HHS Public Access

Author manuscript

Am J Primatol. Author manuscript; available in PMC 2017 June 01.

Published in final edited form as:

Am J Primatol. 2013 November ; 75(11): 1063–1083. doi:10.1002/ajp.22175.

Four Decades of Ground-Breaking Research in the Reproductive and Developmental Sciences: The Infant Primate Research Laboratory at the University of Washington National Primate Research Center

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Abstract

The Infant Primate Research Laboratory (IPRL) was established in the 1970s at the University of Washington as a visionary project of Dr. Gene (Jim) P. Sackett. Supported by a collaboration between the Washington National Primate Research Center and the Center on Human Health and Disability, the IPRL operates under the principle that learning more about the causes of abnormal development in macaque monkeys will provide important insights into mechanisms underlying childhood neurodevelopmental disorders. Over the past forty years, a broad range of research projects have been conducted at the IPRL. Some have described the normal expression of species-typical behaviors in nursery-reared macaques while others have focused on specific issues in perinatal medicine and research. This article will review the unique history of the IPRL and the scientific contributions produced by research conducted in the laboratory. Past and present investigations at the IPRL have explored the consequences of adverse early rearing, low-birth-weight, prematurity, epilepsy, chemical/drug exposure, viral infection, diarrheal disease, vaccine safety, assisted reproductive technologies and perinatal hypoxia on growth and development. New directions of investigation include the production of a transgenic primate model using our embryonic stem cell-based technology to better understand and treat heritable forms of human mental retardation such as fragile X.

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Keywords

infant; Macaque; development; behavior; animal model

This review article details the rich history of scientific accomplishments at the Infant Primate Laboratory (IPRL), a core facility of the Washington National Primate Research Center (WaNPRC) and the Center on Human Development and Disability (CHDD) at the University of Washington in Seattle, Washington. Our story begins with a personal narrative from the founder of the laboratory, Dr. Gene (Jim) P. Sackett, on how the laboratory came to exist and the interdisciplinary vision that has actively sustained the laboratory for over four decades. The narrative is followed by a review of the important research that has been conducted in the laboratory over the years in the areas of breeding, pregnancy and delivery, early rearing effects on development, normative behavioral development, the effects of exposures to environmental pollutants and maternal and childhood medications, infectious disease processes, models for treatments of birth injury, and stem-cell biology and transgenic monkey models.

Our story begins….

Establishing the IPRL-A personal narrative by Dr. Jim Sackett

In February of 1970, Gerry Ruppenthal, Ed Stephenson, and I (Jim Sackett) flew from Madison, Wisconsin, to decide about taking jobs in Seattle at the University of Washington. Starting as an undergraduate, Gerry had worked for Harry and Margaret Harlow at the University of Wisconsin Psychology department Primate Laboratory for over 10 years. Ed was an electrical engineer constructing primate test and measurement equipment for the Harlow lab. I was an Associate Professor of Psychology in my seventh year at Wisconsin learning how to study rhesus monkey behavioral development.

I had been offered a Washington Regional Primate Research Center (WaRPRC) core staff position, a three year new program grant in the Child Development and Mental Retardation Center (CDMRC), and a professorship in the Psychology department. The offer included jobs for Gerry and Ed, lab space, and some equipment funds. We arrived on a 70 degree day with a view of snow covered mountains and Puget Sound. Gerry and Ed, Wisconsinites who had not previously seen the ocean, were awed. We accepted our offers, basked in 75 degree sunshine, and went back to 20 below zero in Madison.

We moved to Seattle in August, 1970. Our University Hospital basement space consisted of a large room to be used for housing infants, an adjoining room for juveniles and adults, a nursery room for newborn monkeys, a food preparation area, a cage washer, a room with caging for pregnant females, five small testing rooms, and an office. Harry Harlow had graciously allowed us to take a number of cages and equipment, and 16 juvenile rhesus monkeys that I had been studying for several years. We were ready to work by the end of 1970.

Initial Goals

Some background history is necessary to understand our early goals. At Wisconsin, Gerry's work with rhesus monkeys involved assisting in time mating procedures, building and maintaining inanimate surrogate mothers, nursery care of newborns, developing observational measurements of infants with and without mothers in their home cages and infants and juveniles in playrooms, conducting learning tests on infants and juveniles and designing cages and methods for housing mother-father-infant family groups. This work was the core of the Harlows' developmental research in the late 1950's and 1960's [see Mears, 1986, for details].

I was brought to Wisconsin by Harlow as a postdoctoral researcher, having worked with normal and developmentally disabled humans and rats but no prior research experience with nonhuman primates. I developed a research program which involved rhesus monkeys that had been reared by other researchers under social isolation, partial isolation, peer only, and mother rearing conditions. The work studied post-rearing social and personal behavior, social preferences, and responses to novelty. I also began studies aimed at understanding the mechanisms producing the abnormal behaviors seen in social isolation reared monkeys. This involved rearing monkeys with social experience from still and motion pictures of monkeys and nonsocial scenes, and included therapy studies attempting to normalize the social behavior of adult isolation reared monkeys [see Sackett 1970; Pratt & Sackett, 1967 for some of this work].

The main species studied in the WaRPRC was the pigtail monkey (*M. nemestrina*), maintained in a 1,000 animal breeding colony in Eastern Washington, a 1-hour flight or 5-hour drive from Seattle. There was also a small colony of crabeating macaques (*M. fascicularis*). The breeders were housed in indoor harem rooms containing one male and 4–6 females with their infants. Juveniles were housed in larger mixed sex groups until attaining reproductive maturity. The facility also had a 300 animal baboon (*P. papio*) breeding colony housed indoors in harem groups or in an outdoor compound.

Our programmatic goal was to develop a research program to study behavioral and physiological mechanisms of deficits in learning performance, social behavior, and curiosity and exploratory behavior following social deprivation rearing. Of special interest were sex differences we had previously found, with females seemingly less affected by social deprivation rearing than males. The aim of our first study was to see if the rearing condition and sex effects in rhesus monkeys could be replicated in pigtails. To do these studies we needed a major piece of missing equipment-a playroom. Gerry designed one based on the Wisconsin model, which was built during the fall of 1970. With the addition of four incubators donated from the University Hospital for nursery rearing newborn monkeys, we were almost ready to go.

In this early period we had a major liability. We knew almost nothing about pigtail macaque behavior. We also knew nothing about the colony breeding and husbandry procedures in Eastern Washington which would provide our research subjects. So we began taking periodic trips to observe pigtail behavior, understand husbandry procedures, and study breeding and pregnancy outcome records. In addition to finding that pigtail behavior really

was different from that of rhesus, we learned a distressing fact that shaped the future of our lab and work. The breeding colony had no nursery. We remedied this situation by developing a “Save-A-Baby” program, with the goal of providing nursery care for naturally occurring and experimentally produced at-risk newborns from the colony. However, we did not know much about caring for at-risk newborns. Although the center veterinarians were helpful, we also turned to the University Hospital Neonatologists and high risk newborn nurses. With this assistance, we developed a high risk newborn primate nursery employing the same procedures as used in the human NICU. By the end of fall, 1970, our nursery began receiving at-risk newborns from the breeding colony. These were usually 3–30 days old, many of low birth weight or born prematurely, others rejected by their mothers or from a mother injured in her harem group. A number of the rejects were medically normal, and they became control subjects for our studies of at-risk monkeys. In some years, the Baby-Save population included 50–60 monkeys. Many of the infants saved through this program developed normally and became juvenile and adult members of the primate center research or breeding colony.

Specialized Husbandry Procedures

The research we planned primarily required rearing healthy monkeys from birth without mothers. There were a number of reasons for motherless rearing. First, our experimental rearing conditions themselves precluded mothers. But as important, most of the measures of growth, behavior, and physiology that we planned could not be done with monkeys separated from their mothers on an almost daily basis. Separation itself could lead to injury of the mother, infant, or human doing the procedure. Separation is traumatic for most mothers and infants, and can have numerous unwanted immediate and delayed behavioral and physiological effects [e.g., Berman et al., 1994; Laudenslager et al., 1990].

Our experience with the Save-a-Baby program showed that a successful high-risk nursery required 24-hour a day personnel for feeding, providing medical care, and measuring vital signs during the first weeks after birth. This became, and continues to be, our standard practice. Newborns separated from their mothers require a temperature controlled environment (isolette) for the 1st 4 days of life. After graduating from an isolette, infants are housed in small single cages with a hanging cloth “surrogate mother,” a heating pad, and a special formula feeder. Monkeys are trained by hand to self-feed, which is usually accomplished by 2–3 weeks of age. Studies were done to develop a milk formula minimizing diarrhea and for scheduling formula replenishing and when to remove the cloth surrogate and to wean from formula. These and other husbandry studies are detailed in the book “Nursery Care of Nonhuman Primates” [Ruppenthal & Reese, 1979].

Socialization and Behavior Testing

From studies at Wisconsin, we knew that lack of socialization results in abnormal personal and social behavior. To overcome this, we adopted a standard socialization procedure in which infants were put daily into a playroom with 2–6 monkeys for 30–45 minutes. The playroom was used for both socialization and to generate social development data. In collaboration with many colleagues, we adapted a number of human developmental assessments for use with macaque monkeys. These included methods for studying skeletal

development, neonatal reflexes and perceptual behavior, vision, recognition memory, and object permanence. They became a standard developmental test battery for all newborns and infants assigned to IPRL research projects and for most “Infant Save” animals. Table 1 lists many of these measures. A complete list of assessments is provided in Ruppenthal & Sackett [1992].

By the end of 1971, it was clear that the future of our lab depended on its use by other researchers and that an interdisciplinary approach was critical to laboratory goals. To foster such an environment, and to meet the husbandry research goals of the WaRPRC and the research goals of the CDMRC, we proposed that our lab be opened to all qualified investigators both at the University of Washington and elsewhere. We named the facility the Infant Primate Research Laboratory at the University of Washington (IPRL). It was good luck that both of our sponsoring centers were preparing their next NIH 5-year grant applications. We included a proposal for the IPRL as an interdisciplinary core facility in both grants-a proposal for joint funding by separate NIH institutes that was rare at the time. The proposal was funded in both centers and the relationship has stood for over 40 years.

Research in Reproductive and Developmental Sciences at the IPRL

Section One: Rearing Conditions and Effects on Biobehavioral Development

The study of how rearing conditions affect development has been an important topic in modern developmental sciences, including contemporary research [Fox & Rutter, 2010]. Early life experience effects on biological and behavioral development have been a major research topic in the IPRL since its beginning. This review describes some of these studies concerning behavior, brain, and effects in adulthood.

Social Isolation Rearing—One initial IPRL research goal was to identify mechanisms of behavioral deficits and abnormal behavior that were previously found in rhesus monkeys (*M. mulatta)* produced by rearing without access to social stimulation. Since our primary species would be pigtail monkeys (*M. nemestrina*), new studies were conducted in the pigtail using the same observers, social isolation rearing cages, measurement methods, and mother-peer and peer-only controls as in the rhesus studies [Sackett, 1972a, 1972b].

The pigtail social isolates were reared for six or nine months from birth [Sackett, et al., 1976]. Post-rearing tests occurred in 30–40 minute daily playroom sessions with each social group containing both isolate and socialized monkeys. Species differences, even between socialized animals, were observed. Socialized pigtails engaged in more social and passive behavior but less exploration of the environment and play with toys. Rhesus isolates spent over 55% of their time in the “isolate syndrome” behaviors of withdrawal, self-clutching, rocking, and stereotyped locomotor activity. Pigtail infants had half of that duration. Rhesus isolates had almost no positive social behavior, while pigtails did engage in some social play and social exploration. Pigtail isolates also had more than double the amount of time in nonsocial exploration than rhesus isolates. These findings questioned the generality of social deprivation rearing effects between the two species and suggested that genetic and possibly prenatal effects were important in determining how rearing conditions affect macaque development.

