Abstract
Explaining the emergence of behavioral organization and functional action patterns during ontogeny represents a challenge for developmental science. Using interlimb coordination in the fetal and neonatal rat as a model behavior, this chapter reviews central mechanisms and sensory regulation of spontaneous limb movement, pharmacological induction of locomotor-like behavior, motor learning and memory, and environmental factors that contribute to the construction of organized motor behavior during perinatal development. Recent experiments indicate that action systems in the fetus emerge under the joint influence of neural resources, biomechanical constraints, proprioceptive feedback, and contingencies posed by the intrauterine environment. This research suggests that experience accruing from feedback about motor performance may play a significant role in the perinatal construction of motor behavior.

Keywords: behavioral organization, fetus, newborn, ontogeny, locomotion, interlimb coordination, motor performance, limb movement, motor learning, L-DOPA, Quipazine

Introduction
Fetal and neonatal rats move and are capable of expressing behavioral organization immediately before and after birth. Yet functional patterns of motor coordination represent a challenge to the motor system, whereby different parts of the animal must move in rather specific spatial and temporal relationships to each other. Although motor activity expressed by the fetus often appears random, uncoordinated, and immature, quantitative approaches to examining motor behavior in the fetus have shown that fetal animals exhibit considerable movement organization, including examples of interlimb coordination, before birth (e.g., Kleven, Lane, & Robinson, 2004; Robinson & Smotherman, 1987; Smotherman & Robinson, 1987).

How the developing central nervous system (CNS) proceeds from initially expressing only spontaneous movement to shortly thereafter expressing functional patterns of motor behavior remains unresolved. Furthermore, how the developing animal continues to express functional patterns of movement with a continuously changing CNS and growing body is a remarkable challenge. This developmental challenge in motor control has been called the “calibration problem,” implying that the developing animal must continually modify or recalibrate its motor execution strategy to satisfy the demands of the biomechanical and/or
environmental context (Robinson & Kleven, 2005). Indeed, evidence is mounting that the developing animal can make use of sensory and movement-related feedback acquired during spontaneous movement to shape functional action patterns (Petersson, Waldenström, Fähraeus, & Schouenborg, 2003; Robinson, 2005; Robinson & Kleven, 2005; also see Khazipov & Buzsáki; Schouenborg; Chapter 20).

Understanding how coordinated movement is expressed and develops during the perinatal period requires characterization of the multiple factors that contribute to motor control: neural resources that must exist to generate organized movement, interactions between different parts of the animal—neural and biomechanical—that constrain and shape movement, and interactions between the animal and the environment in which movement is expressed. This multifactorial approach is complex, but essential for understanding perinatal motor development because these immature animals must maintain stability of motor performance in the face of dramatic changes in body and environment during the perinatal period (i.e., solving the calibration problem). From this approach, several tenets emerge: (1) much of fetal and neonatal behavior is continuous, (2) there are shared mechanisms of behavior during the perinatal period, (3) the developing motor system depends on sensory input to permit stable motor performance, and (4) contingencies in the environment contribute to perinatal behavioral development.

In this chapter, we review empirical evidence on the origins and determinants of motor coordination in the rat. We begin with a brief review of experimental demonstrations of action patterns in the fetus, followed by discussion of the mechanisms of motor coordination in the fetus and the newborn. Finally, we discuss evidence relating to the significance of early experience, plasticity, and interaction with the environment on motor behavior during perinatal development. We hope to promote a broader recognition of the multifactorial nature of motor behavior in the perinatal rat and highlight the role that the organism plays in influencing its own behavioral development.

Perinatal Behavioral Continuity: Moving Beyond the Fetal Freak Show

Anecdotes and descriptions of fetal behavior, obtained by opportunistic observations of human fetuses from prematurely terminated pregnancies (Hooker, 1952), in vivo preparations of animal fetuses (e.g., Angulo y Gonzalez, 1932; Narayanan, Fox, & Hamburger, 1971; Smotherman & Robinson, 1986), and most recently from real-time and 4D ultrasonic imaging of human fetuses (e.g., de Vries & Fong, 2006; de Vries, Visser & Prechtl, 1982; Kurjak et al., 2004), have provided important demonstrations of the ability of animal and human fetuses to express organized patterns of motor behavior. Such descriptive evidence has been instrumental in documenting that many patterns of functional postnatal behavior have their roots in the prenatal period. Developmental continuity between prenatal and postnatal behavior is a fact too often ignored in facile nativist interpretations of infant behavior (e.g., Marcus, 2001; Spelke & Newport, 1998). As a developmental concept, transnatal continuity is inherent in the principles of forward reference (Cognell, 1929) and anticipatory development (Carmichael, 1954; Oppenheim, 1981), which emphasize that behavior, like other functional systems, must begin to develop well before the behavior is needed to function. For example, developmental psychologists take for granted the expression of functional elements of sucking behavior (nipple search and attachment, organized sucking, and milk ingestion) by newborn infants. But the sudden expression of organized sucking behavior within minutes of birth does not imply instantaneous development or expression of a latent capacity constructed by genes; all these elements of sucking behavior are expressed before birth (Robinson et al., 1992; Robinson & Smotherman, 1992a; Smotherman & Robinson, 1992a) and can be evoked by stimuli available to the fetus in utero (Hepper, Wells & Lynch, 2005; Korthank & Robinson, 1998; Myowa-Yamakosho & Takeshita, 2006; Ross & Nijland, 1998). Behavior is not a trivial aspect of prenatal life, nor is the prenatal period inconsequential for the expression of functional behavior after birth.

