

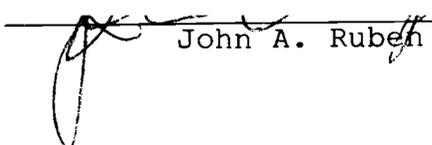
AN ABSTRACT OF THE THESIS OF

Scott R. Robinson for the degree of Doctor of Philosophy in Zoology presented on November 3, 1989.

Title: A Comparative Study of Prenatal Behavioral Ontogeny in Altricial and Precocial Murid Rodents.

Redacted for Privacy

Abstract approved:


John A. Ruben

Using techniques that permit the direct observation of fetuses in vivo, prenatal behavioral development was compared among four species in the rodent family Muridae: the Norway rat (Rattus norvegicus), Mongolian gerbil (Meriones unguiculatus), cotton rat (Sigmodon hispidus), and spiny mouse (Acomys cahirinus). Rattus and Meriones bear altricial offspring, as do nearly all other Murid rodents. But Sigmodon and Acomys have independently evolved the reproduction of precocial young. The behavioral maturity of precocial neonates implies significant differences from altricial rodents in patterns of prenatal development. Specifically, fetal behavioral patterns that are antecedent to postnatal behavior should be accelerated relative to birth in precocial species, while patterns that promote the survival of the fetus in utero (ontogenetic adaptations) should be retained through gestation and therefore appear

retarded relative to antecedent behavior.

Numerous quantitative measures were used to characterize the development of fetal behavior. These included: (a) the incidence of movement in discrete body regions of the fetus and overall fetal activity, (b) the tendency for fetal movements to be temporally clustered in bouts, (c) the emergence of synchronous movement, (d) the sequential patterning of fetal behavior, (e) the response to intraoral chemosensory stimulation, (f) the expression of species-typical behavioral patterns, such as facial wiping and contact-righting, and (g) the behavioral response to experimental occlusion of the umbilical cord.

In precocial species, most patterns of fetal behavior emerged relatively earlier in gestation than in altricial species. However, a few patterns, such as the fetal response to umbilical cord occlusion, were accelerated in altricial species. This developmental pattern was consistent with the predicted shift in the timing of events during prenatal behavioral ontogeny. Consistent patterns of behavioral development observed in all four species further suggested the existence of transient motor patterns that may serve as a developmental bridge between randomly produced movements and coordinated behavioral patterns. The existence of prenatal behavioral heterochrony and behavioral scaffolding imply that early behavioral development is not a unitary process, but involves multiple, independent ontogenetic trajectories.

A Comparative Study of Prenatal Behavioral Ontogeny in
Altricial and Precocial Murid Rodents

by

Scott R. Robinson

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed November 3, 1989

Commencement June 1990

APPROVED:

Redacted for Privacy

Associate Professor of Zoology in charge of major

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Date thesis is presented November 3, 1989

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ACKNOWLEDGEMENTS

Completion of this doctoral program represents, like birth, both an end and a beginning. I would like to single out three persons who I believe contributed most to my intellectual development during my academic gestation. A series of courses in the history and philosophy of science by Dr. Paul L. Farber, who also is a member of my current doctoral committee, exerted a strong influence on me as an undergraduate at O.S.U. and helped to determine my fate as an obligate intellectual. I differentiated as a behavioral biologist under the direction of Dr. Jack P. Hailman at the University of Wisconsin (Madison), and those who are familiar with Jack's work and outlook on science will recognize his influence on my current research. After a delayed implantation, my academic gestation proceeded in the laboratory of Dr. William P. Smotherman at O.S.U. (now at the State University of New York at Binghamton), who provided the support, enthusiasm and experience that allowed this project to come to term.

Thanks to the other members of my Doctoral Committee -- Dr. John E. Morris (Zoology), Dr. Andrew R. Blaustein (Zoology), Dr. Timothy J. Cowles (Oceanography), and especially Dr. John A. Ruben (Zoology) -- who helped to manage a complicated labor. Thanks also to three

Chairpersons in the Department of Zoology -- Dr. Charles E. King, Dr. Christopher J. Bayne and Dr. Jane Lubchenco -- who facilitated the initiation, conduct and completion of my graduate work at O.S.U.

The research reported in this thesis was supported in part by grant HD 16102-06 from the National Institute of Child Health and Human Development (NIH) to William P. Smotherman, and by Instructional Research Awards from Oregon State University to S.R.R. Some of the technical equipment was provided by the Departments of Animal Science, Psychology, and Zoology, and the work was conducted in space made available by the College of Pharmacy and the Department of Zoology. The spirit of interdisciplinary cooperation was important not only in the ideas, but also in the practical conduct of this research.

Finally, I must thank my wife, Karen, and my fitness, John and Amanda, who have steadfastly reminded me that there is life after birth.

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A COMPARATIVE STUDY OF PRENATAL BEHAVIORAL ONTOGENY
IN ALTRICIAL AND PRECOICIAL MURID RODENTS

CHAPTER 1. INTRODUCTION

A. Why Study Fetal Behavior?

Every pregnant mother knows that the fetus she carries moves before birth. The fact of prenatal movement has been known to science at least since the writings of Aristotle. Hieronymus Fabricius [1604, cited in Carmichael 1954], William Harvey [1651], Karl Ernst von Baer [1828] and other early pioneers of biology remarked on the movements of vertebrate embryos or drew inferences about the prenatal formation of "the sensitive soul", to phrase the question in Aristotelian terms. Bichat [1827], who persisted in the peripatetic tradition in the 19th century, concluded from his fetal observations that the fetus subsists in a vegetative existence devoid of sense experience or function. This conclusion was echoed a half-century later in William James's famous allusion to the "blooming, buzzing confusion" of the human newborn [James 1890] and continues to be repeated in modern biology and psychology textbooks. Indeed, a comparison of passages penned by Aristotle and James more than 22 centuries apart illustrates the unity of

scientific viewpoint in addressing the issues of behavior and sensation before birth:

As soon then as the offspring of all animals are born, especially those born imperfect, they are in the habit of sleeping, because they continue sleeping also within the mother when they first acquire sensation.... But nevertheless they are found to wake even in the womb (this is clear in dissections and ovipara), and then they immediately fall into a sleep again. This is why after birth also they spend most of their time in sleep. [Aristotle, De Generatione Animalium, p. 321].

Prior to all impressions on sense-organs the brain is plunged in deep sleep and consciousness is practically non-existent. Even the first weeks after birth are passed in almost unbroken sleep by human infants. It takes a strong message from the sense-organs to break this slumber. [James 1890, p. 456].

Despite this early history (or perhaps because of the negative conclusions), questions regarding the movement and habits of the fetus have received scant attention outside of folklore and popular belief until modern times. The field of behavioral embryology has remained to the present somewhat apart from the mainstream of both developmental and behavioral science.

The first modern attempts to investigate the prenatal development of behavior are generally attributed to Wilhelm Preyer, who summarized his fetal research in an extensive monograph published just over a century ago [1885]. Preyer's novel questions and observations encouraged a later generation of neuroanatomists to develop rudimentary techniques for observing fetuses in vivo [Brown 1915;

Swenson 1926; Avery 1928]. The field of behavioral embryology flourished in the 1920s and '30s in the laboratories of Coghill [Swenson 1926; Coghill 1929; Angulo y Gonzalez 1932], Windle [Windle & Griffin 1931; Windle, O'Donnell & Glasshagle 1933; Windle, Minear, Austin & Orr 1935], Carmichael [Coronios 1933; Carmichael 1934], and Barcroft [Barcroft, Barron & Windle 1936; Barcroft & Barron 1939] among others. This flurry of research was motivated by an interest in the origins of motor reflexes and neuromuscular development. Prenatal reflexology was seen principally as a useful method for identifying stages of neural development [see reviews by Hooker 1952; Carmichael 1954].

The early emphasis on fetal reflexes as a diagnostic tool probably reinforced the impression that fetal motility lacks intrinsic coordination or spatio-temporal organization. This impression, which was stressed in many of the classic studies and in some of the more recent ones [Hamburger 1963; Narayanan, Fox & Hamburger 1971], fostered a traditional viewpoint that prenatal motor activity is random, directionless and without purpose; fetal movement is a mere epiphenomenon of the early development of the nervous system. For reasons of technical simplicity, this neurological perspective became increasingly focussed on avian models of prenatal behavior [see reviews by Hamburger 1963; Kuo 1967; Bekoff 1981; Oppenheim 1981, 1982a] to the virtual exclusion of fetuses: between 1945 and 1980 only

one general study of fetal behavior appeared in the English-language literature [Narayanan, Fox & Hamburger 1971]. In addition, the focus on evoked reflexes and neuroanatomy diverted attention from the ontogeny of spontaneous fetal activity and functional behavior.

The viewpoint that the fetus is a passive passenger during its residence in utero thus has had a long and rich history. Today, however, this viewpoint is undergoing rapid change, due in part to renewed interest in fetal research within a diversity of disciplines ranging from psychobiology and ethology to teratology and pediatric medicine, and in part to the influence of improved technical, analytic and conceptual tools with which to study behavior. Within the past decade, research concerned with the patterning of prenatal behavior has discovered temporal rhythms in fetal activity [Robertson 1985; Robertson, Dierker, Sorokin & Rosen 1982], the emergence of motor coordination before birth [Bekoff & Lau 1980; Robinson & Smotherman 1987, 1988], and the prenatal development of species-typical action patterns [Smotherman & Robinson 1987a]. Research on sensory development in human and nonhuman fetuses has demonstrated prenatal chemosensation [Pedersen, Stewart, Greer & Shepherd 1983; Smotherman & Robinson 1988a] and audition in utero [Birnholtz & Benecerraf 1983; Geubelle 1984; Fifer & Moon 1988]. Research on the importance of experience during prenatal life has documented the ability of fetuses to respond to changes in the intrauterine environment

[Smotherman & Robinson 1986], to learn by association of stimuli [Smotherman & Robinson 1985, 1987b] and to retain prenatal experiences into postnatal life [Smotherman 1982; Stickrod, Kimble & Smotherman 1982; Hepper 1988]. (Many of these research findings are summarized in recent reviews [Robinson & Smotherman in press; Smotherman & Robinson 1987b, 1988b]). It has become manifest that the behavior of mammals has its roots in the prenatal period and that a complete understanding of behavioral development must include investigation of the behavioral capabilities of the fetus.

B. New Tools for Fetal Investigation

The present is a paradoxical time for fetal research. Concern for the healthy development of infants is stimulating the desire of both the public and the professional communities to learn more about the needs of the fetus and prenatal care. Efforts to improve the viability of preterm or low birthweight babies are underscoring the need for more information on the behavioral potential, relationship to environment, and development of the fetus [Krasnegor 1987, 1988]. At the same time, such concerns have fostered an appreciation of the fetus as a sensitive, fragile organism, which has fueled the public debate over issues such as abortion and fetal rights. Recent court cases, in which lawsuits have been filed on

behalf of fetuses against women that have abused drugs during pregnancy or against individuals or corporations that have endangered or compromised fetal viability dramatize this dimension of fetal research [Morris 1986]. The ethical considerations engendered by our changing awareness have come full cycle to restrict the methods that can be used to study human and animal fetuses.

A solution to this paradoxical impediment to fetal research has emerged with the development of noninvasive technological means of monitoring and imaging the fetus in utero. Two important technologies have come to be routinely used in clinical applications in the past 10-20 years. External fetal monitoring, which involves little more than the placement of a sensory transducer around the abdomen of a pregnant woman, enables the recording of fetal heart rate and detection of gross fetal body movements. More precise information about the kinds of movements exhibited by the fetus is provided by real-time ultrasonography, in which the echoes of high-frequency sound pulses are used to define contours and surfaces within the body and thereby produce a video image of the fetus as it moves in utero [Birnholz 1988]. These two innovations have created a window on human fetal development that has replaced earlier approaches, such as examination of aborted fetuses [Humphrey 1953].

Experimentation with human fetuses remains an emotionally charged issue. Recent guidelines for the study of human fetuses and ethical considerations in general have

placed limitations on the study of human fetuses. Moreover, the current generation of indirect methods for monitoring or imaging the human fetus do not provide sufficiently detailed information about the form and patterning of fetal movements. Creation of continuous, fine-grained records of fetal behavior demand direct observation of the fetus. Therefore, comparative study of nonhuman animals remains centrally important to understanding the prenatal development of behavior, even when motivated by human medical concerns.

C. Conceptual Issues in Prenatal Behavioral Ontogeny

1. Determinants of Behavior

A comprehensive understanding of why behavior occurs requires investigation of four distinct types of biological questions. These four determinants of behavior [Tinbergen 1963; Hailman 1967] or levels of analysis [Sherman 1988] are often arrayed in a two-by-two matrix to illustrate the relationship of each determinant to issues of proximate versus ultimate cause, concepts that are traceable to Aristotle's efficient and final causes, and to individuals versus populations as the appropriate units of observation (Table 1.1) [Hailman 1976a]. To anchor these abstractions in a real example, let us ask the question: Why does the fetus move? Valid answers include: (a) because fetal

Table 1.1
Classification of Behavioral Determinants

	Cause	Origin
	<hr/>	<hr/>
Proximate	(a) CONTROL	(b) ONTOGENY
Ultimate	(c) PERPETUATION	(d) PHYLOGENY

movements are spontaneously generated by the nervous system and may be evoked by external stimulation [Hamburger 1973]; (b) because movement by the fetus is a necessary stage in the development of reflexes and more complex behavior expressed by the newborn [Precht1 1986]; (c) because prenatal motility promotes healthy muscle and bone growth and prevents many kinds of congenital defect [Moessinger 1988]; and (d) because embryonic motility is phylogenetically ancient, occurring in all vertebrate classes [Carmichael 1954]. Each of these proposed answers may or may not be true. In fact, the possibility that all four answers simultaneously can be true is ensured by recognizing the independence of different levels of analysis.

Parsing behavioral determinants in this way has proven useful in underscoring differences among approaches to framing and testing research hypotheses. It also has provided a potential resolution to a number of apparently sterile disputes in the literature. But placing emphasis on independence among different determinants has tended to obscure necessary points of connection between them. Darwin, of course, was the first to recognize that natural selection, which probably is the most important mechanism for perpetuating a trait within a population, can lead to behavioral evolution [1859]; natural selection and evolution continue to provide the major organizational framework within ethology and behavioral ecology [Alcock 1988; Krebs &

Davies 1984]. Recent years also have seen resurgent interest in the relationship of ontogeny to phylogeny [Gould 1977; Raff & Kaufmann 1983]. The logical relationship of ontogeny to other determinants has less often been discussed, but will be important in our investigation of fetal behavior.

2. Ontogeny and Control

Of fundamental importance to understanding fetal development, or developmental issues in general, is an epigenetic perspective. Epigenesis is hardly a new idea. The debate between adherents of an early epigenetic philosophy and their preformationist counterparts shaped the early history of embryology [Gould 1977; Oppenheim 1982b]. Indeed, the epigenesis-preformation dichotomy survives in modern guise as an implicit foundation underlying the recurrent dispute over nature and nurture as alternative and mutually-exclusive factors shaping the development of behavior [Oyama 1985]. This dispute is at once perplexing and frustrating, because it long since has admitted of a solution [Kuo 1967; Hinde 1968; Lehrman 1970]. Behavioral development consists of a phenotypic series that is the product of a dynamic interplay between the organism, its environment, and its genetic endowment, not a gradual elaboration of an architectural plan preconfigured in the genes nor an inscription of a creative environment upon a

blank slate.

If ontogeny can be represented as a succession of phenotypes, then the general plan of ontogenetic research entails (a) documentation of distinguishable phenotypes in the sequence, and (b) investigation of the processes involved in transforming one phenotype into the next. Identification and description of phenotypes is straightforward when dealing with morphological characters, but subtly more complex when dealing with behavior. Behavior does not have an independent physical existence; it is a transient expression of a complicated system of neurons, muscles and other effector organs. Therefore the phenotype of interest in ontogeny is not the instantiation of a particular act, but the underlying control system governing behavior [Hailman 1982]. It is possible to address this control system at many levels, ranging from scrutiny of the details of neurobiology and physiology to more general theories of motivation and stimulus-response relationships. The present study will adopt an explicitly cybernetic view of behavioral control [Ashby 1956], treating the fetus as a machine with operationally defined states and inputs and stochastically organized outputs. From such a cybernetic perspective one can readily quantify developmental changes in behavioral control. It eventually may be possible to extend this framework to embrace transformational rules as well, thereby uniting it with the concept of epigenesis [Hailman 1982].

3. Ontogeny and Perpetuation

The developing organism and the series of behavioral phenotypes it expresses do not exist in a vacuum. An important implication of epigenesis is that ontogenetic change occurs within a predictable succession of environments or ontogenetic niches. The notion of ontogenetic niche as a predictable ecological and social environment associated with a period during the life history of an animal [West & King 1987; West 1988] long has been appreciated with reference to invertebrates and lower tetrapods that have larval stages which subsequently undergo metamorphosis. The environments encountered by caterpillars and tadpoles often are radically different from those faced by adult butterflies and frogs. Viewing the milieu within a shelled egg as a transient ontogenetic niche also has been usefully applied to oviparous amniotes, especially birds [e.g., Gottlieb 1971]. Yet few organisms experience more profoundly different environments during development than placental mammals. Conceptualizing the fetus as an organism that resides in and interacts with a unique environment is only beginning to influence the study of mammalian behavioral development [Smotherman & Robinson 1988b].

Foremost among the implications of the concept of ontogenetic niche is the existence of ontogenetic adaptations: specialized structures or functions that

promote survival for a brief portion of the life of an organism [Oppenheim 1981; Alberts & Cramer 1988]. The placenta, umbilical cord, and modifications of the fetal circulatory system are classic examples. Nonspecialists often overlook the fact that these structures are derived from embryonic and not maternal tissues and therefore must be viewed as anatomical adaptations of the fetus to the intrauterine environment [Oppenheim 1982a, 1984]. An important mode of adaptation of mature animals to their environment is behavior. Immature animals, such as newborn rodents, existing in a transient "breast and nest" habitat, also exhibit unique behavioral adaptations such as suckling [Hall & Williams 1983] and huddling [Alberts & Cramer 1988]. The conjunction of these ideas raises an interesting and relatively unexplored question: Does the fetus exhibit behavioral adaptations to its intrauterine niche? Developmental studies have tended to focus on issues of behavioral continuity and early antecedents of postnatal behavior [Hall & Oppenheim 1987]. However, Smotherman and Robinson [1987c, 1988c] recently have described the behavioral response of the rat fetus to experimentally induced occlusion of the umbilical cord, which may be an example of a fetal adaptation to accidental cord compression in utero. This question will be explored further in the present study.

A second class of ontogenetic adaptation includes stages of organization that facilitate the development of

more complex structures. The origin of complexity is a problem that has seized the attention of experimental embryologists since the early research of Spemann. Epigenesis requires that an organism remain intact and viable through all intermediate stages between the relatively unorganized zygote and the complex adult. One means of bridging beginning and end points is the production of intermediate structures that serve no purpose other than to provide a substrate for further development. Just as scaffolding is erected and later dismantled in the construction of an arch, transient ontogenetic structures may be important in the developmental construction of organic complexity.

Although not usually phrased in these terms, classic embryology abounds with examples of morphological scaffolding. The gastrula of early embryogenesis well illustrates this point. Gastrulation is a phylogenetically ancient stage in the development of multicellular animals. It is the point of origin of differentiated germ layers that not only serve as building materials but also provide the source of inductive interactions that drive embryological development. Although attempts repeatedly have been made to identify the gastrula with hypothetical primordial organisms, the most famous of which may be Haeckel's "Gastraea" [Gould 1977], all have failed. The gastrula exists exclusively as scaffolding in the ontogeny of the metazoa. Robinson and Smotherman [1988] recently have

suggested that transient ontogenetic scaffolding may be important in early behavioral development as well. This question will be addressed further in the present study.

4. Ontogeny and Phylogeny

Just as ontogeny can be represented as a succession of phenotypes, evolution can be thought of as a succession of ontogenies; what are modified through natural selection are not the endpoints of development, but the ontogenetic trajectories that lead to altered endpoints. Although the modern synthesis of evolutionary theory does not formally incorporate developmental processes [Mayr 1982], it is now widely appreciated that alterations in ontogenetic trajectories may be an important mechanism of evolutionary change [Raff & Kaufman 1983]. Heterochrony, a phyletic change in the rate or timing of developmental events [de Beer 1958], is probably the best documented mode of interaction between ontogeny and phylogeny [McKinney 1988]. The term itself implies comparison; a rate must be measured against a standard. Hence, identification of heterochrony requires that the timing of two or more processes be offset in different species. Classical examples of heterochrony involve altered allometric relationships between size and shape of morphological features during growth [Gould 1977]. More recently, behavioral heterochrony has been implicated in the evolution of domestic breeds from wild progenitors

[Price 1984; Miller 1988].

There is no theoretical reason to expect that heterochrony would be less common before birth than after birth. Indeed, the wide variation in the degree of sensory and motor maturity expressed by newborn mammals implies striking changes in the rate of development before birth [Brunjes 1983]. Ornithologists have long recognized a fundamental life-history distinction between birds that hatch in a helpless condition, featherless and with eyes closed, unable to leave the nest, and wholly dependent on their parents for food and care (altricial species), and other birds that are covered with downy feathers, leave the nest soon after hatching, and are relatively independent of parental care (precocial species) [Nice 1962]. Like so many other concepts in biology, recognition of these two modes of early development can be traced to Aristotle's distinction between young born "perfect" (precocial) or "imperfect" (altricial) [Aristotle, De Generatione Animalium]. The utility of the distinction between altricial and precocial offspring has led to its application to patterns of mammalian reproduction [Eisenberg 1981], life-history strategies such as r- and K-selection [Horn 1978; Bekoff & Byers 1985], and the evolution of behavioral complexity in tetrapods [Burghardt 1988]. Altricial-precocial differences also have been suggested as a major source of variation in patterns of prenatal development [Carmichael 1954; Oppenheim 1972], although the question has received scarcely any

empirical attention in mammals.

Comparison of patterns of behavioral development in altricial and precocial fetuses thus holds the greatest promise for identifying behavioral heterochrony during the prenatal period. Moreover, specific patterns of heterochrony may be predicted. Because precocial species are born in a mature state, one should expect a sequence of behavioral phenotypes to be compressed and shifted to earlier points in ontogeny as measured relative to birth. This temporal shift is illustrated by the developmental series A_1 - A_6 depicted schematically in Figure 1.1. However, accelerated development should apply only to patterns of fetal movement that are antecedent to postnatal behavior. Ontogenetic adaptations of the fetus should be relatively unaffected by an evolutionary shift from altricial to precocial development and remain most closely correlated with changes in the fetal environment. The relative stability of fetal adaptations is represented by the series B_1 - B_6 in Figure 1.1. Thus, in species that differ along the altricial-precocial dimension, antecedent behavior should be accelerated (precocial) or retarded (altricial) in development relative to the timing of parturition and relative to ontogenetic adaptations that must be maintained through term. Documentation of this form of behavioral heterochrony therefore should provide a unique method of distinguishing adaptive and antecedent behavior expressed by the fetus.

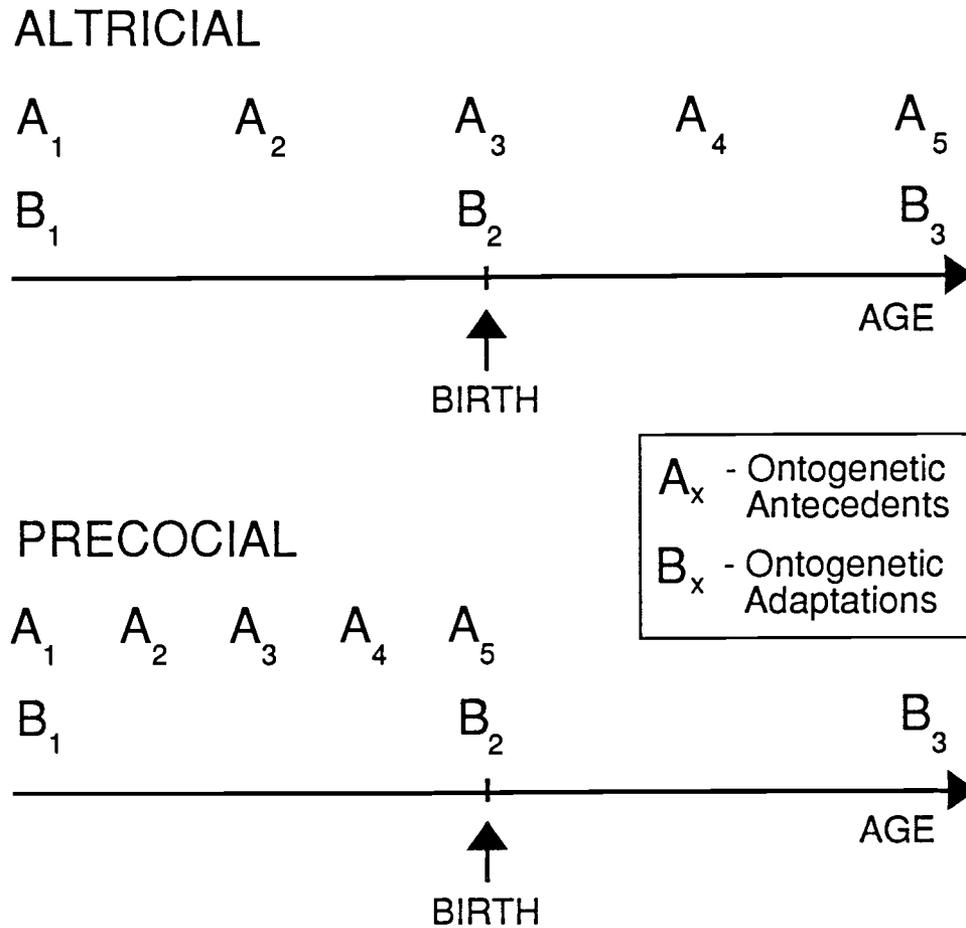


Figure 1.1. Schematic diagram of predicted pattern of heterochrony in altricial and precocial species. A_x symbols represent the sequence of emergence of ontogenetic antecedents of later behavior. B_x symbols represent the sequence of emergence of behavioral ontogenetic adaptations. Note that A_x are shifted to relatively earlier points in gestation, relative to B_x , in precocial species.

These, then, are the principal themes that will be addressed in the present study: epigenesis and the origin of behavioral organization, ontogenetic adaptation, behavioral scaffolding, and heterochrony in the evolution of altricial and precocial species. In the next chapter, I will attempt to elaborate a comparative method suitable to address these questions.

CHAPTER 2. COMPARATIVE DESIGN AND METHODS

A. Logic of Comparative Study

1. Why compare fetal behavior in different species?

Although the past few years have seen remarkable progress in prenatal behavioral research, the field is still primarily in the stage of describing the capabilities and range of variation in fetal behavior. Fetuses of only a handful of species have been examined at all, and most of these have not been re-examined since the classic days of fetal reflexology (Table 2.1). Moreover, species have been selected for fetal research on the basis of their availability and ease of maintenance in the laboratory. Imbedded in the classic literature also are implicit notions of a scala naturae [Hodos & Campbell 1969]. Modern concepts of evolution and systematics have had little impact on fetal research. Therefore, a primary need remains to simply describe how prenatal behavioral development varies among mammalian taxa.

The comparative method long has been appreciated as an important tool for distinguishing between homologous and analogous structures. Homologous structures are corresponding attributes in different species that are similar owing to an unbroken chain of inheritance from a

Table 2.1

General studies of fetal behavior. Species are listed by common and Latin name. For each study, the method employed for preparing the pregnant female and the principal behavioral objective of the study (evoked reflexes versus spontaneous fetal movements) are specified.

SPECIES & METHOD EMPLOYED	FOCUS OF STUDY	AUTHOR & YEAR
<u>NORWAY RAT (<i>Rattus norvegicus</i>)</u>		
cesarean delivery	evoked reflexes	Lane 1917
cerebral anemia	spontaneous/evoked	Swenson 1926
cerebral anemia	spontaneous/evoked	Angulo y Gonzalez 1932
cerebral anemia	evoked reflexes	Windle et al. 1935
spinal transection	spontaneous/evoked	Narayanan et al. 1971
chemomyelotomy	spontaneous	Narayanan et al. 1982
spinal transection & chemomyelotomy	spontaneous	Smotherman et al. 1984
lidocaine spinal blockade	spontaneous	Smotherman & Robinson 1986
<u>HOUSE MOUSE (<i>Mus musculus</i>)</u>		
spinal transection	spontaneous	Kodama & Sekiguchi 1984
<u>GUINEA PIG (<i>Cavia porcellus</i>)</u>		
cesarean delivery	evoked	Avery 1928
cervical transection	evoked	Carmichael 1934

(table continued)

Table 2.1 (continued)

SPECIES & METHOD EMPLOYED	FOCUS OF STUDY	AUTHOR & YEAR
RABBIT (<u>Oryctolagus cuniculus</u>)		
decerebration	evoked	Pankratz 1931
CAT (<u>Felis catus</u>)		
decerebration	spontaneous/evoked	Windle & Griffin 1931
decerebration	spontaneous/evoked	Windle et al. 1933
decerebration	spontaneous/evoked	Coronios 1933
SHEEP (<u>Ovis aries</u>)		
general/spinal anesthesia	evoked reflexes	Barcroft et al. 1936
general/spinal anesthesia	spontaneous/evoked	Barcroft & Barron 1939
HUMAN		
cesarean delivery	evoked	Minkowski 1928
aborted fetuses	evoked	Hooker 1952
real-time ultrasonography	spontaneous	deVries et al 1982,1985
external monitoring	spontaneous	Edwards & Edwards 1970
external monitoring	spontaneous	Robertson 1985

common ancestor. Analogous structures, in contrast, arise through evolutionary convergence because similar functional demands often favor similar adaptive structural solutions [Ghiselin 1976; Hailman 1976a]. These concepts have formed the core of two traditions in comparative biology [Mayr 1982; Ridley 1983]: homology (plesiomorphy, or primitive characters) has been emphasized in efforts to discover phyletic relationships among taxa (e.g., cladism), while analogy (apomorphy, or derived characters) has been emphasized in the study of convergent adaptation (e.g., behavioral ecology). It is the latter of these traditions -- the study of adaptation and common functional demands -- that is of particular interest in the present context.

The logical potential for extracting information about adaptation from comparative study is illustrated in the following example. First, if comparisons are planned correctly, then species selected for study may be distinguished in each of two orthogonal dimensions: phyletic relationship and functional circumstance. Table 2.2 depicts a comparative study in which two categories are recognized in each dimension. If cross-species comparison in this hypothetical study were to reveal a structure that appears only in adjacent horizontal cells of this matrix (cells A and B or C and D), then one may conclude that the structure is derived from common ancestry within a phyletic lineage. In other words, the structures, as expressed in different species, are likely homologous. Conversely, if

Table 2.2

Possible comparisons in idealized study involving species from two phyletic lineages in two types of ecological circumstance or life-history pattern

Phyletic relationship	Ecology or Life History	
	Type 1	Type 2
Lineage 1	A	B
Lineage 2	C	D

corresponding structures appear only in adjacent vertical cells (cells A and C, or B and D), then their similarity may be attributed to common selection pressures arising from shared ecological circumstances or life-history traits. The existence of such attributes provides evidence for similar adaptations produced through evolutionary convergence. As many authors have pointed out, this style of comparative reasoning is just as applicable to behavior as it is to anatomy [Hailman 1976b; Maynard Smith & Holliday 1979; Jarman 1982; Ridley 1983; Clutton-Brock & Harvey 1984]. Further extension of carefully designed comparative analysis to problems of fetal behavior should yield information on the evolution and function of prenatal behavioral patterns.

A final justification for comparative study is relevant for much of the current research on fetal behavior that is motivated by a desire to learn more about human fetuses and preterm infants. The study of human fetal behavior has expanded greatly with the advent of indirect methods for monitoring fetal activity, such as tocodynamometry [Edwards & Edwards 1970; McLeod, Brien, Loomis, Carmichael, Probert & Patrick 1983; Robertson, Dierker, Sorokin & Rosen 1982; Robertson 1985] and real-time ultrasonography [Birnholtz 1988; de Vries, Visser & Precht1 1982, 1984, 1985; Precht1 1985]. Nevertheless, these methods do not provide the detail of direct fetal observation nor the control of experimental manipulation. Direct observation and experimentation with human fetuses is further limited by

legal and ethical considerations. For these reasons, animal models remain necessary elements of fetal research, even from a biomedical perspective. Systematic comparative analysis of fetal behavior should facilitate meaningful interpretation of research involving animal models.

2. Why Limit Comparison to Murid Rodents?

Non-systematic selection of subject species, so evident in early studies of fetal behavior, greatly diminishes the power of comparative analysis. Four of the best studied species (rats, Rattus norvegicus; cats, Felis catus; sheep, Ovis aries, and humans) are members of different mammalian orders and differ markedly in life-history, physiology, and patterns of reproduction. Moreover, different methods and research questions have been applied to each of these species. Rats, for example, have figured prominently in studies of neurological development in mammalian fetuses, while sheep have been more extensively used in studies of prenatal physiology. Yet how are we to compare these two isolated points among mammalian taxa? Rats and sheep differ in body size, nutritional needs, uterine and placental anatomy, number of offspring in each pregnancy, length of gestation, and many other parameters of known importance to fetal development [Dawes 1968; Eisenberg 1981]. In spite of these confounds, several writers have ascribed differences in fetal behavior in these two species to the degree of

sensory-motor competence at birth: rat pups are altricial and lambs are precocial [e.g., Carmichael 1954].

Many of the same criticisms can be applied to a comparison of rats and Guinea pigs (Cavia porcellus), another rodent that has been employed in fetal research. In addition to bearing precocial offspring, Guinea pigs are larger than rats and bear fewer, larger offspring that develop over a longer period of gestation. With the fetal behavior of so few mammalian species adequately described, comparative study at present will provide stronger inferences if most biological parameters are held relatively constant. Ideally, closely related species should be compared that exhibit similar body size, gestation length, litter size and reproductive physiology, but differ along an altricial-precocial dimension.

One of the recent conceptual advances in comparative method is the recognition of how phyletic relationships must be incorporated into the design of comparative studies. Recently, several critics have argued that species cannot be considered independent in comparative studies if the characters under study are shared by evolutionary descent [Ridley 1983; Felsenstein 1985]. To illustrate this difficulty, consider a hypothetical comparative study of ten altricial species (all members of genus G_1) and ten precocial species (all members of genus G_2). A comparison of developmental patterns among these 20 species may well reveal a striking correlation with altricial-precocial life

history. But the effective sample size is two, not twenty, because each life history pattern is shared by all members of the same genus. If the objective of comparative study is to identify adaptation, or in other words the common functional demands that give rise to similar structural attributes through evolutionary convergence, then the species selected for study must represent independent evolutionary events. Careful comparative analysis therefore uses best estimates of phylogeny to identify species (or higher taxa) that have independently derived a character of interest, because only independently derived characters can be used to assess evolutionary convergence [Ridley 1983].

Because research on fetal behavior is labor intensive, requiring time-consuming breeding procedures, cross-sectional sampling and involved surgical techniques, a broad range of species cannot reasonably be surveyed in a restricted period of time. Therefore, the selection of subject species must be limited to a few species that exhibit optimal combinations of life-history traits and phyletic relationships. In the research design outlined below, four species of rodents in the family Muridae will be examined. In each species, fetuses of varying gestational age will be observed under different environmental circumstances to further the principal goals of this project. In brief, these goals are: (1) to describe how fetal behavior changes during gestation in several closely-related species, providing information on the range of

variation in patterns of ontogeny; (2) to employ stochastic models to aid in measuring behavioral organization and identifying corresponding stages of prenatal behavioral development; (3) to compare corresponding stages of development among altricial and precocial species to investigate the influence of heterochrony on patterns of behavioral ontogeny in utero; and (4) to use heterochrony as a tool to help distinguish patterns of prenatal behavior that are functional in utero from patterns that are antecedent to postnatal function.

3. Comparative Design

The requirements of rigorous comparative analysis of altricial and precocial fetuses can be satisfied within the rodent family Muridae. I will follow the taxonomic recommendations of Anderson and Jones [1984] that the Muridae subsume the family Cricetidae, including most of the New World rats and mice. Thus constituted, the Muridae is one of the largest of vertebrate families, comprising several hundred genera distributed throughout the Old and New Worlds. Virtually all Murid species bear altricial offspring, which is the presumptive ancestral condition. But species in at least three genera (Acomys, Sigmodon, Ototylomys) have independently derived varying degrees of precociality. Spiny mice (Acomys cahirinus) bear furred offspring whose eyes open within minutes of birth. Cotton

rats (Sigmodon hispidus) are only slightly less precocial; their young open their eyes within 24 hours of birth. In contrast, laboratory rats (Rattus norvegicus) and Mongolian gerbils or jirds (Meriones unguiculatus) bear typically altricial offspring whose eyes remain closed for two weeks or more after birth (Table 2.3). Acomys and Rattus are members of the subfamily Murinae; Sigmodon and Meriones are members of the former family Cricetidae, and thus occupy a different lineage within the Muridae. These four species therefore satisfy the requirements established above for a complete comparative design (Table 2.4).

Study of these four species will permit several kinds of pairwise comparisons that will begin to parse some of the factors that often are confounded in studies of fetal development. Each species corresponds to one of the cells of the idealized comparative study depicted in Table 2.2: Acomys (A), Rattus (B), Sigmodon (C) and Meriones (D). Because precociality evolved independently in Acomys and Sigmodon, similar patterns of fetal behavior shared by these species, but not shared with Rattus or Meriones which bear altricial young, would constitute evidence of evolutionary convergence in prenatal ontogeny. Patterns that are similar between Rattus and Acomys, or between Meriones and Sigmodon, are more closely tied to phyletic relationship than to life-history. Finally, patterns that are similar between Rattus and Sigmodon, or between Acomys and Meriones, may reflect the influence of body size on fetal development.

Table 2.3

Taxonomy and developmental characteristics of four rodent species

FAMILY MURIDAE				
	Murid lineage		Cricetid lineage	
	<u>Acomys</u>	<u>Rattus</u>	<u>Sigmodon</u>	<u>Meriones</u>
	<u>cahirinus</u>	<u>norvegicus</u>	<u>hispidus</u>	<u>unquiculatus</u>
Subfamily	Murinae	Murinae	Sigmodontinae	Gerbillinae
Gestation	38 days	21 days	27 days	24 days
Litter size	2	8	7	7
Neonate	precocial	altricial	precocial	altricial
Adult mass	80 g	250 g	150 g	60 g

Table 2.4
Comparative design of the present study

Phyletic relationship	Developmental Pattern	
	Altricial	Precocial
Murid lineage	<u>Rattus</u>	<u>Acomys</u>
Cricetid lineage	<u>Meriones</u>	<u>Sigmodon</u>

B. SUBJECTS

Subject fetuses were the progeny of each of four species listed in Table 2.3. Breeding adults were maintained in 33 X 38 X 10 cm polycarbonate shoebox cages with hardwood bedding. Cages were kept in a temperature- and humidity-controlled colony room exposed to a 12-hr light:12-hr dark photoperiod regime (lights on at 0700). Adults were provided with food and water ad libitum. In all housing and experimental procedures, animal subjects were treated in accordance with guidelines for animal care established by the National Institutes of Health, the International Society for Developmental Psychobiology, and the Animal Behavior Society. Housing and breeding requirements varied slightly among species, however.

1. *Rattus norvegicus*

Female Sprague-Dawley rats were bred to Long-Evans males (Charles River Laboratories, Wilmington, MA) to produce fetuses for study. This cross, which has been utilized extensively in previous studies of fetal behavior in our laboratory [e.g., Smotherman & Robinson 1985; Smotherman & Robinson 1986; Smotherman & Robinson 1988b], reliably produces large litters of healthy offspring. Females were housed in groups of three per cage. During breeding, a single male was introduced into the cage for a

period of four days. Vaginal smears were collected daily from each female; the first day in which sperm were evident was designated day 0 of gestation. By this method, parturition consistently occurred on day 21 of gestation. Females remained in groups of three until the day of fetal observation, when they were rehoused individually several hours before surgical preparation.

2. Sigmodon hispidus

Cotton rats were reared at OSU from breeding stock obtained from a colony maintained at the National Institutes of Health (Bethesda, MD). Juveniles were housed in groups, but sexually mature female and male cotton rats were housed individually to minimize fighting. Fetuses of known gestational age were produced by introducing breeding males into female cages for a 24 hr period immediately after parturition or by collecting and examining vaginal smears daily over a longer breeding period (4-7 days). Fetuses from these timed matings were used to construct standard growth curves to date pregnancies from a smaller number of untimed matings. Gestation length under these conditions of breeding was 27-28 days.

3. Meriones unguiculatus

Female and male outcrossed Mongolian gerbils

(Tumblebrook Farms, West Brookfield, MA) were housed as permanent breeding pairs. Gerbils exhibit a reliable post-partum estrus, permitting pregnancies to be timed by counting the day immediately following parturition as day 0 of gestation. The timing of pregnancies was corroborated for many breeding pairs by observation of post-partum copulation and copulatory plugs. Standard growth curves constructed from fetuses of confident gestational age were used to date a few uncertain pregnancies. Gestation length was 24 days.

4. *Acomys cahirinus*

Female and male spiny mice were obtained as an entire breeding colony from the University of Virginia, Charlottesville (from the laboratory of Dr. Peter C. Brunjes, Department of Psychology). Breeding pairs were established by pairing juvenile males and females before sexual maturity. (Pairing of sexually mature adults typically leads to fighting and injury.) Pregnancies were timed following the same procedures used for gerbils, with day 0 of gestation designated as the first day following parturition or the day of observation of a copulatory plug. Gestation length was 37-38 days.

C. Methods of Data Acquisition

Direct fetal observation necessitates (a) humane desensitization of the pregnant female without rendering fetuses immobile through general anesthesia, (b) surgical preparation of the uterus and fetuses to bring them into direct view, and (c) maintenance of healthy subject fetuses throughout the period of observation. In addition, behavioral study is aided by (d) controlled manipulation of the local physical and sensory environment of the fetus. A technical ability to view the fetus is incomplete, however, without (e) a correspondingly precise method of description and quantification of fetal movement.

1. Preparation of Pregnant Females

The principal difficulty with devising ways to directly observe live fetuses is satisfying the conflicting needs of maintaining a normal physiological environment for the fetus while minimizing discomfort and trauma for the mother. The first attempts to view animal fetuses involved study of dying fetuses obtained from abortions. At the other extreme, researchers of the 1920s and '30s often studied fetuses while mothers were under the effects of complete general anesthesia (a practice that persists today in the study of fetal physiology). Because general anesthetics readily cross the placenta, fetuses within anesthetized

mothers are also anesthetized and consequently show little behavior. Excluding classical methods, such as decerebration produced by ligation of the carotid arteries [Angulo y Gonzalez 1932], two techniques were generally available prior to the 1980s that permitted direct observation of fetuses while circumventing the effects of general anesthesia. Physical transection of the spinal cord was developed early in fetal research [Windle & Becker 1940] and has been employed in recent studies of fetal motor activity [Narayanan, Fox & Hamburger 1971; Kirby 1979; Kodama & Sekiguchi 1984]. A number of variant techniques are available for transecting the spinal cord, but most involve some degree of surgical incision through the skin, dorsal musculature and vertebral column to gain access to the spinal cord. Physical transection of the spinal cord is effective in producing posterior paralysis and eliminating sensation in the lower limbs and abdomen.

An alternative procedure developed in the 1960s -- chemomyelotomy -- achieves the same end as spinal transection with a simple intraspinal injection of ethanol [Basmajian & Ranney 1961]. Chemomyelotomy results in complete interruption of nervous transmission within the spinal cord at the site of injection and paralysis of the hindquarters and lower abdomen. This procedure also has been used effectively in the study of fetal behavior [Narayanan, Narayanan & Browne 1982; Smotherman, Richards & Robinson 1984]. A reversible alternative to chemomyelotomy,

which involves intraspinal injection of lidocaine and epinephrine, has become available more recently [Smotherman, Robinson & Miller 1986]. Lidocaine-induced spinal anesthesia is evidently similar to chemomyelotomy in its effects on the mother and fetuses in rats [Smotherman & Robinson 1986], but is shorter lasting, remaining effective for only 60-90 minutes. To permit longer observation sessions, obviate likely species differences in the duration of lidocaine anesthesia, and facilitate comparison with previous research, chemomyelotomy was selected for use in this study.

To administer chemomyelotomy to pregnant rats, the following procedure was employed. The rat was placed in a 1.0-l jar suffused with 20 ml of anesthesia-grade ethyl ether until fully anesthetized. Once insensate, the rat was removed from the jar and placed in a prone position on a surgical table. A small area of fur overlying the lower thoracic and lumbar region of the rat's dorsum was removed with surgical clippers. To aid visual guidance during administration of the chemomyelotomy, a small midline incision (5-10 mm) was made in the skin, not extending into the musculature, overlying the upper lumbar portion of the spinal column. An injection of 100% ethanol was then delivered by inserting a 25 ga. needle, oriented perpendicular and dorsal to the spine, into the space between the first and second lumbar vertebrae. This site was identified by counting dorsal spinal processes posterior

to the last pair of ribs. The tip of the needle was directed caudally and was moved through a lateral arc within the spinal canal during the injection, which consisted of a 2-3 s pulse of 100 ul of ethanol. The head and thorax of the rat was slightly elevated immediately after injection to prevent rostral flow of ethanol within the spinal canal.

Successful administration of chemomyelotomy is indicated by the rat exhibiting hyperventilation, extension of both hindlimbs, and nipple erection. The procedure results in irreversible spinal blockade that permits surgical procedures in the lower abdomen without the further use of general anesthesia. Attempts to locate the desensitized area by probing the rat's abdomen suggest that the ethanol injection exerts its effects slightly rostral to the site of injection. In developing this technique, I have found that injection at a more rostral location results in a much higher incidence of respiratory arrest. Injection at this site, however, is fully adequate to desensitize the lower half of the rat's body and reliably produces a prepared rat that does not respond to surgical manipulation of the lower abdomen or uterus [Smotherman & Robinson in press].

Variations on this procedure were found to be necessary in adapting chemomyelotomy to other rodent species. Cotton rats proved to be very sensitive to ether anesthesia and were removed from the jar promptly upon the cessation of movement. Ethanol was injected at the same spinal site, but

the volume of injection was reduced to 50 ul and was administered with a 28 ga. needle. Gerbils also received a spinal injection of reduced volume with a 28 ga. needle consisting of 50 ul of ethanol. In all other respects, the chemomyelotomy procedure employed for these two species was identical to that described for rats.

Spiny mice presented a different set of problems and the procedure was further modified to produce an acceptable preparation. The spiny hairs in the dorsal fur of the pregnant spiny mouse were resistant to rapid removal with clippers and obscured visual guidance of the needle through dorsal incisions. Consequently, chemomyelotomy was administered in this species without first making a small incision in the dorsal skin. Instead, spinal landmarks were located by inserting a fine dissecting needle through the dorsal skin and using it as a tactile probe to locate dorsal spinal processes. The dissecting needle then was inserted into the musculature along the anterior edge of a spinal process to locate the space between the first and second lumbar vertebrae. When located, this probe served as a tactile guide for placement of a 30 ga. hypodermic needle. Injection consisted of 30 ul of ethanol into the spinal canal. As with the other species, the tip of the needle was directed caudally and was slowly rotated within the spinal canal during the actual injection. The outward effects of this modified procedure in spiny mice appeared identical to chemomyelotomy in the other three species and resulted in

healthy females that evinced no response to lower abdominal surgical manipulations.

2. Preparation of Subject Fetuses

Once the pregnant female had been prepared with chemomyelotomy, it was placed in a plexiglas holding apparatus that elevates the head and body 45° above the horizontal [Smotherman, Richards & Robinson 1984]. The rat was secured in the apparatus with a velcro jacket placed around the thorax. Although the hindlimbs were immobilized by the spinal procedure, the rat was still capable of upper trunk movements that indirectly moved the lower body. To completely immobilize the lower body, the hindlimbs were secured to the holding device with strips of duct tape. To prevent self-inflicted injury of the female, the forelegs were similarly immobilized with tape. Within a few minutes in this position, females became quiet and showed little or no signs of discomfort, even during extended observation sessions.

After securing the prepared female, the lower ventrum was shaved and a low mid-ventral incision performed. The length of incision varied with species and gestational age, being just large enough to permit each horn of the uterus to be externalized from the abdomen. For rats near term, incisions of 20-30 mm were necessary; incisions as small as 10 mm were sufficient for smaller fetuses. The prepared

female and holding device then were placed in a warm water bath (maintained at $37.5^{\circ} \pm 0.5^{\circ}$ C) containing a buffered isotonic saline solution, Locke's solution [Galigher & Kozloff 1971]. Water depth was adjusted to meet the tip of the rat's sternum, leaving the abdomen below and the thorax and head well above the water surface. Both horns of the uterus, and all resident fetuses, were externalized through the abdominal incision into the saline bath. This manipulation was accomplished taking care to avoid placing strain on points of uterine attachment at the ovarian ends or at the cervix. The female and fetuses remained undisturbed in this situation, without further manipulation, for a period of 15-20 min. This accommodation period was employed to ensure that the female and fetuses completely recovered from any lingering effects of early ether anesthesia. Previous reports have indicated that a delay of 15 min is adequate to eliminate residual effects of brief ether administration [Smotherman, Richards & Robinson 1984; Smotherman & Robinson 1986].

Fetuses were observed in one of two physical conditions: in utero and ex utero. Fetuses were visible in utero through the semi-transparent wall of the uterus after immersion in the saline bath. Although the uterus is nearly opaque early in gestation, it thins and becomes more transparent as gestation proceeds. I found that observation in utero was feasible in all four species at the time of earliest fetal motility. Because the uterus is not

surgically disturbed, observation of fetuses in utero most closely approximates normal intrauterine conditions during gestation.

To provide a clearer and more detailed view of fetal movements, fetuses also were delivered and observed outside the uterus (ex utero). A small transverse incision (10-20 mm) was made in the uterine wall just posterior to the subject fetus. The incision was located on the side of the uterus opposite to sites of placental attachment and location of uterine blood vessels. A small cut also was made in the chorion and amnion near the head of the subject fetus, and the fetus gently delivered through the uterine incision into the saline bath. In this way the subject fetus was suspended within the water bath while the chorion, amnion and placenta remained within the uterus. The ex utero condition does not impair fetal viability [Smotherman, Richards & Robinson 1984; Smotherman & Robinson 1986, Smotherman & Robinson in press]. However, care was taken to prevent twisting of the umbilical cord or pressing of adjacent fetuses within the uterus against the umbilical cord or placenta, which can lead to occlusion of umbilical blood circulation or placental separation. To guard against these events, the umbilical cord was routinely monitored throughout observation sessions. I found that observation of fetuses ex utero, without signs of fetal distress, was possible in all four species at all gestational ages except the earliest age of testing. At this early age, fetal

tissues were sufficiently delicate that umbilical cord occlusion was likely following fetal delivery from the uterus. For this reason, observation of fetuses ex utero was not conducted at the earliest ages of testing for any of the four species.

Because fetuses are no longer limited by the confining envelopes of the amnion, chorion and uterine wall, observation ex utero provides a means of assessing the effect of intrauterine restraint on fetal activity at different gestational ages. Delivery from the uterus also provides convenient access to the fetus for experiments requiring physical or surgical manipulation. Two methods of manipulating the physical or sensory environment of subject fetuses, namely intraoral infusion of a chemosensory solution (lemon extract) and experimental occlusion of the umbilical cord, required delivery of subject fetuses ex utero; these techniques will be described in detail in Chapters 8 and 9. Finally, for measurement of fine motor abilities of fetuses, the clearer view afforded by observation ex utero facilitates creation of detailed real-time video-tape records of fetal behavior.

3. Observation Protocol

Fetuses of each species were observed under different experimental conditions at regular intervals during gestation. In Rattus, Sigmodon, and Meriones, observations

were conducted daily beginning at the earliest age at which spontaneous fetal movement was apparent (hereafter designated as "inception") and continuing through the last day of gestation (designated "term"). In Acomys, owing to a limited number of available breeding dams and small litter size, fetuses were observed on gestational days 22 (inception), 26, 28, 30, 32, 34, and 37 (term). Four conditions of observation were employed: in utero, ex utero, intraoral infusion, and umbilical clamp. In utero and ex utero observations were conducted in 30-min sessions, during which external physical and acoustic stimuli were minimized. Infusion and clamp tests were conducted in shorter sessions (4-min and 3-min, respectively), which will be described in greater detail in Chapters 8 and 9. A minimum sample size of five fetal observations was collected in each combination of age by condition. Actual sample sizes, conditions and ages of observation are summarized in Table 2.5.

Whenever data are collected from multiple offspring of the same mother, treatment effects may be confounded with litter effects [Abbey & Howard 1973; Chapman & Stern 1977]. In previous work with rat fetuses, no evidence of litter effects on behavioral variables has been reported [Robinson & Smotherman 1987; Smotherman, Richards & Robinson 1984; Smotherman & Robinson, 1985, 1986, 1987a, 1988a]. Nevertheless, in Rattus, Sigmodon, and Meriones only one observation in each condition was conducted within a litter,

Table 2.5

Sample sizes of fetuses studied under different combinations of species, gestational age and condition of observation (in utero versus ex utero)

RATTUS	day	16	17	18	19	20	21						
		_____	_____	_____	_____	_____	_____						
in utero		5	5	5	5	5	5						
ex utero		0	5	5	5	5	5						
MERIONES	day	18	19	20	21	22	23	24					
		_____	_____	_____	_____	_____	_____	_____					
in utero		5	5	5	5	5	5	5					
ex utero		0	5	5	5	5	5	5					
SIGMODON	day	17	18	19	20	21	22	23	24	25	26	27	
		_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	
in utero		5	5	5	7	6	5	5	5	5	6	6	
ex utero		0	5	5	7	10	5	5	5	6	6	6	
ACOMYS	day	22	26	28	30	32	34	37					
		_____	_____	_____	_____	_____	_____	_____					
in utero		5	5	5	5	5	5	5					
ex utero		0	5	5	5	5	5	6					

each involving a different individual fetus. In utero observations were restricted to fetuses within unmanipulated uterine horns (i.e., horns that had not yet contributed fetuses for ex utero observation). The small number of available subject fetuses in Acomys required that some fetuses be observed in more than one experimental condition. When this was necessary, data from a particular individual were collected in the following strict sequence of conditions: in utero, ex utero, intraoral infusion, umbilical clamp. If two or more fetuses were present, observations were alternated among subjects. In no instance did an individual fetus contribute more than one observation to a particular condition.

In each condition, one subject fetus was observed during a session (focal animal sampling). The subject was illuminated with cool light conducted from the source by fiber-optic lightguides. A video-tape camera was positioned vertically over the subject fetus and the entire observation session recorded (VHS format, SP recording speed). Simultaneously, I directly viewed the fetus during the session, categorized each instance of fetal movement and recorded these events on an audio-tape recorder. Audio-tape records were subsequently transcribed by entering fetal behavior codes into a microcomputer programmed to function as a real-time event recorder. Other computer programs were developed to count the frequency of events in different behavioral categories and measure other aspects of temporal

and sequential organization of fetal movement. Source codes for these programs were written in Turbo Pascal (Borland Associates) and are available from the author upon request.

4. Description and Quantification

Categories of fetal behavior were defined by the region(s) of the body that moved. Five body regions were distinguished: forelimb (F), rearlimb (R), head (H), trunk (T) and mouth (M). Bilateral and unilateral movements of a particular body region (e.g. forelimbs) were not routinely distinguished. During the course of this study, finer behavioral distinctions within these regional categories were recognized. For instance, in some analyses trunk movements were further divided into curl (C), which involved ventral or lateral flexion of the trunk, and stretch (S), which involved dorsal extension or straightening of the trunk. Especially among older fetuses, coordinated behavioral patterns were apparent, such as facial wiping (W) and paw-licking (L). These and other action patterns will be dealt with in greater detail in subsequent chapters.

This behavioral coding scheme was developed to allow different categories of fetal movement to occur either singly or in various combinations. To unambiguously describe complex patterns of behavior within this scheme, a few terms will need to be defined. A behavioral event is an instance of fetal movement, involving one or more body

regions, that occurs at one time. A component or act is a single body region involved in a particular event. A simple event comprises a single component. The first letter of the component's name serves as a unique coding label for simple events (F, R, H, M, T). In contrast, a synchronous event comprises two or more components that have a simultaneous onset. As a convention, each category of synchronous movement is uniquely represented by the combination of first letters of its components. Thus, the synchronous movement involving forelegs, head and rearlegs is represented as FHR. Because synchronous movements are defined as components that have apparently simultaneous onset, no order or serial relationship is implied by the sequence of letters in the categorical label (i.e., FHR is equivalent to HFR). Of course, a synchronous event cannot comprise the same component twice (i.e., FF is impossible).

The sum of events in each category of movement provided one means of measuring fetal behavior. To describe more inclusive patterns of fetal activity, several summary indices were used. The sum of all events across all categories was used as one index of overall fetal activity (Event Activity); the sum of all acts was used as another (Component Activity). Similarly, the sum of all synchronous events was used to measure the incidence of Synchronous Activity. Other special-purpose indices will be described later in the relevant chapters. However, one index that was used in a variety of contexts deserves mention here.

Entropy or uncertainty, symbolized by convention as H , was adapted from information theory usage [Shannon & Weaver 1949] to describe the equitability of distribution of events among different categories of movement. H is a useful statistic for expressing the information required to predict an event from an array of possible outcomes. H is expressed in bits per event, representing the number of dichotomous guesses required on average to specify an outcome, and is calculated by the general expression:

$$H = \sum_{i=1}^N P_i (-\log_2 P_i) \quad (2.1)$$

where N is the number of behavioral categories and P_i is the probability that a given event will fall in category i . The values assumed by H will vary from a minimum of 0 to a maximum of $\log_2 N$ (when all categories are equally probable). Adjustments in the calculation of H will be discussed in the chapters appropriate to the specific application of this index of uncertainty.

5. Post-observation Measurements

At the conclusion of the last observation for a particular litter, the prepared dam was removed from the saline bath, released from the holding device and quickly euthanized by cervical dislocation. Each conceptus, comprising fetus, placenta and extra-embryonic membranes,

was removed promptly from the uterus, preserving information on the relative position of its placental attachment within the uterine horn. Whenever possible, conceptuses were removed with intact membranes to permit measurement of amniotic fluid volume (this, of course, was not possible following ex utero observation of a particular fetus). A conceptus was placed on a moist paper towel and rolled over to remove adherent blood or water from the saline bath, then weighed to the nearest 0.01 g. Each fetus was weighed twice more after (a) removal of amniotic fluid and (b) removal of placenta, umbilical cord and membranes to provide separate measurements of the mass of amniotic fluid, placenta (and membranes) and fetus. Because the specific gravity of amniotic fluid is nearly equal to water, the mass of amniotic fluid measured in this way was taken as a direct estimate of amniotic fluid volume in ml. The sex of each fetus was also noted by visual inspection of anogenital distance (males have larger AG distances). This method was considered reliable only for older fetuses (after day 18 in Rattus, day 19 in Sigmodon, day 21 in Meriones, and day 26 in Acomys). Fetuses were euthanized after collection of these physical data.

6. Data Analysis

A variety of quantitative techniques were employed to assess fetal behavioral organization. Within each species,

frequency counts in event categories and summary indices were initially analyzed by two-factor (Age X Condition) between-subjects ANOVA. When the interaction of Age by Condition was significant, post-hoc tests for simple effects were conducted by one-factor ANOVA (Age) or t-test (Condition) and subsequent comparison of individual group means performed by the method of Newman-Keuls [Winer 1971]. Comparisons of group means collapsed across Conditions followed significant main effects of Age. Because analyses involved multiple dependent variables, a conservative alpha level (0.01) was considered significant in these tests. Specialized procedures were employed for measuring temporal patterning, movement synchrony and sequential organization of fetal behavior. These procedures will be detailed in Chapters 5, 6 and 7.

CHAPTER 3. ENVIRONMENTAL CONTEXT OF FETAL MOVEMENT

A. Introduction

Conditions within the uterus usually are portrayed as unchanging and unconditionally benign. But the environment in which the fetus resides and grows actually is subject to considerable variation during the course of gestation, between successive pregnancies, and among different mothers [Smotherman & Robinson 1988a]. The most obvious source of variation derives from the embryo itself: quantitative changes in growth result in qualitative alterations in the physical environment in utero. As a rough method of measuring these physical changes, data were collected on fetal body mass, placental mass, and amniotic fluid volume from all four subject species, with sampling beginning at the inception of movement and continuing through term.

B. Overview of the Fetal Environment

Murid rodent fetuses develop within a duplex uterus; the two horns of the uterus are independent and meet the vagina at separate cervixes. Each horn is loosely suspended by mesenteries within the peritoneum, but is more or less fixed in position at the ovarian and cervical ends. The wall of the uterus, composed predominantly of smooth muscle,

is elastic, permitting physical deformation during fetal movement. Each conceptus (including the embryo or fetus, placenta, umbilical cord, extraembryonic membranes and amniotic fluid), with the rare exception of monozygotic twins, bears a separate site of placentation. Placentae appear to be spaced at regular intervals within the horn. Early in gestation, adjacent conceptuses are physically separate, resembling a string of pearls. As growth proceeds, the uterus thins and distends; in rodents, at least, this thinning results in semi-transparency by the time of the first fetal movements, permitting visual inspection of fetuses in utero by a human observer. By the last third of gestation, adjacent conceptuses come into direct physical contact, with the formation of a circular zone of contact as adjacent chorionic membranes are pressed together. It is likely that chemical exchange across the chorion and amnion is possible at this time. Perhaps the best evidence of intrauterine chemical exchange is the "masculinization" effect of female fetuses that are exposed to testosterone produced by male siblings in adjacent uterine positions [Meisel & Ward 1981; Richmond & Sachs 1984; vom Saal 1984].

Each fetus remains attached to its own placenta throughout gestation. The placenta is the sole means of life support for the fetus, providing an interface between maternal and fetal blood supplies for the exchange of oxygen, nutrients, waste products of cellular respiration,

and a variety of chemical compounds of relatively low molecular weight, including hormones. The placenta of Murid rodents is of the hemochorial type, with a single tissue layer separating maternal and fetal circulation. Early in gestation the placenta nearly envelopes the conceptus, further isolating it from adjacent siblings; by the last third of gestation the edge of the placenta withdraws from the chorion and assumes its mature discoid shape.

The fetus is connected to the placenta by means of the umbilical cord, which by the fetal period of gestation (which may be defined as beginning with the inception of movement) comprises two arteries (conducting deoxygenated blood from the fetus) and one vein (conducting oxygenated blood to the fetus). The umbilical cord is flexible and allows some freedom of movement, but nevertheless tethers the fetus and ultimately restricts the range of motion. Tensile strain on the cord apparently contributes to its steady increase in length during the fetal period [Moessinger, Blanc, Marone & Polsen 1982].

Two embryonic membranes, the chorion (outer) and amnion (inner), form nested envelopes around the fetus, creating a space filled with amniotic fluid. (A much smaller volume of exocoel fluid fills the space between the amnion and chorion.) The primary constituent of amniotic fluid is water, which undergoes rapid turnover through the competing processes of fetal ingestion and micturition [Marsh, King & Becker 1963; Wirtschafter & Williams 1957]. Amniotic fluid

also contains a complex assortment of proteins and other compounds, some of which are important nutritional or immunological factors for the fetus [Abbas & Tovey 1960; Lev & Orlic 1972]. The composition of amniotic fluid apparently undergoes dramatic change during late gestation, exhibiting alteration in protein and urea content [Wirtschafter & Williams 1957; Tam & Chan 1977] and a substantial increase in fluid viscosity [Marsh, King & Becker 1963]. Qualitative changes in viscosity near term were noted in all four species examined in the present study.

C. Fetal Body Mass

Body mass underwent profound change in all four species. In Rattus, fetal mass increased nine-fold (9.0X) from the onset of fetal movement on day 16 through term on day 21 (Table 3.1). This compares with increases of 7.9X, 11.1X and 13.5X in Meriones (Table 3.2), Sigmodon (Table 3.3), and Acomys (Table 3.4), respectively. Fetal body growth was roughly exponential during the period of measurement, which is consistent with previous reports of fetal growth rates [Knox & Lister-Rosenoer 1978]. Statistical tests for age effects are not presented because the age of some subjects was determined by comparison of their body mass (or more precisely the mean mass of their litter) to growth curves obtained from litters of known gestation. Fetal mass therefore should be considered as a

Table 3.1

Fetal body mass (g), placenta mass (g), and amniotic fluid volume (ml) for Rattus. Values for placenta and amniotic fluid represent mean \pm SEM.

GESTATIONAL AGE	MEAN BODY MASS	RANGE BODY MASS	PLACENTA MASS (g)	AMNIOTIC FLUID VOLUME (ml)
16	0.61	0.56 - 0.67	0.45 \pm .02	0.48 \pm .01
17	1.03	0.87 - 1.29	0.48 \pm .02	0.57 \pm .02
18	1.72	1.59 - 1.96	0.61 \pm .01	0.69 \pm .03
19	2.87	2.68 - 3.13	0.70 \pm .02	0.61 \pm .04
20	4.37	3.93 - 4.94	0.78 \pm .06	0.35 \pm .06
21	5.49	5.19 - 5.70	0.88 \pm .03	0.18 \pm .02

Table 3.2

Fetal body mass (g), placenta mass (g), and amniotic fluid volume (ml) for Meriones. Values for placenta and amniotic fluid represent mean \pm SEM.

GESTATIONAL AGE	MEAN BODY MASS	RANGE BODY MASS	PLACENTA MASS (g)	AMNIOTIC FLUID VOLUME (ml)
18	0.35	0.33 - 0.36	0.32 \pm .03	0.24 \pm .01
19	0.64	0.56 - 0.78	0.32 \pm .01	0.29 \pm .01
20	1.17	1.04 - 1.42	0.37 \pm .01	0.34 \pm .01
21	1.60	1.51 - 1.73	0.37 \pm .01	0.31 \pm .02
22	2.03	1.92 - 2.11	0.35 \pm .01	0.26 \pm .03
23	2.40	2.21 - 2.57	0.37 \pm .01	0.21 \pm .04
24	2.78	2.61 - 3.02	0.41 \pm .03	0.08 \pm .02

Table 3.3

Fetal body mass (g), placenta mass (g), and amniotic fluid volume (ml) for Sigmodon. Values for placenta and amniotic fluid represent mean \pm SEM.

GESTATIONAL AGE	MEAN BODY MASS	RANGE BODY MASS	PLACENTA MASS (g)	AMNIOTIC FLUID VOLUME (ml)
17	0.58	0.45 - 0.71	0.27 \pm .01	0.74 \pm .04
18	0.81	0.66 - 0.88	0.31 \pm .01	0.82 \pm .04
19	1.02	0.89 - 1.15	0.37 \pm .03	0.71 \pm .13
20	1.88	1.71 - 2.26	0.51 \pm .05	0.64 \pm .09
21	2.25	2.12 - 2.45	0.46 \pm .01	0.76 \pm .07
22	2.74	2.38 - 2.94	0.50 \pm .02	0.69 \pm .04
23	3.34	3.05 - 3.53	0.49 \pm .02	0.64 \pm .06
24	4.44	4.03 - 4.70	0.50 \pm .02	0.32 \pm .05
25	5.02	4.81 - 5.23	0.57 \pm .04	0.35 \pm .00
26	5.45	5.00 - 5.80	0.48 \pm .02	0.15 \pm .04
27	6.44	5.94 - 6.98	0.73 \pm .08	0.13 \pm .12

Table 3.4

Fetal body mass (g), placenta mass (g), and amniotic fluid volume (ml) for Acomys. Values for placenta and amniotic fluid represent mean \pm SEM.

GESTATIONAL AGE	MEAN BODY MASS	RANGE BODY MASS	PLACENTA MASS (g)	AMNIOTIC FLUID VOLUME (ml)
22	0.50	0.36 - 0.69	0.25 \pm .02	0.37 \pm .03
26	1.11	0.96 - 1.35	0.37 \pm .03	0.49 \pm .02
28	1.59	1.49 - 1.77	0.43 \pm .02	---
30	2.46	2.22 - 2.86	0.54 \pm .04	0.57 \pm .15
32	3.06	2.84 - 3.88	0.63 \pm .08	---
34	4.77	4.42 - 5.52	0.66 \pm .04	0.42 \pm .05
37	6.73	6.23 - 7.37	0.83 \pm .02	0.18 \pm .02

supplemental defining characteristic of gestational age. Toward this end, Tables 3.1-3.4 list the range of variation in body mass rather than the standard error for each age. In these tables, mean values for body mass were calculated by first obtaining the average individual mass within each litter and then finding the mean and range of these litter scores.

D. Placental Mass

The placenta also exhibited continual growth during the last third of gestation in Rattus, but the rate of growth was much more linear (Table 3.1). A significant effect of gestational age was evident in the one-way ANOVA examining placental mass ($F_{5,22} = 23.9, p < .001$).

In Meriones, there was imperceptible change in placental mass from day 18 through term ($F_{6,25} = 1.9, p > .05$) in spite of steady growth of the fetus (Table 3.2).

The pattern of placental growth appeared more complicated in Sigmodon (Table 3.3). The significant overall ANOVA ($F_{10,76} = 10.5, p < .001$) indicated that changes in placental mass occurred during this period of gestation. Post-hoc comparisons by the method of Newman-Keuls suggested that the placenta underwent steady growth from day 17 through day 20, but approached an asymptote in mass thereafter.

Placental growth appeared monotonic and linear in

Acomys over the fetal period ($F_{6,27} = 29.1, p < .001$; Table 3.4).

E. Amniotic Fluid Volume

From a behavioral viewpoint, changes in amniotic fluid volume may be the most interesting component of the physical environment in utero, because (a) amniotic fluid provides a space surrounding the fetus that permits fetal movement with relatively little expenditure of energy and (b) abrupt changes in fluid volume occur late in gestation [Marsh, King & Becker 1963; Tam & Chan 1977].

Amniotic fluid appeared to increase and then decrease in volume in Rattus (Table 3.1). A significant effect of gestational age was evident ($F_{5,22} = 25.1, p < .001$). Post-hoc comparisons revealed a significant increase in amniotic fluid volume from day 16 through day 18. Fluid volume remained near this peak for a day, then declined dramatically through term. The volume of fluid on day 21 was only 26% of the peak achieved on day 18.

Significant variation in amniotic fluid volume during the last third of gestation also was evident in Meriones ($F_{6,23} = 7.0, p < .001$; Table 3.2). However, post-hoc examination indicated that this variation was entirely attributable to a sudden decrease in fluid volume on the last day of gestation. No significant fluctuation in fluid volume occurred over the period of days 18-23.

In Sigmodon, amniotic fluid was already near its peak volume at the beginning of the fetal period (day 17; Table 3.3). The significant change in fluid volume ($F_{10,45} = 10.8$, $p < .001$) was found to be due to a steady decrease after day 23. At term (day 27), amniotic fluid volume was only 16% of the peak on day 18.

Amniotic fluid was sampled at five ages in Acomys: days 22, 26, 30, 34, and 37 (Table 3.4). Over this period, fluid appeared to vary in volume, although the effect of age was marginally significant ($F_{4,10} = 3.8$, $p = .04$). Post-hoc tests found a significant difference only between the peak of fluid volume on day 30 and its nadir (32% of peak volume) on day 37.

Preliminary efforts to compare changes in amniotic fluid volume were complicated by differences in absolute gestational age among the four species studied. Depicting age-dependent changes is a general problem in the comparative study of growth processes, solutions for which have been approached in various qualitative [e.g., Gould 1977] or quantitative [e.g., Eisenberg 1981] ways. Most commonly, corresponding points during development have been set as equal, such as the time of conception or the time of birth. Because the focus in the present study is development during the fetal period, ranging from the inception of movement through term, an alternative method was adopted for the purpose of graphical presentation. For each species, actual gestational age was converted to a

fraction of the fetal period, referred to as relative fetal age, ranging from 0 (inception) to 1.0 (term). Changes in a dependent variable were then plotted against this common age scale. (This method of compensating for variation in gestation length also will be important for the discussion in Chapter 10.)

Absolute differences in amniotic fluid volume also were apparent across species. At the inception of movement, fluid volume was greatest in Sigmodon (.74 ml) and least in Meriones (.24 ml). To compensate for absolute differences, amniotic fluid volume measured at each age was expressed as a percentage of the maximum mean volume of amniotic fluid for each species. The resultant plot of changes in relative amniotic fluid volume as a function of relative fetal age is presented in Figure 3.1. Inspection of this graph indicates that despite differences in gestation length and absolute size, changes in amniotic fluid volume were quite similar across species. In all four species, fluid volume remained roughly stable until halfway through the fetal period, when fluid began to disappear sharply. At term, fluid volume ranged from 16% (Sigmodon) to 32% (Acomys) of maximum.

F. Free Space In Utero

The principal physical components of the fetal environment discussed above, namely fetal mass, placental mass and amniotic fluid volume, all undergo change during

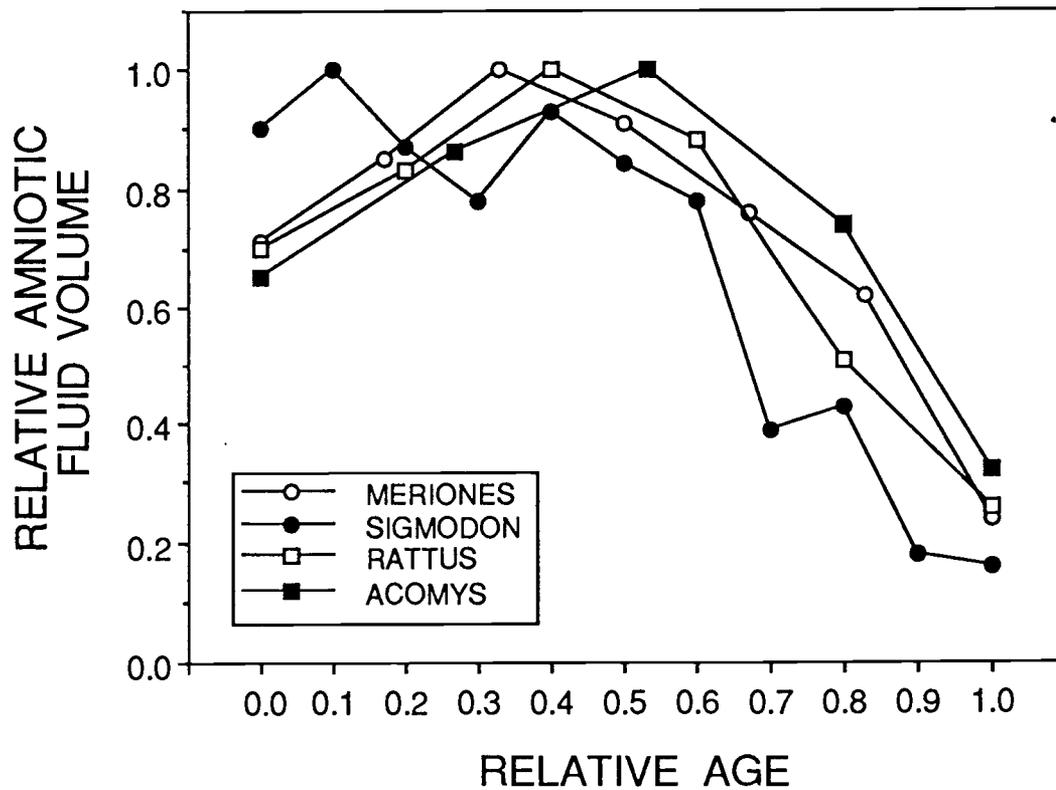


Figure 3.1. Changes in relative amniotic fluid volume during late gestation in four Murid species. Fluid volume is portrayed on the ordinate as the observed mean volume for a particular age divided by the maximum mean volume observed between the inception of movement and term. Relative age is expressed as a fraction of the fetal period for each species, with the inception of movement equal to 0.0 and term equal to 1.0. See text for further discussion.

late gestation. Because fetal growth is approximately exponential, in contrast to the linear growth of the placenta (or lack of growth in Meriones), the fetus constitutes an expanding fraction of the whole conceptus as gestation proceeds. If amniotic fluid is also considered, this trend is accentuated owing to the decrease in fluid volume evident in all four species near term. These conflicting patterns of growth could have important consequences for fetal movement through the relative decrease in free space surrounding the fetus in which movement may occur. To measure the combined effects of fetal, placental and amniotic fluid growth or diminution, an index of free space surrounding the fetus was calculated. Free space was defined as the volume of amniotic fluid divided by the sum of the volumes of amniotic fluid, placenta and fetus. It was assumed for simplicity of calculation that the specific gravities of amniotic fluid, placenta and fetus are equal, which is within the accuracy of mass measurements employed in this study.

This index of free space varied significantly with gestational age in Rattus ($F_{5,22} = 176.0, p < .001$). Free space diminished monotonically over the period from a maximum of 31% on day 16 to a minimum of 3% on day 21. The rate of decline was greatest from day 19 through term.

Free space also decreased steadily from day 18 through term in Meriones ($F_{6,23} = 59.7, p < .001$). This trend occurred in spite of the relative stability of placenta mass

and amniotic fluid volume, indicating that the major component of the index is increasing fetal mass, accentuated by disappearing amniotic fluid near term. Free space declined from a maximum of 26% to a minimum of 3% at term.

In Sigmodon, free space exhibited a significant decline from 46% on day 17 to 2% on days 26 and 27 ($F_{10,45} = 73.0$, $p < .001$). The sharpest relative decrease occurred between day 23 and day 24.

The same trend of diminishing free space was evident among the five calculable ages in Acomys ($F_{4,10} = 32.5$, $p < .001$). Free space was greatest (32%) on day 22 and least (3%) on day 37.

Changes in free space in all four species as a function of relative fetal age are depicted in Figure 3.2. Although Sigmodon began the fetal period with a higher ratio of amniotic fluid to total conceptus volume, free space was essentially equal across species during the last two-thirds of the fetal period.

G. Litter Size and Distribution

Litter size differed among the four species and varied among pregnancies within species. Table 3.5 summarizes litter size characteristics for each of the species studied. Rattus clearly had the largest average litter size and Acomys the smallest.

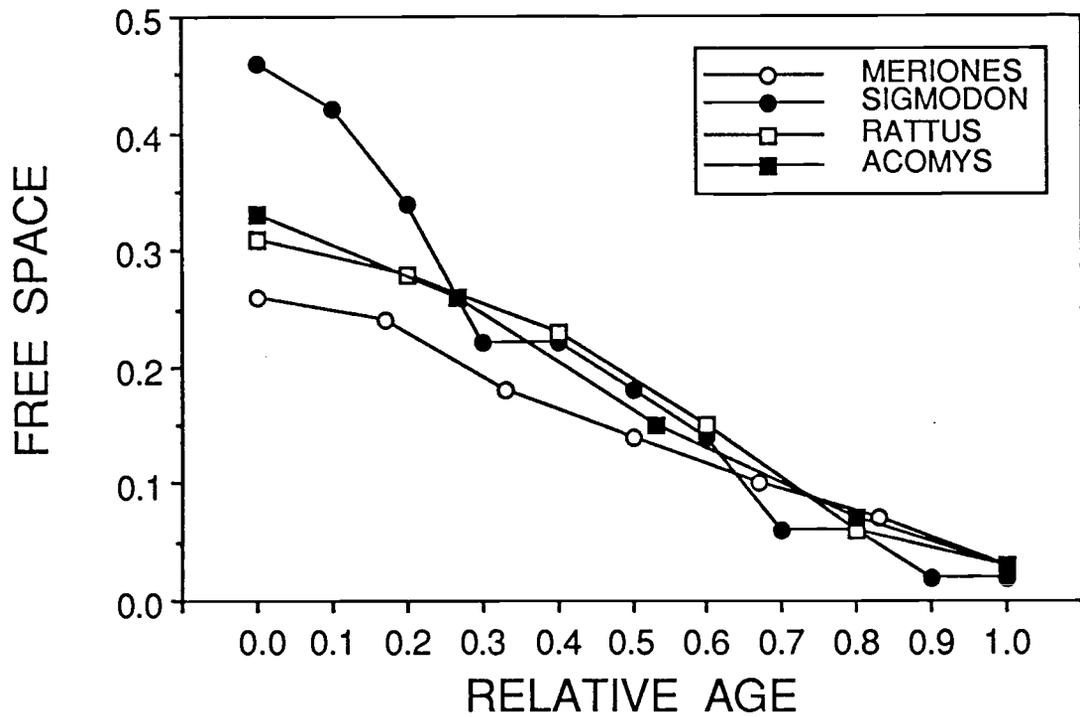


Figure 3.2. Changes in an index of intrauterine free space during late gestation in four Murid species. Free space is calculated as the volume of amniotic fluid divided by the sum of the volumes of the fetus, placenta and amniotic fluid. Gestational age for each species is plotted on a relative scale as in Figure 3.1.

Table 3.5
Litter size for four Murid species

SPECIES	MEAN	SEM	RANGE	N
RATTUS	13.3	0.5	6-17	30
MERIONES	7.2	0.3	4-9	35
SIGMODON	6.1	0.2	1-11	93
ACOMYS	2.0	0.1	1-4	34

It is likely that the problem of intrauterine restraint brought about through diminishing free space during gestation is compounded by variation in litter size [Barr, Jensh & Brent 1970]. Between pregnancies, the number of fetuses varied over a 2- to 11-fold range. Perhaps more important is variation in uterine density within a pregnancy. In Muroid rodents, fetuses reside in a duplex uterus. The number of fetuses in each horn of the uterus appears to vary independently [Smotherman & Robinson 1988a]. Table 3.6 summarizes variation in litter size between uterine horns. As may be seen in this table, the majority of litters in all four species comprise fetuses distributed unequally between the two uterine horns. The discrepancy, as expected, is greater in Rattus, which also has a larger average litter size. Curiously, Acomys also exhibits a marked discrepancy between horns in spite of the small litter size.

H. Discussion

The various measures of growth presented in this chapter confirm that physical parameters of the fetal environment undergo substantial change during the last days of gestation in both altricial and precocial species. The combined effect of these changes necessarily is the reduction of free space within the uterus and the consequent induction of physical restraint of fetal movement. If

Table 3.6

Discrepancy in number of fetuses between uterine horns

SPECIES	MEAN	SEM	RANGE	% > 1	N
RATTUS	3.9	0.6	0-11	67	30
MERIONES	2.0	0.3	0-5	51	35
SIGMODON	1.9	0.1	0-6	53	93
ACOMYS	1.3	0.1	1-4	50	34

fetuses are responsive to their surrounding conditions, their behavior should be modified by the changing physical environment in utero. This general hypothesis will imply numerous specific predictions in following chapters as the behavior of fetuses observed in utero and ex utero is characterized and compared.

CHAPTER 4. GENERAL DESCRIPTION OF FETAL ACTIVITY

A. Introduction

Because fetal movements were defined (and perceived) as discrete events, simple counts of the frequency of events in various movement categories provide the most basic level of description of fetal behavior.

B. Fetal Movement Components

Five body regions were distinguished for the purpose of quantifying fetal movement: forelimbs, rearlimbs, head, trunk and mouth. Each was analyzed in a two-factor ANOVA to assess the main effects of gestational Age and Condition of observation and their interaction. The level of significance was established at $p < .01$, although I will also report the results of tests that yielded marginally significant results ($.01 < p < .05$). For each category of behavior, an additional one-factor ANOVA was performed to incorporate in utero data from the inception of movement (the earliest age tested). In the following descriptions, effects that are not explicitly mentioned were not significant.

1. Forelimbs

Forelimb activity was the most consistently abundant form of fetal movement observed in all four species. Early forelimb movements were almost invariably unidirectional in form, consisting of simple flexion in a single joint (shoulder or elbow). With advancing age, the diversity of forelimb movements expanded, including relatively coordinated actions involving both limbs and multiple joints in shifting vectors of limb motion. Forelimb activity was observed at the inception of movement and was present through term in all individual fetuses studied.

In Rattus, movements involving the forelimb region were the most prevalent kind of fetal behavior at all ages, ranging from 70% of all events on day 16 to 54% on day 20. Forelimb activity subjectively appeared to increase to an early peak followed by a slight decline in frequency (Figure 4.1), but the overall Condition by Age ANOVA revealed no significant change in the abundance of forelimb movements during the period of days 17-21. A secondary analysis of in utero data incorporating observations conducted on day 16 also found no significant variation in forelimb activity with age. The main effect of Condition was significant ($F_{1,40} = 29.1, p < .001$), indicating that fetuses observed ex utero performed forelimb movements more frequently than fetuses in utero (Table 4.1).

Forelimb movements also were relatively abundant in

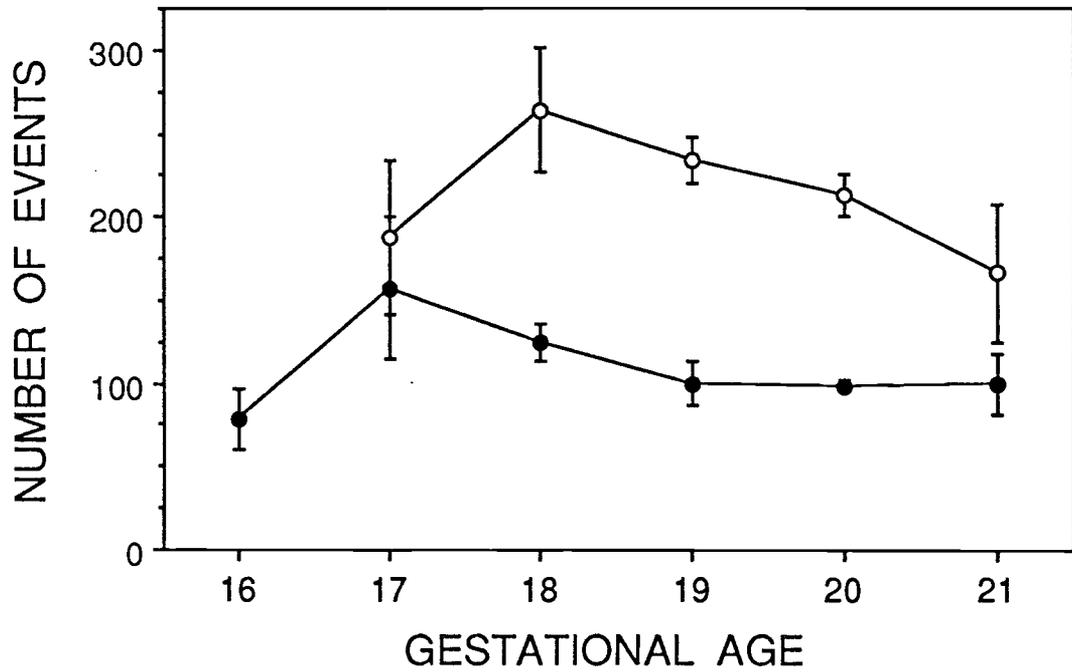


Figure 4.1. Incidence of forelimb movement during gestation in *Rattus*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

Table 4.1

Summary of post-hoc comparisons for Rattus. Comparison of group means (Newman-Keuls) followed significant main effects ($p < .01$), or simple effects where the interaction was evident. Direction of significant difference between Conditions or Ages is indicated by '<' or '>', non-significance by '='. Ages are presented in an ordered sequence; ages not appearing in the sequence did not differ from adjacent ages.