To further study macaque species differences, crab-eating monkeys (*M. fascicularis*) were reared for 6–7 months under the same isolation conditions, with one month of post-rearing playroom social testing [Sackett et al., 1981]. Only a single post-rearing month was available for the crab-eating monkeys, as they were sacrificed afterwards for brain anatomy research (see below). Comparisons among the three species were obtained from behavior measures during the rearing and post-rearing playroom sessions. This was done using a novel electronic data acquisition system developed in the IPRL [Sackett, et al., 1973]. The results showed that all three species differed both during and after rearing. In isolation, rhesus spent over 50% of their time in "isolate syndrome" activity, while crab-eating monkeys spent 25% and pigtails less than 5%. Pigtails and crab-eating monkeys spent over 50% of their isolation observation time in exploration, rhesus spent under 15% in this activity. In the playroom, crab-eating monkeys had about half of the almost 60% isolate syndrome time as rhesus, with pigtails exhibiting the least. The most surprising finding was that crab-eating monkeys did not differ from their social controls in the positive social behaviors of play and sex or in nonsocial exploration, although they did have the same amount of social fear and withdrawal behavior as rhesus. These results showed a clear dissociation of isolate, exploratory, and social behavior among these species. This suggests that a single process arising from deprived rearing cannot explain this pattern. Rather, a combination of genotype differences, interacting with prenatal and/or postnatal development factors, needs to be considered [Sackett, 1982]. Such mechanisms were subsequently identified by Suomi [2006].

Self-directed and stereotyped motor behavior is a major post-rearing effect of social isolation seen in all macaque species that we have studied. Theoretically, such behavior might be caused by an autonomic nervous system imbalance between sympathetic and parasympathetic activity. To assess this idea, a study of auditory evoked heart rate responses in isolate and socially reared pigtail monkeys was undertaken [Martin et al., 1988]. Baseline heart-rate did not differ after adaptation to a sound chamber test environment. When tested with white noise signals, however, isolates failed to show the normal biphasic response of heart-rate acceleration followed by deceleration, with subsequent return to baseline. Isolates exhibited only heart-rate acceleration in the 10 seconds after stimulus onset. This finding suggests that the differences in autonomic nervous system functioning between socialized and isolate monkeys may be the result of deficits in inhibitory control. Failure to inhibit arousal in response to a stimulus change might be a mechanism related to the persistence of social isolation rearing effects [e.g., Sackett et al., 1999].

Pair Rearing—In the 1980's, government regulations were proposed stating that all infant primates reared without mothers should be housed socially in pairs in order to maximize their psychological well-being. Rearing in pairs had been shown in 1960's research to produce abnormal clinging behavior and deviant behavior persisting into young adulthood [e.g., Harlow & Harlow, 1965; Chamove, et al., 1973]. Believing the validity of results from earlier studies revealing poor outcomes following pair rearing, IPRL investigators conducted two pair rearing studies to replicate the earlier work and support our standard housing method.

Study one compared pigtail monkeys raised in pairs with singly caged monkeys from week 3 through month 4 [Ruppenthal, et al., 1991]. All animals received daily 4-monkey playroom sessions with their cage mate and another pair. They also received all age-appropriate IPRL developmental tests. The results showed that mutual clinging, fear and withdrawal dominated the time budgets of the animals raised exclusively in pairs. Individually-caged monkeys in 4-monkey playgroups rarely showed these behaviors, engaging in much greater levels of play, exploration, and varied activity. At 8–10 months of age, pairs and individually caged monkeys were housed together in a large pen containing 7–8 other animals including their cage mate. Pair reared monkeys were subordinate, rarely played, and spent most of their time huddled on the cage floor, completely failing to adapt to group living.

A second study housed 8 monkeys in two groups of rotating pairs from 30 days through 6 months of age [Novak & Sackett, 1997]. Every 2–3 days the pairs were changed, with each animal paired with every other animal an equal number of times over the rearing period. For 30–40 minutes each day all four animals of each group were put together by removing the walls between their cages, similar to the daily playroom grouping of all IPRL animals. Home cage tests compared the rotating pairs with the continuous pairs of study one during months 2–4. There were no significant differences between the groups in clinging or in the frequency of varying their behavior, and the rotating pairs actually had less time spent in social exploration and play than the continuous pairs. Behavioral observations during month 6 compared the rotating pairs with the continuous pairs and with singly caged controls during their playroom sessions. Again there was no difference between rotating or continuous pairs in time spent clinging, social explore and play, both conditions being much lower in these positive social behaviors than singly cage controls. Taken together, these two studies reinforced the 1960’s results and our view that our single cage rearing with daily socialization and developmental testing is a better nursery rearing procedure than pair rearing.

Nursery Rearing-Good or Bad for Juvenile and Adult Life?—For over 35 years, the standard IPRL rearing method has called for housing infants in single cages from which they could see and hear many other animals and also humans engaged in a variety of husbandry and data collection activities. Spanning the ages of 25 days to 10–12 months, IPRL infants also have daily socialization in playroom groups and a series of developmental tests involving growth, perception and cognition. Many of these tests include interacting with humans and almost all involve willingness to respond positively to novel and complex stimulation. These procedures are quite different from protocols for nursery rearing in most other colonies. Typically, nonhuman primates reared in nurseries live in single cages, pairs, or small groups of age-mates. Nursery reared primate infants usually receive minimal human handling and few opportunities to experience varied and complex stimulation outside of their home cage or playroom. This leads to the common expectation that nursery rearing conditions will produce lifelong behavioral, emotional, physiological, and health problems [e.g., Parker & Maestripieri, 2011]. To assess if such lifelong effects are observed in IPRL nursery reared infants, investigators turned to the Primate Center’s electronic animal records system (ARS). The ARS contained data on animal locations and which animals were housed together, growth, clinical treatments and other health records, survival and causes of death.

Because monkeys in the breeding colony were generally housed in harem groups, data could also be collected concerning opportunities for pregnancy and sire identification, as well as pregnancy outcomes and offspring survival. Over the years 1976–1996, 506 nursery reared pigtail monkeys were relocated at about 14 month of age from the IPRL to the primate center breeding colony at Medical Lake, Washington. Their success as juvenile and adult breeding colony members was compared with 1,187 natives who were reared with their mothers in harem groups [Sackett, et al., 2002]. As juveniles, monkeys of both sexes lived together in all-native or mixed native-IPRL groups. As adults, IPRL females lived in harem groups, while most IPRL males lived in all male groups as the colony managers rarely allowed them to mate.

The results of this unique large sample research project were clear for the population of 2–10 year old animals studied. There were no yearly rearing differences in overall loss from sample for any reason, indicating no population sampling bias between origin groups. There were no differences in (1) survival, (2) body weight for either sex until age 7, when IPRL females became heavier than native females, (3) clinical treatments for any disorder, (4) reason for treatment among the three primary categories of infection, inflammation, or diarrhea, and (5) in receiving bite wounds while in a social group. The bite wound category is especially important because it was our only measure reflecting behavior. Bite wounding in juvenile and harem groups occurred mainly to subordinate individuals, and is probably a good indirect measure of social status in this colony. By this inference, IPRL monkeys were not more likely to be subordinate in status, and thus equally able to compete within their social groups.

Of course, successful reproduction is one of the main reasons for maintaining a primate breeding colony. IPRL females did not differ from the native monkey 90% pregnancy rate over parities 1–5. They also did not differ by parity in fetal losses due to abortion, stillbirth, or offspring death in the 30 day neonatal period. Because of colony manager biases against using nursery reared males as harem breeders, only 10 IPRL males had breeding opportunities. They did produce 239 pregnancies, 196 by two males while in harem groups, the rest during single-cage timed mating. This study clearly showed that IPRL pigtail monkeys can adapt to social living in large groups without ill health or growth effects, and that they are as capable of reproduction as mother-reared, breast-fed, early socialized pigtails. Perhaps future studies will determine what properties of IPRL rearing procedures produce the positive developmental effects that other types of nursery procedures seemingly do not.

Section Two: Studies of Breeding, Pregnancy and Delivery

Premature delivery is the leading cause of infant mortality in the United States and an important source of behavioral development problems. Prematurity rates have slowly increased, reaching over 12% of live births by 2007 [Klebanoff & Keim, 2011]. Low birth weight (LBW) is also a major correlate of human developmental problems. In 2009 the United States LBW rate was 8.2% according to the National Center for Health Statistics. This was similar to the LBW rate in our primate center Medical Lake pigtail monkey breeding colony, defined as at or below the 10^{th} percentile for sex. By 1975, many of these

LBW newborns were reared in the IPRL. Some were probably born prematurely, but gestational age at birth was usually not known for most LBW newborns. These newborns received almost identical care as that given to at-risk human babies.

The IPRL test battery measures revealed differences between LBW and normal weight newborns and infants [Sackett, 1981a]. Achieving diurnal cyclicity of basic life functions, heart and respiration rate, and body temperature was much slower in LBW neonates, as were postnatal development of sleep wakefulness cycles. The rate of weight gain was slower for LBW monkeys during the neonatal and infancy periods, especially for males. Attaining motor coordination was much slower for LBW neonates, averaging over 40 days to attain full self-feeding ability without human assistance compared with 20 days for controls. Low-birthweight neonates slept more. They also engaged in less self and environment exploration when awake. During initial playroom socialization at 30–60 days of age, LBW monkeys had less positive contact with other infants and more socially elicited fear than controls, differences persisting through six months of age [Worlein & Sackett, 1997]. Cognitive deficits were also found for LBW infants [Gunderson, et al., 1989]. Even as juveniles, LBW monkeys performed poorer than controls on difficult learning tasks [Fredrickson, et al., 1987]. Like human infants, these data showed that LBW monkeys were physiologically, physically, and behaviorally delayed in development, with important cognitive deficits persisting into the juvenile period.

The Prematurity in Primates Project—Studies of social deprivation had revealed species and sex differences, as well as large individual differences in outcomes, leading investigators to conclude that prenatal and genetic factors interacting with rearing experiences might cause such effects. Poor pregnancy outcomes, including fetal loss, prematurity and low birth weight are also related to genetic or familial factors and prenatal stress [e.g., Beydoun & Saftlas, 2008]. These factors motivated work on an ontogenetic model of poor prenatal and perinatal outcomes and their relationships to postnatal developmental problems. The model was based on selective breeding of pigtail monkeys with histories of excellent or poor pregnancy outcomes [Sackett, 1984]. It was also based on the many findings in humans and animals that stress during pregnancy can result in poor pregnancy outcomes and deviant offspring health and behavioral development [see Coe et al., 2010, for review of nonhuman primate prenatal risk factors].

The Primate Center computerized records were used to identify female and male breeders at high or low risk for fetal loss, stillbirth, prematurity or low birth weight, and offspring neonatal death. Forty-seven High Risk females had their conceptions end in three of three or 50% of four or more of these outcomes; 5 high risk males achieved poor outcomes in at least 50% of their 30 or more conceptions regardless of mating with high or low risk females; 54 low risk females had zero of three or one of 4 or more conceptions end in a poor outcome; 7 low risk males had 10% or fewer poor outcomes. These males were bred within and between the female risk groups, with high and low risk females. Conceptions were produced by placing the female in the male's cage for 24–72 hours, yielding good estimates of gestation length. Stress was produced on about one-third of 320 conceptions by hand-capturing the pregnant female from her cage for a daily 5-minute period from 30 gestation days until 30

days before the estimated end of pregnancy. Few animals adapted to capture over this period. The remaining pregnancies were undisturbed [Sackett, 1981a].

Selective Breeding Results: Pregnancy Outcome—Selective breeding fetal losses on non-stressed pregnancies supported the retrospective results [Sackett, 1990]. Overall, high risk females had 2.64 times higher risk of fetal loss than low risk females, while high risk males had 4.86 times higher risk than low risk males. Without clear evidence of genetic abnormalities or teratogenic effects from drugs or poisons, males are rarely considered as a source of fetal loss in humans. This study clearly showed that a small percentage of breeding males in a primate population can be an important cause of this outcome.

