California, San Francisco
Laboratory Animal Resource Center
Room 401B, Box 0564
San Francisco, CA 94143-0564
Tel: 415-502-8295
Fax: 415-502-8252
E-mail: pthorsen@larc.ucsf.edu

Van Belle, Sari

University of Wisconsin-Madison
Department of Zoology
145 Noland Hall, 250 North Mills Street
Madison, WI 53706
E-mail: sarievanbelle@primatesmx.com

Wang, Xiaoqin, Ph.D.

Johns Hopkins University
School of Medicine
720 Rutland Avenue, Ross 424
Baltimore, MD 21205
Tel: 410-614-4547
Fax: 410-614-9599
E-mail: xwang@bme.jhu.edu

Yamamoto, Marie Emilia

Universidade Federal do Rio Grande do Norte,
Natal, Brazil
E-mail: emilia.yamamoto@uol.com.br

Zahed, Sophia Katerina

University of Wisconsin-Madison
Psychology Dept.
1202 W. Johnson St.
Madison, WI 53706
Tel: 608-262-7550
Fax: 608-262-4029
E-mail: skzahed@wisc.edu

Zemba, Lindsay

National Institutes of Health
Laboratory of Comparative Ethology
16701 Elmer School Rd, Bldg 112, Rm 205
Dickerson, MD 20842
Tel: 301-496-0835
Fax: 301-496-0066
E-mail: zembal@mail.nih.gov

List of Attendees – Contact Information



Ziegler, Toni, Ph.D.

Wisconsin National Primate Research Center

University of Wisconsin-Madison

1220 Capitol Court

Madison, WI 53715-1299

Tel: 608-263-3507

Fax: 608-263-3524

E-mail: ziegler@primate.wisc.edu