Mammalian fetuses are born after a period of physiological dependency on the mother, and because the fetus's life support derives from the placental connection to the mother's uterus, researchers face a significant challenge to gain experimental access to fetal subjects for behavioral study. Thus, in nearly all cases, proper experimental designs are feasible only with the use of nonhuman animals. The principal animal models in current use to study fetal development involve large domestic animals, such as sheep or pigs, which can support extensive chronic instrumentation and longitudinal research designs (Moore & Hanson, 1992),
or laboratory rodents, such as rats and mice, that can be prepared for direct behavioral observation and permit efficient collection of statistical samples in cross-sectional experimental designs (Brumley & Robinson, 2005; Kleven & Ronca, 2009; Robinson & Smotherman, 1992b).

Our laboratories have concentrated attention on the rat fetus as a robust model of prenatal behavioral development. Experimental access to the rat fetus is provided by methods that permit exteriorization of the uterus and fetal subjects. The pregnant rat is prepared by chemical or pharmacological blockade of the spinal cord at the high lumbar level, obviating the need for general maternal anesthesia, which suppresses fetal activity (Smotherman & Robinson, 1991a). When immersed in a physiological saline bath at maternal body temperature (37.5°C), this maternal preparation provides clear visual and physical access to individual fetuses. Fetal rats can be observed through the semitransparent wall of the uterus (in utero), after delivery from the uterus into the saline bath while remaining within the amniotic sac (in amnion), or after removal of the amniotic and chorionic membranes that surround the fetus (ex utero). Each condition of observation presents different advantages: preparation in utero most closely approximates environmental conditions during normal pregnancy, while preparation in amnion and ex utero provide progressively clearer visualization and more direct experimental access to individual fetal subjects. These methods permit test sessions lasting up to 2 h that may include video recording of fetal motor behavior, experimental presentation of chemical or tactile stimuli, biomechanical constraint of movement, or manipulation of CNS function by peripheral or central drug administration, surgical lesion, or transection of the brainstem or spinal cord. Developmental changes in fetal behavior are measured by sampling from different pregnancies at different gestational ages from the inception of fetal movement on E16 (embryonic age 16 days postconception) through term (E22).

The advent of methods for studying the behavior of the exteriorized rodent fetus has provided a window on fetal development that has revealed an extensive repertoire of behavior that can be expressed before birth. Fetuses of all mammalian species exhibit spontaneous movement, but much of this motor activity superficially appears random, uncoordinated, and purposeless (Hamburger, 1973). However, application of statistical methods for quantifying temporal patterning (Kleven et al., 2004; Robertson & Bacher, 1995; Robertson, Dierker, Sorokin, & Rosen, 1982; Robertson & Smotherman, 1992c), detailed video analysis of movement patterns (Bradley, Solanki, & Zhao, 2005; Chambers et al., 1995; Johnston & Bekoff, 1999; Robinson & Smotherman, 1991a; Watson & Bekoff, 1990), and experimental presentation of ecologically relevant sensory stimuli (Robinson & Smotherman, 1992b; Robinson et al., 1992; Ronca & Alberts, 1995) has uncovered behavioral organization that was not recognized in classic studies of fetal development (Carniello, 1954; Hamburger, 1963). Some of these behavioral attributes are apparently unique to the fetus and may represent ontogenetic adaptations that promote survival and healthy development before birth (Oppenheim, 1982; Smotherman & Robinson, 1990). But most examples of fetal behavior—including simple reflexes, body orientation and posture, and components of locomotion, grooming, suckling, ingestive behavior, and species-typical reactions to appetitive and aversive stimuli—foreshadow functional action patterns of the infant or the adult. Many of the behavioral capacities that have been documented in this descriptive phase of behavioral research on the rat fetus have been the subject of previous reviews (Alberts & Ronca, 1996; Robinson & Brumley, 2005; Robinson & Smotherman, 1992d).

Basic identification of the range of phenomena to be explained is a necessary first step in any area of scientific inquiry, and characterization of the behavioral capacities of mammalian fetuses has been crucial to our beginning to understand principles of perinatal behavioral development. However, demonstration that a behavior pattern can be expressed by the fetus does not explain how that behavior develops before birth. Much of the attraction of classic and more recent descriptions of fetal behavior is the carnival-like novelty of revealing that a particular behavior can be expressed at such an early age. There is little doubt that such novelty can help draw attention to the theoretical implications of behavioral development before birth. But in themselves, mere demonstrations of behavioral expression in the fetus represent little more than a fetal freak show.