Pregnancy outcomes of high risk females did not show a differential effect of stress, regardless of male risk. However, low risk females did have a significant rise in fetal loss under capture stress regardless of male risk.

Reproductive histories had revealed that the live-born LBW rate for high risk females (40%) and males (36%) was considerably higher than that for low risk females (8%) or males (6%) [Sackett, 1981a]. However, in the breeding study neither female nor male risk groups differed in producing LBW offspring, although offspring of low risk parents were heavier at birth than offspring of high risk parents. The lack of LBW differences could be due to the high abortion rate of high risk monkeys who might have been LBW, or to the single cage housing of this study rather than the harem group housing during the reproductive history period

An important finding occurred in studying the parturition process [Goodlin & Sackett, 1983]. Compared with low risk females, high risk females spent less time in postures unique to labor, were more likely to deliver after midnight indicating longer labors, and had infants with more bruising and lower Apgar scores. These parturition effects suggested that high risk females had more difficult labors than low risk females. Thus, offspring of high risk females could be at greater risk for developmental problems since abnormal labor and delivery is an important risk factor for child development problems.

Selective Breeding Results: Offspring Development—Data at birth for 50–80 live-born offspring were reviewed by Sackett [1981a, 1984]. Offspring of high risk females and males where less mature at birth based on birthweight and evaluation of skeletal maturity even though females did not have shorter gestations [Newell-Morris et al., 1980],. Offspring of stressed females displayed greater asymmetry of the finger dermal ridges finger prints, supporting the idea that asymmetry may measure perturbed prenatal development [Newell-Morris, et al. 1989].

Offspring were reared under standard IPRL nursery conditions. Absolute body weight and growth velocity in early infancy were both influenced by parental risk. Weight was highest and velocity greatest when both females and males were low risk, with weight and velocity lowest when both were high risk [Sackett, 1981a, 1984]. Other early measures produced similar results. When both parents were at high compared with low risk, offspring of high

risk parents took longer to achieve self-feeding, had more days receiving medical treatments, and spent less time awake and active in their nursery cages.

Behavior in playroom social groups from 30 days through eight months was reviewed by Worlein & Sackett [1995]. No parental risk effects were found in social development, but prenatal stress effects were apparent. Offspring of stressed females were more fearful during their initial introduction to the novel playroom environment. Results of observations throughout the 8-month test period indicated that infants of stressed females played as much as infants from non-stressed females but they initiated fewer social interactions and engaged in less social exploration.

Offspring were studied from 3–7 months of age in the Wisconsin General Test Apparatus for discrimination and reversal learning task performance, object quality learning set, and for breaking a response set formed in choosing between four boxes to find the one box containing food. While parental risk and maternal stress did not affect learning on the relatively simple black-white discrimination and reversal tasks, infants of females who were stressed during pregnancy failed to perform (balked) twice as often as infants of non-stressed females. Maternal stress also influenced performance on the set breaking task. Infants of females who were stressed during pregnancy were less capable of shifting their behavioral strategies when environmental contingencies required change. Parental risk did influence performance on the learning set task. Infants of high risk parents had a significantly lower percentage of correct responses (59.7%) than offspring with low risk parents (69.1%) at the end of this task [see Sackett, 1981a, 1984 for details].

Mechanisms of Parental Risk

Males: To investigate the contributions of the male to infant developmental outcome, karyotypes, sperm counts and morphology, hormones, red cell polymorphisms, and virus and bacteria assays of high versus low-risk males were studied [Sackett, 1990]. No measure revealed differences. Thus, the mechanisms by which high risk males detrimentally affected pregnancy outcome and offspring development were unknown. They remain unknown due to two factors. First, males aged over the course of the study and either died or were removed from breeding due to infirmities. Second, after years of removing individual females from harem groups for excessive fetal losses, this research led the breeding colony managers to remove the male instead. This had the beneficial effect of lowering overall fetal losses, but kept us from continuing the research by identifying new high risk males.

Females: A collaboration with Dr. Norman Klein and colleagues at the University of Connecticut produced an important clue about maternal risk. Blood serum from our females with histories of low or excessive fetal losses was added to rat embryo cultures [Klein et al., 1982]. Embryos cultured on high risk blood had growth and morphological abnormalities not seen in those cultured on low risk blood, indicating that high risk blood contained a factor or factors teratogenic to rat embryo development. A subsequent study determined that antibodies to laminin, a cell adhesion protein, were responsible for the high risk monkey blood effect on rat embryo serum [Carey, 1986]. A final study immunized low risk monkeys with laminin [Weeks et al., 1989]. Although their blood had previously not affected rat

embryos, it was teratogenic after immunization. This study also laminin immunized low risk females that had no prior pregnancy losses. After immunization they failed to produce a live-born offspring in over two years of mating. This work proved that laminin antibodies were one cause of monkey abortions, and suggested the theory that antibodies to basic proteins could be an important mechanism for primate fetal loss and perhaps also other prenatal and postnatal developmental problems.

Additional Studies—In addition to the above, IPRL investigators have examined a number of important aspects of conception, gestation and birth. Pigtailed macaques exhibit visual signaling of estrus status through sex-skin swellings and coloration. Early work [Steiner et al., 1977] specifically described levels of sex hormones, ovulation, and sex skin changes, results which are used today to manage a timed-mating colony. Additional details of estrus cycling involving weaning and body weight on amenorrhea were reported by Maninger et al. [2000]. Two studies in the late 1970's investigated physiological correlates of pregnancy in the female, specifically in steroid-binding proteins [Schiller et al., 1978] and high density lipoproteins [Rudel at al., 1981]. The availability of timed-mated (known conception date) pregnancies, primarily for the production of healthy full-term infants, has resulted in production of an IPRL literature on fetal development. Sirianni et al. [1975] produced early work on anthropometrics of newborn pigtails, providing a baseline system which the IPRL continues to use to this day. Sirianni and Newell-Morris [1980] and Sirianni et al. [1981] described the craniofacial growth of fetal pigtails taken by cesarean-section. DeVito et al. [1989] published volumetric growth measures for major brain divisions in fetal pigtails. This work was followed by Conrad et al. [1989] which described the use of ultrasound to diagnose pregnancy at an early fetal age, and Conrad et al. [1995] which described estimation of fetal growth and gestational age estimation, also using ultrasound, and again, establishing normative values that continues to be used in the IPRL to this day.

A great deal of research in the IPRL has focused on risk factors associated with poor pregnancy outcome. Sackett [1981b] dealt with an early concern about moving pregnant females, finding no detrimental effects of air shipment from Eastern Washington on pregnancy outcomes in pigtailed or long-tailed macaques or savannah baboons. Goodlin & Sackett [1983] described normal parturition in pigtails, including labor, delivery and immediate newborn status. This work established a quantitative system for describing research-related issues in labor and delivery still used in the IPRL. The heritability of birth weight was estimated in 3,562 captive pigtails using three decades of breeding and pedigree records by Ha and colleagues [Ha et al., 2002]. The model demonstrated a strong ($h(2) = 0.51$) heritable component of birth weight overall. The correlative effects of social housing factors like group size, stability, and composition on pregnancy outcome have also been explored [Ha et al., 1999]. Presence of the male during pregnancy was the strongest predictor of successful pregnancy outcome, followed by group size and stability, and the proportion of other pregnant females in the group during gestation. This original work has led to a long-term research program designed to investigate individual risk factors like age, parity and earlier pregnancy outcome history and the relationship between aggression levels within social groups and pregnancy failure rates. Stockinger et al. [2011] found that the total number of previous births and the proportion of previous pregnancies resulting in a

cesarean-section best predicted poor outcome while Ha et al. [2011] confirmed that social dynamics, and especially higher aggression levels within groups, resulted in a higher rate of poor pregnancy outcomes. This work has resulted in further research that is currently underway in the WaNPRC breeding colonies, looking to integrate and experimentally confirm these findings.

Section Three: Characterization of Normative Behavioral Development

An early and fundamental objective of the IPRL has been to maintain, summarize and report normative data on all aspects of the development of nonhuman primate infants, with a focus on pigtail macaque monkeys. Domains of study include physical growth, early reflexes, visually-guided reaching, cognitive development, temperament, motor milestones and social behavior. For over forty years, researchers at the IPRL have been studying infant macaque monkeys to better understand the biobehavioral mechanisms that control normal and abnormal development. Scholarly manuscripts published from investigators at the IPRL have established many biological and neurobehavioral norms for nursery reared macaque infants.

Infant Physical Development—The measurement of physical growth at the IPRL allows changes in both stature and bodyweight to be documented. Newell-Morris & Tarrant [1978] described general features of hand and foot ossification, results which would be subsequently used to estimate developmental status and prematurity. Newell-Morris & Sirianni [1982] continued this work, describing measures of humerus bone growth in both fetal and infant pigtails using trichromatic bone labels. Finally, and in a more general sense, Sackett & Ruppenthal [1992] published a frequently-cited paper on normative growth of nursery-raised pigtails infants, documenting effects of sex and birth weight, but interestingly, also illustrating the critical importance of feeding schedules on physical growth. During the decades of research in the IPRL, other studies of morphology and biomechanics have occasionally appeared. Spelman et al. [1979] reported on conduction velocities of the ulnar nerve as a function of gestational age in infant pigtails and Johnston [1980] described the ability of infant pigtails to compensate for substrate elasticity in leaping behavior.

Infant Neurobehavioral Development—Macaque monkey infants and human infants share certain limitations and abilities, particularly during the first months of life. Both lack language, display poorly developed motor skills, and have prolonged periods of infancy during which a complex repertoire of behaviors develop. Data have shown that there are important similarities between macaque monkeys and human infants in certain key aspects of development. Perceptual-cognitive skills (including measures of visual orientation, tracking, visual acuity, object permanence, and visual recognition memory) develop at about a 4:1 ratio between the two species; 4 weeks in the human infant being roughly equivalent to 1 week in the macaque. To measure and more closely study neurobehavioral development during infancy and beyond, a standardized battery of assessments has been developed at the IPRL. This test series covers key aspects of cognitive, motor and social development in a controlled laboratory setting and is described below:

Newborn Health Assessment and Congenital Malformation Exam—To evaluate newborn health status, a Newborn Assessment modeled after the Apgar rating scale used

with human infants [Apgar, 1953] is performed as soon as possible after birth and again at approximately one-half hour after birth. Heart rate, respiration, rectal temperature, muscle tone, activity and skin color are evaluated using predefined codes from 0 to 2. In addition to the Newborn Assessment, a systematic examination of the newborn for major birth defects takes place after the initial assessment. The exam includes observations of the skin (pigmentation, rash, bruises, edema), head (molding, fontanel, depressions, overriding suture), face (symmetry, mouth, eyes, ears, teeth), trunk (sternal retraction, spine, breasts, neck), genitalia (vagina, anus, penis, scrotum) and extremities (digits, limbs). Previous studies have indicated that normal newborn monkeys exhibit composite APGAR scores between 10 and 12 at the initial and follow-up tests. Newborn monkeys delivered via cesarean section, like human infants, typically have lower initial APGAR scores [Burbacher et al, 2004a].

Neonatal Behavioral Assessment Scale—A behavioral assessment procedure modeled after the Brazelton Neonatal Behavioral Assessment Scale for human infants is used to evaluate neonatal reflexes, reactivity and responses to the environment [Brazelton & Nugent, 1995]. The assessment consists of items aimed at evaluating seven different reflexes (hand clasp, foot clasp, grasping, righting, rooting, sucking and snout) and four behavioral responses (visual orientation, visual following, startle and auditory orient). Neonates are examined every other day during the first two postnatal weeks. Kroeker et al. [2007] provided normative values for 255 healthy IPRL-reared infants and described a sophisticated method for analysis of censored milestone data. In general, the hand clasp response was the first reflex observed and was present on postnatal day 1. All other neonatal reflexes were in place by 3 to 5 days after birth. Neurobehavioral responses were slower to develop but visual exploration patterns that included orienting and tracking were present by one week of postnatal age.