BEHAVIOR	INTERACTION (A X C)			
	MAIN EFFECTS		SIMPLE EFFECTS	
	AGE	CONDITION	AGE	CONDITION
Forelimb		In < Ex		
Rearlimb			Ex: 17<18=21	18: In < Ex 19: In < Ex 20: In < Ex 21: In < Ex
Head		In < Ex		
Trunk Curl			In: 16>17>19=21 Ex: 17>18=21	17: In < Ex 18: In < Ex 19: In < Ex 20: In < Ex 21: In < Ex
Mouth		In < Ex		

(table continued)

Table 4.1 (continued)

BEHAVIOR	MAIN EFFECTS		INTERACTION (A X C)	
			SIMPLE EFFECTS	
	AGE	CONDITION	AGE	CONDITION
Event Activity		In < Ex		
Component Activity		In < Ex		
Entropy			In: 16<17=20>21	17: In < Ex
			Ex: 17<18<19=21	18: In < Ex
				19: In < Ex
				20: In < Ex
				21: In < Ex

Meriones, accounting for over 60% of all fetal movements from day 19 through term. An age effect was not evident in the overall ANOVA nor in a secondary analysis including day 18 in utero. The effect of Condition was significant ($F_{1,48} = 40.3, p < .001$; Figure 4.2); more forelimb activity was expressed ex utero than in utero (Table 4.2).

The absolute frequency of forelimb movements varied considerably during gestation in Sigmodon, although the relative abundance remained quite stable at about 60% of all events. Post-hoc tests following the significant main effect of Age ($F_{9,95} = 4.26, p < .001$) indicated that forelimb activity increased from days 19 to 21, remained at a plateau through day 25, then decreased until day 27 (Table 4.3). Although the interaction was not significant, the trend was most noticeable among fetuses observed ex utero (Figure 4.3). The expression of forelimb movements on day 18 in utero did not differ from day 19. A significant main effect of Condition also was apparent, with more forelimb activity occurring ex utero than in utero.

The interaction of Age by Condition was significant in Acomys ($F_{5,49} = 4.0, p = .008$). In utero, forelimb movements varied significantly with age, nearly quadrupling in frequency from day 22 to day 26 and declining again after day 32. A significant simple effect of Age also was evident ex utero, with a sharp rise to a peak on day 28 followed by a decrease in frequency through term (Figure 4.4). Forelimb movements were more abundant ex utero than in utero at all

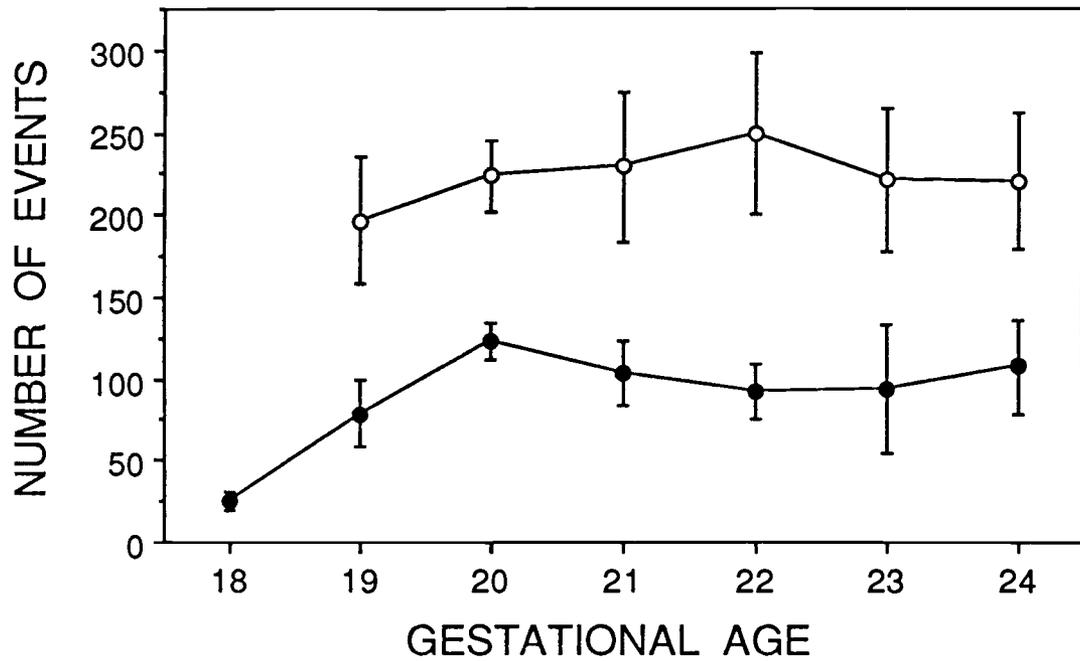


Figure 4.2. Incidence of forelimb movement during gestation in Meriones. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

Table 4.2

Summary of post-hoc comparisons for Meriones. Conventions as in Table 4.1.

BEHAVIOR	MAIN EFFECTS		INTERACTION (A X C)	
			SIMPLE EFFECTS	
	AGE	CONDITION	AGE	CONDITION
Forelimb		In < Ex		
Rearlimb		In < Ex		
Head		In < Ex		
Trunk Curl		In < Ex		
Mouth		In < Ex		
Event Activity		In < Ex		
Component Activity		In < Ex		
Entropy		In < Ex		

Table 4.3

Summary of post-hoc comparisons for Sigmodon. Conventions as in Table 4.1.

BEHAVIOR	MAIN EFFECTS		INTERACTION (A X C)	
			SIMPLE EFFECTS	
	AGE	CONDITION	AGE	CONDITION
Forelimb	18=19<21=25>27	In < Ex		
Rearlimb	18=19<21=25>26=27	In < Ex		
Head	18=19<21=25>27	In < Ex		
Trunk Curl		In < Ex		
Mouth			In: 17=24<25>26=27	19: In < Ex
			Ex: 18=23<24>25=27	20: In < Ex
				21: In < Ex
				22: In < Ex
				24: In < Ex
Event Activity	18=19<21=25>27	In < Ex		
Component Act.	18=19<21=25>27	In < Ex		
Entropy	18=19<25=27	In < Ex		

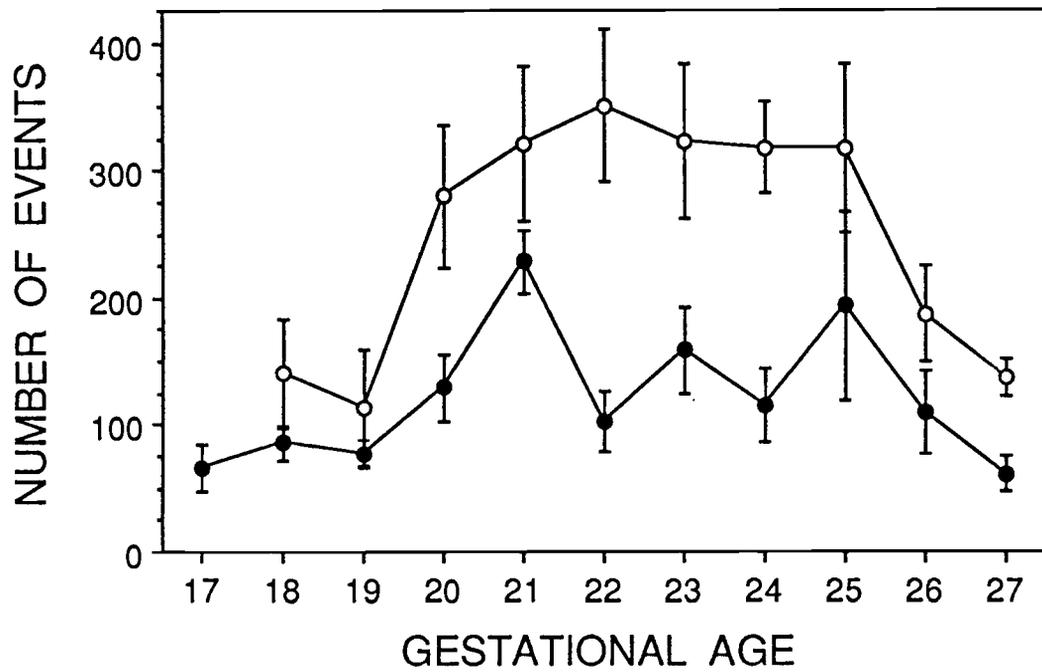


Figure 4.3. Incidence of forelimb movement during gestation in Sigmodon. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

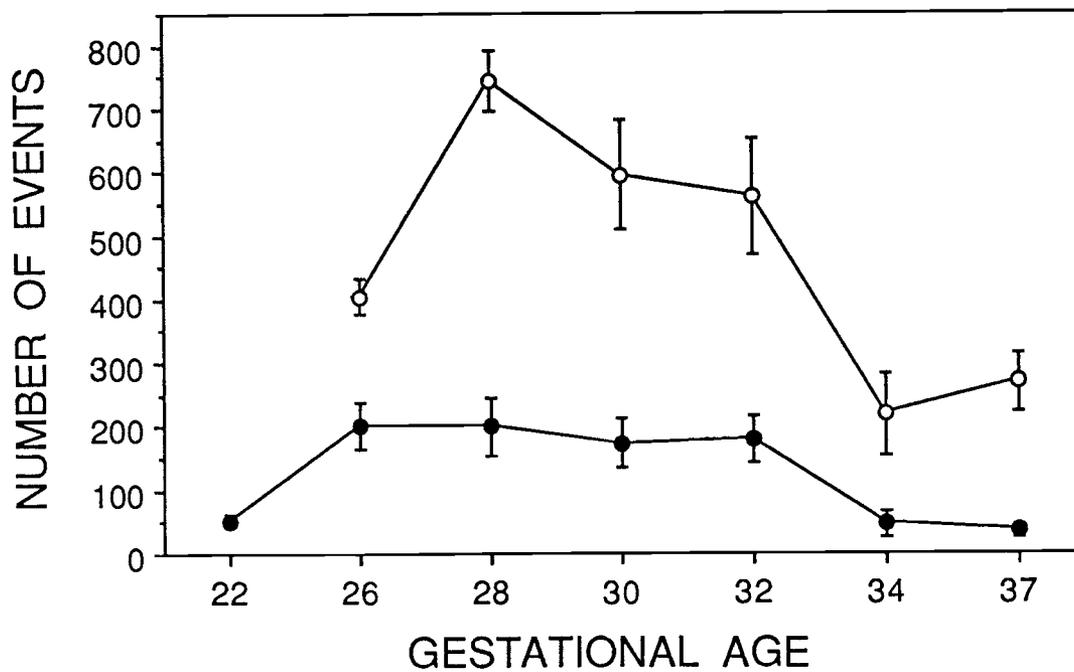


Figure 4.4. Incidence of forelimb movement during gestation in *Acomys*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

ages (Table 4.4). Overall, forelimb activity accounted for 50% (day 34) to 81% (day 22) of all fetal movements.

2. Rearlimbs

Rearlimb movements were less diverse than forelimb. Most events consisted of simple extension of one or both legs, generally directed caudally. Movement was observed to occur at pelvis, knee and ankle. Among older fetuses motion by both rearlimbs occasionally resembled alternated stepping movements.

Movements by one or both rearlimbs constituted an important but variable element of the fetal repertoire in Rattus, ranging from 5% (day 16) to 44% (day 21 ex utero) of all fetal activity. A significant interaction between Age and Condition was found ($F_{4,40} = 5.2, p=.008$). In utero, rearlimb movements were virtually non-existent on day 16 and exhibited little change in occurrence over the next five days of gestation (Figure 4.5). But ex utero rearlimb movements increased steadily in frequency through term; post-hoc tests indicated that significantly less rearlimb activity occurred on day 17 than on all subsequent ages. The simple effect of condition also was dependent upon age, with more events occurring ex utero than in utero on days 18-21.

The pattern of change of rearlimb activity appeared virtually identical in Meriones, with rearlimb movements

Table 4.4

Summary of post-hoc comparisons for Acomys. Conventions as in Table 4.1.

BEHAVIOR	INTERACTION (A X C)			
	MAIN EFFECTS		SIMPLE EFFECTS	
	AGE	CONDITION	AGE	CONDITION
Forelimb			In: 22<26=32>34=37	26: In < Ex
			Ex: 26<28=32>34=37	28: In < Ex
				30: In < Ex
				32: In < Ex
				34: In < Ex
				37: In < Ex
Rearlimb			In: 22<28=37	26: In < Ex
			Ex: 26<28=32>34=37	28: In < Ex
				30: In < Ex
				32: In < Ex
				34: In < Ex
Head				37: In < Ex
			In < Ex	

(table continued)

Table 4.4 (continued)

BEHAVIOR	MAIN EFFECTS		INTERACTION (A X C)	
			SIMPLE EFFECTS	
	AGE	CONDITION	AGE	CONDITION
Trunk Curl			In: 22>26=37	26: In < Ex
			Ex: 26<28>30=34>37	28: In < Ex
				30: In < Ex
				32: In < Ex
				34: In < Ex
				37: In < Ex
Mouth		In < Ex		
Event Activity	26<28=32>34=37	In < Ex		
Component Act.			In: 22<26=32>34=37	26: In < Ex
			Ex: 26<28=32>34=37	28: In < Ex
				30: In < Ex
				32: In < Ex
				34: In < Ex
				37: In < Ex
Entropy		In < Ex		

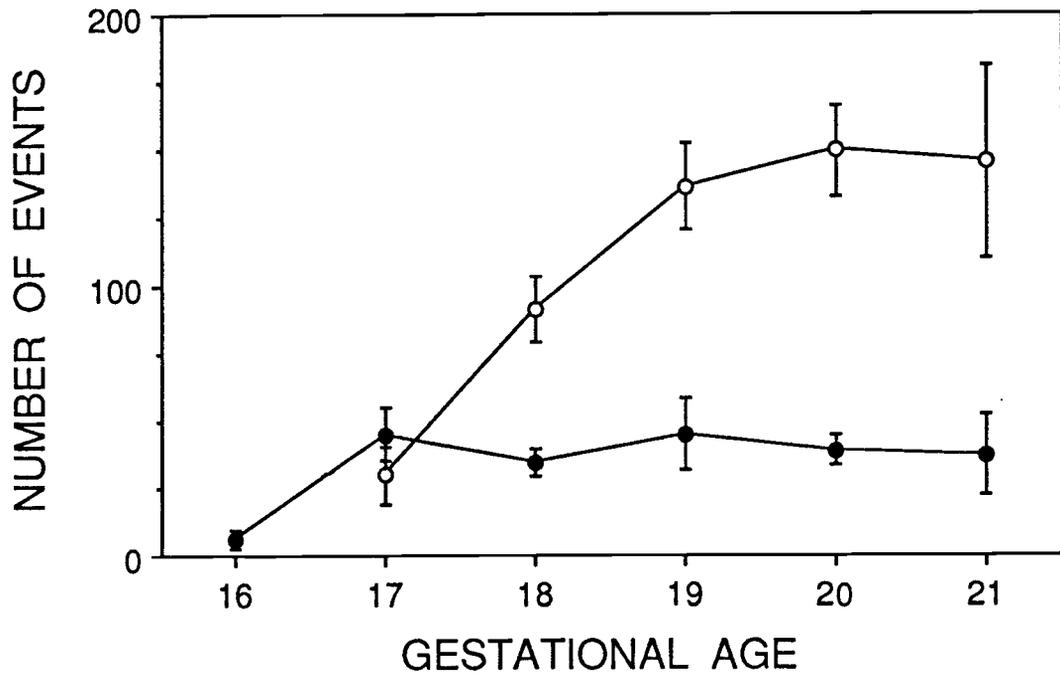


Figure 4.5. Incidence of rearlimb movement during gestation in *Rattus*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

varying from 9% (day 19 ex utero) to 45% (day 24 ex utero) of overall activity (Figure 4.6). A significant main effect of Condition was indeed evident ($F_{1,48} = 47.8, p < .001$) with fewer rearlimb movements in utero than ex utero (Table 4.2). However, greater variability between subjects was probably responsible for a nonsignificant interaction and marginally significant main effect of Age ($F_{5,48} = 2.5, p = .041$).

The expression of rearlimb movements in Sigmodon appeared strongly modal. Significant main effects of Age ($F_{9,95} = 5.6, p < .001$) and Condition ($F_{1,95} = 55.9, p < .001$) were found. Post-hoc tests indicated that rearlimb activity remained at a low level through day 19, increased significantly until day 21, remained at this higher level of incidence until day 25, then decreased through term (Table 4.3). Examination of Figure 4.7 suggests that the expression of rearlimb movements ex utero was primarily responsible for the age effect, although the interaction of Age by Condition was marginally significant ($F_{9,95} = 2.29, p = .022$). More events occurred ex utero than in utero. Overall, rearlimb movements constituted 18% (day 18) to 35% (day 22) of fetal activity, although the representation of rearlimb differed considerably at term (6% in utero versus 35% ex utero).

In Acomys, rearlimb movements were a more consistent element of the repertoire, appearing in 30% (day 26) to 41% (day 37) of behavioral events. The absolute frequency of rearlimb movements varied widely with both age and condition

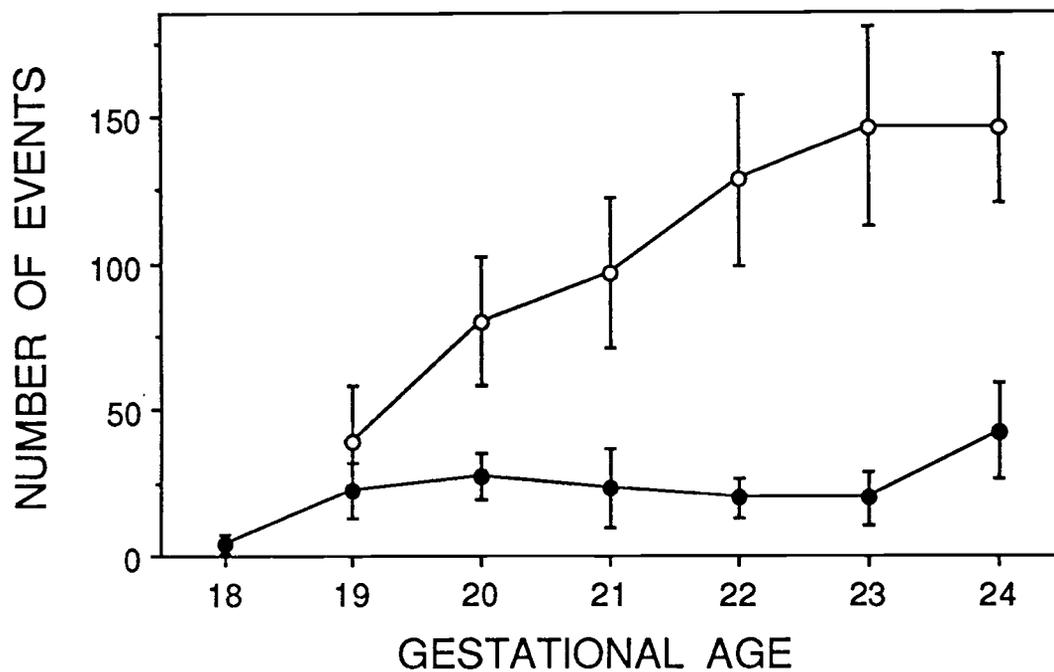


Figure 4.6. Incidence of rearlimb movement during gestation in *Meriones*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

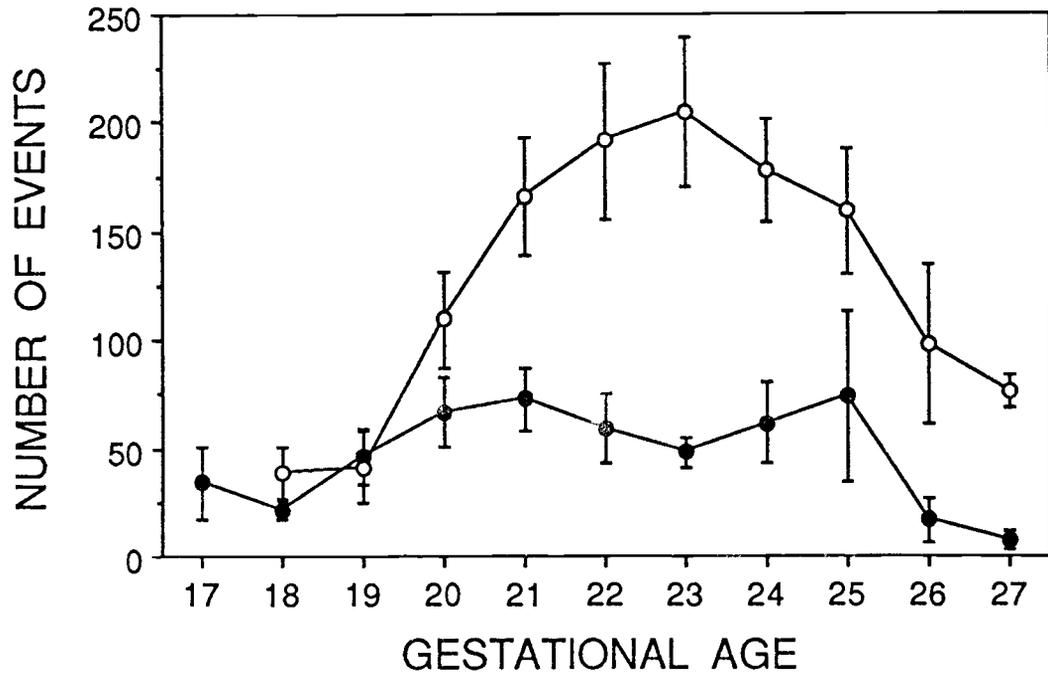


Figure 4.7. Incidence of rearlimb movement during gestation in Sigmodon. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

(Figure 4.8), as indicated by the significant interaction ($F_{5,49} = 4.8, p=.002$). In utero, rearlimbs were moved less often on day 22 than all subsequent ages. Ex utero, rearlimb activity increased sharply to a peak on day 28, remained at about this level through day 32, then declined to days 34-37 (Table 4.4). On all days tested, fewer events occurred in utero than ex utero.

3. Head

Head movements consisted of dorsal, lateral, or ventral flexion of the neck or, less commonly, rotation of the head relative to the trunk. Dorsiflexion, resulting in rostral extension of the nose and mouth, was the most common form of head movement, particularly during the first few days after inception of movement.

The incidence of head movement followed an ontogenetic trajectory quite similar to that of forelimb movement in Rattus (Figure 4.9). No significant variation with age was detected in the overall ANOVA, but the main effect of Condition was significant ($F_{1,40} = 96.1, p<.001$). Head movements were more than four-times as common ex utero as in utero and constituted 41% of all activity ex utero compared to 24% in utero.

Head activity also failed to change with age in Meriones (Figure 4.10). Overall, head movements accounted for 19% of all events in utero and 31% ex utero. The

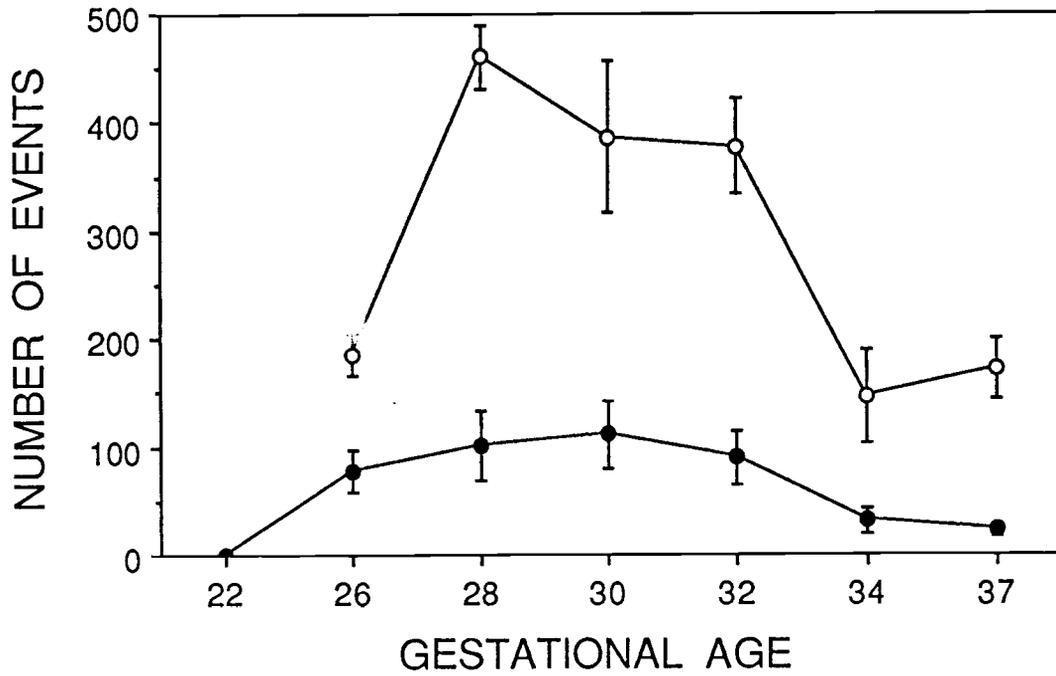


Figure 4.8. Incidence of rearlimb movement during gestation in *Acomys*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

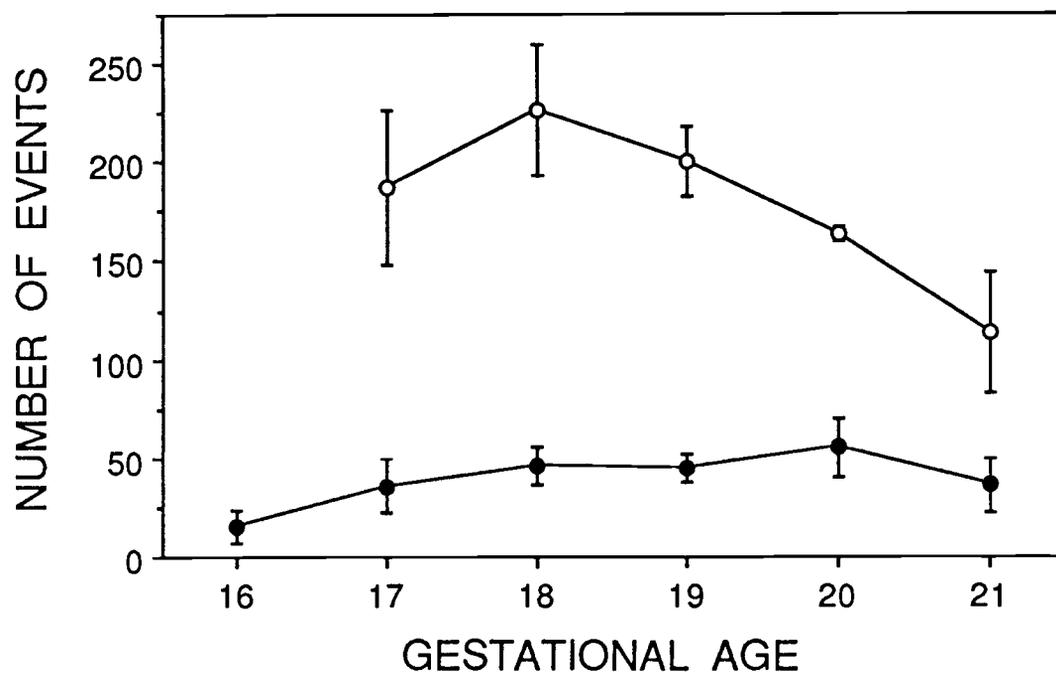


Figure 4.9. Incidence of head movement during gestation in *Rattus*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

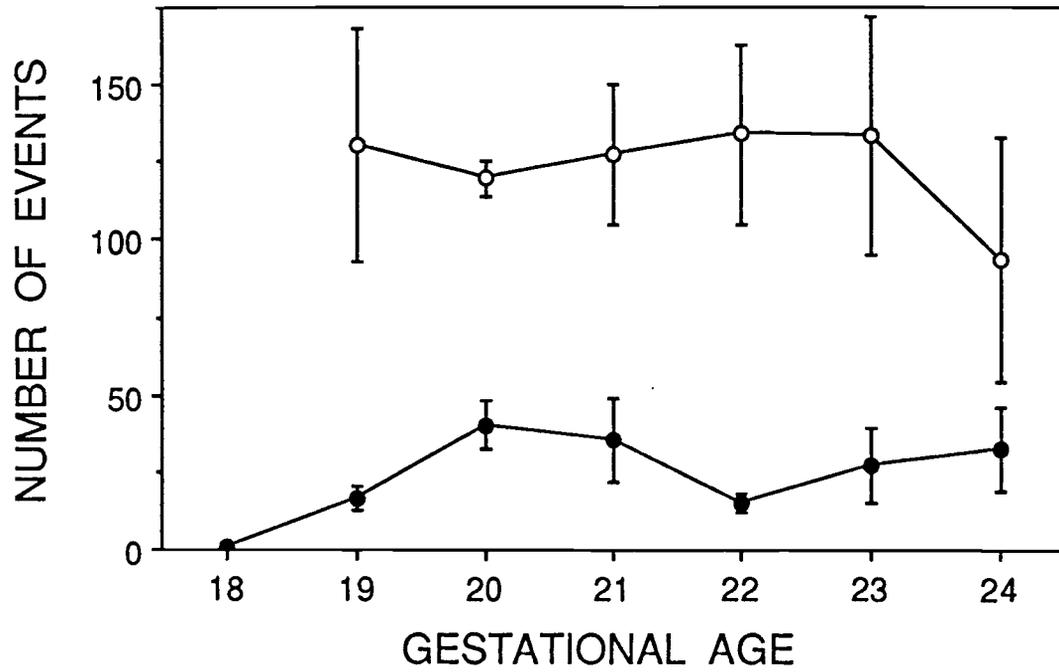


Figure 4.10. Incidence of head movement during gestation in *Meriones*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

absolute frequency of head movement differed between Conditions ($F_{1,48} = 50.4, p < .001$) with a four-fold increase ex utero relative to in utero.

In Sigmodon, a significant main effect of Age was evident ($F_{9,95} = 2.71, p = .004$). As is illustrated in Figure 4.11, the pattern of gestational change in head movements was nonlinear. Post-hoc analyses indicated that head activity remained at fairly low levels through day 19, occurred at an elevated rate over days 21-25, then dropped to a lower incidence by day 27 (Table 4.3). This pattern seemed more evident ex utero (Figure 4.11), but the interaction of Age by Condition was not significant. The main effect of Condition was also significant ($F_{1,95} = 19.9, p < .001$), with head movements occurring more frequently ex utero than in utero. Secondary analysis of in utero data indicated that head movements were less common at the inception of movement (day 17) than one day later. Head movement occurred in about 25% of all events, both in utero and ex utero.

Ontogenetic change in head activity was less evident in Acomys. The incidence of head movement remained nearly constant in utero, and the apparent peak of activity on day 28 ex utero did not differ significantly from other ages (Figure 4.12). However, the main effect of Condition was significant ($F_{1,49} = 124.2, p < .001$), with more than a seven-fold difference in activity between fetuses in utero and ex utero (Table 4.4). Head movements ranged from 18% of all

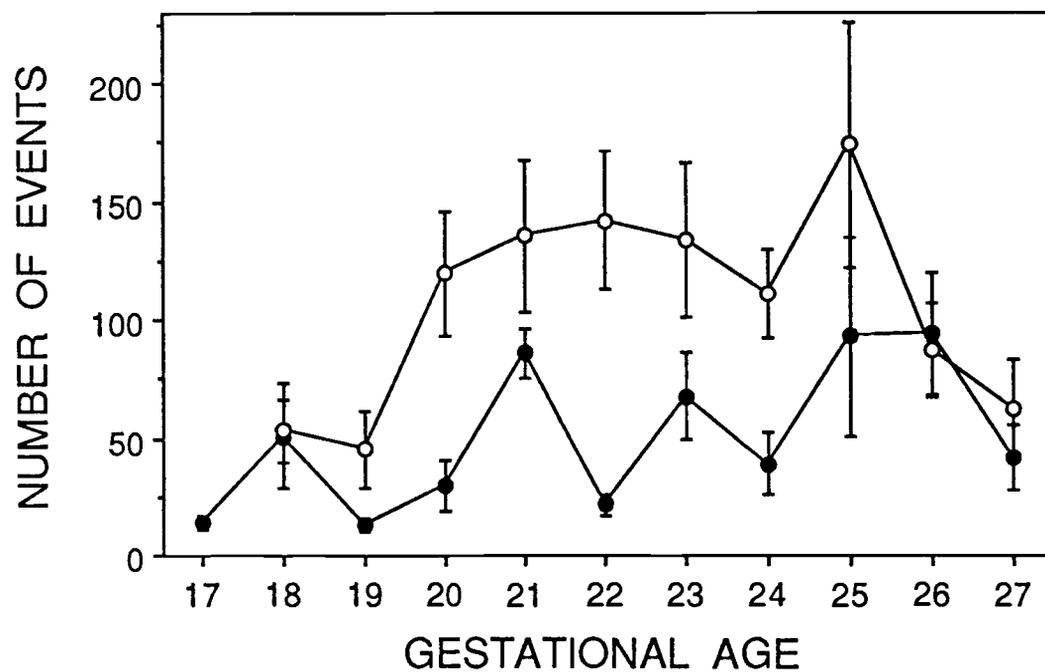


Figure 4.11. Incidence of head movement during gestation in Sigmodon. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

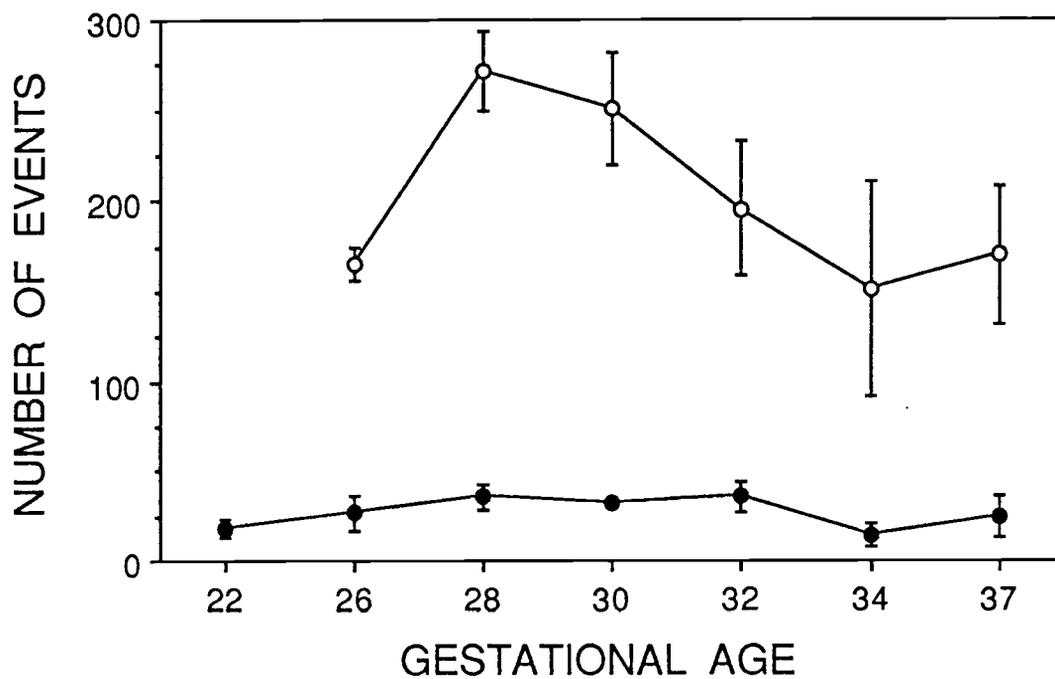


Figure 4.12. Incidence of head movement during gestation in *Acomys*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

events on day 26 to 36% on day 37 and were relatively as well as absolutely more abundant ex utero than in utero.

4. Trunk

Two general categories of trunk movement were distinguished: curl and stretch. Stretches involved dorsal flexion of the trunk, resulting in an apparent straightening of the fetus from its slightly C-shaped resting posture. Other forms of trunk flexion, including ventral "tucking", lateral "bending" and rotary "twisting" movements were classified as curls. Lateral curling movements have been described as the earliest motor elements exhibited by vertebrate embryos, and indeed were exhibited by fetuses of all four species on the day of movement inception. Twisting motions and other complex trunk movements tended to occur later in gestation.

In Rattus, curls varied widely in relative abundance, ranging from 15% of all events on day 16 to only 2% on day 21. The overall ANOVA revealed a significant interaction between Age and Condition ($F_{4,40} = 5.36, p=.005$). Post-hoc comparisons indicated that fewer curls occurred in utero than ex utero from day 17 through term (Table 4.1). In utero, curls were most abundant on day 16, declined steadily until day 19, and continued to occur at low frequency through term. Ex utero, curls were much more abundant on day 17 than all subsequent ages (Figure 4.13).

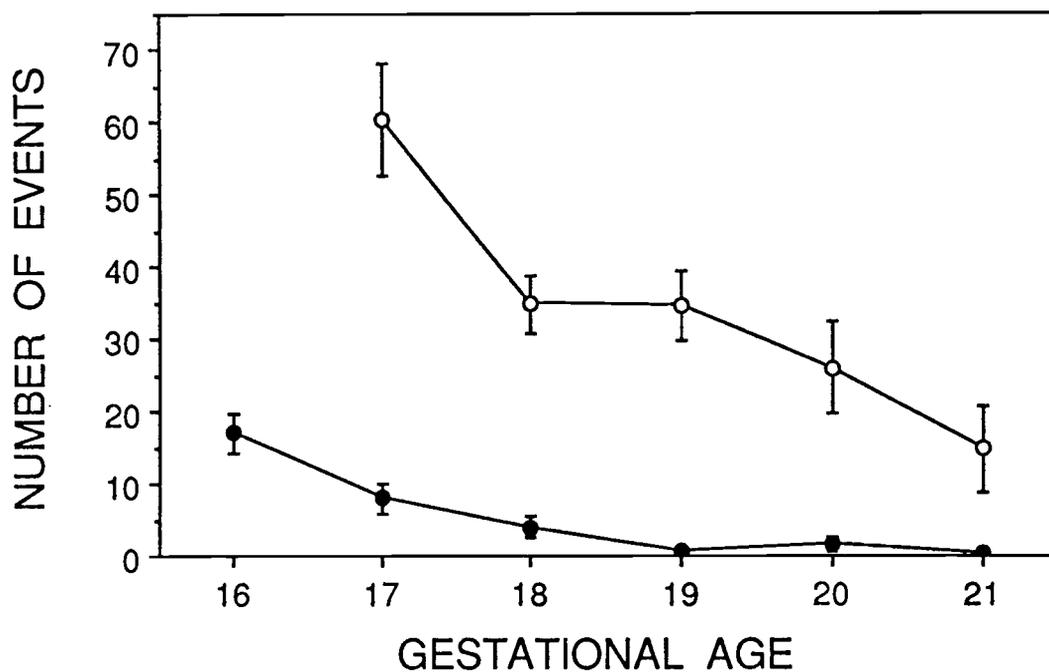


Figure 4.13. Incidence of trunk curl movement during gestation in *Rattus*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

Age-related changes in the occurrence of curls were less well-defined in Meriones. The main effect of Age was marginally significant ($F_{5,48} = 3.2, p=.014$). This effect was due almost entirely to the elevated incidence of curls on day 19 (Figure 4.14). An analysis to assess simple effects in utero, which incorporated data from day 16, indicated that curls occurred more frequently at the inception of movement than at subsequent ages. A significant main effect of Condition also was evident ($F_{1,48} = 42.7, p<.001$), with more curls occurring ex utero than in utero. Curls were relatively more common early (34% on day 18; 8% on day 19) than later in gestation (only 1% by day 20).

Curl activity was relatively uncommon in Sigmodon, amounting to no more than 8% of all events (on day 17). No significant variation with age was detected in the overall ANOVA, but the main effect of Condition was significant ($F_{1,95} = 44.4, p<.001$). More curls occurred ex utero than in utero (Figure 4.15).

Curls also were relatively rare in Acomys, occurring in 13% of events on day 22 but no more than 3% of events thereafter. The interaction of Age by Condition was significant ($F_{1,49} = 131.4, p<.001$). Post-hoc comparisons revealed that more curls occurred on day 22 than all other ages in utero (Table 4.4). Ex utero, the incidence of curl movements increased significantly from day 26 to day 28, declined to an intermediate level across days 30-34, then

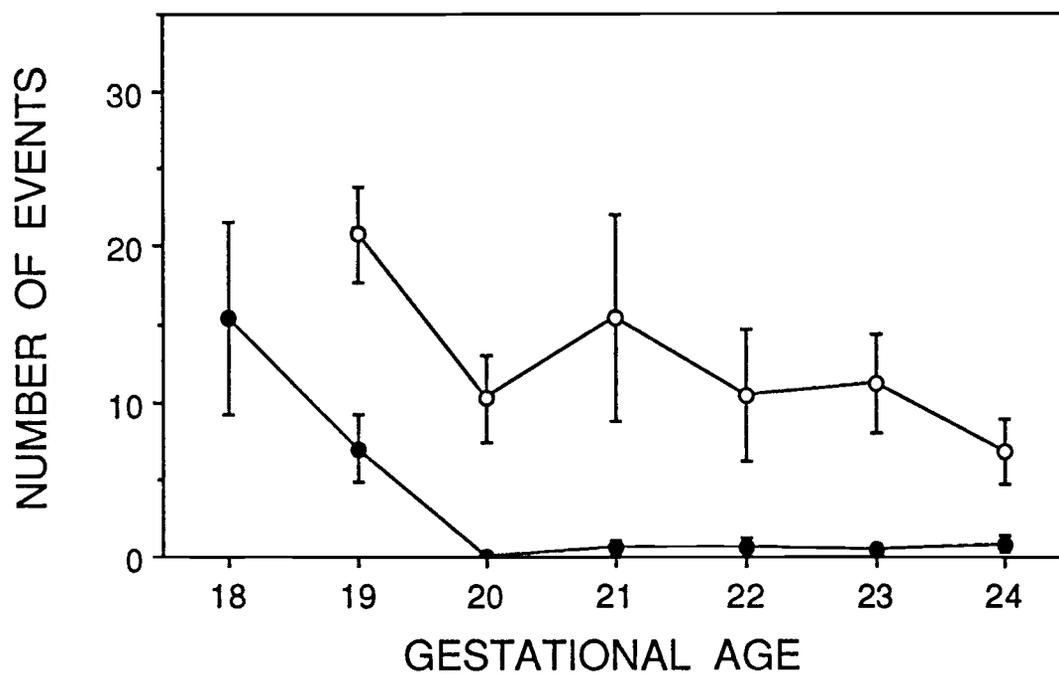


Figure 4.14. Incidence of trunk curl movement during gestation in Meriones. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

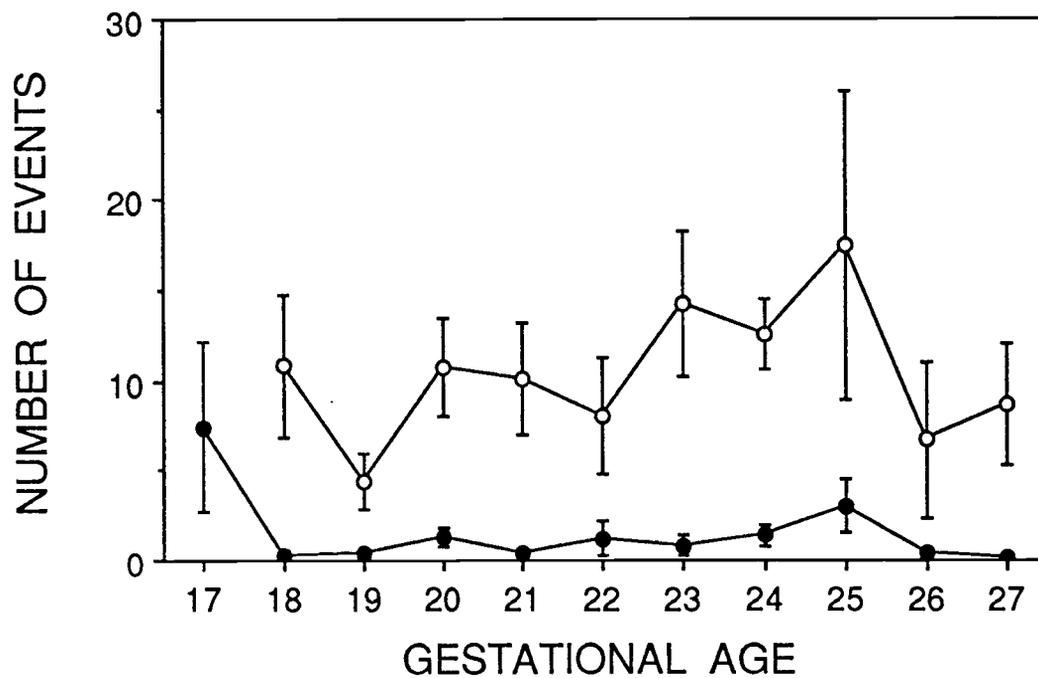


Figure 4.15. Incidence of trunk curl movement during gestation in Sigmodon. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

diminished at term (Figure 4.16). More curls occurred ex utero than in utero at all ages from day 26 through term.

Stretch movements of the trunk were rare in all four species and were not subjected to ANOVA. In Rattus, no stretches were observed at all, either in utero or ex utero. Stretches were nearly as rare in Meriones, being expressed by none of the fetuses in utero and only three fetuses (10%) ex utero. Stretching movements were more abundant in the two precocial species. In Sigmodon, two fetuses (3%) in utero and 23 fetuses (38%) ex utero exhibited stretching; no stretches were observed before day 20. Collapsing these data across ages, more stretches were observed ex utero than in utero ($\chi^2_1 = 22.7, p < .001$). In Acomys, two fetuses (6%) in utero and 20 fetuses (65%) ex utero performed stretches, but none were observed prior to day 28. Collapsing across ages, more stretches occurred ex utero than in utero ($\chi^2_1 = 25.6, p < .001$). The occurrence of stretching movements and its distribution across gestational ages and conditions in each species is summarized in Table 4.5.

5. Mouth

Mouth movements included any instance in which the mouth was opened and closed. Early mouth movements were just that: a mechanical opening and closing, often performed in rhythmic succession within temporally delimited bouts (see Chapter 5). Among older fetuses, especially in

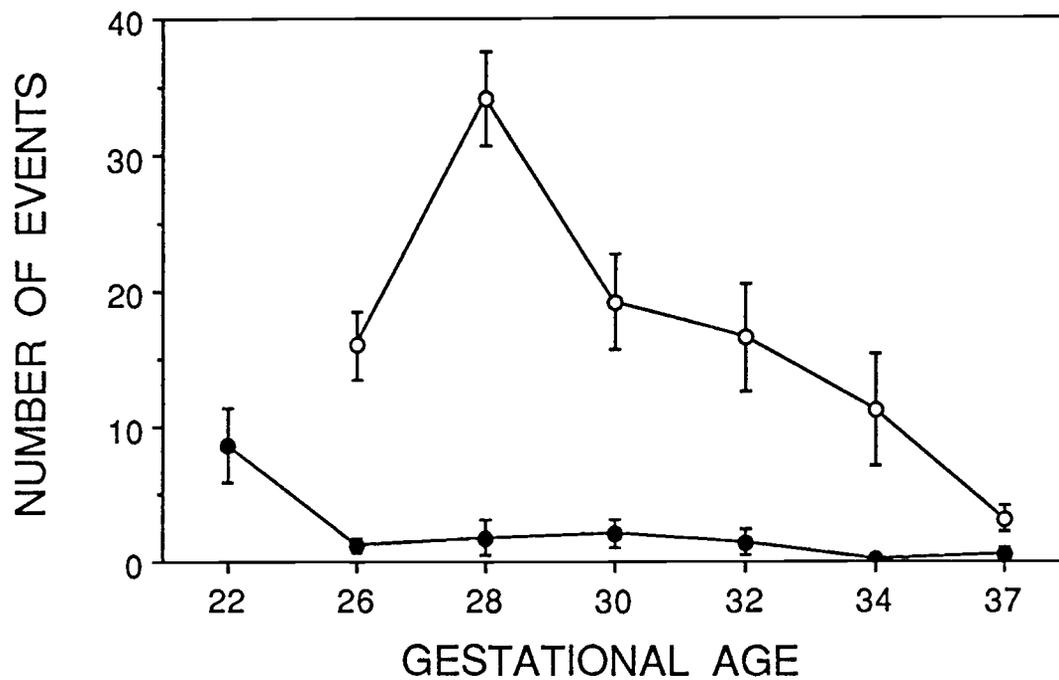


Figure 4.16. Incidence of trunk curl movement during gestation in *Acomys*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

Table 4.5

Number of subjects of various ages exhibiting Stretch movements of the trunk in four rodent species.

RATTUS	day 16	17	18	19	20	21						
		_____	_____	_____	_____	_____	_____					
in utero		0	0	0	0	0	0					
ex utero			0	0	0	0	0					
MERIONES	day 18	19	20	21	22	23	24					
		_____	_____	_____	_____	_____	_____	_____				
in utero		0	0	0	0	0	0	0				
ex utero			0	0	1	0	0	0	2			
SIGMODON	day 17	18	19	20	21	22	23	24	25	26	27	
		_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
in utero		0	0	0	0	1	0	0	0	1	0	0
ex utero			0	0	3	4	4	3	4	4	1	0
ACOMYS	day 22	26	28	30	32	34	37					
		_____	_____	_____	_____	_____	_____	_____				
in utero		0	0	0	2	0	0	0				
ex utero			0	5	5	4	2	4				

precocial species, a greater diversity of mouth movements was apparent. More complicated movements included tongue protrusion, a variety of oral-facial postures, and licking.

The frequency of mouth movements expressed by Rattus fetuses varied considerably. Visual inspection of the incidence of mouth movement suggested a peak of activity on day 19 (ex utero; Figure 4.17), but the overall ANOVA showed the main effect of Age to be only marginally significant ($F_{4,40} = 2.73, p=.042$). No significant variation was found in utero, including data from day 16. The main effect of Condition was significant ($F_{1,40} = 20.6, p<.001$). More mouth movements occurred ex utero than in utero. Mouth movements were consistently uncommon, ranging from 1% (day 17 in utero) to 8% (day 21 ex utero) of all events.

Mouth movements were similarly uncommon among Meriones fetuses, comprising 1% (in utero) to 4% (ex utero) of events (Figure 4.18). No significant variation with age was evident, but the main effect of Condition was significant ($F_{1,48} = 15.5, p<.001$). Mouthing occurred more often ex utero than in utero.

In Sigmodon, mouth movements exhibited extreme fluctuations in frequency (Figure 4.19). The relative abundance of mouth movements varied from 0% (day 19 in utero) to 17% (day 24 ex utero) of all events. A significant interaction of Age by Condition was found ($F_{9,95} = 2.77, p=.004$). Post-hoc comparisons revealed just a single age in utero and ex utero that produced the effect.

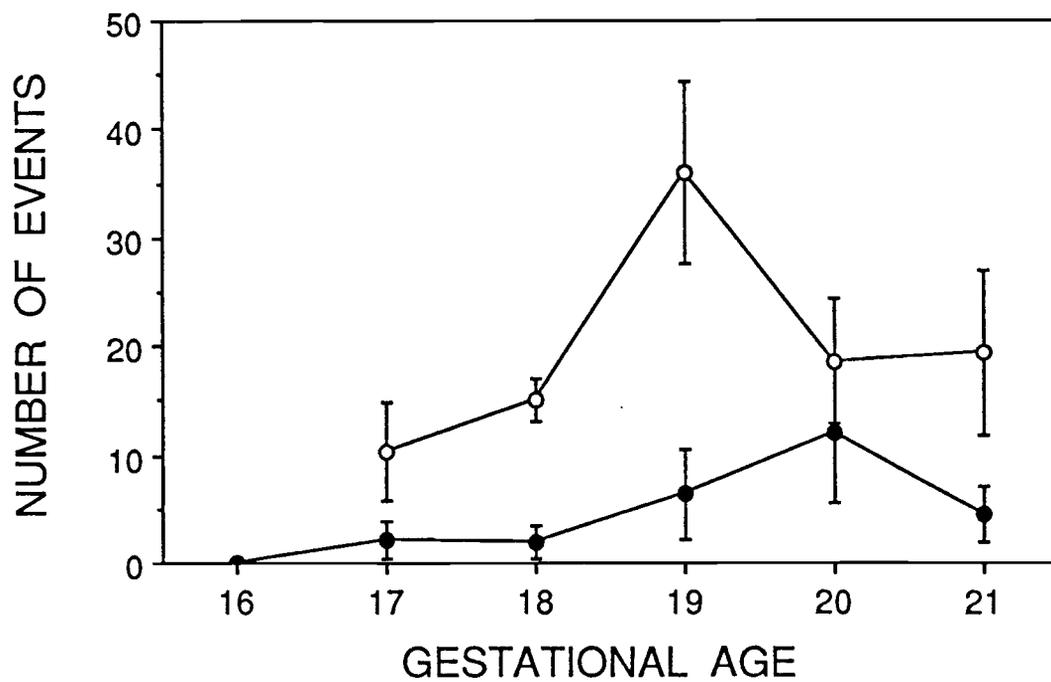


Figure 4.17. Incidence of mouth movement during gestation in *Rattus*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

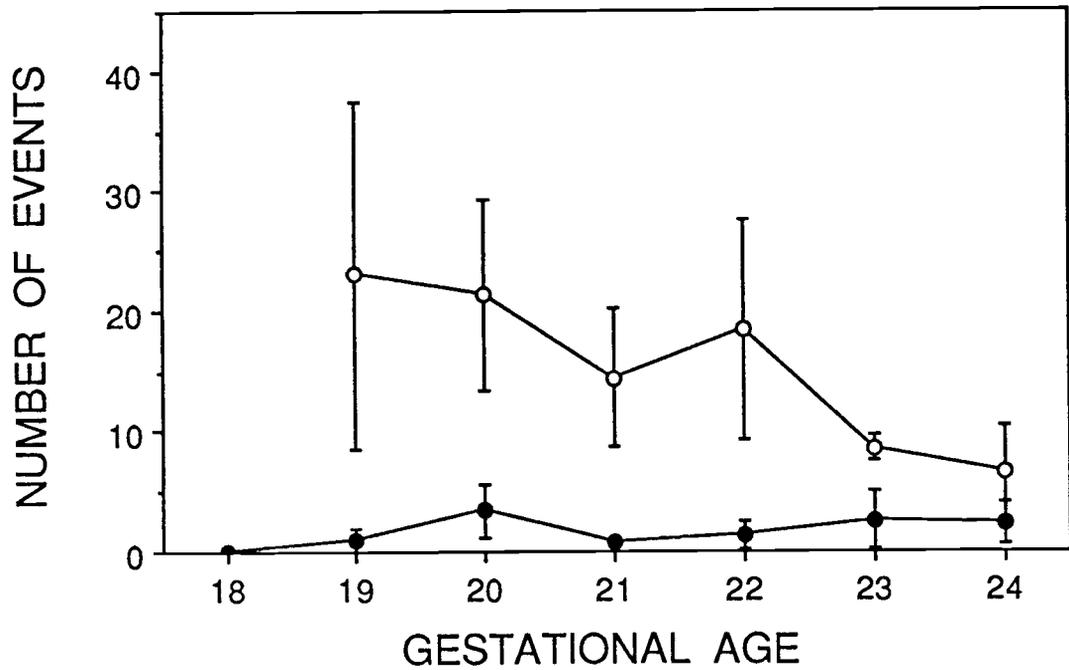


Figure 4.18. Incidence of mouth movement during gestation in *Meriones*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

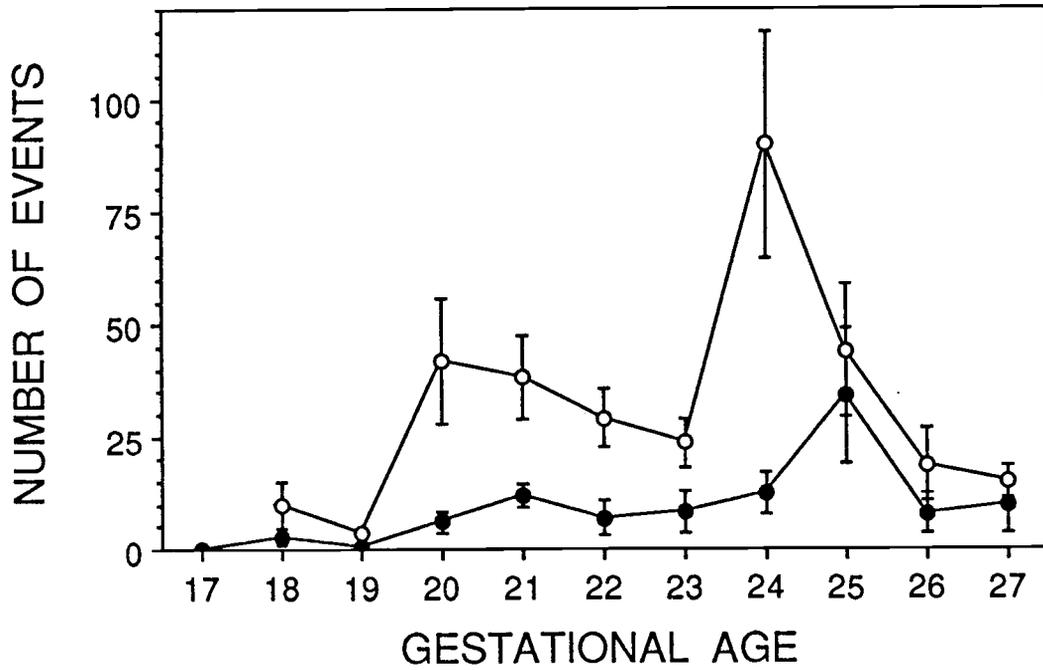


Figure 4.19. Incidence of mouth movement during gestation in *Sigmodon*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

In utero, mouth movements were more frequent on day 25 than all precedent and subsequent ages. Ex utero, mouth movements occurred more often on day 24 than any other age (Table 4.3). Significantly more mouth movements were observed ex utero than in utero on days 19-22 and day 24.

Mouth movements were relatively uncommon in Acomys, occurring in less than 5% of events overall. Age-related changes in the incidence of mouth movements were virtually nonexistent in utero and non-significant ex utero (Figure 4.20). The main effect of Condition was significant ($F_{1,49} = 32.0, p < .001$); more mouth activity occurred ex utero than in utero.

C. Overall Fetal Activity

Two measures of overall fetal activity were employed: Event Activity and Component Activity. Component activity differs from Event Activity only in the treatment of synchronous movements; an instance of synchronous movement involving two body regions (e.g., FR) is counted as one event and two components. Event Activity is synonymous with "Whole Activity" [Smotherman, Richards & Robinson 1984; Smotherman & Robinson 1986] and is roughly equivalent to the sum across all behavioral categories employed by other investigators [Narayanan, Fox & Hamburger 1971; Narayanan, Narayanan & Browne 1982; Kodama & Sekiguchi 1984].

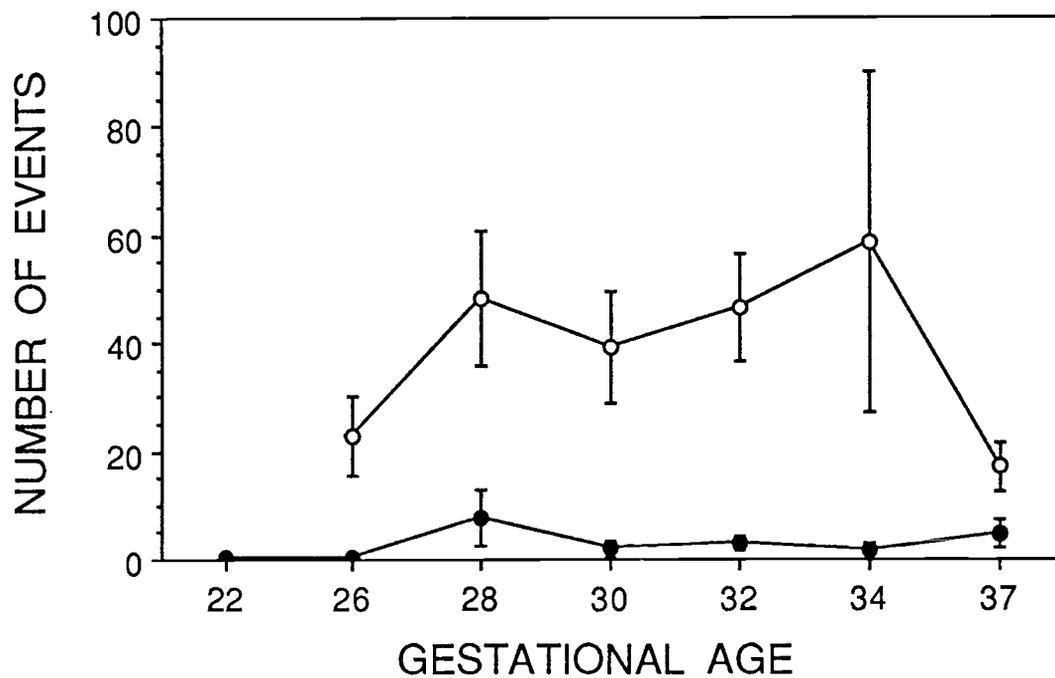


Figure 4.20. Incidence of mouth movement during gestation in *Acomys*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

1. Event Activity

The total number of behavioral events in Rattus varied as a function of Condition ($F_{1,40} = 64.7, p < .001$), but did not show significant variation with Age (Figure 4.21). Nearly twice as many events occurred ex utero as in utero.

Meriones exhibited the same pattern of development in event activity (Figure 4.22). No significant effect of Age was apparent, but nearly three times as many events occurred ex utero as in utero ($F_{1,48} = 59.9, p < .001$).

In Sigmodon, both the main effect of Age ($F_{9,95} = 4.33, p < .001$) and Condition ($F_{1,95} = 41.5, p < .001$) were significant. Post-hoc comparisons indicated that overall activity was relatively low through day 19, increased over the period of days 21 through 25, then decreased from day 25 to term (Table 4.3). More events occurred ex utero than in utero (Figure 4.23).

Event activity also varied as a function of both Age ($F_{5,49} = 11.2, p < .001$) and Condition ($F_{1,49} = 131.1, p < .001$) in Acomys. The interaction was only marginally significant ($F_{5,49} = 2.7, p = .032$). Post-hoc comparisons conducted on main effects indicated that activity was significantly higher during days 28-32 than on day 26 or days 34-37 (Table 4.4). Fetuses were more active ex utero than in utero (Figure 4.24).

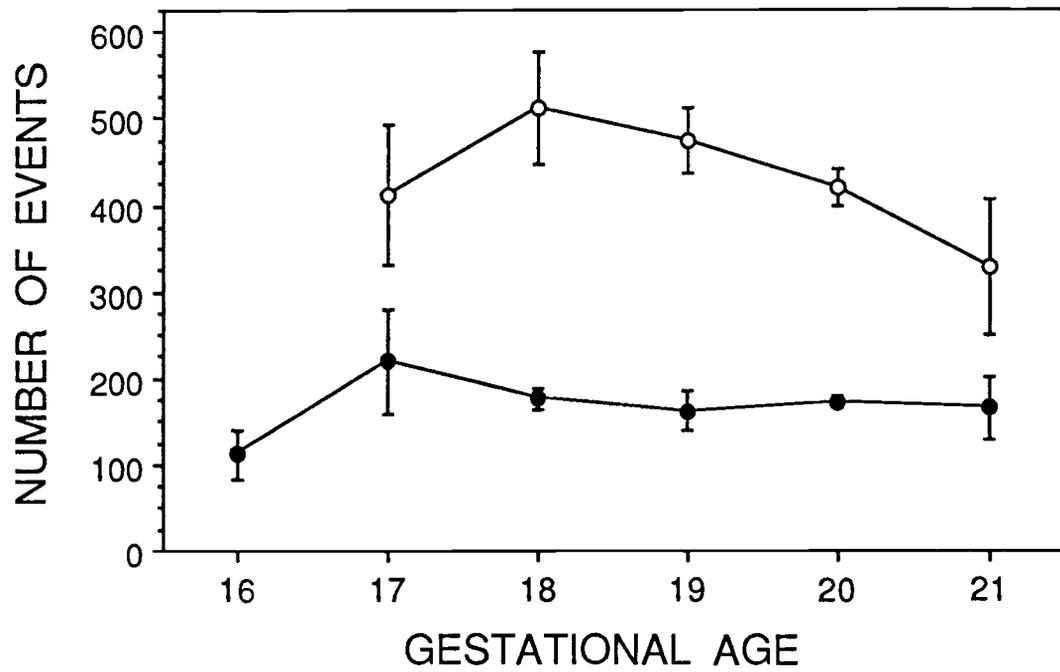


Figure 4.21. Incidence of event activity during gestation in *Rattus*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

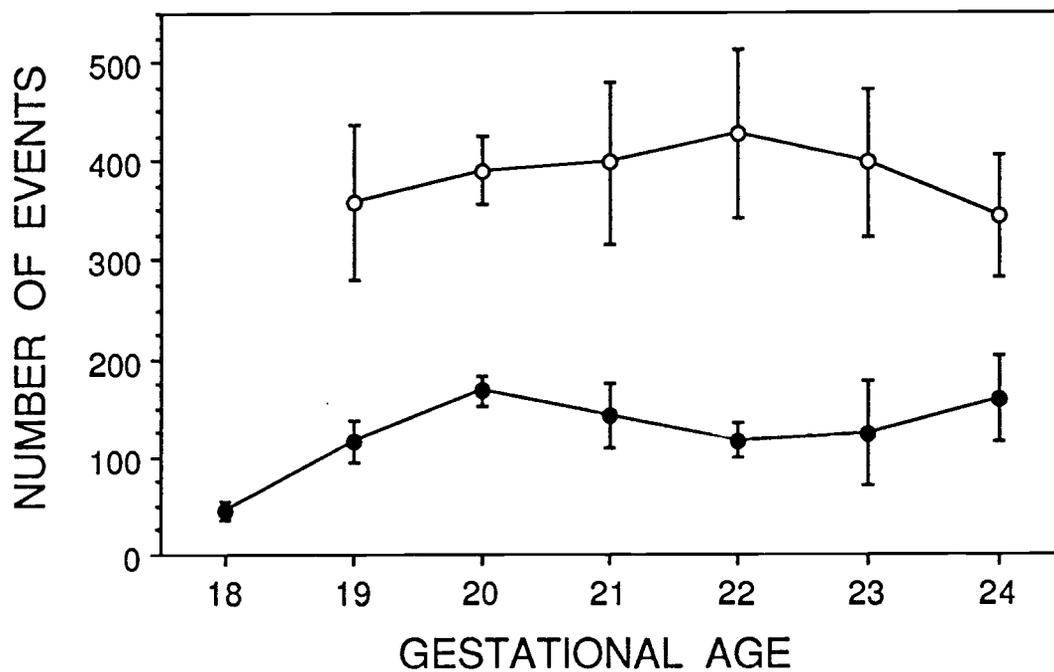


Figure 4.22. Incidence of event activity during gestation in Meriones. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

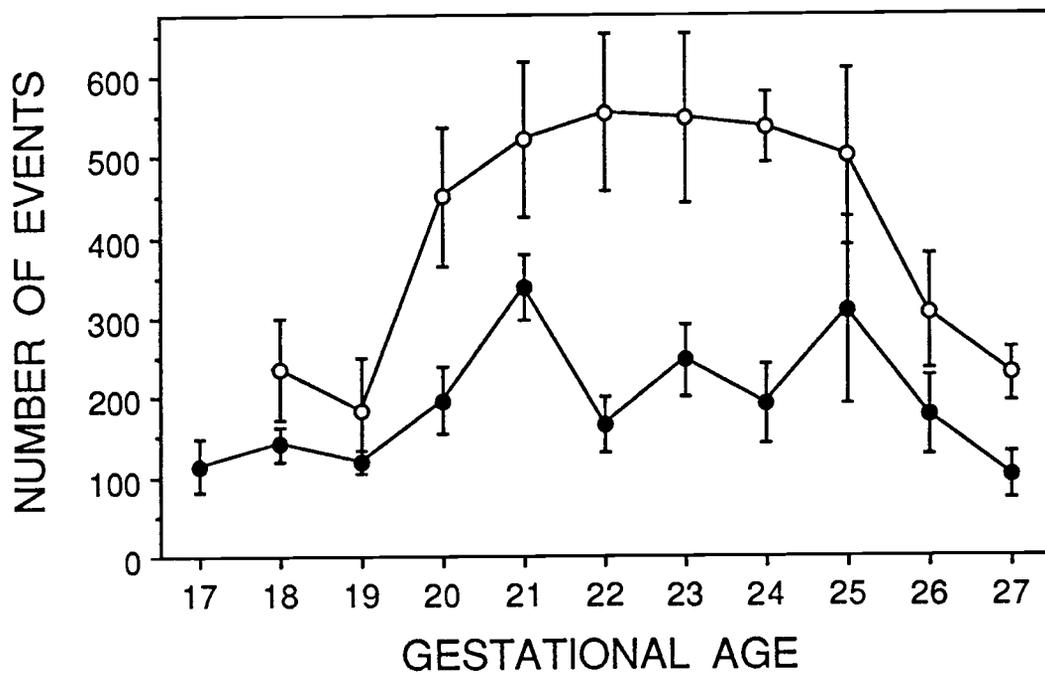


Figure 4.23. Incidence of event activity during gestation in Sigmodon. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

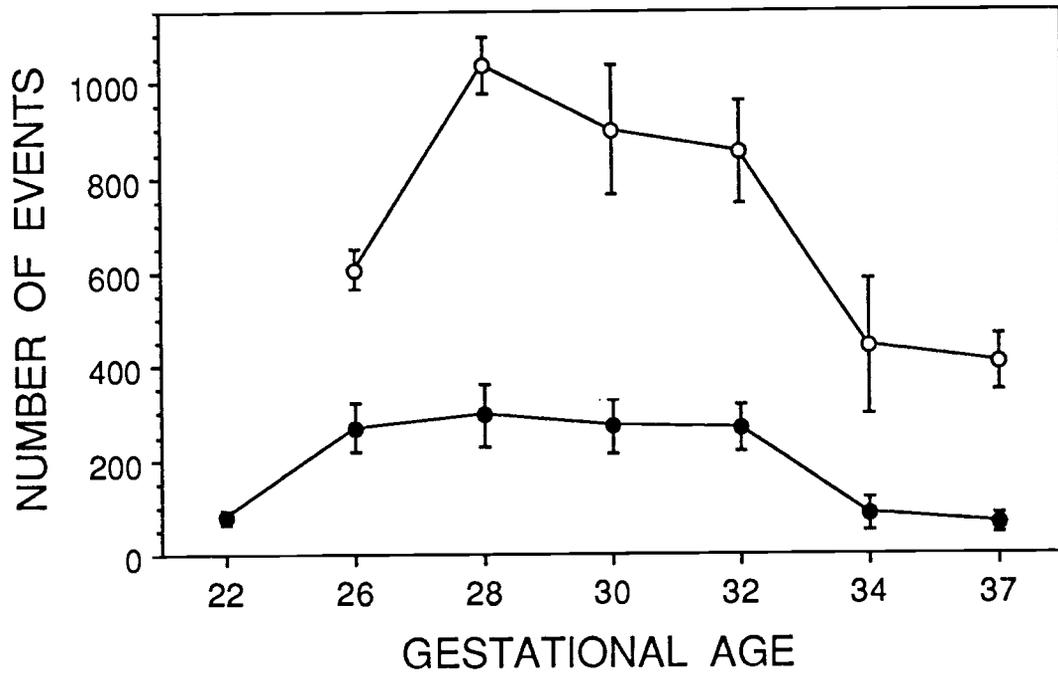


Figure 4.24. Incidence of event activity during gestation in *Acomys*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

2. Component Activity

As may be expected, patterns of variation in Component Activity closely paralleled changes described for Event Activity. In both Rattus and Meriones, only the main effect of condition was significant (Rattus: $F_{1,40} = 77.0$, $p < .001$; Figure 4.25; Meriones: $F_{1,48} = 59.2$, $p < .001$; Figure 4.26). In Sigmodon, the total number of acts increased before day 21 and decreased after day 25 ($F_{9,95} = 4.4$, $p < .001$; Figure 4.27); activity ex utero exceeded activity in utero ($F_{1,95} = 43.2$, $p < .001$).

Only in Acomys did the pattern of change of component activity differ slightly from that described above for event activity. The interaction of Age by Condition was found to be significant ($F_{5,49} = 3.7$, $p = .002$). In utero, the total number of acts increased from day 22 to 26, remained elevated during the period of days 26-32, then decreased until term (Figure 4.28). Ex utero, the total number acts increased from day 26 to 28, remained elevated until day 32, then decreased through day 37. More acts occurred ex utero than in utero at all ages (Table 4.4).

D. Discussion

1. Replication of previous findings

The data reported here for Rattus represent an

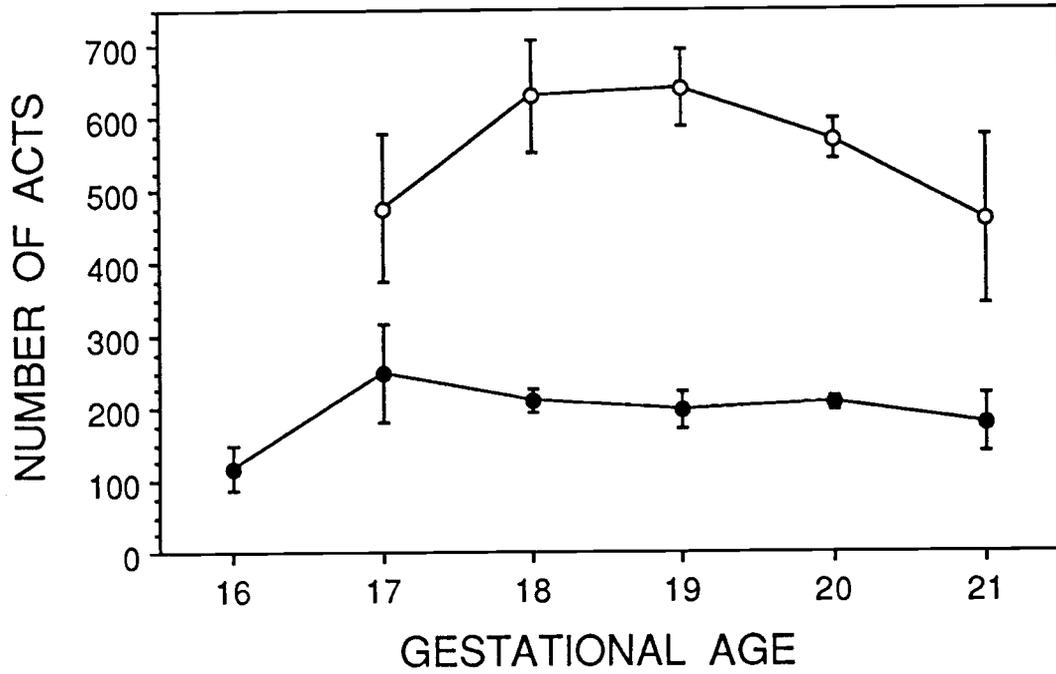


Figure 4.25. Incidence of component activity during gestation in Rattus. Closed circles represent mean \pm SEM number of acts per 30 min for fetuses in utero; open circles represent fetuses ex utero.

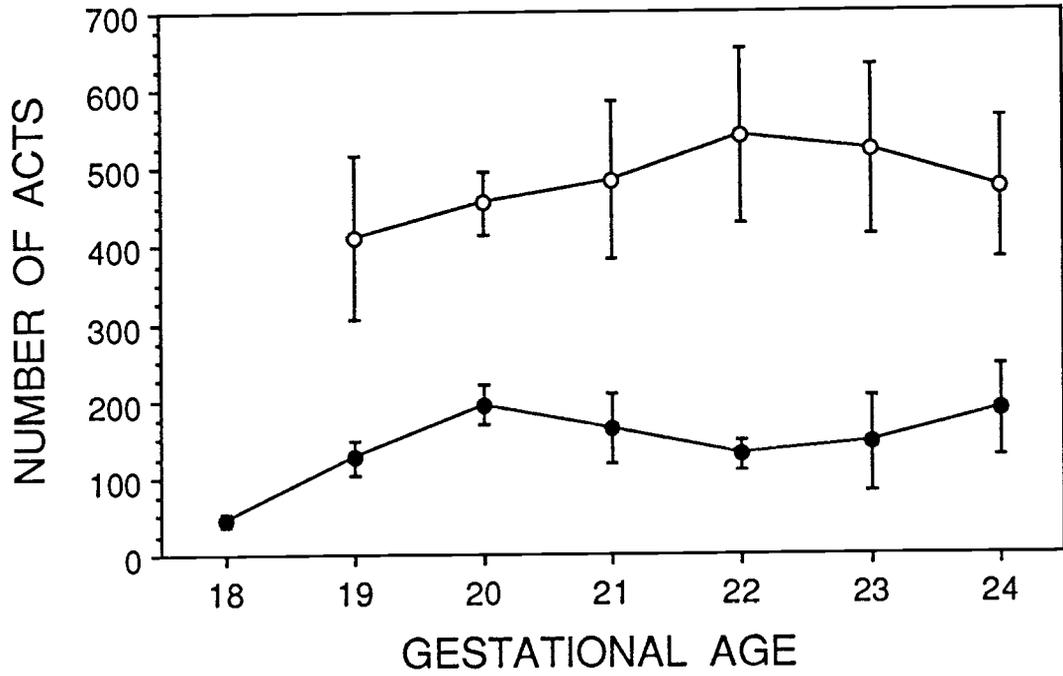


Figure 4.26. Incidence of component activity during gestation in Meriones. Closed circles represent mean \pm SEM number of acts per 30 min for fetuses in utero; open circles represent fetuses ex utero.

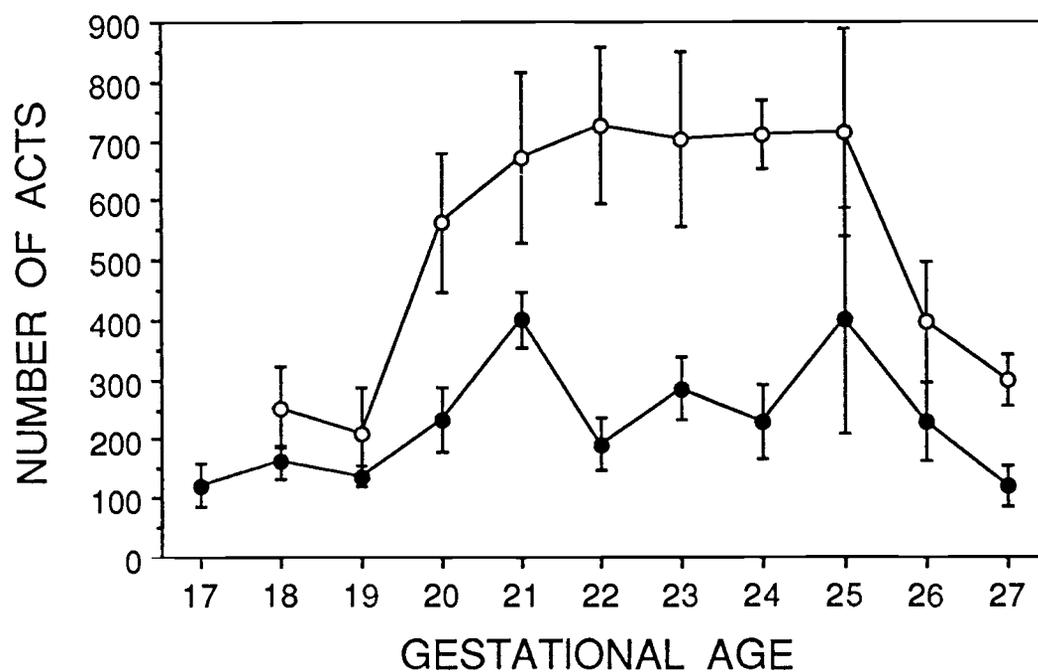


Figure 4.27. Incidence of component activity during gestation in Sigmodon. Closed circles represent mean \pm SEM number of acts per 30 min for fetuses in utero; open circles represent fetuses ex utero.

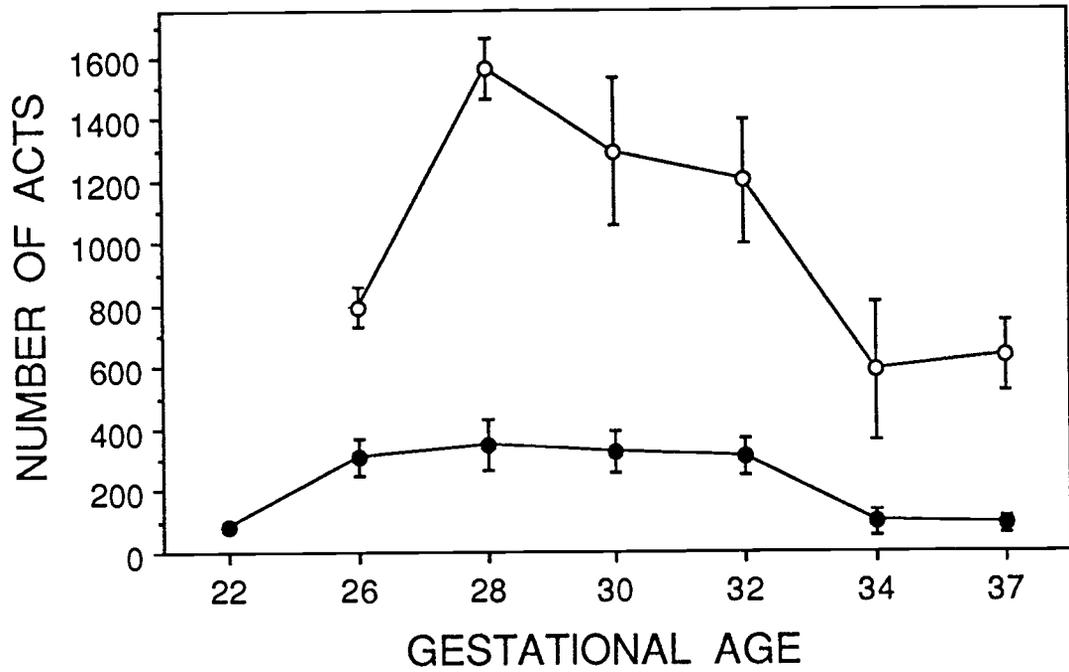


Figure 4.28. Incidence of component activity during gestation in *Acomys*. Closed circles represent mean \pm SEM number of acts per 30 min for fetuses in utero; open circles represent fetuses ex utero.

independent data set from previous reports [Smotherman, Richards & Robinson 1984; Smotherman & Robinson 1986]. Generally speaking, they replicate the findings of those reports, although minor differences in patterns of motor development were apparent. The most noticeable difference was the existence of fewer significant Age main effects or interactions in the present study. Smotherman & Robinson [1986] reported developmental changes for forelimbs, rearlimbs, head, and mouth, as well as whole activity (event activity) and component activity; the present study reports age-related changes only for rearlimbs and trunk curl. This apparent difference bears scrutiny.

Close comparison of the graphs presented in the previous report and this study suggest that the differences between the studies are more apparent than real. The shapes of the developmental curves in both studies are very similar for forelimbs, rearlimbs, head and both measures of overall activity. The incidence of mouth movements was described as showing a sharp developmental peak in both studies, although the age of that peak was identified as day 20 in the previous report and day 19 in this study (and yet a third pattern was reported by Smotherman, Richards & Robinson [1984], suggesting that mouth activity is extremely variable). Moreover, the absolute levels of fetal activity are comparable in the two studies. For example, at the peak of activity on day 19 ex utero, Smotherman & Robinson [1986] reported a mean of about 135 acts per 10 minutes, compared

to 419 acts in 30 minutes reported in this study. These seeming differences are most likely due to chance variation in sampling; the relatively small sample sizes employed in the present study doubtlessly underlie the absence of several potentially significant effects.

One difference between the studies remains and seems less trivial. The 1986 report described a bimodal pattern of development for trunk curls *ex utero*, with a high incidence early (day 17) and a secondary peak near term (days 20-21); this study replicated the early peak, but failed to find the late peak. I believe this difference may be attributed entirely to improvements in surgical protocols that have been instituted in the past three years. As we mentioned in the previous report, fetuses at term exhibit a placental connection that is much more fragile than at younger ages. Unless preventive measures are employed, it is likely that partial placental separation will occur, especially as adjacent siblings within the uterus press against the placenta of the subject fetus. Both studies explicitly included only outwardly healthy fetuses in their analyses. Yet it remains possible that the late peak in curls (a known fetal response to hypoxia; see Chapter 9) reported in the previous study was induced by undetected compromise of the placenta or umbilical cord. Such compromise was obviated in the present study by surgical methods for isolating the subject fetus and its placenta from adjacent siblings. In fact, no blood loss was observed

from any subject in this study.

2. Effects of intrauterine restraint

It was evident from the behavior of fetuses in utero and ex utero that physical conditions present at the time of observation are a potent influence on the expression of prenatal behavior. Virtually all categories of fetal movement in all four species were affected by removing the restraining influence of the uterine wall and extraembryonic membranes. Fetuses always exhibited more activity ex utero than in utero, regardless of the category of behavior under scrutiny.

However, this robust finding of a Condition effect does not identify the underlying mechanism responsible for differences in fetal activity in utero and ex utero. Two general kinds of mechanisms may be postulated. First, under conditions of restraint, greater energy expenditure by the fetus may be required for movements to be expressed. Thus, low energy movements may be passively inhibited by a restraining environment, in effect filtering the expression of motor activity. Sensory responsiveness and internally mediated behavioral adjustment by the fetus are unnecessary for passive inhibition to occur. An alternative possibility is that fetuses actively monitor conditions within the uterus, sense the difference between the environments in utero and ex utero, and actively adjust their motor activity

in accordance with environmental conditions. Consciousness or intentionality are not necessary elements of such active responsiveness, but sensory feedback is. The present data cannot distinguish between these competing hypotheses, and further discussion of this question will be deferred to later chapters.