The existence of fetal behavior raises questions, generates hypotheses, and poses challenges for research, but it does not in itself provide an explanation for the development of behavior before birth. For instance, close examination of the expression of action patterns in immature animals is often fluid and variable, fragmented or incomplete, and far
less coordinated or stereotyped than the same or similar action patterns expressed by the adult. Oral grasping of an artificial nipple in the rat fetus is not the same as functional suckling by the neonatal rat; facial wiping by the fetus is not identical to grooming by the adult rat; and stepping evoked by dopaminergic or serotonergic agonists is not isomorphic with walking locomotion. To move beyond simple descriptions and demonstration proofs of fetal competence, it is essential that investigators apply an experimental approach to study the determinants of perinatal behavioral development. Understanding how these early behavioral forms are related to and contribute to the development of functional adult behavior remains a largely unrealized challenge for future research.

**Multiple Determinants of Action Systems in the Perinatal Rat**

As in other aspects of fetal behavior, demonstration proofs of behavioral capacities before birth have been important for understanding the developmental trajectory of organized action. Their main contribution has been the identification of the age at which a measure of coordinated movement or an action pattern that resembles mature behavior can first be recognized to occur in the immature animal. Such demonstrations also indicate the developmental status of perceptual, motor, and/or neural systems that are necessary to initiate, coordinate, orient, and regulate organized action. With this information as a foundation, the broader field of fetal research can begin to turn its attention to identifying and explaining the causal mechanisms of behavioral development.

Here we offer a process-oriented developmental framework for understanding the development of action systems in the rat that is based on recognizing the multiple determinants of influence on motor performance. With this approach, we can move beyond mere demonstration proofs of behavioral capacities and begin to (1) provide a more mechanism-based and multifactorial explanation for the development of action systems and (2) reveal the role that experience and plasticity plays in shaping motor performance during perinatal behavioral development. This approach entails recognizing that motor organization is more loosely structured in the fetus and neonate and that the organism plays an active role in the development of coordinated movement. It also requires understanding the relationships among local neural resources, propriospinal interactions, kinesthetic feedback, biomechanical constraints, and environmental contingencies that the organism encounters during development and the expression of movement. Much of our effort to date has focused on interlimb coordination as a model system for addressing general problems in the development of action systems.

**Endogenous Rhythmic Pattern Generators and Local Neural Mechanisms**

In the rat, rudimentary forms of interlimb coordination are apparent during spontaneous motor activity expressed during the fetal period. Spontaneous movements are first expressed by the rat fetus on E16 and continue to occur at different rates and in different parts of the body throughout the prenatal and early postnatal periods (Corner, 1977; Gramsbergen, Schwartz, & Prechtl, 1970; Robinson, Blumberg, Lane, & Kreber, 2000; Seelke, Karlsson, Gall, & Blumberg, 2005; see Chapter 20). Early descriptions of fetal movement noted the nearly simultaneous movement of different parts of the body, variously referred to as “regional,” “complex,” or “synchronous” motor activity (Narayan et al., 1971; Robinson & Smotherman, 1988; Smotherman & Robinson, 1986). Kleven et al. (2004) provided more quantitative measurement of the occurrence of synchronous limb movements during the period of fetal motility, from the inception of spontaneous movement to birth (E16–E22). At the inception of spontaneous movement, synchronous limb movements or interlimb synchrony was at chance levels for all limb pairs. Between E18 and E20, the occurrence of synchronous forelimb movements increased; thus, the forelimbs became more strongly coupled over this period. Synchrony profiles for the hindlimbs were significantly different from chance at E19, with the hindlimbs becoming more tightly coupled at E20. Intersegmental synchrony began to exhibit tight interlimb coupling at E20 as well. Thus, interlimb synchrony reveals that motor coordination may not be present when the rat fetus expresses its first spontaneous movements, but shows steady improvement as interlimb coupling increases during the late prenatal period (Kleven et al., 2004; Robinson & Smotherman, 1987).

From decades of research examining the neural mechanisms of spontaneous movement in various vertebrate species, it is clear that mechanisms within the spinal cord are a major determinant in initiating, modulating, and organizing spontaneous motor activity during the perinatal period. For
instance, spontaneous movements in the embryonic chick are correlated with activity in the spinal cord (Provine, 1972), which continues to be expressed even after all afferent feedback has been eliminated (Narayanan & Malloy, 1974a, 1974b). In the rat fetus, spontaneous movement continues to be expressed and organized into multilimb bouts following a high cervical spinal transection (Robinson et al., 2000). Following a midthoracic spinal transection, both the forelimbs and hindlimbs continue to express spontaneous movement (Blumberg & Lucas, 1994), with activity continuing to show cyclic organization (Robertson & Smotherman, 1990). Thus, there is ample evidence showing that the earliest movements and forms of motor organization expressed by perinatal animals are generated by local mechanisms within the spinal cord.