Neonatal Activity—A numerical five point rating scale used at the IPRL to assess sleep-wakefulness and vital signs, i.e., respiration, heart rate and temperature, is collected every four hours, 7 days/week, while infants reside in the nursery. These parameters of neonatal physiology provide key health data to veterinary personnel and nursery caretakers. Previous studies have indicated that infants begin regulating sleep-wakefulness rhythms shortly after birth [Ruppenthal & Reese, 1979]. On postnatal day 1 and 2, infants spend about 70% of their time sleeping and have a resting heart rate of ~230 beats/min. Some evidence of cyclic curves for both sleep and wakefulness is present by the end of week 1 but pronounced cyclicity in activity level is not observed until week 3. Temperature and respiration cyclicity are present by the end of week 1 but the basic 8-hour heart rate cycle is not present until 3 weeks of age. By postnatal week 4, respiration and heart rate cycles are highly correlated and rise and fall together.

Temperament—Temperament is typically defined as the characteristics of an individual's personality or nature that are present at birth and have a genetic/biological basis [Saudino, 2005]. Certain aspects of temperament such as behavioral inhibition have been linked with an increased risk of mental disorders. Infants reared at the IPRL are evaluated on a longitudinal measure of temperament developed for macaque infants. During temperament

ratings, infants are assessed for reactivity, emotionality and sociability while being removed from their home-cage for daily weighing. Response and latency to capture, vocalizations, activity level and emotional reactivity are scored. Because temperament is best expressed when the subject is faced with an external demand or stimulus, an unfamiliar data collector dressed in unusual, brightly-colored clothes is sent into the laboratory to weigh the animals once a week and response to this novelty is recorded by an independent observer. Heath-Lange et al. [1999] published the original form of the IPRL temperament assessment with a comparison of results across four species of monkeys while Sussman & Ha [2011] published a more detailed analysis of a larger data set describing developmental and cross-situational stability in the outcomes. Reactivity and boldness were identified as the primary components of infant macaque temperament and the data revealed a trend toward calmer and bolder behavior with age.

Visually-Guided Reaching—To evaluate early reaching and eye-hand coordination, testing on a simple object retrieval task at the IPRL begins at 14 days of age. Infants are encouraged to reach for and pick up a small attractive toy that has been dipped in applesauce and placed on a platform directly in front of them. Successful pick up of the test stimulus is recorded. Mastery of this task ensures that animals have achieved the requisite motor skills for the object permanence test. Previous studies have indicated that normal macaque infants are able to successfully grasp and pick up an object by approximately 30 days of age [Burbacher et al., 1986, Ha et al., 1997].

Object Permanence Assessment—Object permanence is the conceptual understanding that objects continue to exist even when they cannot be seen, heard, or touched and represents an important milestone in early cognitive development [Piaget, 1954]. This ability develops in parallel stages in both human and macaque infants and is considered a measure of early representational abilities and spatial memory. The development of object permanence is assessed through the presentation of 1) no hide, 2) partial hide, 3) full-hide and 4) A not B trials using both a screen and well testing platform. Basic Piagetian object permanence typically develops in macaque infants by about 4 months of age [Burbacher et al., 1986, Ha et al., 1997].

Visual Acuity Assessment—A forced-choice preferential looking technique adapted from human infancy research is used to evaluate the development of visual acuity over the first twelve weeks of life [Teller, 1983]. The preferential looking technique relies on the strong visual preference observed in primate infants for viewing patterned stimuli over homogenous gray fields. Visual acuity is determined by showing infants various black and white gratings (stripes) paired with a homogeneous gray field of equal intensity. The tester must observe the visual orientation responses of the infant and indicate the location (left or right) of the stripes. By varying the width of the stripes, stimuli related to visual acuity from 0.5 to 28 cycles/degree (20/23 to 20/1400 Snellen) can be presented over the first 12-weeks of life. IPRL investigators have shown that visual acuity development is similar across human and macaque infants [Booth, et al., 1985]. Both demonstrate acuity of about 1 cycle/degree (20/600 Snellen equivalent) near birth but infant monkey acuity develops about four

times faster than human infants. Acuity develops gradually in both species and reaches functional maturity by 3–5 years in humans and about 1 year in macaques.

Visual Recognition Memory Assessment—The Visual Paired-Comparison test methodology is used to study emerging visual recognition memory skills in both human and nonhuman primate infants [for review see Burbacher & Grant, 2012]. Both human and macaque infants are capable of demonstrating recognition after familiarization study times as short as 5 seconds and delays periods as long as 24 hours [Fagan, 1973, 1974, Gunderson & Swartz, 1985]. The primary outcome measure is known as the novelty preference score and reflects the amount of time the infant spends actively looking at novel rather than familiar test stimuli. Normal, full-term macaque and human infants typically provide overall novelty preference scores between 60 and 65%. Infants at the IPRL are evaluated on this procedure over multiple test sessions during the first two months of life. Normal full-term macaques display a visual preference for novelty as early as three weeks of postnatal age [Gunderson & Sackett, 1984].

Social Behavior Assessment—Social behavior is an important aspect of development in young nonhuman primates and forms the basis for later reproductive success and placement within the social hierarchy. Social behavior is studied at the IPRL to document how infant monkeys initiate and respond to social overtures in playrooms equipped with ramps, swinging chains and shelves. The development of species-typical social behavior is coded in real time by a trained data collector 3 days/week in stable, mixed-sex playgroups. The codes identify individual animals and measure major classes of behavior such as play, fear, aggression, grooming, withdrawal and exploration. At 8 months of age, infants reared in the IPRL exhibit relatively normal species-specific behavioral profiles, exhibiting high levels of play (~32%) and low levels of disturbance behaviors (<2%) [Worlein & Sackett, 1997]. Play and social behaviors emerge early in development, increase over time and occupy a significant portion of the infants' time budgets.

Motor Milestones—The purpose of the Motor Milestones Assessment is to provide an evaluation of the development of gross motor skills in young monkeys during open play sessions. In humans, examples of motor milestones might include age at sitting up, crawling and walking. Infants are observed 5 days/week and the ages at which they successfully master jumping or climbing up the playroom's various ramps, chains and shelves are recorded. Gross motor responses are evaluated over the first nine months of life by observing infants in the playroom. Infants are generally capable of climbing up a ramp in the playroom by 25 days of age and can release their grasp when hanging from a shelf or chain around day 43.

Learning and Memory—Wisconsin General Test Apparatus—The measurement of emerging learning and memory skills is fundamental to characterizing the development of cognition. Beginning at approximately 145 days of postnatal age, infants begin testing on a standard series of learning and memory assessments utilizing the Wisconsin General Test Apparatus (WGTA). The series begins with several adaptation sessions to acclimate the animal to the testing environment and proceeds sequentially from simple to complex tests of

learning abilities. The IPRL battery includes Object Discrimination and Reversal, Hamilton Search and Set Breaking and Learning Set. A recent publication by Ha and colleagues [Ha et al., 2011] examined performance on Object Discrimination, Object Discrimination Reversal and Hamilton Search in over 200 infant macaques reared at the IPRL. Results indicate that infants can be effective problem solvers and require about 4 test sessions to solve the object discrimination task and 7 test sessions to solve the object discrimination reversal. Performance on the discrimination task predicts performance and latency on the reversal task, providing evidence of mental continuity between the two test measures. In contrast, infants were unable to effectively solve the Hamilton Search task, lacking the cognitive maturity to develop and then break successful search strategies. In a separate study, Mandel & Sackett [2008] analyzed Learning Set data from the IPRL historical normative archives and found strong evidence of both within- and between-problem learning. This finding indicates that nursery-reared infants are capable of demonstrating strategy based solutions on formal learning tests by about 6 to 8 months of age.

Learning and Memory—Touch-sensitive Computer Learning—One of the major efforts in developing new learning procedures has been the continuing evolution of the IPRL computerized touch-sensitive testing system [initial studies in Mandel & Sackett, 2008, 2009]. The current computerized test battery includes Color Discrimination and Reversal, Hamilton Search, Learning Set, Nonmatch-to-Sample, Delayed Nonmatch-to-Sample, Delayed Match–to-Sample, Serial List Learning and Color-cued Conditional Concept Learning. The computer-based IPRL system can assess simple discrimination learning in infant macaques as young as 90 days of age, a methodological achievement never before attained with touch sensitive technology. Initial studies compared performance on the WGTA with performance using computer-based methods and found comparable rates of learning on tests of Discrimination and Reversal as well as Nonmatch-to-Sample. These results support the conclusion that young macaque monkeys can perform well in a computerized test environment using touch-sensitive technology. Tasks currently under development are focused on spatial memory, working memory and complex concept acquisition.

Section Four: Prenatal and Childhood Exposures to Developmental Neurotoxicants

There is a growing scientific awareness of the unique risks that prenatal exposure to neurotoxicants such as environmental chemicals pose to the health and well-being of infants and children [Landigran & Goldman, 2011]. Children can be more susceptible to chemically-induced impairment because their nervous systems are rapidly developing. Exposure to a neurotoxicant may interfere with developmental processes that are ongoing in the infant and child but not in the adult [Rice & Barone, 2000]. Significant and sometimes persisting effects of early toxicant exposure on postnatal health and behavior have been documented for a range of agents that include environmental chemicals, maternal and childhood medications and drugs of abuse [Grandjean & Landrigan, 2006]. Whereas human epidemiology research is important for establishing relationships between neurotoxicant exposures and health effects in children, comparative work with animals such as the macaque monkey provides a critical link in modeling the developmental consequences of

exposure as well as investigating the mechanisms of neurotoxicant injury [Grant & Rice, 2008, Paule et al, 2012].

Environmental Chemicals—The IPRL has a strong history of work in helping to define the maternal reproductive and infant developmental effects of early neurotoxicant exposure. For example, studies conducted in the IPRL have focused on the early, latent, and long-range effects of in utero exposure to methylmercury (MeHg). MeHg is a bioaccumulative, environmental pollutant found in many of the world's oceans, lakes and rivers. Catastrophic events involving human MeHg poisoning revealed that the developing fetus is at the greatest risk for adverse effects. To evaluate the long-tem effects of MeHg exposure on maternal reproduction and infant neurobehavioral development, a longitudinal research program at the IPRL was initiated using Macaca Fascicularis monkeys. Adult females were orally exposed to 0, 50, 70 or 90 ug/kg/day MeHg throughout pregnancy. Reproductive dysfunction was observed in treated females such that increased mercury concentrations were associated with decreased fertility and increased early spontaneous abortion [Burbacher et al., 1984].

Infants born to MeHg exposed mothers were of normal birthweight and did not show evidence of major or minor congenital malformations; however, neurobehavioral deficits across multiple functional and sensory domains were documented. Infants exposed to MeHg exhibited delays in the attainment of object permanence and deficits in visual recognition memory scores on an adapted version of the Fagan Test of Infant Intelligence [Burbacher et al., 1986, Gunderson et al., 1986, 1988]. The development of social behavior was also disrupted by gestational exposure to MeHg. Exposed infants displayed reduced levels of social play behaviors and increased levels of nonsocial, passive behaviors [Burbacher et al., 1990]. While exposure was not related to changes in physical growth during infancy, treated males displayed a latent effect of treatment on weight gain during puberty [Grant-Webster et al., 1992]. As adults, MeHg-exposed animals did not show evidence of deficits in learning abilities or memory [Gilbert et al., 1996]. However, significantly reduced contrast sensitivity thresholds, especially at higher spatial frequencies, were observed during adulthood (11–14.5 years of age) [Burbacher et al., 2005a]. These findings indicate that levels of MeHg exposure that do not produce signs of toxicity in the pregnant mother can have significant effects on emergent cognitive processing in young infants as well as permanent, irreversible effects on adult spatial vision in primates.