3. Substrates of fetal activity

In all four species, simple events involving every principal body region (head, forelimbs, rearlimbs and trunk) were observed at the inception of movement. There is no evidence of a pre-neurogenic period of motility in mammals and all evidence indicates that the earliest movements of the fetus are generated by neural commands originating within the central nervous system [Oppenheim & Haverkamp 1986]. Therefore, at least the rudiments of the neural substrates subserving motor activity in basic regions of the body are present at inception.

But the present findings also suggest that the neural substrates governing fetal movements continue to develop during the days following inception. In most cases, limb movements (especially rearlimbs) increased in frequency after their emergence and trunk movements decreased. These findings imply that either (a) existing neural substrates are modified, or (b) new substrates are incorporated into the control system as gestation proceeds. The near absence

of age-related changes in Meriones may constitute an important exception to this trend and could imply a general retardation of behavioral development in this species. Conversely, the significant reductions near term in several categories of movement evident ex utero in Acomys and Sigmodon, but apparently absent in Meriones and Rattus, may indicate the emergence of central inhibitory processes controlling behavior. The ability to suppress as well as excite movements is an important aspect of early behavioral development, which may be accelerated in precocial fetuses. These issues will be addressed more fully in the following chapters.

CHAPTER 5. TEMPORAL PATTERNING OF FETAL MOVEMENT

A. Introduction

One of the important characteristics of behavior, as expressed by mature animals, is its organization in time. In virtually all animals, fluctuations in activity occur on multiple time scales. Analysis of the temporal patterning of behavior has been a favored method of ethologists for detecting and measuring changes in internal states (such as "motivational" states) underlying the expression of behavior [Nelson 1973; McFarland 1974; Colgan 1989]. On a brief time scale, temporal patterning, in the form of synchronized activation of different muscle groups and body parts, is fundamental to the coordinated movement involved in all organized motor behavior. This chapter will describe two opposing aspects of temporal patterning of fetal behavior: the random distribution of behavioral events in time, and the tendency for movements to occur in clusters or bouts. A third aspect of temporal patterning -- the synchronization of fetal movements -- will be treated in Chapter 6.

B. Log Survivor Analysis

To an observer, fetal behavior does not seem to be distributed randomly in time. Events appear to occur in

bouts. A bout may be defined as a temporal cluster of events: within a bout, successive events are separated by brief time intervals; intervals are longer between bouts. However, subjective appraisal may result in erroneous conclusions about the existence of temporal patterning, as observers tend to see patterns even in random temporal distributions. But objective definition of bout structure is possible by quantitative analysis of the length of intervals between successive events.

If the occurrence of a behavioral event in a given time series is independent of the occurrence of earlier events (a defining characteristic of events in a Poisson process), then the distribution of time intervals between successive events will follow a negative exponential function [Nelson 1964; Fagen & Young 1978]. Such a distribution typically is presented as a log survivor curve, in which the ordinate displays the log of the cumulative number of intervals of length equal to or greater than t and the abscissa displays the length of interval (t). On a semilogarithmic plot such as this, a negative exponential distribution will fall along a straight line, with the slope of the line being proportional to the probability of an event occurring within a specified period of time.

The protocol employed in this study for recording fetal behavior permits this method of quantitative temporal analysis. Each instance of fetal movement was treated as a discrete point event which occurred in a time series. The

time of occurrence of each event was recorded to the nearest second, enabling the length of inter-event intervals to be measured ± 1 s. Separate plots of the cumulative distribution of interval lengths were obtained for each subject fetus. Combined plots of data for all subjects within each combination of Age by Condition within each species also were prepared to simplify presentation.

Upon visual inspection of the various log survivor curves, it was apparent that the overall similarity of the observed distributions of intervals to an exponential model was quite good (Figures 5.1 - 5.4). To quantitatively assess how well the temporal distribution of fetal behavior could be described by a random process, observed survivor curves were compared to the best-fitting negative exponential distribution. Traditionally, this theoretical exponential distribution is generated by conducting a least-squares linear regression on the logged values of the observed cumulative distribution [Slater 1974; Fagen & Young 1978]. This method yields the best-fitting line for the log-transformed data, but not the best-fitting negative exponential distribution for the raw frequency data [Machlis 1977]. A regression line consistently underestimates the predicted number of short intervals, yielding the impression of bout structure where none may exist.

The negative exponential distribution that best fits an observed distribution was estimated by iterative approximation. A computer program was developed to employ

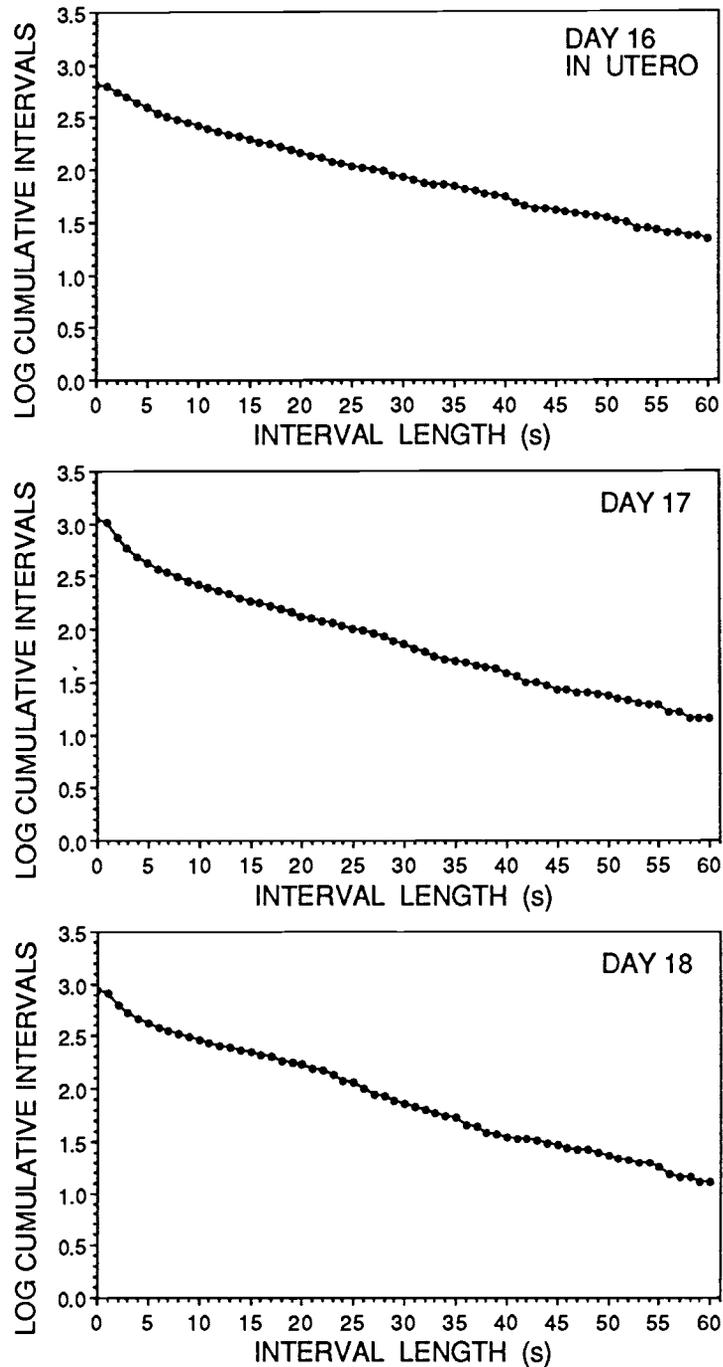


Figure 5.1. Log survivor curves of intervals between successive fetal movement events in Rattus. Points on the curve represent the cumulative number of inter-event intervals equal to or greater than a given duration. Each graph presents data for fetuses in one condition on one day of gestation. (Figure continued on next three pages.)

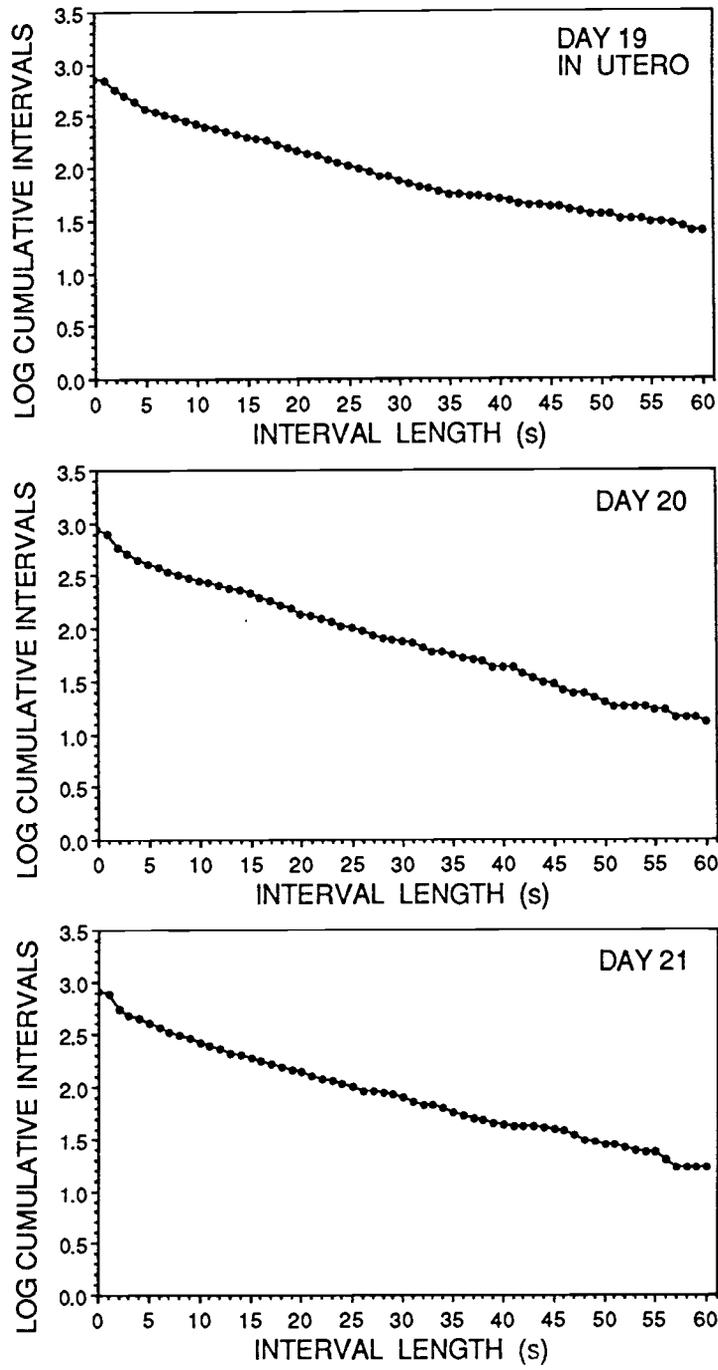


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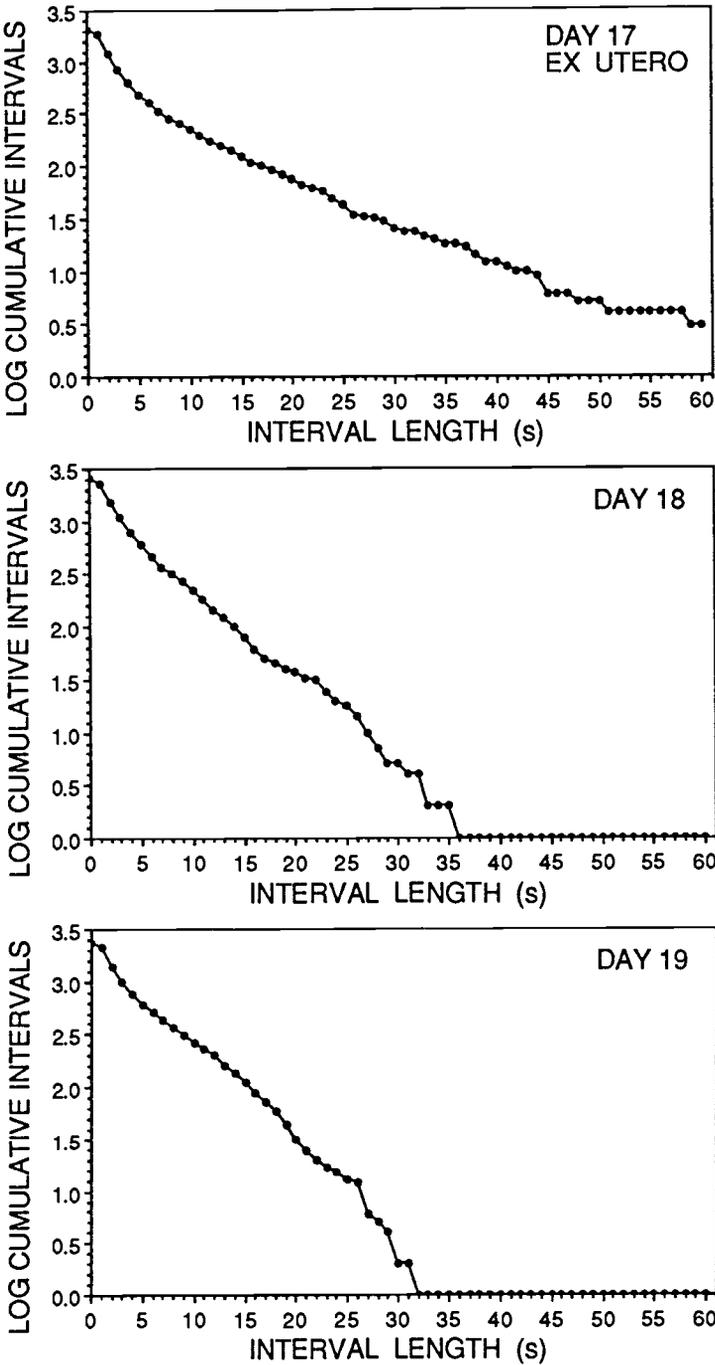


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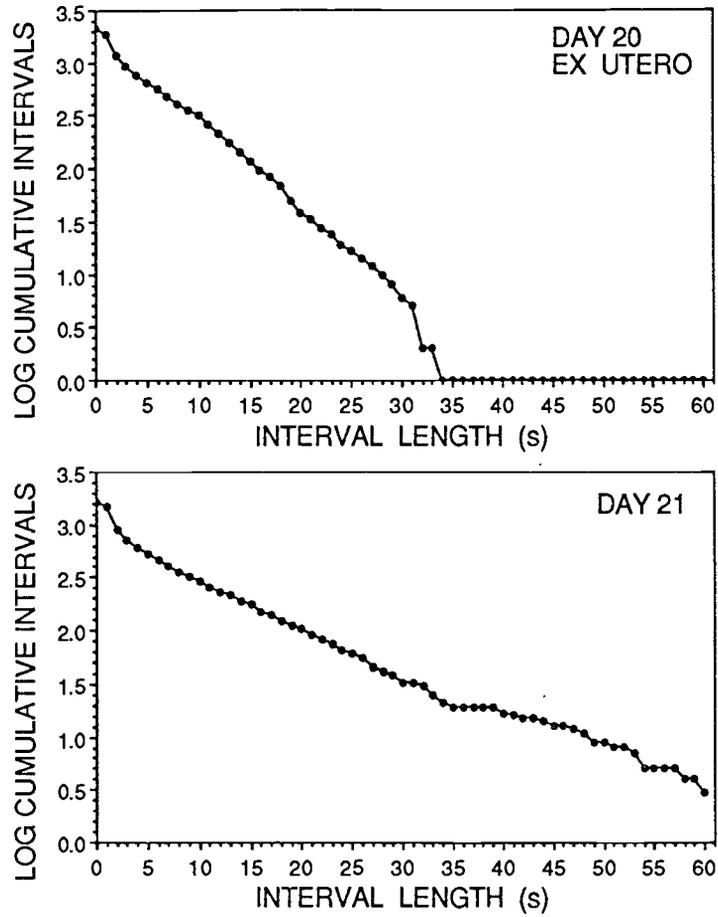


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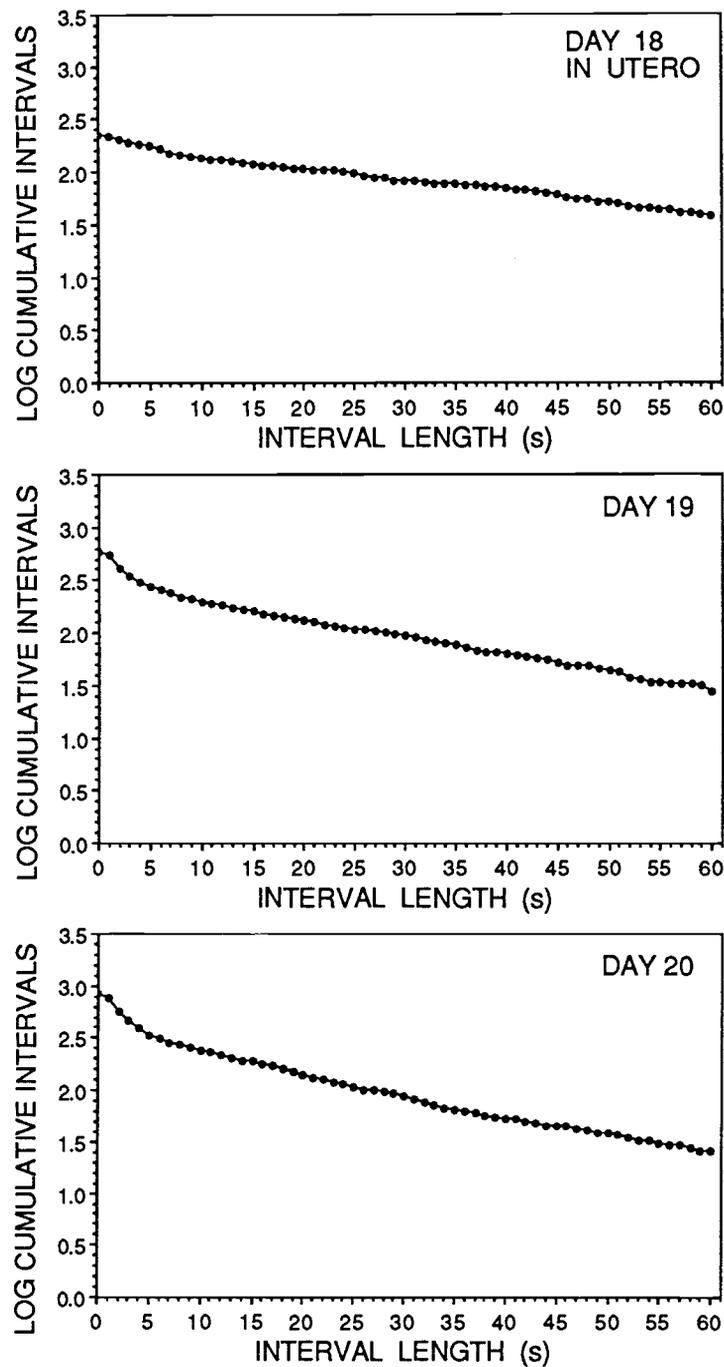


Figure 5.2. Log survivor curves of intervals between successive fetal movement events in Meriones. Points on the curve represent the cumulative number of inter-event intervals equal to or greater than a given duration. Each graph presents data for fetuses in one condition on one day of gestation. (Figure continued on next three pages.)

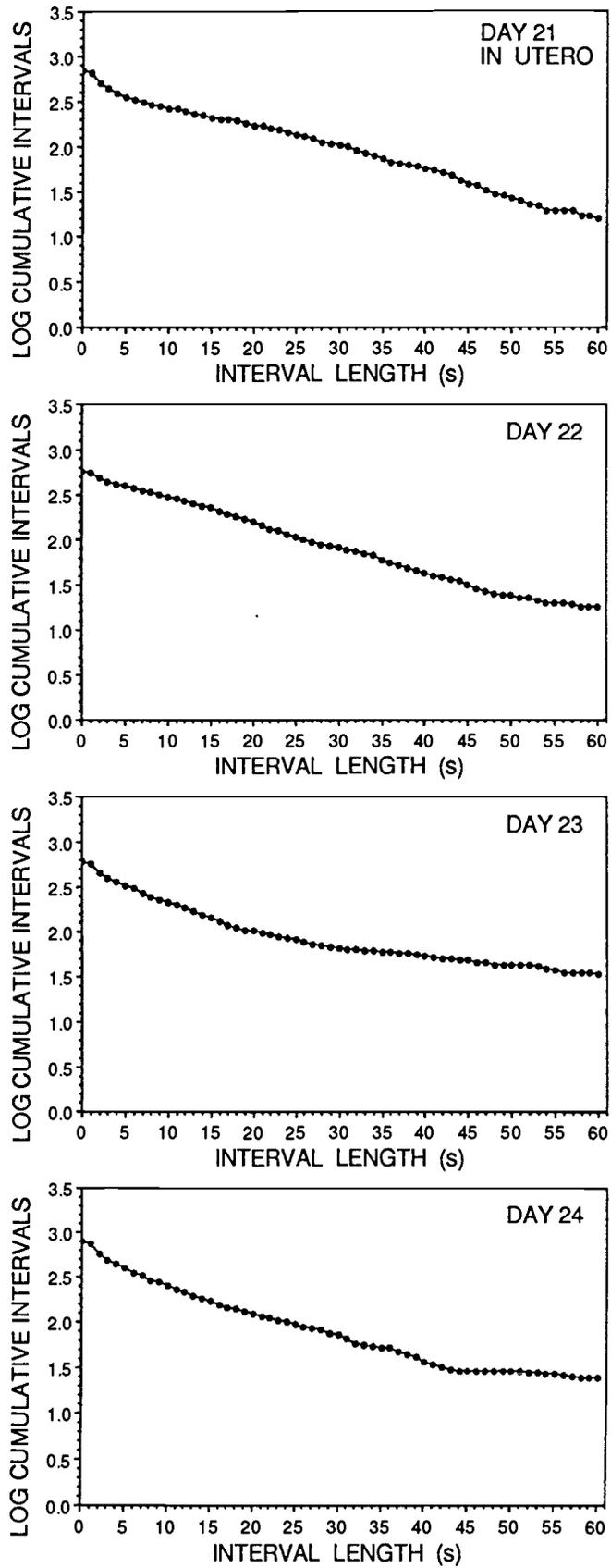


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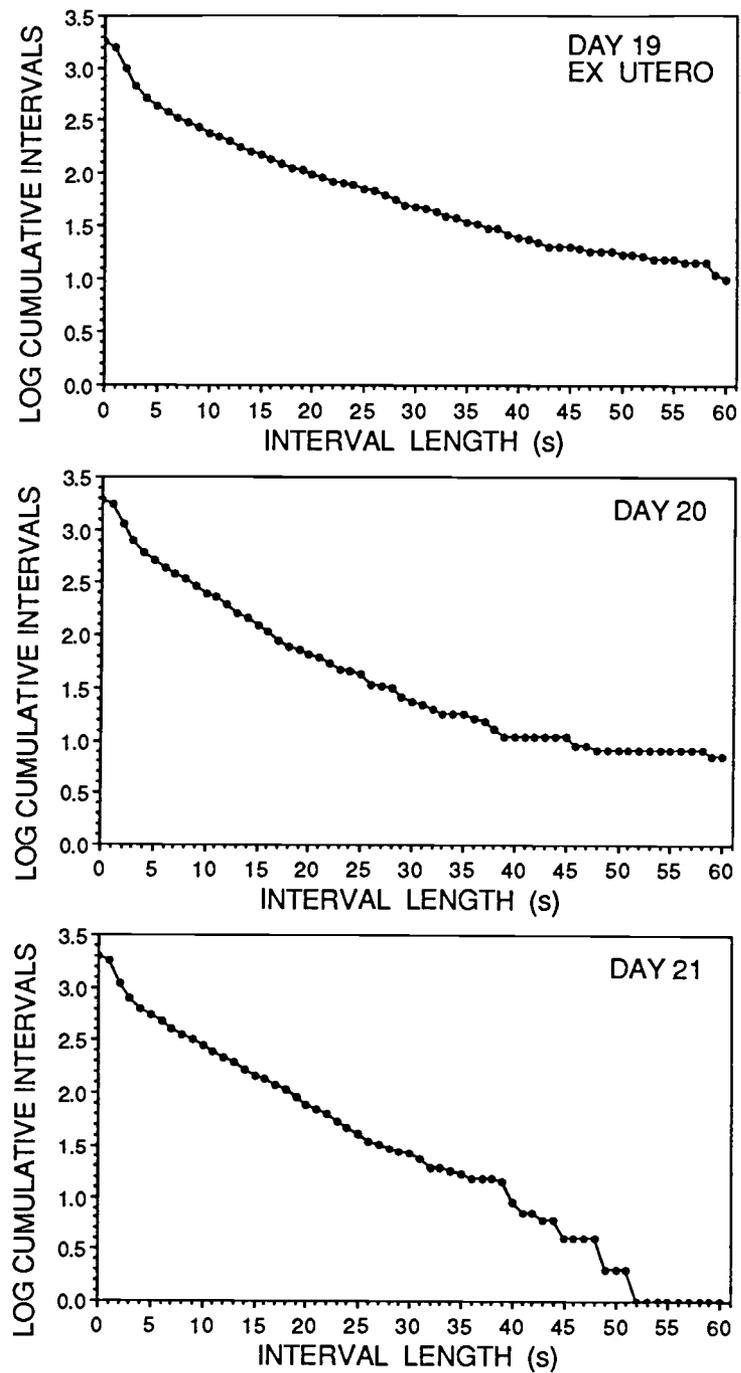


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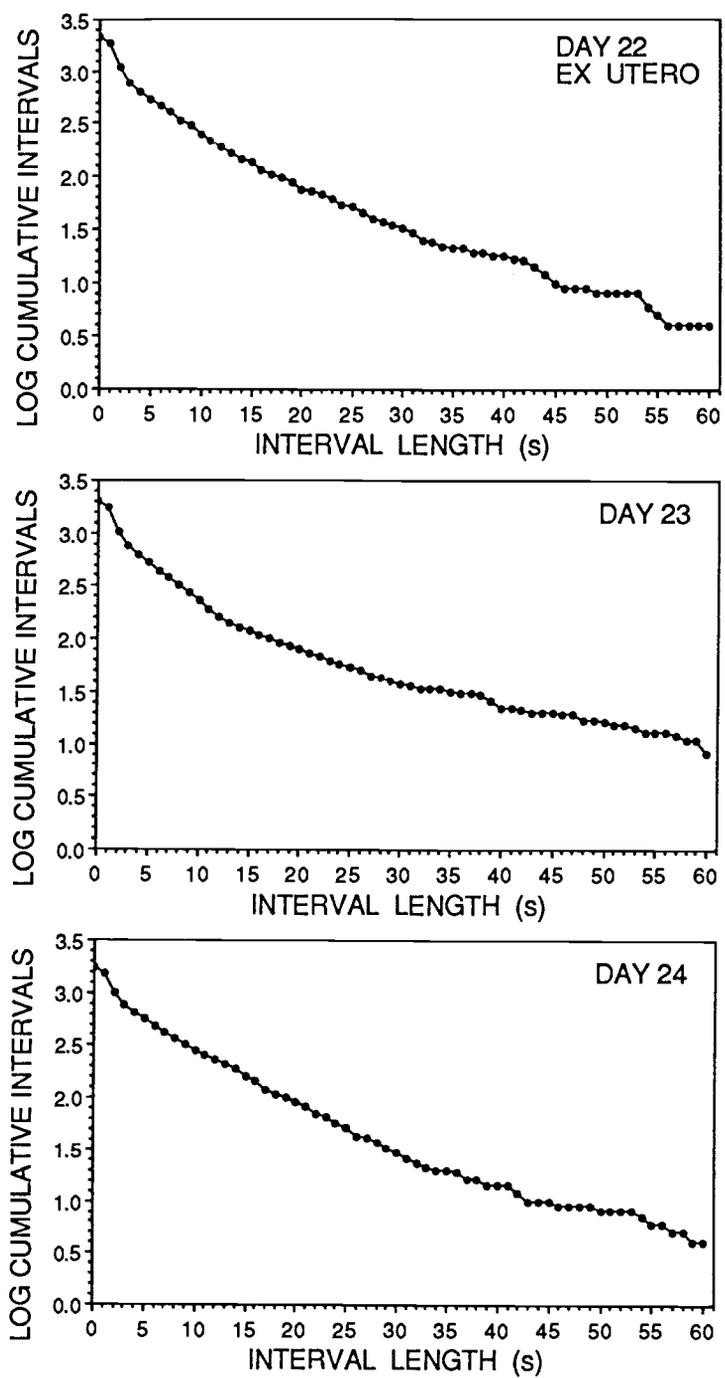


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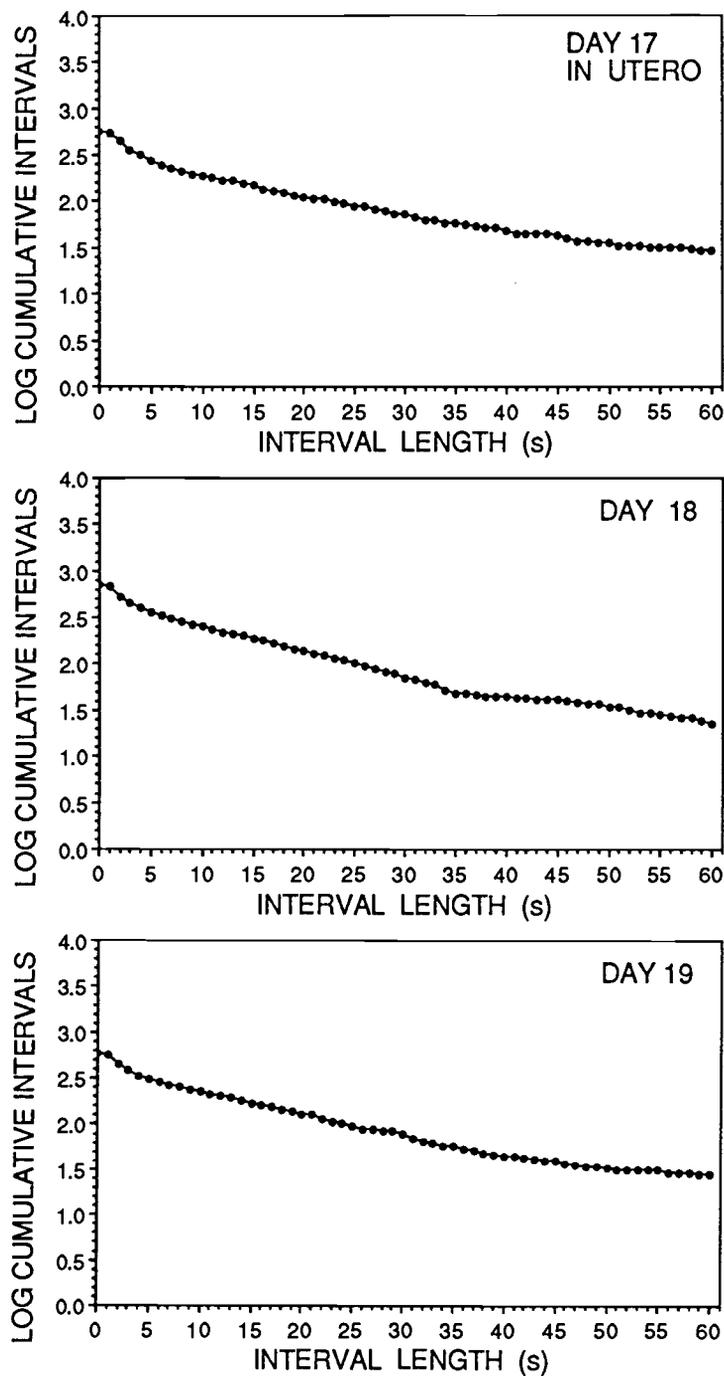


Figure 5.3. Log survivor curves of intervals between successive fetal movement events in Sigmodon. Points on the curve represent the cumulative number of inter-event intervals equal to or greater than a given duration. Each graph presents data for fetuses in one condition on one day of gestation. (Figure continued on next five pages.)

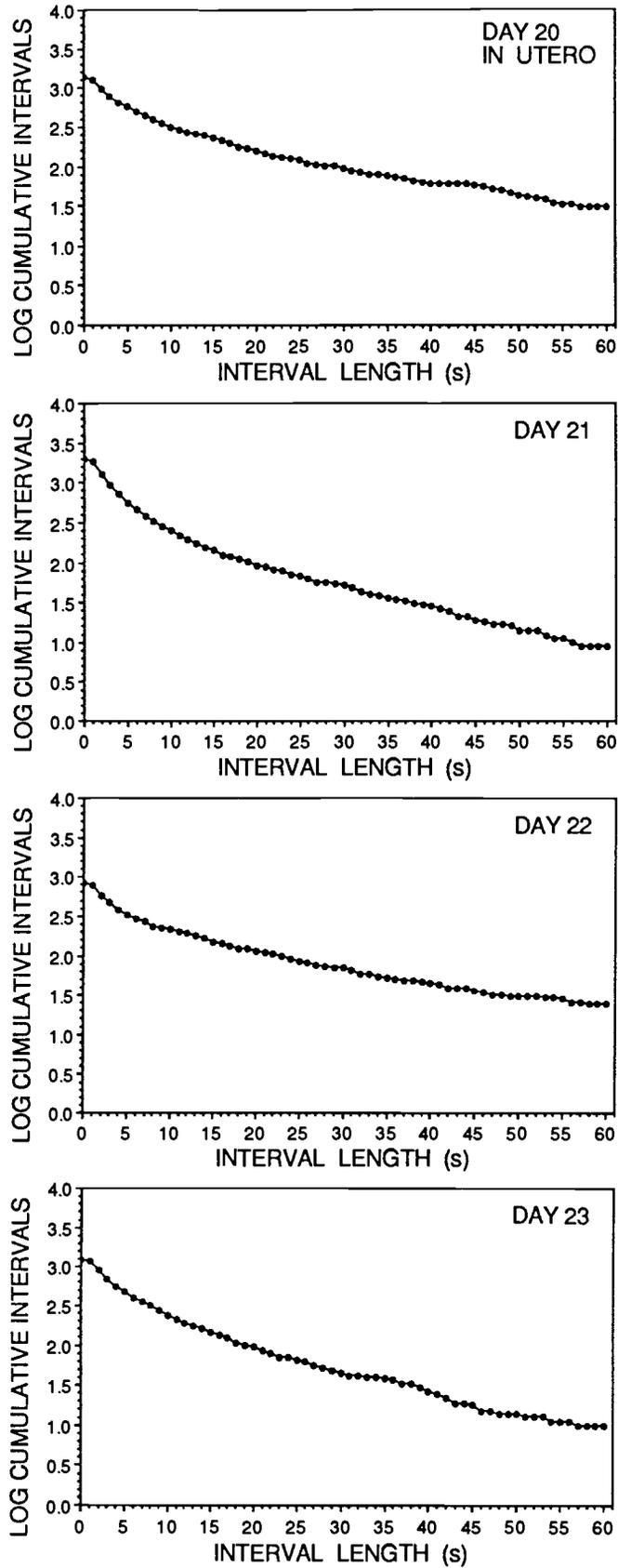


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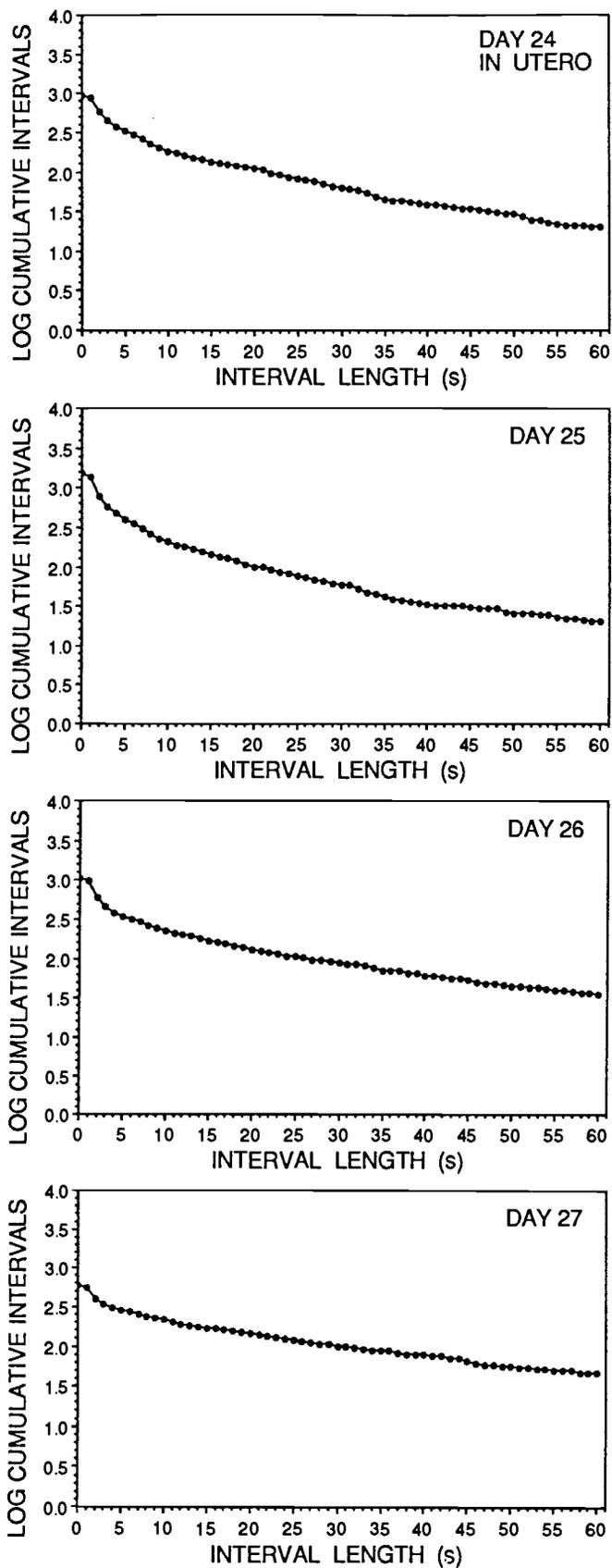


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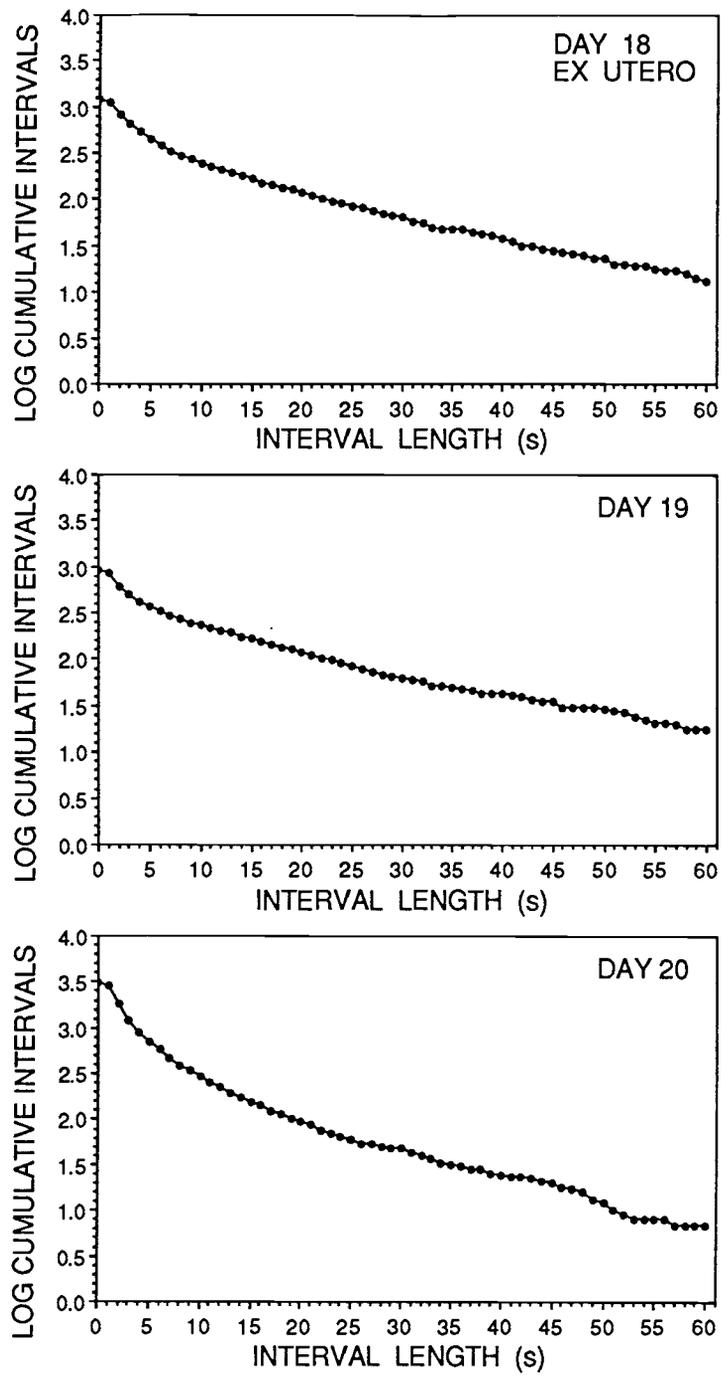


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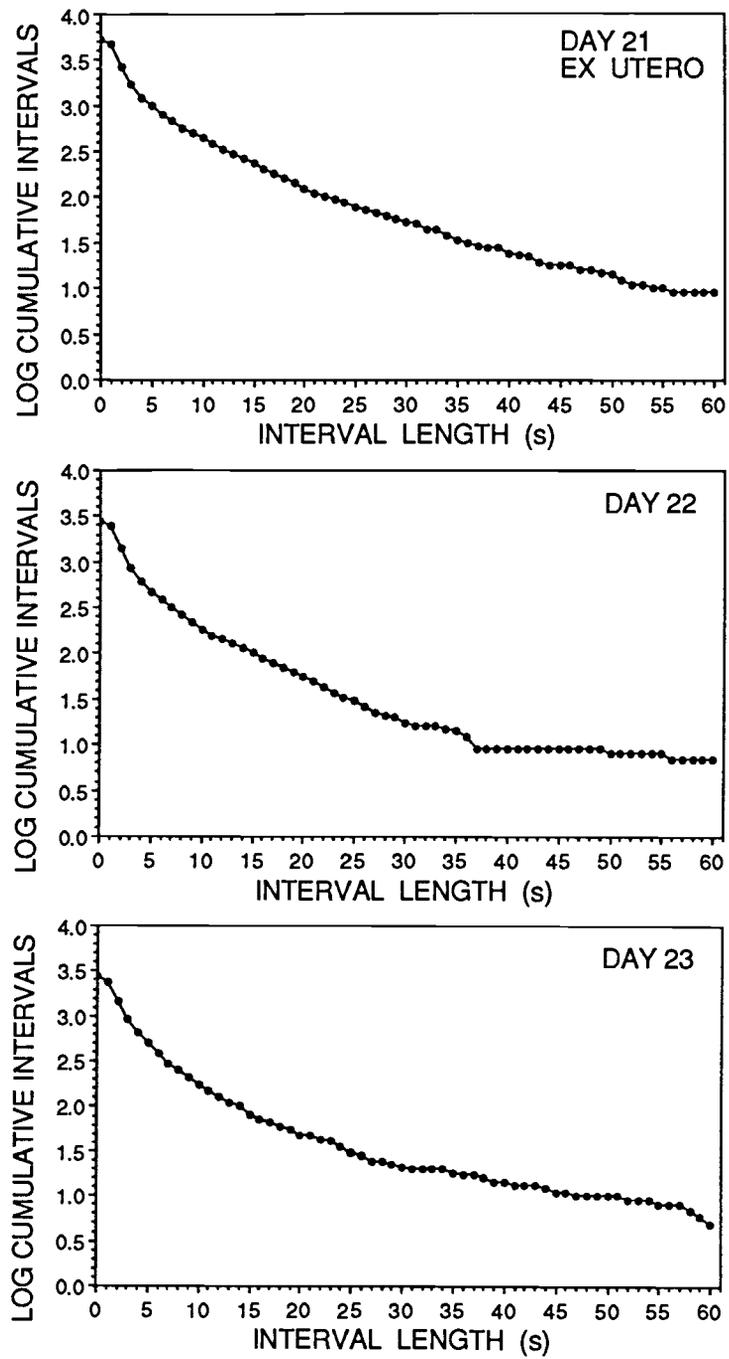


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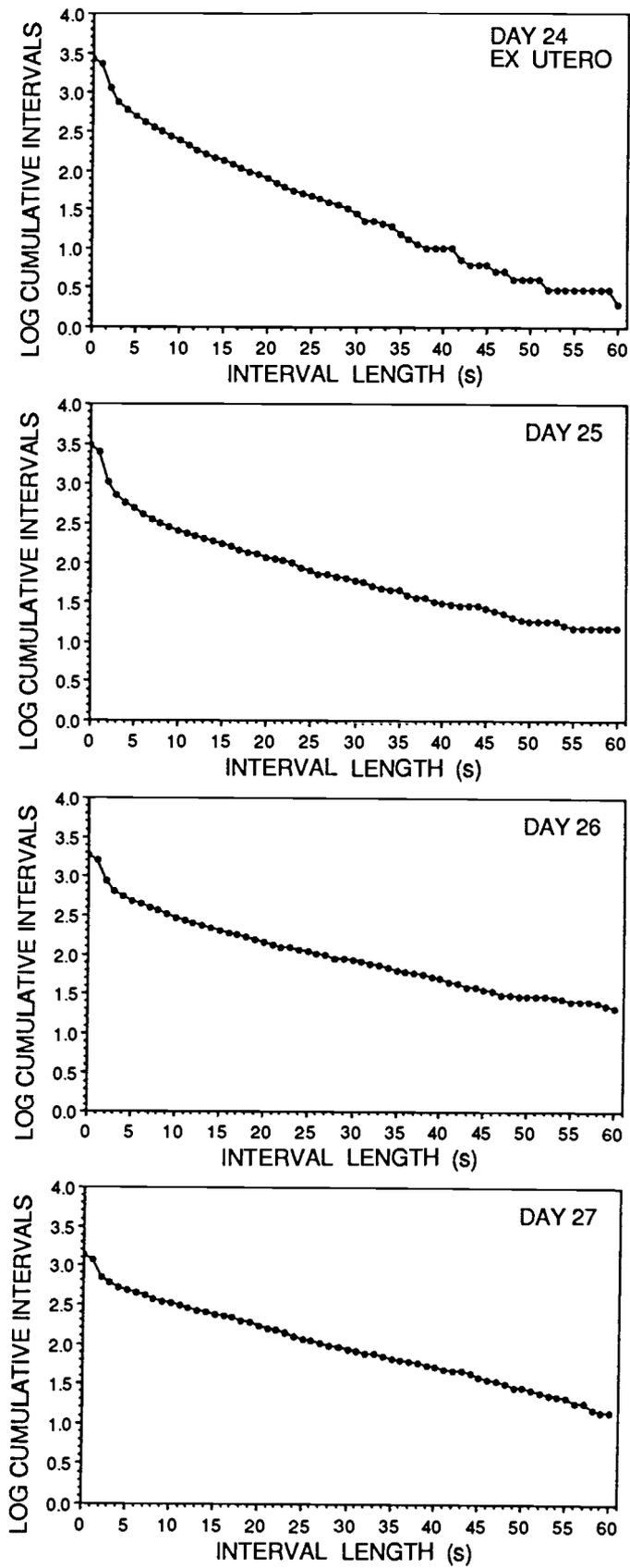


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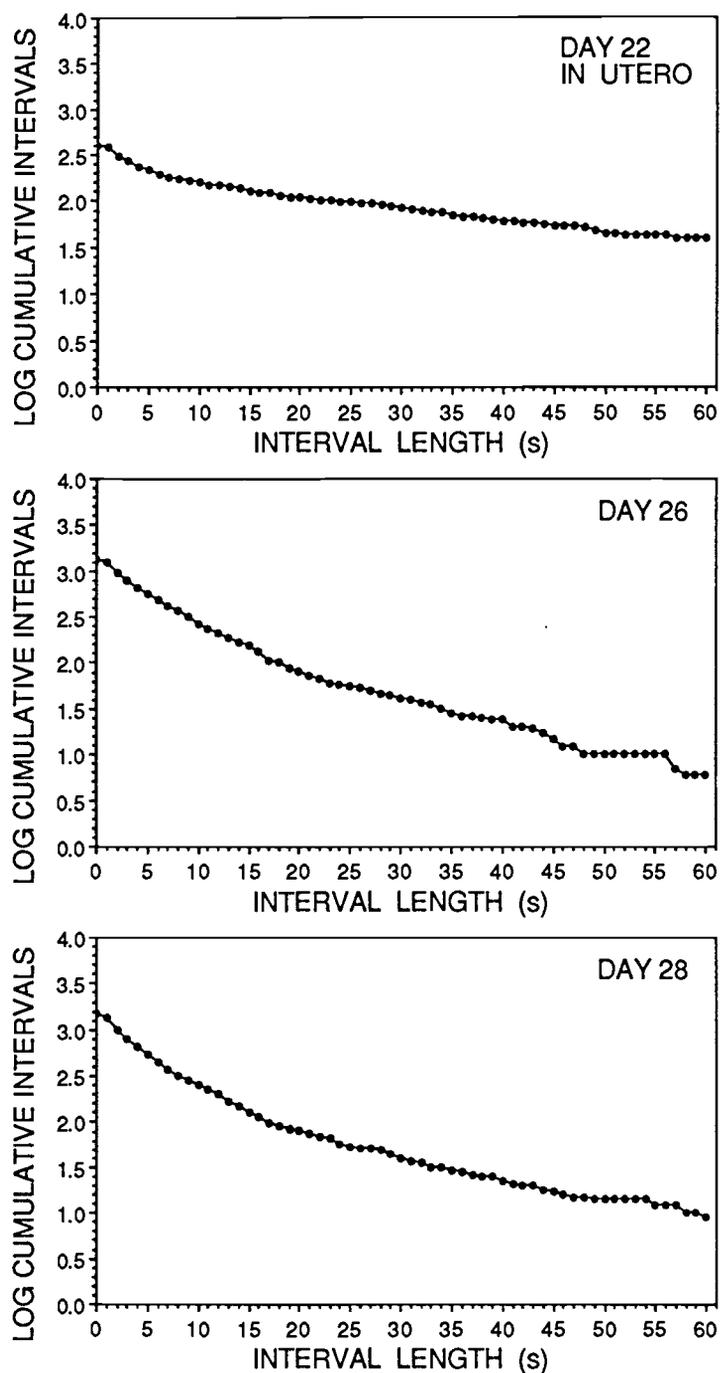


Figure 5.4. Log survivor curves of intervals between successive fetal movement events in Acomys. Points on the curve represent the cumulative number of inter-event intervals equal to or greater than a given duration. Each graph presents data for fetuses in one condition on one day of gestation. (Figure continued on next three pages.)

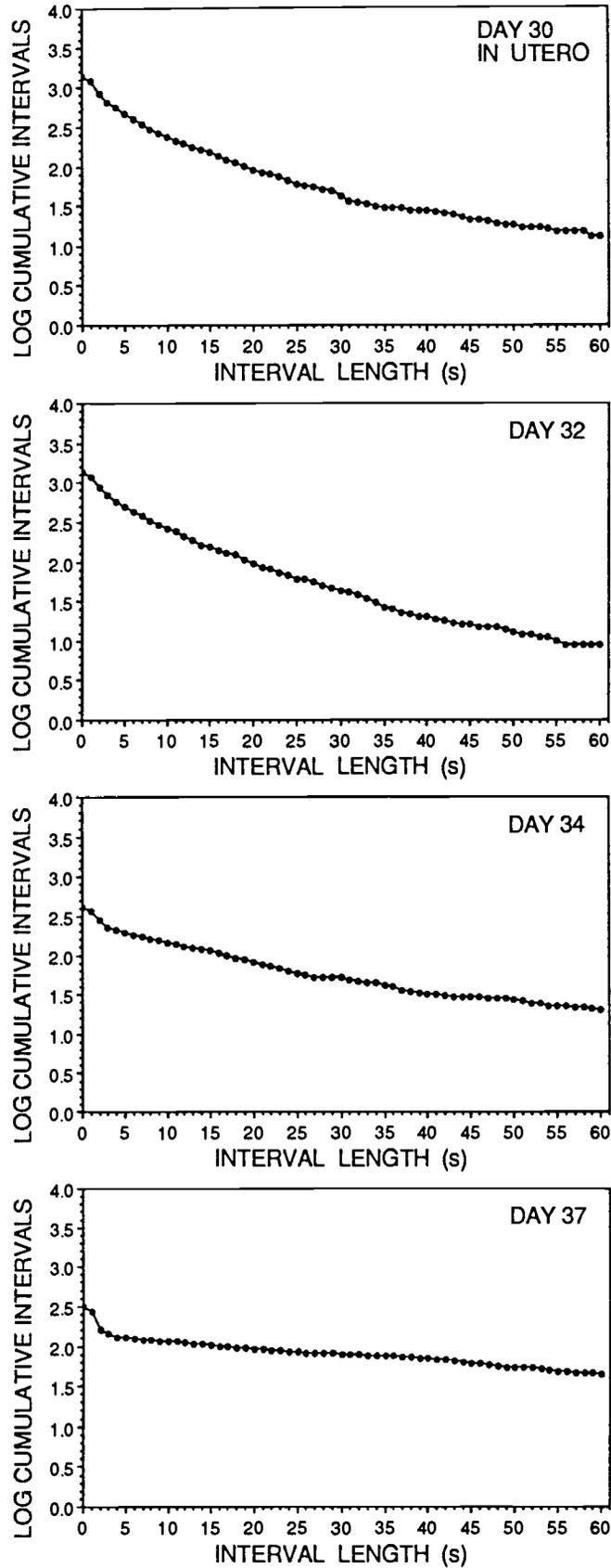


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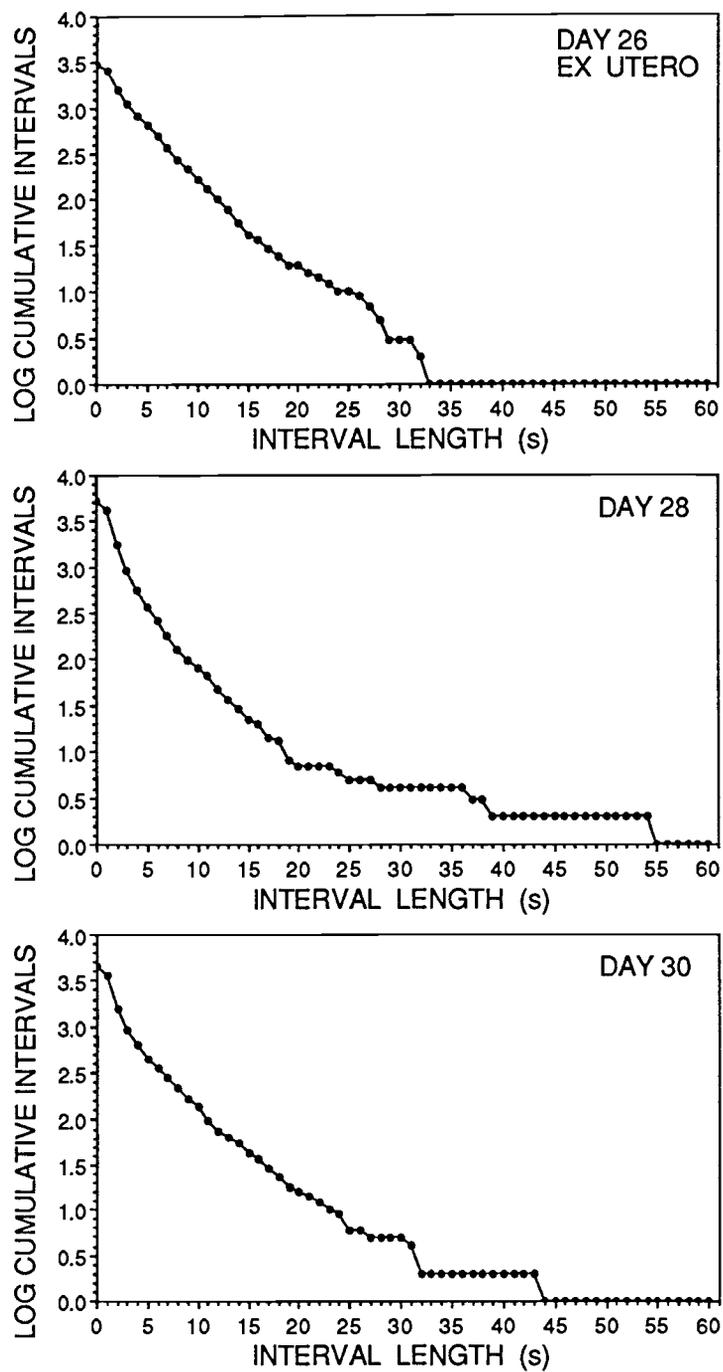


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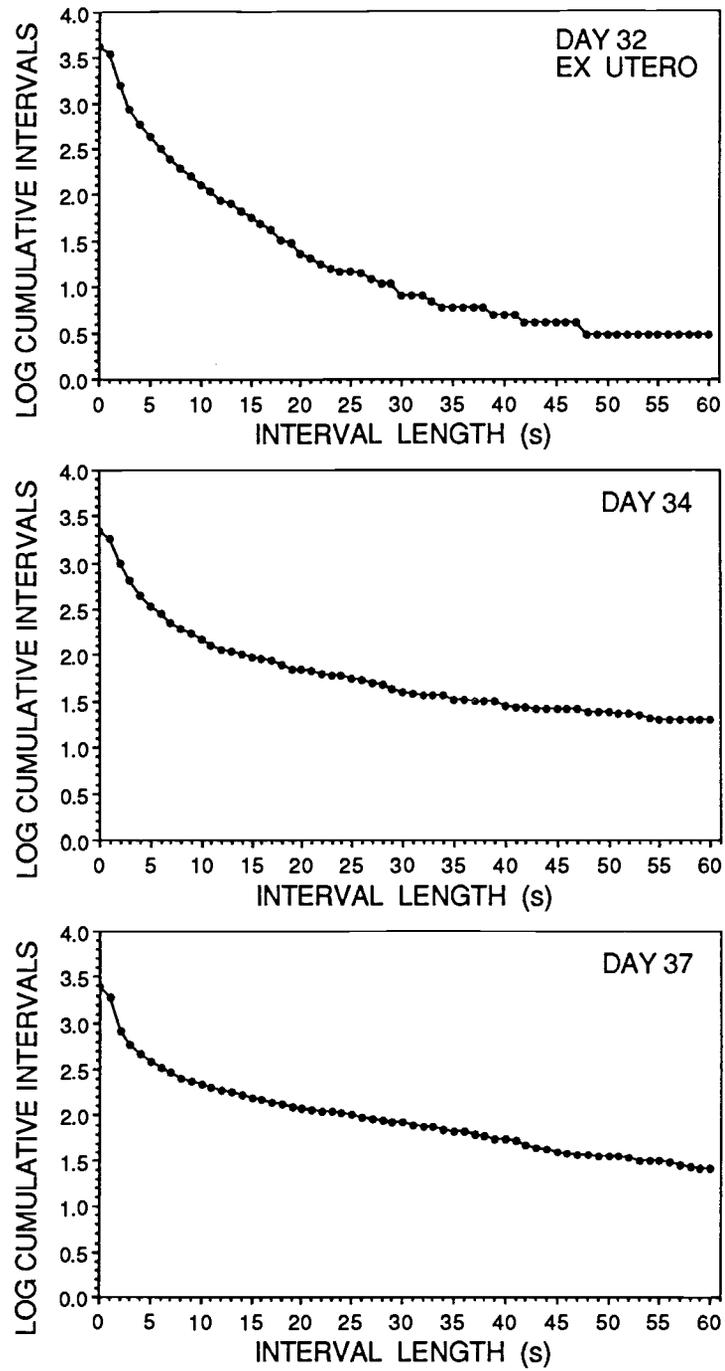


Figure 5.4 continued.

the following least-squares algorithm: (a) a hypothetical line was generated from given values for the slope and Y-intercept (initially estimated from the regression line); (b) a predicted exponential distribution of interval lengths was obtained by calculating the antilog of points at integer units along the hypothetical line; (c) the fit of the predicted distribution to the observed distribution was estimated by calculating the total discrepancy (i.e., the sum of the squares of the differences between observed and predicted interval lengths); (d) if the total discrepancy thus obtained was less than that of the previously tested hypothetical distribution, then the slope and Y-intercept were incremented and steps a-c repeated; (e) if the discrepancy was greater than that previously calculated, then the previous slope and Y-intercept were accepted as parameters of the best-fitting negative exponential distribution. This technique, similar to a method developed by Machlis [1977], does not provide a biased estimate of short interval lengths. Once obtained, a Pearson product-moment correlation coefficient was calculated as a quantitative measure of the agreement between the predicted exponential distribution and the observed (untransformed) raw frequency distribution of interval lengths.

Observed correlations were uniformly high across ages and conditions in all four species (Table 5.1). In Rattus, an average correlation of 0.989 was obtained (range: 0.929 - 0.997), which accounts for about 98% of the variation in the

Table 5.1

Median correlation (Pearson r) between negative exponential and observed frequency distributions of inter-event intervals for four species

RATTUS	day 16	17	18	19	20	21						
	_____	_____	_____	_____	_____	_____						
in utero	.987	.986	.983	.994	.986	.991						
ex utero		.984	.991	.991	.993	.987						
MERIONES	day 18	19	20	21	22	23	24					
	_____	_____	_____	_____	_____	_____	_____					
in utero	.976	.956	.973	.982	.990	.988	.992					
ex utero		.956	.987	.987	.975	.974	.985					
SIGMODON	day 17	18	19	20	21	22	23	24	25	26	27	
	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
in utero	.969	.983	.982	.983	.976	.966	.983	.955	.964	.938	.966	
ex utero		.980	.985	.986	.979	.971	.984	.946	.911	.953	.947	
ACOMYS	day 22	26	28	30	32	34	37					
	_____	_____	_____	_____	_____	_____	_____					
in utero	.973	.996	.993	.977	.982	.974	.869					
ex utero		.993	.961	.972	.951	.945	.871					

observed distribution. Comparable average r values were found for Meriones (0.979; range: 0.892 - 0.998), Sigmodon (0.967; range: 0.818 - 0.997), and Acomys (0.958; range: 0.744 - 0.999). The overall close fit of the interval data to an exponential model indicates that a substantial proportion of fetal activity at all ages is generated by a temporally random process. In other words, the probability that an event will occur is nearly independent of the time since the occurrence of the last event.

However, it appeared that virtually every log survivor curve differed in a systematic way from the predicted straight line. The tail of each distribution was generally straight, but the left end of the distribution rose above the line, giving the entire curve a characteristic concave appearance. The concave shape at the head of a log survivor curve indicated that short intervals were produced more frequently than predicted by a single exponential process. Further, the amount of overproduction of short intervals appeared to vary with gestational age and condition of observation. For example, the curve for Rattus fetuses on day 16 in utero was exceedingly close to a straight line, but exhibited noticeable deviation on day 17 (Figure 5.1). Similarly, the degree of curvature was greater on day 21 ex utero than in utero. Comparable variation was also apparent in the log survivor curves for Meriones, Sigmodon, and Acomys (Figures 5.2 - 5.4). Concave log survivor curves with systematic deviation at short intervals often have been

reported in the literature on temporal analysis of behavior of adult animals [Nelson 1964; Dawkins & Dawkins 1974; Slater 1974; Machlis 1977; Slater & Lester 1982]. They are evidence that behavioral activity is organized in bouts.

It seemed for each species that the youngest fetuses observed did not exhibit bout organization, but that older fetuses did. To test this hypothesis, the observed interval distribution was compared to the predicted negative exponential distribution by means of a Kolmogorov-Smirnov goodness of fit test [Roscoe 1975]. The percentage of subjects that exhibited a temporal pattern significantly different than predicted by an exponential model is listed in Table 5.2. In all cases where a significant difference was found, the maximum deviation of the observed distribution occurred in the left-most region of the curve ($t < 10$ s), indicating that bout organization was present.

Several important patterns emerged from this analysis. First, in every combination of age by condition except two, at least two subject fetuses exhibited a temporal pattern of activity organized in bouts. The two exceptions were observed in Rattus and Meriones at the inception of movement. At this early age in altricial species, a single exponential process was sufficient to account for the observed temporal pattern of behavior in every subject. The lack of significance could not be attributed to the amount of activity (the sample size used in the Kolmogorov-Smirnov test). Evidently, bout structure has not yet developed at

this age in altricial species. In contrast, two of five precocial Sigmodon fetuses and all five Acomys fetuses exhibited a temporal pattern of activity indicative of bout organization at the earliest age tested.

Most fetuses observed after the inception of movement exhibited activity that clustered in bouts. In a few cases, the lack of a significant result appeared to be due to a small number of fetal movements from which to base a distribution. This was most likely for one fetus on day 27 in utero in Sigmodon and one fetus on day 34 in utero in Acomys. If all remaining subjects are considered fair tests of the random model, then an additional trend is apparent in Table 5.2. In general, more fetuses ex utero exhibited bout structure than fetuses in utero. This condition effect was evident in Rattus (19 of 25 fetuses in utero versus 24 of 25 fetuses ex utero; $\chi^2_1 = 4.2$, $p=.04$), Meriones (20 of 30 in utero versus 28 of 30 ex utero; $\chi^2_1 = 6.7$, $p=.01$), and Acomys (20 of 30 in utero versus 30 of 31 ex utero; $\chi^2_1 = 9.4$, $p=.002$), but was not significant in Sigmodon (49 of 55 in utero versus 54 of 60 ex utero). In Meriones, fewer fetuses observed in utero exhibited bout structure during the last three days of gestation than during the three preceding days (days 19-21: 13 of 15 fetuses, days 22-24: 7 of 8 fetuses; $\chi^2_1 = 5.4$, $p=.02$).

C. Development of Bout Structure

Although bout organization of fetal activity was apparent at all ages after the inception of movement, examination of the log survivor curves suggested that further quantitative development of bout organization occurred during subsequent gestation. Specifically, it appeared that the portion of the curve that deviated from the predicted straight line became restricted to briefer intervals while the magnitude of the deviation became more pronounced. This trend was most evident in Acomys (Figure 5.4), for which the head and tail of the interval distribution often appeared quite distinct.

There are two general ways to interpret concave log survivor curves. First, intervals may be generated by a single time-dependent process. In other words, the probability of an event occurring may be directly proportional to the time elapsed since the preceding event. Such a process does produce distributions that deviate systematically from a straight line on a semilog plot. One method for distinguishing such a process is to plot the log cumulative distribution against a log time scale. On a log-log plot, a process with a proportional time-dependency will appear as a straight line. However, preliminary analyses showed that log-log plots of these fetal data result in convex curves. This finding indicates that the principal process generating longer intervals in these distributions

clearly is a negative exponential function or related function (such as the log-normal distribution [Schleidt 1974; Machlis 1977]).

A second general interpretation is that the observed distribution is the product of two or more superimposed processes responsible for generating intervals between bouts (the tail of the distribution) and intervals within bouts (the deviant head of the distribution) [van Liew 1962]. This approach has been applied with success to the temporal analysis of behavior [Nelson 1964; Slater 1974; Machlis 1977]. Indeed, some of the fetal log survivor curves looked remarkably like two exponential distributions with a clear point of intersection (e.g., Figure 5.4, day 37 ex utero). This interpretation of concave log survivor curves suggested an additional analysis of variation in the curvature of the observed distribution at short interval lengths.

To measure this variation objectively, a second computer program was developed to implement the following iterative algorithm. (a) A particular interval length was selected as a bout criterion (BCI; initially $BCI = 1$). (b) Two regression lines were calculated, one comprising intervals from 0 s to the BCI, the second comprising intervals from the BCI through the tail of the distribution. (c) The difference between the slopes of the two lines, D , was calculated. (d) BCI was incremented by 1 s and steps a-c repeated. Iteration continued through the first 30 s of the distribution. (e) The BCI yielding the greatest

difference between the slopes of the two regression lines (D_{\max}) was accepted as the objective BCI of the distribution. The absolute value of D_{\max} was taken as an index of the curvature of the observed log survivor distribution at short intervals. It should be emphasized that this procedure is not intended to identify the pair of negative exponential functions that best fit the data. D_{\max} is simply used as a quantitative estimate of the relative strength of bout organization in the overall temporal pattern of activity for each individual fetus.

1. Deviations at short interval lengths

For each species, the scores obtained for D_{\max} were analyzed in a two-factor (Age X Condition) ANOVA (excluding data from the day of movement inception). Following significant interactions, post hoc analyses for simple effects were conducted. Treatment means within each condition were compared by the method of Newman-Keuls to describe the pattern of ontogenetic change in bout structure.

In Rattus, the interaction between Age and Condition was significant ($F_{4,40} = 7.3, p < .001$). Post hoc analysis of simple effects showed significant variation of D_{\max} with gestational age in utero. Comparison of means revealed that D_{\max} showed a significant reduction in magnitude on day 19 relative to both earlier and later ages, indicating that the

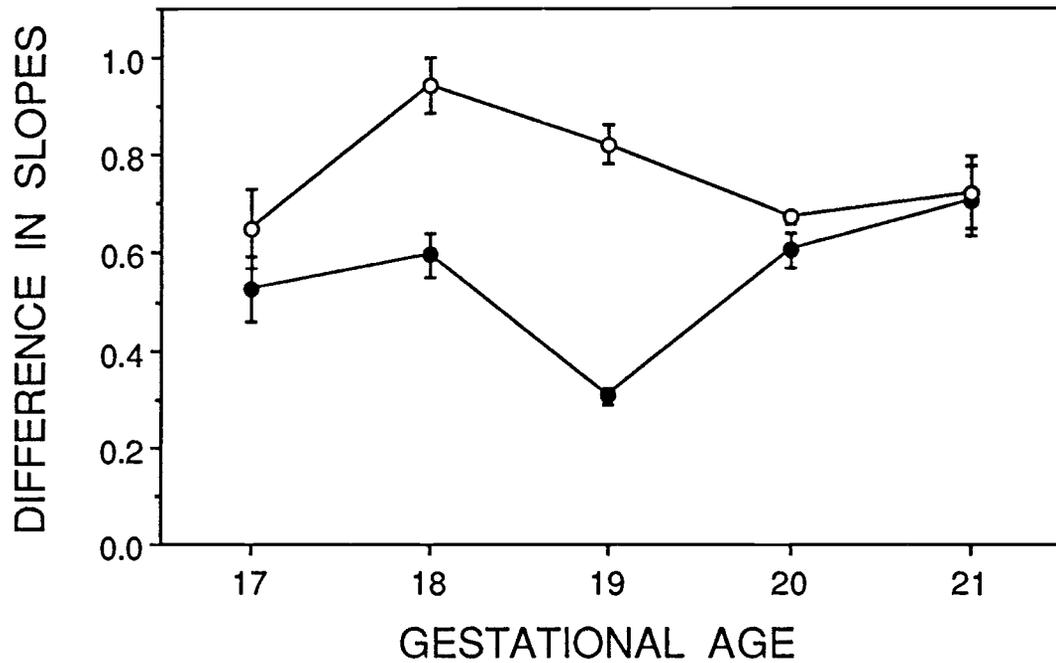


Figure 5.5. Gestational changes in the difference between slopes of short-interval and long-interval regression lines (D_{\max}) in Rattus. Closed circles represent fetuses observed in utero; open circles represent fetuses ex utero. See text for details on calculation of D_{\max} .

expression of bout organization of motor activity was sharply reduced at this age (Figure 5.5). However, this effect may have been due to only three of five fetuses exhibiting a significant deviation from the exponential model on day 19 (Table 5.2). Fetuses observed ex utero also exhibited significant change in D_{\max} with age. Deviation from a random model increased on day 18 relative to earlier and later ages.

A significant interaction also was evident in Meriones ($F_{5,48} = 16.9, p < .001$). As in Rattus, fetuses observed in utero exhibited a significant reduction in the magnitude of D_{\max} midway through gestation, on day 22, relative to both earlier and later ages (Figure 5.6). At this age, only two of five fetuses exhibited significant deviation from the exponential model (Table 5.2). Ex utero, overall bout organization was significantly reduced on day 24 relative to earlier ages.

Bout organization varied as a function of both Age and Condition in Sigmodon ($F_{9,95} = 14.8, p < .001$), but the patterns of developmental change were noticeably more complex than was the case in Rattus or Meriones (Figure 5.7). Both in utero and ex utero, post hoc tests revealed two significant peaks in the magnitude of D_{\max} during gestation. In utero, D_{\max} was significantly elevated on day 21 and again during days 24-25 relative to all other ages. Ex utero, this index of bout structure increased from day 19 to 20 and reached a peak on day 21. D_{\max} declined over days

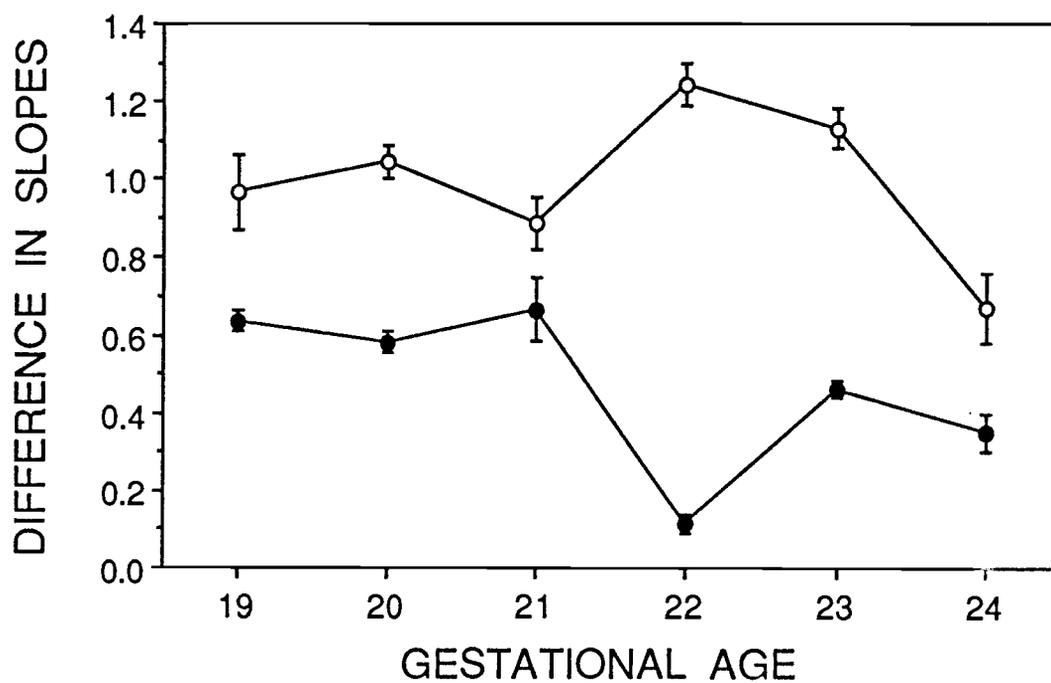


Figure 5.6. Gestational changes in the difference between slopes of short-interval and long-interval regression lines (D_{max}) in *Meriones*. Closed circles represent fetuses observed in utero; open circles represent fetuses ex utero. See text for details on calculation of D_{max} .

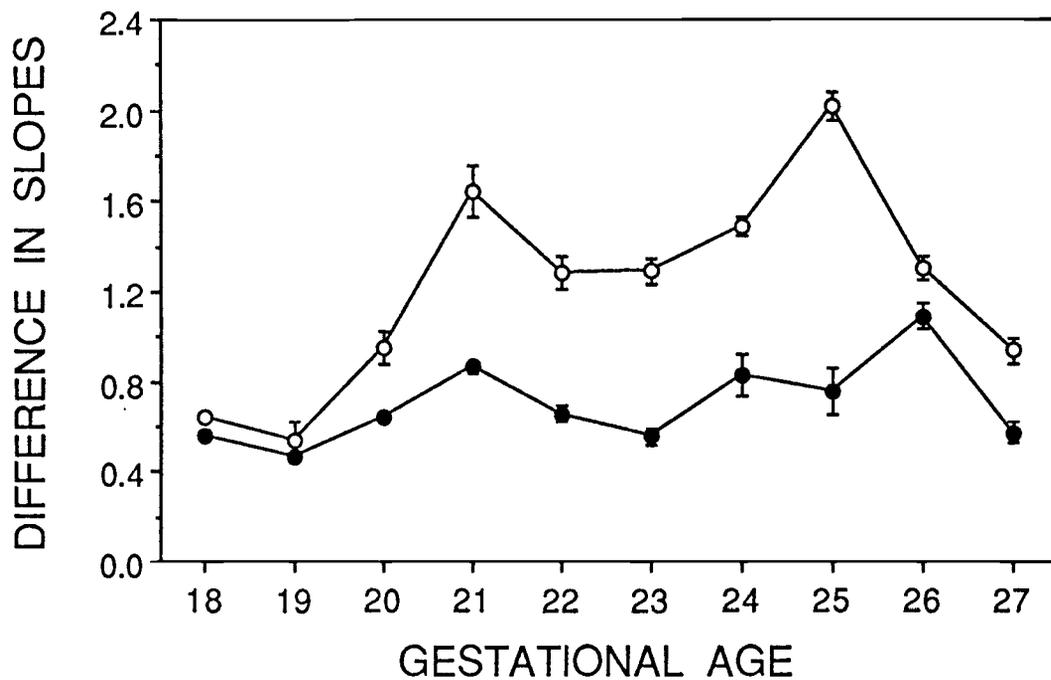


Figure 5.7. Gestational changes in the difference between slopes of short-interval and long-interval regression lines (D_{\max}) in Sigmodon. Closed circles represent fetuses observed in utero; open circles represent fetuses ex utero. See text for details on calculation of D_{\max} .

22-24, then increased sharply to a second peak on day 25, declining thereafter.

Finally, a significant interaction was found in Acomys ($F_{5,49} = 16.0, p < .001$). Post hoc tests revealed a fairly stable expression of bout organization in utero over days 26-30, a significant decline in D_{\max} on day 32, followed by a significant increase through term (Figure 5.8). A more complex pattern of developmental change in bout structure was evident ex utero. D_{\max} increased from day 26 to day 28, decreased to a lower level over days 30-32, decreased further on day 34, then returned to about the level of days 30-32 on day 37. Overall, this produced a sharp peak in the expression of bout organization on day 28, followed by a general decline through term.

2. Objective determination of the bout criterion

A second useful measure that was calculated by the algorithm described above was an objective definition of the Bout Criterion Interval, or BCI. Identification of a BCI is important for further analysis of intervals within bouts or intervals between bouts, and various methods have been employed by other investigators to identify an objective BCI [cf. Machlis 1977; Fagen & Young 1978; Slater & Lester 1982]. Typically, some method is used to estimate the point on the log survivor curve where the slope changes most abruptly; the algorithm employed in the present study may be

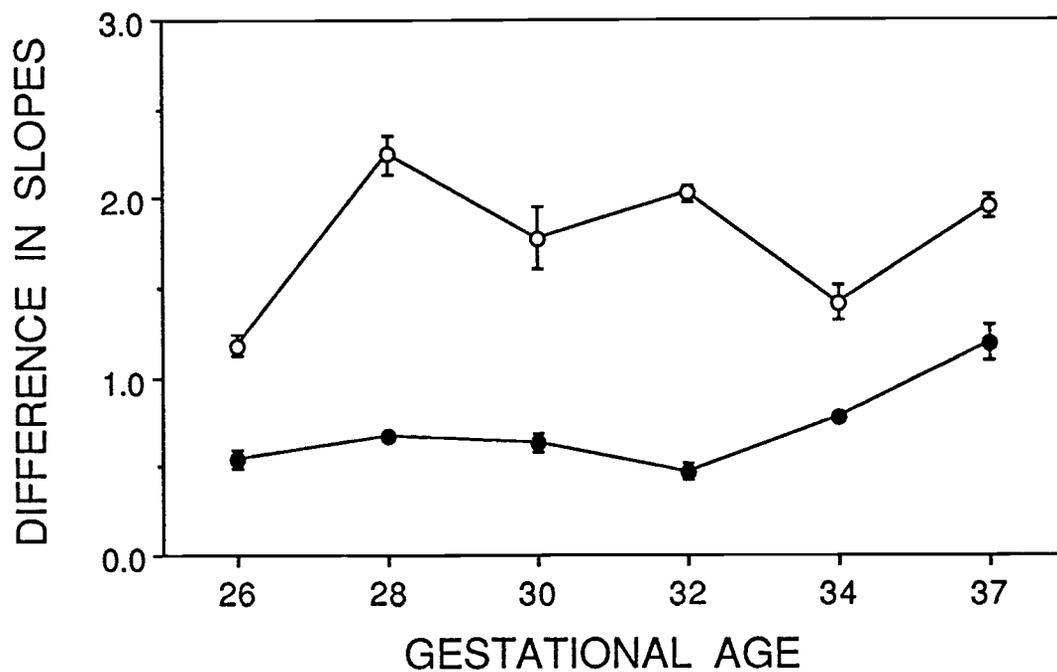


Figure 5.8. Gestational changes in the difference between slopes of short-interval and long-interval regression lines (D_{\max}) in *Acomys*. Closed circles represent fetuses observed in utero; open circles represent fetuses ex utero. See text for details on calculation of D_{\max} .

seen as a variant of this approach. This procedure will tend to identify a BCI that is between the point that minimizes errors in the assignment of intervals to within bout or between bout categories and the point that minimizes errors in total time assigned to these two categories [Slater & Lester 1982]. Most importantly, the current procedure is objective and operational, and therefore is suitable for developmental comparisons.

Initial scrutiny of the BCI values suggested a developmental trend consisting of progressive restriction of the steep portion of the curve to shorter intervals. To quantitatively test this hypothesis, BCI scores were analyzed within each condition by a Median test [Siegel 1956]. The median BCI values within each combination of Age and Condition for each species are presented in Table 5.3.

In Rattus, significant change in BCI over days 17-21 occurred in utero ($\chi^2_4 = 14.9, p=.005$); the BCI expressed by fetuses on days 20 and 21 was significantly less than the BCI on day 17. No significant change in BCI was evident ex utero. In Meriones, BCI did not vary as a function of gestational age either in utero or ex utero.

Among the precocial species, however, BCI clearly changed with age. Among Sigmodon fetuses, significant trends for BCI to decrease progressively with age were found both in utero ($\chi^2_9 = 18.8, p=.027$) and ex utero ($\chi^2_9 = 17.9, p=.036$). Generally, BCI was identified at longer intervals over days 20-23 than at subsequent ages in both conditions.

Table 5.3

Median objective Bout Criterion Interval (BCI, in s) for four species

RATTUS	day 16	17	18	19	20	21						
in utero	6	4	3	5	2	2						
ex utero		5	5	4	3	2						
MERIONES	day 18	19	20	21	22	23	24					
in utero	2	3	3	3	2	2	3					
ex utero		3	4	3	3	3	2					
SIGMODON	day 17	18	19	20	21	22	23	24	25	26	27	
in utero	3	3	3	4	5	4	6	3	3	3	3	
ex utero		3	2	3	3	3	3	3	2	2	2	
ACOMYS	day 22	26	28	30	32	34	37					
in utero	4	4	5	3	3	3	2					
ex utero		4	3	3	3	3	2					

In Acomys, a similar trend for reduction of BCI in older fetuses was evident both in utero ($\chi^2_5 = 16.6, p=.005$) and ex utero ($\chi^2_5 = 11.2, p=.048$). In this species, BCI occurred at longer intervals on days 26-28 (in utero) or day 26 (ex utero) than at older ages.

D. Discussion

The finding that log survivor curves of behavioral events performed by Rattus fetuses indicate systematic overproduction of short intervals after day 16 of gestation replicates an earlier temporal analysis of fetal behavior in rats reported by Smotherman & Robinson [1986]. The analyses reported in this chapter, however, considerably extend the conclusions of the earlier report. Three general conclusions regarding the temporal patterning of fetal behavior seem warranted.

First, a negative exponential model, when correctly formulated, agreed quite well with the overall distribution of inter-event intervals in all four species. This fact indicates that a single underlying process -- most probably one that has characteristics of a Poisson process -- is primarily responsible for generating movement events. This conclusion is underscored by the finding that the temporal pattern of activity expressed by altricial species at the inception of movement (day 16 in Rattus; day 18 in Meriones) actually did not differ from the predictions of a negative

exponential model.

Second, systematic deviation from the exponential model was apparent in the majority of fetuses. This deviation consisted of overproduction of events separated by very brief intervals. In other words, events tended to occur in bouts. The emergence of bout organization occurred relatively early: on the day of movement inception in precocial species and one day later in altricial species. It seems most parsimonious to assume that a second generative process is responsible for the overproduction of short intervals. It is plausible that a second, steeper negative exponential process [Fagen & Young 1978] or a related function [Machlis 1977] emerges to generate events within bouts. These events are superimposed upon the distribution of intervals produced by the shallower exponential function that generates gaps between bouts.

Third, bout organization continued to develop after its emergence. This was evident in two different parameters, which appeared to follow different ontogenetic trajectories. The magnitude of the overproduction of short intervals, estimated as the difference between the slopes of two regression lines on either side of a Bout Criterion Interval, generally increased to a peak and then declined late in gestation. The decline in the magnitude of discrepancy occurred earlier in precocial species than in altricial species, although Sigmodon exhibited a secondary peak and decline just before term. Changes in the BCI,

identified as the point of maximum curvature on the observed log survivor distribution, were strongly evident in precocial species, in which objective bouts became progressively more restricted to briefer interval lengths as gestation proceeded. A similar developmental trend was less strongly expressed in Rattus, which only showed significant change in utero, and was apparently absent in Meriones. Neither the changes in overproduction of short intervals nor in BCI could generally be attributed to age-related differences in overall fetal activity. Therefore, these two aspects of temporal patterning appear to represent independent dimensions of emerging behavioral organization in the fetus.

CHAPTER 6. DEVELOPMENT OF MOVEMENT SYNCHRONY

A. Introduction

An important aspect of emerging behavioral organization during the prenatal period is movement synchrony. Virtually all organized behavioral patterns require close coordination of many motor units distributed through multiple regions of the body. Movement synchrony -- the simultaneous onset of movement in two or more body regions -- may be viewed as the simplest form of motor coordination.