In addition to its role in generating spontaneous movement, the spinal cord also houses the basic neural circuitry involved in the production of locomotion. In the in vitro spinal cord preparation, the spinal cord is extracted from the animal's body and pinned down in a bath chamber. Typically, all nerve processes are cut and recording electrodes are placed on select ventral roots (VRs). Variants of this preparation include leaving the brain stem intact with the spinal cord, leaving some nerve processes intact, and leaving the hindleg(s) attached to the spinal cord. Bath application of pharmacological agents, such as monoamines and excitatory amino acids, is effective in evoking locomotor-like activity in this preparation. Limb muscle, motor neuron, or VR recordings from the hindleg nerves show that the pattern of electrical activity induced by these agents is similar to that during locomotion—mainly extensor and flexor alternation on one side of the cord coupled with alternation on the contralateral side. Because the activity of the spinal cord generally corresponds to the pattern of hindleg activity during locomotion, alternation of motor neuron or VR activity between sides of the spinal cord is often referred to as "fictive locomotion.”

Fictive locomotion can be induced in the developing rat spinal cord. Bath application of NMDA, 5-hydroxytryptamine (5-HT), acetylcholine, or dopamine to the in vitro perinatal spinal cord evokes alternated activity in contralateral hindlimb VRs and muscles of the hindlimbs (Asutara, Abraham, Iwahara, Garcia-Rill, & Skinner, 1991; Cazalets, Grillner, Menard, Crémeaux, & Clarac, 1990; Cazalets, Sqaill-Houssaini, & Clarac, 1992; Cowley & Schmidt, 1994; Iizuka, Nishimaru, & Kudo, 1998; Kiehn & Kjaerulf, 1996; Kudo & Yamada, 1987; Smith, Feldman, & Schmidt, 1988; see also Chapter 10). These substances, however, do not evoke identical patterns of activity, indicating that the spinal mechanisms involved in locomotion can be modulated by the action of different neuroactive drugs. Recently, interneurons within specific spinal segments that mediate fictive locomotion of the hindlimbs have been identified in the isolated spinal cord of the neonatal rat in vitro (for review, see Kiehn & Butt, 2003).

Experiments with the isolated spinal cord of the rat fetus have shown that spinal locomotor networks undergo substantial developmental transitions, both in motor pattern output and in intrinsic properties, shortly before birth (Kudo, Nishimaru, & Nakayama, 2004). For instance, between E14.5 and E16.5, 5-HT induces rhythmic synchronization of extensor-dominant and flexor-dominant lumbar VRs (Iizuka et al., 1998; Nakayama, Nishimaru, & Kudo, 2001). The 5-HT pattern of VR discharge on E17 is highly variable (Nishimaru & Kudo, 2000). On E18.5, 5-HT induces alternation between the right and left hindlegs (Gree, Smith, & Feldman, 1992), although flexors and extensors are active in the same leg (Iizuka et al., 1998). By E20.5, 5-HT induces the fictive locomotor pattern (Iizuka et al., 1998). Coordinative changes in VR activity (from synchrony to alternation) are seen at the same ages during NMDA-induced VR activity as well (Ozaki, Yamada, Iizuka, Nishimaru, & Kudo, 1996).

Interestingly, there also is a switch from GABAergic to glycinergic inhibition during the prenatal period, with GABAergic synaptic transmission being prevalent in the spinal cord on E17/E18 and glycinergic transmission becoming more common during the neonatal period (Gao, Stricker, & Ziskind-Conhaim, 2001). During 5-HT-induced motor activity, glycine is an excitatory transmitter at E14.5/E15.5 (Nakayama et al., 2001). After E17.5/E18.5, glutamate becomes the main excitatory transmitter and glycine becomes the main inhibitory neurotransmitter during 5-HT-induced rhythmicity. Results from experiments that applied glycine receptor antagonists during 5-HT or NMDA-induced fictive locomotion suggest that glycine-mediated inhibition is responsible for changes in the pattern of motor output from synchrony on E16.5 to alternation on E20.5 (Iizuka et al., 1998; Nakayama et al., 2001; Ozaki et al., 1996).
In summary, experiments with the in vitro spinal cord preparation have shown that mechanisms that generate and coordinate locomotor-like activity are present and functional during the late fetal and early neonatal period in the rat (Clarac, Brocard, & Vinay, 2004). For a more comprehensive review of neural mechanisms involved in the development of locomotion, see Chapter 10.

**Locomotor-like Behavior of the Perinatal Rat**

Although the basic mechanisms for locomotion may be present at birth in the rat, behavioral experiments using whole animal preparations, such as the in vivo rat fetus and newborn, suggest that locomotor coordination is not crystallized in the spinal cord at this time. Because rats do not typically exhibit spontaneous episodes of quadrupedal walking until approximately 2 weeks postnatal (due to weak muscle strength and poor postural control), locomotor behavior is assessed in these young subjects in vivo by experimentally evoking locomotor-like patterns of limb coordination called "stepping" behavior.