Using the successful model developed for the MeHg studies, a longitudinal study to characterize maternal reproductive performance and infant development following exposure to methanol (MeOH) was launched in the IPRL. MeOH is a clean-burning liquid fuel that is being evaluated in the private and federal arena as an alternative to petroleum-based motor fuels such as gasoline. If MeOH fuel were used on a widespread basis, significant human exposure could occur. Studies were initiated to define the maternal toxicokinetics of MeOH exposure via inhalation as well as effects on reproduction and infant development. Adult female Macaca fascicularis monkeys were exposed to 0, 200, 600 or 1,800 ppm MeOH vapor for approximately 2.5 hours/day, 7 days/week [Burbacher et al., 2004a]. Methanol exposure did not alter menstrual cycles, conception rate or birth status outcomes [Burbacher et al., 2004b]. The mean length of pregnancy was however, reduced in MeOH-exposed females by 6–8 days when compared to control monkeys. This finding suggests that

maternal MeOH exposure might have an impact on the fetal neuroendrocrine system; disrupting the biochemical events that control the timing of birth.

Infants born to MeOH exposed females were normal in birthweight and showed no evidence of physical birth malformations. No MeOH-related effects were observed on neonatal reflexes, gross motor development, spatial memory, concept learning, or social behavior. However, delays in the development of visually-guided reaching in exposed male infants and depressed performance on the visual recognition memory task in both exposed male and female infants were observed [Burbacher et al., 1999]. These research findings suggest that inhaled, low-dose MeOH exposure may be related to subtle disruptions in the motor and cognitive development of exposed primate infants. Physical growth and survival were studied in these unique animals from 1 to 4 years of age. Several monkeys with higher exposures to MeOH developed an unusual wasting syndrome that, despite skilled veterinary intervention, resulted in their deaths. Results of full-field electroretinography indicate that prenatal MeOH exposure did not result in gross changes in the phototransduction cascade mediated by photoreceptors. Under scotopic conditions, in which the a-wave is largest, a-wave analysis of the leading edge did not indicate significant effects due to MeOH exposure. There were however, small but significant reductions in the b-wave amplitude under the light-adapted (photopic) condition for the MeOH exposed offspring [Burbacher, unpublished data].

Maternal and Childhood Medications—Therapeutic agents used during pregnancy and childhood can pose a risk to the developing nervous system by interfering with normal developmental processes [Isoherranen & Burbacher, 2008]. Studies have demonstrated that early exposure to prescription and non-prescription medication can result in adverse treatment effects, ranging from overt congenital dysmorphia to subtle changes in neurobehavioral development over time.

Antiseizure Medication—In the United States, approximately 1 million women of child-bearing age have been diagnosed with epilepsy. This medical condition requires lifelong medication to control seizures and, during pregnancy, a delicate balance must be struck between maternal benefits and fetal risks. Studies at the IPRL provided important information on the effects of antiseizure medication exposure on infant macaque monkeys. In the initial studies, adult Macaca mulatta females were exposed to phenytoin throughout pregnancy [Phillips & Lockard, 1985]. Study results indicated that third-trimester high-level phenytoin exposure had a significant deleterious impact on neonatal reflexes (nursing, grasping clasping) and the early expression of gross motor abilities. The authors extended this initial experimental work, switching to Macaca fascicularis monkeys and examining the health effects of both phenytoin and/or stiripentol across pregnancy [Phillips & Lockard, 1993]. Infants exposed to phenytoin alone or in combination with stiripentol showed signs of hyperexcitability and had difficulty orienting to visual stimuli and remaining focused during cognitive testing. In contrast, infants exposed solely to stiripentol did not show any deleterious treatment effects.

In a more comprehensive look at the maternal medications used to control epileptic seizures, carbamazepine in monotherapy or carbamazepine plus stiripentol polytherapy were

evaluated [Phillips & Lockard, 1996]. Throughout pregnancy, phenytoin, stripentol, and carbamazepine plasma levels were maintained between 4–12, 4–10, and 1–6 micrograms/ml, respectively (for both monotherapy and polytherapy conditions). Confirming previous results, infants receiving phenytoin in mono- or polytherapy exhibited behavioral signs of hyperexcitability which interfered with early cognitive testing. Infants exposed to stiripentol and/or carbamazepine during gestation were unaffected and did not display signs of hyperexcitability during postnatal behavioral assessment.

Drugs of Abuse—The use of alcohol (ethanol) during pregnancy is the greatest single cause of preventable mental retardation in the world. Depending on the frequency and severity of alcohol consumption patterns, mothers who continue to drink during pregnancy may place their fetus at increased risk for congenital defects, intellectual deficits and abnormal physical growth patterns [Ornoy & Ergaz, 2010]. Studies at the IPRL aimed at elucidating the reproductive and neurobehavioral risks associated with binge drinking followed by abstinence, a pattern that represents women who stop drinking when they discover they are pregnant or at some point in the pregnancy. Adult female Macaca nemestrina were treated with 1.8 gm/kg ethanol once per week for the first 3, 6, or 24 weeks (full gestation) of pregnancy. Results demonstrated that a single binge exposure or a series of binge exposures during the first 6 to 8 weeks of pregnancy can result in significant physical and neurobehavioral abnormities in exposed offspring [Clarren & Astley, 1992, Clarren et al, 1992]. Ethanol exposure was related to deficits in visual recognition memory, delays in the attainment of object permanence and reduced composite scores of learning and memory skills. Study results showed that maternal peak blood ethanol values are most predictive of teratogenic risk and an intermittent pattern of heavy binge drinking is associated with the greatest expression of neurotoxicity in exposed offspring. The greatest number of craniofacial alterations in infants exposed to ethanol occurred when exposure took place on gestation day 19 or 20 [Astley et al., 1999]. This suggests that a critical period for craniofacial defects may exist in nonhuman primates as well as humans. Ethanol-induced skeletal changes were difficult to detect at birth, increased substantially at 6 months and then gradually lessened as the animals reached 12 and 24 months of age. As these animals aged, magnetic resonance procedures were conducted to collect images of the brain [Miller et al., 1999]. Prenatal exposure to relatively high levels of ethanol was associated with a significant increase in the size of the corpora callosa and in the number of colossal axons. The authors suggested that fetal ethanol exposure may interfere with the normal neural pruning process that occurs during gestation and that this structural overgrowth of axons and dendrites may underlie some of the long-term and persistent cognitive deficits observed in exposed infants and children.

Antiviral Medication—Studies of maternal medications at the IPRL have provided important information on the safety of antiviral treatment with Zidovudine (Azidothymidine or AZT) during pregnancy and the effects of fetal exposure on behavioral development. Zidovudine was the first drug to be approved for treatment of HIV infection but there were important questions about the safety of this medication for the developing fetus, particularly those in their first trimester. To provide data on the safety and maternal-fetal pharmacokinetics of AZT in a primate model, pregnant Macaca nemestrina monkeys were

exposed to 1.5 mg/kg AZT every 4 hours across pregnancy (via gastric catheter) [Ha et al., 1994, Tuntland et al., 1998, Ha et al., 1998]. Results indicated that placental transfer of AZT is passive and that the drug is extensively transferred to the fetal compartment. The concentration of AZT in amniotic fluid was higher than in fetal plasma because it is eliminated slowly from the amniotic cavity. Data from the IPRL provided key information that demonstrated when AZT is orally administered during pregnancy in a primate model, the average fetal dose will be ¾ of the maternal dose. Exposed females developed an asymptomatic macrocytic anemia that disappeared when AZT treatment was discontinued. Infants born to exposed females were also slightly anemic at birth. Exposed infants displayed a transient delay in the development of neonatal reflexes (rooting, snout) and had difficulty solving a simple color discrimination learning problem although performed as well as control infants on other, more difficult learning tasks. Overall, few toxic effects were found in exposed females and infants and at the dose studied, no significant adverse health effects from prenatal exposure to AZT were predicted for pregnant women.

Neuroleptic Drugs—Neuroleptic drugs (also known as antipsychotics) are widely used to treat children who exhibit a number of behavioral pathologies and symptoms. These conditions include psychotic and mood disorders, severe mental retardation, autism, hyperactivity and those who engage in self-injurious behaviors [Olfson et al., 2006]. Treatment can begin in early childhood (3–4 years of age) but serious side effects have been reported. Recently, atypical antipsychotic drugs with less severe side effects have been used in pediatric clinical treatment but the safety and efficacy of these drugs for children and adolescents has not been well-established. A project at the IPRL was undertaken to evaluate the effects of two drugs in this class, *risperidone* and *quetiapine*, in young, normally developing male Macaca nemestrina monkeys [Sackett et al., 2010]. Males received a daily dose of risperidone (0.25 mg/kg) or quetiapine (2 mg/kg) for 4 months. A subset of the animals was switched to a higher dose for an additional 4 months of exposure. Subjects were tested on a comprehensive developmental test battery that included measures of (1) social, cognitive, emotional, perceptual and motor behavior; (2) physical assessments of health, somatic growth, bone mineralization, and hormonal function; and (3) high-resolution anatomical and chemical magnetic resonance brain imaging. The findings from this study indicate that risperidone and quetiapine are safe for normal skeletal growth and weight gain in young macaque monkeys. Thyroid function in exposed animals also remained at normal levels. Although a transient effect, low bone density was found in some animals receiving low-dose risperidone. This suggests that it may be important to monitor children on risperidone for diminished bone mineralization. To evaluate cognitive development, animals were assessed with measures of two-object discrimination and learning set throughout drug exposure and after treatment was discontinued [Mandell et al., 2011]. While animals were actively receiving treatment, exposure to risperidone or quetiapine was not associated with impairment on the learning tests. However, after cessation of the drug, risperidone-exposed subjects engaged in preservative behaviors that suggest neurologic impairment in the form of cognitive inflexibility and deficits in adaptive behavior. This suggests that there may be latent effects of risperidone on learning and development that are expressed after cessation of treatment.

Childhood Vaccines—Thimerosal is an antibacterial and fungistatic agent composed of ethylmercury and thiosalicylate that is used as a preservative in vaccines both in the United States and around the world. Given that methylmercury is an established developmental neurotoxicant, concerns about the use of thimerosal in vaccines given to infants and children have been voiced. By 2001, most childhood vaccines in the United States were formulated without thimerosal (with the exception of multi-dose inactivated influenza vaccines) but thimerosal preserved vaccines continue to be used in developing countries [Dorea et al., 2009]. In response to safety concerns about thimerosal, an investigative team at the IPRL lead by Tom Burbacher conducted a comparative toxicokinetic study of thimerosal and methylmercury in neonatal primates [Burbacher et al., 2005b]. Results of the study showed that methylmercury is not a suitable reference for ethylmercury-based toxicokinetics and that the two forms of mercury produce a different pattern of distribution in the blood and brain of infants. A separate project aimed at the development of a nonhuman primate model for studying the neurobehavioral consequences of childhood vaccines with and without thimerosal is on-going at the IPRL [Hewittson et al., 2010]. Thus far, study results have documented abnormal early neurodevelopmental responses in male infant rhesus macaques receiving a single dose of thimerosal-containing Hepatitis B vaccine at birth. Importantly, the effects of thimerosal exposure appear to be exacerbated when infants are born with a lower birthweight or a shortened gestational age.

Section Five: Infectious Disease Processes

Enteric Pathogens—Diarrheal diseases are significant contributors to morbidity and mortality in both human and non-human primates. Factors affecting disease transmission and acquisition of immunity are difficult to study in humans due to a number of confounding factors [Quinn et al., 1984]. Prospective studies of experimental infection in humans, although possible for healthy adults [Chappell et al., 2006], are not possible in the most at risk populations (infants and immunocompromised individuals) for ethical reasons. Nonhuman primate infants suffer from many of the same diarrheal diseases as human infants. In many cases symptoms are indistinguishable from those seen in human infants and young children [Miller et al., 1990a]. This makes nonhuman primates an excellent model for studying agents of diarrheal infection. The IPRL has played an important role in studying two enteric pathogens (Campylobacter and Cryptosporidium) which cause diarrheal illness in both humans and nonhuman primates [Wilson et al., 1984; Navin & Juranek, 1984]

Campylobacter—Campylobacteriosis is caused by a bacterium. It is the most common cause of diarrhea world wide [http://www.who.int/mediacentre/factsheets/fs255/en/] and one of the most common causes of diarrheal illness in the United States [http://www.cdc.gov/nczved/divisions/dfbmd/diseases/campylobacter/]. In the US, it is estimated to affect over 2.4 million persons each year. Although campylobacter infection does not commonly cause death, it can lead to other serious long-term consequences. For example, in approximately 1 in every 1000 persons campylobacterosis will lead to Guillian-Barre syndrome, a serious autoimmune condition that causes paralysis, can last several weeks and usually requires intensive hospital care [Hardy et al., 2011; http://www.cdc.gov/nczved/divisions/dfbmd/diseases/campylobacter/].