The behavioral coding and data recording scheme employed in the present study is well suited to detect and characterize the occurrence of synchronous movement (see Chapter 2 for further elaboration of this protocol). In this chapter, developmental changes in the overall frequency of movement synchrony, as well as patterns of synchrony involving specific categories of movement, will be described. Throughout these descriptive sections, it will be useful to recall that each behavioral category (forelimbs, rearlimbs, head, trunk, mouth) represents movement of a distinct region of the body and is uniquely represented by the first letter of its name (F, R, H, T, M). For instance, a movement event comprising forelimbs and head will be referred to by the shorthand label: "FH".

Prior to about 1980, published descriptions of fetal

behavior commonly emphasized the apparent "randomness" and "lack of organization" inherent in fetal motility [e.g., Angulo y Gonzalez 1932; Windle 1944; Narayanan, Fox & Hamburger 1971; Hamburger 1973]. Yet randomness per se has seldom been subjected to empirical scrutiny [cf., Bekoff & Lau 1980; Provine 1980]. The quantitative analysis of movement synchrony in rodent fetuses permits objective evaluation of these claims. In addition to describing observed patterns of synchronous movement, this chapter will present several stochastic models that are intended to test different aspects of the null hypothesis that movement synchrony results from chance association of individual component acts. Predictions from these models serve as an objective standard against which the emergence of simple coordinated behavior can be measured.

B. Occurrence of Synchronous Movement

The number of synchronous movement events observed over a 30-min session was obtained for each fetal subject. In addition, these counts were converted to a percentage of the total number of movement events (Event Activity) as a measure of the relative abundance of synchronous movements. Raw frequency and relative abundance data were analyzed in the same manner as described earlier for simple components and overall activity (Chapter 6).

1. Raw frequency of synchronous movement

Among Rattus fetuses, synchronous movements occurred rarely on day 16 of gestation, but were commonly observed thereafter. Results of the two-way ANOVA revealed a main effect of Condition ($F_{1,40} = 103.9, p < .001$) and a marginally significant interaction of Age and Condition ($F_{4,40} = 2.8, p = .038$). The one-way ANOVA including data from day 16 in utero confirmed that the incidence of movement synchrony varied significantly with age ($F_{5,24} = 5.43, p = .002$). A post-hoc comparison of means (Newman-Keuls) indicated that movement synchrony was least common on day 16, increased significantly over the period of days 17-20, then decreased in occurrence on day 21 (Figure 6.1). A marginally significant simple effect of age was evident ex utero. This was attributed to the increase in synchronous movements between days 17-18; no significant change in frequency occurred on day 21 (Table 6.1). At all ages, synchronous movement occurred more often ex utero than in utero.

A significant main effect of Condition was noted in Meriones ($F_{1,48} = 46.4, p < .001$). Although the incidence of movement synchrony appeared to be sharply reduced on day 18 in utero, variation among subjects was high and no significant effect of Age was found (Figure 6.2). Movement synchrony was more frequent ex utero than in utero.

The overall ANOVA revealed significant main effects of Age ($F_{1,95} = 43.4, p < .001$) and Condition ($F_{9,95} = 4.7,$

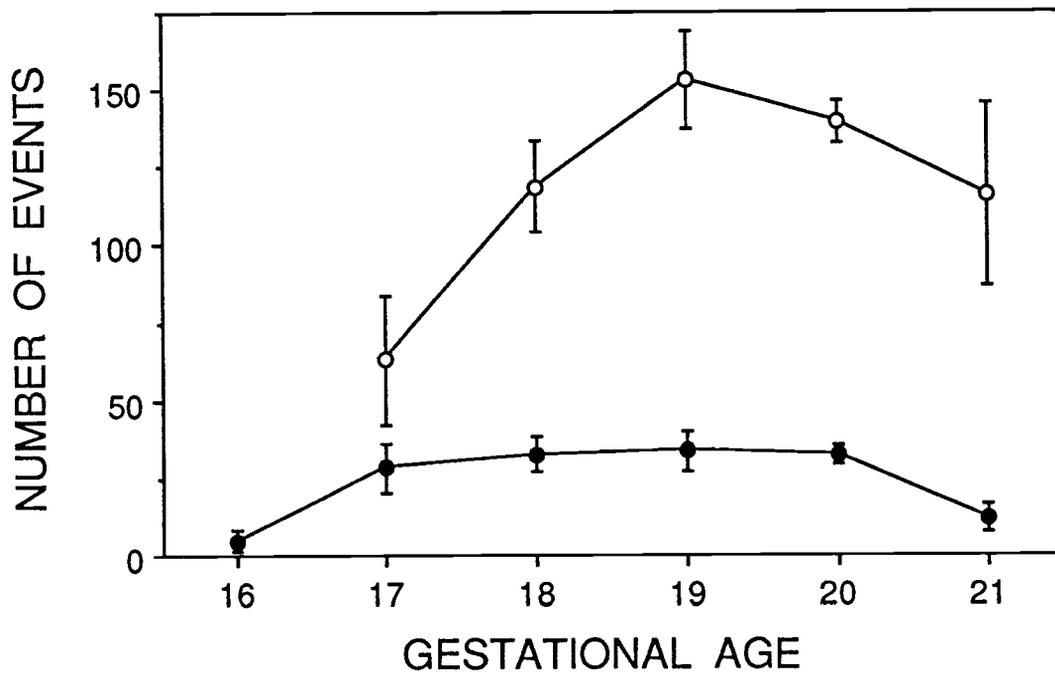


Figure 6.1. Incidence of movement synchrony during gestation in *Rattus*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

Table 6.1

Summary of post-hoc comparisons in analyses of frequency of synchronous movement for four rodent species. Comparison of group means (Newman-Keuls) followed significant main effects ($p < .01$), or simple effects where the interaction was evident. Significance and direction of difference between Conditions or Ages is indicated by '<' or '>'; non-significance by '='. Ages are presented in an ordered sequence; ages not appearing in the sequence did not differ from adjacent ages.

SPECIES	MAIN EFFECTS		INTERACTION (A X C)	
	AGE	CONDITION	AGE	CONDITION
	RATTUS	In: 16<17=20>21	In < Ex	
MERIONES		In < Ex		
SIGMODON	18=19<21=25>27	In < Ex		
ACOMYS			In: 22<28=37	26: In < Ex
			Ex: 26<28=32>34=37	28: In < Ex
				30: In < Ex
				32: In < Ex
				34: In < Ex
				37: In < Ex

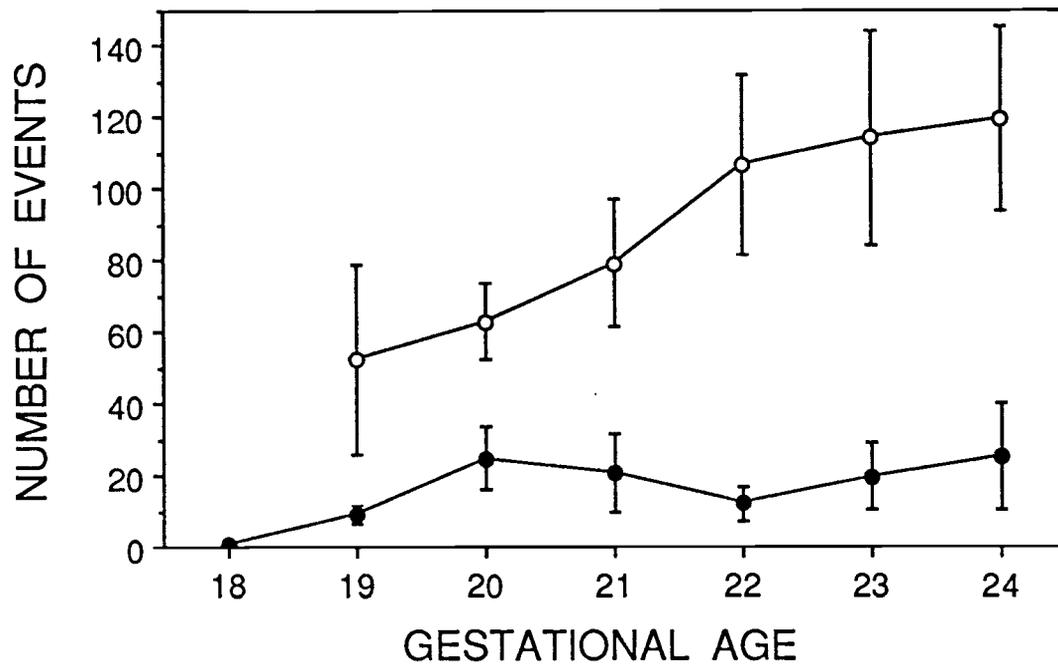


Figure 6.2. Incidence of movement synchrony during gestation in Meriones. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

$p < .001$) in Sigmodon. Post-hoc comparison of Age means collapsed across conditions indicated that movement synchrony increased in frequency early in gestation, remained roughly stable across most ages, then decreased significantly between the peak on day 25 and term (Table 6.1). Synchronous events occurred nearly three times as often ex utero as in utero (Figure 6.3).

In Acomys, movement synchrony varied as a function of both Age and Condition, as indicated by the significant interaction ($F_{5,49} = 5.3, p < .001$). Significant simple effects of age were evident both in utero and ex utero. Synchronous movements were almost non-existent on day 22 and were significantly more common in utero at later ages (Figure 6.4). Ex utero, synchrony increased after day 26, exhibited a high frequency of occurrence between days 28-32, then declined on days 34-37 (Table 6.1). At all ages, synchronous movements were much more common ex utero than in utero.

2. Relative abundance of movement synchrony

When expressed as a percentage of the total number of behavioral events, developmental changes in the expression of movement synchrony generally became much more apparent. In Rattus, the relative abundance of synchronous movements exhibited a significant interaction between Age and Condition ($F_{4,40} = 12.3, p < .001$). Simple effects of Age

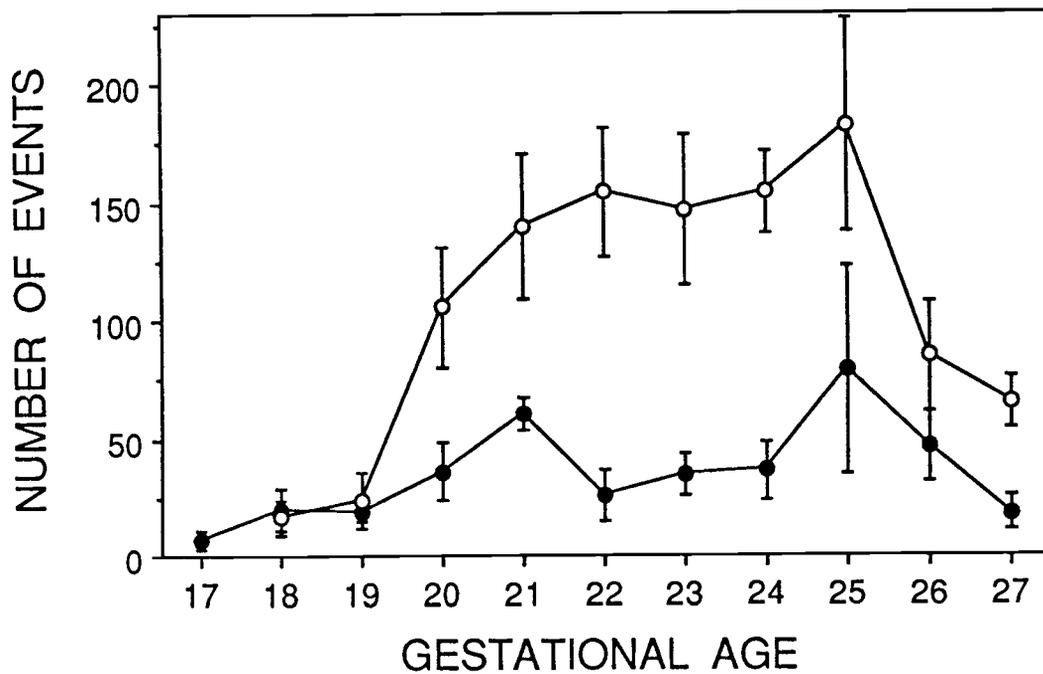


Figure 6.3. Incidence of movement synchrony during gestation in *Sigmodon*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

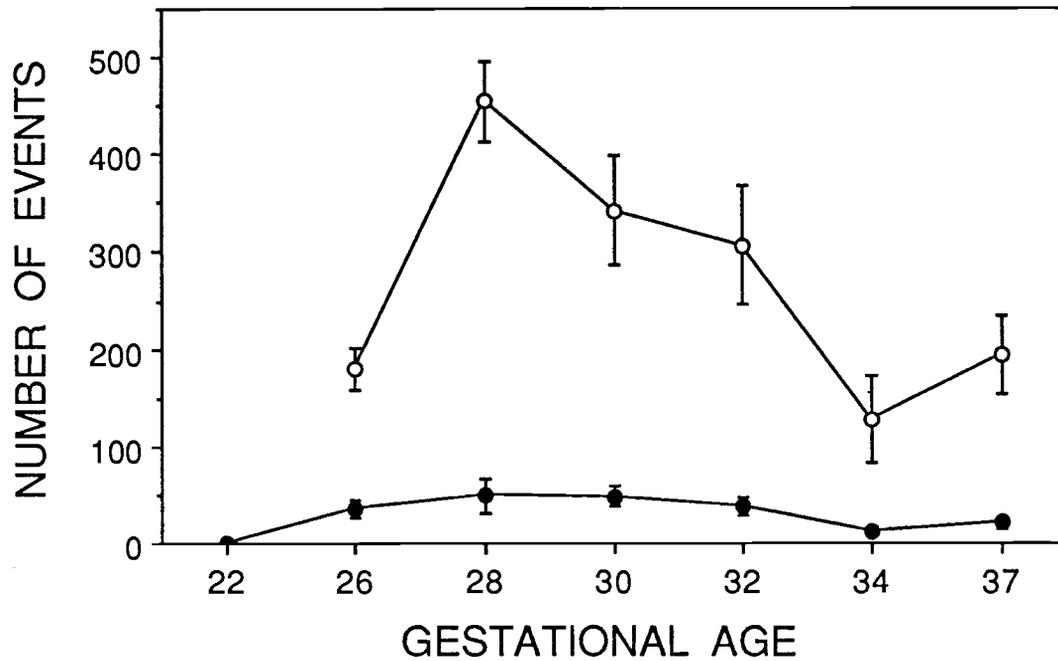


Figure 6.4. Incidence of movement synchrony during gestation in *Acomys*. Closed circles represent mean \pm SEM number of events per 30 min for fetuses in utero; open circles represent fetuses ex utero.

were revealed by post-hoc analyses both in utero and ex utero (Table 6.2). Fetuses observed in utero performed a smaller proportion of synchronous movements on days 16 and 21 than at other ages (Figure 6.5). Fetuses ex utero exhibited an increase in synchrony relative to overall activity over days 17-19 and synchrony remained relatively common through term. On days 19-21, the relative abundance of synchronous movements was greater ex utero than in utero.

In Meriones, the main effect of Condition was evident ($F_{1,48} = 46.4, p < .001$), with more events involving synchronous movement ex utero than in utero. Although synchrony was relatively uncommon in utero early in gestation, no significant variation as a function of fetal age was found (Figure 6.6).

A significant interaction between Age and Condition was indicated in Sigmodon ($F_{9,95} = p < .001$). Post-hoc tests for simple effects revealed no significant age-related variation in the relative abundance of movement synchrony in utero. However, synchronous movement events ex utero increased sharply in relative abundance from days 18-19 to a peak on day 25 (Figure 6.7). Simple condition effects were mixed, but through the middle portion of the fetal period indicated that synchronous movements were relatively more common ex utero than in utero (Table 6.2).

In Acomys, the overall ANOVA revealed significant main effects of Age ($F_{5,49} = 6.8, p < .001$) and Condition ($F_{1,49} = 100.8, p < .001$); the interaction was only marginally

Table 6.2

Summary of post-hoc comparisons in analyses of frequency of relative abundance of synchronous movement for four rodent species. Conventions as in Table 6.1.

SPECIES	INTERACTION (A X C)			
	MAIN EFFECTS		SIMPLE EFFECTS	
	AGE	CONDITION	AGE	CONDITION
RATTUS			In: 16<17=20>21	19: In < Ex
			Ex: 17<18<19=21	20: In < Ex
				21: In < Ex
MERIONES	19<20=24	In < Ex		
SIGMODON			Ex: 18=19<20<25=27	19: In > Ex
				21: In < Ex
				22: In < Ex
				23: In < Ex
				24: In < Ex
ACOMYS				25: In < Ex
				27: In < Ex
	26=34<37	In < Ex		
	In: 22<26=34<37			

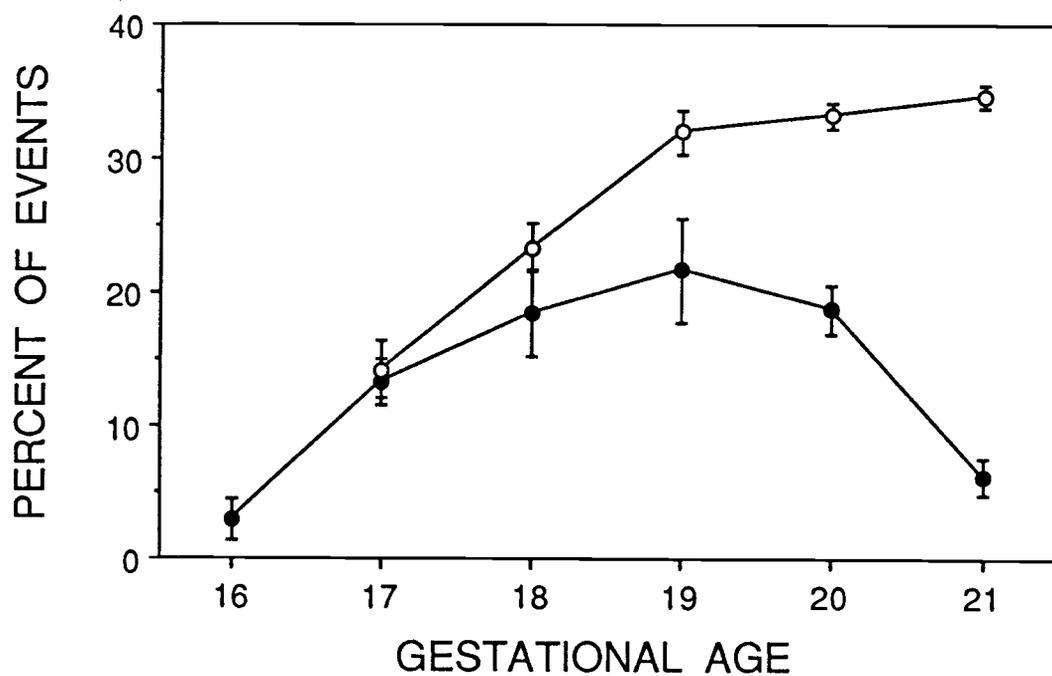


Figure 6.5. Movement synchrony expressed as a percentage of event activity during gestation in *Rattus*. Closed circles represent mean \pm SEM values for fetuses in utero; open circles represent fetuses ex utero.

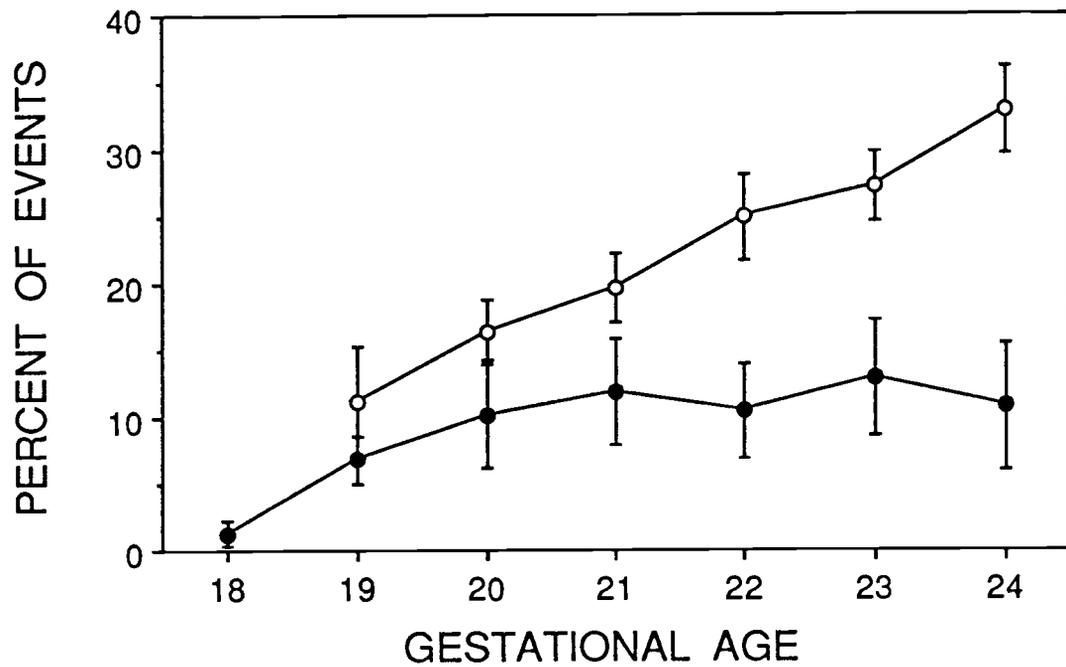


Figure 6.6. Movement synchrony expressed as a percentage of event activity during gestation in Meriones. Closed circles represent mean \pm SEM values for fetuses in utero; open circles represent fetuses ex utero.

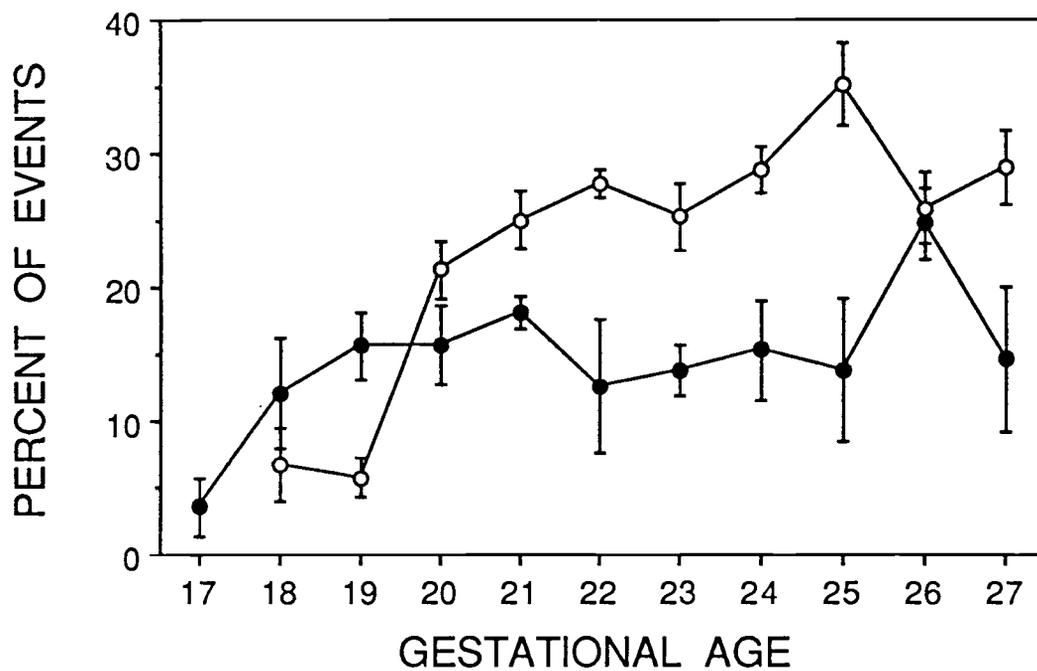


Figure 6.7. Movement synchrony expressed as a percentage of event activity during gestation in *Sigmodon*. Closed circles represent mean \pm SEM values for fetuses in utero; open circles represent fetuses ex utero.

significant. Post-hoc comparison of group means collapsed across conditions indicated that the relative abundance of synchronous movement decreased on day 37 (Table 6.2).

Further analysis of in utero data including day 22 also showed a significant effect of Age ($F_{6,28} = 7.4, p < .001$), with synchronous movements occurring least often on day 22, increasing in relative occurrence over days 26-34, and decreasing again on day 37 (Figure 6.8). On average, the proportion of events involving synchrony ex utero was more than double the rate in utero.

C. First Order Random Model of Movement Synchrony

Many investigators have noted that fetuses occasionally move two or more parts of the body at the same moment, a performance that seems to indicate central coordination of movement. However, co-occurrence does not necessarily imply coordination. The simultaneous occurrence of movement in two or more body regions could occur by coincidence. Especially in immature organisms that exhibit imperfectly coordinated behavior, the null hypothesis that movement synchrony arises through chance association of independent movement events must be considered. To test the null hypothesis of randomness, it is necessary to compare observed performances of synchrony against an objective, quantitative standard. The use of formal stochastic models to generate testable predictions about the incidence of

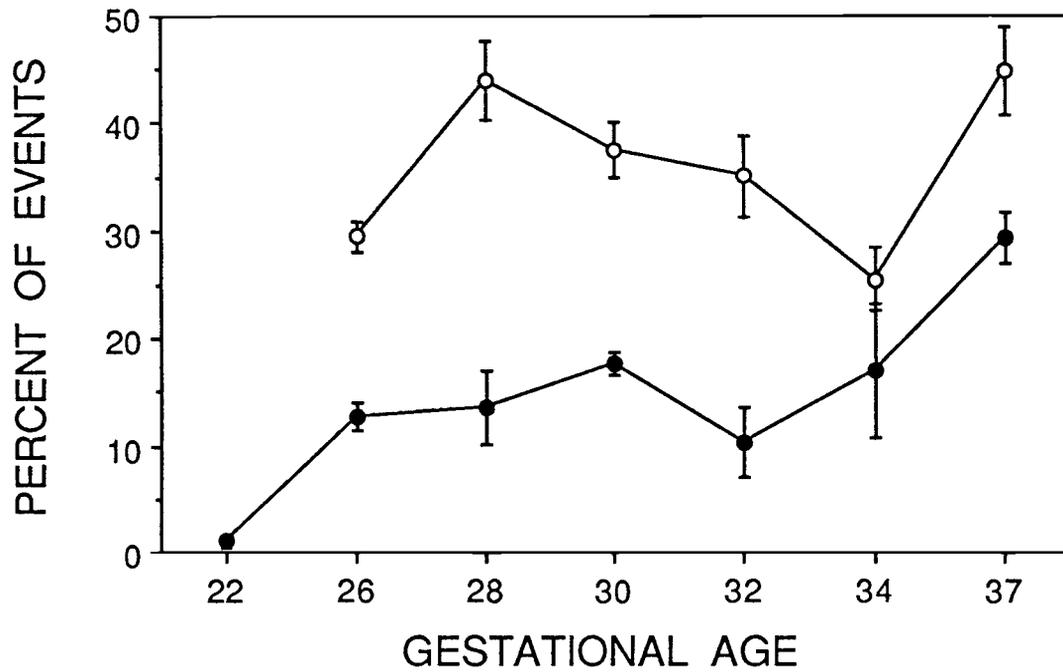


Figure 6.8. Movement synchrony expressed as a percentage of event activity during gestation in *Acomys*. Closed circles represent mean \pm SEM values for fetuses in utero; open circles represent fetuses ex utero.

movement synchrony can provide such a standard [Robinson & Smotherman 1988].

1. General characteristics of a random model

The mathematical approach adopted in the present study represents an important modification of an earlier random model applied to rat fetuses [Smotherman & Robinson 1986]. In the original model, the expected probability of a synchronous movement (P_s) was calculated as the joint probability of movement of two or more body regions. From basic probability theory, a joint probability is the product of two simple probabilities; thus, the simple probability (P) of a movement event involving a single component is squared to yield the joint probability of co-occurrence of two components. More generally, P^n is the probability of co-occurrence of n components. Predictions generated by a single-process product model of this sort are especially sensitive to the method of estimating P . In the previously reported case, P was estimated as the total number of movement events divided by the number of 1-s time bins (B) in the observation session.

As it happens, values for the parameters selected in the original model were fairly accurate in predicting the incidence of movement synchrony in rat fetuses [Smotherman & Robinson 1986]. But preliminary tests suggested that the underlying assumptions of a product model of this sort are

grossly violated under conditions of high fetal activity, when the estimator of P approaches 1.0. Specifically, the original random model was not robust when applied to Sigmodon and Acomys. Revision of the model was considered necessary for general application across species.

The problem of estimating P directly can be eliminated by assuming a single process that follows a Poisson distribution. The Poisson is an extension of the binomial distribution to the special case where P is very small, the number of time bins is very large, and their product (M) is a value of moderate size [Bhattacharyya & Johnson 1977]. M represents the rate or average number of times that an event occurs per unit time. Given the Poisson parameter M , the probability of x events occurring within a unit time can be calculated.

A formal postulate of the Poisson distribution is that events are instantaneous and the chance of two or more events occurring simultaneously is zero. Therefore, to adapt the Poisson to the issue of synchrony, only the onset of movement was regarded as instantaneous; it was considered possible for entire movement events, having finite duration, to overlap in time and appear synchronous. As a criterion of synchrony, the occurrence of two events within the same 1-s time bin was counted as an instance of synchronous movement involving two components. Thus, the predicted probability of synchronous movement (P_s) was calculated as the probability of two or more simple events, generated by a

Poisson process with parameter M , occurring within a 1-s bin:

$$P_S = 1 - (e^{-M} + e^{-M} M) \quad (6.1)$$

Multiplying P_S by the total number of 1-s bins (B) in a 30-min observation session (1800) yields a prediction of the total number of synchronous movements that should occur by random association.

The predicted incidence of synchronous movement was calculated separately for each individual fetus. Rather than subjecting each of these predictions to a separate statistical test, observed frequencies of synchronous movement were converted to a binomial test z-score. Each z-score was calculated thus:

$$z = (f_S - NP_S) (NP_S Q)^{-0.5} \quad (6.2)$$

In equation 6.2, f_S is the observed frequency of synchronous movements, N is the observed total number of movement events, P_S is the predicted probability of synchronous movement, and Q is $1 - P_S$, the predicted probability of simple events [Bakeman & Gottman 1986]. It may be noted that the z-score thus obtained is precisely equal to the square root of the goodness-of-fit Chi-Square with one degree of freedom computed from observed and expected frequencies of synchronous and simple events.

Although some critics have objected to assigning

probability values to binomial z-scores calculated in this manner [e.g., Gottmann & Bakeman 1979], caution is recommended only in samples where N is small, a situation that did not apply to the great majority of subjects. At the least, these z-scores provide an objective basis for comparison of the magnitude of deviation of observed frequencies of synchronous movement from predictions generated by the first order random model. Because the method of generating predicted frequencies yields a liberal estimate of random association, statistical significance can be inferred only from those comparisons that indicate the observed number of synchronous movements exceeds the prediction. Therefore, a one-tailed value of z ($z = 2.33$, $p = .01$) was used as the criterion of significant difference. To simplify comparison, a composite z-score was obtained by calculating the mean of individual z-scores within each combination of Age by Condition [Roscoe 1975].

2. Single-process estimate of M: overall fetal activity

The hypothesis of random association can be addressed at several levels of behavioral organization. At the simplest level (first order), no distinctions are made among categories of synchronous movement. The occurrence of all movement events are assumed to result from a single Poisson process. Initially, the parameter of this process, M, was estimated by dividing total Event Activity by the number of

1-s bins (B) within a 30-min observation session.

Composite z-scores, reflecting the relative agreement of observed and predicted frequencies of synchronous movement, are depicted in Figure 6.9 for Rattus. In this graph (and others to follow), two horizontal lines are plotted to facilitate interpretation of the relative magnitude of individual z-scores. The solid line displays $z = 0$, for which predicted and observed values are equal. The dashed line is positioned at the criterion value of z ($z = 2.33$); calculated z-scores above this line indicate that significantly more synchronous movements occurred within that combination of gestational Age and Condition than were predicted by the single-process random association model.

As is evident in Figure 6.9, synchronous movements occurred more frequently than could be attributed to random association among the majority of Rattus fetuses. At the inception of movement (day 16), synchronous movements occurred rarely and well within the predictions of the random model. Over days 17-20, movement synchrony was more abundant than predicted both in utero and ex utero. A significant decline in synchrony occurred on day 21 in utero, but was not evident ex utero at this age.

The same general pattern was evident in Meriones, which exhibited movement synchrony within levels predicted by random association on days 18-19 (Figure 6.10). Synchrony occurred more frequently than predicted at more advanced ages in both conditions. In fact, the incidence of

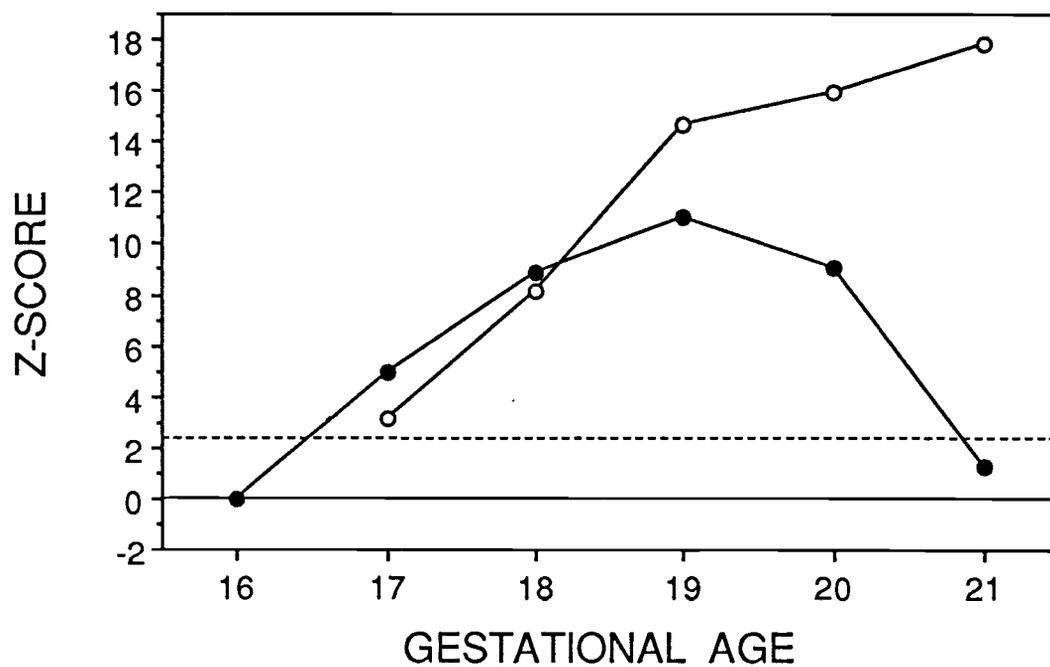


Figure 6.9. Composite z-scores depicting the relative agreement between the observed incidence of synchronous movement and the first order random association model (single process) in Rattus. The dashed line represents the criterion value of z ($z = 2.33$).

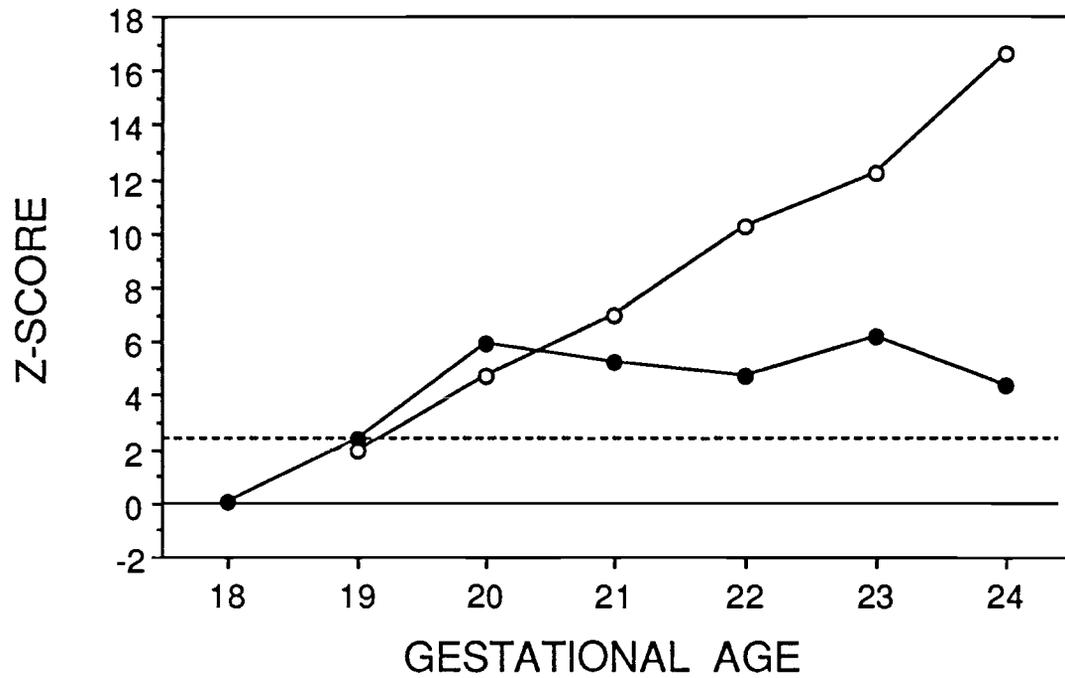


Figure 6.10. Composite z-scores depicting the relative agreement between the observed incidence of synchronous movement and the first order random association model (single process) in Meriones. The dashed line represents the criterion value of z ($z = 2.33$).

synchronous movements remained above random levels at term in utero, in contrast to the trend in Rattus.

In Sigmodon, the random model accurately predicted the incidence of synchrony at the inception of movement and on day 18 ex utero (Figure 6.11). Fetuses in all other groups expressed synchronous movements more often than predicted. Overall, a peak in the incidence of synchrony was evident on days 25-26. Although it appeared that synchronous movements declined in frequency on day 27 in utero, their rate of production remained above predictions of the random model.

Virtually identical results were obtained for Acomys (Figure 6.12). The random model based on a single Poisson process was sufficient to explain the occurrence of synchrony only at the inception of movement (day 22). Older fetuses all expressed synchronous movements more often than predicted by chance association.

3. Dual-process estimate of M: between and within bouts

The foregoing analysis demonstrated that the null hypothesis of random association provided an adequate explanation of the occurrence of synchrony at the inception of movement. Among older fetuses, though, the incidence of synchrony generally exceeded predictions generated by a single Poisson process. This finding may suggest the early emergence of centrally coordinated movement, but it does not rule out the possibility that synchronous movements arise

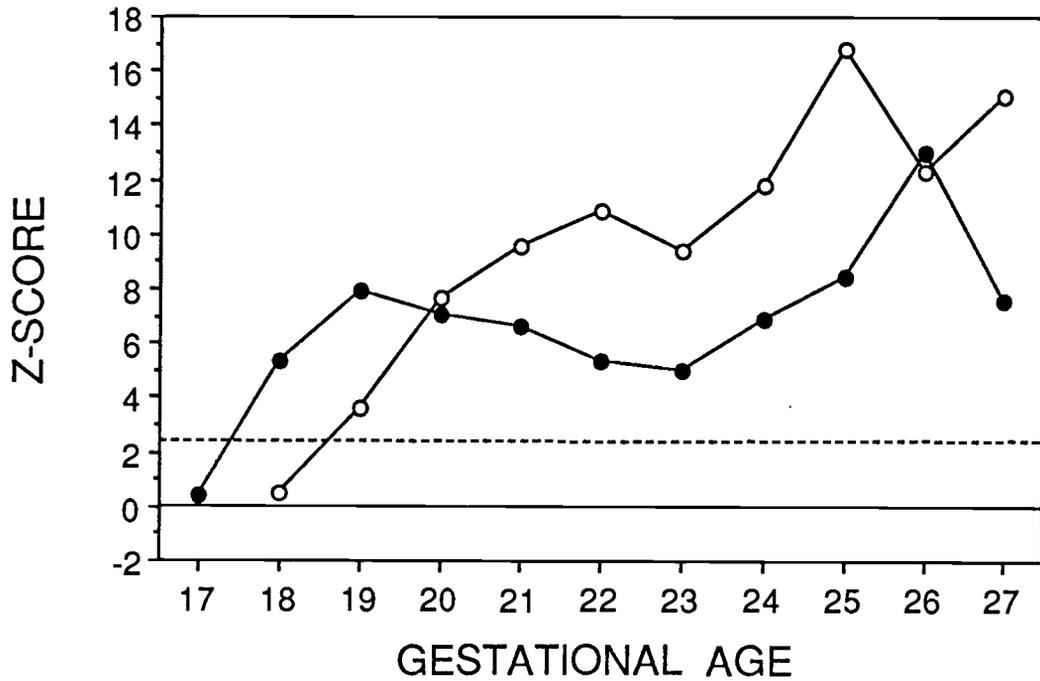


Figure 6.11. Composite z-scores depicting the relative agreement between the observed incidence of synchronous movement and the first order random association model (single process) in Sigmodon. The dashed line represents the criterion value of z ($z = 2.33$).

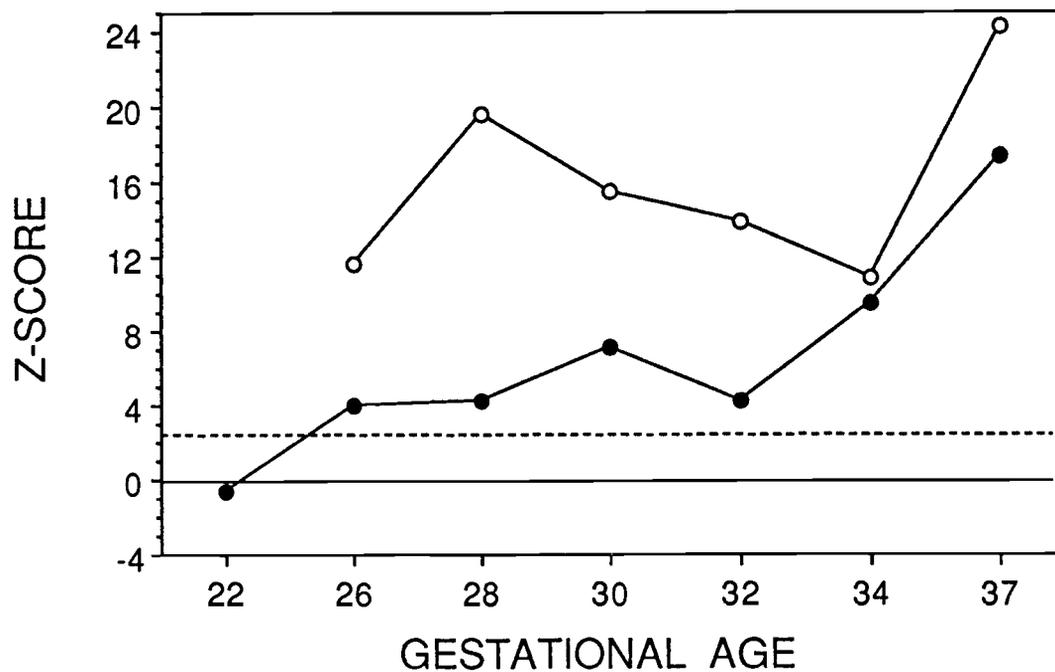


Figure 6.12. Composite z-scores depicting the relative agreement between the observed incidence of synchronous movement and the first order random association model (single process) in *Acomys*. The dashed line represents the criterion value of z ($z = 2.33$).

incidentally through random association of independent events. The reason why the predictions of a single process random model are inconclusive is fundamental: after the inception of movement, events tend to occur in bouts.

If events were generated by a single random process, then they would exhibit a random distribution in time. In fact, the exponential model of temporal patterning introduced in the previous chapter is derived from an assumption that events are generated by a single Poisson process. But at least two processes may be inferred from the observed distribution of inter-event intervals, one that generates intervals between bouts, and a second that generates shorter intervals within bouts [Nelson 1964; Machlis 1977].

If events tend to occur in clusters, then the chance of accidental temporal overlap within a bout is greatly increased. The existence of bout structure, therefore, leads to a greater predicted number of synchronous movements that arise from random association. To account for the effect of nonrandom temporal patterning, a second random model was developed that incorporates two Poisson processes. The parameter of the first process, M_1 , is the rate of activity generated between bouts. M_1 was estimated from the cumulative number of events predicted by the best-fitting negative exponential distribution of interval lengths, as obtained by the iterative algorithm described in Chapter 5.

The parameter of the second Poisson process, M_2 , is the

rate of activity within bouts. M_2 was estimated by calculating the difference between the observed number of intervals of a given length and the number of intervals predicted by the best-fitting negative exponential distribution. This difference, d_b , was obtained and summed across intervals 0-5 s in length. Of course, short intervals, and therefore events within bouts, were not generated over the entire course of an observation session. To obtain an accurate estimate of M_2 , the total elapsed time within bouts, t_b , was calculated by multiplying the number of intervals by interval length summed over all intervals 0-5 s in length. M_2 was then computed as d_b divided by t_b .

The predicted probability of synchronous movement was calculated separately for each Poisson process from equation 6.1, with the between-bout parameter M_1 yielding P_{S1} and the within-bout parameter M_2 yielding P_{S2} . Calculation of expected frequencies of synchronous movement differed slightly for the two processes. Because the between-bout process may be considered to produce some events at all interval lengths [Slater & Lester 1982], the contribution of the first process to the overall number of synchronous movements was calculated as the product of P_{S1} and B , the total number of bins in the observation session. The contribution of the within-bout process was obtained by multiplying P_{S2} by t_b . Thus, the overall frequency of synchronous movement predicted by a dual-process model of random association was given by the sum of the contributions

derived from the between-bout and the within-bout processes. As with the single process model discussed above, the observed incidence of synchronous movement was compared to predictions after conversion to a binomial test z-score.

The general expectation that movement synchrony was underestimated by the single-process model was confirmed. Predicted levels of synchronous movement were nearly always greater if nonrandom temporal patterning was incorporated into the random model. In Rattus, the effect of employing a dual-process model was to delay the emergence of nonrandom synchronous movement (Figure 6.13). Predictions of the random model accounted for the incidence of synchrony at the inception of movement and day 17, but generally not thereafter. A significant decrease in movement synchrony again was evident at term in utero.

The implications of a dual-process random model were very different for Meriones. The earliest age for which the observed incidence of movement synchrony exceeded predictions was day 21 ex utero (Figure 6.14). On days 19-20 ex utero, synchronous movements occurred at rates within predicted levels generated by the random model. After day 21, the incidence of synchrony remained above random levels ex utero. However, the hypothesis of random association was generally sufficient to account for synchronous movements in utero. Fetuses expressed synchronous movements more often than predicted only at one age in utero: day 23.

The dual-process model revealed a yet more complex

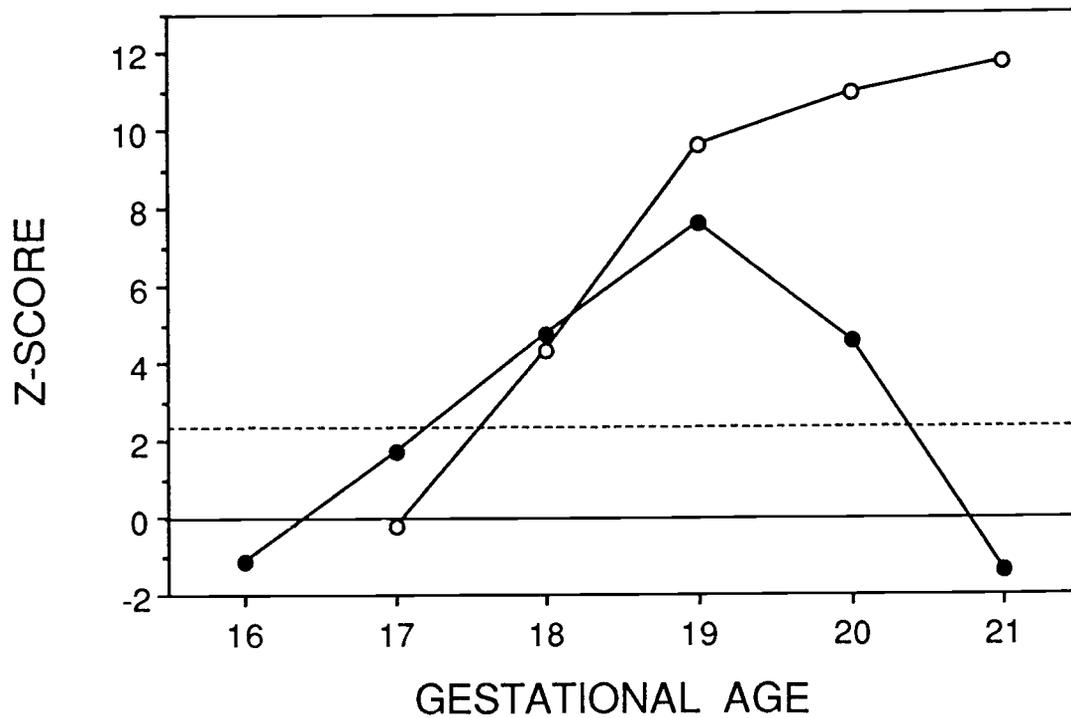


Figure 6.13. Composite z-scores depicting the relative agreement between the observed incidence of synchronous movement and the first order random association model (dual process) in Rattus. The dashed line represents the criterion value of z ($z = 2.33$).

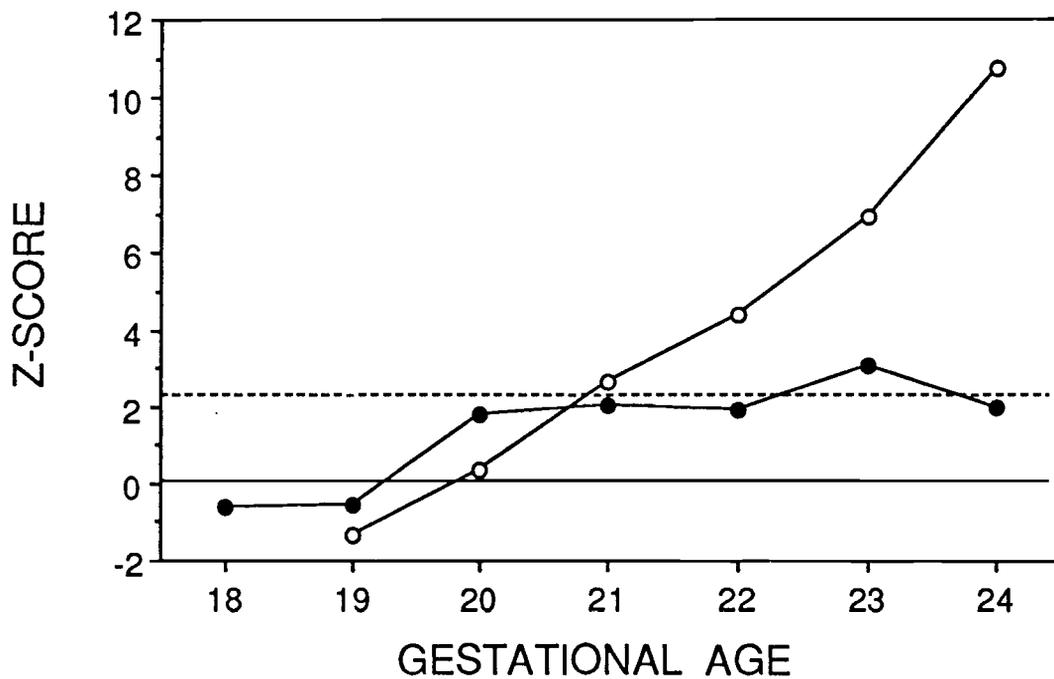


Figure 6.14. Composite z-scores depicting the relative agreement between the observed incidence of synchronous movement and the first order random association model (dual process) in *Meriones*. The dashed line represents the criterion value of z ($z = 2.33$).

pattern of expression of movement synchrony in Sigmodon (Figure 6.15). The random association model accurately predicted the expression of synchronous movement at the inception of movement and on day 18 for fetuses in both conditions. Ex utero, synchrony rose above random levels on day 20 and continued to be expressed significantly more than predicted through term. In utero, however, the expression of movement synchrony was bimodal, occurring above chance levels on days 19-20 and again on days 24-26. At all other ages in utero, including day 27, the incidence of synchronous movement did not differ significantly from levels predicted by chance.

The bimodal pattern of expression of movement synchrony also was evident in Acomys (Figure 6.16). All fetuses observed ex utero performed synchronous movements more often than could be explained by chance association. In utero, the dual-process random model adequately predicted the incidence of synchrony from the inception of movement through day 28. Synchronous movements occurred more often than predicted on day 30 and again on day 37, but did not differ from predictions on days 32-34 in utero.

D. Patterns of Linkage among Components

The analyses presented in sections B and C above treat all instances of synchronous movement as equivalent; no distinctions are drawn among different categories of

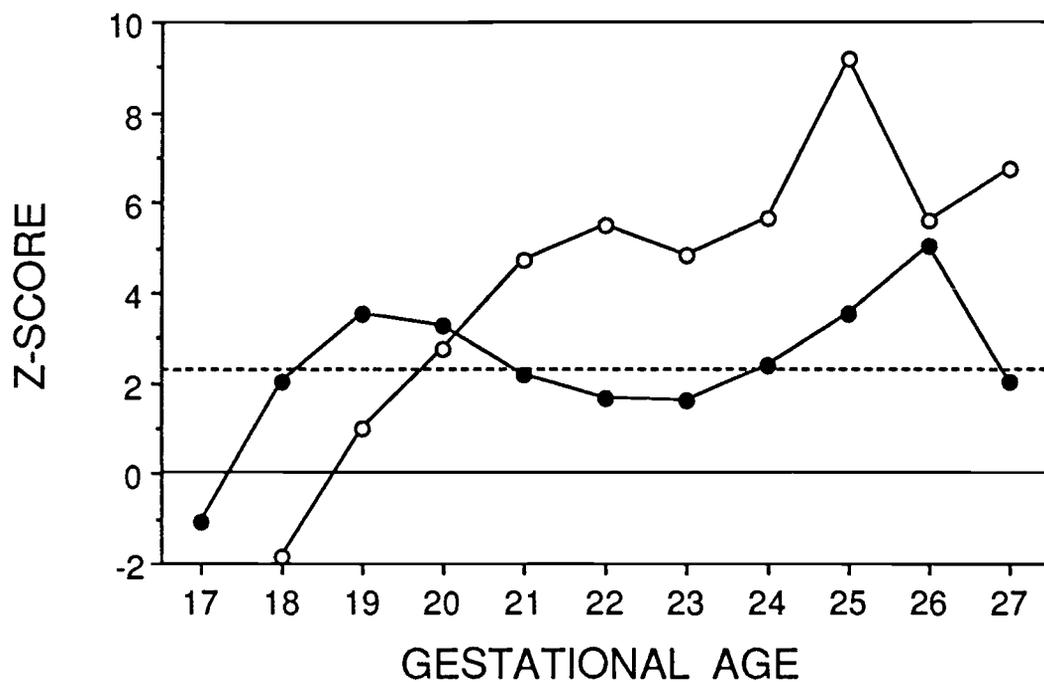


Figure 6.15. Composite z-scores depicting the relative agreement between the observed incidence of synchronous movement and the first order random association model (dual process) in Sigmodon. The dashed line represents the criterion value of z ($z = 2.33$).

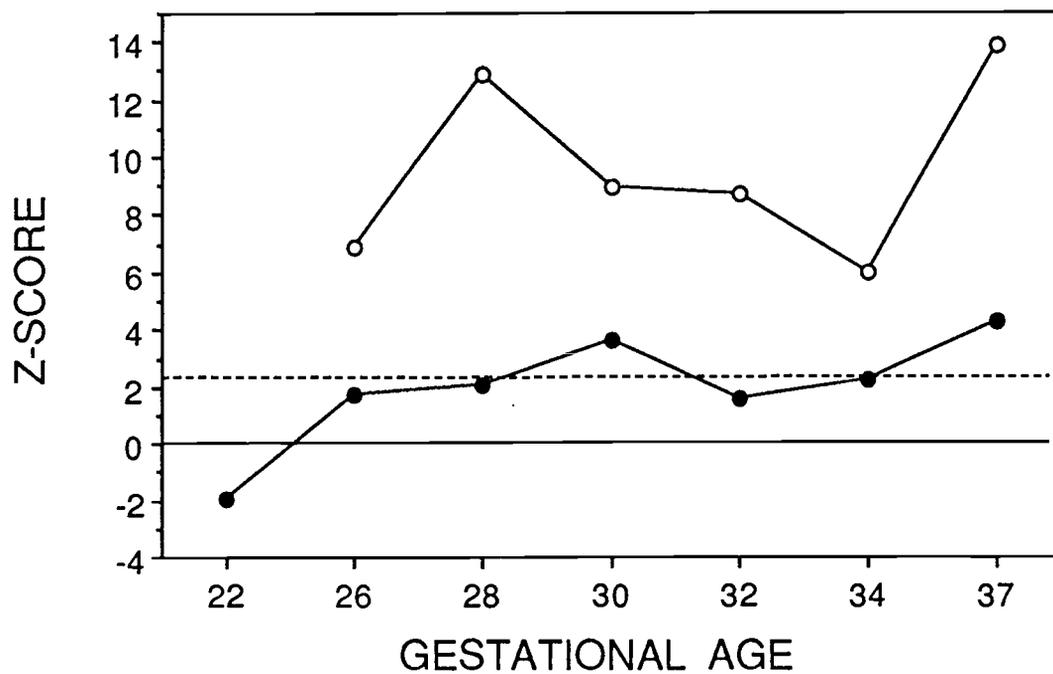


Figure 6.16. Composite z-scores depicting the relative agreement between the observed incidence of synchronous movement and the first order random association model (dual process) in *Acomys*. The dashed line represents the criterion value of z ($z = 2.33$).

synchrony or among the individual components that are associated in synchronous events. It was apparent, however, that certain combinations of components were associated in synchronous events much more often than other combinations. As a preliminary approach to description of more detailed patterns of synchronous movement, a concept of linkage was formulated. An instance of synchronous linkage was defined as the co-occurrence of a pair of component movements. In synchronous events consisting of two components, only one linkage relationship, specified by the unique pair of components, was considered present. In synchronous events comprising three or more different components, a different linkage relationship was specified by each unique pair of constituent components. Thus, in the synchronous event FHR, three patterns of linkage are apparent: F-H, F-R, and H-R.

By recognizing five basic body regions (F, R, H, T, M), ten categories of linkage are possible. The average frequency of occurrence of each category was calculated for subjects in each Age by Condition group. Rather than comparing average linkage scores within isolated categories to assess change across ages or conditions, it was considered more important to examine developmental changes in the overall pattern of linkage relationships. To visually display the linkage pattern for a particular age, an association diagram [Sustare 1978] was constructed that depicted all five regional components and pairwise linkages among them. The occurrence of linkage was represented by a

line connecting two components. To exclude rare categories, only linkage categories that exhibited a mean frequency of one instance per 30-min of observation were included in the diagram. Because synchronous movements were very uncommon in all four species at the inception of movement, this age was not included in the diagrams. This method of graphically describing patterns of synchronous movement has been reported previously in studies of rat fetuses [Robinson & Smotherman 1987, 1988].

Linkage diagrams depicting patterns of synchronous movement in Rattus are presented in Figure 6.17. It is apparent from inspection of these diagrams that two categories of linkage predominated throughout gestation: F-H and F-R. Other patterns, such as F-M and H-M, were expressed consistently but at lower frequency. The linkage H-R was evident at most ages, particularly ex utero. However, closer scrutiny indicated that virtually all of the instances of H-R linkage occurred within synchronous movements consisting of three or more components; the doublet HR, in contrast to FH and FR, was exceedingly rare. The linkages expressed in utero appeared to represent a subset of the linkages expressed ex utero.

The linkage diagrams constructed for Meriones were generally similar in appearance to Rattus (Figure 6.18). The linkages F-H, F-R and H-R were observed at all ages ex utero, F-M and H-M at most ages. As in Rattus, linkage relationships appeared simplified in utero, with only F-H

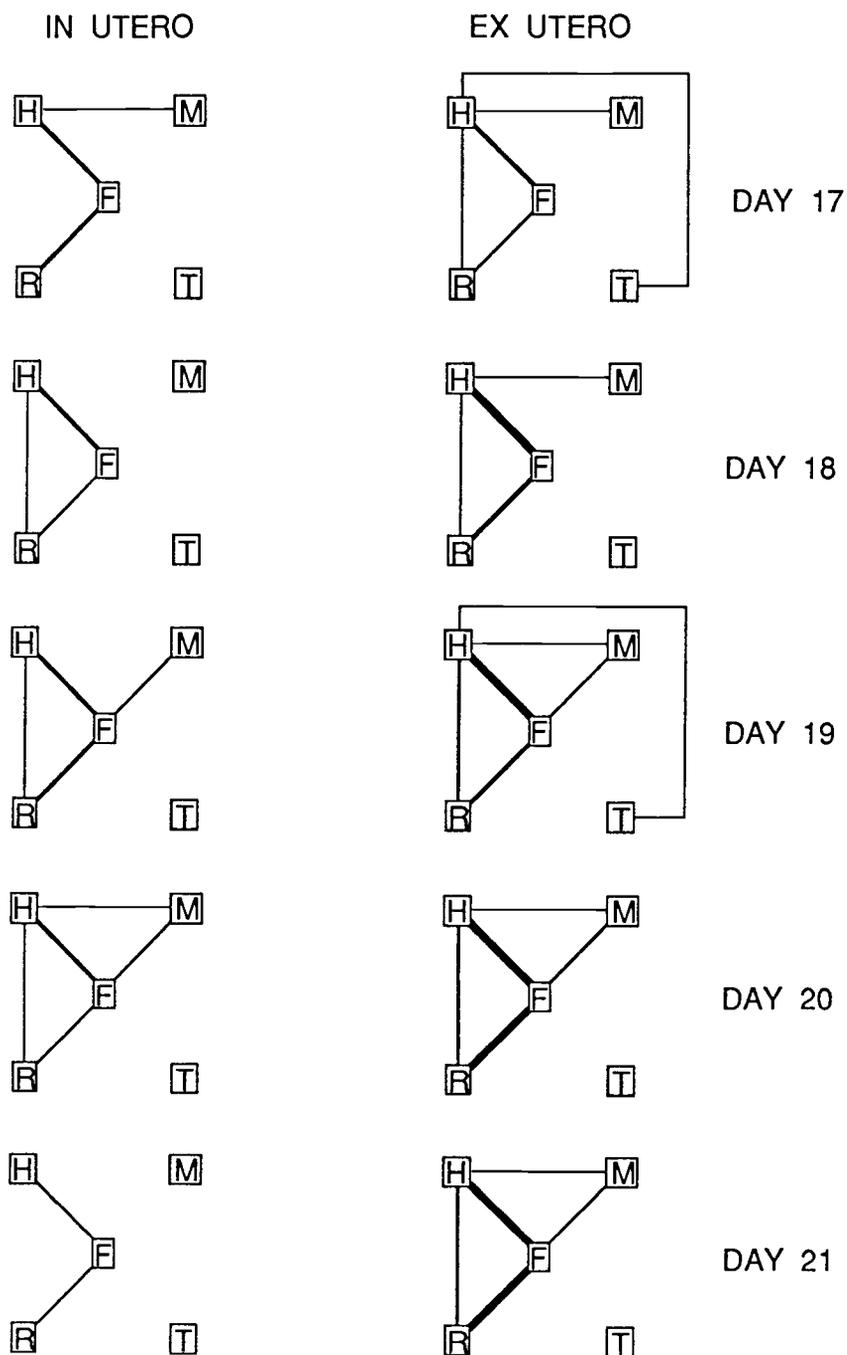


Figure 6.17. Patterns of synchronous linkage between pairs of movement categories in *Rattus*. Separate linkage diagrams are presented for each age and condition of observation. Lines connecting two categories represent a mean frequency of synchronous movement exceeding once per 30-min. Wider lines represent greater frequency of linkage.

Figure 6.18. Patterns of synchronous linkage between pairs of movement categories in Meriones (next page). Separate linkage diagrams are presented for each age and condition of observation. Lines connecting two categories represent a mean frequency of synchronous movement exceeding once per 30-min. Wider lines represent greater frequency of linkage.

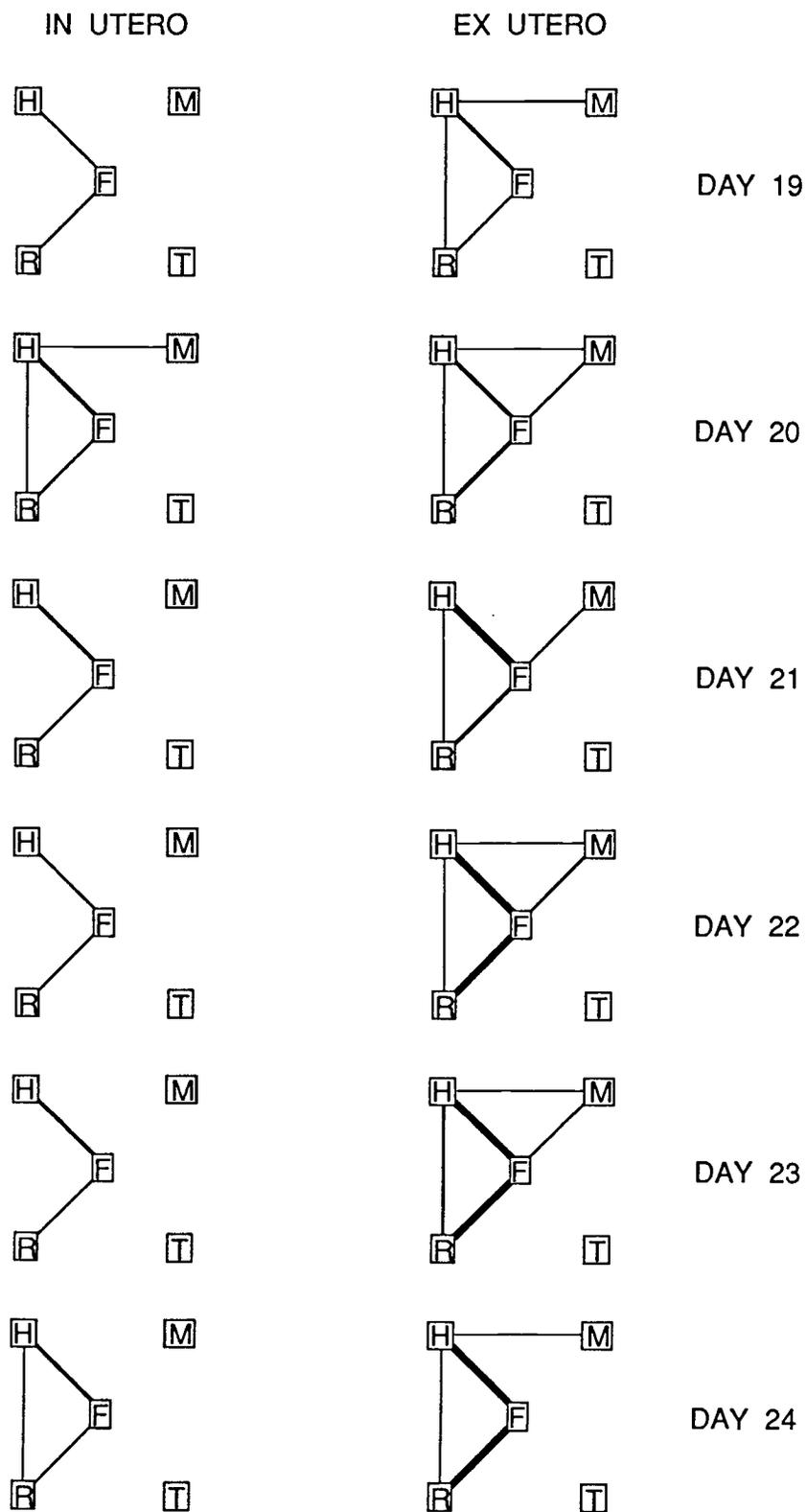


Figure 6.18 continued.

and F-R expressed at most ages.

A trend of increasing complexity of the diagrams with gestational age was noticed in Sigmodon (Figure 6.19). As was evident in the altricial species, linkage relationships in utero were fewer in number, giving the entire diagram the appearance of relative simplicity. The extent of the difference in utero and ex utero, however, was accentuated with advancing age. On day 21, for example, the patterns of linkage expressed in different conditions were identical. By day 24, linkage relationships were about the same in utero, but greatly expanded ex utero. In fact, on days 24-25 ex utero, virtually every pairwise combination of components was observed more often than the criterion frequency (once per 30-min). The overall pattern of linkage became greatly simplified over the last two days of gestation. For most ages and conditions, F-H and F-R were clearly the most abundant linkages between component movements.

Complex patterns of synchronous linkage were evident at an early age in Acomys (Figure 6.20). Nearly all combinations of components were linked on day 28 ex utero and remained so through day 34. As in other species, linkage diagrams for fetuses in utero were relatively simpler in appearance and generally exhibited less change through gestation. F-H and F-R were far more abundant than other linkages.

The subjective impression of overall simplicity or

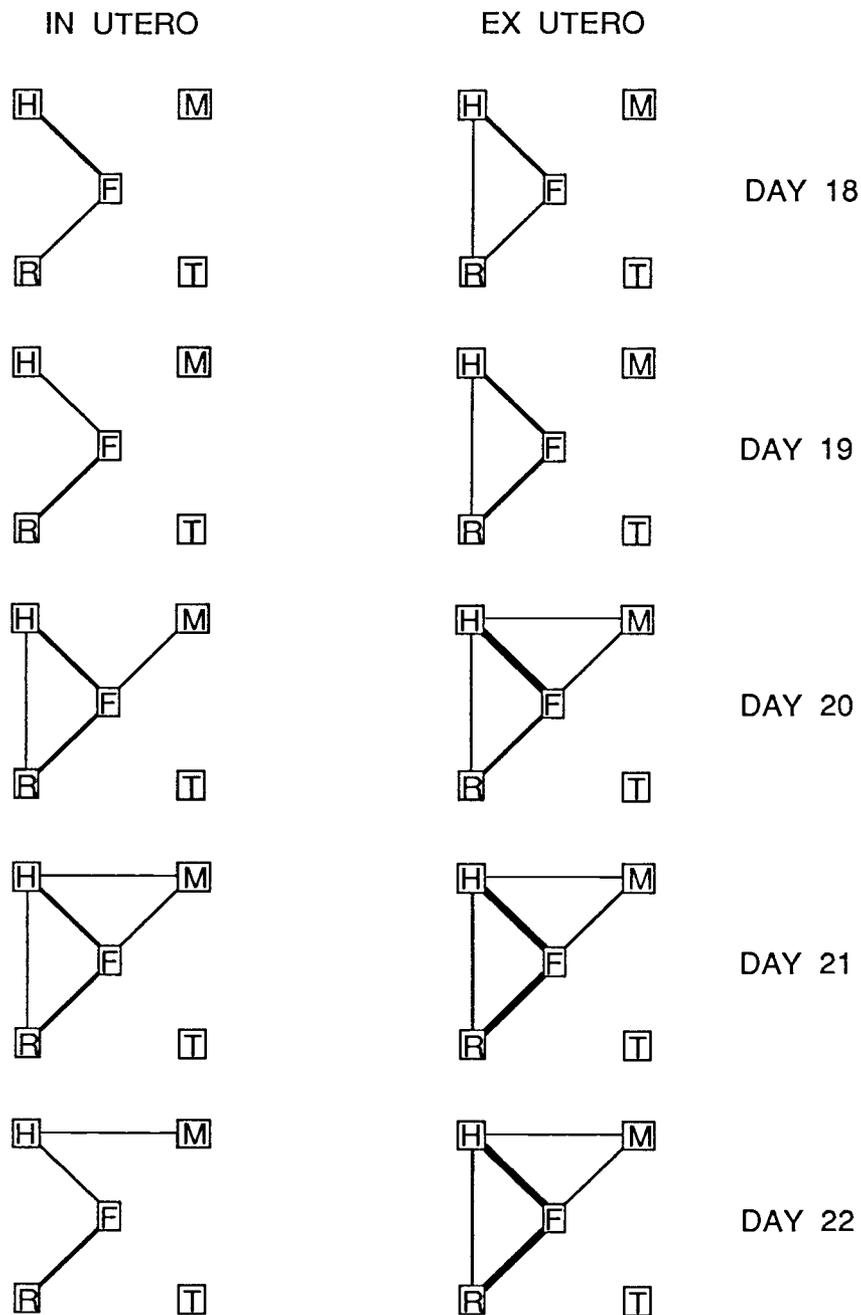


Figure 6.19. Patterns of synchronous linkage between pairs of movement categories in Sigmodon. Separate linkage diagrams are presented for each age and condition of observation. Lines connecting two categories represent a mean frequency of synchronous movement exceeding once per 30-min. Wider lines represent greater frequency of linkage. (Figure continued on next page.)

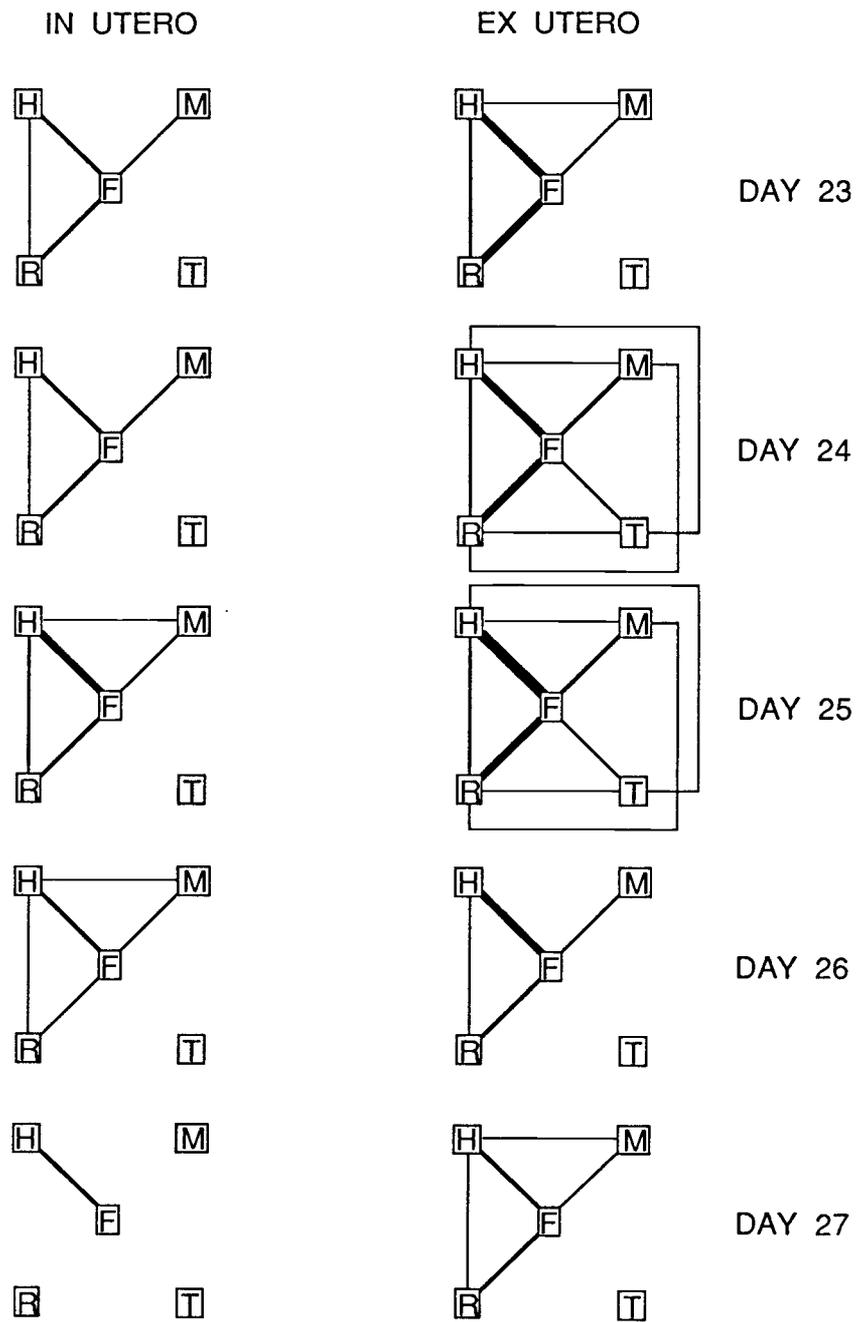


Figure 6.19 continued.

Figure 6.20. Patterns of synchronous linkage between pairs of movement categories in Acomys (next page). Separate linkage diagrams are presented for each age and condition of observation. Lines connecting two categories represent a mean frequency of synchronous movement exceeding once per 30-min. Wider lines represent greater frequency of linkage.

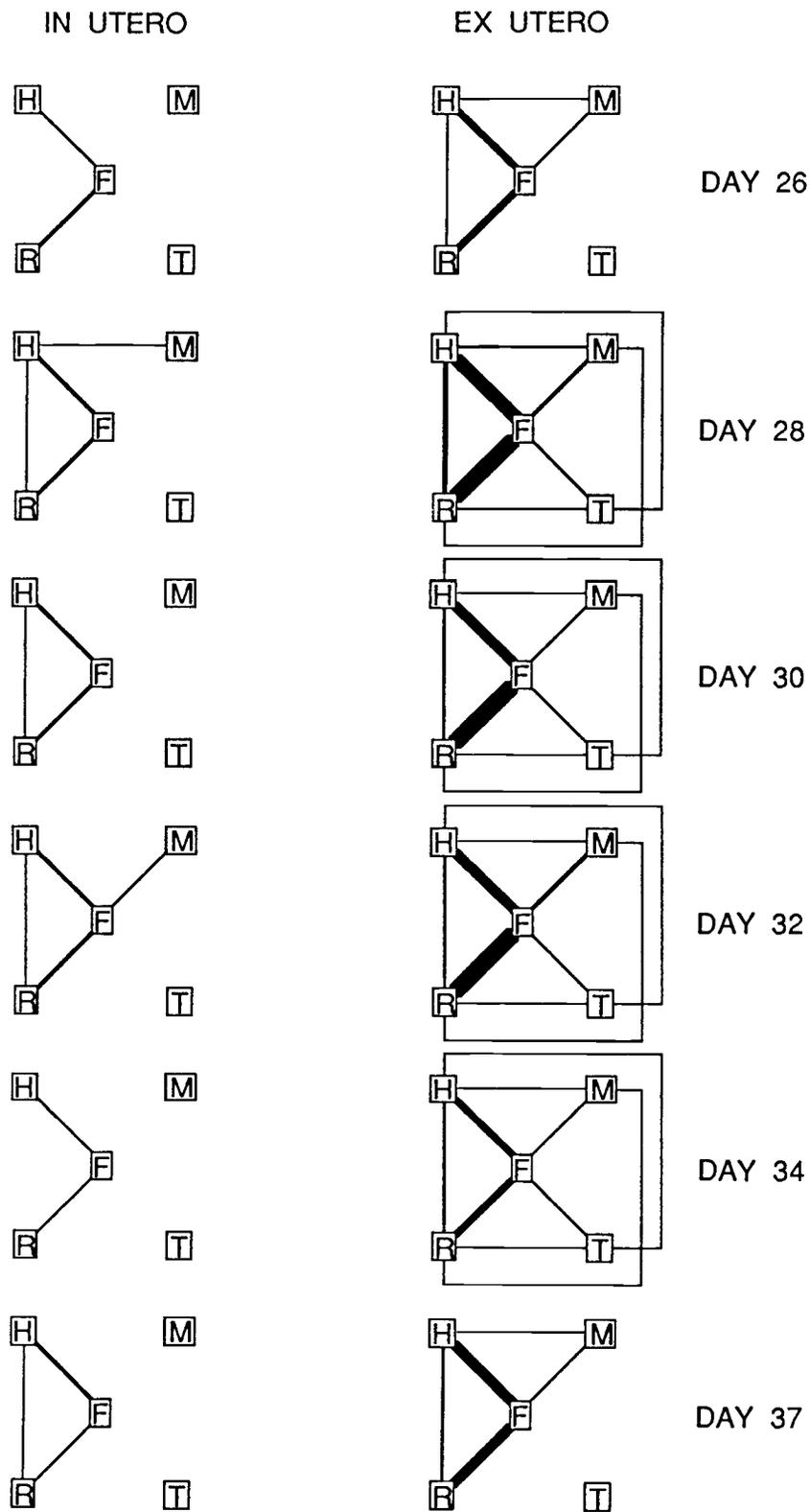


Figure 6.20 continued.

complexity of the linkage diagrams is simply a function of the number of linkages expressed. Fluctuations in the number of observed linkages may be taken as an approximate quantitative descriptor of changes in the overall pattern of movement synchrony across ages and conditions. Figure 6.21 depicts variation in the number of linkages expressed during gestation for Rattus. The number of linkages, parallel to the overall number of synchronous movements, was generally greater ex utero than in utero. The most striking change depicted in this graph occurred between days 20-21, when the number of linkages expressed ex utero remained stable, but dropped precipitously in utero.

The trend for differential expression of movement synchrony in utero and ex utero was clearly evident in Meriones (Figure 6.22). However, within each condition the number of linkages did not generally increase or decrease with age. At term, the number of linkages expressed in utero did not decrease as in Rattus.

Although age-related change in the number of linkages was relatively subtle in the altricial species, it was quite pronounced in Sigmodon (Figure 6.23). Linkage relationships were relatively similar over days 18-21, but diverged strikingly after day 23. The peak in number of linkages expressed ex utero on days 24-25 was not evident in utero. However, the decrease in linkages ex utero on day 26 was followed one day later by a comparable decrease in utero.

A similar pattern of development of linkage

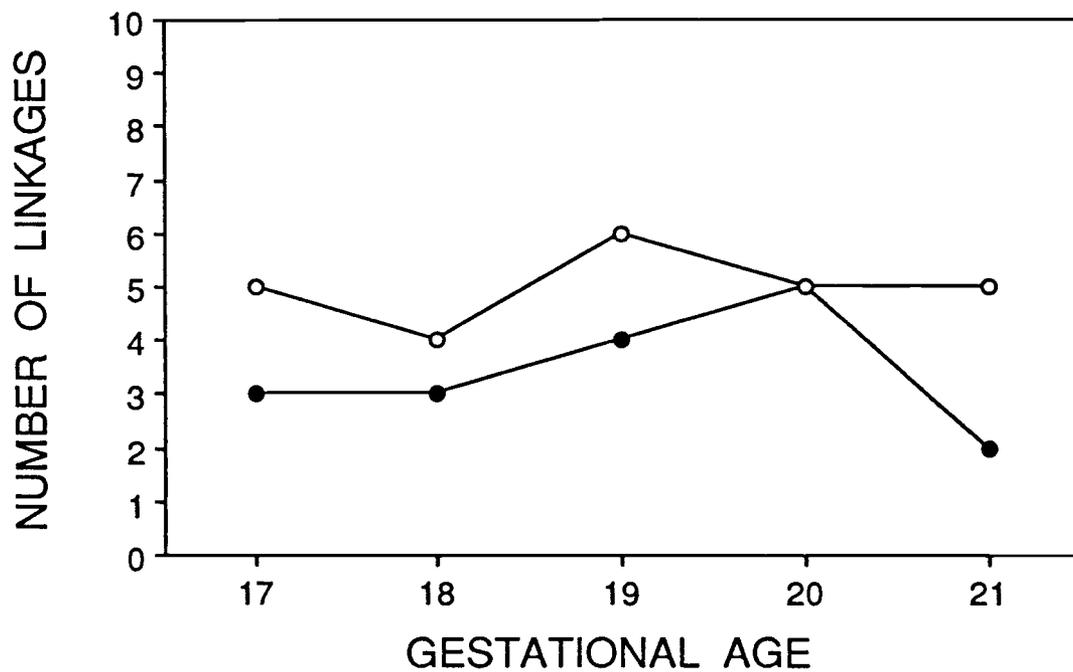


Figure 6.21. Number of synchronous linkages between pairs of movement categories exceeding once per 30-min in Rattus. Closed circles represent fetuses observed in utero; open circles represent fetuses ex utero.

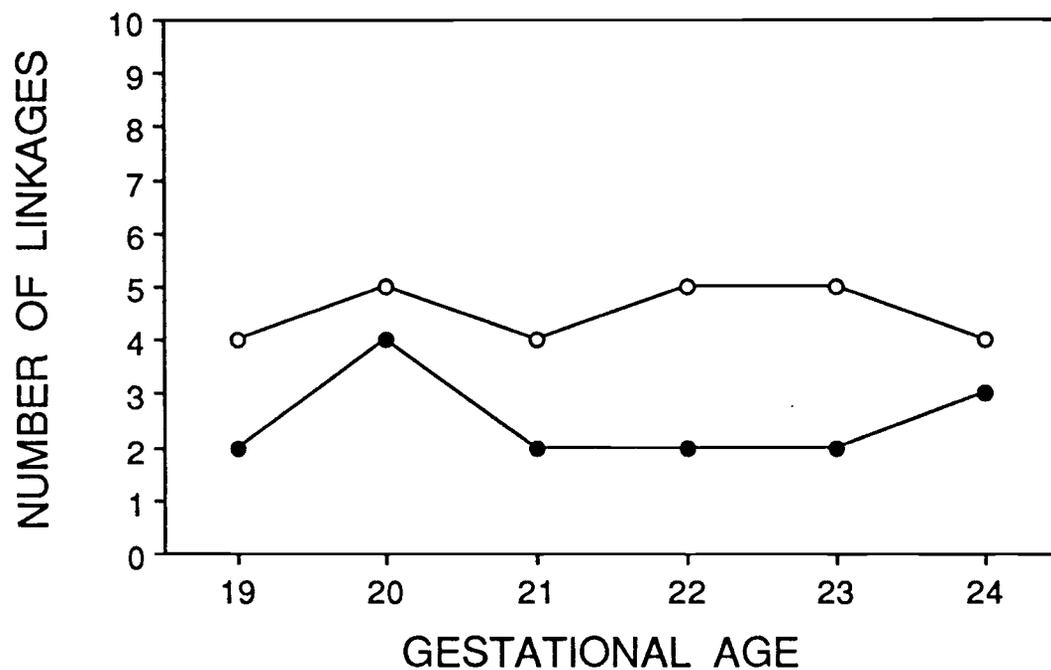


Figure 6.22. Number of synchronous linkages between pairs of movement categories exceeding once per 30-min in Meriones. Closed circles represent fetuses observed in utero; open circles represent fetuses ex utero.