Alternated stepping behavior is occasionally expressed during spontaneous motor activity in the near-term fetus (Bekoff & Lau, 1980). However, stepping can be reliably evoked in the newborn rat using pharmacological agents (i.e., serotonergic or catecholaminergic drugs) or strong sensory stimulation such as a tail-pinch (Norreel, Pfieger, Pearlstein, Simeoni-Alias, Clarac, & Vinay, 2003), placement of the pup on a cold surface (Altman & Sudarshan, 1975), or exposure to the scent of bedding and nesting materials (Fady, Jamon, & Clarac, 1998; Jamon & Clarac, 1998).

Presumably, pharmacological stimulation of neurotransmitter systems and forms of sensory-induced stepping in the immature rat either directly or indirectly impinge on spinal locomotor mechanisms. In the case of stepping behavior induced by serotonergic agonists, such as quipazine, it appears that 5-HT receptors directly activate spinal locomotor networks. Thus, a midthoracic spinal cord transection, which effectively eliminates communication between the brain and caudal levels of the spinal cord, does not eliminate quipazine-induced hindlimb stepping in the E20 rat fetus (Brumley & Robinson, 2005). This finding suggests that local 5-HT receptors in the spinal cord mediate 5-HT-induced stepping behavior in the young rat (Brumley & Robinson, 2005; McEwen, Van Hartesveldt, & Stehouwer, 1997).

Using quipazine to elicit stepping in fetal and newborn rats, we found that hindlimb stepping increased and coordination improved from E20 to P1 (postnatal day 1; 24 h after birth), though not in a strictly linear fashion (Figure 9.1). Stepping behavior also can be induced in perinatal rats by administration of the catecholamine precursor L-DOPA (Heberling & Robinson, 1997; McEwen, Stehouwer, & Van Hartesveldt, 1994; Van Hartesveldt, Sickles, Porter, & Stehouwer, 1991). Limb coordination during L-DOPA-induced stepping also is highly variable in E20 and E21 fetuses, but by P0 (day of birth), the stepping pattern is more constrained and rhythmically alternating (Robinson & Kleven, 2005). It should be noted that unlike 5-HT, which acts more directly at the level of spinal locomotor mechanisms, the effects of L-DOPA on stepping appear to depend on descending pathways from midbrain locomotor regions (McEwen et al., 1997).

The findings from 5-HT and L-DOPA-induced stepping provide convergent evidence that interlimb coordination during locomotor-like behavior is relatively immature and unstable during the perinatal period, but does exhibit developmental change (i.e., improvement). Such change is likely due to both central and peripheral influences on central locomotor generating circuitry. In conjunction with the results of in vitro spinal cord preparations and studies of spontaneous motor activity, studies of evoked locomotor-like activity suggest that the earliest forms of motor coordination are generated and regulated by the spinal cord, are expressed by the fetus and newborn, and show developmental change during both the prenatal and postnatal periods.

**Proprioception and Interlimb Dynamics**

One of the implications of the effectiveness of pharmacological probes, such as L-DOPA and 5-HT agonists, to activate locomotor behavior in perinatal animals is that local spinal networks are not completely autonomous, but are influenced by communication between different levels of the spinal cord and between the spinal cord and the brain. Accordingly, results from both behavioral and neurophysiological experiments, which involved performing surgical transection of the spinal cord, indicate that propriospinal projections influence interlimb coordination during spontaneous and locomotor activity. For instance, Robinson et al. (2000) showed that spontaneous limb activity in fetal and neonatal rats is organized into multilimb
bouts, whereby the probability of a limb movement occurring within 0.2 s of another limb movement is elevated until four limb movements have occurred. Thus, spontaneous limb activity in perinatal rats often involves reciprocal excitation among limbs coupled with a brief refractory period after each limb movement. Multilimb bout organization persists following cervical spinal transection, showing that descending influences from the brain are not necessary for this form of interlimb coordination (Robinson et al., 2000). This bout organization suggests a role for intraspinal connections as a regulating factor in the expression of spontaneous activity.

Additional evidence for functional propriospinal pathways in the perinatal rat comes from studies of fictive locomotion that have examined coordination between rostral and caudal spinal locomotor-generating circuits (e.g., Ballion, Morin, & Viau, 2001; Juvin, Simmers, & Morin, 2005). Ballion and colleagues (2001) reported coordination between forelimb (C7–T1) and hindlimb (L2–L5) VRs during 5-HT/NMA-induced rhythmic activity in the P1–P4 rat in vitro brain stem–spinal cord preparation. In this study, fictive locomotion was evoked in each region of the cord separately (cervicothoracic and thoracolumbar), albeit at different burst frequencies. When the spinal cord was left
intact, the slower rostral and faster caudal VR burst frequencies became coordinated and exhibited an intermediate burst frequency. Similarly, Robertson and Smotherman (1990) found that midthoracic spinal transection significantly slowed cyclic forelimb activity but did not slow cyclic hindlimb activity in the E20 rat fetus. Together, these studies suggest that propriospinal pathways entail ascending as well as descending influences on interlimb coordination in the perinatal rat.