Studies in the IPRL investigating the prevalence of Campylobacter infection indicated that 70% of infants housed in the lab were infected by at least one strain of Campylobacter by 18 months of age [Russell et al., 1988]. A prospective study of Campylobacter infection was conducted on 4 infants (3 ½ to 4 ½ months old) that were specific pathogen free for both Campylobacter and Shigella [Russell et al., 1989]. These infants were experimentally infected with two *C jejuni* serotypes that were obtained from outbreaks of human *C jejuni* infections [Blaser et al., 1979; Glass et al., 1983]. All 4 infants developed acute symptoms similar to those seen in humans that were characterized by fluid diarrhea, bloody stools, fecal leukocytes and acute colitis. Symptoms lasted from 7 to 11 days. Rechallenge of these infants with the same strains did not result in clinical symptoms [Russell et al., 1989]. Similarly, juveniles that had been previously infected with multiple strains of Campylobacter species, did not show clinical symptoms. The authors concluded that Campylobacter infection in *M nemestrina* caused clinical and pathologic sequelae similar to those found in humans and that prior infection resulted in protection against subsequent diarrheal symptoms. These data may in part explain why travelers from developed countries (who don't often encounter these enteric pathogens) often experience diarrheal symptoms upon exposure in developing countries, while inhabitants of these areas do not experience symptoms, since they have been exposed to multiple strains during development.

Another prospective study looked at *C cinaedi* and *C fennelliae* infection in nonhuman primate infants [Flores et al., 1990]. These strains of Campylobacter are not commonly found in nonhuman primates and are isolated primarily from homosexual men. Infection with these agents is often associated with bacteremia (a serious and potentially fatal systemic infection of the blood) in immunocompromised humans, although an exact causal relationship has not been established. Nine M. nemestrina infants were orally challenged with *C cinaedi* (n=3), *C. fennelliae* (n=4) or *C jejuni* (n=2) and one infant served as a control and received sterile broth. Data indicated that infection with both *C. fennelliae* and *C cinaedi* produced clinical symptoms that were similar to *C jejuni* (as described above). Gut colonization and excretion persisted for 3 weeks at relatively high concentrations despite resolution of acute signs of infection. Since excretion lasts for such an extended period of time in this model it may explain the propensity for these organisms to be transmitted sexually among homosexual men [Quinn et al., 1984]. Infection with *C cinaedi* and *C fennelliae* also resulted in bacteremia for all of the challenged infants. The authors hypothesized that since the infantile immune system is underdeveloped, these infants represented an immunocompromised state. Therefore, this model is especially relevant since the causal relationship of bacteremia accompanying campylobacter infection in immunocompromised humans (such as those infected with HIV) has been difficult to characterize due to simultaneous infection with other potential pathogens [Hunter & Nichols, 2002; Quinn et al., 1984]. Thus, development of a nonhuman primate model is an important step in providing the pathogenic role of these agents and may also explain the occurrence of bacteremia in immunosuppressed individuals.

Cryptosporidiosis—Cryptosporidis is a diarrheal disease caused by a protozoan (Cryptosporidia). Oocysts secreted from infected individuals do not require a secondary vector, can survive for lengthy periods outside a host and are resistant to many disinfectants

[http://www.cdc.gov/parasites/crypto/gen_info/infect.html]. Cryptosporidiosis is one of the most important diarrheal pathogens affecting people worldwide and has been found to be an especially significant contributor to pediatric diarrheal morbidity in developing countries [Fayer & Unger, 1986; Shirley et al., 2012]. Cryptosporidis also represents a significant risk to imunocompromised individuals such as those infected with HIV [Nissapatorn & Sawangjaroen, 2011]. Because cryptosporidiosis often occurs in outbreaks (contaminated drinking or recreational water, daycare centers, etc.) it is classified as a reportable disease to the Centers of Disease Control (CDC). CDC surveillance estimates that there are approximately 13 cases diagnosed yearly for each 100,000 persons in the US population [http://www.cdc.gov/parasites/crypto/].

A study of experimental infection with Cryptosporidium in *M nemestrina* infants was conducted at the IPRL. Infants (n=4) who were 25 to 50 days old were inoculated via nasogastric tube with either 2×10^5 oocysts (n=2) or only 10 oocysts (n=2). Infection resulted in clinical enteritis and fecal passage of large numbers of oocysts in all four infants [Miller et al., 1990b]. Surprisingly, there was no apparent dose-related effect on either the clinical illness or the duration of time that oocysts were excreted. This indicates that a very small number of oocysts can produce full blown diarrheal infection and the ability to infect other individuals. Infants in both groups were ill for 1–2 weeks and continued to shed oocysts for up to 56 days post infection, well after symptoms resolved. After clearance of the infection (at least three negative stool examinations over a period of at least two weeks) the infants were rechallenged with 2×10^5 or 100 oocysts from the same stock used for the original challenge. None of the rechallenged infants evinced clinical symptoms. However, three did shed a very small number of oocysts intermittently for 10–35 days after rechallenge. In a small study of two infants, Miller et al. [1991], found that breast feeding by disease resistant females did not confer immunity or attenuate experimental clinical illness in their infants. Thus, breast feeding in human populations may not confer immunity to infants. Collectively, these studies established an experimentally reproducible nonhuman primate model of cryptosporidial disease that closely resembles that of human infection. Of special import is the demonstration that a very small amount of this infectious agent can cause full-blown disease.

Models of HIV/AIDS—The specter of AIDS continues to haunt the world. At the end of 2010, 34 million people were living with human immunodeficiency virus (HIV) and 3.4 million of these are infants and children less than 15 years of age. Each day approximately 1,000 children are newly infected with HIV [http://www.slideshare.net/UNAIDS/unaids-world-aids-day-report-2011-core-slides-10250153]. Most of these infections are transmitted from mother to child during pregnancy, during labor/delivery, or through breast milk [Torpey et al., 2012]. In developed countries, improved obstetrical management and intensive anti-retroviral therapy both pre and postnatally have dramatically reduced neonatal HIV transmission and have made HIV infection a chronic managed disease [Buchholz et al., 2010]. However, it must never be forgotten that these benefits remain largely unavailable in developing countries, which represent 90% of all new HIV infections in infants. Without treatment, one third of HIV infected infants die before their first birthday [Newell et al., 2004].

HIV is a lentivirus which belongs to a subfamily of the family of retroviruses (Retroviridae). Retroviruses do not consist of DNA as do most viruses. They consist of RNA that is duplicated in the host cell to make DNA which is then incorporated into the host's genome where at a later date it will instruct the cell to assemble infective virus. Lentiviruses usually lead to death in an infected individual and are characterized by a long incubation period to overt disease and the ability to infect nondividing cells.

To date, the nonhuman primate model appears to be the best animal model of HIV infection [Van Rompay, 2012]. Nonhuman primate models of HIV infection have utilized a number of lentiviruses including SIV (a naturally occurring nonhuman primate HIV-like virus), various SHIVs (pathogenic chimeric viruses made from a combination of HIV and SIV genes) and HIV-2 $_{287}$ (a human HIV virus passaged through nonhuman primates to increase pathogenicity [McClure et al., 2000]. Macaques infected with these viruses exhibit virological, immunological, pathological and central nervous system effects that are similar to those seen in HIV infected humans [Hirsch & Lifson, 2000; Sopper et al., 2002]. Studies of HIV infection in infants and children are difficult due to a host of confounds such as maternal drug use, lack of prenatal care, poverty, maternal illness, etc. [Coscia, et al., 2001; Drickover, et al., 2001; Mellins et al., 1994; Robertson, et al., 2010; Wilkins et al., 1990]. Studies in nonhuman primates can circumvent these confounding factors. In addition, prospective studies involving experimental infection with a lentivirus that would never be possible in the human population can be conducted in nonhuman primates. The IPRL has played a significant role in investigating causes of maternal fetal/infant lentiviral transmission, treatments to lessen the chances of transmission and the postnatal consequences of infection in a nonhuman primate model.

Maternal Transmission—Since most HIV infections in infants occur through vertical transmission from the mother it is imperative to know when and how infection occurs as well as maternal factors (timing of infection, viral load, CD4 count, etc.) that can influence transmission. This is especially important in guiding ameliorative therapies in developing nations where ongoing pre- and postnatal treatment strategies may not be available.

A study conducted at the IRPL investigated the maternal-fetal/infant transmission rate of a highly pathogenic lentivirus (SHIV-SF162P3) when females were inoculated intravenously during pregnancy. Infants delivered vaginally and were allowed to nurse for 6 months. Therefore this study models the experience of human infants in developing countries where infants are exposed to virus prenatally and perinatally by exposure to infected maternal fluids and postnatally by breast feeding. Jayaraman et al. [2004] inoculated 10 pregnant *M nemestria* females with 100MID_{50} of SHIV-SF162P3 during the second trimester. All females became infected following inoculation. Four of the resulting nine births were infected (transmission rate of 44.4%) a similar rate to that seen in humans [Buholholz et al., 2010]. One of these infections occurred in utero and three peri- or postnatally. The in utero-infected infant exhibited high viremia, rapid CD4 lymphocyte cell loss [Jayaraman et al., 2007] which was comparable to what has been reported in HIV in utero-infected infants [Drickover et al., 1994]. The authors concluded that this model could be useful in answering questions regarding the role of maternal infection, maternal immune response and risk of

transmission to the infant as well as the efficacy of various drug regimens administered pre and postnatally.

A unique chronic catheterization model was developed in the IPRL by Dr. Rodney Ho and colleagues that allowed simultaneous investigation of the time-course of lentivirus infection in the female, and response of the fetus to this infection during pregnancy [Ho et al., 1996; Ho et al., 2001]. This model involved implanting catheters in the fundus of the uterine cavity as well as the femoral vein and artery of pregnant nonhuman primates at 120 days of gestation. During this procedure, catheters were also implanted in the carotid artery and internal jugular vein of the fetus [for a complete description of this procedure see Ho et al., 1996]. These tethered females were inoculated intravenously with HIV-2_{287} (a highly pathogenic lentivirus) between 124 and 143 days of gestation. It was found that all females became infected and developed an intense viremia followed by a precipitous decrease in CD4 lymphocytes which is typical of this lentivirus. Amniotic fluid of the females became virus positive between 7 and 14 days post infection and all infants born to these females were infected prenatally.

This model was then used to examine the efficacy of a triple drug therapy (Zidovudine, Didanosine, Indinavir) during gestation in preventing maternal fetal transmission [Ho et al., 2000]. In this tether study, 8 females were inoculated with HIV-2_{287} between 131 and 142 days of gestation. Five females received the triple drug therapy administered three times daily through a gastric catheter and 3 control females received no therapy. All females became infected, but viral loads were much lower in those females receiving drug treatment. Virus was detected in the amniotic fluid of all control females and was followed by detection of virus in the fetus within 9 days. Infant viremia also continued after birth. Although virus was also detected in the amniotic fluid of 2 of the 5 drug treated females their fetuses remained virus free. Repeated testing of these infants until they were euthanized at 1–2 years of age indicated that they remained uninfected. This study demonstrated the efficacy of triple drug therapy in preventing maternal/fetal transmission. Current guidelines now recommend triple drug therapy for pregnant women infected with HIV http://whqlibdoc.who.int/publications/2010/9789241599818_eng.pdf. In developed countries this has dramatically reduced the incidence of maternal transmission to the infant and has changed HIV1-infection in children from a fatal illness to a chronic disease with decreased mortality and improved quality of life [Bucholz et al., 2010].