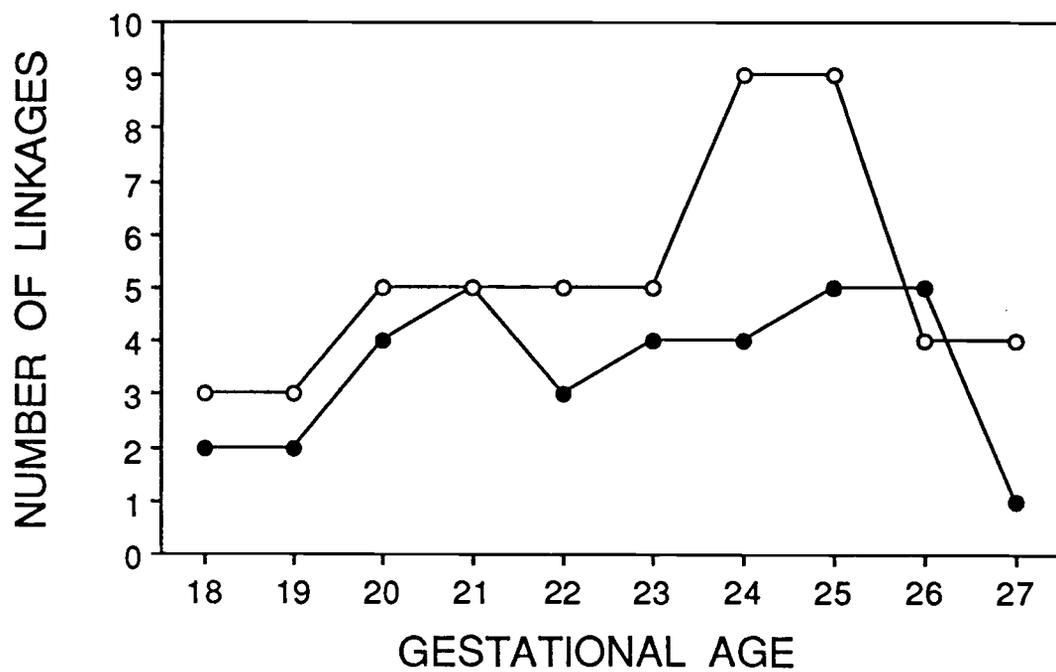


Figure 6.23. Number of synchronous linkages between pairs of movement categories exceeding once per 30-min in *Sigmodon*. Closed circles represent fetuses observed in utero; open circles represent fetuses ex utero.

relationships was evident in Acomys (Figure 6.24). At all ages, the number of linkages expressed was greater ex utero than in utero. Although no overall increase or decrease was evident in utero over the course of gestation, the number of linkages observed ex utero increased sharply on day 28 to a broad peak that persisted through day 34. The decline in linkage relationships expressed on day 37 ex utero was not evident in utero.

Considered as a whole, patterns of synchronous linkage appeared to be most affected by (a) the conditions under which fetuses were observed (in utero versus ex utero), and (b) the developmental status of the species (altricial versus precocial). In almost all cases, more linkages were observed ex utero than in utero. Because the predominant linkages, namely F-H and F-R continued to be expressed in both conditions, the principal effect of the in utero environment was to constrain or inhibit expression of rarer patterns of synchronous linkage. The condition effect was most pronounced in precocial species, which exhibited a developmental peak in the number of linkages expressed ex utero that was not apparent in altricial species. This finding may indicate a greater diversity as well as greater abundance of synchronous movements in precocial species.

E. Second Order Random Model of Movement Synchrony

The first order random models presented in section C



Figure 6.24. Number of synchronous linkages between pairs of movement categories exceeding once per 30-min in Acomys. Closed circles represent fetuses observed in utero; open circles represent fetuses ex utero.

were developed to test the null hypothesis that the total frequency of synchronous movements is a function solely of the overall incidence of fetal movement events. Examination of linkage relationships among pairs of body regions, presented in section D, indicated that more detailed distinctions among categories of synchronous movement are warranted. The conjunction of these two ideas raises the question of whether the relative abundance of different categories of synchrony is predictable on the basis of random association of component movements. A second order random model has previously been described which addresses this question in rat fetuses [Robinson & Smotherman 1987]. A slightly modified version of this model was applied to all four rodent species in the present study.

1. Characteristics of the second order random model

Unlike a first order random model, the predictions of a second order model are not dependent on the overall rate of fetal activity. Rather, predictions are made about the relative distribution of events among different categories of synchronous movement. To apply a second order random model in the present study, ten categories of synchronous movement were selected for quantitative testing, including seven doublets (FH, FM, FR, FT, HM, HR, HT) and three triplet combinations of components (FHM, FHR, FHT). Observed and expected frequencies of all other potential

combinations of components were too small to permit meaningful statistical comparison. In overview, the model involves estimation of the probability of occurrence of a particular synchronous category as the product of the probabilities of occurrence or non-occurrence of all five components.

As inputs to the model, simple probabilities of occurrence were obtained for each regional component (F, R, H, M, T). The simple probability of movement (P_r) involving the regional component r was estimated as the observed frequency of component r divided by the total number of fetal movement events:

$$P_r = \frac{f_r}{\sum_{i=1}^n f_i} \quad (6.3)$$

In equation 6.3, f_r is the observed number of movements of component r and n is the total number of recognized components ($n = 5$). The complement Q_r of these simple probabilities was also calculated for each component as $1 - P_r$.

Probabilities of component movements and their complements were used to compute joint probabilities of movement involving two or more components. For example, the joint probability of synchronous movement involving only components 1 and 2 was defined as $P_{1,2} = P_1 P_2 Q_3 Q_4 \dots Q_n$. In addition to producing joint probabilities which estimated

the likelihood of synchronous movement involving two or more components (e.g., $P_{1,2}$), this method of calculation also produced joint probabilities of non-synchronous events (involving movement of one component in isolation) and of non-events (involving no movement). The probability of non-events was calculated for logical completeness as $Q_0 = Q_1 Q_2 Q_3 \dots Q_n$. Obtaining a value of Q_0 permitted computation of the overall probability of simple (non-synchronous) events, and the complement of this probability plus Q_0 thus yielded the overall probability of synchronous movement (P_s).

Once values for these various probabilities were obtained, the only remaining step in the second order random model was to generate a predicted frequency of synchronous movements for each category involving a specified set of regional components. For example, the predicted frequency ($f_{1,2}$) of the synchronous category including components 1 and 2, but no other components, was calculated as:

$$f_{1,2} = \frac{P_{1,2} f_s}{1 - Q_0 + \sum_{i=1}^n \frac{P_i Q_0}{Q_i}} \quad (6.4)$$

where f_s is the observed total frequency of synchronous movement. The term in the denominator after the summation sign is the probability of non-synchronous movement involving component i . Thus, the denominator as a whole provides a value for the probability of overall synchronous

movement, P_s .

The net result of this second order model is to generate a predicted frequency for each focal category of synchronous movement that reflects the relative abundance of its constituent components, but which is not directly related to the overall frequency of fetal movement. The model is internally consistent, because predicted frequencies calculated for all possible combinations of components sum to the observed number of synchronous movements.

As discussed in section C, observed frequencies of movement in each focal category of synchrony were compared to predictions generated by the model after transformation to a binomial test z-score. Each z-score was calculated in accordance with equation 6.2, with the necessary modifications that f_s represents the observed frequency of movement in the focal synchronous category s , P_s is the predicted probability of synchronous movement in category s , Q is $1 - P_s$, and N is the total number of synchronous movements. As above, composite z-scores were calculated as the average of the individual z-scores within each Age by Condition group for each of the ten focal categories of synchrony. Because the second order model was based on the expected relative abundance of different categories among overall synchronous activity, observed frequencies that differed from predictions in either direction (greater or less) could be considered. However, because the overall

number of comparisons was large (10 categories for each combination of Age by Condition), greater emphasis was placed on the overall patterns of results than on the significance of particular comparisons. A less stringent two-tailed criterion for significance was judged most appropriate for outlining these patterns ($z = 1.96$, $p = .05$).

2. Results of second order random model

Across all four species the second order model appeared to be broadly predictive of the distribution of movements among ten focal categories of synchrony. In Rattus, composite z-scores indicated deviation from model predictions in 19 of 110 comparisons (17%). A pattern of deviation was apparent in four of the ten focal categories of synchronous movement. The category FH (Fore-Head) consistently occurred more often than predicted among fetuses observed ex utero and were more abundant in utero on days 18-20 of gestation (Figure 6.25). The synchronous movement category FR also exceeded predictions ex utero on the last two days of gestation, but increased incidence was not apparent in utero (Figure 6.26). HR movements occurred less often than expected on days 19-21 ex utero (Figure 6.27). FT movements also appeared to be reduced relative to predicted frequencies on days 17-20 ex utero. Isolated marginal differences were observed on day 18 for the category FHR (less frequent) and day 17 for FHT (less frequent).

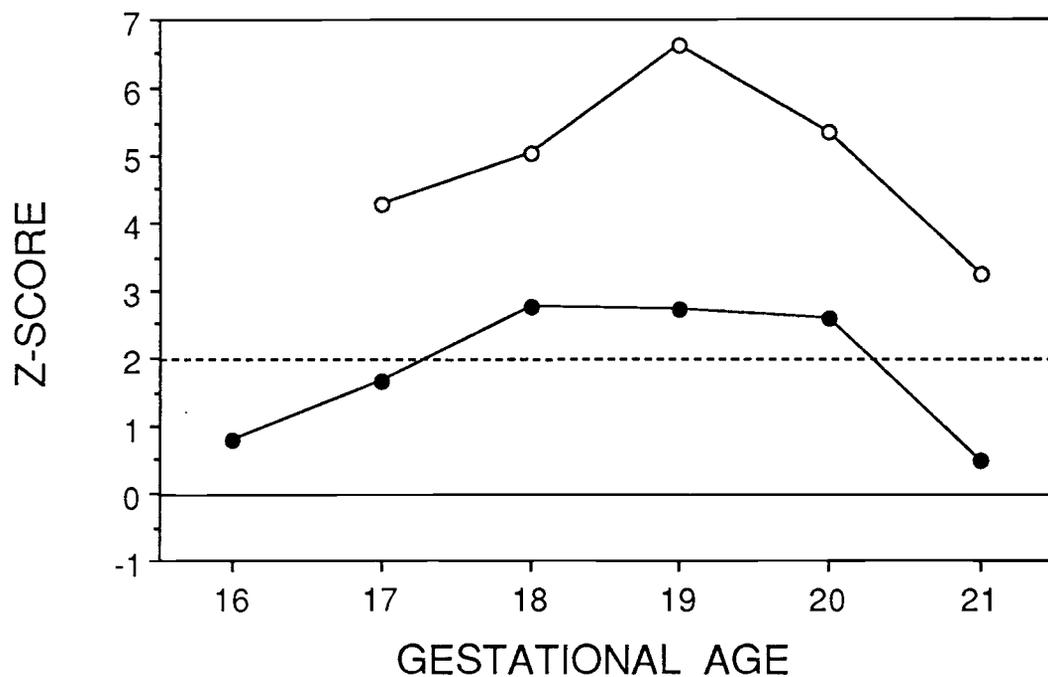


Figure 6.25. Composite z-scores depicting the relative agreement between the observed incidence of forelimb-head (FH) synchrony and the second order random association model in Rattus. The dashed line represents the criterion value of z ($z = 1.96$).

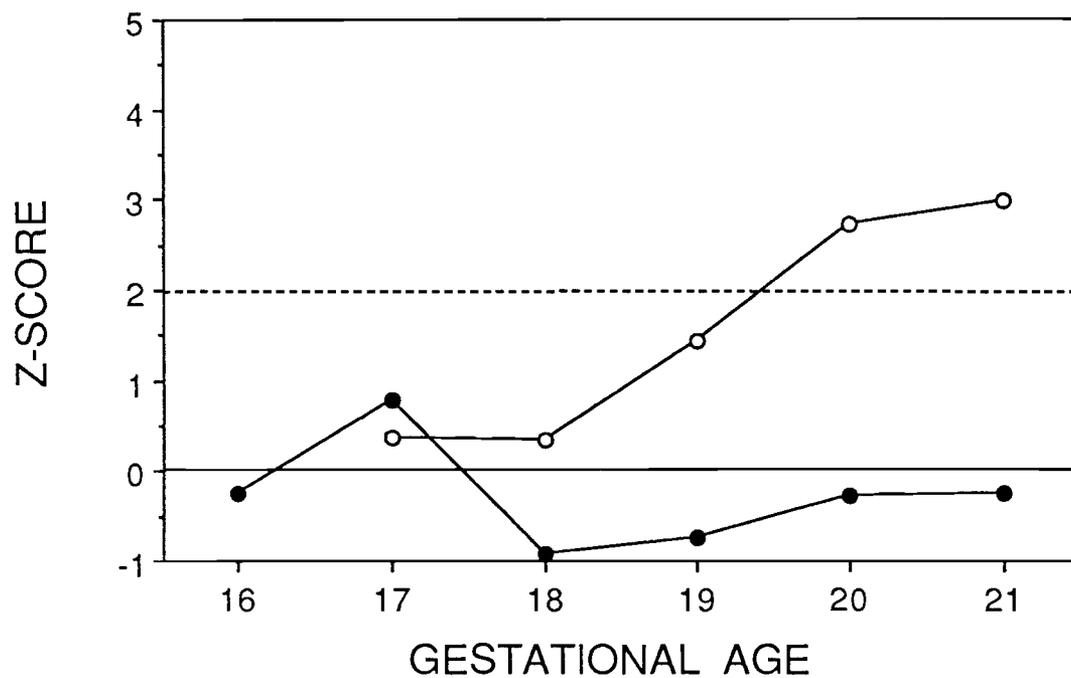


Figure 6.26. Composite z-scores depicting the relative agreement between the observed incidence of forelimb-rearlimb (FR) synchrony and the second order random association model in *Rattus*. The dashed line represents the criterion value of \bar{z} ($z = 1.96$).

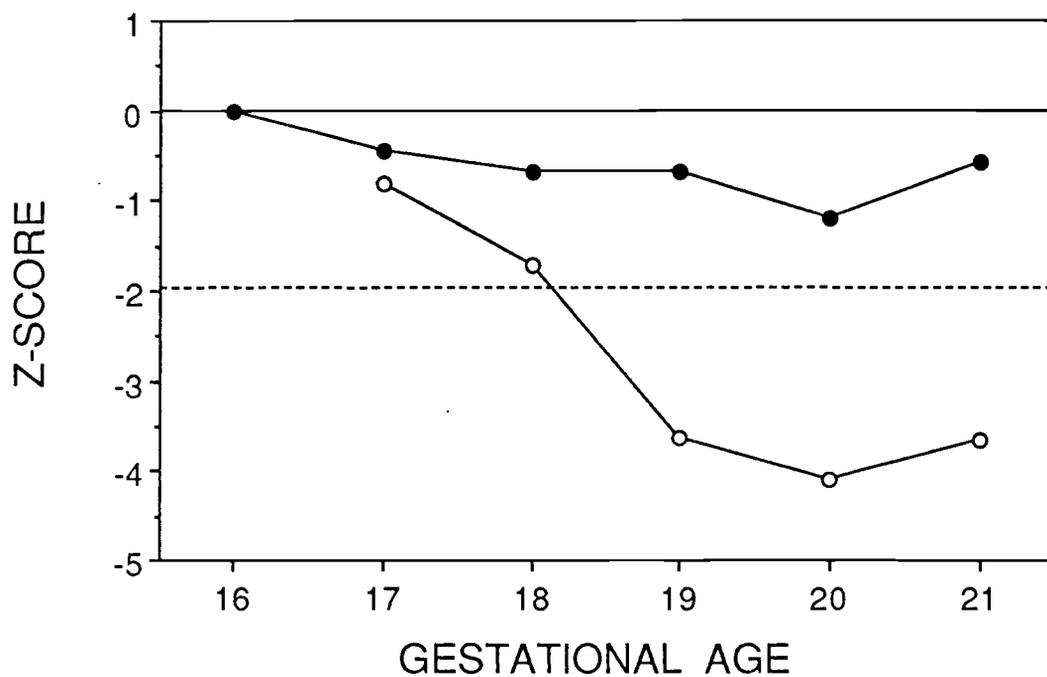


Figure 6.27. Composite z-scores depicting the relative agreement between the observed incidence of head-rearlimb (HR) synchrony and the second order random association model in Rattus. The dashed line represents the criterion value of z ($z = 1.96$).

Comparisons of observed frequencies of synchronous movement with model predictions revealed similar developmental trends in Meriones. Overall agreement with the model was good, with 15 of 130 comparisons (12%) indicating deviation from a random model. All of these differences were observed ex utero. Increases relative to predictions were evident for FH movements on days 19-23 (Figure 6.28) and FR movements on days 22-24 (Figure 6.29). An isolated increase in the category HM was indicated on day 19 of gestation. As in Rattus, HR movements were less common than predicted after day 19 (Figure 6.30).

In Sigmodon, 26 of 210 comparisons (12%) indicated deviation from predictions of the second order model. Most of these differences involved the same three categories identified in the altricial species. FH movements were more abundant than predicted on days 21-27 in utero, and days 20-27 ex utero (Figure 6.31). FR movements were not elevated in utero, but occurred more often than expected on days 20 and 24 ex utero (Figure 6.32). HR movements were less abundant than predicted on days 21-27 ex utero (Figure 6.33). Finally, the category FHR exhibited a reversal from a reduction in incidence on day 23 to an increase over model predictions on day 25 ex utero.

Deviations from the random model were somewhat more common in Acomys, occurring in 26 of 130 comparisons (20%). FH movements exhibited an increase over expected on days 28-32 in utero and on days 26 and 30-34 ex utero (Figure 6.34).

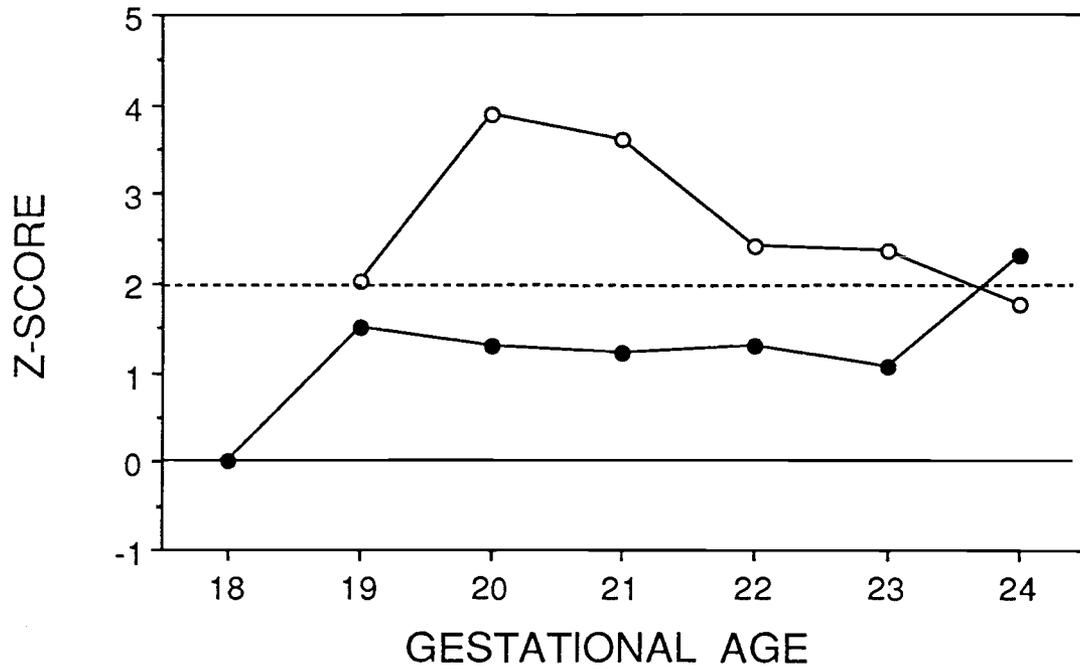


Figure 6.28. Composite z-scores depicting the relative agreement between the observed incidence of forelimb-head (FH) synchrony and the second order random association model in Meriones. The dashed line represents the criterion value of z ($z = 1.96$).

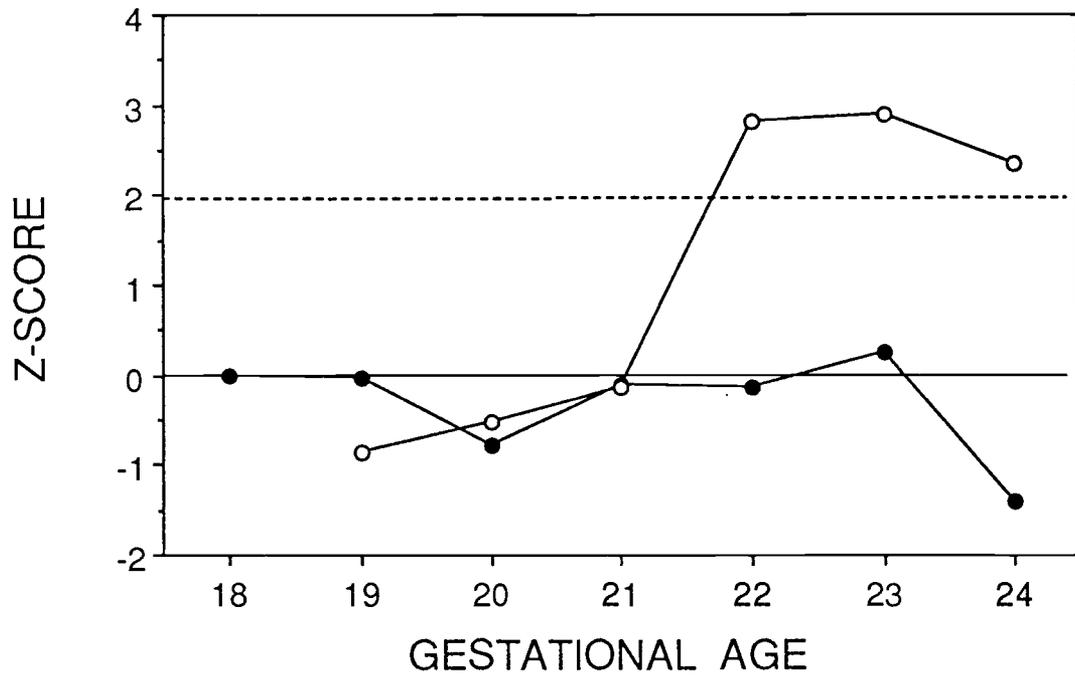


Figure 6.29. Composite z-scores depicting the relative agreement between the observed incidence of forelimb-rearlimb (FR) synchrony and the second order random association model in Meriones. The dashed line represents the criterion value of z ($z = 1.96$).

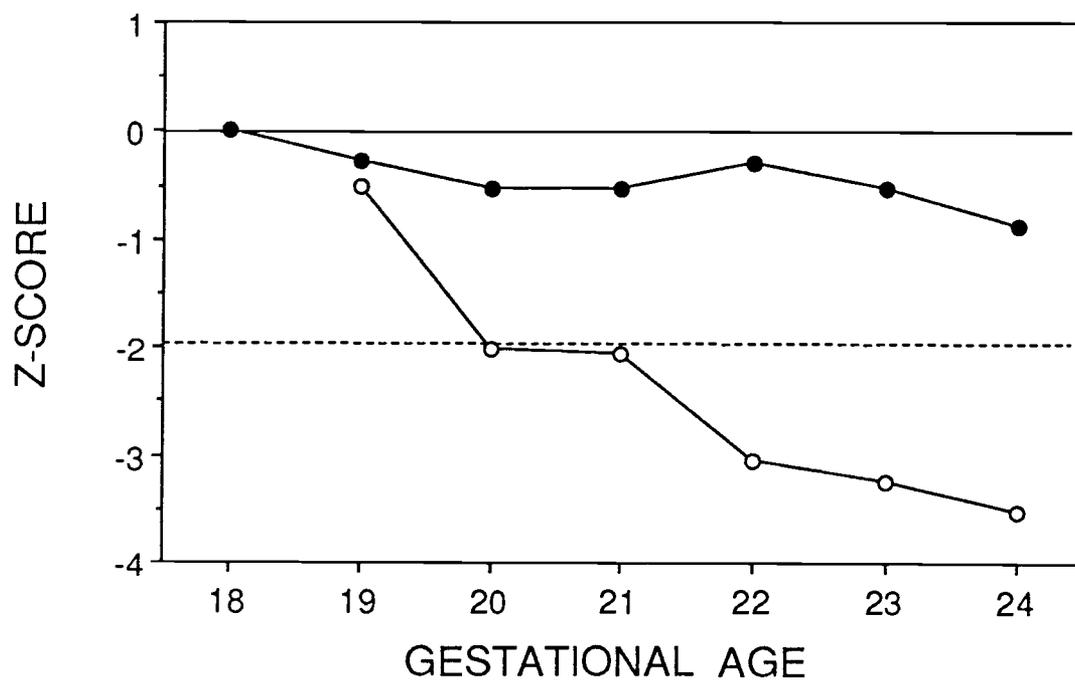


Figure 6.30. Composite z-scores depicting the relative agreement between the observed incidence of head-rearlimb (HR) synchrony and the second order random association model in Meriones. The dashed line represents the criterion value of z ($z = 1.96$).

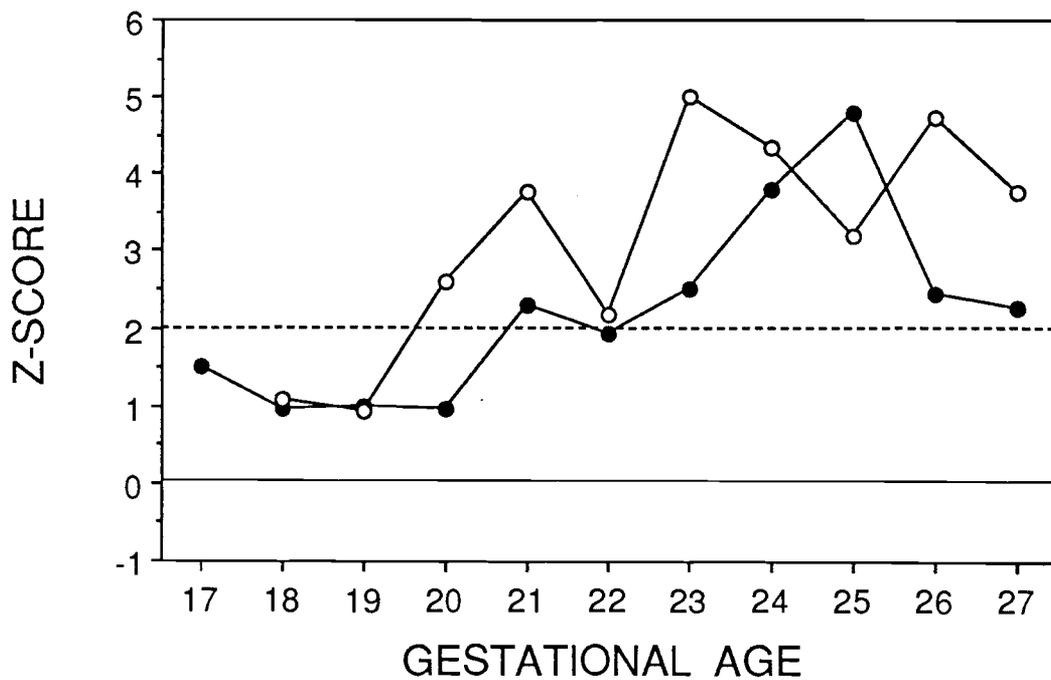


Figure 6.31. Composite z-scores depicting the relative agreement between the observed incidence of forelimb-head (FH) synchrony and the second order random association model in Sigmodon. The dashed line represents the criterion value of z ($z = 1.96$).

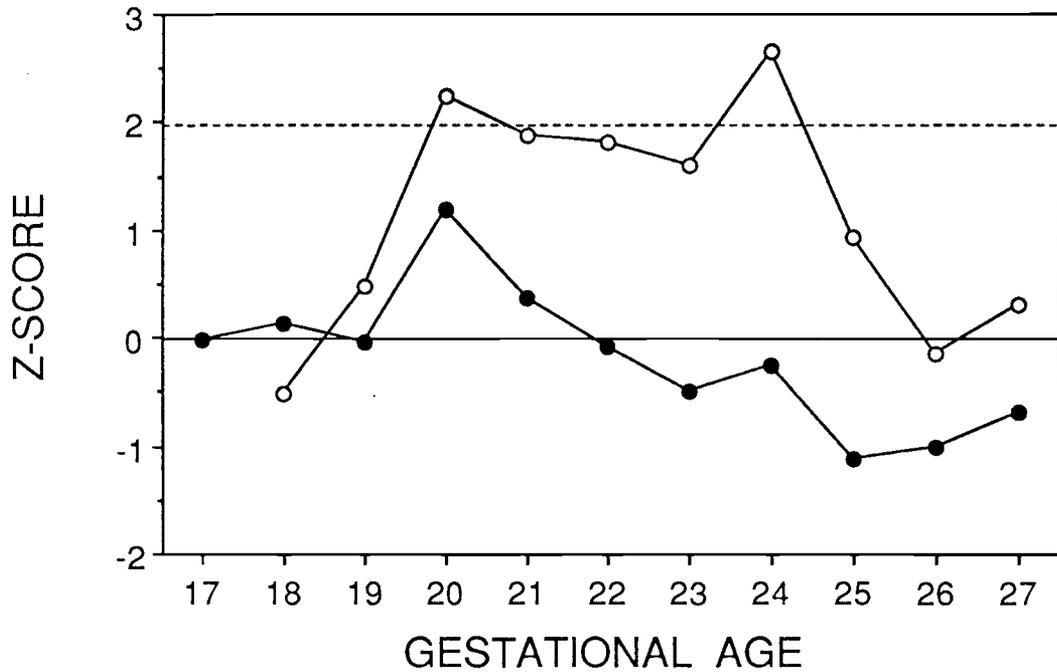


Figure 6.32. Composite z-scores depicting the relative agreement between the observed incidence of forelimb-rearlimb (FR) synchrony and the second order random association model in *Sigmodon*. The dashed line represents the criterion value of z ($z = 1.96$).

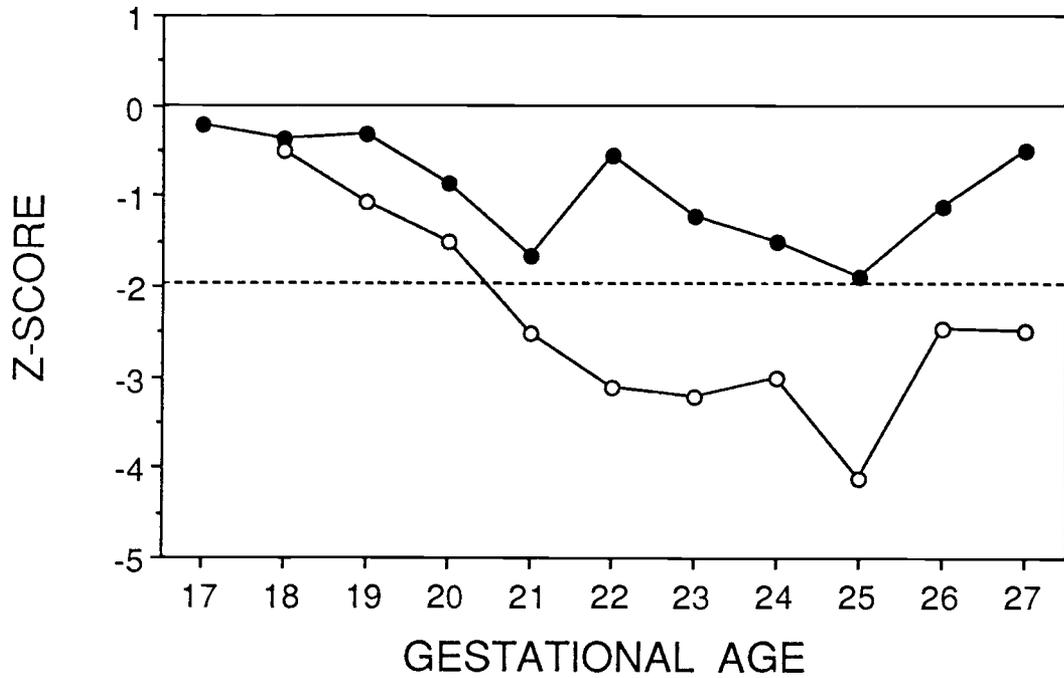


Figure 6.33. Composite z-scores depicting the relative agreement between the observed incidence of head-rearlimb (HR) synchrony and the second order random association model in *Sigmodon*. The dashed line represents the criterion value of z ($z = 1.96$).

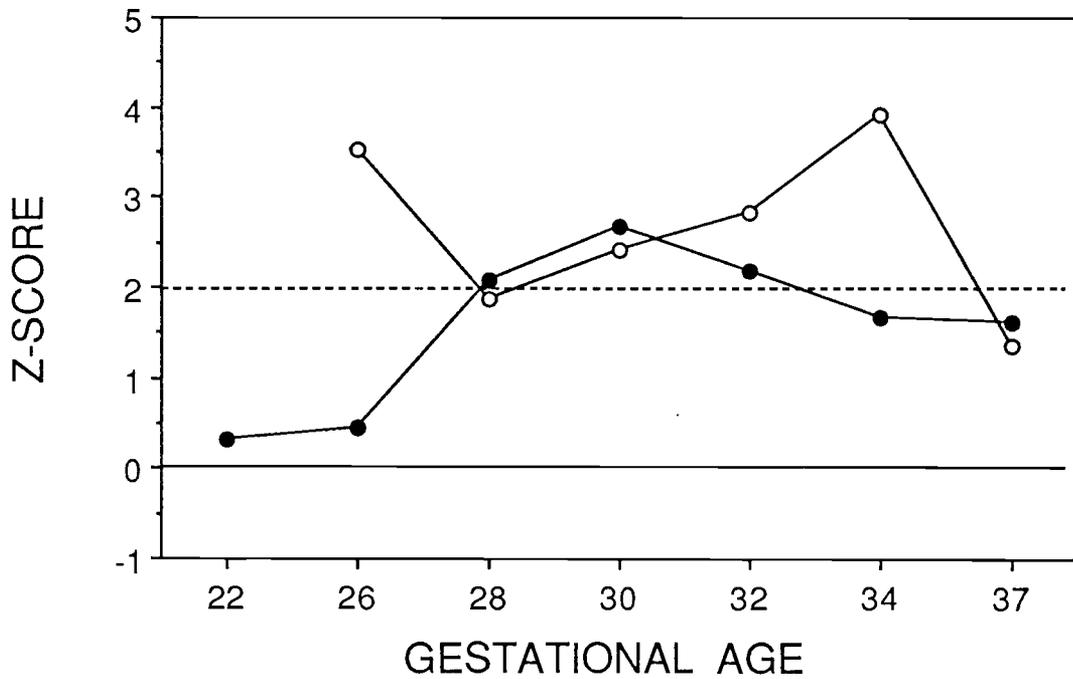


Figure 6.34. Composite z-scores depicting the relative agreement between the observed incidence of forelimb-head (FH) synchrony and the second order random association model in *Acomys*. The dashed line represents the criterion value of z ($z = 1.96$).

FR movements did not differ from predictions in utero, but consistently exceeded predictions at all ages ex utero (Figure 6.35). Three other categories of synchronous movement differed at some point during gestation from model predictions, all ex utero. The category HR occurred less often than predicted at all ages (Figure 6.36). FT movements also were reduced on days 26-30. Finally, as in Sigmodon, the triplet category FHR exhibited a reversal during development, occurring less often than predicted on day 26, but more often on days 28, 30 and 37 (Figure 6.37).

In overview, the dominant patterns revealed by the second order synchrony model were overproduction of FH and FR movements and underproduction of HR, and to a lesser extent FT, synchronous movements. In precocial species, the category FHR switched from fewer to more than expected. The remaining five focal categories failed to exhibit systematic deviation from predictions generated by the random model as a function either of gestational age or condition of observation.

F. Behavioral Diversity

With the emergence of synchronous movement during gestation, the potential behavioral repertoire of the fetus expands considerably. If each unique combination of components is treated as a separate behavioral category, then a set of five simple movements gives rise to a

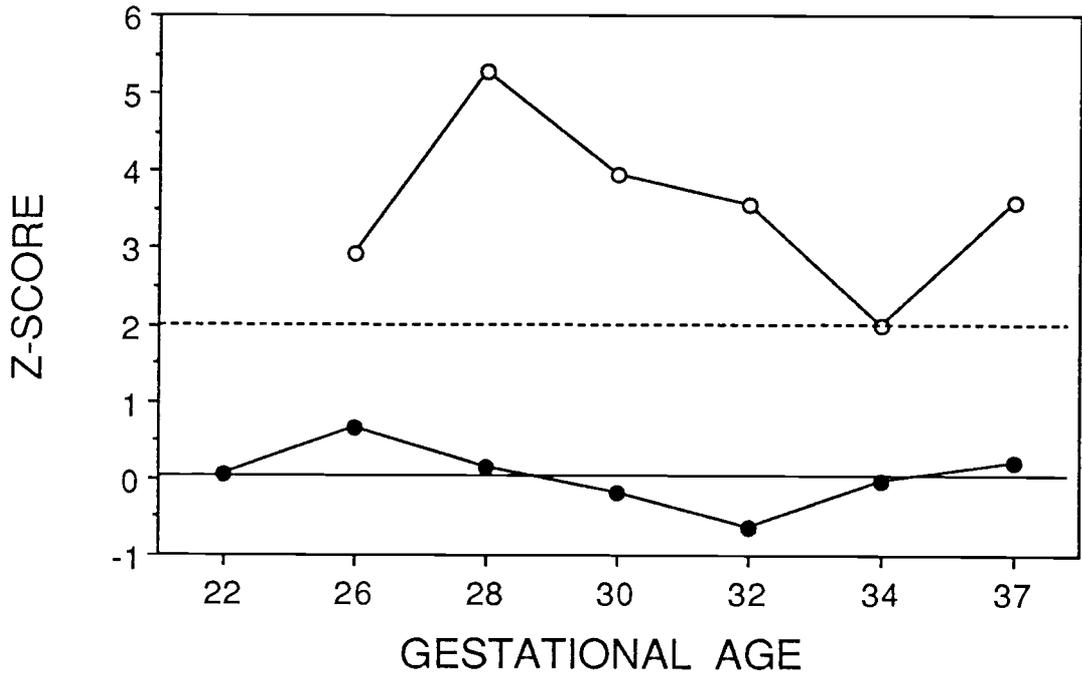


Figure 6.35. Composite z-scores depicting the relative agreement between the observed incidence of forelimb-rearlimb (FR) synchrony and the second order random association model in *Acomys*. The dashed line represents the criterion value of \bar{z} ($z = 1.96$).

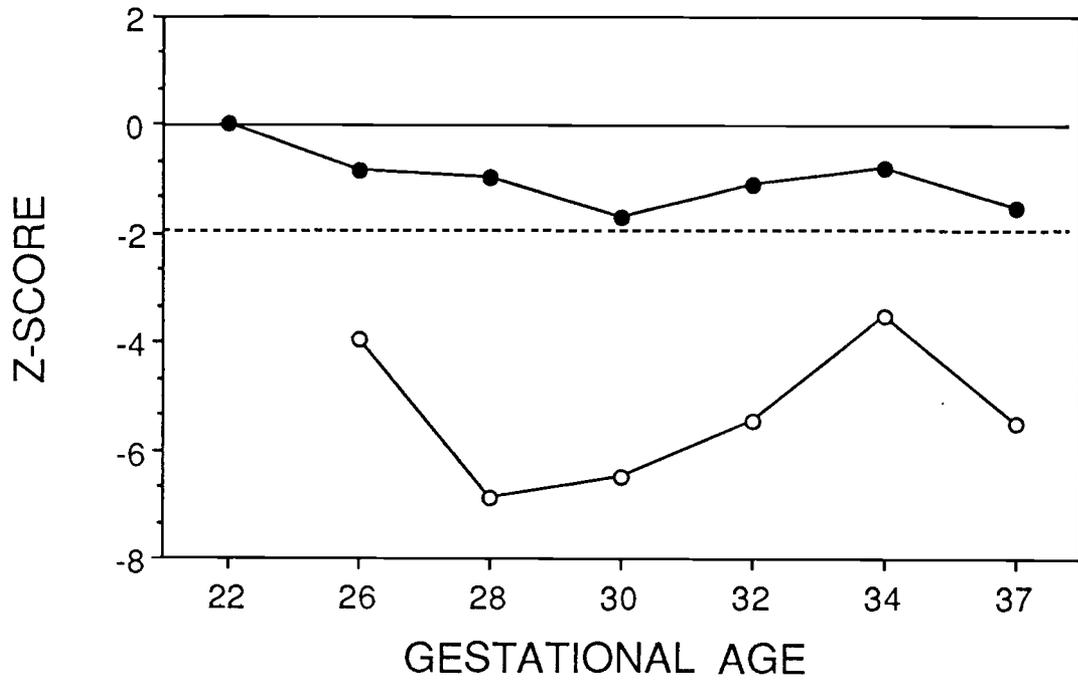


Figure 6.36. Composite z-scores depicting the relative agreement between the observed incidence of head-rearlimb (HR) synchrony and the second order random association model in *Acomys*. The dashed line represents the criterion value of z ($z = 1.96$).

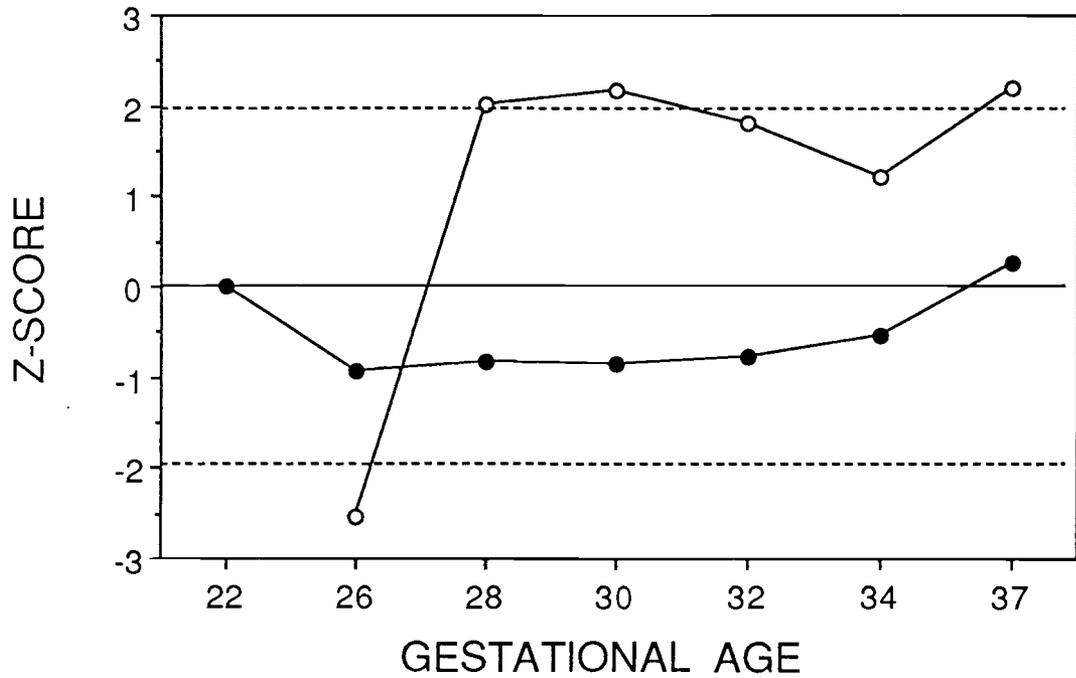


Figure 6.37. Composite z-scores depicting the relative agreement between the observed incidence of forelimb-head-rearlimb (FHR) synchrony and the second order random association model in *Acomys*. The dashed line represents the criterion value of z ($z = 1.96$).

potential repertoire of 31 kinds of simple and synchronous events. Of course, not all of these combinations were expressed by fetuses, but it seemed that both the number of categories and the average abundance of movements in different categories increased during gestation.

As a quantitative measure of changes in behavioral repertoire, an index of diversity was calculated using the standard information theory measure of entropy described in Chapter 2 [Shannon & Weaver 1949]. Briefly, entropy or H describes the average uncertainty facing a naive observer in specifying the next event to occur. Thus H incorporates information about both the number of categories and the relative abundance of events across categories. Entropy scores were calculated for each fetal subject and analyzed by two-factor ANOVA in the same way as general activity measures (Chapters 2 & 4).

The information theory index of entropy varied significantly as a function of both Age ($F_{4,40} = 5.3$, $p=.006$) and Condition ($F_{1,40} = 72.1$, $p<.001$) among Rattus fetuses. The interaction between Age and Condition was marginally significant ($p=.017$). An additional one-way ANOVA including in utero data for day 16 fetuses also was significant. Post-hoc analyses (Newman-Keuls) revealed that behavioral diversity increased in utero from day 16 to 17, remained relatively stable through day 20, then decreased on day 21 (Figure 6.38). Following the significant simple effect of Age ex utero, post-hoc tests indicated that

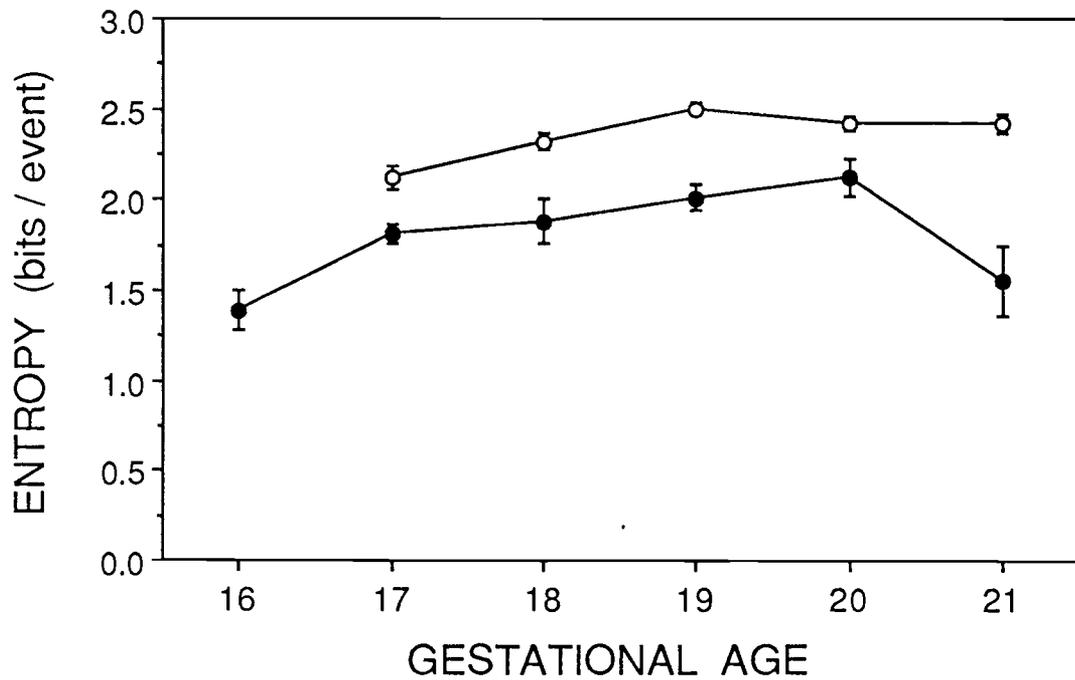


Figure 6.38. Entropy scores, expressed in bits per event, reflecting changes in overall fetal behavioral diversity in Rattus. Closed circles represent fetuses observed in utero; open circles represent fetuses ex utero.

diversity increased steadily from day 17 to a plateau on days 19-21 (Table 6.3). Behavioral diversity was greater ex utero than in utero at all ages.

In Meriones, only the significant effect of Condition was evident ($F_{1,48} = 51.5, p < .001$). Fetal behavior was less diverse in utero than ex utero (Figure 6.39).

Significant main effects were found for both Age ($F_{9,95} = 2.9, p = .003$) and Condition ($F_{1,95} = 63.1, p < .001$) in Sigmodon (Table 6.3). Post-hoc comparison of means collapsed across conditions indicated a significant increase in entropy scores between days 19 and 24. Diversity was greater ex utero than in utero (Figure 6.40).

The main effect of Condition ($F_{1,49} = 79.0, p < .001$) was significant in Acomys. Entropy scores were less in utero than ex utero. The interaction of Age by Condition also was marginally significant ($p = .031$). An additional one-way ANOVA including day 22 fetuses indicated significant variation in behavioral diversity with age in utero (Figure 6.41). Comparison of means showed that diversity was less on day 22 than on days 28-37 (Table 6.3).

G. Discussion

The findings of the present study confirm and extend data previously reported on the development of motor synchrony in Rattus [Smotherman & Robinson 1986; Robinson & Smotherman 1987]. Specifically, (a) overall changes in the

Table 6.3

Summary of post-hoc comparisons in analyses of behavioral diversity for four rodent species. Conventions as in Table 6.1.

SPECIES	MAIN EFFECTS		INTERACTION (A X C)	
			SIMPLE EFFECTS	
	AGE	CONDITION	AGE	CONDITION
RATTUS			In: 16<17=20>21	17: In < Ex
			Ex: 17<18<19=21	18: In < Ex
				19: In < Ex
				20: In < Ex
				21: In < Ex
MERIONES		In < Ex		
SIGMODON	18=19<25=27	In < Ex		
ACOMYS		In < Ex		

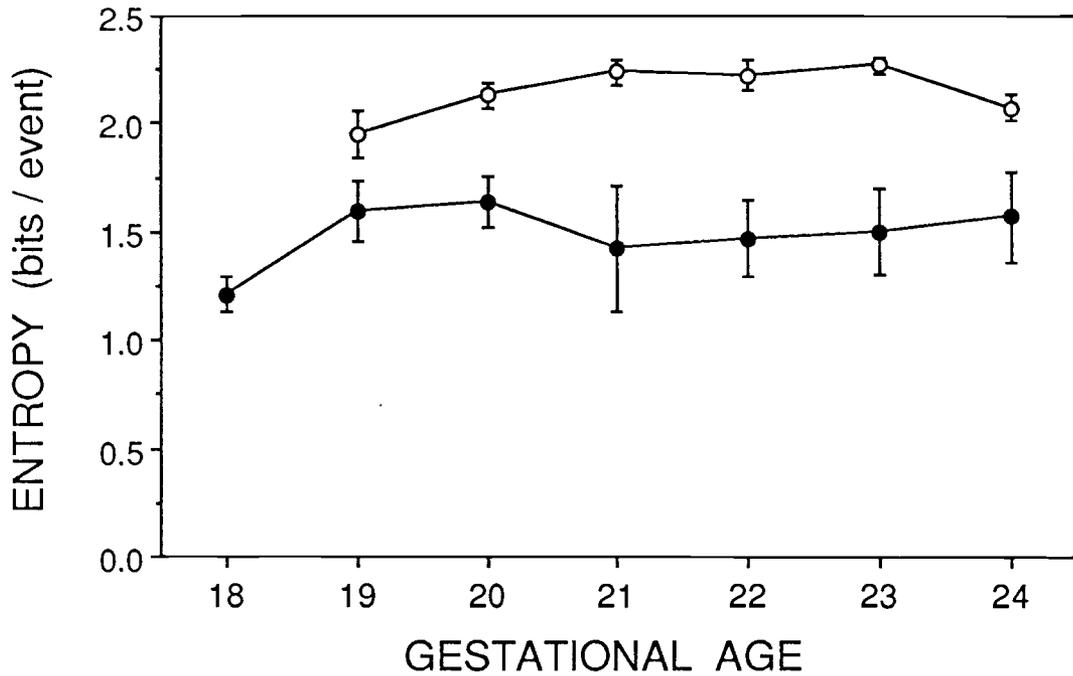


Figure 6.39. Entropy scores, expressed in bits per event, reflecting changes in overall fetal behavioral diversity in *Meriones*. Closed circles represent fetuses observed in utero; open circles represent fetuses ex utero.

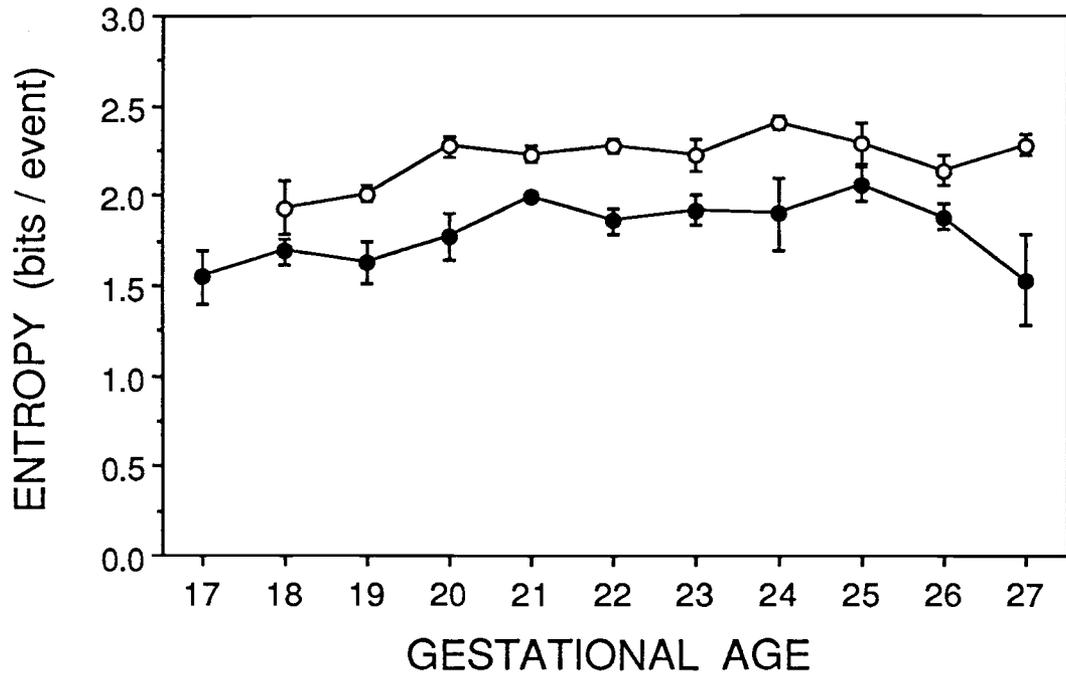


Figure 6.40. Entropy scores, expressed in bits per event, reflecting changes in overall fetal behavioral diversity in *Sigmodon*. Closed circles represent fetuses observed in utero; open circles represent fetuses ex utero.

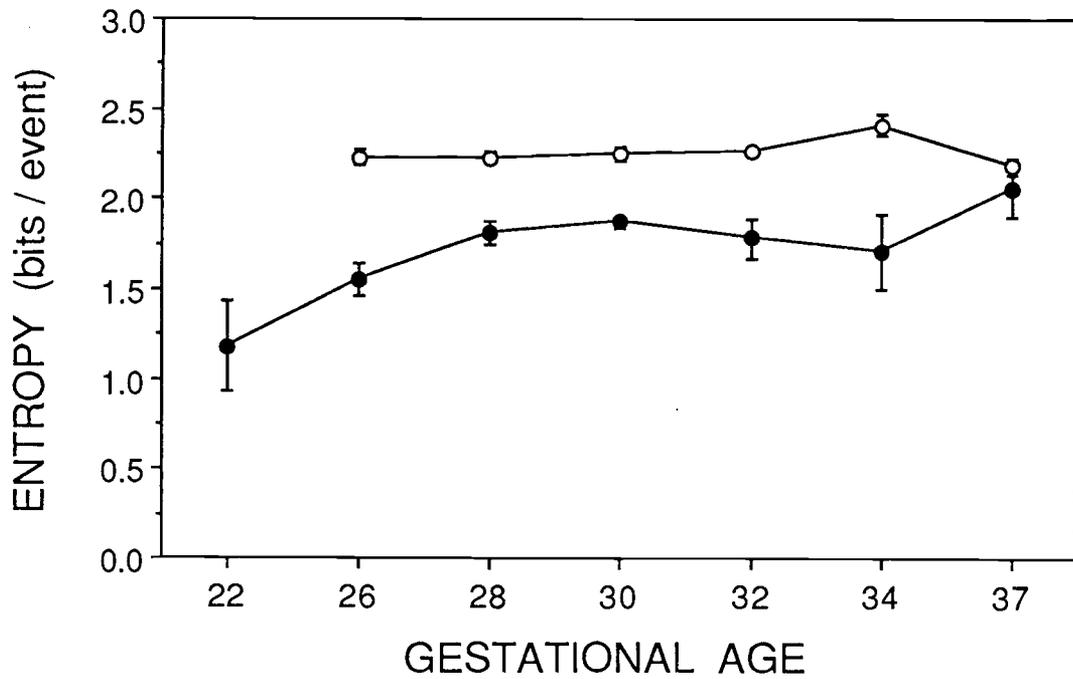


Figure 6.41. Entropy scores, expressed in bits per event, reflecting changes in overall fetal behavioral diversity in *Acomys*. Closed circles represent fetuses observed in utero; open circles represent fetuses ex utero.

frequency of synchronous movements were comparable, with the possible exception of more abundant instances of synchrony on day 17 ex utero in the present study; (b) overall levels of movement synchrony did not increase significantly above chance levels (dual-process model) until day 18 of gestation; (c) the incidence of synchrony remained high ex utero through term, but decreased absolutely and relative to predictions of a random model among fetuses observed in utero on day 21; (d) synchronous movements were less abundant and reflected fewer patterns of linkage between body regions with fetuses in utero than ex utero; (e) the most common categories of synchronous movement involved foreleg-rearleg (FR) and foreleg-head (FH), both of which occurred more frequently than chance association would predict; (f) certain other categories of synchrony, notably head-rearleg (HR), occurred less often than predicted by chance after day 18.

Many of these corroborative findings were the product of stochastic models designed to investigate the production of synchronized movements. The models elaborated in this chapter were modified considerably from previous reports [Smotherman & Robinson 1986; Robinson & Smotherman 1987] to permit extension to other species. The fact that similar conclusions were reached regarding the behavior of Rattus fetuses argues that the new versions of these stochastic models are comparable, but more robust. For example, incorporation of information about temporal patterning in

the dual-process Poisson model described in section C permits application of the model under a much broader range of fetal activity conditions than was possible with original probability estimates.

The emergence of synchronous movement during prenatal development in Meriones, the other representative of an altricial species, was in most respects very comparable to Rattus. Differences that were apparent were consistent with the general view that the behavioral development of Meriones proceeds at a slightly slower rate than Rattus. The earliest age at which synchronous movements exceeded chance levels (dual-process model) was slightly delayed relative to Rattus, and fewer cases of synchronous linkage were expressed, particularly in utero. The finding that synchronous movements expressed in utero at term occur at rates no greater than chance association is a notable extension, as it is consistent with the interpretation that physical restraint within the uterus suppresses the expression of coordinated behavior [Smotherman & Robinson 1986, 1988b].

By the same measures, movement synchrony emerged relatively earlier in gestation in precocial species than altricial species. The results of the first order random models were especially interesting in this respect, as they indicated two different peaks in synchrony in both Sigmodon and Acomys. The existence of two peaks, which was most evident among fetuses in utero, suggests the emergence of

different behavioral processes. The early peak may indeed correspond to the single peak expressed by altricial fetuses, and the second peak to behavioral events ordinarily restricted to the postnatal period in altricial species. If this view is correct, then the decline in synchrony to levels below chance during the ages between peaks, evident in both Sigmodon and Acomys, may correspond to the decline in synchrony at term in Rattus and Meriones. In other words, the expression of coordinated movement by precocial fetuses may be more sensitive to physical restraint at intermediate ages when fetal motor abilities remain relatively immature.

The results of the second order random models may bear on this question. Two categories of synchronous movement -- FH and FR -- consistently occurred at frequencies significantly above chance association. By this criterion, these two synchronous categories probably should be considered independent behavioral patterns, distinct from the simple components they comprise. In three of the four species, FH movements emerged above the background of chance at an earlier gestational age than FR movements. (In Acomys, FH and FR emerged simultaneously on day 26). The timing of the developmental emergence of FH suggests that it was primarily responsible for the initial increase of overall motor synchrony above the level of random association.

At more advanced gestational ages, the synchronous

category HR occurred at levels significantly less than predicted by the chance association model. This finding suggests the development of central mechanisms capable of inhibiting the expression of motor behavior by the fetus. As in the expression of FH and FR movements, the suppression of HR movements occurred at relatively earlier ages in precocial species than altricial species. In precocial species, the triplet category FHR also exhibited initial suppression, and did not increase above chance levels until late in gestation. The emergence of the HR suppression occurred at or just before the reduction in overall synchrony late in gestation in altricial species and between peaks of synchrony in precocial species. This pattern of emergence suggests that the development of central motor inhibition, responsible for suppressing the production of HR movements (and FHR in precocial species), may be a necessary precursor to the reduction of overall synchronous activity under conditions of increasing fetal restraint.

Conversely, the increase in FHR movements above levels predicted from chance association, which was restricted to precocial species late in gestation, coincided with the second peak in overall synchronous movement. The emergence of FHR and more complicated patterns of synchronous movement also occurred during a general reduction in the number of synchronous linkages and overall fetal activity. Thus, the two precocial species appeared to express stages in the development of coordinated behavior that are absent during

the prenatal period in altricial species. Specifically, the simultaneous expression of activational and inhibitory processes in the control of fetal behavior probably reflects the relatively greater motor maturity of Sigmodon and Acomys fetuses during the latter half of the fetal period.

CHAPTER 7. SEQUENTIAL ORGANIZATION OF FETAL BEHAVIOR

A. Introduction

Sequential organization of movement is a fundamental property of behavior. On a fine level, coordinated action requires the sequential activation (and inhibition) of different motor units. On a coarser time scale, most postnatal behavior, and goal-directed behavior in particular, is organized in functional sequences. Much can be inferred about the underlying mechanisms controlling behavior from sequential analysis [Hailman & Sustare 1973; Fentress & McLeod 1986]. In this chapter, a sequential model will be elaborated to describe overall sequential organization and specific patterns of serial dependency in spontaneous fetal behavior.

B. Markov Sequential Model

Most ethological analyses have employed a Markov model in dealing with sequential relationships [Hailman & Sustare 1973; Fagen & Young 1978; Bakeman & Gottman 1986]. A Markov process exists when a system can express any of a finite number of discrete states, but knowledge of the immediately preceding state completely determines the next state to occur. A more generally useful concept is the Markov chain,

which allows for stochastic, rather than deterministic, relationships between successive states. Any ordered pair of events that exhibits a nonrandom dependency may be considered a link in a Markov chain.

Application of a Markov sequential model to behavior requires that the stream of behavior be parsed into a manageable list of exclusive and exhaustive categories. The set of five basic components (F, R, H, M, T) provides only a partial list, because each denotes only a simple event comprising a single component. The findings of the synchrony analyses reported in Chapter 6 indicated that several categories of synchronous movement, notably FH and FR, occurred more often than predicted by chance association and should be considered discrete behavioral categories. However, it is inappropriate to consider all possible combinations of components that occur in synchronous events as discrete categories because (a) most combinations can be accounted for as transient, random associations of independent events, and (b) inclusion of all combinations produces a list of categories that requires an unrealistically large sample size for sequential analysis.

The problem of sample size is central in the analysis of behavioral sequences. The minimum sample size required is necessarily related to the number of recognized categories of behavior. Given n categories, the number of possible ordered pairs of categories is n^2 . As a rule of thumb, $5n^2$ is considered marginally sufficient and $10n^2$ is

fully sufficient to permit statistically valid inferences to be drawn about sequential relationships [Fagen & Young 1978]. In the present study, if all possible synchronous combinations of components were considered independent, the resulting matrix would contain more than 900 different pairwise transitions, requiring a sample of 4500-9000 events.

To reduce this list to a manageable size, preliminary sequential analyses were conducted on an independent data set obtained in a previous study of behavior of fetal rats [Smotherman & Robinson 1986; Robinson & Smotherman 1988]. Based on these preliminary analyses, a list of eight behavioral categories was selected for use in sequential analysis in the present study. Four of these categories included only simple events and were defined by their component category: forelimb (F), rearlimb (R), head (H), and trunk (T). A fifth category comprised all events in which mouth movements (M) occurred, including both simple and synchronous events. Two synchronous categories were defined to distinguish forelimb-head movements (FH) and forelimb-rearlimb movements (FR), as suggested by the findings of Chapter 6. Finally, to provide an exhaustive list, all other synchronous movements were pooled to form an eighth category: Other (O).

Once the list of movement categories was established, fetal behavioral data were summarized by tallying the frequency of all sequential transitions between ordered

pairs of the eight categories in a transition matrix. An example of a transition matrix is presented in Table 7.1. A separate matrix was created for each species for each combination of age and condition of observation, with data pooled across all subjects within an age by condition group. These transition matrices constituted the common starting point for all subsequent sequential analyses reported below. As a convention, different sequential transitions will be symbolically represented by the code for the preceding event separated by a slash from the succeeding event (e.g., F/FR denotes the simple event F followed immediately by the synchronous event FR).

C. Overall Sequential Organization

1. Chi-square analysis of transition matrix

The null hypothesis in sequential analysis assumes that transitions between successive events are governed by chance. The null hypothesis most simply can be tested by application of a Chi-square test of independence to the entire transition matrix [Fagen & Young 1978]. Expected frequencies in each cell of the matrix were calculated by multiplying row and column totals and dividing by the grand total for the matrix. This method of generating expected frequencies is appropriate for studies in which it is possible for each behavioral category to succeed itself

Table 7.1

An example of a transition matrix as employed in sequential analyses. This matrix summarizes sequential transitions between pairs of events for Rattus fetuses observed ex utero on day 21 of gestation. Entries represent the observed frequency of transition between immediately precedent and subsequent event categories. Underlined entries represent self-recursive transitions. Transition matrices for all other ages and conditions for all four species are presented in Appendix A.

PRECEDENT EVENT	SUBSEQUENT EVENT							
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	<u>62</u>	65	43	3	15	44	45	10
Rearlimb	66	<u>103</u>	59	17	10	66	37	26
Head	51	58	<u>41</u>	11	9	44	34	7
Trunk	11	6	16	<u>3</u>	2	9	10	11
Mouth	13	11	12	33	<u>3</u>	16	7	1
FR	45	45	53	11	17	<u>45</u>	21	10
FH	26	75	17	17	8	9	<u>38</u>	19
Other	13	21	13	4	2	14	17	<u>10</u>

(e.g., H/H or FR/FR). Owing to the definition of categories as discrete events, self-recursion was logically (and empirically) possible in the present study. A separate Chi-square test was conducted for each transition matrix. A conservative alpha level of .01 was selected as the criterion for significance in these tests (df = 49 in all tests). The results of Chi-square analyses are presented for all species, ages and conditions in Table 7.2.

In Rattus, the observed frequencies of sequential transition expressed at the inception of movement among fetuses observed in utero did not differ from the null model of random sequential association. Sequential organization also was lacking among fetuses in utero on day 17 of gestation, although fetuses observed ex utero at the same age exhibited a significant deviation from independence in the overall pattern of sequential transitions. Serial dependency in two-event transitions was evident in all other combinations of age and condition.

A random sequential model also was sufficient to account for observed patterns of transition in Meriones at the inception of movement and on days 20 and 23 of gestation, but only among fetuses observed in utero. Chi-square values indicated a significant deviation from independence at all ages ex utero.

The same general pattern of results was apparent among precocial Sigmodon and Acomys fetuses. Both species exhibited independence of sequential transition at the

Table 7.2

Patterns of significant difference from a random sequential model as indicated by Chi-square tests of independence applied to entire transition matrices. Results are presented as NS (not significant), * (significant at .01 level), and ** (significant at .001 level).

RATTUS	day 16	17	18	19	20	21						
in utero	NS	NS	*	**	**	**						
ex utero		**	**	**	**	**						
MERIONES	day 18	19	20	21	22	23	24					
in utero	NS	*	NS	**	**	NS	**					
ex utero		**	**	**	**	**	**					
SIGMODON	day 17	18	19	20	21	22	23	24	25	26	27	
in utero	NS	*	NS	**	**	**	**	**	**	**	**	**
ex utero		**	*	**	**	**	**	**	**	**	**	**
ACOMYS	day 22	26	28	30	32	34	37					
in utero	NS	NS	**	**	**	**	*					
ex utero		**	**	**	**	**	**					

inception of movement and at one other early gestational age in utero (day 19 in Sigmodon; day 26 in Acomys). But significant differences from the random model were evident at all ages among fetuses observed ex utero.

The consistent finding across these tests was that little or no sequential organization of fetal motor behavior exists at the inception of movement, but that serial dependencies emerged quickly in both altricial and precocial species over the next one or two days of gestation. Moreover, the expression of sequential patterning appeared to be facilitated by observing fetuses ex utero. Aside from documenting deviation from independence, however, Chi-square tests provided little information about subsequent changes in overall sequential organization.

2. Information theory analysis

Developmental changes in sequential structure implied by the foregoing analyses can be more precisely described through the mathematics of formal information theory [Shannon & Weaver 1949]. The logic of using information measures to interpret sequential data is well discussed by Hailman [1977]. Briefly, entropy scores are calculated to reflect the total uncertainty in a transition matrix at various levels of description. The maximum entropy of a matrix, denoted H_0 , is defined solely in terms of the number of recognized event categories (N): $H_0 = \log_2 N$. With eight

behavioral categories, $H_0 = 3.00$ bits per event in all matrices.

At a more detailed level of description, the frequencies of events in each behavioral category are incorporated into the calculation of entropy. First-order entropy, denoted H_1 , is calculated by equation 2.1, where P_i is equal to the overall frequency of category i divided by the total number of all events. Because H_1 incorporates information about the relative frequency of different behavioral categories, but does not include information about sequences, it is comparable to the index of behavioral diversity employed in Chapter 6.

A third level of description considers information about sequential transitions between pairs of events. Second-order entropy, denoted H_2 , is calculated as a weighted average of the entropies for each row in the transition matrix:

$$H_2 = \sum_{j=1}^N P_j H_j \quad (7.1)$$

In equation 7.1, P_j is the simple probability of a transition occurring in row j of the matrix (i.e., the probability that the preceding event will be category j), and H_j is the entropy, calculated from equation 2.1, for row j . (In applying equation 2.1 to compute H_j , P_i is the conditional probability of occurrence of category i , calculated as the frequency of events in cell ij divided by

the total number of events in row j .) When the observed frequencies in a transition matrix are completely described by row and column totals (meaning that preceding and succeeding events are independent), $H_1 = H_2$.

Several derived measures are useful in interpreting calculated values of entropy. The difference between H_0 and H_1 , denoted I_1 , is the reduction in the amount of uncertainty (information gain) that results from consideration of the frequency of events in different behavioral categories. Similarly, the difference between H_1 and H_2 , denoted I_2 , is the information gain achieved by considering two-event transitions. When I_2 is large relative to I_1 , the system is said to be semi-Markovian. Semi-Markovian sequential organization has been described in many previous ethological studies and may be generally characteristic of postnatal behavior [Hailman 1977].

Developmental changes in the sequential organization of fetal behavior in Rattus is summarized in Figure 7.1, which depicts the ratio of I_2 to I_1 from the inception of movement through term. Larger values for this ratio are indicative of greater sequential organization; values that approach 1.0 approximate a semi-Markov process. Shortly after the inception of movement in Rattus, the ratio was small, suggesting that overall sequential organization was relatively weak. About day 19, however, I_2 increased relative to I_1 . Peak values for the ratio occurred on day 19 ex utero and day 20 in utero. A substantial decrease in

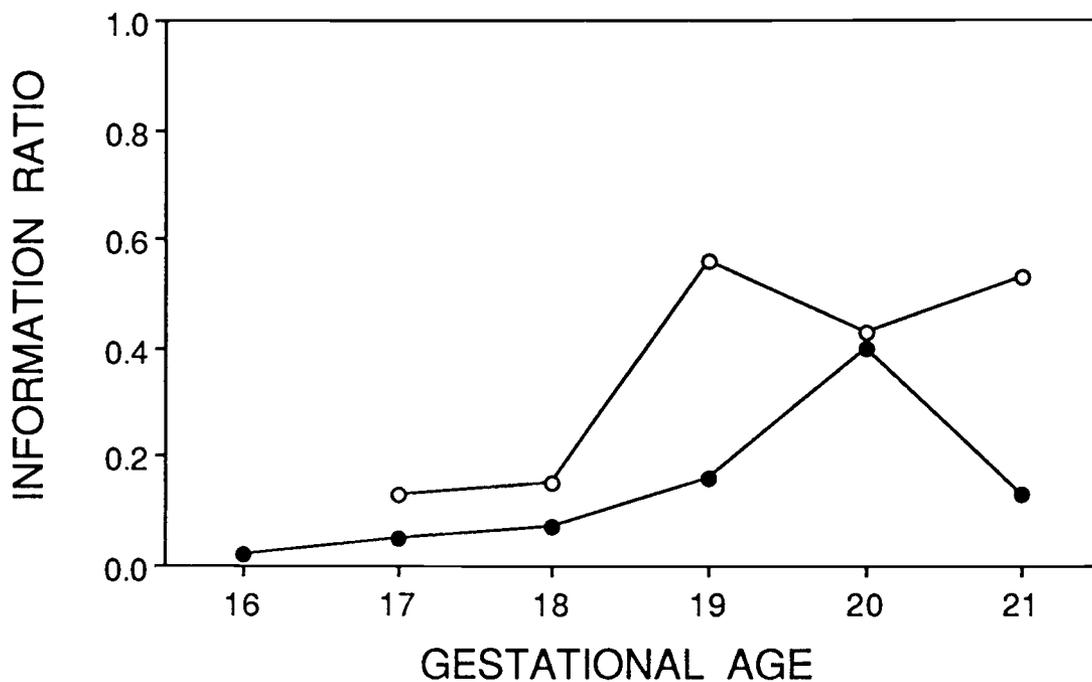


Figure 7.1. Gestational changes in the information ratio I_2 / I_1 in Rattus. Higher values of this ratio reflect greater overall sequential organization of fetal behavior. Closed circles represent values for fetuses in utero; open circles represent fetuses ex utero.

overall sequential organization was evident at term in utero, but was not apparent ex utero.

In Meriones, changes in sequential structure reflected by the ratio $I_2:I_1$ followed a similar pattern (Figure 7.2). Values for the ratio remained low through much of the fetal period, but exhibited an increase just before term. Peak values occurred on day 24 in utero and one day earlier ex utero, but did not approach the magnitude exhibited in Rattus. Although sequential structure was evident in the prenatal motor behavior of Meriones, it appeared to be much less well developed than in Rattus.

Among precocial Sigmodon fetuses, overall sequential organization remained relatively low until day 24 of gestation, when an increase in the ratio $I_2:I_1$ was evident both in utero and ex utero (Figure 7.3). Peak values for the ratio occurred on day 25 in both conditions and decreased over the last two days of gestation. At the peak of sequential organization, overall fetal activity in Sigmodon more closely approximated a semi-Markov process than was evident in either altricial species.

Much the same developmental pattern was exhibited by Acomys fetuses (Figure 7.4). Values for the ratio $I_2:I_1$ remained low in utero until relatively late in gestation, but were much higher ex utero as early as day 26. An increase in the ratio was evident on day 34 in both conditions, and peak values were expressed at this age ex utero and at term in utero. As in Sigmodon, overall

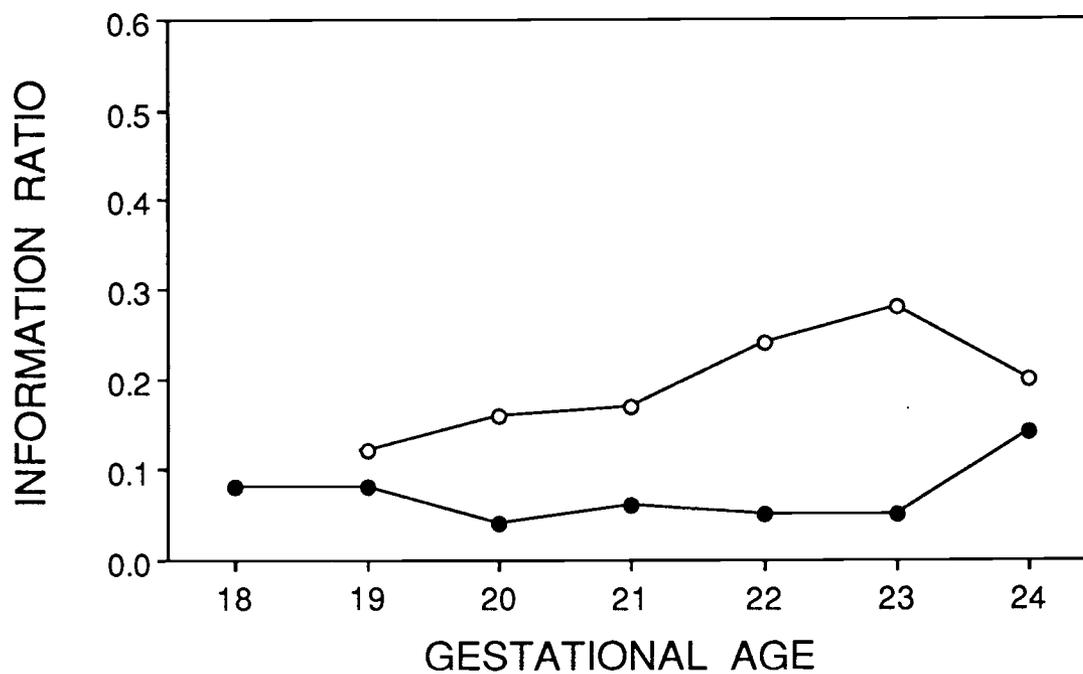


Figure 7.2. Gestational changes in the information ratio I_2 / I_1 in Meriones. Higher values of this ratio reflect greater overall sequential organization of fetal behavior. Closed circles represent values for fetuses in utero; open circles represent fetuses ex utero.

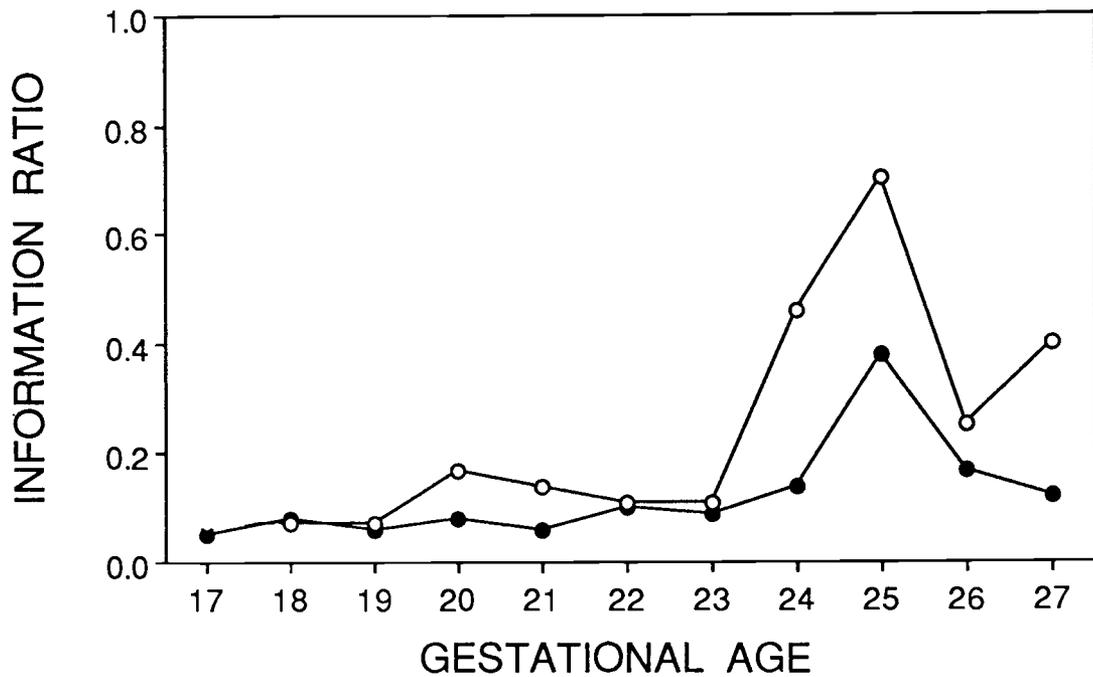


Figure 7.3. Gestational changes in the information ratio I_2 / I_1 in Sigmodon. Higher values of this ratio reflect greater overall sequential organization of fetal behavior. Closed circles represent values for fetuses in utero; open circles represent fetuses ex utero.

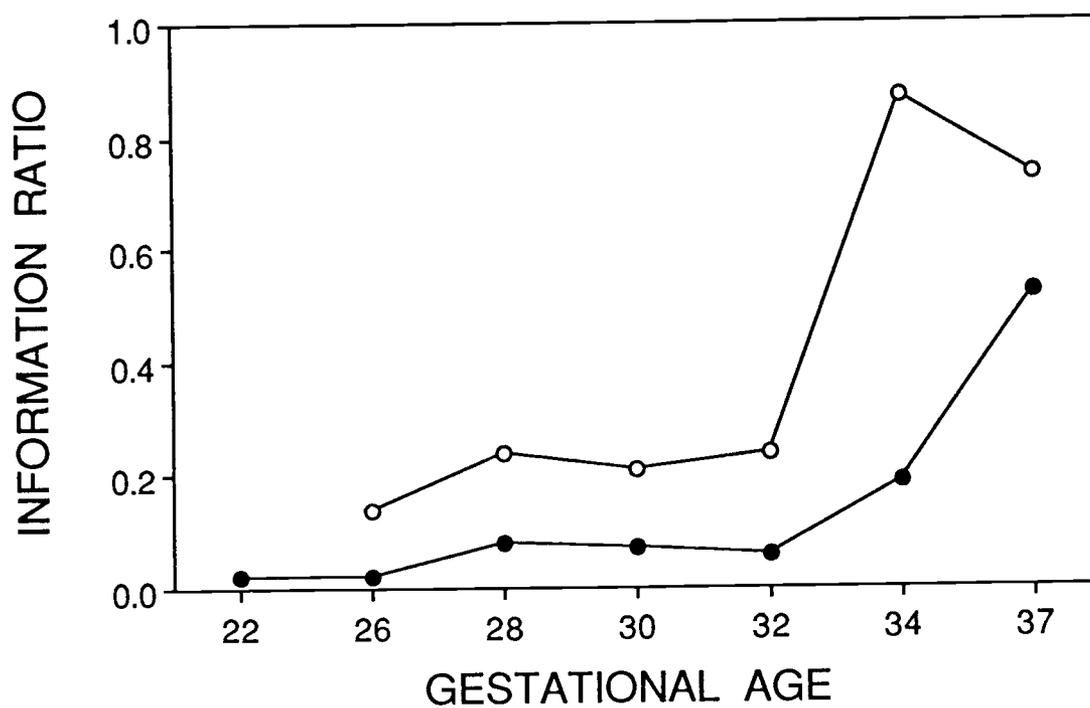


Figure 7.4. Gestational changes in the information ratio I_2 / I_1 in *Acomys*. Higher values of this ratio reflect greater overall sequential organization of fetal behavior. Closed circles represent values for fetuses in utero; open circles represent fetuses ex utero.

sequential organization at its maximum expression approached the character of a semi-Markov process.

D. Patterns of Event Sequences

The emergence of sequential organization in fetal activity must be due to the overproduction of certain event sequences. Numerous methods have been employed to identify and describe specific event sequences that are responsible for the nonindependence found in a transition matrix [Fagen & Young 1978; Bakeman & Gottman 1986]. Several different approaches were explored in the present study in an effort to further characterize sequential patterns in spontaneous fetal behavior.

1. Self-recursive transitions

Initial inspection of the transition matrices across ages and conditions suggested a simple organizational 'rule' that could have accounted for much of the observed deviation from a random model. This rule was self-recursion. At nearly all ages, fetuses appeared to show a marked propensity to repeat the same category of movement in a sequence of two or more events. This was manifested as an overproduction of transitions along the diagonal of the matrix. To test the hypothesis that self-recursion occurred more often than should be expected by chance, the sum of

event transitions in these cells of the matrix was compared to expected frequencies by calculation of a binomial test z-score (see Chapter 6, section C, for discussion). The z-score was computed thus:

$$z = (f - NP) (NPQ)^{-0.5} \quad (7.2)$$

In equation 7.2, f is the observed frequency in the focal category of sequential transition (in this case, the sum of cells along the diagonal), N is the total number of event sequences, P is the expected probability of occurrence of the focal category of transition as determined from row and column totals, and Q is the complement of P . A one-tailed value of z ($z = 2.33$, $p = .01$) was considered significant.

In Rattus, the frequency of self-recursion did not differ from chance levels on days 16 or 17 of gestation (Figure 7.5). However, fetuses observed both in utero and ex utero exhibited self-recursive event sequences more often than expected from day 18 through term. This developmental pattern was virtually identical to the pattern of deviation from independence in the entire matrix, described above (Table 7.2). Although all categories of movement exhibited some self-recursion, the majority of recursive transitions were accounted for by three categories in utero (F, R, M) and only two categories ex utero (FR, M).

No clear pattern was apparent in the significant overproduction of self-recursion during gestation in

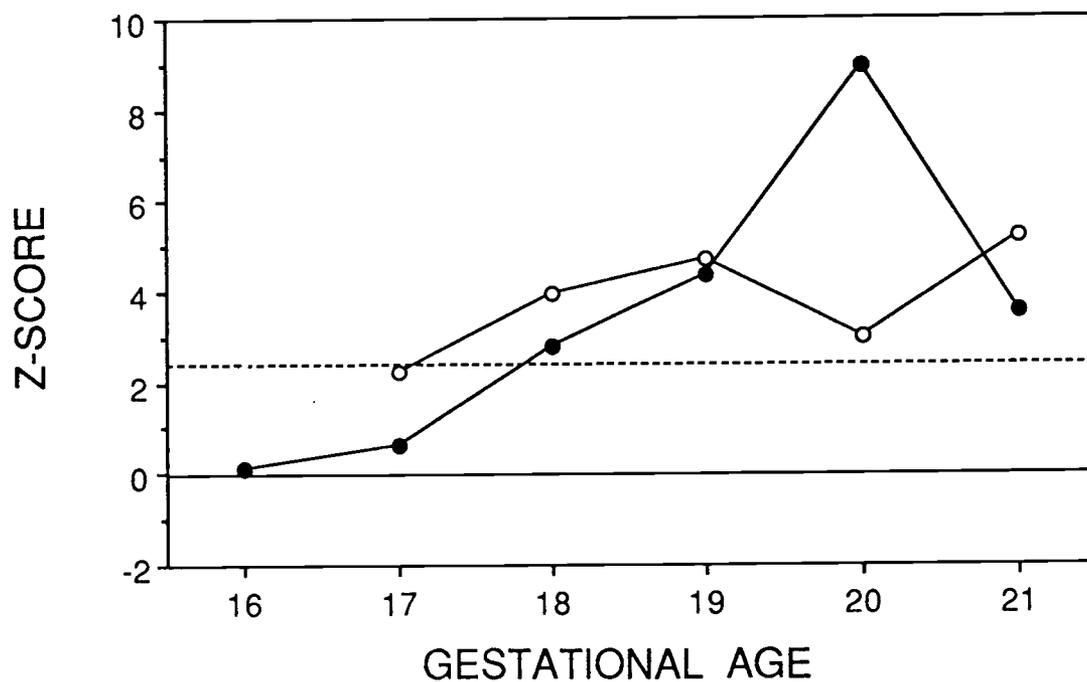


Figure 7.5. Composite z-scores depicting the relative agreement between the observed incidence of self-recursive event sequences and the expected incidence based on random sequential association in Rattus. The dashed line represents the criterion value of z ($z = 2.33$).