Experiments with human infants and newborn rats in which an external weight has been applied to a single limb during spontaneous activity provide evidence that proprioceptive feedback influences the frequency and coordination of spontaneous movement. At 6 weeks of age, an external ankle weight attached to one leg of a human infant decreases the number of spontaneous kicks in the weighted leg but increases the number of kicks in the nonweighted leg (Thelen, Skala, & Kelso, 1987; Vaal, van Soest, & Hopkins, 2000). By 12–16 weeks of age, the kick rate of the nonweighted leg increases proportionately to the weight (25%–100% of the mass of the calf) added to the other leg (Ulrich, Ulrich, Angulo-Kinzler, & Chapman, 1997). These experiments show that human infants express compensatory changes in spontaneous kicking behavior in response to bilateral asymmetries in leg mass and suggest that proprioceptive feedback may serve to regulate the quantity and quality of movement during spontaneous motor activity.

We have tested the idea that proprioceptive feedback influences expression of spontaneous limb activity during the perinatal period in an experiment in which an external weight was added to one hindlimb of PI rats (Hynes, Bromley, Oetken, & Robinson, 2003). Weights were calibrated to approximate 0%, 50%, or 100% of the average mass of a hindlimb and consisted of small lead disks attached to a strip of tape that was wrapped around the circumference of the limb at the ankle. As shown in Figure 9.2, the presence of the limb weight did not depress activity of the weighted limb. However, changes in the frequency of hindlimb movements were evident during the 30-min period during which the weight was attached. In subjects exposed to 50% or 100% limb weights, the nonweighted hindlimb showed more activity than the weighted limb (Figure 9.2B). These findings imply that neonatal rats compensate for a static load (weight) on a single limb, but as a result of this compensation, the nonweighted limb in the same girdle is also affected. Thus, limb weighting is a useful experimental perturbation of the immature motor system that has been shown to reveal compensatory changes in limb movement as well as intrinsic dynamics between limbs during spontaneous activity. Whether the spinal cord mediates homeostatic-like regulation in spontaneous activity is unclear. But the evidence from studies discussed previously on the propriospinal mediation of interlimb coordination implies this may be plausible.

Behavioral experiments with human infants and newborn rabbits provide convergent support for the idea that proprioceptive feedback also may contribute to plasticity in the development of organized action patterns. Thelen (1994) probed the plasticity of the motor system in 3-month-old human infants in a variation on the well-known conjugate reinforcement learning paradigm. In its typical application, conjugate reinforcement takes advantage of the propensity of young infants to exhibit spontaneous kicking when lying in a supine posture; this kicking behavior typically involves movement synergies resembling stepping movements, including alternation between left and right legs (Thelen, 1985; Thelen & Fisher, 1983). To evoke conjugate reinforcement, one end of a ribbon is attached to an ankle of the infant as it lies supine and the other end of the ribbon is attached to a mobile suspended overhead. Vigorous kicking of the tethered leg by the infant results in shaking of the mobile, which infants typically find reinforcing, resulting in an increase in the rate of kicking (Kraebel, Fable, & Gerhardtstein, 2004; Rovee-Collier, Morrongiello, Aron, & Kupersmidt, 1978; Rovee & Rovee, 1969). In the variation of the above experiment performed by Thelen and colleagues, an elastic yoke was used to create a physical linkage between both ankles of the infant. A ribbon then was attached to one leg and the overhead mobile in the conventional manner. In this configuration, infants learned to kick both legs in a synchronous, in-phase pattern in order to vigorously shake the mobile (Thelen, 1994).

Comparable motor plasticity has been reported in the development of locomotion in newborn rabbits. Shortly after leaving the nest around 10 days after birth (P10), rabbit neonates exhibit an alternated quadrupedal walking gait. By P20, this alternated stepping is replaced by synchronized hopping. If the rabbit is prepared with a spinal transection at P10, the alternated hindlimb coordination persists, suggesting that the emergence of in-phase
Figure 9.2 Hindlimb response of neonatal rats to a unilateral weight. The external weight was attached at the ankle with a small strip of tape and was calibrated to 50% or 100% of the average mass of the hindlimb; control subjects (0% weight) were exposed to the tape only. (A) Activity of the weighted hindlimb during and after exposure to a unilateral hindlimb weight. Points depict mean hindlimb movements per 5 min; vertical lines show S.E.M. Vertical dashed lines at 5 and 35 min indicate the times when the weight was added or removed from the hindlimb, respectively. (B) Activity of the nonweighted hindlimb expressed as a percentage of weighted hindlimb activity.

Leg movements are dependent on intact supraspinal resources in the CNS. However, the developmental outcome was influenced by daily motor training between P10 and P20, which was accomplished by attaching the hindfeet of the rabbit to the pedals of a training device. When training consisted of in-phase rotation of the pedals, the rabbits learned a hopping gait, but when training was alternated (like a bicycle), they retained the immature, alternated walking pattern (Viala, 2006; Viala, Viala, & Fayein, 1986). As the authors concluded, these findings imply that proprioceptive feedback from the hindlimbs may have a structuring effect during early development on the spinal networks involved in controlling interlimb coordination during locomotion.