Oral Mucosal Infection Model—It is thought that the majority of human infant HIV infections occur during parturition through mucosal exposure to HIV-containing maternal secretions or later through oral or gastrointestinal exposure to infected breast milk [Krivine et al., 1992; Little et al., 2012; Mofenson, 1997; O'Shea, 1998]. A study was conducted at the IPRL that investigated viral dynamics following oral mucosal exposure to HIV-2_{287} as a model of peri-/postnatal infection [Herz et al., 2002]. Within 24 hours of vaginal delivery (to noninfected females), 4 infants were orally exposed to a cell free viral suspension of HIV2_{287} at a dose of either 1000 TCID50 or 10000 TCID50. The two infants at the lower dose did not become infected, but those exposed to the higher dose developed viremia within a week following exposure, followed by rapid CD4 depletion. One of the two infected infants had a much higher plasma free viral load (10^7 vs 10^6 copies per ml). This infant

exhibited clinical symptoms much sooner and was euthanized at day 16 post delivery due to CD4 depletion and clinical onset of simian AIDS. The two non-infected lower-dose infants were re-exposed to $HIV2_{287}$ at 2.5 months of age (1 by the oral route at a dose of 10000 TCID50 and one by the rectal route at 1000 TCID50). These infants became infected at the second exposure and displayed viral dynamics and CD4 depletion similar to the previously infected infants. The same relationship of higher viral load correlating with more rapid CD4 depletion and onset of clinical disease was seen in one of the re-exposed infants. This finding is important because data from human infants also demonstrate a strong correlation between high viral load and rapid disease progression [Dickover et al., 1998; Shearer et al., 1997; Mutasa et al., 2012]. These data suggested that anti-retroviral treatment capable of decreasing viral load during initial infection could have a significant impact on slowing disease progression. Subsequent studies in humans have indicated that the earlier infants receive Highly Active Retroviral Therapy (HART) the better their outcomes. HIV infected children who receive treatment within the first three months of life experience a fourfold reduction in disease progression and mortality compared to those infants who receive treatment later [Buhholz et al., 2010].

Postnatal Developmental Consequences of HIV Infection—Central nervous system involvement in HIV infection is especially prevalent in infants and children where the brain is a primary site of HIV infection [Epstein, 1988]. It is not commonly recognized how devastating neurological impairments can be in HIV infected infants and children. These deficits encompass virtually every realm of behavioral functioning and can include cognitive delay and mental retardation, fine and gross motor impairments, deficits in attention and concentration, hyperactivity, delays in acquisition of social behaviors and language as well as disturbances in mood including apathy and depression [see Worlein et al., 2005]. Studies of CNS sequelae due to HIV infection in infants and children are difficult due to confounds including prenatal maternal drug use, lack of prenatal care, poverty, or maternal illness, [Coscia et al., 2001; Mellins et al., 1994; Wilkins et al., 1990; Robertson et al., 2010]. Studies in nonhuman primates can circumvent these confounding factors.

Studies were conducted in the IPRL investigating central nervous system effects of lentivirus infection as a nonhuman primate model of pediatric neuroAIDS. In these studies, infants were removed from their mothers at birth and reared in the IPRL virus suite. Rearing protocols in the virus suite were based on those used at the IRPL [Ruppenthal & Sackett, 1992] with several special accommodations for these physiologically fragile infants [for a complete description of rearing practices see Worlein et al., 2006]. Since both human and nonhuman primate lentivirus-infected infants grow at a significantly slower rate [Isanka, 2009], infants were fed formula that was supplemented with a high calorie, vitamin enriched nutritional supplement. Toys, diapers and cloth surrogate covers were disinfected between uses to guard against secondary infections which can be devastating in immunosuppressed infants. To ensure normal behavioral development infants were socialized daily in a large, easily disinfected cage with like-virus infected infants and non-infected controls. As a result, virus infected infants grew at a rate comparable to normal nursery reared infants and did not receive significantly more veterinary treatments than noninfected controls [Worlein et al.,

2006]. Therefore developmental consequences evinced by these infants could be ascribed to viral effects on the CNS rather than general illness or debilitation.

One study investigated the effects of postnatal infection. *M. nemestrina* infants were inoculated with HIV-2_{287}, postnatally at approximately 1 month of age [Kinman et al., 2004]. Inoculation occurred either intravenously or intrathecally (into the CSF). Disease progression was evaluated by virological assessment of blood and cerebral spinal fluid (CSF), CD4 lymphocytes in blood, and quinolinic acid levels in CSF (a surrogate marker of neuronal cell damage). Both routes of inoculation produced detectable viral RNA in CSF and productive infection in the blood. Detection of virus in CSF correlated with a 4–8 fold rise in CSF quinolinic acid levels. All HIV-infected infants experienced a severe and rapid decline in CD4 lymphocytes within 10 weeks of infection. HIV-infected infants, particularly those infected by the intravenous route, exhibited significant delays in reaching cognitive milestones and acquiring both fine and gross motor milestones when compared to noninfected controls. These deficiencies closely mimic those exhibited by HIV infected human infants [Van Rie et al., 2008; 2009].

A second study investigated the effects of prenatal infection on postnatal behavioral development. Subjects were 20 infant pigtail macaques; 8 controls born to uninfected females, and 12 infants whose females had been inoculated and infected with HIV-2_{287} in the third trimester of pregnancy [Worlein et al., 2005]. Seven infants were infected prenatally, as measured by polymerase chain reaction (PCR) at birth and five were not infected (as measured by PCR and coculture on repeated testing). Infected infants attained cognitive and motor milestones at significantly later ages than controls, thus exhibiting developmental delays that were similar to human infants infected with HIV. Developmental deficits were not correlated with viral dose given to the female, maternal CD4 lymphocyte levels at parturition or infant viral RNA levels at birth. Deficits were negatively correlated with proportions of CD4 lymphocytes in the infants at birth and this correlation was even greater 2 weeks after birth indicating poorer performance in those infants with a more rapid disease progression. Since developmental delay was significantly related to disease progression, these data suggest that postnatal therapies starting shortly after birth could be effective in ameliorating developmental sequelae in infected infants. Subsequent studies in humans have shown that early retroviral treatment strategies substantially improve survival in HIV-1 infected infants and children as well as ameliorating deficits in cognitive and motor development [Diener et al., 2012; Van Rie et al., 2009]. Early treatment is now recommended for all HIV infected infants by the World Health Organization [http://www.who.int/hiv/pub/paediatric/infants2010/en/index.html].

Thus, the nonhuman primate model developed at the IPRL has represented a useful model of pediatric neuroAIDS which can be used to study mechanisms causing neural damage as well as the efficacy of ameliorative therapies in the absence of the myriad of confounds that can compromise the results from human studies.

Section Six: Innovative Treatments for Birth Injury

The first neonatal intensive care unit (NICU) at the University of Washington opened in 1963. At the time, it was one of just a few in the country. Prior to this time there was little

interest in the specialized care of newborns, and this was reflected in the high mortality rate of approximately 20/1000 live births. A new focus on newborn care developed in the 1960s due to several important events: 1) A White House Conference on Children in 1961 addressed infant morbidity, 2) a large multicenter Collaborative Project on Cerebral Palsy was undertaken by the National Institute of Neurological Diseases and Blindness (National Institutes of Health) and, 3) the National Institute of Child Health and Human Development (NICHHD) was formed in 1962 under President John F. Kennedy. When President Kennedy's prematurely born son died of hyaline membrane disease (HMD) in August 1963, this further focused attention on the health of the newborn. This confluence of events spurred several improvements in neonatal care, and during the 1960s, the ability to warm, nourish, monitor and oxygenate babies rapidly improved. Mechanical ventilation for infants with respiratory distress was introduced in the early 1960s. Increasingly, preterm babies survived, but pulmonary morbidity was a huge problem causing both morbidity and mortality. The availability of the Primate Center offered an unparalleled opportunity to model neonatal diseases to better understand their pathophysiology and study new therapeutic interventions. Thus began a longstanding collaboration between the Division of Neonatology and the Primate Center.

Use of the nonhuman primate to model neonatal human diseases has focused on 3 main areas at the WaNPRC: 1) lung development and preterm respiratory distress syndrome, 2) control of respiration, and 3) perinatal hypoxic-ischemic brain injury. In the late 1970s, developmental studies of lung morphology and physiology were completed by Hodson and colleagues [Hodson et al., 1977, Palmer et al., 1977, Prueitt et al., 1979]. Preterm *M. nemestrina* were found to be an excellent model in which to study the pathogenesis and treatment of infant respiratory distress syndrome (IRDS), or as it used to be called, HMD [Prueitt et al., 1979, Jackson et al., 1985]. The composition of pulmonary surfactant and how it changes with fetal maturity and postnatally with HMD was studied [Jackson et al., 1986, 1988]. The pulmonary inflammatory response to preterm delivery and mechanical ventilation was studied [Jackson et al., 1988, 1987] along with lung repair mechanisms [Jackson et al., 1990a,b]. Prenatal steroids were found to accelerate pulmonary development and surfactant production with an increase in lung compliance [Kessler et al., 1982, Truog et al., 1983]. High frequency oscillation was tried as an alternative to conventional mechanical ventilation [Truog et al., 1984, Jackson et al., 1991, Truog et al., 1992]. In an attempt to further improve pulmonary outcomes, exogenous surfactant was next added to high frequency oscillatory ventilation [Hodson, 1991, Jackson et al., 1994a]. The lung injury response was further investigated, and the change in lung proteoglycans with HMD were described [Juul et al., 1993, Juul et al., 1994]. Finally, liquid ventilation with perfluorocarbons was attempted [Jackson et al., 1994b, Tarczy-Hornoch et al., 1996]. These studies were preclinical, and were complemented by clinical studies in critically ill neonates [Leach et al., 1996, Hoekstra et al., 1991, Ferrara et al., 1991].

Studies were also done to improve our understanding of how neonates controlled respiration [Guthrie et al., 1980, 1981, Woodrum et al., 1981, Watchko et al., 1988, Watchko et al., 1987, Mayock et al., 1998, Mayock et al., 1986]. This was important not only for the respiratory problems that accompanied prematurity, and critically ill term neonates, but also because sudden infant death was still a major, and possibly preventable, cause of infant

mortality. Over this time period, for a variety of reasons, including routine administration of prenatal steroids for preterm birth, surfactant administration, and improved ventilators, mortality for preterm infants decreased markedly.

In 2000, the focus of the neonatal division research changed to improving neurodevelopmental outcomes of high risk babies. At this point, mortality in neonates born prematurely had improved markedly with improvements in prenatal and postnatal care. In fact, babies that were extremely premature (23 and 24 weeks of gestation) were now surviving. Unfortunately, approximately 50% of survivors from extreme prematurity suffered significant long term neurodevelopmental impairment with intellectual or physical disability, cerebral palsy (CP), hydrocephalus, or seizures.

Another group of vulnerable infants were term babies with hypoxic-ischemic brain injury. Hypoxic-ischemic encephalopathy (HIE) affects 1.3–4.7/1000 liveborn infants per year in the U.S., and contributes to 23% of neonatal deaths globally [Kurinczuk et al., 2010, Wu et al., 2004]. Similar to extreme prematurity, sequelae of moderate to severe HIE include intellectual disability, CP, hydrocephalus, and seizures. Loss of productivity, dependency, recurrent use of medical and rehabilitation services, and reduced life expectancy all exacerbate the burden [Wang et al, 2008, CDC MMWR, 2004].