Meriones (Figure 7.6). More instances of self-recursion occurred than expected both early and late during the fetal period in utero, and at most ages ex utero. Further, there was no close correspondence between self-recursion and ages at which a significant deviation from independence in the overall matrix was found. As in Rattus, only a few categories in utero (F, M) and ex utero (FR, M) appeared to occur at significantly elevated levels of self-recursion.

Among Sigmodon fetuses, self-recursion was relatively common after day 22 in utero and on days 20-22 and 24-27 ex utero (Figure 7.7). Ages for which self-recursion was evident also exhibited significant deviation from independence in the overall matrix. In utero, overproduction of self-recursive movements were distributed among five categories of behavior (F, R, FH, FR, M). Ex utero, a high incidence of self-recursion appeared to be restricted to four categories of movement (R, FH, FR, M).

Self-recursive transitions occurred at chance levels before day 28 in Acomys (Figure 7.8). At all subsequent ages ex utero, and all ages except term in utero, self-recursion occurred more often than expected. This developmental pattern corresponded closely to patterns of nonindependence in the entire transition matrix. Elevated levels of self-recursion involved a similar set of movement categories both in utero (F, R, FR, M) and ex utero (F, R, FH, FR, M).

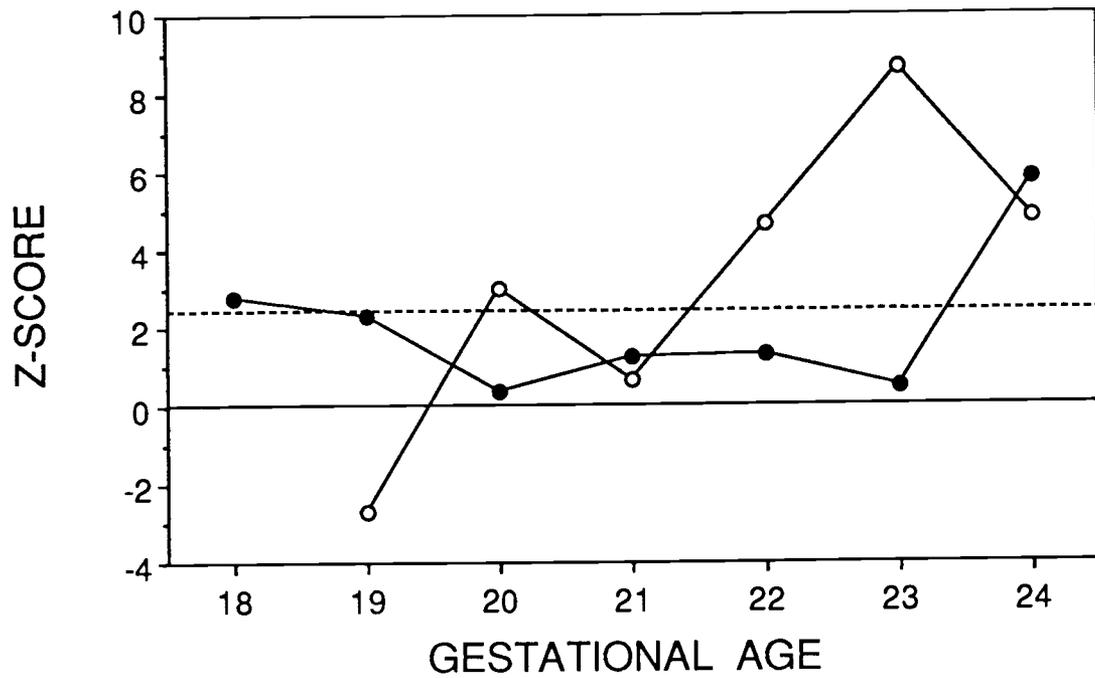


Figure 7.6. Composite z-scores depicting the relative agreement between the observed incidence of self-recursive event sequences and the expected incidence based on random sequential association in Meriones. The dashed line represents the criterion value of z ($z = 2.33$).

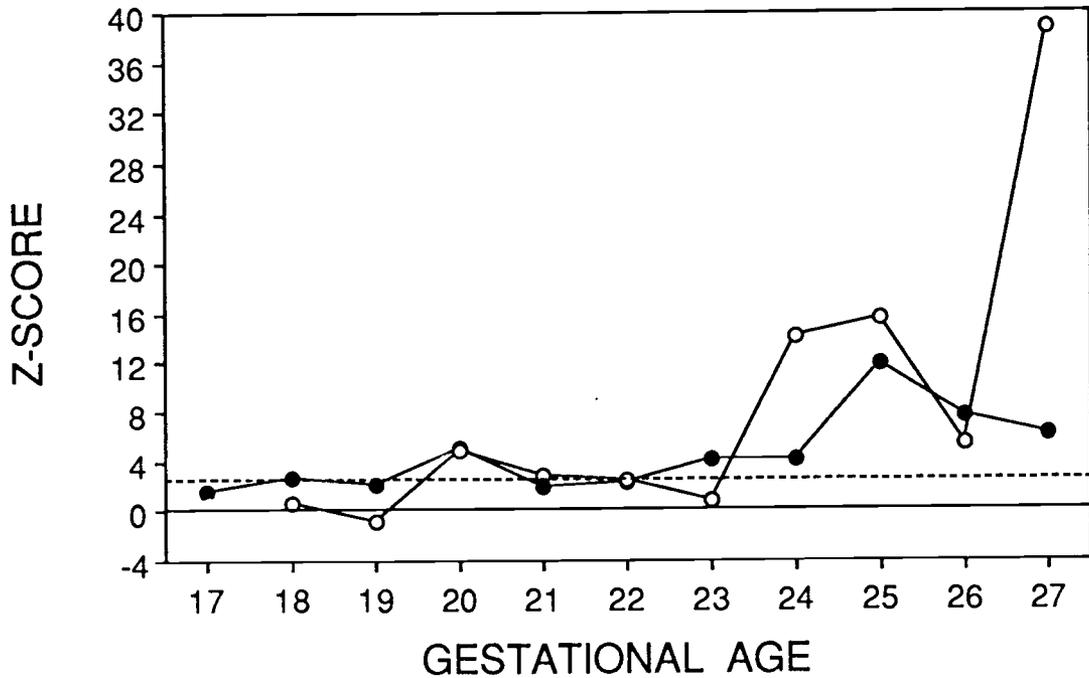


Figure 7.7. Composite z-scores depicting the relative agreement between the observed incidence of self-recursive event sequences and the expected incidence based on random sequential association in Sigmodon. The dashed line represents the criterion value of z ($z = 2.33$).

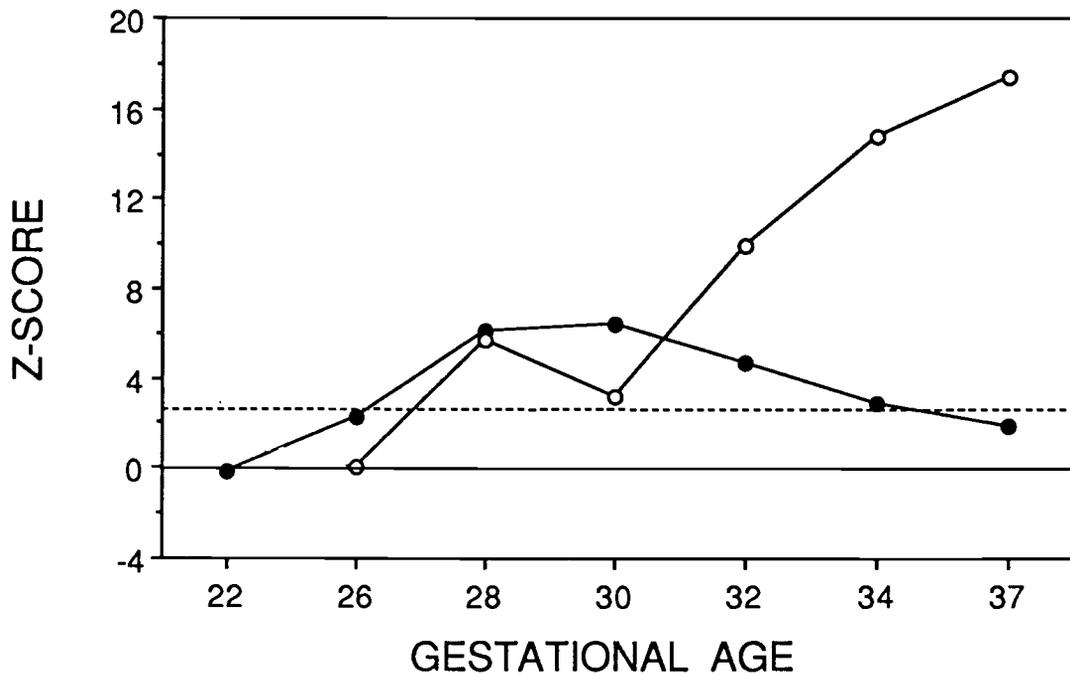


Figure 7.8. Composite z-scores depicting the relative agreement between the observed incidence of self-recursive event sequences and the expected incidence based on random sequential association in *Acomys*. The dashed line represents the criterion value of z ($z = 2.33$).

2. Sequential asymmetry

Another method was employed for identifying patterns of sequential transition other than self-recursion. Each transition matrix was constructed to be symmetrical; excluding self-recursion, every pair of behavioral categories were connected in two cells of the matrix, representing the two different directions of sequential transition between events. For example, the categories F and R yield two categories of two-event transition: F/R and R/F.

If left to chance association, the frequencies of transition in each pair of cells in the matrix should be equal. That is, there is a .50 probability that a sequential transition involving F and R will occur in the direction F/R, and .50 that it will occur in the opposite direction. Each transition matrix was systematically examined to identify pairs of cells that deviated from the predicted pattern of symmetry. Significance was judged by comparing the observed frequencies to a 50:50 distribution by means of a Chi-square goodness of fit test (df=1) [Siegel 1956]. The occurrence of significant asymmetry within the matrix was taken as evidence of preferred sequential relationships between pairs of event categories.

Numerous examples of asymmetric sequential transition were identified in all four species. In Rattus, sequential asymmetry was found among fetuses observed in utero only on

day 21, but at all ages ex utero (Table 7.3). A total of 19 cases of asymmetry were identified out of 308 comparisons (6%). Ten different event sequences exhibited a preferred direction of occurrence, but two sequences in particular stood out as consistent and relatively abundant. F/FH occurred more frequently than the reverse direction at term in utero and at four of five ages ex utero. FH/R also occurred over the last four days of gestation ex utero.

Comparisons of sequential symmetry revealed a similar developmental pattern in Meriones. A total of 23 of 364 comparisons (6%) indicated a significant directionality in sequential relationship (Table 7.4). Eleven different event sequences exhibited asymmetry, but as in Rattus, the most consistent event sequences were F/FH and FH/R. These two patterns accounted for nearly half of the observed differences. F/FH was asymmetric over the entire period of days 19-24. FH/R emerged slightly later (day 20) and was expressed significantly more often than random through term. Only one case of sequential asymmetry was evident in utero (on day 23); all others occurred with fetuses ex utero.

In Sigmodon, 34 of 588 comparisons (6%) indicated significant sequential asymmetry, an overall result identical to the findings for altricial species. Eleven categories of event transition exhibited asymmetry, but 24 of the significant comparisons were accounted for by only three event sequences (Table 7.5). F/FH exhibited a preferred direction on days 24-25 in utero and days 20-25 ex

Table 7.3

Asymmetric event sequences that occur significantly more often in the indicated direction in Rattus. Significant Chi-square tests are indicated by * ($p=.05$) or ** ($p=.01$), with $df=1$.

Condition	Age	Precedent	Subsequent	Chi-square
In Utero	21	F	FH	4.8 *
		FH	H	8.9 **
Ex Utero	17	F	FH	13.9 **
		FH	H	6.4 *
		M	F	5.8 *
	18	FH	R	7.8 **
		FR	FH	14.2 **
	19	F	FH	9.8 **
		FH	R	10.6 **
		H	F	4.2 *
	20	F	FH	7.0 **
		FH	R	18.0 **
		R	H	6.1 *
	21	F	FH	5.1 *
FH		R	12.9 **	
FR		FH	4.8 *	
H		FH	6.7 **	
R		T	5.3 *	
		R	FR	4.0 *

Table 7.4

Asymmetric event sequences that occur significantly more often in the indicated direction in Meriones. Conventions as in Table 7.3.

Condition	Age	Precedent	Subsequent	Chi-square	
In Utero	23	FH	FR	9.0 **	
Ex Utero	19	F	FH	4.3 *	
		FH	F	5.9 *	
		H	M	10.1 **	
		M	F	4.8 *	
	20	F	FH	5.6 *	
		FH	M	7.1 **	
		FH	R	7.8 **	
		H	M	7.4 **	
		M	F	7.5 **	
		F	FH	10.9 **	
	21	FH	R	5.7 *	
		22	F	FH	7.7 **
			FH	R	11.0 **
			R	FR	7.8 **

table continued

Table 7.4 (continued)

Condition	Age	Precedent	Subsequent	Chi-square
Ex Utero	23	F	FH	4.1 *
		FH	R	17.9 **
		FR	H	5.4 *
		FR	FH	4.5 *
		R	H	5.4 *
	24	F	FH	5.0 *
		FH	R	11.5 **
		FR	FH	3.9 *

Table 7.5

Asymmetric event sequences that occur significantly more often in the indicated direction in Sigmodon. Conventions as in Table 7.3.

Condition	Age	Precedent	Subsequent	Chi-square	
In Utero	24	F	FH	4.2 *	
		FH	R	8.1 **	
	25	F	FH	8.4 **	
		FH	R	8.3 **	
		FR	H	9.0 **	
		H	F	7.3 **	
Ex Utero	20	R	FR	5.5 *	
		F	FH	6.4 *	
		FH	R	21.7 **	
		H	M	5.1 *	
		21	F	FH	5.7 *
			F	T	6.6 *
	FH		R	16.2 **	
	22	FR	F	F	5.5 *
			R	FR	13.4 **
		F	FH	8.9 **	
		FH	R	17.3 **	
		R	FR	11.3 **	

table continued

Table 7.5 (continued)

Condition	Age	Precedent	Subsequent	Chi-square
Ex Utero	23	F	FH	4.2 *
		FH	R	9.3 **
		R	FR	10.1 **
	24	F	FH	4.1 *
		FH	R	11.5 **
		R	FR	4.9 *
	25	F	FH	9.9 **
		FH	R	19.8 **
		FR	H	8.1 **
		R	FR	11.9 **
	26	FH	R	4.1 *
		FR	H	3.9 *
R		FR	5.2 *	
27	FH	R	10.3 **	
	FR	FH	10.7 **	
	R	M	6.4 *	

utero. FH/R also occurred at significant frequencies on days 24-25 in utero and from day 20 through term ex utero. A third pattern, R/FR, was apparent on day 25 in utero and over the period of days 21-26 ex utero. Overall, 7 sequences were identified in utero and 27 ex utero.

Of the four species, deviations from the random symmetry model were most common in Acomys. Overall, 38 of 364 comparisons (10%) and 14 different categories of transition exhibited asymmetry, but five specific event sequences accounted for 24 of the observed deviations from the random model. As in the other three species, F/FH and FH/R were consistently asymmetric over virtually all ages ex utero (Table 7.6). R/FR, which also was noted in Sigmodon, was significant on days 28-32 and again at term. The sequence FR/H was evident at all ages except term ex utero. Finally, the sequence FR/FH occurred more often than predicted on day 28 and days 32-37 ex utero. Only one case of asymmetry was observed in utero (on day 26).

In all four species, most cases of sequential asymmetry were the result of overproduction of events in the preferred direction. For example, the event sequence FH/R occurred significantly more often than predicted by row and column totals at all ages where asymmetry was apparent, but R/FH occurred at the level of chance. However, the event sequence FR/FH, which exhibited asymmetry at several ages in the two precocial species, actually was expressed at an absolute frequency at or slightly below predictions derived

Table 7.6

Asymmetric event sequences that occur significantly more often in the indicated direction in Acomys. Conventions as in Table 7.3.

Condition	Age	Precedent	Subsequent	Chi-square
In Utero	26	H	FH	4.3 *
Ex Utero	26	F	FH	11.9 **
		FH	M	9.3 **
		FH	R	17.4 **
		FR	H	13.0 **
		H	F	6.3 *
	28	M	F	5.0 *
		T	FR	5.6 *
		F	FH	12.2 **
		FH	R	39.7 **
		FH	M	4.6 *
	30	FR	FH	7.7 **
		FR	H	9.8 **
		H	F	11.0 **
		R	FR	10.2 **
		R	T	4.0 *
30	F	FH	13.5 **	
	FH	R	49.6 **	

Table 7.6 (continued)

Condition	Age	Precedent	Subsequent	Chi-square
Ex Utero	30	FH	H	19.6 **
		FR	FH	4.2 *
		FR	H	7.5 **
		M	F	4.2 *
		R	FR	5.9 *
	32	F	H	6.9 **
		F	FH	14.4 **
		FH	R	22.3 **
		FR	FH	8.7 **
		FR	H	4.9 *
	34	R	FR	17.3 **
		F	FR	4.6 *
		FH	R	10.0 **
		FR	FH	8.9 **
		FR	H	5.6 *
	37	H	FH	5.9 *
		F	FH	4.8 *
		FH	R	18.8 **
		FR	FH	4.1 *
		H	FH	8.2 **
		R	FR	20.1 **

from row and column totals in the overall transition matrix. The complementary event sequence, FH/FR, almost always occurred at levels much below the predictions of random association. The underproduction of FH/FR was most evident *ex utero*, especially in precocial species. Significant reductions in FH/FR were evident from day 20 through term in Sigmodon and day 26 through term in Acomys, compared to days 23 through term in Meriones and days 18 through term in Rattus. Thus, unlike most other instances of asymmetric sequential relationship, FR/FH appeared to involve inhibition of sequential transitions proceeding in the opposite direction.

E. Discussion

Considering the findings of the Chi-square and information analyses together, it appeared that all four species began to express sequentially organized motor activity shortly after the inception of movement. However, sequential structure remained a relatively minor aspect of overall behavioral organization until late in gestation, when a second sharp increase in sequential patterning occurred. Although the time-course of developmental changes in sequential organization appeared similar in altricial and precocial species, the degree of sequential structure, as indicated by the magnitude of the ratio $I_2:I_1$, was more pronounced in precocial species, particularly during the

peak in late gestation.

Sequential patterning of behavior, as evidenced by the overproduction (or underproduction) of specific event sequences, also was more pronounced in precocial species. Event sequences that involved self-recursion were found in all species and emerged relatively early in gestation, roughly coincident with the initial deviation of the overall transition matrix from chance association. The overproduction of self-recursive event sequences was intensified in precocial species, especially among fetuses observed *ex utero*, as gestation proceeded. However, self-recursion, which is a relatively simple form of sequential relationship, did not appear sufficient to account for the peak in sequential organization that occurred late in gestation in all four species (Figures 7.1-7.4). It seems likely that the emergence and strengthening of specific event sequences, such as the consistent triad F/FH, FH/R, R/FR, was responsible for the expansion of sequential organization that occurred just before term.

Finally, an effect of fetal restraint in utero was evident in the expression of sequential behavioral organization. Information measures in particular indicated a pronounced difference between the sequential behavior of fetuses observed in utero and *ex utero*. In part, this effect may be partly attributable to reduced behavioral diversity in utero (see Chapter 6), which would tend to inflate values of I_1 and reduce the magnitude of the ratio

$I_2:I_1$. Additionally, certain event sequences, especially those involving synchronous movements, may be differentially affected by physical restraint within the uterus.

CHAPTER 8. RESPONSE TO INTRAORAL INFUSION

A. Introduction

The last four chapters have addressed the issue of behavioral organization in undisturbed fetuses. Apart from preparing the environment in which fetuses were observed (in utero or ex utero), subject fetuses were not explicitly manipulated. Documenting that behavioral organization can be expressed under relatively constant conditions is important, because it counters the view that the fetus is a passive object during its development in utero. It is equally important, however, to address the question of how fetuses respond to various forms of sensory stimulation.

The study of evoked fetal activity, which refers to motor responses elicited by stimuli controlled by the investigator, has played a prominent role in the history of fetal research, often overshadowing the study of "spontaneous" behavior (see Table 2.1 in Chapter 2). Probing fetal sensory responsiveness can be a useful method for uncovering otherwise hidden behavioral organization. In this chapter and the next, the response of rodent fetuses to two very different forms of sensory stimulation will be described.

The present chapter describes the behavior of fetuses following controlled exposure to a taste/odor stimulus.

Smotherman & Robinson [1987a, 1987b, 1988a] have reported that a solution prepared from lemon extract (or one of several other strongly-scented botanical extracts, such as orange or mint) when infused directly into the mouth of a rat fetus consistently elicits a distinctive behavioral response. One aspect of the response is an increase in fetal activity that quickly rises to a peak and dissipates more slowly over the course of about one minute [Smotherman & Robinson 1988a]. Of perhaps greater interest is the expression of facial wiping, a stereotypic action pattern that exhibits an abrupt developmental emergence on day 20 of gestation [Smotherman & Robinson 1989].

Facial wiping in fetal rats consists of placement of one or both forelimbs in contact with the side of the head, generally just behind the eyes, and sweeping the limb(s) in a rostral direction along the face. The movement form of facial wiping is similar to overhead limb strokes during facial grooming in mature rats, suggesting that prenatal facial wiping is developmentally continuous with adult grooming behavior. The action patterns employed in grooming or comfort behavior are highly conserved during evolution; virtually all mammals with nonspecialized anatomy employ the forelimbs in grooming movements that are directed toward the head and face [Eisenberg 1981]. If facial wiping, as expressed by fetal rats, is ontogenetically related to adult grooming behavior, then it should be present in the repertoire of other fetal rodents. Moreover, as an

antecedent of postnatal behavior, prenatal facial wiping should exhibit a developmental time course that is relatively accelerated in precocial species.

B. Methods

A minimum sample of five subject fetuses was tested at each gestational age. The ages and actual sample sizes of fetuses that received infusions are presented in Table 8.1. Pregnant females and subject fetuses were prepared for observation ex utero, following the same procedures employed in 30-min observations. In addition, after delivery into the saline bath immediately before the test session, each subject was fitted with a fine intraoral cannula to permit controlled presentation of a chemosensory test solution.

Installation of the cannula was accomplished using procedures developed for application to neonatal rats [Hall & Rosenblatt 1977; Kehoe & Blass 1985] and subsequently adapted for use with rat fetuses [Smotherman & Robinson 1988, in press]. Briefly, a slender steel wire was inserted through the fetus's lower jaw and tongue at a point immediately posterior to the intermolar eminence. A cannula fashioned from a 2-3 cm length of PE-10 polyethylene tubing (0.61 mm diameter) was fitted over the tapered end of the wire, and using the wire as a guide, pulled back through the tongue and lower jaw. The oral tip of the cannula was formed into a flange that rested on the dorsal surface of

Table 8.1

Number of subjects of various ages employed in lemon infusion experiments.

RATTUS	day 17	18	19	20	21					
	_____	_____	_____	_____	_____					
	5	5	9	11	9					
MERIONES	day 21	22	23	24						
	_____	_____	_____	_____						
	5	5	5	9						
SIGMODON	day 18	19	20	21	22	23	24	25	26	27
	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
	5	6	11	13	5	5	5	5	7	7
ACOMYS	day 26	28	30	32	34	37				
	_____	_____	_____	_____	_____	_____				
	7	5	8	5	5	6				

the tongue and prevented removal. Throughout this installation procedure, which required only 30-60 s, care was exercised to maintain the subject fetus under the surface of the water and avoid placing strain on the umbilical cord. Rat fetuses prepared in this way do not evince any lasting response to the installation procedure or to the presence of the cannula itself [Smotherman & Robinson 1988a].

To deliver the infusion, the free end of the cannula was connected to a longer piece of PE-50 tubing, which in turn was attached to a 23-ga. needle on a micrometer syringe containing the test solution. The connecting piece of PE-50 tubing was sufficiently long to avoid restricting fetal movement. This system permits precise infusion (± 1 ul) of a test solution to a fetus without otherwise interrupting ongoing activity. The test solution employed in the present study was prepared as a well-mixed 1:3 dilution of pure lemon extract (Schilling brand) in isotonic saline. This particular solution, which has a strong olfactory component, has been found to be very consistent in eliciting facial wiping and other distinctive behavioral responses from rat fetuses [Smotherman & Robinson 1987a, 1988a] and neonates [Smotherman & Robinson 1989].

Individual subjects were tested during a 4-min (240 s) observation session, during which two infusions were delivered. The two infusions, each consisting of 20 ul of the lemon solution delivered in a 1-2 s pulse, occurred

within a 5-s window at the end of the first and third minutes of the session. Subjects were observed continuously through the session and their behavior recorded following the same protocol described in Chapter 2.

C. Fetal Activity Following Infusion

To measure the overall fetal response to lemon infusion, frequency counts of fetal movements were summed to yield a component activity score for each subject. Overall activity of fetuses during the 1-min period following the first lemon infusion was broken into 12 5-s intervals for analysis. Activity also was tabulated for the 5-s window in which the infusion occurred. A final activity score (designated 'pre-infusion') was calculated as the mean activity expressed during the 11 5-s intervals before infusion. For each age tested, fetal activity across these 14 intervals was analyzed in a one-way repeated measures ANOVA. Where a significant main effect of Intervals was evident ($\alpha = .05$), a planned comparison using a one-tailed Dunnett t-test [Roscoe 1975] was conducted to determine whether the post-infusion interval exhibiting the highest rate of fetal movement was significantly greater than the pre-infusion baseline. A fetal activity response to lemon infusion was judged to be present only if this planned comparison was significant. To assess age-related changes in the magnitude of fetal response to lemon

infusion, activity scores for the six 5-s intervals immediately following infusion were summed for each age. This combined 30-s activity score was compared across ages in a one-way ANOVA.

The results of the repeated measures ANOVAs and planned comparisons for Rattus are summarized in Table 8.2. Significant increases in overall activity after infusion were evident at all five gestational ages. The pattern of change in activity after infusion was consistent with previous reports: activity quickly increased during the first 5-15 s after infusion, then decreased over the next 20-30 s, eventually returning to pre-infusion levels (Figure 8.1). The amount of fetal movement after infusion also varied with age ($F_{4,34} = 3.7, p=.014$); fetuses on days 17 and 21 were less active after infusion than fetuses on days 18-20.

Owing to their small size, Meriones fetuses were tested only on days 21-24 of gestation. Except on day 24, lemon infusion elicited little or no fetal response (Table 8.3). At this age, the characteristic spike in activity immediately after infusion followed by a gradual decline in fetal activity was apparent (Figure 8.2). Although the number of movements expressed after infusion looked to be greater on day 24, fetal activity did not vary significantly with fetal age.

Sigmodon fetuses expressed responsiveness to lemon infusion at a relatively early age. The slight increase in

Table 8.2

Summary of statistical effects in lemon infusion tests with Rattus fetuses. Results of the hypothesis tests employing the Dunnett t statistic are presented as NS (not significant), * (significant at the .05 level), or ** (significant at the .01 level).

AGE	F SCORE	DF	P VALUE	PEAK INTERVAL	DUNNETT T	RESULT
17	3.5	13,52	<.001	15	3.46	**
18	4.5	13,52	<.001	15	4.12	**
19	4.5	13,104	<.001	15	4.13	**
20	6.4	13,130	<.001	5	5.50	**
21	2.8	13,104	<.001	5	3.53	**

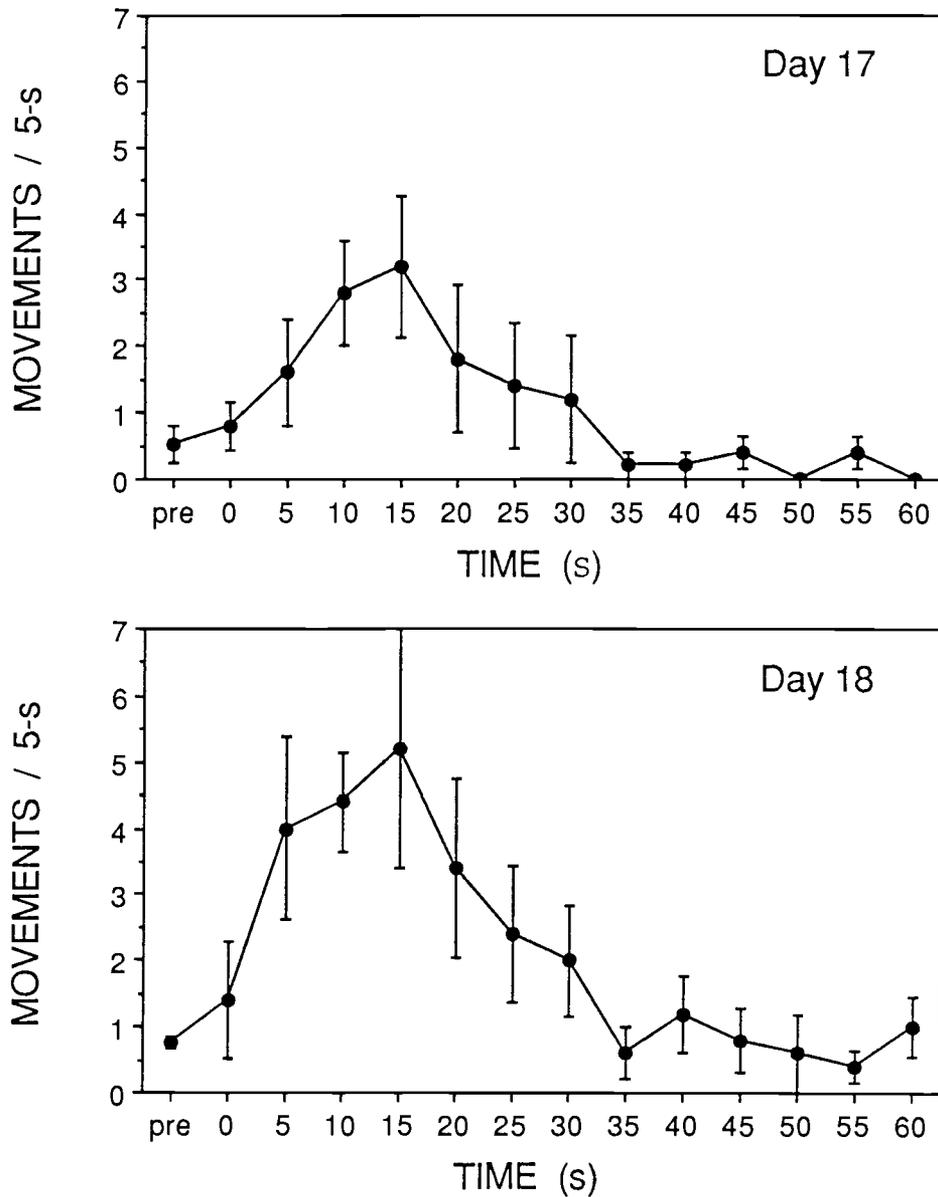


Figure 8.1. Changes in fetal event activity over the 60-s period immediately following infusion of lemon in *Rattus*. Points represent mean values; vertical bars represent SEM. Separate graphs are presented for five days of gestation. (Figure continued on next page.)

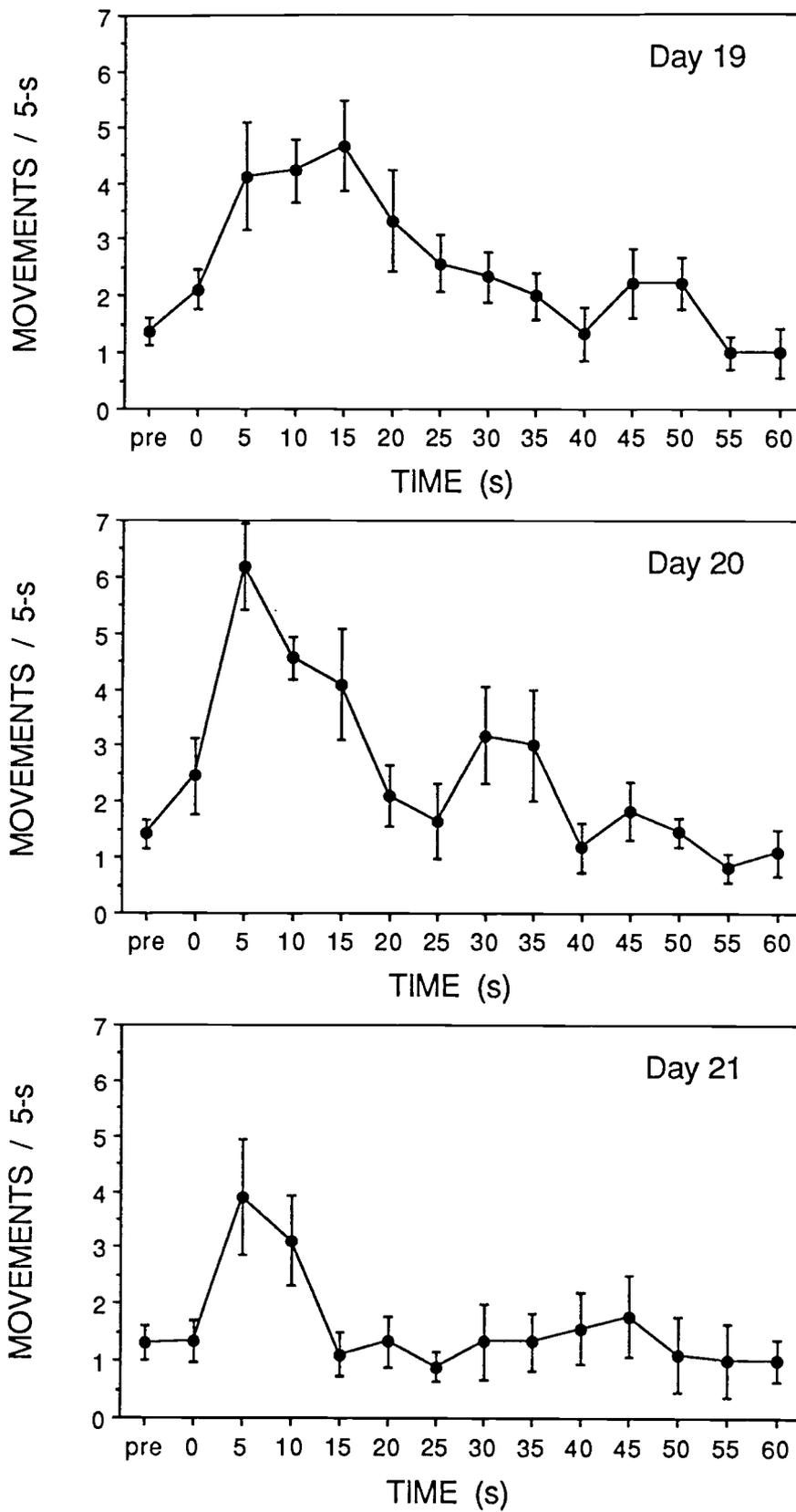


Figure 8.1 continued.

Table 8.3

Summary of statistical effects in lemon infusion tests with Meriones fetuses.

AGE	F SCORE	DF	P VALUE	PEAK INTERVAL	DUNNETT T	RESULT
21	0.7	13,52	.714	--	--	NS
22	0.7	13,52	.750	--	--	NS
23	0.5	13,52	.916	--	--	NS
24	2.1	13,104	.022	5	2.74	*

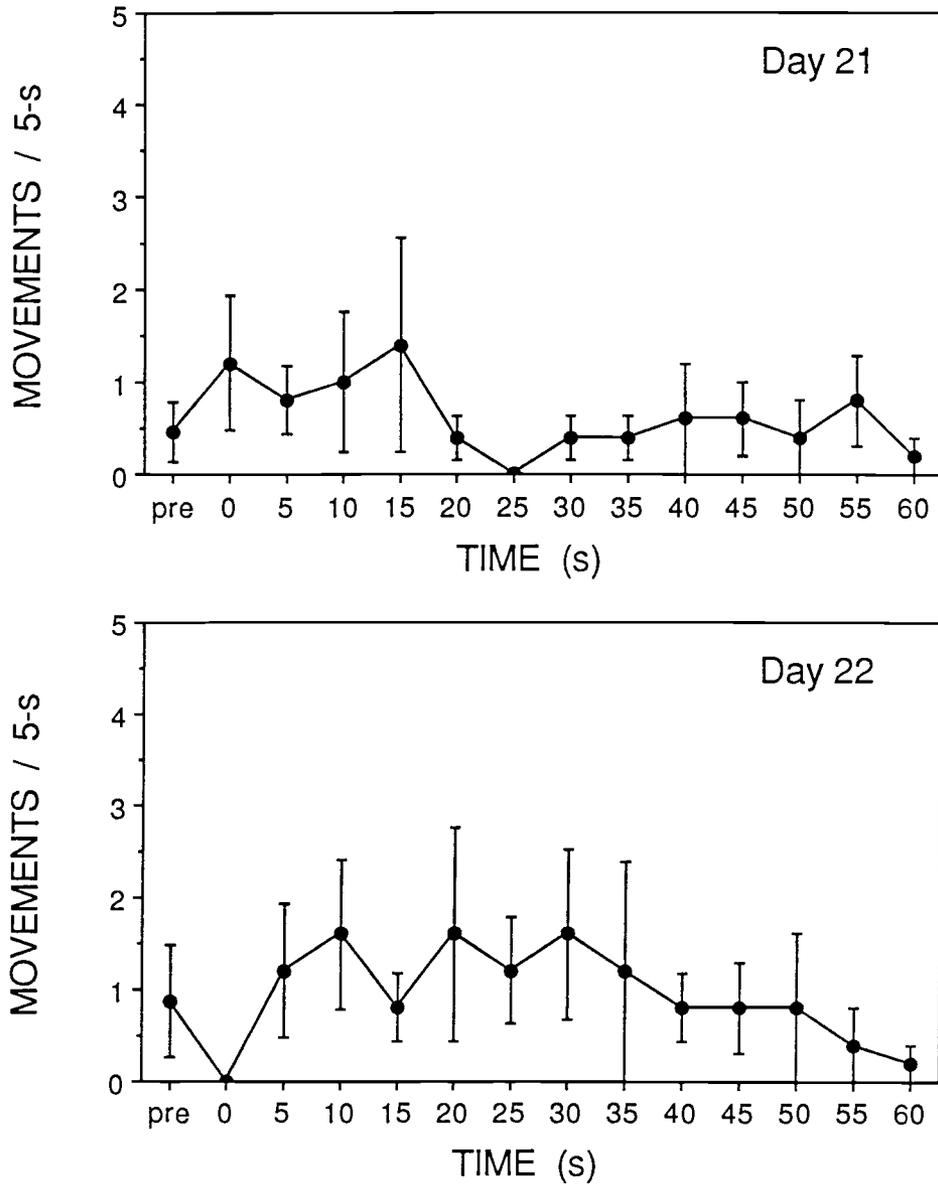


Figure 8.2. Changes in fetal event activity over the 60-s period immediately following infusion of lemon in Meriones. Points represent mean values; vertical bars represent SEM. Separate graphs are provided for four days of gestation. (Figure continued on next page.)

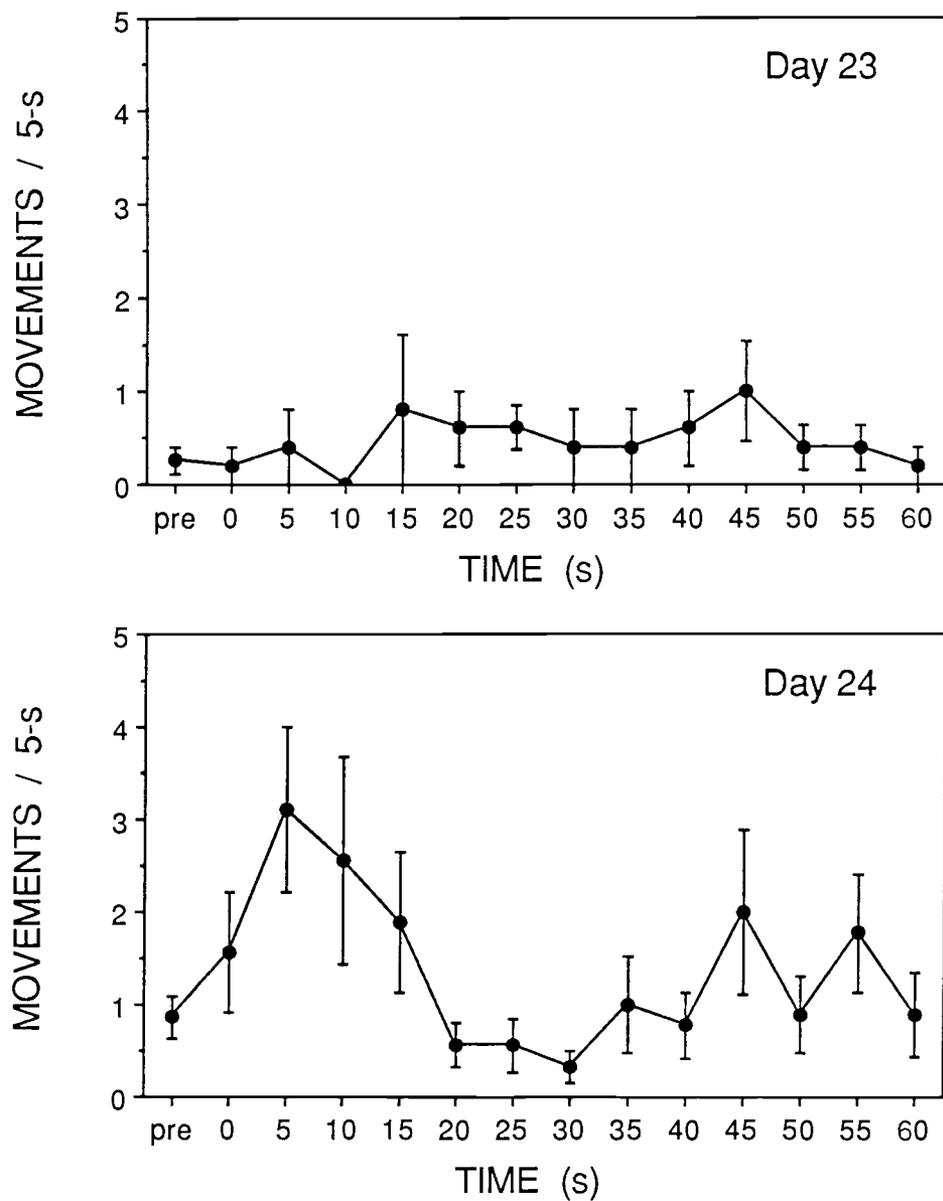


Figure 8.2 continued.

activity on day 18 was not significant, but at all subsequent ages fetal activity increased sharply after infusion (Table 8.4). The pattern of response appeared slightly different than that of Rattus. The rate of fetal movement began to rise during the interval of infusion and reached a peak only 5 s after infusion. By the third post-infusion interval (15 s), fetal activity returned to near baseline levels (Figure 8.3). Examination of 30-s activity scores indicated that the total number of fetal movements also varied as a function of age ($F_{9,59} = 3.89, p < .001$). Post-hoc comparison of means revealed that activity after infusion was significantly reduced on days 18-19 relative to later ages.

In Acomys, lemon infusion elicited a strong activity response at all ages tested (Table 8.5). The temporal pattern of activity change was very similar to that described for Sigmodon: the rate of fetal movement was noticeably elevated during the interval of infusion, increased to a peak within 5 s, and diminished to near-baseline levels only 15-20 s after infusion (Figure 8.4). Comparison of 30-s activity scores at different ages revealed no significant change in the magnitude of fetal response during gestation.

D. Facial Wiping

Facial wiping in response to lemon infusion exhibited

Table 8.4

Summary of statistical effects in lemon infusion tests with Sigmodon fetuses.

AGE	F SCORE	DF	P VALUE	PEAK INTERVAL	DUNNETT T	RESULT
18	1.7	13,52	.087	--	--	NS
19	2.3	13,65	.016	5	2.99	*
20	5.5	13,130	<.001	5	4.89	**
21	10.8	13,156	<.001	5	7.91	**
22	5.7	13,52	<.001	5	6.29	**
23	4.3	13,52	<.001	5	5.26	**
24	2.4	13,52	.014	5	3.73	**
25	4.4	13,52	<.001	5	4.79	**
26	9.4	13,78	<.001	5	7.34	**
27	3.6	13,78	<.001	5	4.01	**

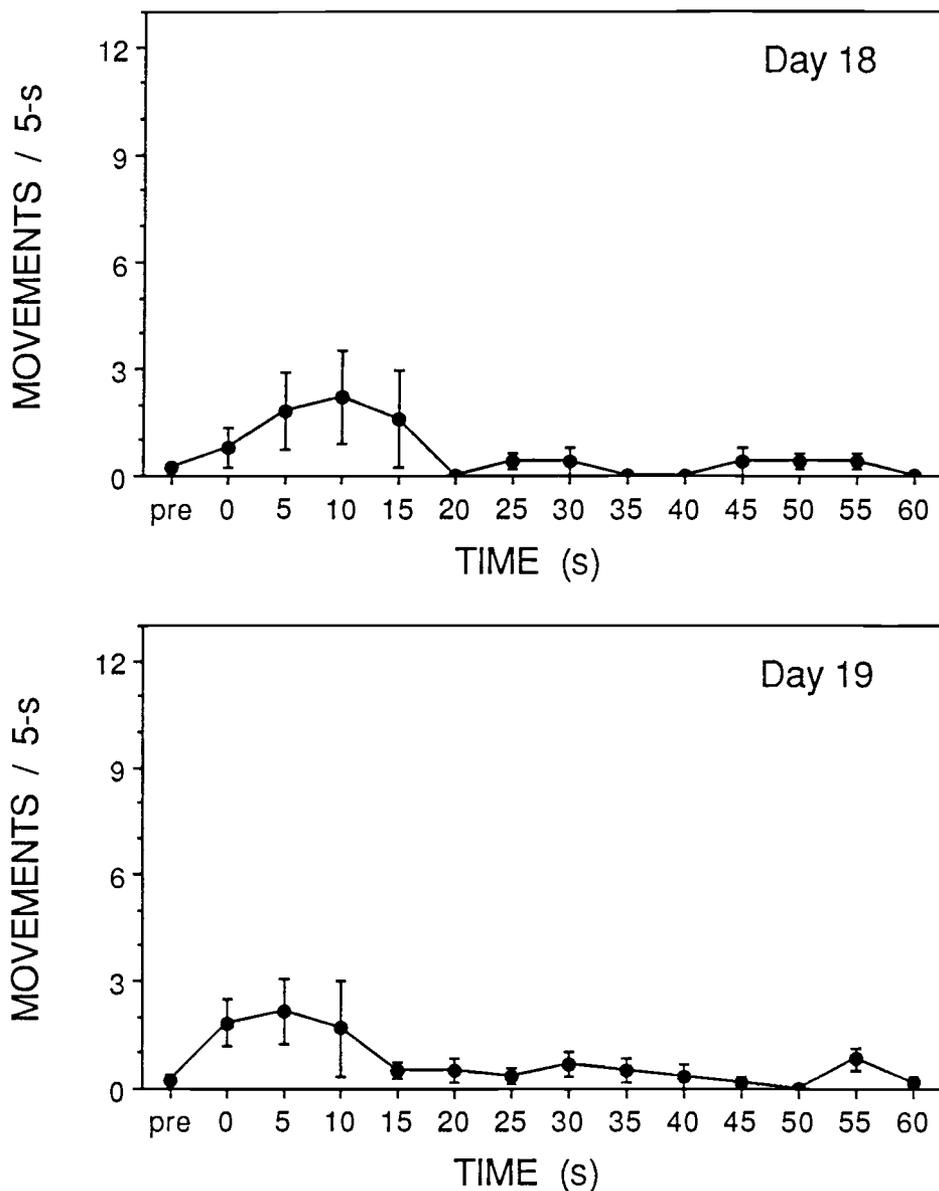


Figure 8.3. Changes in fetal event activity over the 60-s period immediately following infusion of lemon in Sigmodon. Points represent mean values; vertical bars represent SEM. Separate graphs are provided for ten days of gestation. (Figure continued on next three pages.)

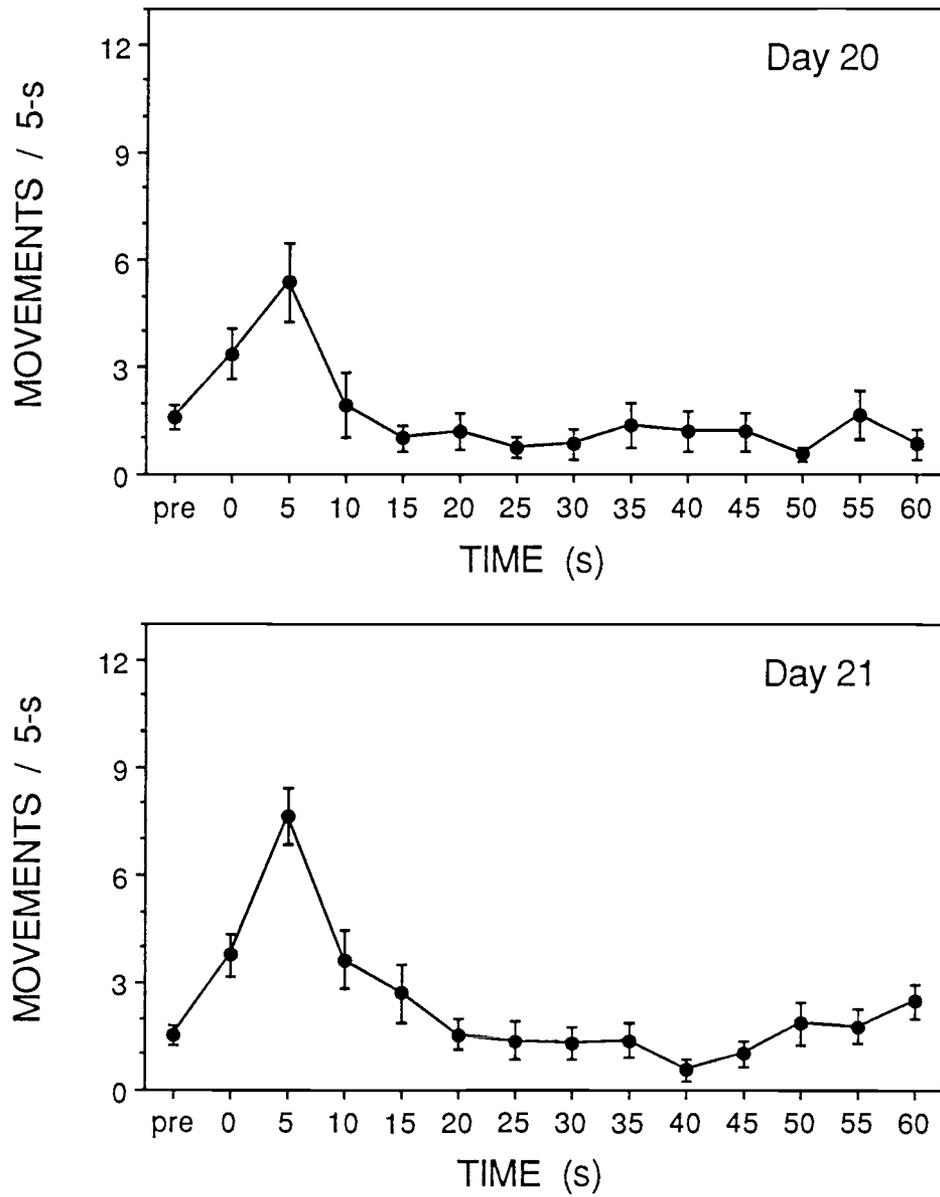


Figure 8.3 continued.

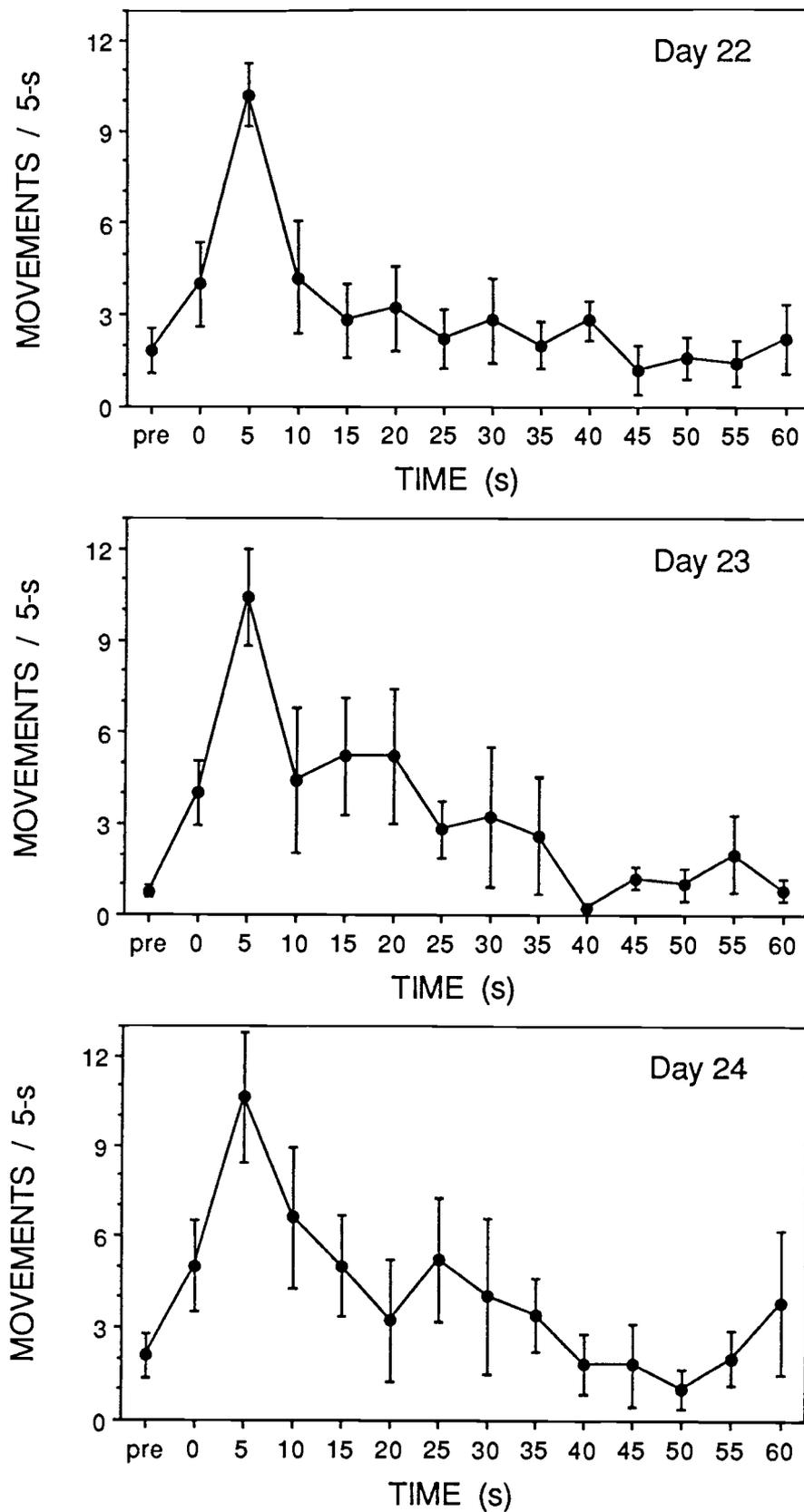


Figure 8.3 continued.

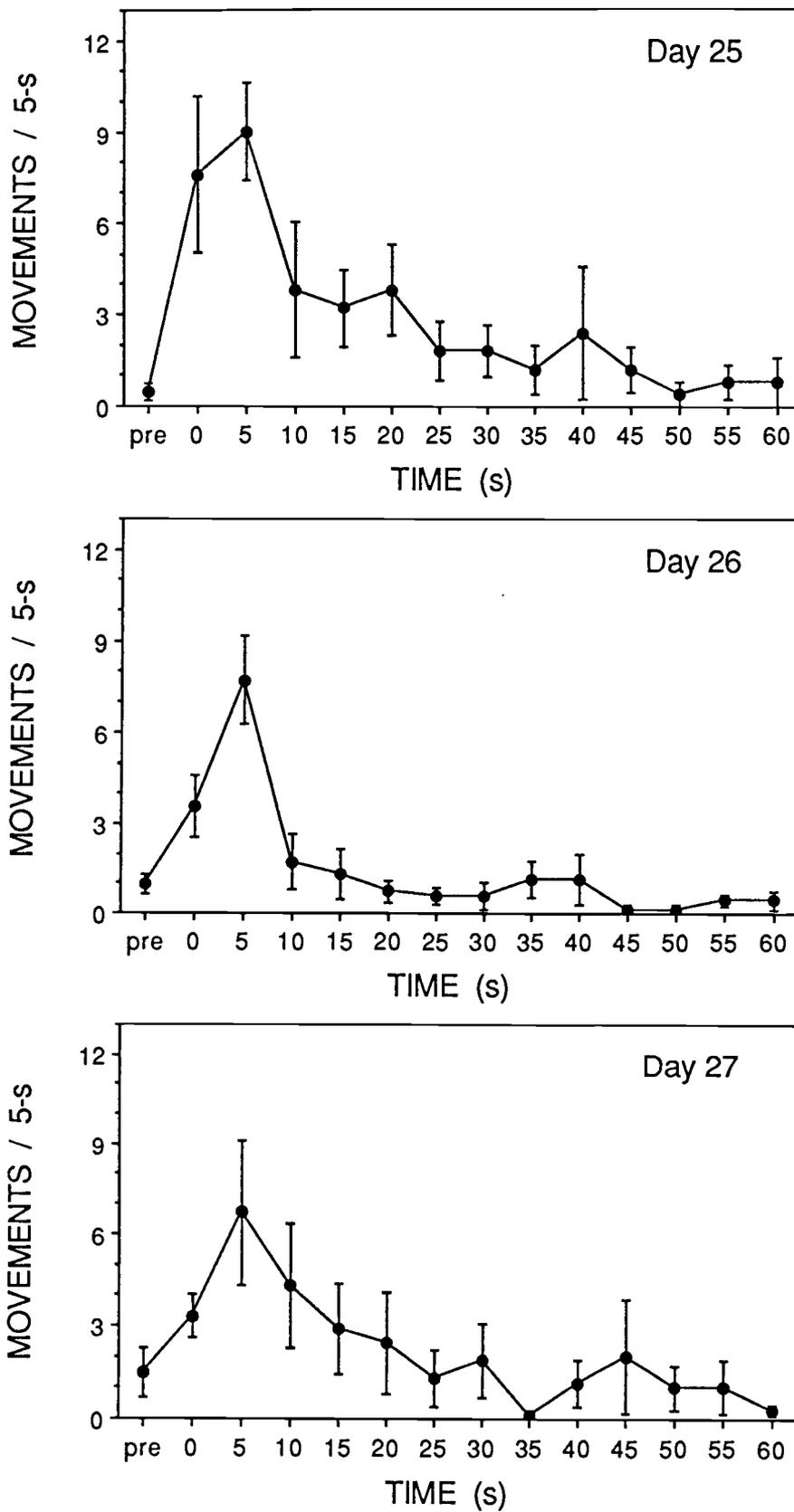


Figure 8.3 continued.

Table 8.5

Summary of statistical effects in lemon infusion tests with Acomys fetuses.

AGE	F SCORE	DF	P VALUE	PEAK INTERVAL	DUNNETT T	RESULT
26	12.6	13,52	<.001	5	6.46	**
28	9.0	13,91	<.001	5	6.67	**
30	7.2	13,52	<.001	5	6.03	**
32	5.9	13,52	<.001	5	5.07	**
34	12.82	13,65	<.001	5	7.68	**
37	3.3	13,78	<.001	5	4.19	**

Figure 8.4. Changes in fetal event activity over the 60-s period immediately following infusion of lemon in Acomys (next page). Points represent mean values; vertical bars represent SEM. Separate graphs are provided for six days of gestation. (Figure continued on following page.)

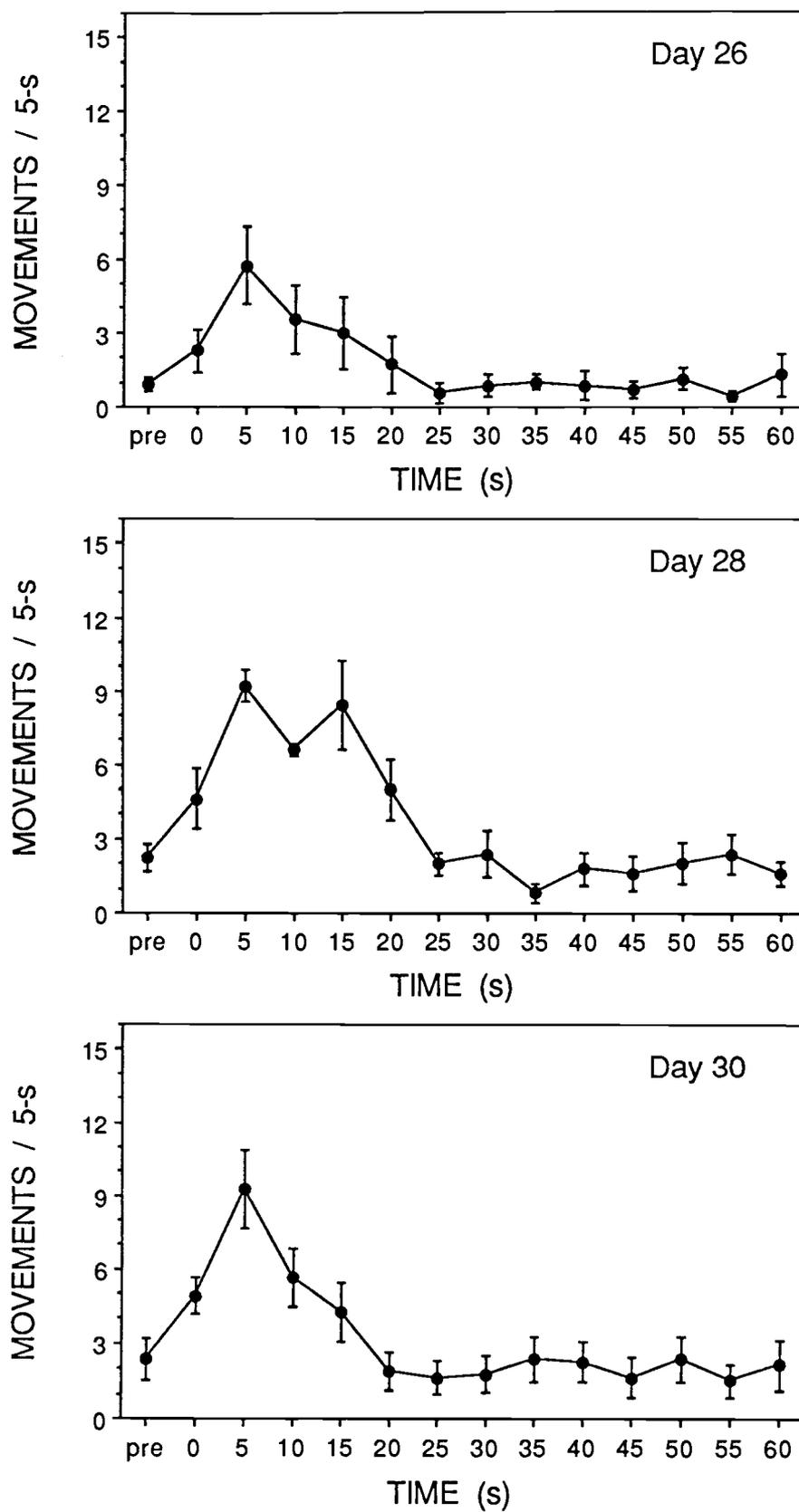


Figure 8.4 continued.

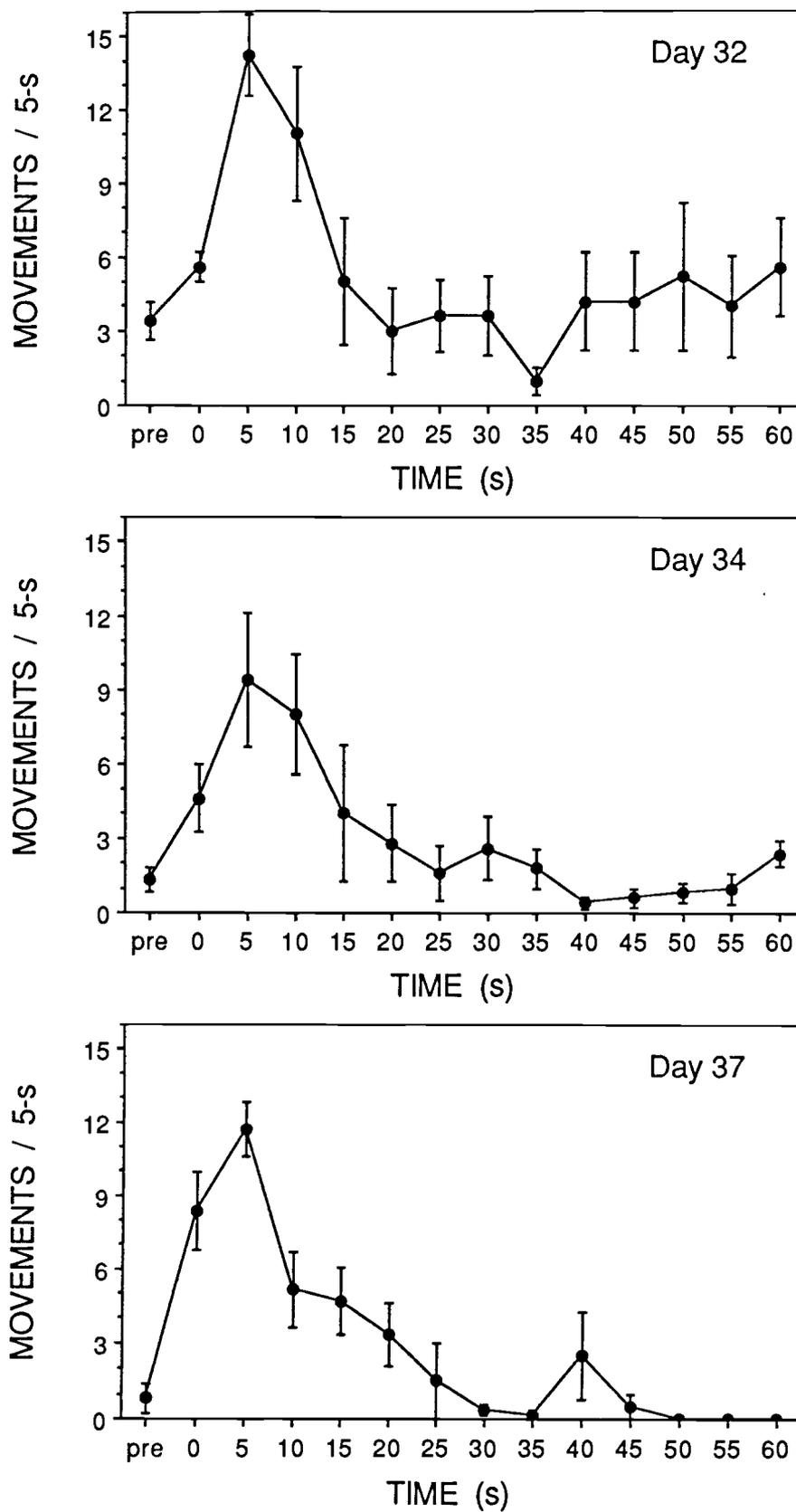


Figure 8.4 continued.

very regular patterns of expression in all four species. In Rattus, facial wiping was not observed on days 17-19, but was performed by 18 of 20 fetuses tested on the last two days of gestation (Figure 8.5). The apparent difference in the ages of expression of facial wiping was confirmed by a Chi-square test of independence ($\chi^2_4 = 32.7, p < .001$). This finding corresponds precisely to previous descriptions of the developmental emergence of this action pattern.

The occurrence of facial wiping was very consistent across individuals. Wiping typically appeared in a flurry of multiple strokes during the phase of elevated fetal activity. Comparison by t test indicated that the number of strokes performed during the initial bout of wiping did not differ between days 20 and 21; an average of 6.5 ± 0.8 facial wipes (mean \pm SEM) occurred in the first bout. The mean latency between infusion and the first facial wipe, measured to the nearest 1-s from videotape records, was 5.6 ± 0.7 s on day 20 and 7.4 ± 1.3 s on day 21. The difference in mean latency to wipe was not different between these two ages.

In Meriones, facial wiping was not observed until day 24, when about half of the subjects exhibited wipes in response to lemon infusion (Figure 8.6). To permit statistical comparison, days 21-23 were collapsed together, creating a 2 X 2 matrix. This comparison revealed a significant difference between ages ($\chi^2_1 = 10.5, p = .001$). On day 24, a mean of 1.8 ± 0.2 strokes occurred in the

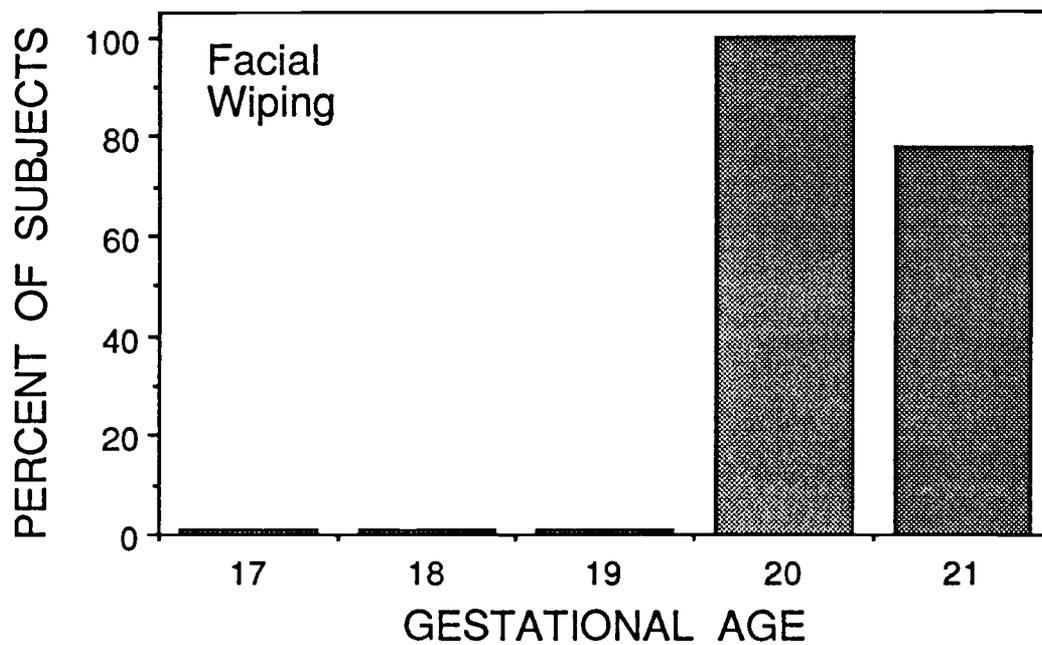


Figure 8.5. Percentage of fetal subjects that exhibited facial wiping in response to infusion of lemon at different ages in Rattus.

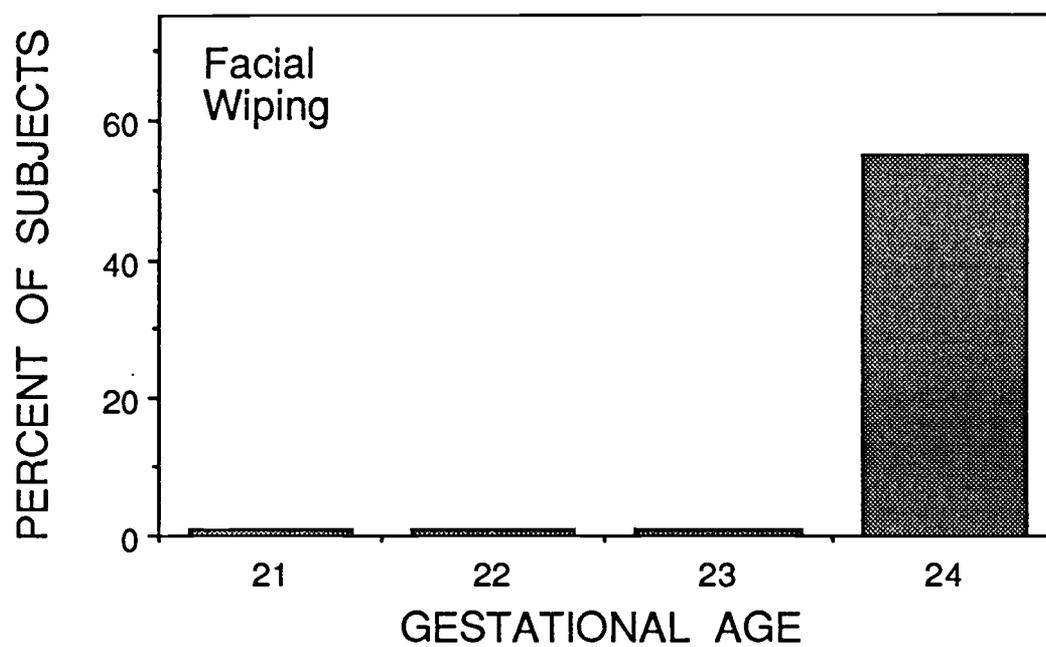


Figure 8.6. Percentage of fetal subjects that exhibited facial wiping in response to infusion of lemon at different ages in Meriones.

initial wiping bout. The latency between infusion and the first facial wipe was 8.6 ± 4.0 s.

Facial wiping also was elicited by lemon infusion in Sigmodon, but at a relatively earlier age (Figure 8.7). Wiping was not expressed on days 18-19 or on days 24-27, but was exhibited by the majority of fetuses tested on days 20-23. To compare the incidence of facial wiping at different ages, adjacent ages with sample sizes less than 10 were collapsed together, yielding the following age groups: 18+19, 20, 21, 22+23, 24+25, 26+27. The expression of wiping varied significantly among these six age groups ($\chi^2_5 = 47.1, p < .001$). Particular note should be taken of the absence of facial wiping in the response of Sigmodon fetuses to infusion after day 23.

The number of strokes performed during the initial bout of facial wiping did not vary with fetal age; overall 2.4 ± 0.3 strokes occurred in this bout. However, variation was apparent in the latency between infusion and the first facial wipe (in s): 4.3 ± 1.9 (day 20), 13.5 ± 2.2 (day 21), 17.0 ± 3.8 (day 22), 3.0 ± 0.0 (day 23). Collapsing days 22-23 for analysis, a one-way ANOVA indicated that wiping latency was significantly less on day 20 than on subsequent days ($F_{2,24} = 3.9, p = .035$).

The pattern of developmental expression of facial wiping was virtually identical in Acomys (Figure 8.8). Wiping was not expressed on day 26 or on days 32-37, but occurred in response to lemon infusion on days 28 and 30.

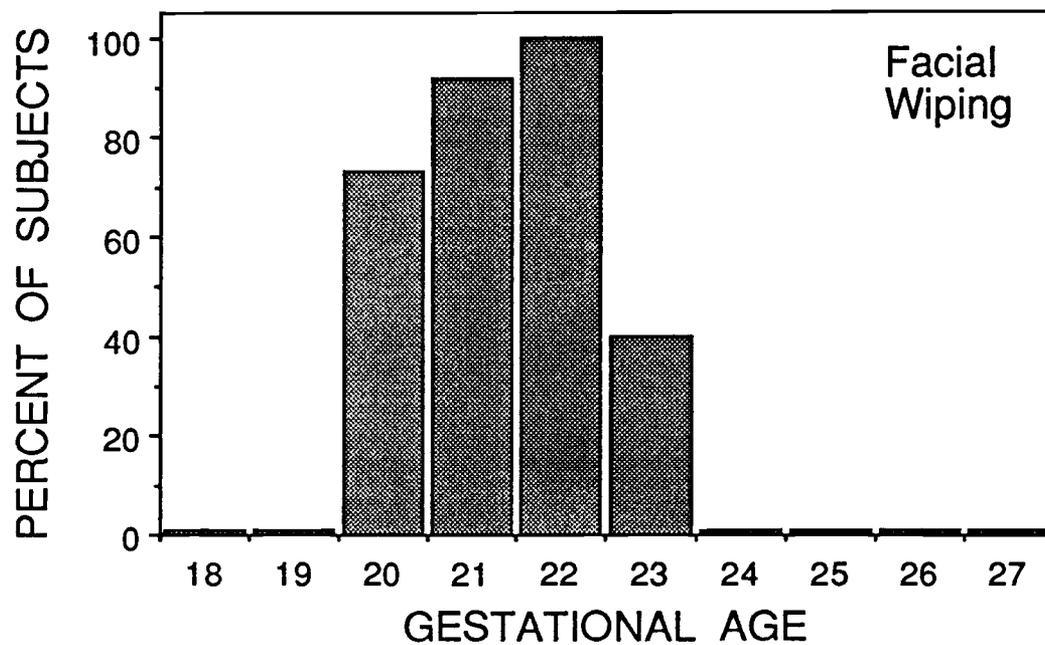


Figure 8.7. Percentage of fetal subjects that exhibited facial wiping in response to infusion of lemon at different ages in Sigmodon.

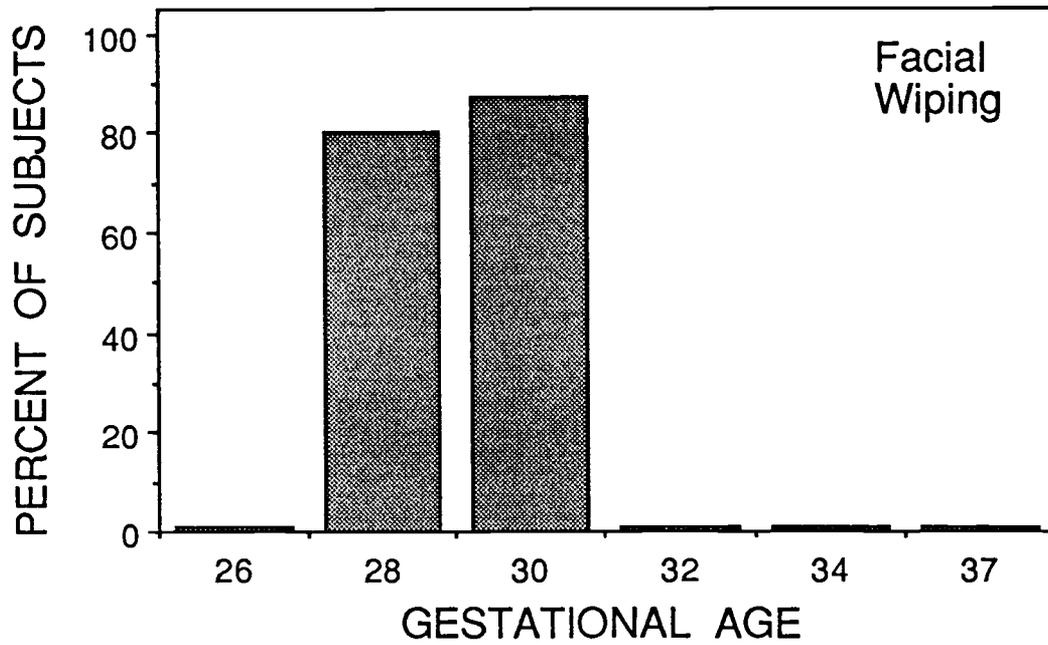


Figure 8.8. Percentage of fetal subjects that exhibited facial wiping in response to infusion of lemon at different ages in Acomys.

To analyze this pattern, samples from adjacent ages were collapsed together, creating three age groups: 26+28, 30+32, 34+37. Even when the two ages for which wiping was apparent were split in this fashion, significant age-related change in the expression of the wiping response was evident ($\chi^2_2 = 8.2, p=.017$). As in Sigmodon, facial wiping was expressed only during a narrow window relatively early in gestation. An overall average of 4.2 ± 1.0 strokes occurred in the initial bout of wiping, which did not vary between days 28 and 30. The delay between infusion and the first facial wipe also did not differ significantly as a function of fetal age. On day 28, wiping latency was 8.3 ± 4.0 s; on day 30 it was 11.7 ± 2.3 s.

E. Other Responses to Infusion

Older fetuses of precocial species exhibited different responses to infusion than facial wiping. The pronounced increase in activity consisted predominantly of vigorous, sometimes even violent, limb and body movements. Later examination of videotape records suggested that these movements were similar in form to two patterns of postnatal behavior: contact righting and quadrupedal locomotion.

The righting reflex is a fundamental behavioral pattern of terrestrial vertebrates that involves actively returning the body to a prone posture after displacement. Contact righting is the form of this behavior that occurs when the

body is in contact with a solid substrate (as distinct from air-righting, which is governed by a vestibular sense). The development of contact righting has long been appreciated to have roots in the prenatal period [Windle & Fish 1932], particularly in precocial species [Carmichael 1934, 1954].

A principal component of the contact righting response of neonatal rodents is ventriflexion or torsion of the body trunk around its long axis [Altman & Sudarshan 1975]. To quantify righting behavior in response to infusion, the incidence of both trunk torsion and successful attainment of a prone body position during the post-infusion interval were determined. A positive trunk torsion response was scored if the fetus's shoulders and hips were rotated at least 45° relative to one another in the 60-s interval following infusion. Similarly, a positive prone position was scored if the fetus attained a position within 45° of prone, measured relative to the substrate on which the fetus rested, for at least 1-s during the 60-s post-infusion interval.

Another pattern of behavior that is exhibited to varying degrees by neonatal mammals is quadrupedal locomotion. Mature walking locomotion involves coordination of stepping movements by all four limbs in a characteristic pattern of foot contact [Halbertsma 1983]. In young animals, particularly in altricial species, interlimb coordination is first apparent within a girdle: cyclic movements of contralateral limbs are strictly alternated,

with each limb about 50% out of phase with the other limb. Thus, the left limb remains in contact with a substrate (the stance phase) as the right limb is flexed and moved forward (the swing phase). Alternated stepping movements by limbs in the pectoral or pelvic girdles previously have been described for 20-day-old rat fetuses [Bekoff & Lau 1980] and are spontaneously produced by many neonatal mammals, even when removed from contact with a substrate (so-called air-stepping) [Bradley & Smith 1988].

In the present study, contralateral limb movements that resembled stepping were observed during spontaneous fetal activity. In fact, a substantial proportion of synchronous FR movements (discussed in Chapter 6) appeared to exhibit trajectories and phase relationships that were similar to the stepping motion of walking. Stepping movements were relatively common among older fetuses of precocial species, but also were identifiable among altricial fetuses near term. To quantify quadrupedal locomotor behavior during infusion experiments, a positive locomotor response was scored if the fetus exhibited activity of all four limbs while in a prone position, with contralateral limbs moving in an alternated (stepping) pattern.

Scoring the occurrence of trunk torsion, quadrupedal locomotion, and maintenance of a prone body position was accomplished with the aid of videotape playback. Videotape records created during infusion experiments were scrutinized at reduced speed to determine fetal responses. Time

measurements, accurate to less than 1 s, were facilitated by digital stopwatch recordings stamped on the videotape records. Videotapes were examined at all ages tested for all four species.

Organized responses other than facial wiping were absent in both Rattus and Meriones. Among Rattus fetuses, only one subject (out of 39) exhibited trunk torsion, and none attained a prone position or exhibited a locomotor response during the post-infusion interval. In Meriones, none of the subjects exhibited any of these responses.

Observation of Sigmodon fetuses originally suggested the existence of other organized responses to infusion, and not surprisingly, the occurrence of trunk torsion, prone position and locomotor activity was objectively confirmed in this species. Trunk torsion was absent earlier than day 21 and increased sharply in frequency between days 21 and 22 (Figure 8.9). With adjacent ages collapsed for analysis (in the same way as in the wiping analysis), the incidence of trunk torsion exhibited significant variation with age ($\chi^2_5 = 37.1, p < .001$).

The attainment of a prone position during the post-infusion interval was observed in a single fetus on days 20, 21 and 22, but occurred consistently after day 23 (Figure 8.10). Age related changes in prone position also were significant ($\chi^2_5 = 28.9, p < .001$). Although some younger fetuses successfully righted themselves, they seemed less capable of maintaining a prone position. To test this idea,

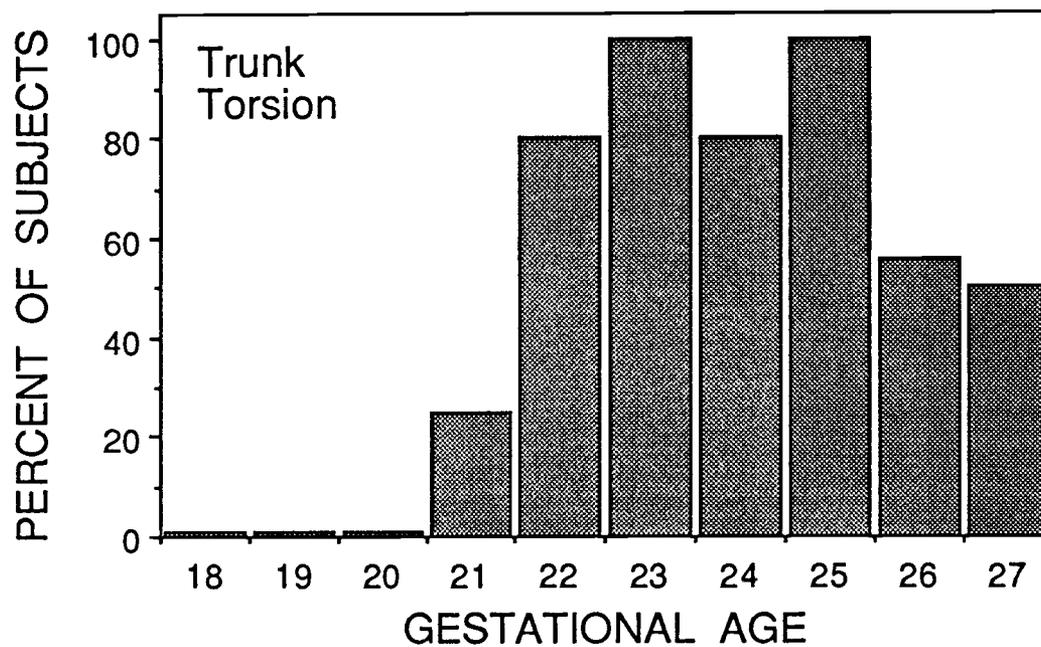


Figure 8.9. Percentage of fetal subjects that exhibited trunk torsion, a component of the righting reflex, in response to infusion of lemon in Sigmodon.