Kinesthetic Feedback in Prenatal Motor Development

Neuroembryological studies of motility in chicken embryos have clearly demonstrated that prenatal movements are not simple reflexive responses to random stimulation, but are the product of spontaneous activity in the CNS (Hamburger, Wenger, & Oppenheim, 1966), especially in brachial and lumbosacral regions of the spinal cord (Landmesser & O’Donovan, 1984; Narayanan & Hamburger, 1971). Although the expression of motility in the absence of proprioception implies that sensory feedback about movement is not important in neurobehavioral development before birth (Haverkamp & Oppenheim, 1986), evidence from nonmammalian embryos is divided
in its implications for the role of experience in prenatal motor development. Classic and modern experiments in which amphibian larvae were raised in water containing an immobilizing drug found no lasting impairment in swimming behavior when the larvae were replaced in fresh water (Haverkamp & Oppenheim, 1986; Matthews & Detwiler, 1926), although these findings have been disputed by studies in which larval behavior was quantified more carefully (Fromme, 1941). In contrast, abundant evidence has emerged in recent years to support the opposite conclusion about the importance of proprioceptive feedback in early neuromotor development. Spontaneous activity of motor units (motor neurons and their associated muscle fibers) is necessary for the normal processes of cell death, synapse elimination, and restructuring of neuronal connectivity within the motor system that occur during perinatal development. Reduced neuronal activity results in sparing of supernumerary motor neurons, while externally applied stimulation accelerates the rate of cell death (Oppenheim, 1991; also see Chapter 5). Activity in the motor system also is thought to be responsible for the reduction of polysynaptic innervation of muscle fibers (Navarrete & Vrbova, 1993). Moreover, the amount of activity is not as important as the pattern of activity; bursts of electrical stimulation are more effective in promoting selective attrition of synapses than a steady rate of stimulation (Thompson, 1983). Knockout mice deficient in the gene for the trkC receptor exhibit gross motor deficits (Klein et al., 1994), which is likely due to the absence of muscle spindles at birth (Helgren et al., 1997; Hory-Lee, Russell, Lindsey & Frank, 1993; Kucera, Emnors, Walors, & Jaenisch, 1995; Snider, 1994). These findings suggest that many functional properties of the motor system are shaped by proprioceptive feedback arising from patterned motor activity during prenatal development (Thompson, 1983).

Although earlier studies suggested that avian embryos, and by logical extension mammalian fetuses, are largely unresponsive to proprioceptive stimulation (Hamburger et al., 1966; Oppenheim, 1972), more recent experimental work has provided compelling evidence that embryonic behavior is strongly influenced by proprioceptive cues. For example, hatching behavior in the chick embryo is triggered by proprioceptive stimuli generated by the posture of the neck (Bekoff & Kauer, 1984). Changes in buoyancy associated with reduced amniotic fluid volume, or experimental restraint of ankle movement, exert dramatic effects on the patterning of chick embryonic movement (Bradley, 1997, 2001; Bradley & Sebelski, 2000). Anatomical evidence suggests that muscle spindles begin to differentiate from primary myotubes within the period of fetal motility in the rat (E16–E18) and appear to be part of complete afferent circuits by E19 or E20 (Kucera, Walors, & Reichler, 1989). Electrical recording from primary afferent nerves in the fetal rat has provided more direct evidence that proprioceptors respond to changes in limb position as early as E18 (Fitzgerald, 1987), before sensory end organs are fully differentiated. These changes in neuroanatomy occur at the same time as dramatic changes in behavioral organization, including increases in motor activity (Smotherman & Robinson, 1986), expression of action patterns such as oral grasping of a nipple (Robinson et al., 1992), facial wiping (Robinson & Smotherman, 1991a), alternated stepping (Brumley & Robinson, 2005), and the capacity for motor learning (Robinson, Kleven, & Brumley, 2008).

**INTERLIMB YOKE TRAINING: A MODEL OF MOTOR LEARNING IN THE PERINATAL RAT**

The spontaneous movements expressed by the fetus and newborn rat often appear uncoordinated, yet coordinated action patterns that foreshadow functional patterns of adult behavior can be evoked by specific neurochemical or sensory stimulation. How do these action systems develop before birth, and what role may experience play in shaping their development? Although perinatal rats are less mature in their physical and neural development than 10-day-old rabbits or 3-month-old human infants, the limb training methods pioneered by Viala et al. (1986) and Thelen (1994) discussed earlier presented an experimental opportunity to evaluate plasticity in interlimb coordination in immature rats. As adapted for fetal and neonatal rodents (Robinson, 2005), the yoke training paradigm involves use of an interlimb yoke fashioned from suture thread and polyethylene tubing to create a physical linkage between two limbs. With the yoke in place, active movement by one limb results in passive movement of the yoked limb, thereby altering kinesthetic feedback and producing gradual changes in interlimb coordination during spontaneous motor activity.