To address one of the major contributors to CP and severe neurodevelopmental impairment in an animal model that could closely replicate some causes of HIE, a nonhuman primate model of perinatal asphyxia was developed. The nonhuman primate model provides a unique opportunity to study brain injury in a large animal model whose brain structure is similar to humans [Juul et al., 2007, Jacobson Misbe et al., 2011]. In particular, use of this model allows for multiple assessments that would not be possible in human infants, including sequential blood sampling, repeated MRIs, weekly neurodevelopmental assessments, and ultimately, confirmation of these findings by necropsy evaluation. This model was engaged to test various neuroprotective strategies, and to discover much needed biomarkers of hypoxic ischemic brain injury. To date, studies at the IPRL have described the metabolome of normal birth [Beckstrom et al., 2012] and discovered new acute biomarkers of acute hypoxia-ischemia [Beckstrom et al., 2011]. This multi-modal approach, which combines timed evaluation of circulating and urinary proteins (proteomics) and metabolites (metabolomics) with sequential brain imaging, will increase the likelihood of identifying reliable biomarkers to diagnose the degree of injury and improve prognosis by tracking the response to treatment after neonatal brain injury.

Section Seven: New Initiatives/Future Directions

Stem Cells, Chimeras and Transgenic Non-human Primates—Assisted reproductive technologies and embryonic stem cell (ESC) sciences, have revolutionized the bioengineering of animal models of human disease and have been instrumental in allowing scientists to understand molecular mechanisms controlling genes known to be associated with developmental disorders. A widely used approach for the production of bioengineered mouse models is the generation of mouse ESC-chimeras. Here the cells of a pre-implantation stage embryo are combined with genetically distinct and pluripotent ESCs to produce an animal with two different cell populations. This has been particularly useful for

creating gene targeted disease models and testing cell transplant therapies in the mouse (Kiel et al., 2008, Coffman et al., 1993, Huijbers et al., 2011, Stillwell et al., 2009, Kolodziejska et al., 2008). Significant emphasis is currently being placed on advancing the development of mammalian stem cell chimeras in the rat, pig and nonhuman primate to unequivocally demonstrate pluripotency and enhance our understanding of the biomedical utility of ES/iPS cell lines in these species (Simerly et al., 2011, Vassiliev et al., 2010, Yamamoto et al., 2012). The physiological, morphological and genetic similarities between the human and nonhuman primate make the nonhuman primate a singularly exceptional model system for some human diseases. The nonhuman primate is the only laboratory species in which there are several well established ES/iPS cell lines displaying similar morphological and molecular characteristics to human ES/iPS cells. The generation of nonhuman primate stem cell-chimeras represents a very powerful and unique tool for use in two distinct areas of human disease therapeutics and pre-clinical animal model development.

1. **The study of ES/iPS cell based transplant therapies:** Large animal stem cell chimeras would allow for an immunohistocompatible assessment of the therapeutic potential of differentiated native or transgenic (Tg) ES/iPS cells for inducible models of human disease such as spinal cord injury, cardiac ischemia and diabetes. To date, most transplantation studies using human ES/iPS cell-based grafts have been performed in immunocompromised rodents and it is doubtful whether the results of such experiments parallel an allogeneic or even an isogeneic clinical situation (Kerr et al., 2010, Pearl et al., 2011). Moreover, animal size limits the range of functional assays that can be conducted such as the reliable and clinically relevant detection of lung and/or heart function improvements in mice following stem cell transplantation. Hence, transplantation of ES and iPS cell-derived cells into larger animals, such as the nonhuman primate, is necessary to (a) assess clinically relevant functional improvements provided by stem cells after transplantation, (b) compare and contrast the tolerability, integration/efficacy and safety of ES and iPS cell-derived tissue following transplantation, (c) determine the relative benefits/limitations of allogeneic versus autologous stem cell transplantation in a clinically meaningful setting, (d) assess the risk concerning potential teratoma and tumor formation in a clinically relevant immunology setting and (e) facilitate comparison of genetically identical ES and iPS cell based transplants within the same animal.

2. **The generation of clinically relevant nonhuman primate transgenic models of human disease**: ES/iPS cells are amenable to site-specific/targeted genetic modification, thereby potentially providing a more stable and efficient method for generating nonhuman primate models of human genetic disorders compared to previous attempts using lentiviral injection and somatic cell nuclear transfer (Chan et al., 2001, Yang et al., 2008). While ESC from species other than the mouse have been historically less amenable to gene targeting strategies, recent work in the rat, human and the marmoset have demonstrated transgene efficiencies and stability equivalent to the mouse following the refinement of ESC culture conditions and gene targeting methods (Zwaka & Thomson, 2003,

Urbach et al., 2004, Buehr et al., 2008, Ying et al., 2008, Shiozawa et al., 2011, Tong et al., 2010, Khan et al., 2010, Khan et al., 2011, Asuri et al., 2012).

One of the most exciting new directions for the IPRL is a project aimed at generating nonhuman primate stem cell chimeras as a model system of fragile X and associated disorders. Fragile X syndrome (FXS) is the most common heritable form of human mental retardation affecting approximately 1 in 4000 males and 1 in 6000 females and is the most common known cause of autism or “autistic-like” behaviors (Crawford et al., 2001). Fragile X is a trinucleotide repeat disorder that encompasses a range of genetic conditions, all of which result as a function of changes within the *FMR1* gene due to CGG repeat expansion that results in abnormal production and/or expression of *FMR1* mRNA and protein, FMRP (Verheij et al., 1993). Individuals with FXS carry the full mutation CGG repeat sequence while individuals with the pre-mutation are considered fragile X carriers (1 in 800 males and 1 in 260 females). The pre-mutation is associated with late onset fragile X-associated tremor/ataxia syndrome in males (Hagerman & Hagerman, 2004) and fragile X-associated primary ovarian insufficiency and early onset menopause in females (Sherman, 2000).

Understanding of the pathological mechanisms of fragile X and the development of therapies requires a detailed understanding of *FMR1* gene and FMRP function in a clinically relevant model system. To date, there are no known naturally occurring animal models of fragile X, including nonhuman primates (Deelen et al., 1994, Arocena et al., 2003). *In vivo* models include genetically modified species including the mouse (*Mus sp.*; (Bakker et al., 1994) and fruit fly (*Drosophilia melanogaster*; (Wan et al., 2000), while *in vitro* models use tissues obtained from experimental models and human clinical subjects (Urbach et al., 2010, Eiges et al., 2007, Frumkin et al., 2010). However, CGG repeat length, repeat stability and repeat methylation status underpin the spectrum of clinical pathologies and heritability issues associated with the disorder and current model systems are significantly limited in their ability to address the areas of gene/protein function and intergenerational gene transmission observed in the human.

Utilizing advances in adeno-associated virus (AAV) mediated gene targeting of ESCs, studies are underway to produce for the first time nonhuman primate *in vitro* and *in vivo* model systems of fragile X. Generated Tg fragile X ESC lines will have the same genetic background as the control ESC lines providing a powerful tool for comparative *in vitro* studies into the cellular/molecular basis of normal and fragile X genotypes, something that cannot be achieved with individualized ES/iPS cell lines derived from the human. Our ultimate goal however, is to generate liveborn nonhuman primate Tg stem cell-chimeras for advancing our understanding of the molecular mechanisms, inter-generational propagation and therapeutic sensitivity of FXS and fragile X associated disorders resulting from germline stable CGG repeat expansions of the *FMR1* gene.

The full-scale development of a nonhuman primate model of fragile X necessitates a long-term approach. Firstly, development and validation of the platform technology is required. Here we have already established the methodology for producing nonhuman primate ESC-chimeric embryos that show robust integration of ESCs within the pre-implantation embryo. We have also developed embryo transfer techniques in surrogate females that support high

rates of embryo implantation and liveborn outcomes. Secondly, the resources must be available to provide individualized care and behavioral analysis of experimental infants for model validation. To meet this need, all nonhuman primate chimeric infants will undergo state-of-the-art neurobehavioral analysis utilizing the resources available through the IPRL. In characterizing the nonhuman primate fragile X model, it will be important to evaluate and compare fragile X and control (neurotypical) infants with a carefully-selected neurobehavioral test battery that focuses on the functional areas most affected by this genetic syndrome in the human. To assess the possibility of visual-spatial integration and motor coordination deficits such as those found by Diep *et al*, (2012), a challenging test of gross and fine motor dexterity and visually-coordinated reaching will be administered to infants. Infants are assessed on the accuracy, timing and quality of individual reaches and method of pick up. Using the computerized, touch-sensitive learning test methodology that is available at the IPRL, infants will be trained on a spatial-discrimination and successive reversal tests. These tests require focused attention to spatial locations, cognitive flexibility, and inhibitory control. Abnormalities in visual motion processing have increasingly been targeted as a core feature of the fragile X cognitive phenotype and have been recently documented in human infants with FXS (Farzin et al, 2008). Similar impairments in human fragile X pre-mutation infants have also been observed (S. Rivera, personal communication). To measure the presence of a visual motion impairment, infants will be evaluated on a forced-choice contrast detection task. As with human infants, this is accomplished through the psychophysical measurement of eye tracking to static and moving second-order stimuli at different contrast levels. The precise, quantitative measurement of eye tracking is available through the IPRL (Ono et al., 2012). Additionally, infants will be evaluated using the IPRL standardized battery of physical and neurobehavioral assessments which includes tests of sensory and motor skills, individual and social behavior, temperament and object and spatial learning and memory (Burbacher and Grant, 2000).

Nonhuman primates represent a unique model system for furthering our understanding of ES/iPS cell pluripotency supporting *in vitro* and *in vivo* studies focused on the development of nonhuman primate stem cell-chimeras both as Tg models of human developmental disorders and as clinically relevant models for the assessment of stem cell-based transplant therapies. The production of nonhuman primate models using our ESC-based technology will support translational medical advances with respect to understanding the genetic and environmental contributions to developmental disorders as well as complex behavioral traits associated with a given pathology. Furthermore, the development of safe and functional therapeutic strategies involving ESC-derived cell, tissue and organ transplantation models will complement insights from human ESC research and assist in reducing the current knowledge gap in this ever expanding field.

Looking Forward—From describing newborn reflexes to engineering embryonic stem cells, the IPRL has a long and rich history in scientific achievement. The scientific opportunities that are available in the laboratory have drawn investigators and students from the fields of psychology, biology, zoology, anthropology, medicine, neuroscience and nursing. The lab has developed a popular education program that allows university students into the laboratory for “hands on opportunities” to learn about young macaque monkeys and

scientific methods. This program, the brain child of Dr. Jim Sackett, has inspired many young students to actively pursue advanced study in the developmental sciences. The future direction of the IPRL is focused on identifying the mechanisms of childhood disease and disability through a multidisciplinary lens. New research will build on the long and rich history of studies described herein as well as forge new frontiers in the area of genetic disorders and the influence of environmental-gene interactions on normal and abnormal development.

Acknowledgments

We would like to thank the staff of the IPRL for the expert care they have given to the animals over the years. In particular, we note the enduring and significant contributions of staff members Noelle McKain, Caroline Kenney, Brenda Crouthamel, Britni Curtis, Sherry Caffery and Coleen Walker-Gelatt. We also extend our sincere appreciation to Susan Parker for her expert assistance with preparation of the manuscript.

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Table 1

Some of the measures in the standard IPRL test battery used to study growth and behavior of neonatal, infant, and yearling macaques in the IPRL.

Measure	Age Tested
Infant Birth Evaluations	Day 1–2
Weight Gain	Birth to Exit from IPRL
Nursery Reflex and Sensory Ability Assessments	Birth to 20 days
Age At Self-Feeding Anthropometrics	Milestone at 10–15 days Birth - 9 months
Infant Status and Diurnal Cycles	Birth to 20 days
Visual Acuity	Month 1–4
Visual Recognition Memory	14 Days to 60 days
Object Permanence	30 to 180 days
Home Cage Behavior	Birth to Exit from IPRL
Social Behavior	28 Days to Exit from IPRL
WGTA or Touch Screen Learning Series	3 to12 Months
Behavior Living in a Social Group	10 to 24 Months or Exit from IPRL