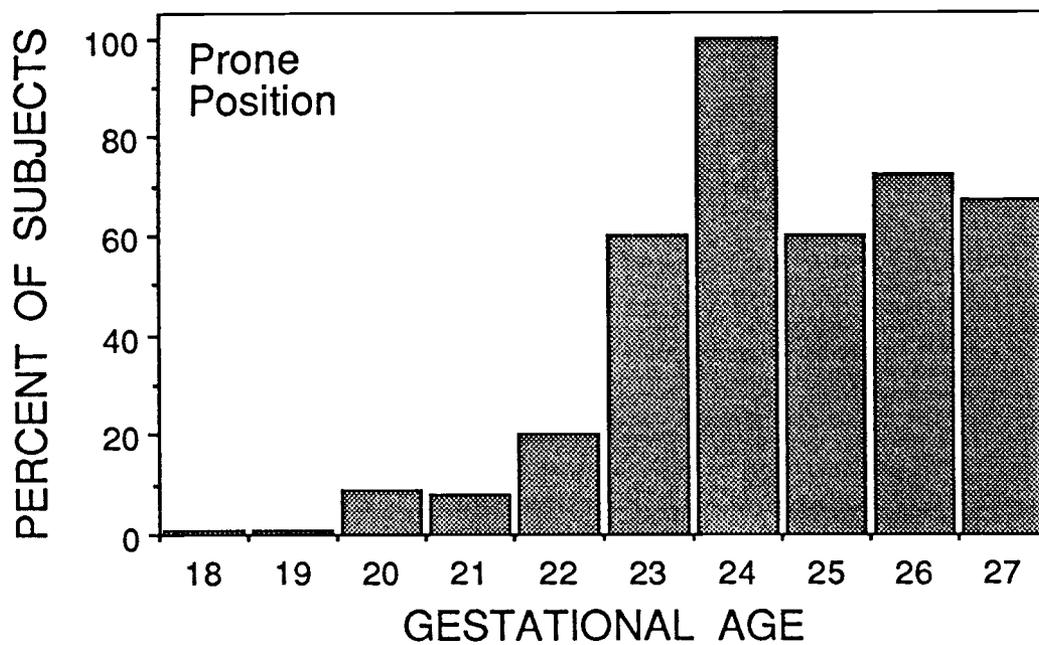


Figure 8.10. Percentage of fetal subjects that attained a prone position during the 60-s following infusion of lemon in Sigmodon.

the duration of the prone position was obtained from videotape records; duration scores were measured from the moment of the fetus attaining a prone posture until the fetus moved into a lateral or supine position or until the end of the 1-min post-infusion interval. (Thus, scores of continuous prone positions were truncated at 60 s.) To permit analysis, data from fetuses younger than 24 days were collapsed into a single category (N = 6) and compared to two groups of older fetuses. One-way ANOVA confirmed that the duration of prone position was less on days 20-23 (14.5 ± 2.5 s) than on days 24-25 (56.1 ± 1.6 s) or days 26-27 (56.4 ± 2.6 s) ($F_{2,20} = 93.7, p < .001$). This finding suggested that dynamic postural maintenance, as distinct from the active righting response itself, emerged about day 24.

The incidence of a quadrupedal locomotor response to infusion also varied with gestational age in Sigmodon. A locomotor response was not observed before day 24, but occurred in about half of the subjects observed on each of the subsequent days. Comparing the incidence of quadrupedal locomotion during the post-infusion interval before day 24 versus on or after day 24 revealed a significant deviation from independence ($\chi^2_1 = 22.5, p < .001$).

Acomys fetuses exhibited the same general developmental trend in other organized responses to infusion, but fetuses performed trunk torsion, righting and locomotor responses less consistently. Trunk torsion was performed by 8 of 20

fetuses (40%) on days 26-30, which was significantly less than the 12 of 16 fetuses (75%) that exhibited torsion on days 32-37 ($\chi^2_1 = 4.4$, $p=.036$; Figure 8.11). The incidence of prone positions after infusion showed a similar trend, with 0 of 20 successfully righting on days 26-30 compared to 11 of 16 fetuses (69%) on days 32-37 ($\chi^2_1 = 19.8$, $p<.001$; Figure 8.12). When expressed, prone positions seemed longer on day 37 than at earlier ages, but the apparent trend was not significant (duration days 32-34 = 19.4 ± 9.5 s; day 37 = 47.3 ± 8.5 s; $t_9 = 2.19$, $p=.056$). A locomotor response was observed only on day 37 (day 37 versus earlier ages: $\chi^2_1 = 29.9$, $p<.001$).

F. Discussion

The developmental pattern of expression of facial wiping by rodent fetuses could be viewed as a behavioral curiosity. But I believe that facial wiping among altricial and precocial species raises issues that are fundamental to understanding the prenatal development of behavior.

1. Emergence of the facial wiping response

All four species observed in this study exhibited a general increase in activity and expressed facial wiping in response to lemon infusion. Emergence of the wiping response was relatively abrupt. At the earliest age that

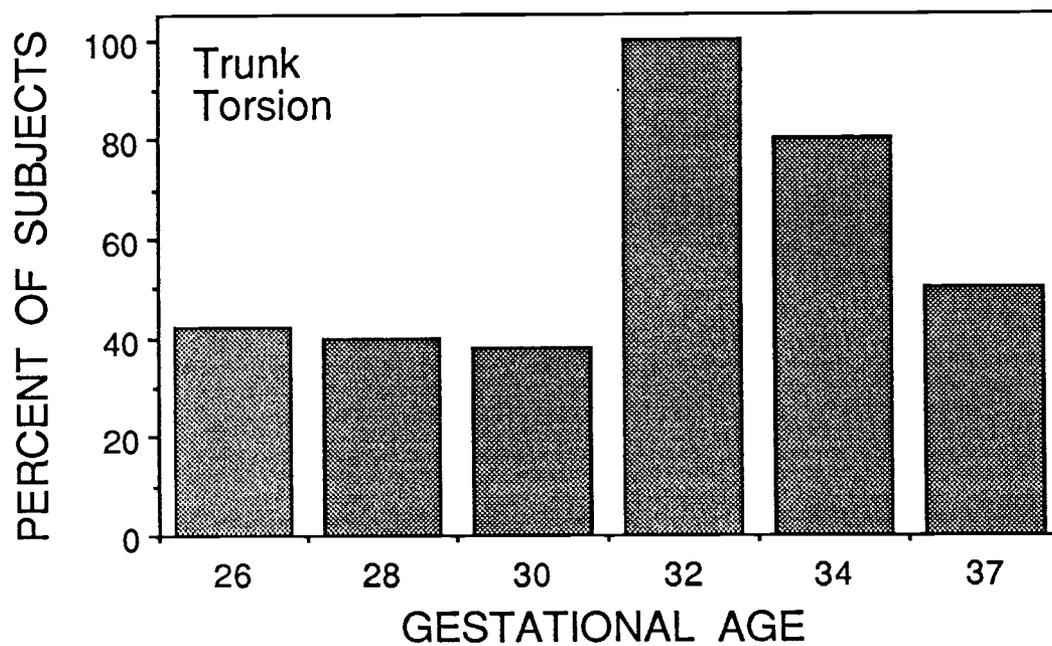


Figure 8.11. Percentage of fetal subjects that exhibited trunk torsion, a component of the righting reflex, in response to infusion of lemon in Acomys.

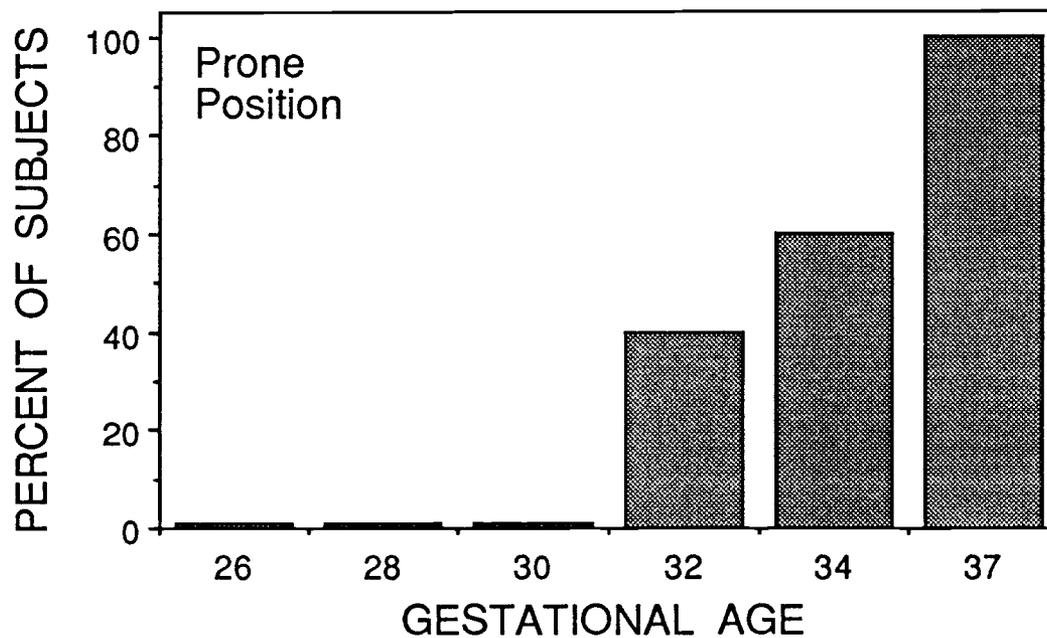


Figure 8.12. Percentage of fetal subjects that attained a prone position during the 60-s following infusion of lemon in Acomys.

facial wiping was observed in each species, the majority of individual fetuses expressed the wiping response. These findings represent a replication of results previously reported for Rattus and confirmation of the general prediction that facial wiping is a conservative pattern of behavior that should be expressed across taxa.

What mechanism might account for the sudden developmental appearance of an organized behavioral pattern such as facial wiping? It is clear that facial wiping is a response to sensory stimulation. The close temporal association of wiping with the moment of infusion indicates that the fetus senses the lemon infusion and actively responds. Moreover, evidence obtained from experiments with rat fetuses strongly suggests that fetal responsiveness to lemon infusion in general, and the wiping response in particular, is controlled by a chemical sense, most likely olfaction mediated by the accessory olfactory bulb [Robinson & Smotherman in press]. The sudden appearance of facial wiping could, in principle, be explained by the establishment of critical neural pathways associated with the onset of sensory function. However, in Rattus, Sigmodon and Acomys, a behavioral response to lemon infusion was evident before the emergence of facial wiping. Further, conditioned aversions to similar botanical extracts can be established in rat fetuses several days earlier than the appearance of the wiping response [Smotherman & Robinson 1985]. The sudden emergence of facial wiping during

gestation therefore cannot be ascribed to the onset of sensory function in three species, but remains a viable hypothesis for Meriones.

A second possibility is that neural elements responsible for generating the motor pattern become functional about the time that facial wiping is first expressed. In a general sense, it must be possible to map behavioral development onto coincident development of the nervous system. In a more specific sense, though, this hypothesis may be testable if the neuronal pattern generator for the facial wiping response can be identified and its development independently charted. This prospect offers an interesting and challenging objective for future research.

A third hypothesis asserts that facial wiping can be subdivided into component movements, all of which are expressed prior to the emergence of a recognizable wiping response. The principle assumption of this model is that sudden emergence of an organized pattern of behavior is due to a relatively small developmental step, namely the coordination of pre-existing components, rather than the relatively large step involving simultaneous, rapid construction of the entire pattern.

Some data from the present study lend support to a "coordinated component" hypothesis. Facial wiping involves coordination of forelimb trajectory with head position. The coordination of head and forelimb movements was one of the earliest forms of synchronized behavior to develop, and in

all four species forelimb-head synchrony emerged prior to the appearance of facial wiping (Chapter 6). Overall fetal activation in response to lemon infusion also preceded facial wiping in all but Meriones. Forelimb and head were the predominant components in this general increase in fetal activity after infusion. More specific evaluation of the coordinated component model must await detailed motion analysis of fetal responses to infusion before and after the onset of facial wiping.

2. Disappearance and developmental continuity

The developmental disappearance of the prenatal facial wiping response is, in some ways, a more intriguing problem than its emergence. Is prenatal facial wiping developmentally continuous with elements of adult grooming behavior and aversive response? The answer to this question depends upon what is meant by continuity. Clearly, facial wiping is not expressed continuously from the time of its prenatal emergence through adulthood. In precocial fetuses (Sigmodon and Acomys), facial wiping disappears from the fetal response to infusion well before term. Although the fetuses of altricial species (Rattus and Meriones) express facial wiping at term, the wiping response disappears shortly after birth [Smotherman & Robinson 1989; Robinson, personal observations]. The temporal discontinuity apparent in both precocial species (prenatal disappearance) and

altricial species (postnatal disappearance) casts doubt upon the relationship between fetal and adult facial wiping.

However, the wiping response to lemon infusion reappears in Rattus pups toward the end of the second week of life [Smotherman & Robinson 1989]. The postnatal reappearance of the wiping response coincides with the emergence of spontaneous autogrooming behavior [Richmond & Sachs 1980], the expression of adultlike responses to aversive or noxious substances [Grill & Berridge 1985; Johanson & Shapiro 1986] and the ability of pups to maintain an upright posture with the hindlimbs [Altman & Sudarshan 1975]. Because lemon infusion is effective in eliciting facial wiping in juvenile pups, and because juvenile and adult grooming movements exhibit virtually the same temporal and spatial form as fetal facial wiping, the underlying control systems and neural basis for facial wiping behavior are almost certainly isomorphic.

The behavioral context in which facial wiping occurs also is similar, at least in precocial species, during prenatal and postnatal life. Overhead limb strokes executed during postnatal grooming behavior, which are topographically similar to fetal facial wiping, are associated temporally and sequentially with other grooming movements. For example, overhead strokes often alternate with bouts of small paw movements in the vicinity of the mouth, some of which are involved with paw-licking [Fentress & Stilwell 1973; Fentress 1978; Golani & Fentress 1985].

Similar paw-licking movements were observed in the present study during spontaneous fetal activity. Specifically, paw-licking was observed in Sigmodon on day 23 and all subsequent ages through term. (A total of 13 fetuses exhibited paw-licking). In Acomys, paw-licking was observed from day 28 through term (total of 15 fetuses), and on many occasions occurred in temporal association with spontaneous facial wiping. Other components of postnatal grooming, such as scratching the body with the rearleg, also were noted on occasion in precocial, but not altricial, fetuses near term. The existence and association of other patterns of grooming behavior with fetal facial wiping strengthens the interpretation of the wiping response as developmentally continuous with postnatal behavior.

Moreover, recent experiments have demonstrated that facial wiping can be elicited, under special conditions, during the early neonatal period when it ordinarily is absent [Smotherman & Robinson 1989]. Immersion of rat pups in a buoyant fluid medium during an infusion trial results in reinstatement of the wiping response during the early neonatal period (day 1 postpartum). Placement of immersed pups on an underwater substrate inhibits the expression of wiping. Providing external supports for an upright body posture also facilitates facial grooming in neonatal mice [Golani & Fentress 1985]. These experimental findings suggest that the wiping response may be analogous to neonatal stepping movements and walking in humans [Thelen &

Fisher 1982]: the environment present at the time of testing constrains the expression of the motor pattern. In a terrestrial environment, the newborn experiences greater mass loading on the body and limbs, which are no longer supported by a buoyant fluid. In human neonates, increasing leg mass initially outpaces muscular growth, and the stepping movements evident at birth disappear from the repertoire until, about 2-3 months later, muscle development has progressed sufficiently to overcome gravitational constraints [Thelen 1988; Thelen, Fisher & Ridley-Johnson 1984].

An environmental constraint hypothesis, as formulated above, presumes that the developmental disappearance of a motor pattern is a passive consequence of the changing physical relationship between the young animal and its environment. An alternative hypothesis views the disappearance as the result of active choice (in the cybernetic sense) among competing behavioral options. One choice is facial wiping, which involves movement of the forelimbs through a trajectory oriented with respect to the head. Another option is postural maintenance, which in young animals with poorly developed motor abilities requires use of all four limbs to maintain a prone position with respect to a substrate [Altman & Sudarshan 1975]. Forelimbs employed in postural maintenance are not available for facial wiping, and vice versa, suggesting that a hierarchy of behavioral priorities is reflected in the underlying

control system [Fentress 1984].

It is evident in precocial species that the response to lemon infusion is not unitary. At different ages, one or more patterns of behavior, including facial wiping, righting, and locomotion, are evoked by infusion. The facial wiping response disappears at just the time that trunk torsion and successful righting to a prone position develop (days 23-24 in Sigmodon; days 30-32 in Acomys). Prenatal disappearance of the wiping response would not be predicted by a passive environmental constraint mechanism, which presumably would operate only in a terrestrial (i.e., postnatal) environment. But prenatal disappearance is consistent with the hypothesis of competition among incompatible and simultaneously activated behavioral processes.

The exceptions to this hypothesis may well prove the rule: on days 21-23 some Sigmodon fetuses exhibited both righting and facial wiping behavior. Closer scrutiny of the expression of these responses by individual fetuses provides further confirmation of the behavioral competition model. Prior to day 24, Sigmodon fetuses were relatively unable to maintain a prone position after righting themselves: the average duration of prone position was 14.5 s. The latency between infusion and wiping, which was longer on days 21-23 (when righting behavior was elicited by infusion) than on day 20 (and also longer than exhibited by Rattus or Meriones fetuses), was virtually equal in length to the duration of

prone position: 13.0 s. Examination of videotapes indicated that when both patterns of behavior occurred during the post-infusion minute, facial wiping occurred after righting. In fact, wiping occurred soon after the fetus reverted from a prone to a lateral or supine position. Only one day later (day 24), the average length of prone position after infusion was in excess of 56 s, a time that was longer than the duration of elevated fetal activity after infusion. Presumably, the control command to perform facial wiping was no longer present when the prone position was finally abandoned.

A behavioral competition hypothesis also is consistent with the observed occurrence of spontaneous facial wiping in unmanipulated fetuses. In Rattus, Sigmodon and Acomys, facial wiping was occasionally observed in fetuses ex utero during bouts of other motor activity. Spontaneous facial wiping did not occur at earlier gestational ages than reported for infusion experiments, but in the two precocial species wiping did occur occasionally during late gestation. Specifically, facial wiping was observed in Acomys on days 32 and 34 (three fetuses at each age) and in Sigmodon on day 25 (one fetus). As noted above, fetuses at these ages responded to infusion by righting and running, but maintenance of a prone posture and locomotor activity eventually waned. During spontaneous activity, facial wiping occurred during intervals in which maintenance of a prone posture and locomotor responses were not apparent.

3. General conclusions

Two broad inferences may be drawn from this analysis of facial wiping. First, it is plausible that the facial wiping response consists of sub-components that antedate the developmental appearance of the complete response. If so, then the emergence of facial wiping depends upon the development of higher level coordinative structures that control the relationships among the component movements. Future research should be able to objectively identify sub-components of facial wiping and independently chart their ontogeny.

Second, although behavioral organization is recognizable in the response of fetuses to infusion, conflict among different patterns of behavior that are simultaneously activated is also evident. One of the hallmarks of adult behavior is that simultaneous activation of physically incompatible patterns of movement rarely occurs. The occasional expression of ambivalent, blended, uncoordinated or contextually-inappropriate patterns of behavior under specific conditions of stimulation was of great interest to the early students of animal motivation, who erected categories such as "redirected" and "displacement" activities to account for unusual forms of behavioral conflict [Hinde 1970]. Nevertheless, by far the most common response of mature animals to simultaneous

presentation of conflicting stimuli is expression of one response and complete inhibition of the other. If the fetal response to lemon infusion may serve as a general indicator, the ability of fetuses to inhibit all but one response is poorly developed in altricial species and does not develop in precocial species until late in gestation.

CHAPTER 9. RESPONSE TO UMBILICAL CORD OCCLUSION

A. Introduction

One of the likely reasons that fetal behavioral adaptations have received little attention is the uncertain relation of the fetus to its environment. Mature animals must seek food, shelter, protection from predators and mates. But the fetus seems to have all its needs satisfied through a life-support system consisting of the umbilical cord and placenta. Maintenance of this life-support system is crucial to fetal survival and growth. Therefore, one of the most likely places to find behavioral adaptations during the prenatal period is in association with maintenance of a healthy umbilical connection to the placenta.

Indeed, fetuses are at risk of transient occlusion of blood circulation within the umbilical cord during gestation. Acute fetal hypoxia, induced by umbilical cord compression, has been implicated as an important cause of brain damage in human fetuses [Mann 1986] and can occur during unremarkable pregnancies [Itskovitz, LaGamma & Rudolph 1987]. Accidental cord occlusion can occur by twisting of the cord as a result of fetal activity or by pinching the cord between the fetus and a hard part of maternal anatomy (such as the pelvis) or, in species that bear multiple offspring, against an adjacent sibling in

utero.

Previous investigators have reported observations that suggested the existence of a behavioral response of fetuses to umbilical cord occlusion. Many of the early studies of fetal motor development conducted during the 1920s and '30s were criticized by Windle [1944] as lacking in proper controls to avoid the effects of fetal hypoxia; to support his claim, Windle described a predictable sequence of behavioral changes that occur when fetuses become anoxic. More recently, descriptions of a brief period of fetal hyperactivity following transient hypoxia have been reported in rats [Becker, King, Marsh & Wyrick 1964] and dogs [Arshavsky, Arshavskaya & Praznikov 1976].

Smotherman and Robinson [1987c, 1988c] have confirmed and extended these previous reports in a series of quantitative experimental investigations of the response of rat fetuses to transient occlusion of the umbilical cord. Briefly, they describe a three-phase response that follows placement of a microvascular clamp on the umbilical cord, which completely occludes umbilical circulation. This response consists of (a) initial suppression of fetal activity, (b) an intermediate phase of fetal hyperactivity, in which activity increases four- to five-fold over baseline levels, and (c) a final phase of behavioral suppression in which fetal activity drops to near zero levels until removal of the umbilical cord clamp. The critical feature of this pattern is the second phase -- hyperactivity -- which distinguishes

it as an active behavioral response to cord occlusion and not a passive physiological effect of reduced oxygen availability. These characteristics of the umbilical clamp response provide a strong prima facie case that it is a behavioral ontogenetic adaptation of the fetus.

In the present study, the umbilical clamp response was further investigated in Rattus and three other rodent species. Specifically, analyses were planned to determine the presence or absence of fetal hyperactivity following placement of the umbilical cord clamp and the pattern of developmental expression of the hyperactive response among the different species.

B. Methods

A minimum sample of five subject fetuses was tested at each gestational age from one day after the inception of movement (day 26 in Acomys) through term. Actual sample sizes are presented in Table 9.1. Pregnant females and subject fetuses were prepared for observation ex utero, following procedures described in Chapter 2. Testing of each subject occurred during a 3-min (180 s) observation session. The subject was observed without manipulation for an initial period of 55 s to establish a baseline of fetal activity.

At the conclusion of the baseline observation period, a small vascular clamp was placed on the umbilical cord of the

Table 9.1

Number of subjects of various ages employed in experiments on fetal response to umbilical cord occlusion.

RATTUS	day 17	18	19	20	21					
	_____	_____	_____	_____	_____					
	5	5	5	5	5					
MERIONES	day 19	20	21	22	23	24				
	_____	_____	_____	_____	_____	_____				
	5	5	5	5	5	6				
SIGMODON	day 18	19	20	21	22	23	24	25	26	27
	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
	5	5	6	11	5	5	5	5	6	5
ACOMYS	day 26	28	30	32	34	37				
	_____	_____	_____	_____	_____	_____				
	5	5	5	5	5	5				

subject. This clamp was sufficiently strong (about 10-20 g of compression pressure) to completely occlude blood circulation within the umbilical cord without producing permanent damage to the cord [Smotherman & Robinson 1987c, 1988c]. An attempt was made to standardize placement of the clamp 5-10 mm from the point of attachment of the umbilical cord to the subject's abdomen, although this was not practical in all cases.

The beginning of the observation period following placement of the clamp was designated t_0 . The actual time of placement of the clamp varied, but always occurred within the 5-s window preceding t_0 . After the umbilical cord clamp was in place, the subject was observed without further manipulation for an additional 2 min (through t_{120}). The condition of the umbilical cord and clamp was monitored through the observation session. Only sessions in which the clamp remained in place throughout the period $t_0 - t_{120}$ are reported; tests in which the clamp was dislodged were discontinued and replaced with different subjects.

Frequency counts of fetal movements were obtained in the same manner as in nonmanipulative observation sessions (e.g, Chapter 4) and summarized as Component Activity. The 120-s period following placement of the clamp was divided into eight 15-s intervals to facilitate measurement of temporal changes in fetal activity in response to umbilical cord occlusion. A ninth interval, designated 'pre-clamp', was derived from the baseline of fetal activity during the

55-s period preceding placement of the clamp; pre-clamp activity was expressed as the average number of acts per 15-s.

For each age tested, fetal activity scores across successive 15-s intervals were analyzed in a one-way repeated measures ANOVA. Where a significant main effect of Intervals was evident ($\alpha = .05$), a planned comparison using a one-tailed Dunnett t-test [Roscoe 1975] was conducted to determine whether the post-clamp interval exhibiting the highest rate of fetal movement was significantly greater than the pre-clamp baseline. The conclusion regarding the presence or absence of a fetal hyperactive response to umbilical cord occlusion at each age was contingent upon the result of this planned comparison.

C. Clamp Effects on Fetal Activity

Statistical analyses revealed a strong behavioral response to experimental umbilical cord occlusion at all five ages tested in Rattus (Table 9.2). The three-phase pattern of activity suppression, activation, and suppression was clearly evident at each age (Figure 9.1). An additional one-way ANOVA was computed to compare the amount of fetal activity at the peak of the hyperactive response (i.e., the 15-s interval during the post-clamp period with the greatest number of acts; "Peak Interval" in Table 9.2). This analysis revealed significant change in the magnitude

Table 9.2

Summary of statistical effects in umbilical clamp tests with Rattus fetuses. Results of the hypothesis tests employing the Dunnett t statistic are presented as NS (not significant), * (significant at the .05 level) or ** (significant at the .05 level).

AGE	F SCORE	DF	P VALUE	PEAK INTERVAL	DUNNETT T	RESULT
17	4.6	8,32	<.001	60	3.26	**
18	3.7	8,32	.008	90	3.71	**
19	11.4	8,32	<.001	60	5.36	**
20	22.8	8,32	<.001	60	7.62	**
21	3.0	8,32	.014	45	2.86	*

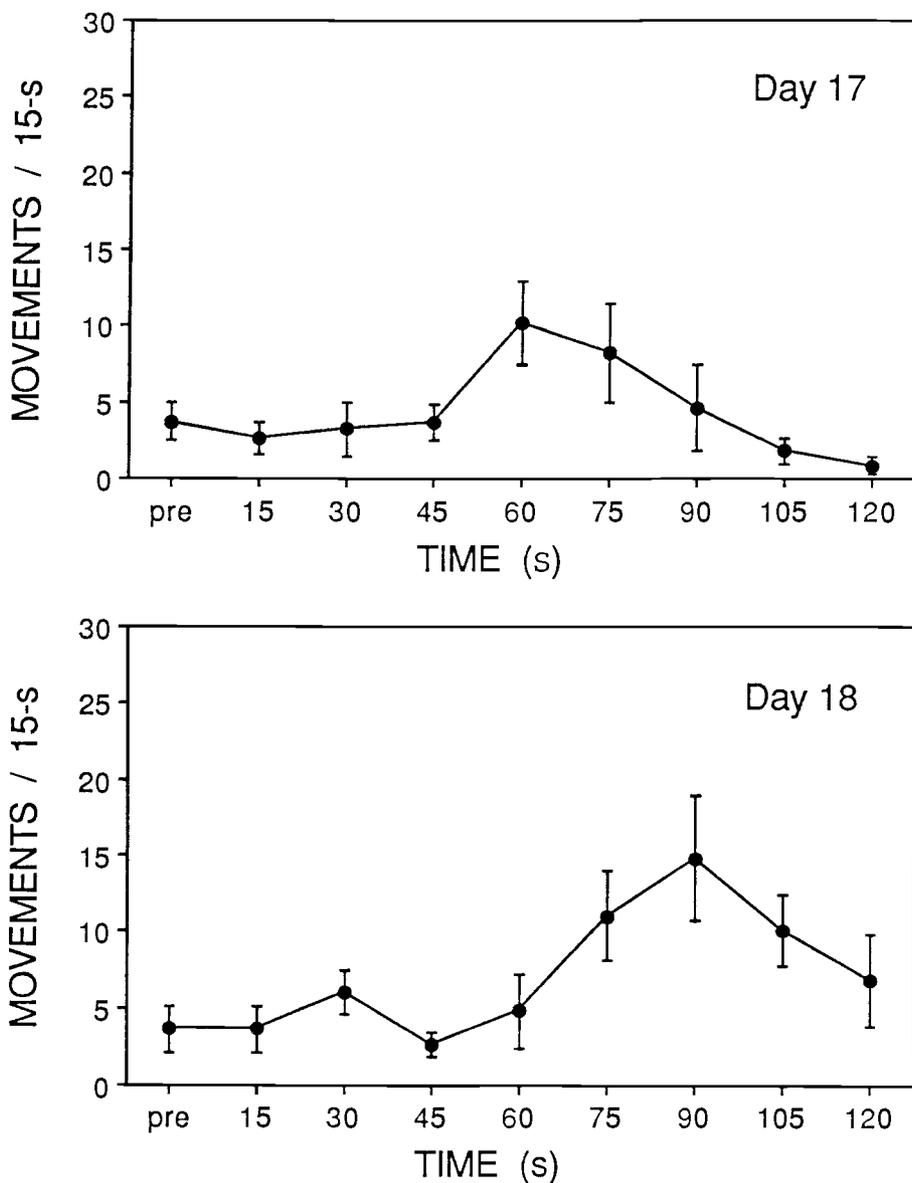


Figure 9.1. Changes in fetal event activity over the 120-s period immediately following placement of the microvascular clamp on the umbilical cord in Rattus. Points represent mean values; vertical bars represent SEM. Separate graphs are provided for five days of gestation. (Figure continued on next page.)

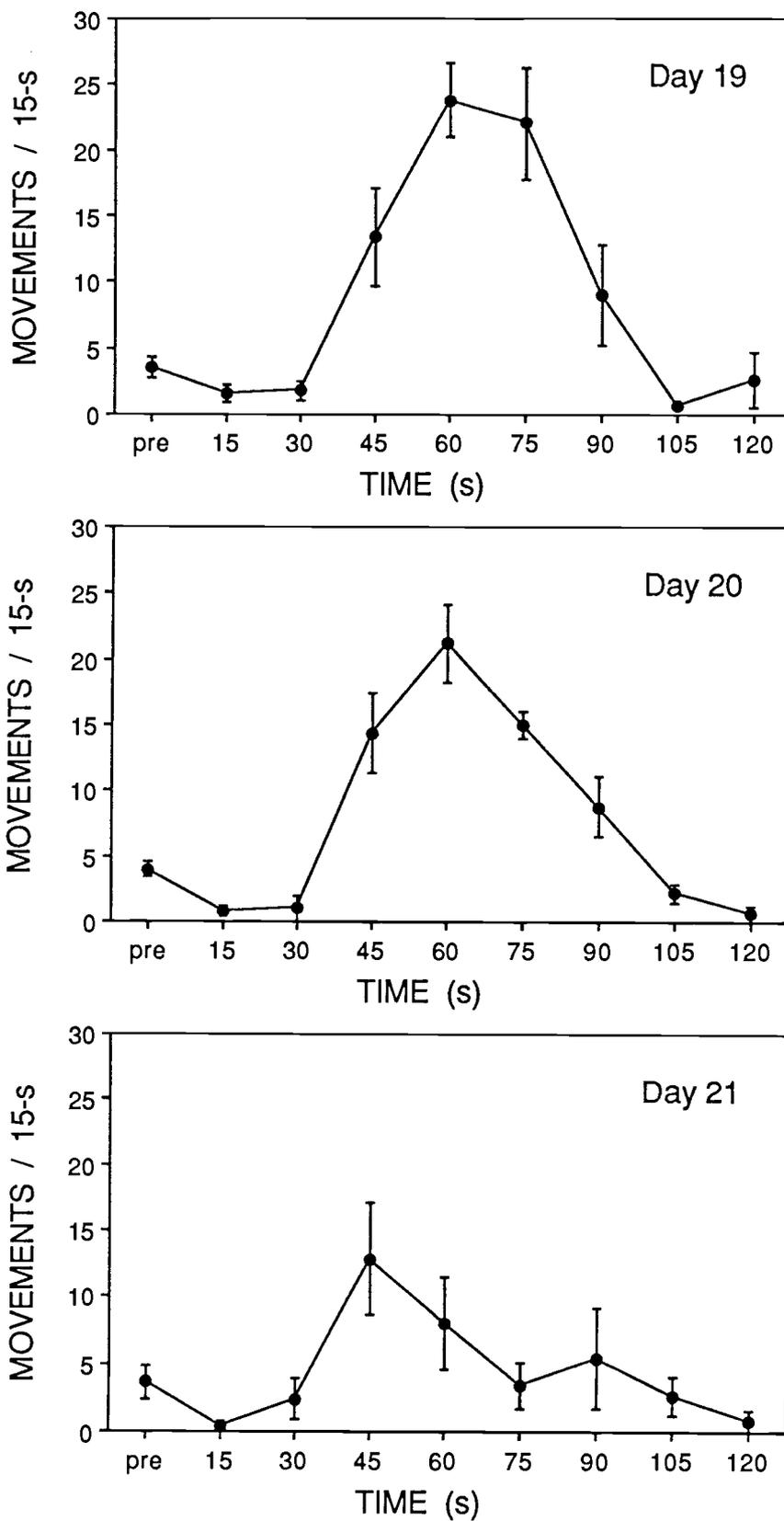


Figure 9.1 continued.

of the clamp response with gestational age ($F_{4,20} = 3.28$, $p=.032$). Further comparison of means indicated only that a significant increase in the magnitude of the response occurred between days 17 and 19.

In Meriones, fetal hyperactivity following placement of the clamp also was evident at all ages tested (Table 9.3). The overall pattern of activity change appeared virtually identical to that exhibited by Rattus, with low rates of movement occurring both before and after the hyperactive phase (Figure 9.2). Post hoc comparison of the amount of fetal activity expressed during the peak of the response indicated no significant variation with gestational age.

Sigmodon fetuses exhibited a behavioral response to umbilical cord occlusion that included a phase of hyperactivity, but this response was not evident at all ages (Table 9.4). No response at all was evident from day 18 through day 21. A significant effect was obtained in the overall ANOVA on day 22, but the planned comparison indicated that the peak of fetal activity during the post-clamp period did not differ significantly from pre-clamp baseline activity. At all subsequent ages, a significant increase in fetal activity was observed following placement of the umbilical cord clamp (Figure 9.3). Post hoc comparison of the peak activity expressed during the last five ages tested suggested variation in the magnitude of the hyperactive response with age ($F_{4,21} = 2.98$, $p=.043$). This effect was due to the reduced level of response observed on

Table 9.3

Summary of statistical effects in umbilical clamp tests with Meriones fetuses

AGE	F SCORE	DF	P VALUE	PEAK INTERVAL	DUNNETT T	RESULT
19	5.4	8,32	<.001	45	3.39	**
20	3.3	8,32	.009	60	3.03	*
21	5.9	8,32	<.001	75	3.88	**
22	8.2	8,32	<.001	75	3.92	**
23	7.2	8,32	<.001	60	4.05	**
24	7.6	8,40	<.001	60	3.46	**

Figure 9.2. Changes in fetal event activity over the 120-s period immediately following placement of the microvascular clamp on the umbilical cord in Meriones (next page). Points represent mean values; vertical bars represent SEM. Separate graphs are provided for six days of gestation. (Figure continued on following page.)

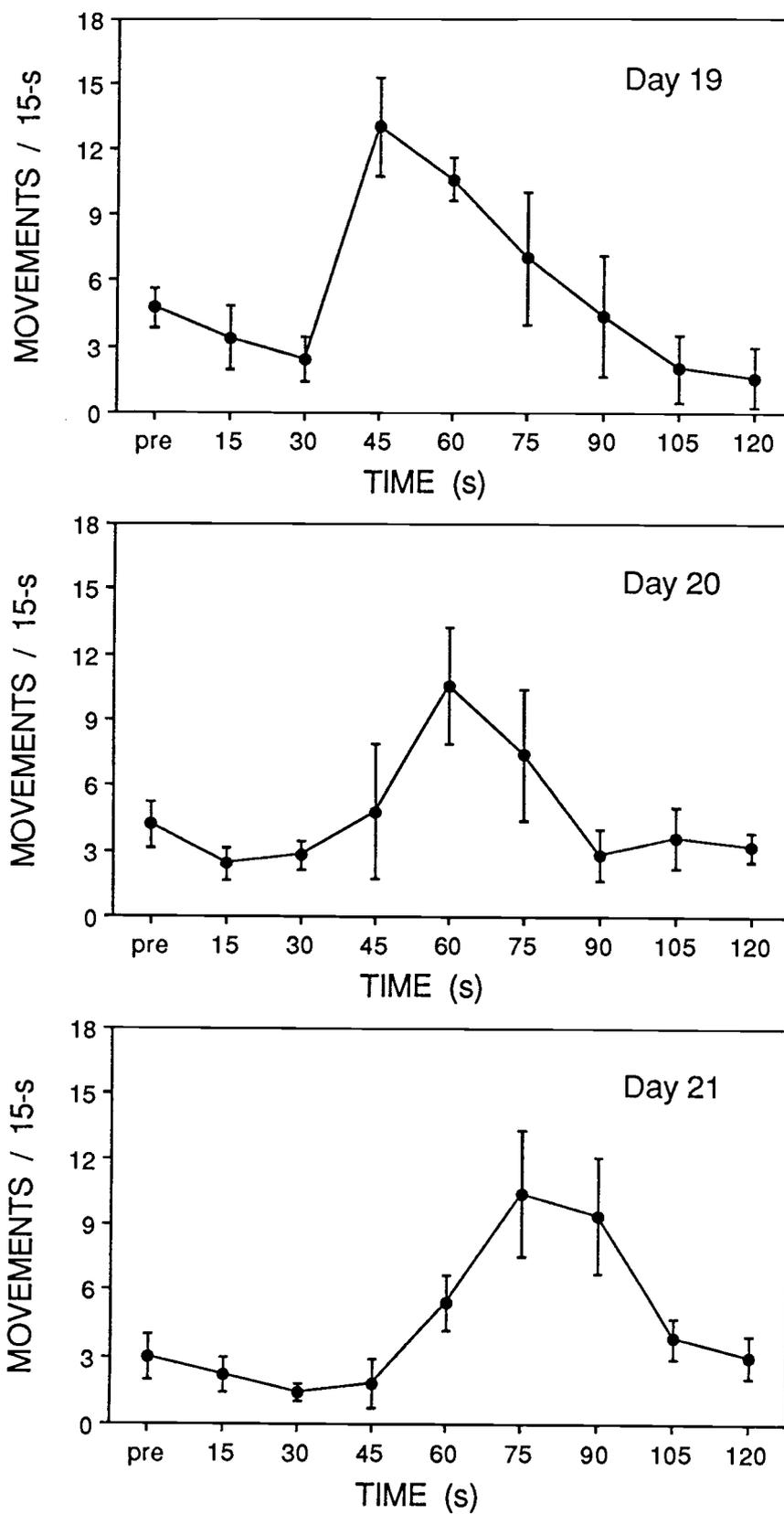


Figure 9.2 continued.

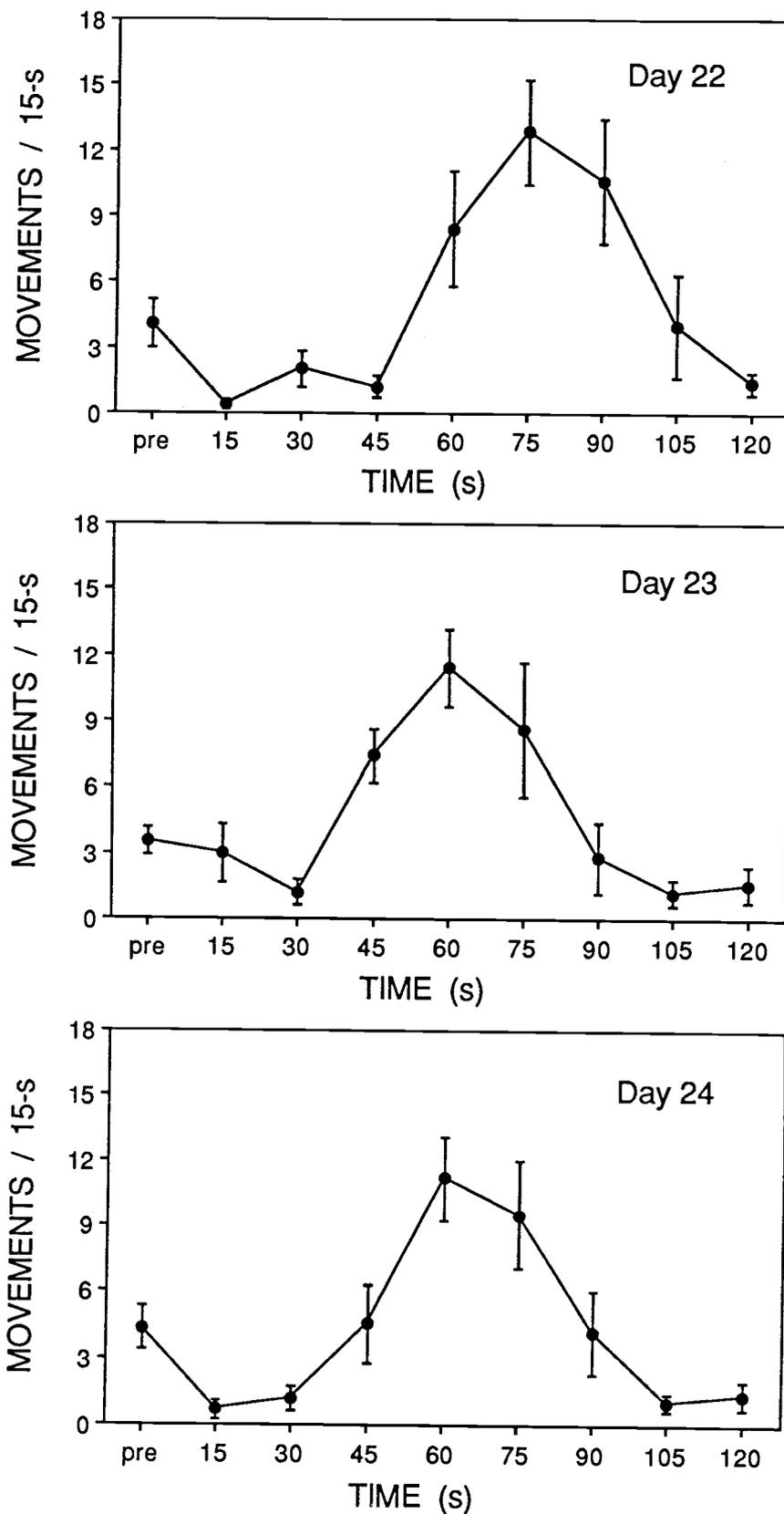


Figure 9.2 continued.

Table 9.4

Summary of statistical effects in umbilical clamp tests with Sigmodon fetuses

AGE	F SCORE	DF	P VALUE	PEAK INTERVAL	DUNNETT T	RESULT
18	0.7	8,32	.681	--	--	NS
19	1.5	8,32	.190	--	--	NS
20	1.6	8,40	.168	--	--	NS
21	1.8	8,80	.089	--	--	NS
22	5.4	8,32	<.001	45	1.35	NS
23	5.8	8,32	<.001	60	2.82	*
24	18.0	8,32	<.001	45	6.06	**
25	14.8	8,32	<.001	45	5.12	**
26	23.3	8,40	<.001	45	8.81	**
27	18.3	8,32	<.001	45	8.90	**

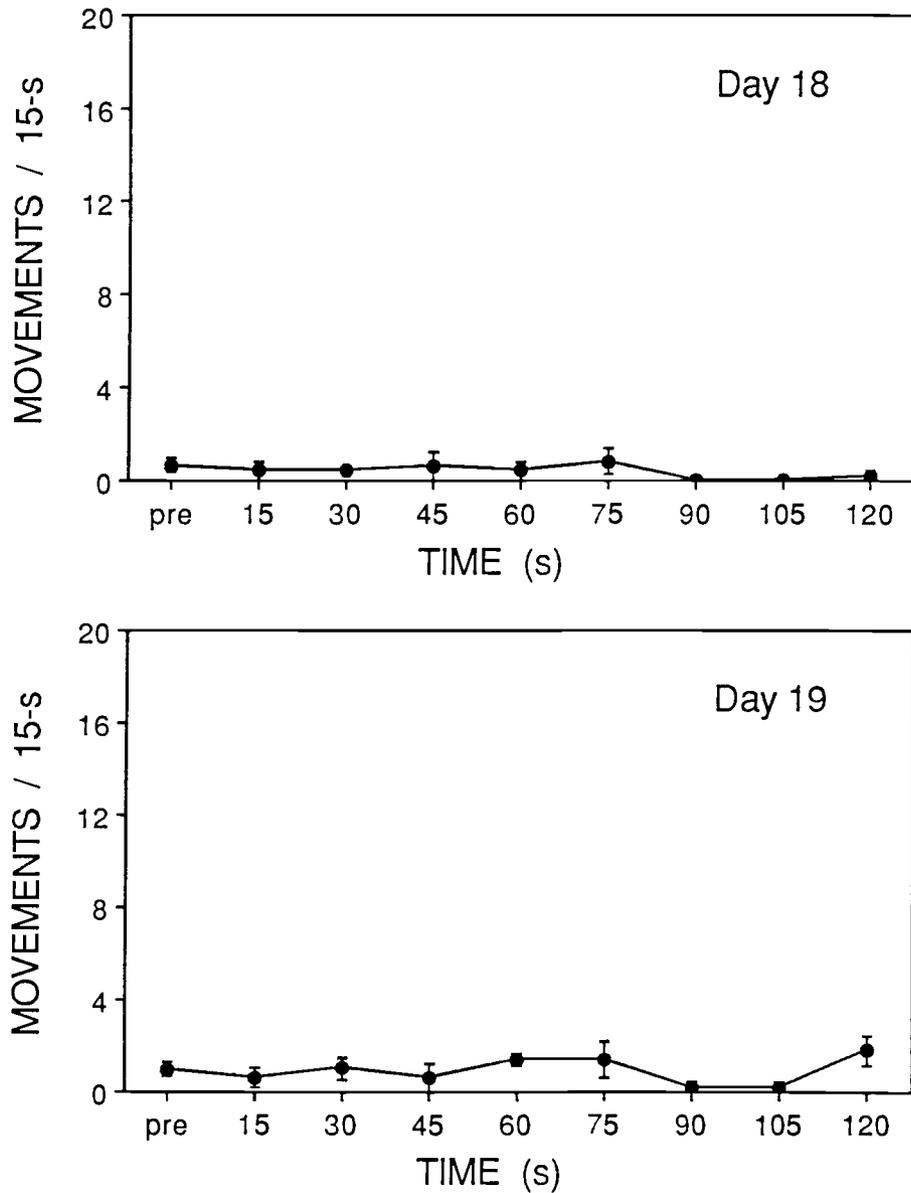


Figure 9.3. Changes in fetal event activity over the 120-s period immediately following placement of the microvascular clamp on the umbilical cord in Sigmodon. Points represent mean values; vertical bars represent SEM. Separate graphs are provided for ten days of gestation. (Figure continued on next three pages.)

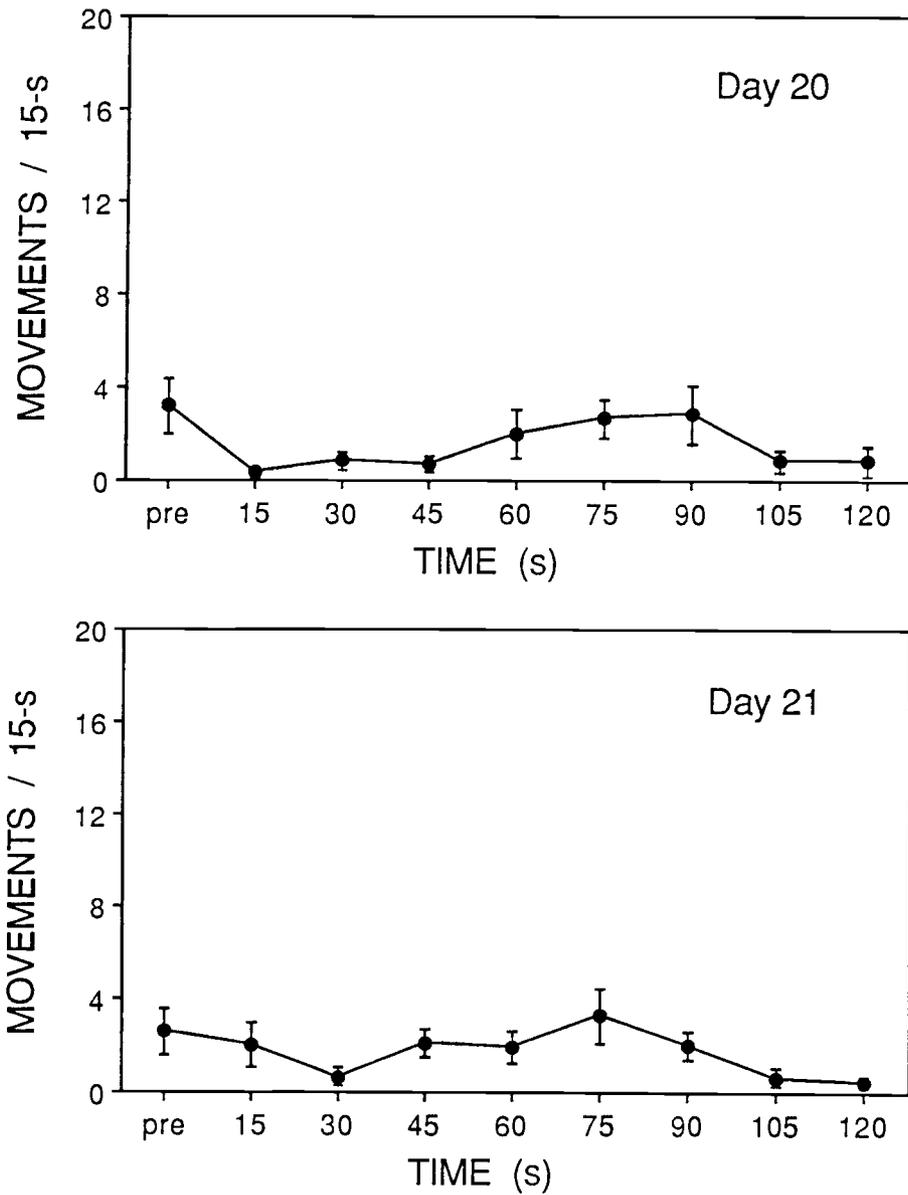


Figure 9.3 continued.

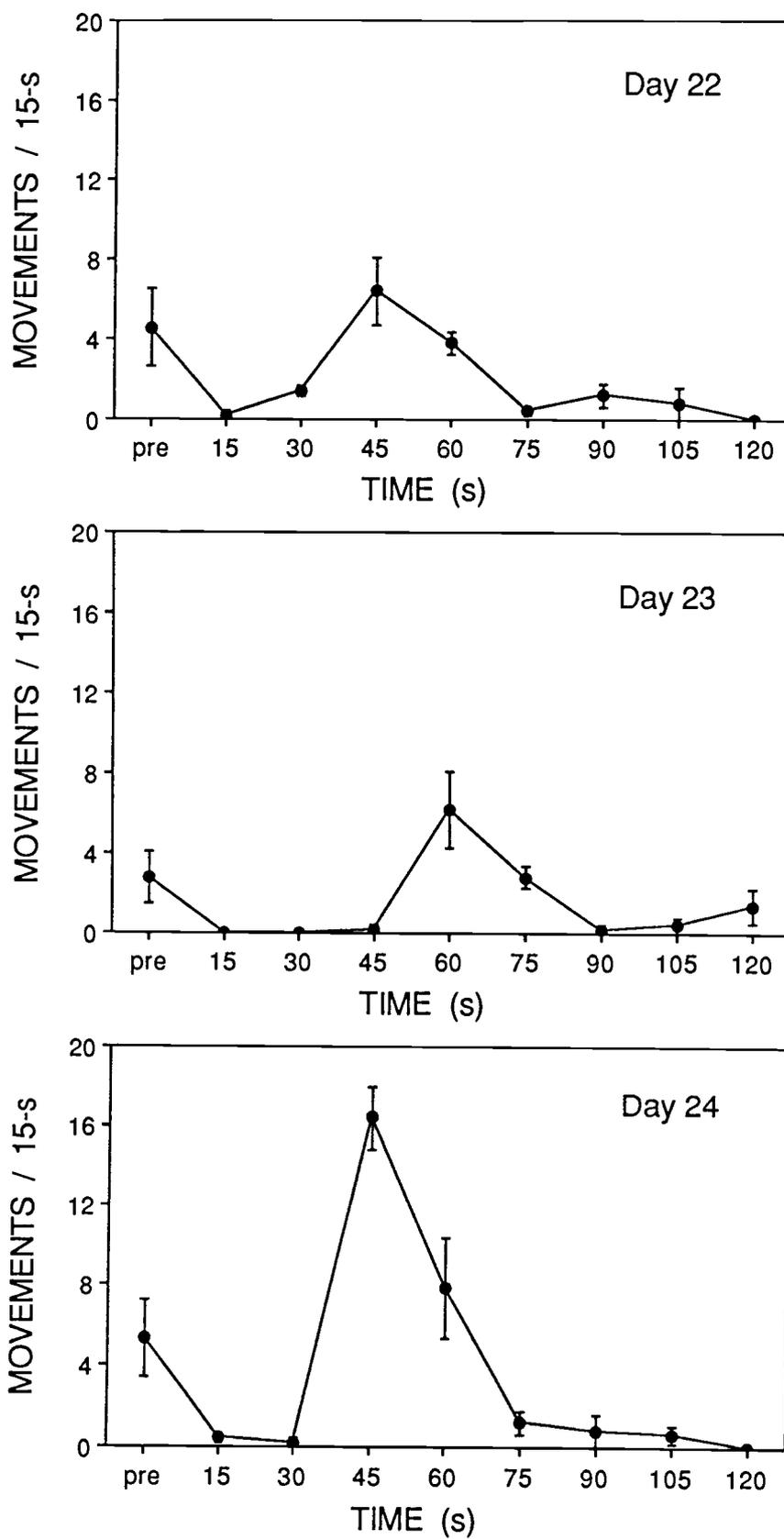


Figure 9.3 continued.

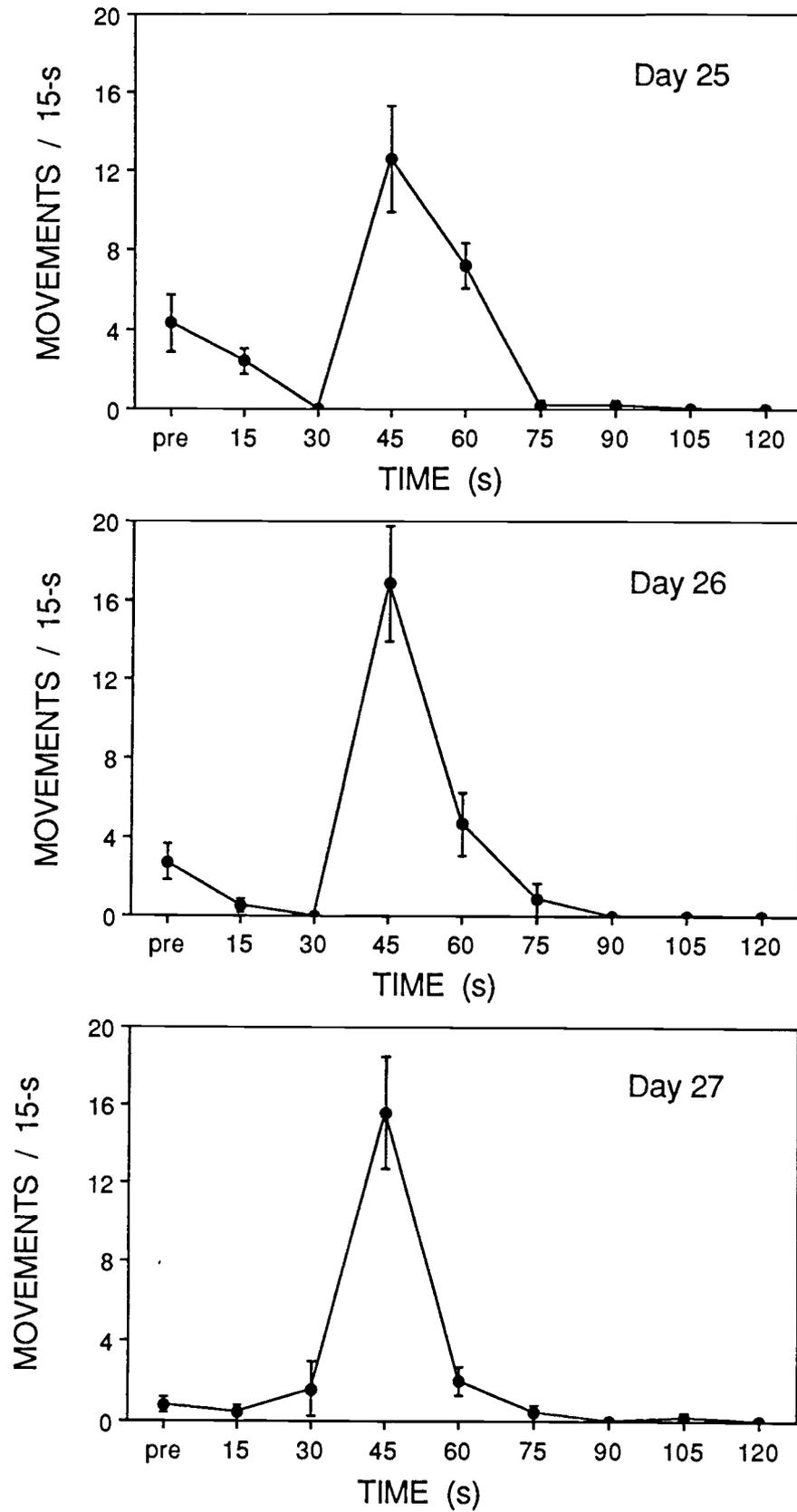


Figure 9.3 continued.

day 23.

The results of umbilical clamp tests were most equivocal in Acomys. No significant response to umbilical cord occlusion was found from day 26 through day 30. Marginally significant main effects and planned comparisons were noted on days 32, 34 and 37 (Table 9.5). However, the number of behavioral acts exhibited during the peak of the hyperactive phase appeared substantially smaller than that seen in the other three species (Figure 9.4). No significant change in the level of peak activity was found across these three days.

D. Motor Patterns Associated with Clamp Response

To further characterize the fetal response to umbilical cord compression, the relative occurrence of different behavioral components was compared across ages in a series of one-way ANOVAs. These components included forelimb, rearlimb, head, trunk and mouth, which together accounted for all acts observed. Relative occurrence was calculated as the frequency of a particular component observed over the 2-min period following placement of the clamp divided by total component activity over the same interval. The analyses incorporated all ages in Rattus and Meriones, but only those ages where a significant increase in activity was noted in Sigmodon (days 23-27) and Acomys (days 32-37).

No significant variation was apparent in the relative

Table 9.5

Summary of statistical effects in umbilical clamp tests with Acomys fetuses.

AGE	F SCORE	DF	P VALUE	PEAK INTERVAL	DUNNETT T	RESULT
26	0.8	8,32	.578	--	--	NS
28	1.8	8,32	.112	--	--	NS
30	0.8	8,32	.619	--	--	NS
32	3.1	8,32	.011	60	2.94	*
34	2.7	8,32	.020	75	2.92	*
37	2.6	8,32	.026	60	3.09	*

Figure 9.4. Changes in fetal event activity over the 120-s period immediately following placement of the microvascular clamp on the umbilical cord in Acomys (next page). Points represent mean values; vertical bars represent SEM. Separate graphs are provided for six days of gestation. (Figure continued on following page.)

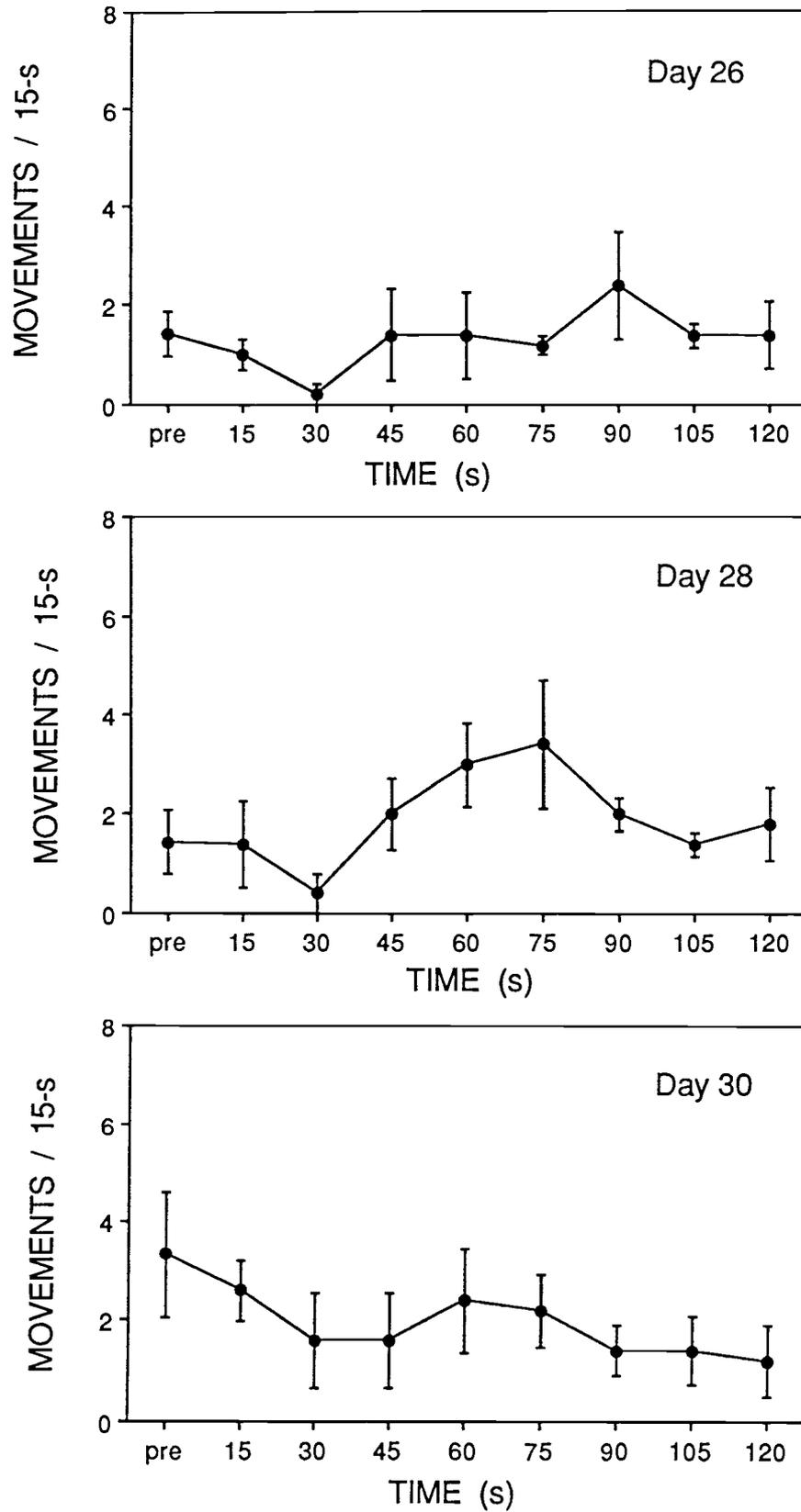


Figure 9.4 continued.

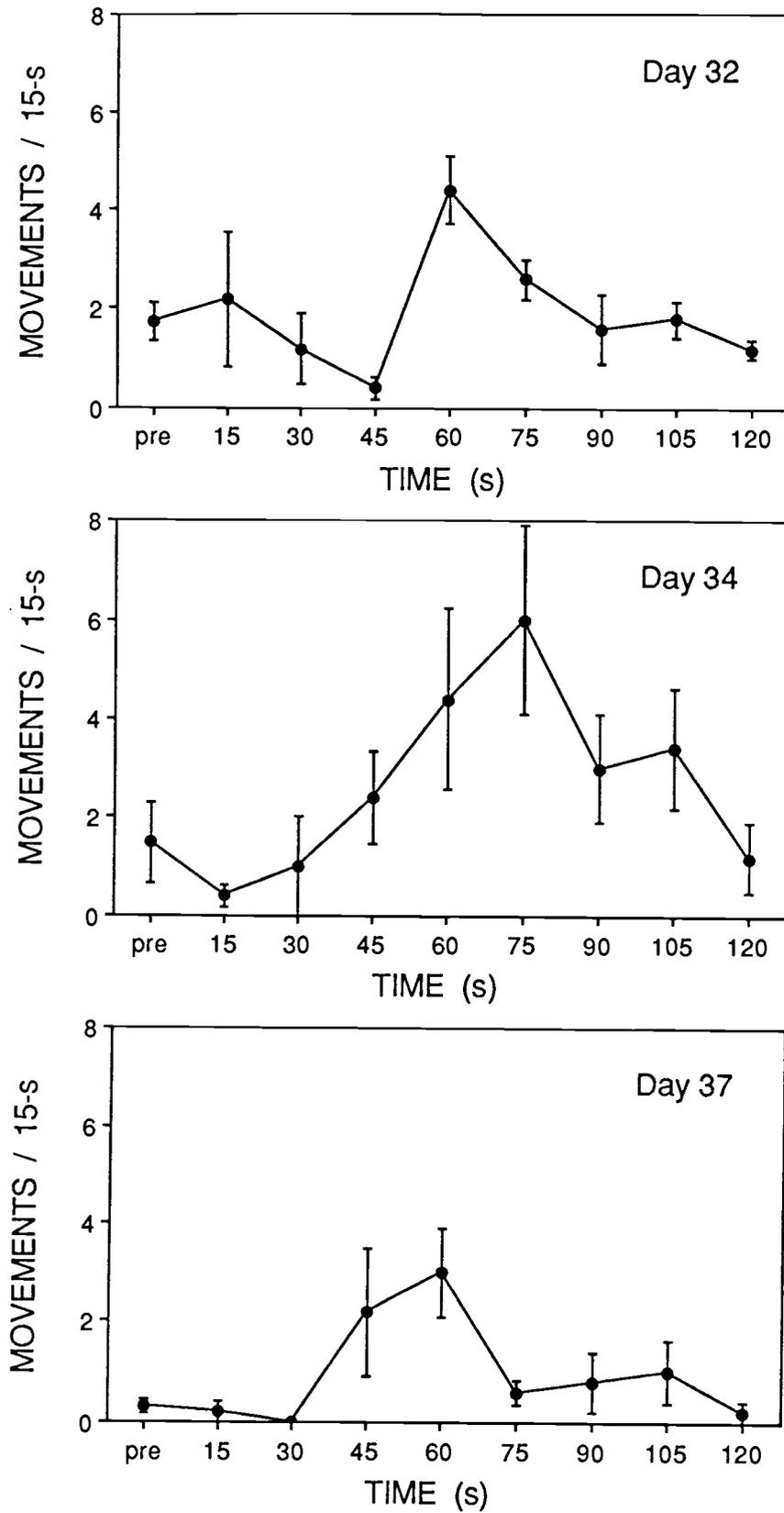


Figure 9.4 continued.

occurrence of forelimb, rearlimb, head or trunk movements over days 17-21 in Rattus. A marginally significant result for mouth activity ($F_{4,20} = 3.3, p=.031$) indicated that mouth movements occurred relatively more frequently on day 21 than on day 17. At no age, however, did mouth movements constitute more than 10% of overall activity. The modal category of behavior at all ages was trunk activity (Figure 9.5), which overwhelmingly consisted of vigorous lateral curling or bending movements. Curls were the most abundant category of movement in 20 of 25 fetuses, including all but one subject over days 18-20. Forelimb and head movements also were relatively common. The majority of head movements involved rapid dorsiflexion of the neck, which resulted in a rostral extension of the head ("head-toss"). Several instances were noted in which forelimbs were extended together in a rostral direction (the "touchdown" posture). Head-tosses and "touchdown" forelimb extension were rarely seen during spontaneous fetal activity.

In Meriones, none of the five categories of movement varied as a function of age (Figure 9.6). The modal category of response involved head movements, which were the most abundant form of movement among 24 of 31 fetuses. Many of these head movements involved rostral extension similar to that observed in Rattus. Forelimb movements also occurred commonly, although the paired rostral extension of forelimbs was not observed. Rearlimb, mouth and, curiously, trunk curl movements were relatively uncommon. In fact,

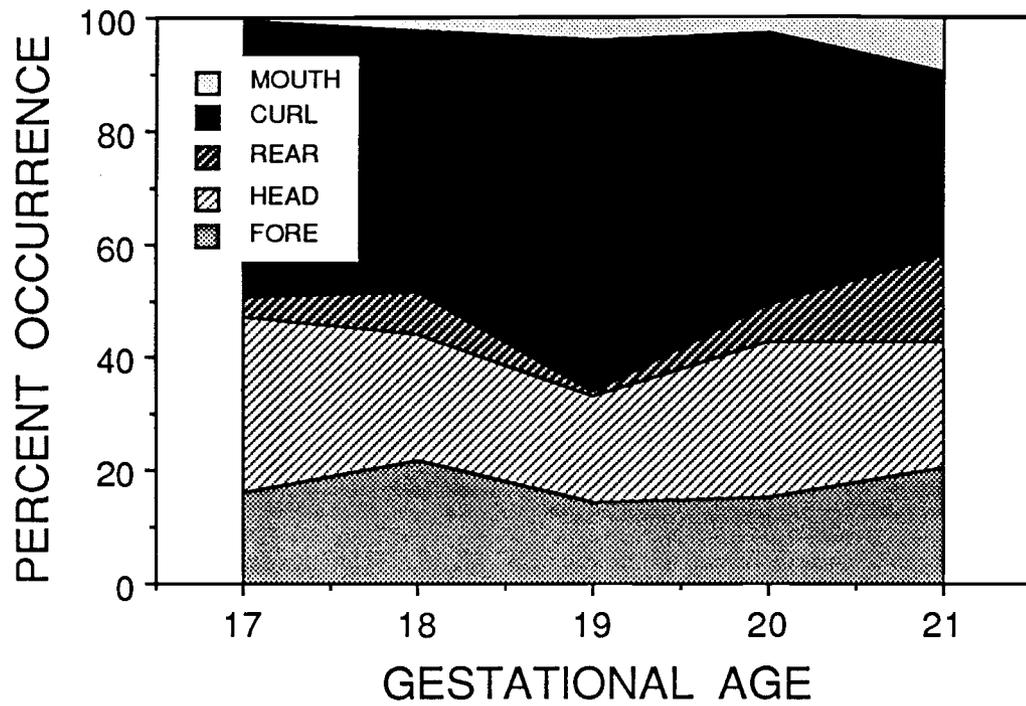


Figure 9.5. Relative abundance of five categories of fetal movement, expressed as a percentage of event activity, following placement of the clamp on the umbilical cord in Rattus. Note the predominance of trunk curl movements.

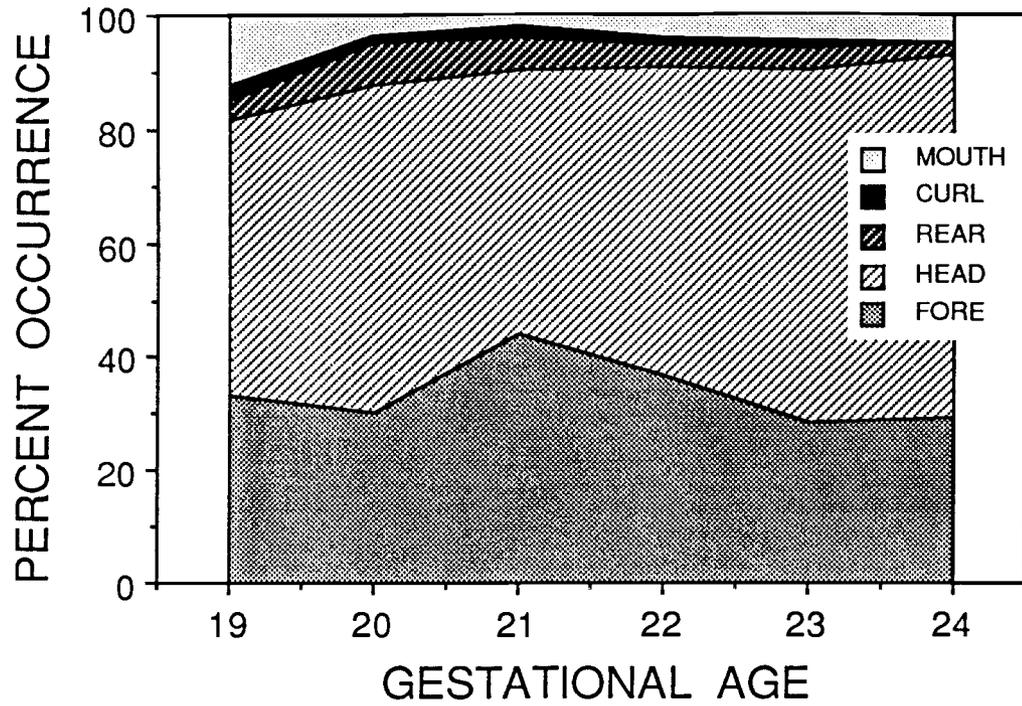


Figure 9.6. Relative abundance of five categories of fetal movement, expressed as a percentage of event activity, following placement of the clamp on the umbilical cord in *Meriones*. Note the predominance of head and forelimb movements and relative absence of trunk curls.

curls overall accounted for less than 2% of fetal activity that occurred in response to umbilical cord compression.

Sigmodon fetuses exhibited relatively more trunk curls on day 23 than at other ages ($F_{4,21} = 5.0, p=.005$). A marginally significant difference was found for the relative occurrence of rearlimb movements ($F_{4,21} = 3.3, p=.032$), which indicated that rearlimb activity was less common on day 23 than at later ages. No significant variation with age was evident for forelimb, head or mouth activity. Overall, the most abundant category of behavior was rearlimb, which was the modal form of movement in 21 of 26 fetuses, including all but one fetus after day 23 (Figure 9.7). Typically, rearlimb movements took the form of a vigorous, synchronous caudal extension of both legs. Often it was noted that each foot was placed on opposite sides of the umbilical cord during the kicking movement, although it was not apparent whether this foot placement was coincidental or coordinated. Trunk movements were the most abundant category of movement on day 23 and the second most common movement at subsequent ages. Trunk movements consisted predominantly of lateral body curls, which appeared very similar to those observed in Rattus. Forelimb and head movements occurred relatively less often and mouth activity was virtually absent.

Analyses of components revealed a marginally significant effect of age on rearlimb activity in Acomys ($F_{2,12} = 4.4, p=.036$). Rearlimb movements were relatively

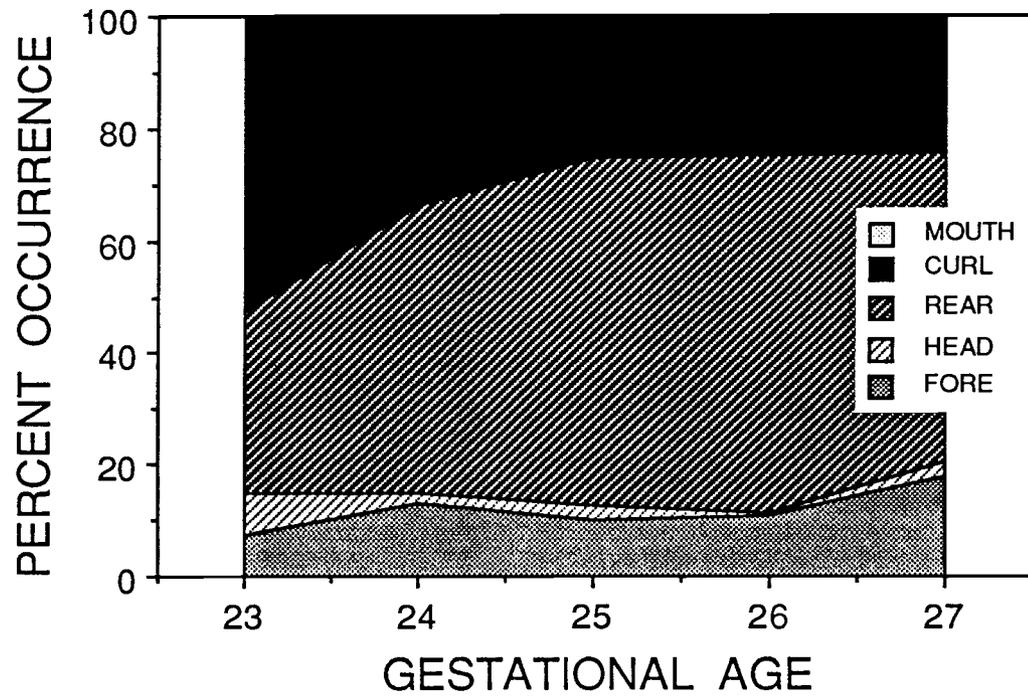


Figure 9.7. Relative abundance of five categories of fetal movement, expressed as a percentage of event activity, following placement of the clamp on the umbilical cord in *Sigmodon*. Note the predominance of trunk curl and rearlimb movements.

more common on day 37 than on day 32. Particularly in older fetuses (days 34-37), rearlimb movements appeared well-organized, involving vigorous extension of both legs or alternated kicking of each leg in a caudal direction. A significant finding was also obtained for mouth activity ($F_{2,12} = 36.2, p < .001$), which decreased in relative occurrence from day 32 through day 37. Mouth movements were the most abundant category of movement on day 32 (modal in all five fetuses) and rearlimb the most abundant on day 37 (modal in four of five fetuses). No clear mode was evident on day 34. Other categories of movement, including forelimb, head and trunk, occurred less often and showed no significant variation with age (Figure 9.8). Forelimb movements, although not numerous, were particularly notable, as they often involved a slow withdrawal of the forelegs to the chest, bringing the paws close to the head and neck, followed by a gradual extension of both forelimbs in a rostral direction along either side of the head. This deliberate movement, which resembled the "touchdown" pattern evident in Rattus, appeared highly coordinated, the fetus often remaining in the extended posture for several seconds. Other organized patterns of movement, such as head tosses and lateral trunk curls, were not evident.

E. Discussion

On the basis of experiments conducted with rat fetuses

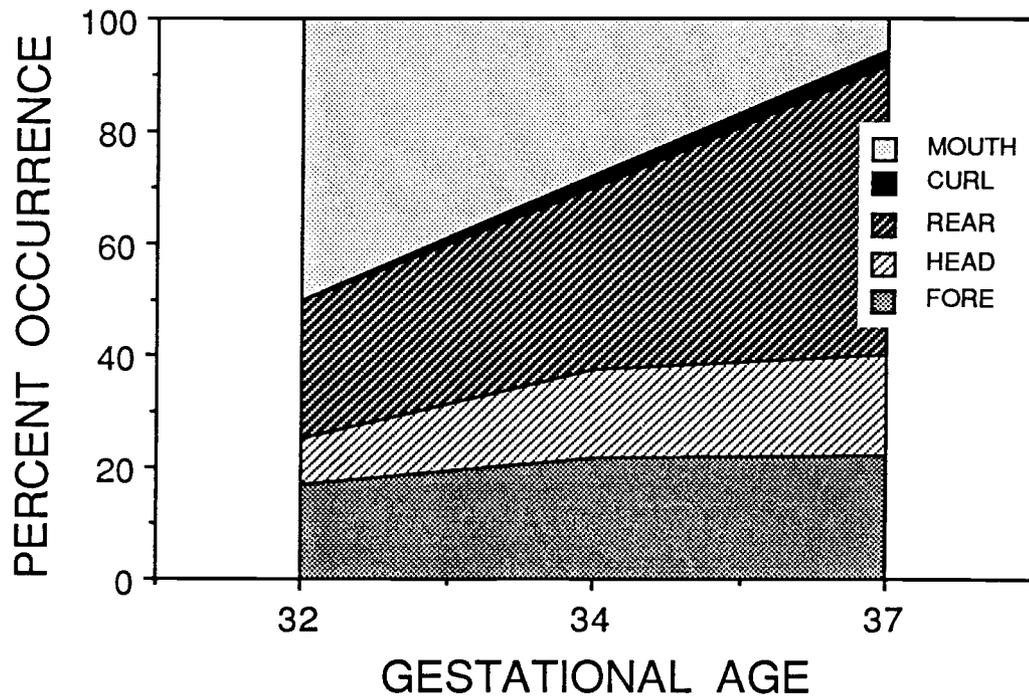


Figure 9.8. Relative abundance of five categories of fetal movement, expressed as a percentage of event activity, following placement of the clamp on the umbilical cord in *Acomys*. Note the predominance of rearlimb and forelimb movements and the relative absence of trunk curls.

over gestational days 19-21, Smotherman and Robinson [1988c] concluded that the umbilical clamp response is a likely candidate for an ontogenetic adaptation that occurs during the prenatal period. The vigorous lateral trunk curls and rostral head extensions described for rat fetuses in previous reports, and replicated in the present study, could well serve to alleviate the cause of accidental cord compression in utero by exerting force against adjacent siblings or hard portions of maternal anatomy. Indeed, vigorous activity during episodes of hypoxia would be maladaptive if it did not help to reverse cord compression because it would more quickly deplete limited oxygen reserves.

If the umbilical clamp response were indeed an ontogenetic adaptation, then it should be expressed in other species as well. Indeed, the present study identified some form of clamp response in all four species. In terms of overall fetal activity and the timing of activity after occlusion of umbilical circulation, the clamp response was very similar among Rattus, Meriones and older Sigmodon fetuses. It is noteworthy that the peak of the second phase response amounted to 23.8 acts in Rattus, 12.8 acts in Meriones, 16.8 acts in Sigmodon, but only 6.0 acts in Acomys. It would appear that hyperactivity, which characterizes the second phase of the clamp response in three species, is less well developed in Acomys.

The finding that qualitatively and quantitatively

different motor patterns were expressed in the clamp response in different species was an unexpected discovery. However, the observed differences evidently are the result of expression of different combinations of components rather than wholly unrelated patterns of behavior. Four organized motor patterns that were rarely or never seen during spontaneous activity or chemosensory stimulation (Chapters 4 & 8) were identified following umbilical cord compression: rapid bouts of lateral trunk curls, head-tosses, synchronous rostral foreleg extensions, and synchronous caudal rearleg extensions. None of these patterns were unique to one species, but none were seen in all four species. Lateral curls were common only in Rattus and Sigmodon. Head-tossing was observed in Rattus and Meriones. Rostral extension of the forelimbs occurred occasionally in Rattus and commonly in Acomys. Synchronous caudal rearleg extension was expressed consistently by Sigmodon and Acomys.

Does the variability of the umbilical clamp response bear on the question of ontogenetic adaptation? Patterns of behavior expressed by mature animals that appear to vary most among species are those that have little functional consequence (and therefore are subject to drift) and those that are most closely attuned to environmental contingencies (e.g., methods of foraging behavior). It is tempting to speculate that the fetal response to umbilical cord compression is a prenatal example of the latter relationship. It must remain for further comparison of

different species to resolve the factors that influence the form of the umbilical clamp response.

The timing of ontogenetic emergence of the clamp response, however, seems more tractable. In Rattus and Meriones, the clamp response emerged at the earliest age tested, only one day after the inception of movement. In Sigmodon and Acomys, emergence of the clamp response was delayed through half of the fetal period, becoming evident only on day 23 (or perhaps day 22) in Sigmodon and day 32 in Acomys. Although the proximate reason for this delay is difficult to identify, it seems clear that the development of this pattern of fetal behavior is relatively retarded in precocial species.

CHAPTER 10. GENERAL DISCUSSION AND CONCLUSIONS

The data reported in the present study represent the first controlled comparative analysis of fetal behavior. As mentioned in Chapter 1, other species have been employed in fetal research, particularly in the classic studies of the 1930s and '40s. Three mammalian species have figured most prominently in modern studies of fetal biology. Human fetuses have received much attention from researchers employing indirect monitoring and ultrasonographic technology to record gross fetal movements. Sheep have proven useful in studies of fetal and placental physiology. Laboratory rats have been the species of choice in studies involving direct observation techniques and true experimental designs to investigate spontaneous motor behavior, sensory function and prenatal learning. Yet the lack of similar methods or research objectives applied to these species has virtually thwarted attempts at meaningful comparison. At the most fundamental level, this study confirms that there is quantitative and qualitative variation in fetal motor behavior among closely related species. This suggests that variation in fetal behavior among more distantly related mammalian taxa is potentially very great.

A. Ontogenetic Antecedents and Ontogenetic Adaptations

On a subjective basis, the motor activity of fetuses does not rival the dynamic, complex character of postnatal behavior. However, the dimensions that define behavior during the postnatal period extend to movements before birth. Fetal movements exhibit temporal, sequential and spatial organization that emerges between the inception of movement and term. Further, fetuses are behaviorally responsive to immediate environmental conditions and sensory stimulation. Expression of these behavioral properties by the fetus implies continuity between prenatal and postnatal life [Bekoff 1988; Smotherman & Robinson 1988d].

Indeed, this study has confirmed and extended a growing list of behavioral attributes that appear to be antecedents of postnatal behavior. This list includes (a) the patterning of activity in objectively defined temporal bouts [Slater 1974; Machlis 1977], (b) the production of synchronously coordinated movements [Bekoff & Lau 1980; Provine 1980], (c) the sequential organization of motor behavior [Briem 1986; Fentress & McLeod 1986], (d) the activational response to sensory stimulation, such as intraoral infusion of novel odorants [Grill & Berridge 1985; Johanson & Shapiro 1986], (e) and the expression of species-typical action patterns. In this last category, for instance, facial wiping and paw-licking were observed that resemble postnatal grooming behavior [Golani & Fentress

1985; Smotherman & Robinson 1989], a righting response was noted that anticipates postnatal patterns for dynamic postural maintenance [Bignall 1974; Altman & Sudarshan 1975], and limb coordination was described that resembles postnatal locomotor movements [Bekoff 1986; Bekoff & Trainer 1979]. With the possible exception of stepping movements, which may be used by the fetus to change orientation within the uterus [Suzuki & Yamamuro 1985], these behavioral attributes develop prior to and in anticipation of their postnatal expression in a functional context.

Other documented patterns of fetal behavior are more difficult to relate directly to postnatal behavior. For example, the fetal response to umbilical cord occlusion appears to have no clear counterpart in the postnatal period. Neonatal rats under conditions of hypoxia exhibit reduced levels of activity and cardiac acceleration [Eden & Hanson 1987]. Yet the response of fetal rats to hypoxia induced by occlusion of umbilical cord circulation is an organized three-phase activity response coupled to transient bradycardia [see also Smotherman & Robinson 1988c]. The vigorous, outwardly directed movements of the fetus during the hyperactivity phase of the clamp response well may increase the probability of alleviating accidental cord compression and thereby promote the survival of the fetus. Accidental cord compression can occur during otherwise unremarkable pregnancies [Mann 1986]. The fetal response to umbilical cord occlusion therefore appears to present a good

case as an ontogenetic adaptation involving prenatal behavior [Oppenheim 1984; Alberts & Cramer 1988].

Other patterns of fetal behavior may be interpreted as possible behavioral adaptations to the intrauterine environment. Chief among these is the fetal response to physical restraint in utero. One of the most robust behavioral effects documented in the present study involves the condition of fetal observation. The behavior of fetuses observed in utero is qualitatively and quantitatively different than that observed ex utero. In general, fetuses ex utero exhibited more activity, greater behavioral diversity, and more pronounced patterning of motor activity than fetuses in utero. Further, the expression of organized action patterns during spontaneous activity was much greater when fetuses were observed outside the uterus.

Several general explanations may be proposed to account for the effect of environmental context on fetal behavior. First, fetuses delivered outside the uterus for observation may be physiologically compromised and exhibit aberrant responses. However, this explanation is contravened by the available evidence. Improved methodologies developed for fetal research [Smotherman & Robinson in press], which were employed in the present study, ensure that the health of fetuses is not impaired by observation ex utero. The behavior of fetuses observed ex utero is distinctly different from the activity of moribund fetuses or fetuses under hypoxic conditions [Windle 1944; Smotherman & Robinson

1987c]. Late in gestation it is possible to deliver fetuses, even after extended observation sessions, which may be cross-fostered to and reared by newly parturient females [Smotherman, Robinson & Miller 1986]. Further, it seems improbable that perturbation of a complex system, such as a developing organism, would result in the false impression of greater organization in many independent variables. Yet fetuses ex utero expressed behavioral organization that was more, not less, similar to postnatal behavior.

If the condition effect is not an artifact, then it probably reflects the behavioral responsiveness of fetuses to increased restraint in utero. The space available for movement is much less in utero than ex utero, and this space diminishes as amniotic fluid disappears and the fetus continues to grow near term. Two possible mechanisms are apparent that could account for reduced behavioral organization under conditions of reduced free space. First, fetal movements may be physically restrained by the elastic wall of the uterus, in effect filtering all but the strongest and most vigorous of fetal movements from overt expression. By this hypothesis, motor commands continue to be generated at the neural level, but immature motor units lack the strength to distend the uterine wall and execute movements. This view is comparable to the explanation proposed by Thelen and colleagues [Thelen & Fisher 1982; Thelen 1988] regarding the postnatal disappearance of neonatal stepping movements. Because the leg mass of human

infants grows faster than leg muscle strength, infants experience a period of development when the legs can no longer be controlled in a gravitational environment. Eliminating the gravitational restraint by immersing the infant's legs in water (analogous to observation of term fetuses ex utero) is effective in reinstating early stepping behavior [Thelen, Fisher & Ridley-Johnson 1984].

The second possible explanation for the condition effect assumes that any reduction of behavioral organization is due to sensory responsiveness of the fetus to its surrounding environment, not to passive environmental masking of fetal movements. Fetuses are responsive to external sensory stimuli; even altricial fetuses are capable of somatosensation and proprioception that would permit them to detect restraint, and potentially adjust to diminishing free space in utero [Gottlieb 1971; Narayanan, Fox & Hamburger 1971]. Such sensory feedback may in turn result in modification of the central commands governing motor activity. Thus, the altered motor organization observed in utero may reflect true behavioral responsiveness of the fetus to environmental conditions. Further, reduction of abundant or vigorous fetal movements in utero may reduce the energy expended to distend the uterus during activity. If this hypothesis proves to be correct, then fetal responsiveness to intrauterine restraint may be interpreted as another example of ontogenetic adaptation.

Are data available to choose between these competing

hypotheses? Direct evidence would require some means of measuring motor commands, such as by EMG recordings. On the one hand, Bekoff [1976] has demonstrated that patterned motor commands are generated by the spinal cord of the chick embryo that are not expressed as overt movements. On the other hand, rodent fetuses are demonstrably capable of inhibiting the expression of certain forms of synchronous movement (HR, HFR) and complex action patterns (facial wiping) in different contexts. It would seem no less plausible that fetuses within the confines of the uterus are capable of comparable behavioral sensitivity to environmental conditions.

B. Scaffolding in the Developmental Construction of Behavior

As originally formulated, the concept of ontogenetic adaptation refers to anatomical, physiological or behavioral attributes that promote the immediate survival or well-being of an immature organism in an ontogenetic niche [Oppenheim 1982]. However, another class of transient ontogenetic attributes may be postulated that exist only to provide a substrate for further development. Anatomical examples of such transient structures abound in the embryological literature. Some, like the mesonephric ducts that give rise to components of the mature reproductive system in mammals, are phylogenetic palimpsests of ancient functional systems. Others, like the gastrula of early embryogenesis, have no

link to past adaptation and serve only as a means of organizing living matter. Structures of the latter sort may be as important in early development as scaffolding is in the construction of an arch. The individual bricks of the arch cannot be set in place without external supports, but once the arch is complete, the support system can be removed.

The metaphor of scaffolding may be useful for understanding certain processes involved in behavioral development. A possible example involves patterns of movement synchrony described in Chapter 6. The results of the second order stochastic models indicated that synchronous movements by very young fetuses were generated essentially by random association; the incidence of motor synchrony could be predicted from overall fetal activity and the relative abundance of motor components. Later in gestation, several categories of synchronous movement were expressed at frequencies significantly greater or less than should be expected by random association, suggesting that their occurrence was the result of central generation or coordination of motor commands.

The sequence in which nonrandom synchronous movements emerged during gestation is particularly interesting. The first category of synchrony to emerge from a background of random association was forelimb-head movements (FH), followed almost immediately (precocial species) or after a period of days (altricial species) by forelimb-rearlimb

movements (FR). As the category FR increased in frequency, the category comprising synchronous head-rearlimb movements (HR) decreased below chance levels. This reduction in HR is the earliest behavioral evidence of central inhibition of motor activity [cf. Barcroft & Barron 1939]. In precocial species, the decrease in HR was accompanied by a corresponding reduction in the triplet category FHR. Note that at this time, different pairwise combinations of the components F, H and R are differentially expressed: FH and FR remained elevated, while HR remained suppressed. FHR, however, reversed its early pattern of expression and eventually exceeded all other synchronous categories in absolute frequency late in gestation in both precocial species.

This chronology of movement synchrony suggests the following developmental interpretation. Originally, synchronous movements are produced by random association of simple motor events. Later, as central coordinative linkages between components are established, certain patterns increase (activation) or decrease (inhibition) in absolute and relative frequency. The early coincident reduction of HR and FHR suggests that these two patterns may initially be governed by the same inhibitory process. Only at a later stage of development, which is exhibited prenatally only by precocial fetuses, is FHR differentiated from HR.

The relationship of these abstract movement categories

to behavioral development is important and fundamental. The coordination of synchronous forelimb and head movements is crucial to the development of mature grooming behavior [Golani & Fentress 1985] and suckling behavior of neonatal rodents [McFarlane, Pedersen, Cornell & Blass 1983]. FR synchrony is equally important in interlimb coordination involved in locomotion [Bekoff & Lau 1980; Grillner 1981]. Indeed, control of head position and orientation is also important in the maintenance of balance during locomotion, so FHR synchrony probably is related to locomotor development as well. However, the establishment of early interlimb coordination (e.g., FR) may be facilitated without the added complication of simultaneous head movements. Thus, the synchronous category FR, which is so evident in the behavior of altricial fetuses near term and precocial fetuses at intermediate ages, may be a transient structure that gives way to or is subsumed by the category FHR at later ages.

A similar interpretation recently has been proposed to explain developmental patterns of early facial grooming in rodents [Golani & Fentress 1985]. Young mice initially exhibit grooming movements involving bouts of forelimb strokes with relatively little involvement of head or trunk. As development proceeds, temporal clustering of strokes disappears and is replaced by individual, spatially restricted strokes as head movements are integrated into the overall sequence. Finally, bout structure of forelimb

strokes re-emerges as smooth coordination of movements involving limbs, head and trunk becomes apparent. The second phase of development described in this sequence involves retrogression in some aspects of overall organization [see also Golani, Bronchti, Moualem & Teitelbaum 1981; Oppenheim 1981], yet it is apparently important in establishing coordination between limbs and head. As such, it may be closely analogous to the FR synchrony exhibited by fetuses. Both transient structures may be examples of behavioral scaffolding.

C. Heterochrony in the Evolution of Fetal Behavior

The foregoing discussion has emphasized common patterns in the prenatal development of behavior in four rodent species. But important behavioral differences also should be expected between species. Particularly if behavioral development consists of multiple ontogenetic pathways, the natural variation inherent in divergent evolution should provide independent descriptions of developmental patterns. This is one of the principal strengths of comparative analysis: to use carefully selected species to disassociate seemingly related processes [Ridley 1983].

The potential for evolutionary change to break up old associations during development is the source of the theoretical prediction of behavioral heterochrony, which was outlined in Chapter 1. Heterochrony involves more than a

simple acceleration or retardation of overall development; it involves a shift in the relative timing of different developmental processes [McKinney 1988]. In the evolution of precocial patterns of development from an altricial ancestral condition, an event that occurred at least twice (Sigmodon and Acomys) in the phylogeny of Murid rodents, heterochrony is predicted in the relative times of emergence of prenatal ontogenetic adaptations and antecedents of postnatal behavior. Specifically, behavioral patterns that are necessary for the well-being of the fetus in utero should be retained until birth in all species, and therefore should appear developmentally retarded relative to the more rapidly developing antecedents of postnatal behavior in precocial species (see Figure 1.1).

Examples of prenatal heterochrony may be gleaned from the recent literature on neural and physiological development in at least two of the four species examined in the present study. Brunjes described patterns of brain development in Acomys, some of which appeared accelerated and others retarded with respect to Rattus. For instance, many indices of neural maturation, such as the degree of myelination of the CNS, indicate that Acomys is much more mature at birth than Rattus [Tessitore & Brunjes 1988]. On the other hand, olfactory bulb growth is most rapid in both species during the period immediately after birth [Brunjes 1983; Leon, Coopersmith, Ulibarri, Porter & Powers 1984], suggesting a heterochronic shift in the timing of different

aspects of neural development. A similar conclusion is suggested by the developmental patterns of enzyme systems in different organs. Enzymes associated with neural function are produced much earlier in development in Acomys than Rattus. But enzymes associated with lung, pancreas and small intestine appear to be more strongly correlated with changes occurring around the time of birth in both species. Because altricial and precocial species alike only begin to breathe and ingest food after birth, the maturation of these enzyme systems appears to be related to the onset of function [Lamers, Mooren, Graaf, Markiewicz & Charles 1985].

Behavioral processes described in the present study provide an independent test of the heterochrony hypothesis. The clearest comparison may be made between two coordinated action patterns that emerge during the prenatal period: facial wiping, which is antecedent to postnatal grooming behavior (Chapter 8), and the behavioral response to umbilical cord occlusion, which is a putative ontogenetic adaptation of the fetus (Chapter 9). Figure 10.1 presents a summary of the expression of these two behavioral patterns during the prenatal period in all four rodent species. Gestational age, depicted along the abscissa, is adjusted in all four species so that the inception of movement lies at the left edge and birth at the right edge of the figure. Facial wiping clearly is expressed relatively later in the two altricial species than in the precocial species. However, the expression of hyperactivity in response to

Figure 10.1. Summary of the expression of two organized behavioral patterns -- facial wiping and the response to umbilical cord occlusion -- by fetuses of four Murid species (next page). The scale depicting gestational age has been adjusted to reflect the same relative time between the inception of movement and term. Note that the early expression of facial wiping is accelerated in the two precocial species. But the emergence of an umbilical clamp response occurs in all species during the last 5-6 days of gestation.

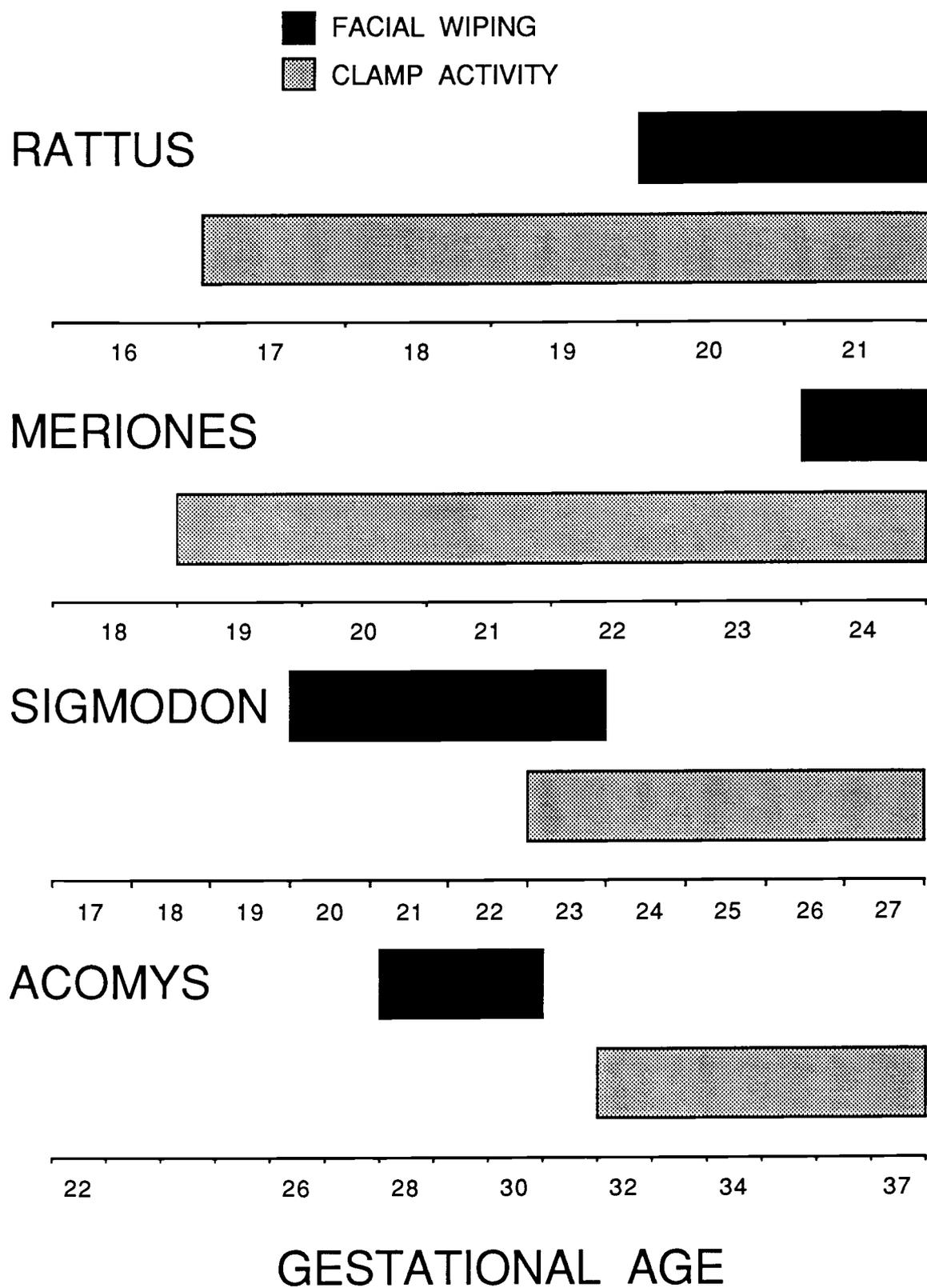


Figure 10.1 continued.

umbilical cord occlusion is absent during the early portion of gestation in Sigmodon and Acomys, resulting in a developmental retardation of the clamp response relative to altricial species. The evident shift in the relative timing of development of these two action patterns is consistent with the predicted occurrence of behavioral heterochrony.

A more general representation of heterochrony is presented in Figure 10.2. This figure portrays the earliest developmental expression of twelve different behavioral processes expressed by rodent fetuses. These patterns do not represent a comprehensive list of prenatal behavior. Rather, examples were selected from each of the principal analyses described in the present study. These included only those behavioral processes expressed by all four species for which pronounced, significant developmental change was evident. These twelve patterns include: (a) the emergence of facial wiping, (b) the emergence of an activational response to umbilical cord occlusion, (c) the expression of activity clustered in temporal bouts, (d) the earliest expression of overall movement synchrony above levels predicted by chance association, (e) the earliest expression of significant overproduction of the synchronous category FH, (f) overproduction of the synchronous category FR, (g) inhibition of the synchronous category HR, (h) asymmetric production of the sequential transition FH/R, (i) asymmetric production of the sequential transition R/FR, (j) the significant reduction in overall synchronous movement to

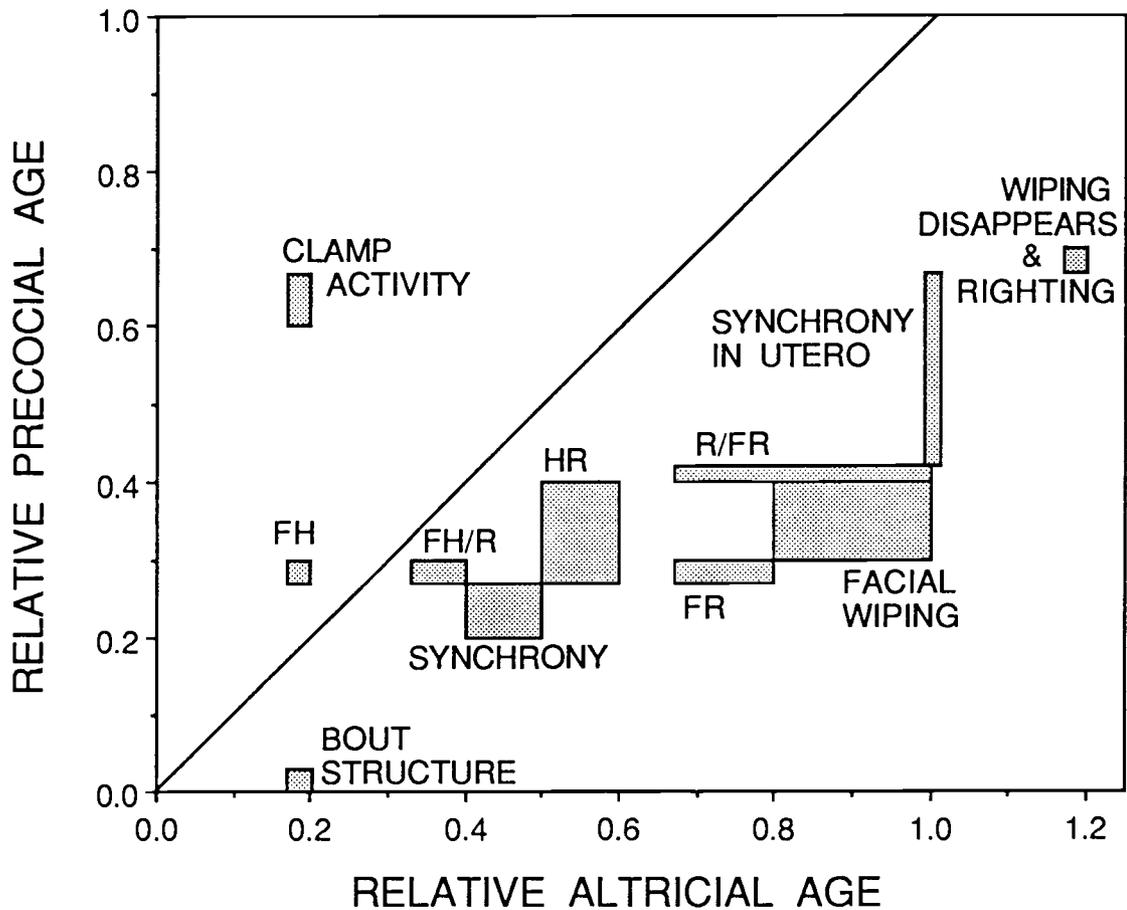


Figure 10.2. Schematic representation of behavioral heterochrony in altricial and precocial fetuses. Twelve different behavioral measures employed in the present study are depicted on the graph. Shaded boxes represent the ontogenetic profile for each measure; each profile is delimited by the relative age of the earliest expression of the behavioral pattern in each of the four species. Age is presented on a relative scale, with the inception of movement equal to 0 and term equal to 1.0. The diagonal line displays the predicted profile if no heterochrony occurs. Measures that appear below the line are relatively accelerated in precocial species; measures that occur above the line are relatively retarded in precocial species. See text for more detailed discussion.

chance levels after emergence in utero, (k) the developmental disappearance of the expression of facial wiping in response to infusion, and (l) the emergence of a contact righting response. The earliest developmental expression of these behavioral patterns is portrayed as a box, the four sides of which are delimited by the earliest and latest relative time of expression in altricial and precocial species. Gestational age is portrayed on a relative scale defined by the inception of movement and birth (see Figure 3.3). Relative gestational age in altricial species is shown along the abscissa; relative gestational age in precocial species is depicted along the ordinate.

If no shift in the developmental timing of these patterns were evident, they should fall along the diagonal line, which defines equivalent relative ages in altricial and precocial species. Patterns that are accelerated in precocial species should appear in the lower right half of this figure; patterns that are relatively retarded in precocial species should appear in the upper left half. As is clear from this symbolic representation, most patterns of prenatal behavior are relatively accelerated in precocial species, falling along a line that approximately bisects the lower half of the figure. This finding is consistent with the view that these patterns are antecedent to or in some way involved in the development of postnatal behavior.

Two exceptions appear in the upper half of the figure.

The first is the fetal response to umbilical cord occlusion, which is clearly expressed at an earlier relative age in altricial species than precocial species. The second pattern is the early overproduction of synchronous forelimb-head movements. The apparent retardation of FH movements in precocial species is an unexpected finding. If the heterochrony model as elaborated in Chapter 1 is correct, and FH movements are indeed shifted to a relatively earlier gestational age in altricial species, then FH movements may play a yet unrecognized role in promoting the well-being of the fetus during the prenatal period. It is premature to speculate what adaptive role FH synchrony may serve. Rather, FH synchrony as an ontogenetic adaptation should be viewed as a hypothesis that may be tested by further investigation of the environmental contexts that promote or inhibit its expression.

The results of the present study thus are consistent with the prediction of behavioral heterochrony. The existence of prenatal behavioral heterochrony implies that the ontogeny of behavior, even during its earliest expression before birth, should not be characterized as a unitary process of motor maturation. Instead, it appears to be more appropriate to view behavioral development as encompassing multiple, independent ontogenetic pathways that sometimes exist in close association and other times result in behavioral competition for expression. This view of behavioral ontogeny is decidedly interactionist [Oyama

1985]. An important task for future research will be to elaborate and further test a multipartite conception of behavioral ontogeny, which may prove to be a point of common ground between traditional ethological [Hinde 1970; Bateson 1987], psychological [Kuo 1967; Hofer 1981] and systems theory [Kugler & Turvey 1987; Thelen, Kelso & Fogel 1987] approaches to understanding behavioral development.

BIBLIOGRAPHY

- Abbas, T. M., & Tovey, J. E. 1960. Proteins of the liquor amnii. British Medical Journal, 2:476-479.
- Abbey, H., & Howard, E. 1973. Statistical procedure in developmental studies on species with multiple offspring. Developmental Psychobiology, 6:329-335.
- Alberts, J. R., & Cramer, C. 1988. Ecology and experience: sources of means and meaning in developmental change. In E. M. Blass (ed.), Handbook of Behavioral Neurobiology, Vol. 9, Behavioral Ecology and Developmental Psychobiology, pp. 1-39. New York: Plenum.
- Alcock, J. 1988. Animal Behavior: An Evolutionary Approach, 4th ed. Sunderland, MA: Sinauer.
- Altman, J., & Sudarshan, K. 1975. Postnatal development of locomotion in the laboratory rat. Animal Behaviour, 23:896-920.
- Anderson, S., & Jones, J. K., Jr. 1984. Orders and Families of Recent Mammals of the World. New York: Wiley.
- Angulo y Gonzalez, A. W. 1932. The prenatal development of behavior in the albino rat. Journal of Comparative Neurology, 55:395-442.
- Aristotle. De generatione animalium. ("On the generation of animals," trans. A. Platt, 1952). Chicago:

Encyclopaedia Britannica.

- Arshavsky, I. A., Arshavskaya, E. I., & Praznikov, V. P. 1976. Motor reactions during the antenatal period correlated with the periodic change in the activity of the cardiovascular system. Developmental Psychobiology, 9:343-352.
- Ashby, W. R. 1956. An Introduction to Cybernetics. London: Chapman & Hall, Ltd.
- Avery, G. T. 1928. Responses of foetal guinea pigs prematurely delivered. Genetic Psychology Monographs, 3:245-331.
- Baer, K. E. von. 1828. Entwicklungsgeschichte der Thiere: Beobachtung und Reflexion. Königsberg: Bornträger.
- Bakeman, R., & Gottman, J. M. 1986. Observing Interaction: An Introduction to Sequential Analysis. Cambridge: Cambridge Univ. Press.
- Barcroft, J., & Barron, D. H. 1939. The development of behavior in foetal sheep. Journal of Comparative Neurology, 70:477-502.
- Barcroft, J., Barron, D. H., & Windle, W. F. 1936. Some observations on genesis of somatic movements in sheep embryos. Journal of Physiology, 87:73-78.
- Barr, M., Jr., Jensch, R. P., & Brent, R. L. 1970. Prenatal growth in the albino rat: effects of number, intrauterine position and resorptions. American Journal of Anatomy, 128:413-428.
- Basmajian, J. V., & Ranney, D. A. 1961. Chemomyelotomy:

- substitute for general anesthesia in experimental surgery. Journal of Applied Physiology, 16:386.
- Bateson, P. P. G. 1987. Biological approaches to the study of behavioural development. International Journal of Behavioral Development, 10:1-22.
- Becker, R. F., King, J. E., Marsh, R. H., & Wyrick, A. D. 1964. Intrauterine respiration in the rat fetus. 1. Direct observations - comparison with the guinea pig. American Journal of Obstetrics and Gynecology, 90:236-246.
- Bekoff, A. 1976. Ontogeny of leg motor output in the chick embryo: a neural analysis. Brain Research, 106:271-291.
- Bekoff, A. 1981. Embryonic development of the neural circuitry underlying motor coordination. In W. M. Cowan (ed.), Studies in Developmental Neurobiology: Essays in Honor of Viktor Hamburger, pp. 134-170. New York: Oxford University Press.
- Bekoff, A. 1986. Ontogeny of chicken motor behaviors, evidence for multiuse limb pattern generating circuitry. In S. Grillner, P. S. G. Stein, D. G. Stuart, H. Forssberg, & R. M. Herman (eds.), Neurobiology of Vertebrate Locomotion, pp. 433-454. Hampshire, U. K.: MacMillan Press.
- Bekoff, A. 1988. Embryonic motor output and movement patterns: relationship to postnatal behavior. In: W. P. Smotherman & S. R. Robinson (eds.), Behavior of the

- Fetus, pp. 191-206. Caldwell, NJ: Telford Press.
- Bekoff, A., & Lau, B. 1980. Interlimb coordination in 20-day-old rat fetuses. Journal of Experimental Zoology, 214:173-175.
- Bekoff, A., & Trainer, W. 1979. The development of interlimb co-ordination during swimming in postnatal rats. Journal of Experimental Biology, 83:1-11.
- Bekoff, M., & Byers, J. A. 1985. The development of behavior from evolutionary and ecological perspectives in mammals and birds. In M. K. Hecht, B. Wallace, G. T. Prance (eds.), Evolutionary Biology, Vol. 19, pp. 215-286. New York: Plenum Press.
- Bhattacharyya, G. K., & Johnson, R. A. 1977. Statistical Concepts and Methods. New York: Wiley.
- Bichat, M. F. X. 1827. Physiological Researches upon Life and Death. (2nd American ed., trans. F. Gold). Boston: Richardson and Lord.
- Bignall, K. E. 1974. Ontogeny of levels of neural organization: The righting reflex as a model. Experimental Neurology, 42:566-573.
- Birnholz, J. C. 1988. On observing the human fetus. In W. P. Smotherman & S. R. Robinson (eds.), Behavior of the Fetus, pp. 47-60. Caldwell, NJ: Telford Press.
- Birnholz, J. C., & Benecerraf, B. R. 1983. The development of human fetal hearing. Science, 222:516-518.
- Bradley, N. S., & Smith, J. L. 1988. Neuromuscular patterns of stereotypic hindlimb behaviors in the first

- two postnatal months. I. Stepping in normal kittens. Developmental Brain Research, 38:37-52.
- Briem, V. 1986. Temporal organisation in the behaviour of newborn infants in active sleep. Behaviour, 99:189-220.
- Brown, T. G. 1915. On the activities of the central nervous system of the unborn foetus of the cat; with a discussion of the question whether progression (walking, etc.) is a "learnt" complex. Journal of Physiology (London), 49:208-215.
- Brunjes, P. C. 1983. Olfactory bulb maturation in Acomys cahirinus: Is neural growth similar in precocial and altricial Murids? Developmental Brain Research, 8:335-341.
- Burghardt, G. M. 1988. Precocity, play, and the ectotherm-endothym transition. In E. M. Blass (ed.), Handbook of Behavioral Neurobiology, vol. 9, Developmental Psychobiology and Behavioral Ecology, pp. 107-148. New York: Plenum.
- Carmichael, L. 1934. An experimental study in the prenatal guinea-pig of the origin and development of reflexes and patterns of behavior in relation to the stimulation of specific receptor areas during the period of active fetal life. Genetic Psychology Monographs, 16:337-491.
- Carmichael, L. 1934. The onset and early development of behavior. In L. Carmichael (ed.), Manual of Child Psychology, 2nd ed., pp. 60-185. New York: Wiley.

- Chapman, R. H., & Stern, J. M. 1977. Failure of severe maternal stress or ACTH during pregnancy to affect emotionality of male offspring: implications of litter effects for prenatal studies. Developmental Psychobiology, 12:255-267.
- Clutton-Brock, T. H., & Harvey, P. H. 1984. Comparative approaches to investigating adaptation. In J. R. Krebs & N. B. Davies (eds.), Behavioural Ecology: An Evolutionary Approach, pp. 7-29. Sunderland, MA: Sinauer Associates.
- Coghill, G. E. 1929. Anatomy and the Problem of Behavior. Cambridge: Cambridge Univ. Press.
- Colgan, P. 1989. Animal Motivation. New York: Chapman and Hall.
- Coronios, J. D. 1933. Development of behavior in the fetal cat. Genetic Psychology Monographs, 14:283-386.
- Darwin, C. 1859. The Origin of Species by Means of Natural Selection. (Reprint of 1st ed., 1979). New York: Avenal.
- Dawkins, M., & Dawkins, R. 1974. Some descriptive and explanatory stochastic models of decision-making. In D. J. McFarland (ed.), Motivational Control Systems Analysis, pp. 119-168. New York: Academic Press.
- Dawes, G. S. 1968. Fetal and Neonatal Physiology: A Comparative Study of Changes at Birth. Chicago: Yearbook Medical Publishers.
- de Beer, G. R. 1958. Embryos and Ancestors. Oxford:

Clarendon Press.

- deVries, J. I. P., Visser, G. H. A., & Prechtl, H. F. R.
1982. The emergence of fetal behavior. I.
Qualitative aspects. Early Human Development, 7:301-322.
- deVries, J. I. P., Visser, G. H. A., & Prechtl, H. F. R.
1984. Fetal motility in the first half of pregnancy.
In H. F. R. Prechtl (ed.), Continuity of Neural Functions from Prenatal to Postnatal Life, pp. 46-64.
Philadelphia: J. B. Lippincott.
- deVries, J. I. P., Visser, G. H. A., & Prechtl, H. F. R.
1985. The emergence of fetal behaviour. II.
Quantitative aspects. Early Human Development, 12:99-120.
- Eden, G. J., & Hanson, M. A. 1987. Maturation of the respiratory response to acute hypoxia in the newborn rat. Journal of Physiology, 392:1-9.
- Edwards, D. D., & Edwards, J. S. 1970. Fetal movement: development and time course. Science, 169:95-97.
- Eisenberg, J. F. 1981. The Mammalian Radiations: An Analysis of Trends in Evolution, Adaptation, and Behavior. Chicago: University of Chicago.
- Fagen, R. M., & Young, D. Y. 1978. Temporal patterns of behaviors: durations, intervals, latencies and sequences. In P. W. Colgan (ed.), Quantitative ethology, pp. 79-114. New York: Wiley.
- Felsenstein, J. 1985. Phylogenies and the comparative

- method. American Naturalist, 125:1-15.
- Fentress, J. C. 1978. Mus musicus: the developmental orchestration of selected movement patterns in mice. In: G. M. Burghardt & M. Bekoff (eds.), The Development of Behavior: Comparative and Evolutionary Aspects, pp. 321-342. New York: Garland Press.
- Fentress, J. C. 1984. The development of coordination. Journal of Motor Behavior, 16, 99-134.
- Fentress, J. C., & McLeod, P. J. 1986. Motor patterns in development. In E. M. Blass (ed.), Handbook of Behavioral Neurobiology, Vol. 8, Developmental Psychobiology and Developmental Neurobiology, pp. 35-97. New York: Plenum.
- Fentress, J. C., & Stilwell, F. P. 1973. Grammar of a movement sequence in inbred mice. Nature, 244:52-53.
- Fifer, W. P., & C. Moon. 1988. Auditory experience in the fetus. In W. P. Smotherman & S. R. Robinson (eds.), Behavior of the Fetus, pp. 175-188. Caldwell, NJ: Telford Press.
- Galigher, A. E., & Kozloff, E. N. 1971. Essentials of Practical Microtechnique, 2nd ed. Philadelphia: Lea and Febiger.
- Geubelle, F. 1984. Perception of environmental conditions by the fetus in utero. In P. O. Hubinont (ed.), Progress in Reproductive Biology and Medicine, Vol. 11, pp. 110-119. Basel: S. Karger.
- Ghiselin, M. T. 1976. The nomenclature of correspondence:

- a new look at "homology" and "analogy". In R. B. Masterton, W. Hodos & H. Jerison (eds.), Evolution, Brain and Behavior: Persistent Problems, pp. 129-142. Hillsdale, NJ: Erlbaum.
- Golani, I., Bronchti, G., Moualem, D., & Teitelbaum, P. 1981. "Warm-up" along dimensions of movement in the ontogeny of exploration in rats and other infant mammals. Proceedings of the National Academy of Science USA, 78:7226-7229.
- Golani, I., & Fentress, J. C. 1985. Early ontogeny of face grooming in mice. Developmental Psychobiology, 18:529-544.
- Gottlieb, G. 1971. Ontogenesis of sensory function in birds and mammals. In: E. Tobach, L. Aronson & E. Shaw (eds.), The Biopsychology of Development, p. 67-128. New York: Academic Press.
- Gottman, J. M., & Bakeman, R. 1979. The sequential analysis of observational data. In M. E. Lamb, S. J. Suomi, & G. R. Stephenson (eds.), Social Interaction Analysis: Methodological Issues, pp. 185-206. Madison, University of Wisconsin Press.
- Gould, S. J. 1977. Ontogeny and Phylogeny. Cambridge: Harvard University Press.
- Grill, H. J., & Berridge, K. C. 1985. Taste reactivity as a measure of the neural control of palatability. In J. M. Sprague & A. N. Epstein (eds.), Progress in Psychobiology and Physiological Psychology, Vol. 11,

- pp. 1-61. New York: Academic Press.
- Grillner, S. 1981. Control of locomotion in bipeds, tetrapods, and fish. Handbook of Physiology, Section 1, The Nervous System, Vol. II, Motor Control, Part 2. pp. 1179-1236.
- Hailman, J. P. 1967. The ontogeny of an instinct. Behaviour Supplement, 15:1-196.
- Hailman, J. P. 1976a. Homology: logic, information, and efficiency. In R. B. Masterton, W. Hodos & H. Jerison (eds.), Evolution, Brain and Behavior: Persistent Problems, pp. 181-198. Hillsdale, NJ: Erlbaum.
- Hailman, J. P. 1976b. Uses of the comparative study of behavior. In R. B. Masterton, W. Hodos & H. Jerison (eds.), Evolution, Brain and Behavior: Persistent Problems, pp. 13-22. Hillsdale, NJ: Erlbaum.
- Hailman, J. P. 1977. Optical Signals: Animal Communication and Light. Bloomington: Indiana University Press.
- Hailman, J. P. 1982. Ontogeny: toward a general theoretical framework for ethology. In P. P. G. Bateson & P. H. Klopfer (eds.), Perspectives in Ethology, Vol. 5, Ontogeny, pp. 133-189. New York: Plenum Press.
- Hailman, J. P., & Sustare, B. D. 1973. What a stuffed toy tells a stuffed shirt. Bioscience, 23, 644-651.
- Halbertsma, J. M. 1983. The stride cycle of the cat: the modelling of locomotion by computerized analysis of

- automatic recordings. Acta Physiologica Scandinavica, Supplement no. 521, pp. 1-75.
- Hall, W. G., & Oppenheim, R. W. 1987. Developmental psychobiology: Prenatal, perinatal, and early postnatal aspects of behavioral development. Annual Review of Psychology, 38:91-128.
- Hall, W. G., & Rosenblatt, J. S. 1977. Suckling behavior and intake control in the developing rat pup. Journal of Comparative and Physiological Psychology, 91:1232-1247.
- Hall, W. G., & Williams, C. L. 1983. Suckling isn't feeding, or is it? A search for developmental continuities. In J. S. Rosenblatt, R. A. Hinde, C. Beer & M. C. Busnel (eds.), Advances in the Study of Behavior, Vol. 13, pp. 219-255. New York: Academic Press.
- Hamburger, V. 1963. Some aspects of the embryology of behavior. Quarterly Review of Biology, 38:342-365.
- Hamburger, V. 1973. Anatomical and physiological basis of embryonic motility in birds and mammals. In G. Gottlieb (ed.), Behavioral Embryology, pp. 51-76. New York: Academic Press.
- Harvey, W. 1651. Anatomical exercises on the generation of animals. (reprint, 1952). Chicago: Encyclopaedia Britannica.
- Hepper, P. 1988. Adaptive fetal learning: prenatal exposure to garlic affects postnatal preferences.

- Animal Behaviour, 36, 935-936.
- Hinde, R. A. 1968. Dichotomies in the study of development. In J. M. Thoday & A. S. Parkes (eds.), Genetic and Environmental Influences on Behaviour, pp. 3-14. New York: Plenum.
- Hinde, R. A. 1970. Animal Behaviour: A Synthesis of Ethology and Comparative Psychology. New York: McGraw-Hill.
- Hodos, W., & Campbell, C. B. G. 1969. Scala Naturae: why there is no theory in comparative psychology. Psychological Review, 76:337-350.
- Hofer, M. A. 1981. The Roots of Human Behavior: An Introduction to the Psychobiology of Early Development. New York: W. H. Freeman.
- Hooker, D. 1952. The Prenatal Origin of Behavior. 18th Porter Lecture Series. Lawrence: University of Kansas Press.
- Horn, H. S. 1978. Optimal tactics of reproduction and life-history. In J. R. Krebs & N. B. Davies (eds.), Behavioural Ecology: An Evolutionary Approach, 1st ed., pp. 411-429. Sunderland, MA: Sinauer.
- Humphrey, T. 1953. The relation of oxygen deprivation to fetal reflex arcs and the development of fetal behavior. Journal of Psychology, 35:3-43.
- Itskovitz, J., LaGamma, E. F., & Rudolph, A. M. 1987. Effects of cord compression on fetal blood flow distribution and O₂ delivery. American Journal of

- Physiology, 252:H100-H109.
- James, W. 1890. Principles of Psychology. (Reprint, 1952). Chicago: Encyclopaedia Britannica.
- Jarman, P. 1982. Prospects for interspecific comparison in sociobiology. In King's College Sociobiology Group (ed.), Current Problems in Sociobiology, pp. 323-342. Cambridge: Cambridge University Press.
- Johanson, I. B., & Shapiro, E. G. 1986. Intake and behavioral responsiveness to taste stimuli in infant rats from 1 to 15 days of age. Developmental Psychobiology, 19:593-606.
- Kehoe, P., & Blass, E. M. 1985. Gustatory determinants of suckling in albino rats 5-20 days of age. Developmental Psychobiology, 18:67-82.
- Kirby, M. L. 1979. A quantitative method for determining the effect of opiates on fetal rats in utero. In Problems of Drug Dependence. NIDA Research Monograph, 27:191-197.
- Knox, W. E., & Lister-Rosenoer, L. M. 1978. Timing of gestation in rats by fetal and maternal weights. Growth, 42:43-53.
- Kodama, N., & Sekiguchi, S. 1984. The development of spontaneous body movement in prenatal and perinatal mice. Developmental Psychobiology, 17:139-150.
- Krasnegor, N. A. 1987. Developmental psychobiology research: A health scientist administrator's perspective. Developmental Psychobiology, 20:641-644.

- Krasnegor, N. A. 1988. On fetal development: A behavioral perspective. In W. P. Smotherman and S. R. Robinson (eds.), Behavior of the Fetus, pp. 227-231. Caldwell, NJ: Telford Press.
- Krebs, J. R., & Davies, N. B. (eds.). 1984. Behavioural Ecology: An Evolutionary Approach, 2nd ed. Sunderland, MA: Sinauer.
- Kugler, P. N., & Turvey, M. T. 1987. Information, Natural Law, and the Self-assembly of Rhythmic Movement. Hillsdale, NJ: Lawrence Erlbaum.
- Kuo, Z.-Y. 1967. The Dynamics of Behavior Development: An Epigenetic View. New York: Random House.
- Lamers, W. H., Mooren, P. G., de Graaf, A., Markiewicz, A., & Charles, R. 1985. Perinatal organ development in rat and spiny mouse: its relation to altricial and precocial timing of birth. In C. T. Jones & P. W. Nathanielsz (eds.), The Physiological Development of the Fetus and Newborn, pp. 41-45. London: Academic Press.
- Lane, H. H. 1917. The correlation between structure and function in the development of the special senses of the white rat. University of Oklahoma Bulletin, no. 140 (8):1-88.
- Lehrman, D. S. 1970. Semantic and conceptual issues in the nature-nurture problem. In L. R. Aronson, E. Tobach, D. S. Lehrman & J. S. Rosenblatt (eds.), Development and Evolution of Behavior, pp. 17-52. San Francisco:

- W. H. Freeman.
- Leon, M., Coopersmith, R., Ulibarri, C., Porter, R. H., & Powers, J. B. 1984. Development of olfactory bulb organization in precocial and altricial rodents. Developmental Brain Research, 12:45-53.
- Lev, R., & Orlic, D. 1972. Protein absorption by the intestine of the fetal rat in utero. Science, 177, 522-524.
- MacFarlane, B. A., Pedersen, P. E., Cornell, C. E., and Blass, E. M. 1983. Sensory control of suckling-associated behaviours in the domestic Norway rat, Rattus norvegicus. Animal Behaviour, 31:462-471.
- Machlis, L. 1977. An analysis of the temporal patterning of pecking in chicks. Behaviour, 63, 1-70.
- Mann, L. I. 1986. Pregnancy events and brain damage. American Journal of Obstetrics and Gynecology, 155:6-9.
- Marsh, R. H., King, J. E. & Becker, R. F. 1963. Volume and viscosity of amniotic fluid in rat and guinea pig fetuses near term. American Journal of Obstetrics and Gynecology, 85, 487-492.
- Maynard Smith, J., & Holliday, R. (eds.). 1979. The Evolution of Adaptation by Natural Selection. London: Royal Society.
- Mayr, E. 1982. The Growth of Biological Thought. Cambridge: Harvard University Press.
- McFarland, D. J. (ed.). 1974. Motivational Control Systems Analysis. New York: Academic Press.

- McKinney, M. L. (ed.). 1988. Heterochrony in Evolution. New York: Plenum Press.
- McLeod, W., Brien, J., Loomis, C., Carmichael, L., Probert, C., & Patrick, J. 1983. Effect of maternal ethanol ingestion on fetal breathing movements, gross body movements and heart rate at 37 to 40 weeks gestational age. American Journal of Obstetrics and Gynecology, 145:251-257.
- Meisel, R. L., & Ward, I. L. 1981. Fetal female rats are masculinized by male littermates located caudally in the uterus. Science, 220, 437-438.
- Miller, D. B. 1988. Development of instinctive behavior. In E. M. Blass (ed.), Handbook of Behavioral Neurobiology, Vol. 9, Developmental Psychobiology and Behavioral Ecology, pp. 415-444. New York: Plenum Press.
- Minkowski, M. 1928. Neurobiologische Studien am menschlichen Foetus. Handbuch die biologische Arbeitsmethoden, Abt. V, Teil 5B, Heft 5, 253:511-618.
- Moessinger, A. C. 1988. Morphological consequences of depressed or impaired fetal activity. In W. P. Smotherman & S. R. Robinson (eds.), Behavior of the Fetus, pp. 163-173. Caldwell, NJ: Telford Press.
- Moessinger, A. C., Blanc, W. A., Marone, P. A., & Polsen, D. C. 1982. Umbilical cord length as an index of fetal activity: experimental study and clinical implications. Pediatric Research, 16:109-112.

- Morris, R. A. 1986. Legal implications for research in the developing organism. Neurotoxicology, 7:3-18.
- Narayanan, C. H., Fox, M. W., & Hamburger, V. 1971. Prenatal development of spontaneous and evoked activity in the rat. Behaviour, 40:100-134.
- Narayanan, C. H., Narayanan, Y., & Browne, R. C. 1982. Effects of induced thyroid deficiency on the development of suckling behavior in rats. Physiology and Behavior, 29:361-370.
- Nelson, K. 1964. The temporal patterning of courtship behaviour in the glandulocaudine fishes (Ostariophysi, Characidae). Behaviour, 24:90-146.
- Nelson, K. 1973. Does the holistic study of behavior have a future? In P. P. G. Bateson & P. H. Klopfer (eds.), Perspectives in Ethology, pp. 281-328. New York: Plenum.
- Nice, M. M. 1962. Development of behavior in precocial birds. Transactions of Linnaean Society of New York, No. 8:1-211.
- Oppenheim, R. W. 1972. Prehatching and hatching behaviour in birds: A comparative study of altricial and precocial species. Animal Behaviour, 20:644-655.
- Oppenheim, R. W. 1981. Ontogenetic adaptations and retrogressive processes in the development of the nervous system and behaviour: a neuroembryological perspective. In K. J. Connolly & H. F. R. Precht (eds.), Maturation and Development: Biological and

- Psychological Perspectives, pp. 73-109. Philadelphia: Lippincott.
- Oppenheim, R. W. 1982a. The neuroembryological study of behavior: progress, problems, perspectives. In R. K. Hunt (ed.), Current Topics in Developmental Biology, vol. 17: Neural Development, Part III, pp. 257-309. New York: Academic Press.
- Oppenheim, R. W. 1982b. Preformation and epigenesis in the origins of the nervous system and behavior: issues, concepts, and their history. In P. P. G. Bateson & P. H. Klopfer (eds.), Perspectives in Ethology, Vol. 5, Ontogeny, pp. 1-100. New York: Plenum Press.
- Oppenheim, R. W. 1984. Ontogenetic adaptations in neural development: toward a more 'ecological' developmental psychobiology. In H. F. R. Prechtl (ed.), Continuity of Neural Functions from Prenatal to Postnatal Life, pp. 16-30. Philadelphia: Lippincott.
- Oppenheim, R. W., & Haverkamp, L. 1986. Early development of behavior and the nervous system: An embryological perspective. In E. M. Blass (ed.), Handbook of Behavioral Neurobiology, Vol. 8, Developmental Psychobiology and Developmental Neurobiology, pp. 1-33. New York: Plenum.
- Oyama, S. 1985. The Ontogeny of Information: Developmental Systems and Evolution. Cambridge: Cambridge Univ. Press.
- Pankratz, D. S. 1931. A preliminary report on the fetal

- movements in the rabbit. Anatomical Record, 48:58-59.
- Pedersen, P. E., Greer, C. A., & Shepherd, G. M. 1986. Early development of olfactory function. In E. M. Blass (ed.), Handbook of Behavioral Neurobiology, Vol. 8, Developmental Psychobiology and Developmental Neurobiology, pp. 163-203. New York: Plenum.
- Pedersen, P. E., Stewart, W. B., Greer, C. A., & Shepherd, G. M. 1983. Evidence for olfactory function in utero. Science, 221:478-480.
- Prechtl, H. F. R. 1986. Prenatal motor development. In M. G. Wade & H. T. A. Whiting (eds.), Motor Development in Children: Aspects of Coordination and Control, pp. 53-64. Dordrecht, Netherlands: Martinus Nijhoff Publishers.
- Prechtl, H. F. R. 1985. Ultrasound studies of human fetal behaviour. Early Human Development, 12:91-98.
- Preyer, W. 1885. Specielle Physiologie des Embryo. Untersuchungen uber die Lebenserscheinungen vor der Geburt. Leipzig: Grieben.
- Price, E. O. 1984. Behavioral aspects of animal domestication. Quarterly Review of Biology, 59:1-32.
- Provine, R. R. 1980. Development of between-limb movement synchronization in the chick embryo. Developmental Psychobiology, 13:151-163.
- Raff, R. A., & Kaufman, T. C. 1983. Embryos, Genes, and Evolution: the Developmental-Genetic Basis of Evolutionary Change. New York: Macmillan.

- Richmond, G., & Sachs, B. D. 1980. Grooming in Norway rats: the development and adult expression of a complex motor pattern. Behaviour, 75, 82-96.
- Richmond, G. & Sachs, B. D. 1984. Further evidence for masculinization of female by males located caudally in utero. Hormones and Behavior, 18, 484-490.
- Ridley, M. 1983. The Explanation of Organic Diversity: The Comparative Method and Adaptations for Mating. Oxford: Clarendon Press.
- Robertson, S. S. 1985. Cyclic motor activity in the human fetus after midgestation. Developmental Psychobiology, 18:411-419.
- Robertson, S. S., Dierker, L. J., Sorokin, Y., & Rosen, M. G. 1982. Human fetal movement: spontaneous oscillations near one cycle per minute. Science, 218:1327-1330.
- Robinson, S. R., & Smotherman, W. P. 1987. Environmental determinants of behaviour in the rat fetus. II. The emergence of synchronous movement. Animal Behaviour, 35:1652-1662.
- Robinson, S. R., & Smotherman, W. P. 1988. Chance and chunks in the ontogeny of fetal behavior. In W. P. Smotherman & S. R. Robinson (eds.), Behavior of the Fetus, pp. 95-115. Caldwell, NJ: Telford Press.
- Robinson, S. R., & Smotherman, W. P. In press. Fetal learning: Implications for the development of kin recognition. In P. G. Hepper (ed.), Kin Recognition.

Cambridge: Cambridge University Press.

- Roscoe, J. T. 1975. Fundamental Research Statistics for the Behavioral Sciences, 2nd ed. New York: Holt, Rinehart and Winston.
- Schleidt, W. M. 1974. How "fixed" is the fixed action pattern? Zeitschrift fur Tierpsychologie, 36:184-211.
- Shannon, C. E., & Weaver, W. 1949. The Mathematical Theory of Communication. Urbana: University of Illinois Press.
- Sherman, P. 1988. The levels of analysis. Animal Behaviour, 36:616-619.
- Siegel, S. 1956. Nonparametric Statistics for the Behavioral Sciences. New York: McGraw-Hill.
- Slater, P. J. B. 1974. The temporal patterning of feeding in the zebra finch. Animal Behaviour, 22:506-515.
- Slater, P. J. B., & Lester, N. P. 1982. Minimising errors in splitting behaviour into bouts. Behaviour, 79:153-161.
- Smotherman, W. P. 1982. Odor aversion learning by the rat fetus. Physiology and Behavior, 29:769-771.
- Smotherman, W. P., Richards, L. S., & Robinson, S. R. 1984. Techniques for observing fetal behavior in utero: a comparison of chemomyelotomy and spinal transection. Developmental Psychobiology, 17:661-674.
- Smotherman, W. P., & Robinson, S. R. 1985. The rat fetus in its environment: behavioral adjustments to novel, familiar, aversive and conditioned stimuli presented in

- utero. Behavioral Neuroscience, 99:521-530.
- Smotherman, W. P., & Robinson, S. R. 1986. Environmental determinants of behaviour in the rat fetus. Animal Behaviour, 34:1859-1873.
- Smotherman, W. P., & Robinson, S. R. 1987a. Prenatal expression of species-typical action patterns in the rat fetus (Rattus norvegicus). Journal of Comparative Psychology, 101:190-196.
- Smotherman, W. P., & Robinson, S. R. 1987b. Psychobiology of fetal experience in the rat. In N. A. Krasnegor, E. M. Blass, M. A. Hofer & W. P. Smotherman (eds.), Perinatal Development: A Psychobiological Perspective, pp. 39-60. Orlando: Academic Press.
- Smotherman, W. P., & Robinson, S. R. 1987c. Stereotypic behavioral response of rat fetuses to acute hypoxia is altered by maternal alcohol consumption. American Journal of Obstetrics and Gynecology, 157:982-986.
- Smotherman, W. P., & Robinson, S. R. 1988a. Behavior of rat fetuses following chemical or tactile stimulation. Behavioral Neuroscience, 102:24-34.
- Smotherman, W. P., & Robinson, S. R. 1988b. The uterus as environment: the ecology of fetal behavior. In E. M. Blass (ed.), Handbook of Behavioral Neurobiology, Vol. 9, Developmental Psychobiology and Behavioral Ecology, pp. 149-196. New York: Plenum.
- Smotherman, W. P., & Robinson, S. R. 1988c. Response of the rat fetus to acute umbilical cord occlusion: an

- ontogenetic adaptation? Physiology and Behavior, 44:131-135.
- Smotherman, W. P., & Robinson, S. R. 1988d. Dimensions of fetal investigation. In W. P. Smotherman & S. R. Robinson (eds.), Behavior of the Fetus, pp. 19-34. Caldwell, N.J.: Telford Press.
- Smotherman, W. P., & Robinson, S. R. 1989. Cryptopsychobiology: The appearance, disappearance, and reappearance of a species-typical action pattern during early development. Behavioral Neuroscience, 103:246-253.
- Smotherman, W. P., & Robinson, S. R. In press. Accessibility of the rat fetus for psychobiological investigation. In H. N. Shair, M. A. Hofer & G. Barr (eds.), Developmental Psychobiology: Current Methodology and Conceptual Issues. New York: Oxford University Press.
- Smotherman, W. P., Robinson, S. R., & Miller, B. J. 1986. A reversible preparation for observing the behavior of fetal rats in utero: spinal anesthesia with lidocaine. Physiology and Behavior, 37:57-60.
- Stickrod, G., Kimble, D. P., & Smotherman, W. P. 1982. In utero taste/odor aversion conditioning in the rat. Physiology and Behavior, 28:5-7.
- Sustare, B. D. 1978. Systems diagrams. In P. W. Colgan (ed.), Quantitative Ethology, pp. 275-311. New York: Wiley.

- Suzuki, S., & Yamamuro, T. 1985. Fetal movement and fetal presentation. Early Human Development, 11:255-263.
- Swenson, E. A. 1926. The Development of Movement of the Albino Rat before Birth. Unpublished Ph.D. Thesis, University of Kansas.
- Tam, P. P. L., & Chan, S. T. H. 1977. Changes in the composition of maternal plasma, fetal plasma and fetal extraembryonic fluid during gestation in the rat. Journal of Reproduction and Fertility, 51:41-51.
- Tessitore, C., & Brunjes, P. C. 1988. A comparative study of myelination in precocial and altricial murid rodents. Developmental Brain Research, 43:139-147.
- Thelen, E. 1988. On the nature of developing motor systems and the transition from prenatal to postnatal life. In W. P. Smotherman & S. R. Robinson (eds.), Behavior of the Fetus, pp. 207-224. Caldwell, NJ: Telford Press.
- Thelen, E., & Fisher, D. M. 1982. Newborn stepping: an explanation for a "disappearing reflex". Developmental Psychology, 18:760-775.
- Thelen, E., Fisher, D. M., & Ridley-Johnson, R. 1984. The relationship between physical growth and a newborn reflex. Infant Behavior and Development, 7:479-493.
- Thelen, E., Kelso, J. A. S., & Fogel, A. 1987. Self-organizing systems and infant motor development. Developmental Review, 7:39-65.
- Tinbergen, N. 1963. On aims and methods of ethology. Zeitschrift fur Tierpsychologie, 20:410-429.

- Van Liew, H. D. 1962. Semilogarithmic plots of data which reflect a continuum of exponential processes. Science, 138:682-683.
- vom Saal, F. S. 1984. The intrauterine position phenomenon: effects on physiology, aggressive behavior, and population dynamics in house mice. In K. J. Flannelly, R. J. Blanchard & D. C. Blanchard (eds.), Biological Perspectives on Aggression, pp. 135-179. New York: Alan R. Liss.
- West, M. J., & King, A. P. 1987. Settling nature and nurture into an ontogenetic niche. Developmental Psychobiology, 20:549-562.
- West, M. J., King, A. P., & Arberg, A. A. 1988. The inheritance of niches: The role of ecological legacies in ontogeny. In E. M. Blass (ed.), Handbook of Behavioral Neurobiology, Vol. 9, Developmental Psychobiology and Behavioral Ecology, pp. 41-62. New York: Plenum Press.
- Windle, W. F. 1944. Genesis of somatic motor function in mammalian embryos: a synthesizing article. Physiological Zoology, 17:247-261.
- Windle, W. F., & Becker, R. F. 1940. Relation of anoxemia to early activity in the fetal nervous system. Archives of Neurology and Psychiatry, 43:90-101.
- Windle, W. F., & Fish, M. W. 1932. The development of the vestibular righting reflex in the cat. Journal of Comparative Neurology, 54:85-96.

- Windle, W. F., & Griffin, A. M. 1931. Observations on embryonic and fetal movements of the cat. Journal of Comparative Neurology, 52:149-188.
- Windle, W. F., Minear, W. L., Austin, M. F., & Orr, D. W. 1935. The origin and early development of somatic behavior in the albino rat. Physiological Zoology, 8:156-185.
- Windle, W. F., O'Donnell, J. E., & Glasshagle, E. E. 1933. The early development of spontaneous and reflex behavior in cat embryos and fetuses. Physiological Zoology, 6:521-541
- Winer, B. J. 1971. Statistical Principles in Experimental Design, 2nd ed. New York: McGraw-Hill.
- Wirtschafter, Z. T., & Williams, D. W. 1957. Dynamics of the amniotic fluid as measured by changes in protein patterns. American Journal of Obstetrics and Gynecology, 74:309-313.

APPENDIX

PRECEDENT EVENT	RATTUS		DAY 17			IN UTERO		
			SUBSEQUENT		EVENT			
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	364	104	60	29	8	38	43	2
Rearlimb	97	24	13	2	1	9	10	1
Head	73	5	14	1	0	4	8	0
Trunk	27	4	3	2	0	2	0	1
Mouth	11	0	0	0	0	0	0	0
FR	37	7	8	1	1	11	1	0
FH	37	13	7	3	1	1	2	0
Other	3	0	0	0	0	0	1	0

PRECEDENT EVENT	RATTUS		DAY 17			EX UTERO		
			SUBSEQUENT		EVENT			
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	180	46	235	76	12	8	107	6
Rearlimb	52	5	31	7	1	4	21	0
Head	256	26	215	72	16	5	47	7
Trunk	87	5	65	95	5	4	21	6
Mouth	27	0	12	4	8	0	1	0
FR	5	2	9	1	0	1	5	0
FH	59	35	75	24	9	1	38	0
Other	5	1	2	9	1	0	1	0

PRECEDENT EVENT	RATTUS		DAY 18			IN UTERO		
			SUBSEQUENT		EVENT			
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	257	55	53	13	3	21	56	5
Rearlimb	60	23	14	2	1	7	13	3
Head	56	12	21	1	1	4	10	1
Trunk	11	3	0	2	0	0	2	0
Mouth	6	0	1	0	1	0	2	0
FR	20	6	5	0	0	2	5	1
FH	46	22	12	0	4	2	21	1
Other	6	2	0	0	0	3	0	1

PRECEDENT EVENT	RATTUS		DAY 18			EX UTERO		
			SUBSEQUENT		EVENT			
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	239	98	198	46	21	30	145	20
Rearlimb	105	41	78	21	3	14	53	6
Head	216	62	188	32	15	28	79	16
Trunk	50	17	29	31	1	9	28	1
Mouth	26	7	15	1	24	0	1	1
FR	21	7	27	9	2	8	17	1
FH	115	86	92	23	7	1	84	5
Other	23	3	9	4	2	3	6	0

PRECEDENT EVENT	RATTUS		DAY 19 SUBSEQUENT EVENT			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	156	53	53	2	9	18	44	3
Rearlimb	52	51	23	1	0	15	18	4
Head	51	20	19	0	1	3	16	3
Trunk	3	0	0	0	0	0	0	0
Mouth	10	2	2	0	15	1	2	0
FR	22	7	5	0	1	6	5	1
FH	40	28	9	0	6	4	13	0
Other	5	2	2	0	0	0	2	1

PRECEDENT EVENT	RATTUS		DAY 19 SUBSEQUENT EVENT			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	92	78	92	28	37	39	97	19
Rearlimb	88	69	82	30	10	39	73	21
Head	122	64	83	18	24	32	69	20
Trunk	32	33	27	8	5	12	34	9
Mouth	32	11	19	6	70	7	27	8
FR	32	26	48	18	6	28	14	4
FH	58	118	61	44	22	9	96	16
Other	26	14	18	8	6	10	15	5

PRECEDENT EVENT	RATTUS		DAY 20			IN UTERO		
			SUBSEQUENT		EVENT			
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	163	48	59	5	8	15	39	4
Rearlimb	49	44	22	0	0	18	9	1
Head	69	19	49	3	5	1	17	1
Trunk	4	1	0	0	0	1	2	0
Mouth	11	1	3	0	42	0	3	0
FR	14	11	7	0	0	3	6	0
FH	30	16	22	0	5	2	17	3
Other	2	3	1	0	0	1	2	1

PRECEDENT EVENT	RATTUS		DAY 20			EX UTERO		
			SUBSEQUENT		EVENT			
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	83	68	69	13	15	59	94	8
Rearlimb	85	92	84	24	12	44	49	30
Head	82	55	61	20	15	56	69	20
Trunk	20	23	20	10	1	11	35	4
Mouth	20	16	10	3	30	9	4	1
FR	47	52	58	15	10	39	14	9
FH	61	101	52	34	8	14	60	9
Other	10	15	24	5	2	12	13	5

PRECEDENT EVENT	RATTUS		DAY 21			IN UTERO		
			SUBSEQUENT		EVENT			
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	236	77	74	2	7	16	24	1
Rearlimb	71	63	18	0	1	6	2	1
Head	97	11	28	0	4	0	3	0
Trunk	1	0	1	0	0	0	0	0
Mouth	10	1	2	0	10	0	0	0
FR	9	7	6	0	0	1	0	0
FH	11	3	16	0	1	0	1	0
Other	0	0	0	0	0	0	2	0

PRECEDENT EVENT	RATTUS		DAY 21			EX UTERO		
			SUBSEQUENT		EVENT			
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	62	65	43	3	15	44	45	10
Rearlimb	66	103	59	17	10	66	37	26
Head	51	58	41	11	9	44	34	7
Trunk	11	6	16	2	3	9	10	11
Mouth	13	11	12	3	33	16	7	1
FR	45	45	53	11	17	45	21	10
FH	26	75	17	17	8	9	38	19
Other	13	21	13	4	2	14	17	10

PRECEDENT EVENT	MERIONES		DAY 19			IN UTERO		
			SUBSEQUENT EVENT					
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	226	48	27	21	2	13	13	0
Rearlimb	43	24	15	5	0	3	3	0
Head	42	7	1	0	3	1	2	0
Trunk	14	5	5	9	0	1	1	0
Mouth	4	0	0	0	0	0	1	0
FR	10	3	4	0	0	0	1	0
FH	11	6	4	0	0	0	2	0
Other	0	0	0	0	0	0	0	0

PRECEDENT EVENT	MERIONES		DAY 19			EX UTERO		
			SUBSEQUENT EVENT					
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	309	84	228	55	53	3	67	5
Rearlimb	94	8	36	9	4	2	24	0
Head	216	29	78	17	35	5	22	4
Trunk	50	5	23	8	1	1	11	1
Mouth	78	6	13	1	14	1	2	0
FR	4	1	5	0	0	0	2	0
FH	45	44	20	11	8	0	32	1
Other	8	0	2	0	0	0	1	0

PRECEDENT EVENT	MERIONES		DAY 20			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	298	66	59	0	8	20	46	2
Rearlimb	69	7	11	0	0	4	11	0
Head	61	6	20	0	7	2	9	1
Trunk	0	0	0	0	0	0	0	0
Mouth	13	2	1	0	1	0	0	0
FR	19	4	3	0	0	1	0	0
FH	36	17	11	0	1	1	11	1
Other	2	0	1	0	0	1	1	0

PRECEDENT EVENT	MERIONES		DAY 20			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	362	140	165	25	19	31	84	5
Rearlimb	157	40	49	8	5	11	43	6
Head	162	40	83	7	18	12	30	1
Trunk	25	7	8	2	0	2	5	2
Mouth	40	5	5	1	50	3	3	0
FR	26	11	18	3	0	1	7	0
FH	56	73	23	5	14	4	24	1
Other	5	2	1	0	1	2	4	0

PRECEDENT EVENT	MERIONES		DAY 21			IN UTERO		
			SUBSEQUENT EVENT					
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	244	43	64	1	4	21	35	0
Rearlimb	46	7	5	0	0	6	10	1
Head	65	5	24	1	0	4	9	0
Trunk	2	0	0	1	0	0	0	0
Mouth	3	1	0	0	0	0	0	0
FR	19	2	9	0	0	3	3	0
FH	30	19	6	0	0	2	9	0
Other	0	0	1	0	0	0	0	0

PRECEDENT EVENT	MERIONES		DAY 21			EX UTERO		
			SUBSEQUENT EVENT					
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	309	135	139	21	21	29	98	16
Rearlimb	135	36	65	13	10	23	55	7
Head	165	61	44	11	9	21	27	5
Trunk	28	10	10	8	2	5	5	3
Mouth	27	3	9	3	23	2	4	1
FR	20	15	31	6	2	8	7	5
FH	57	83	38	7	4	4	35	10
Other	26	1	8	2	1	2	7	4

PRECEDENT EVENT	MERIONES		DAY 22			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	284	46	27	3	1	16	21	0
Rearlimb	45	9	5	0	0	6	4	0
Head	28	8	3	0	2	2	0	0
Trunk	3	0	0	0	0	0	0	0
Mouth	2	0	2	0	3	0	0	0
FR	15	3	4	0	0	2	4	0
FH	20	3	4	0	1	2	2	0
Other	0	0	0	0	0	0	0	0

PRECEDENT EVENT	MERIONES		DAY 22			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	249	147	129	12	26	70	87	11
Rearlimb	136	65	42	10	3	60	41	8
Head	154	27	116	4	12	41	29	4
Trunk	18	8	2	0	2	9	7	2
Mouth	33	4	12	0	35	2	5	1
FR	76	33	44	13	4	38	25	8
FH	54	77	35	8	10	15	24	4
Other	13	3	6	1	0	5	9	1

PRECEDENT EVENT	MERIONES		DAY 23			IN UTERO		
	Fore	Rear	SUBSEQUENT EVENT			FR	FH	Other
			Head	Trunk	Mouth			
Forelimb	229	29	43	0	7	27	35	0
Rearlimb	38	6	10	0	0	4	2	0
Head	47	7	4	1	2	2	11	0
Trunk	1	0	1	0	0	0	0	0
Mouth	8	0	1	0	1	1	2	0
FR	20	5	4	0	0	3	4	0
FH	23	14	12	1	3	0	6	0
Other	0	0	0	0	0	0	0	0

PRECEDENT EVENT	MERIONES		DAY 23			EX UTERO		
	Fore	Rear	SUBSEQUENT EVENT			FR	FH	Other
			Head	Trunk	Mouth			
Forelimb	178	114	75	7	14	80	76	18
Rearlimb	107	115	43	17	4	67	36	11
Head	100	24	136	4	9	34	49	14
Trunk	6	13	9	5	0	5	8	5
Mouth	13	6	10	0	5	3	4	2
FR	82	38	56	10	4	53	19	2
FH	53	82	34	4	5	8	30	11
Other	24	9	6	4	1	14	5	4

PRECEDENT EVENT	MERIONES		DAY 24			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	248	75	36	0	0	19	29	2
Rearlimb	76	50	9	2	1	7	21	3
Head	40	11	11	1	1	4	6	1
Trunk	2	0	2	0	0	0	0	0
Mouth	3	1	0	0	6	0	2	0
FR	12	7	10	0	0	5	2	0
FH	23	25	5	1	3	1	21	1
Other	4	1	1	0	1	0	0	0

PRECEDENT EVENT	MERIONES		DAY 24			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	151	111	57	5	8	88	70	15
Rearlimb	113	69	28	5	3	74	31	14
Head	71	23	43	2	5	42	25	2
Trunk	11	5	4	0	1	5	7	1
Mouth	9	3	2	1	11	6	1	0
FR	92	55	47	7	2	106	21	11
FH	46	64	29	12	2	10	34	5
Other	10	8	5	2	0	10	13	1

PRECEDENT EVENT	SIGMODON		DAY 18			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	160	52	73	0	6	7	33	1
Rearlimb	55	12	14	0	0	3	3	0
Head	76	13	58	1	2	3	20	0
Trunk	1	0	0	0	0	0	0	0
Mouth	7	0	2	0	3	1	1	0
FR	5	5	5	0	1	0	1	0
FH	26	5	22	0	2	2	17	1
Other	0	1	0	0	0	0	1	0

PRECEDENT EVENT	SIGMODON		DAY 18			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	328	96	124	21	14	13	25	2
Rearlimb	96	21	21	2	4	4	11	3
Head	121	19	33	13	15	4	4	0
Trunk	25	5	9	7	2	0	4	0
Mouth	20	4	7	3	12	1	0	2
FR	10	4	9	0	0	2	0	0
FH	20	11	6	6	1	0	4	0
Other	3	2	1	0	0	1	0	0

PRECEDENT EVENT	SIGMODON		DAY 19			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	143	77	24	1	1	28	14	2
Rearlimb	66	63	4	1	1	25	3	1
Head	22	6	5	0	1	1	3	0
Trunk	2	0	0	0	0	0	0	0
Mouth	2	0	1	0	0	0	0	0
FR	38	14	2	0	0	10	1	0
FH	14	5	1	0	0	0	2	0
Other	1	1	0	0	0	1	0	0

PRECEDENT EVENT	SIGMODON		DAY 19			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	209	83	86	11	10	25	24	3
Rearlimb	82	17	21	4	2	8	14	1
Head	81	20	29	2	5	7	12	1
Trunk	11	2	4	3	0	0	1	0
Mouth	7	1	2	2	1	4	1	0
FR	31	6	7	0	0	5	1	1
FH	29	17	6	0	0	1	5	1
Other	2	3	0	0	0	1	1	0

PRECEDENT EVENT	SIGMODON		DAY 20			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	354	112	58	6	14	64	39	6
Rearlimb	123	87	15	2	4	50	13	1
Head	53	24	17	0	3	9	10	3
Trunk	6	3	0	0	0	0	0	0
Mouth	20	5	1	0	10	4	1	1
FR	59	41	18	0	5	25	5	4
FH	30	21	10	0	6	2	3	0
Other	7	3	1	0	0	3	1	0

PRECEDENT EVENT	SIGMODON		DAY 20			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	500	177	170	33	77	132	146	30
Rearlimb	190	57	48	15	29	42	29	9
Head	175	41	75	4	37	36	44	8
Trunk	27	10	9	7	5	5	13	0
Mouth	93	23	20	1	102	24	21	9
FR	139	29	48	6	19	31	9	1
FH	106	77	44	10	19	9	49	7
Other	35	5	7	0	5	4	8	1

PRECEDENT EVENT	SIGMODON		DAY 21			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	519	153	134	2	30	62	113	8
Rearlimb	142	41	48	2	2	25	33	3
Head	141	40	56	0	7	16	24	1
Trunk	1	0	1	0	0	1	1	0
Mouth	32	6	5	0	23	1	3	0
FR	61	19	18	0	3	12	10	1
FH	117	35	19	0	5	8	17	3
Other	9	2	3	0	0	0	2	1

PRECEDENT EVENT	SIGMODON		DAY 21			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	623	373	210	44	116	194	262	45
Rearlimb	376	166	110	19	42	118	105	25
Head	217	97	80	8	22	65	77	22
Trunk	23	26	9	1	7	13	16	3
Mouth	125	37	21	5	127	34	27	8
FR	243	68	85	5	35	89	33	7
FH	210	172	63	12	24	36	78	16
Other	53	21	9	4	9	17	13	4

PRECEDENT EVENT	SIGMODON		DAY 22			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	192	107	25	2	11	28	22	2
Rearlimb	102	56	17	4	4	24	6	1
Head	32	14	3	0	3	4	4	0
Trunk	3	2	1	0	0	0	0	0
Mouth	13	5	2	0	12	1	1	0
FR	35	14	9	0	0	19	3	0
FH	12	14	4	0	3	3	2	0
Other	1	0	0	0	1	1	0	0

PRECEDENT EVENT	SIGMODON		DAY 22			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	375	201	120	13	43	117	120	21
Rearlimb	201	74	63	10	23	79	38	15
Head	145	52	48	3	8	35	38	15
Trunk	6	16	10	0	3	7	4	1
Mouth	34	21	14	2	38	19	14	4
FR	142	42	50	10	11	72	20	15
FH	78	84	28	7	17	21	20	8
Other	28	14	11	2	3	11	10	11

PRECEDENT EVENT	SIGMODON		DAY 23			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	323	87	112	1	13	19	65	3
Rearlimb	91	56	18	1	2	11	7	1
Head	112	22	50	0	6	6	14	2
Trunk	1	2	0	0	0	0	1	0
Mouth	21	3	2	0	11	0	5	0
FR	19	6	6	0	1	5	3	1
FH	50	11	22	2	8	1	17	2
Other	4	2	1	0	1	0	1	0

PRECEDENT EVENT	SIGMODON		DAY 23			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	291	226	103	26	34	118	100	16
Rearlimb	228	127	56	22	22	86	66	15
Head	104	62	33	3	15	41	30	2
Trunk	26	19	6	1	2	12	16	2
Mouth	37	21	10	0	23	9	16	1
FR	136	49	48	11	5	63	27	8
FH	73	106	26	14	15	15	41	10
Other	22	11	5	7	2	3	3	2

PRECEDENT EVENT	SIGMODON		DAY 24			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	173	88	27	2	31	21	53	3
Rearlimb	106	78	17	1	5	19	11	3
Head	33	21	11	1	3	5	5	1
Trunk	0	0	0	0	0	3	3	0
Mouth	30	10	4	0	15	2	1	0
FR	19	12	11	2	4	7	3	0
FH	34	29	9	0	4	1	22	2
Other	3	1	2	0	0	0	3	0

PRECEDENT EVENT	SIGMODON		DAY 24			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	282	146	56	9	116	118	88	21
Rearlimb	162	102	39	10	47	66	22	10
Head	73	32	44	1	17	24	18	8
Trunk	9	20	3	2	1	7	6	3
Mouth	105	57	17	4	225	24	10	6
FR	111	43	30	16	27	72	20	20
FH	63	51	23	7	11	13	51	13
Other	29	8	7	2	4	15	17	8

PRECEDENT EVENT	SIGMODON		DAY 25			IN UTERO		
			SUBSEQUENT EVENT					
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	258	76	54	2	57	27	86	16
Rearlimb	79	60	28	3	10	22	16	4
Head	86	23	37	1	11	2	21	3
Trunk	3	1	4	0	0	1	5	1
Mouth	58	12	13	0	79	2	11	1
FR	24	9	14	2	2	19	9	12
FH	52	37	28	6	16	4	61	11
Other	18	2	4	1	2	14	7	6

PRECEDENT EVENT	SIGMODON		DAY 25			EX UTERO		
			SUBSEQUENT EVENT					
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	250	117	78	20	85	99	162	23
Rearlimb	137	90	42	20	28	59	38	25
Head	92	45	122	5	16	26	42	14
Trunk	20	28	5	5	1	15	12	3
Mouth	72	26	24	7	93	22	12	8
FR	97	27	51	9	18	66	24	30
FH	110	88	29	7	16	16	162	63
Other	58	16	11	15	7	21	38	22

PRECEDENT EVENT	SIGMODON		DAY 26			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	166	20	80	1	23	6	79	0
Rearlimb	30	18	7	0	1	0	16	1
Head	88	11	119	1	6	0	65	2
Trunk	0	0	1	0	0	0	1	0
Mouth	12	6	6	0	12	1	9	0
FR	3	2	2	0	1	0	0	0
FH	69	13	74	0	4	1	67	14
Other	8	2	1	0	0	0	6	4

PRECEDENT EVENT	SIGMODON		DAY 26			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	216	133	64	7	29	53	105	17
Rearlimb	131	84	37	9	24	44	43	7
Head	77	36	29	3	8	6	29	2
Trunk	10	10	1	0	1	6	7	2
Mouth	29	25	11	3	38	2	5	0
FR	56	25	15	9	10	33	10	6
FH	86	64	32	5	2	9	71	12
Other	19	3	1	1	0	11	10	0

PRECEDENT EVENT	SIGMODON		DAY 27			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	131	22	43	0	19	1	42	0
Rearlimb	13	6	9	0	2	0	13	1
Head	51	7	51	0	12	0	16	1
Trunk	0	0	1	0	0	0	0	0
Mouth	26	1	12	0	17	0	2	0
FR	1	0	0	0	0	0	0	0
FH	31	8	22	1	8	0	29	1
Other	3	0	0	0	0	0	0	0

PRECEDENT EVENT	SIGMODON		DAY 27			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	151	99	38	9	28	52	55	5
Rearlimb	95	67	22	11	15	34	22	9
Head	46	19	40	2	7	11	22	2
Trunk	11	4	3	0	3	8	12	3
Mouth	40	4	8	1	30	4	3	1
FR	42	25	17	5	4	23	18	7
FH	43	49	19	14	3	3	35	16
Other	10	7	2	2	0	7	15	3

PRECEDENT EVENT	ACOMYS		DAY 26			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	515	148	33	3	2	81	31	2
Rearlimb	146	58	21	2	0	22	5	0
Head	52	11	7	0	0	10	4	0
Trunk	5	0	1	0	0	0	0	0
Mouth	1	1	0	0	0	0	0	0
FR	74	24	14	0	0	19	2	0
FH	20	13	8	1	0	1	3	0
Other	1	0	0	0	0	0	1	0

PRECEDENT EVENT	ACOMYS		DAY 26			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	415	186	131	19	34	175	171	15
Rearlimb	189	54	49	20	9	77	57	10
Head	175	33	43	8	10	47	45	3
Trunk	23	12	9	1	3	14	14	0
Mouth	55	8	6	2	26	9	7	2
FR	155	56	89	4	7	67	36	5
FH	113	111	31	20	24	27	58	9
Other	20	5	6	2	2	4	5	1

PRECEDENT EVENT	ACOMYS		DAY 28			IN UTERO		
	Fore	Rear	SUBSEQUENT EVENT			FR	FH	Other
			Head	Trunk	Mouth			
Forelimb	449	148	47	4	16	60	36	3
Rearlimb	152	102	14	3	4	53	11	2
Head	38	21	18	0	2	9	7	1
Trunk	3	3	0	2	0	0	1	0
Mouth	14	3	2	0	12	4	4	0
FR	70	40	14	0	2	22	5	1
FH	32	22	1	0	3	5	5	1
Other	4	2	0	0	0	2	0	0

PRECEDENT EVENT	ACOMYS		DAY 28			EX UTERO		
	Fore	Rear	SUBSEQUENT EVENT			FR	FH	Other
			Head	Trunk	Mouth			
Forelimb	414	224	101	26	58	371	200	89
Rearlimb	249	90	66	36	20	159	82	32
Head	154	53	25	12	12	102	30	21
Trunk	36	21	17	1	9	33	30	15
Mouth	79	29	9	3	53	38	20	10
FR	342	107	152	44	35	427	87	64
FH	136	185	24	18	36	54	82	43
Other	73	24	14	22	18	76	48	38

PRECEDENT EVENT	ACOMYS		DAY 30 SUBSEQUENT EVENT			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	330	158	21	5	2	68	38	4
Rearlimb	157	147	21	1	2	42	10	1
Head	32	22	13	0	1	9	4	0
Trunk	5	2	0	0	0	1	2	1
Mouth	3	1	2	0	2	1	1	0
FR	64	34	16	4	1	33	5	3
FH	29	19	7	1	1	3	8	1
Other	4	0	1	0	1	3	1	2

PRECEDENT EVENT	ACOMYS		DAY 30 SUBSEQUENT EVENT			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	385	208	141	15	42	277	163	61
Rearlimb	248	125	83	23	27	164	67	36
Head	156	73	37	14	16	91	77	15
Trunk	11	15	17	0	3	20	22	19
Mouth	63	25	12	1	39	35	16	5
FR	258	123	132	15	36	231	53	50
FH	103	177	31	18	20	34	56	51
Other	69	28	27	21	12	44	36	20

PRECEDENT EVENT	ACOMYS		DAY 32			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	396	158	52	1	8	46	39	3
Rearlimb	159	113	20	1	1	27	12	1
Head	58	19	9	0	1	7	4	2
Trunk	5	1	0	0	0	0	0	0
Mouth	10	1	0	0	3	0	1	0
FR	43	27	10	1	0	23	1	2
FH	29	13	8	3	2	2	9	1
Other	5	2	0	0	0	2	0	1

PRECEDENT EVENT	ACOMYS		DAY 32			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	416	264	96	6	67	262	137	43
Rearlimb	243	216	72	18	17	168	60	31
Head	136	58	37	4	16	72	27	19
Trunk	12	12	9	2	8	13	19	9
Mouth	88	23	9	4	61	29	16	3
FR	248	100	101	24	32	260	52	45
FH	81	124	31	19	28	26	58	27
Other	65	27	15	7	4	33	26	22

PRECEDENT EVENT	ACOMYS		DAY 34			IN UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	76	47	21	0	1	13	12	1
Rearlimb	48	44	6	1	1	13	4	2
Head	16	12	10	0	2	2	4	0
Trunk	0	0	0	0	0	0	1	0
Mouth	1	2	0	0	5	0	0	0
FR	14	5	8	0	0	5	1	0
FH	13	9	0	0	0	0	2	0
Other	2	0	0	0	0	0	1	0

PRECEDENT EVENT	ACOMYS		DAY 34			EX UTERO		
	Fore	Rear	Head	Trunk	Mouth	FR	FH	Other
Forelimb	165	97	79	7	38	54	44	18
Rearlimb	118	111	58	6	29	45	47	20
Head	81	55	76	6	35	36	61	17
Trunk	8	6	10	0	2	5	10	6
Mouth	43	27	34	2	164	14	6	2
FR	34	32	59	10	8	40	18	7
FH	36	83	37	12	13	4	46	20
Other	16	22	13	5	4	10	20	7

PRECEDENT EVENT	ACOMYS		DAY 37			IN UTERO		
	SUBSEQUENT EVENT					FR	FH	Other
	Fore	Rear	Head	Trunk	Mouth			
Forelimb	16	14	12	0	6	8	14	3
Rearlimb	18	16	11	2	0	9	10	1
Head	13	8	9	0	3	8	10	3
Trunk	1	1	1	0	0	0	0	0
Mouth	5	1	2	1	10	1	4	0
FR	12	8	6	0	1	3	1	2
FH	7	17	11	0	3	1	12	2
Other	3	3	2	0	0	2	2	1

PRECEDENT EVENT	ACOMYS		DAY 37			EX UTERO		
	SUBSEQUENT EVENT					FR	FH	Other
	Fore	Rear	Head	Trunk	Mouth			
Forelimb	145	58	60	1	14	88	78	28
Rearlimb	62	58	53	8	9	65	46	16
Head	59	51	97	0	14	54	83	27
Trunk	3	4	5	0	2	6	8	1
Mouth	18	12	13	1	45	7	5	2
FR	99	23	76	9	10	214	39	41
FH	53	98	50	6	8	23	116	61
Other	33	10	31	4	0	56	42	33