In the initial demonstration of yoke training in the rat fetus, E20 fetal subjects were fitted
either with a thread that was immediately bisected (unyoked), or which remained in place for the first 30 min of a 60-min training and test session (yoked). Limb activity was scored from videotape records, taking care to record each instance of conjugate limb movement, which was defined as a movement in which both limbs initiated movement simultaneously and followed parallel spatial trajectories. Yoked subjects showed a pronounced increase in the occurrence of conjugate limb movements during the 30 min of yoke training. After the yoke was bisected with scissors, thereby removing the physical linkage between hindlimbs and marking the beginning of the test period, conjugate hindlimb movements continued to be expressed at rates well above those expressed by unyoked controls (Robinson, 2005).

Variations on this basic experimental design successfully demonstrated that E20 fetuses exhibited an increase in conjugate forelimb movements during training when both forelimbs were yoked, and an increase in conjugate movements of an ipsilateral forelimb–hindlimb pair during yoke training of ipsilateral limbs. Transnatal continuity in yoke learning also was confirmed by testing neonatal rats in a similar yoke training situation (Figure 9.3). P1 rats were suspended in a harness from a horizontal support, which allowed all four legs to move freely without the constraints imposed by contact with a surface. In this posture, yoke training of the hindlimbs resulted in a nearly identical pattern of conjugate hindlimb activity during and after training as was originally reported in E20 fetuses (Figure 9.3A). To assess the specificity of the pattern of interlimb coordination enforced during training, P1 rats also were trained when both hindfeet were attached to a rigid arm that rotated around a central pivot. This “see-saw” training device enforced an antiphase (alternated) pattern of hindlimb movement during the 30-min training period, after which both feet were removed from the training device for the remainder of the

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**Figure 9.3** Motor learning in P1 rat pups exposed to hindlimb training with different enforced patterns of interlimb coordination. (A) Conjugate hindlimb movements during and after in-phase hindlimb training (yoked) or a control condition involving unconnected loops of thread (unyoked). (B) Alternated hindlimb movements during and after antiphase hindlimb training (yoked) or an unyoked control condition. Vertical dashed lines indicate the onset and termination of exposure to the interlimb yoke (yoked subjects) or unconnected loops of thread (unyoked). Note that both training regimes resulted in significant increases in the enforced pattern of interlimb coordination, but alternated hindlimb movements during antiphase training were expressed at a much higher rate.
test session. Antiphase limb training resulted in a dramatic increase in alternated hindlimb movements in yoked pups, but no significant change in hindlimb alternation in unyoked controls (Figure 9.3B). The responsiveness of perinatal rats to different configurations and coordinative patterns of yoke training thus demonstrates that the E20 rat fetus and P1 rat pup can detect changes in proprioceptive feedback induced by the interlimb yoke, modify its interlimb coordination to adjust to specific patterns of movement constraint induced by the yoke, and continue to express changes in interlimb coordination for 15–30 min after the yoke is removed (Robinson, 2005). Notably, this type of learning appears to be mediated by feedback from spontaneous movements, and occurs without any form of explicit reinforcement.

Yoke training in the fetal and neonatal rat exhibits many of the essential features one would expect of true motor learning. (1) Changes in interlimb coordination occur gradually during the period of training and do not resemble a sudden stepwise increase as one might expect of a simple reflexive response to limb restraint (Robinson, 2005; Robinson & Klevén, 2005). (2) Trained patterns of limb activity persist at elevated levels after removal of the interlimb yoke, gradually returning to baseline levels within 20–30 min in E20 fetuses and P1 pups. Thus the effects of yoke training and removal of the yoke resemble conventional patterns of acquisition and extinction during associative learning (Robinson, 2005; Robinson et al., 2008). (3) Changes in interlimb coordination are specific to the limbs that experience yoke training. Conjugate forelimb movement does not increase when the hindlimbs are yoked, conjugate hindlimb movement does not increase when the forelimbs are yoked, and conjugate movement of left forelimb and hindlimb does not increase when the right forelimb and hindlimb are yoked (Robinson, 2005). (4) Changes in interlimb coordination are specific to the pattern of movement permitted during training. In-phase yoke training selectively promotes conjugate movements of the yoked limbs, whereas antiphase yoke training selectively promotes alternated limb movement (Marciano-Reilk, Woller, & Robinson, 2005). (5) When tested in a second training session 30 min after initial conjugate yoke training, perinatal rats show a steeper increase of conjugate hindlimb activity (savings), indicating that yoke training results in effects that are longer lasting than the overt expression of conjugate hindlimb movements (Robinson, 2005).

(6) Savings is evident in P2 subjects when exposed to a second training session 24 h after initial training on P1 (Robinson, Woller, Khetarpal, Fromm, & Brumley, 2004). Savings after a 30-min or 24-h retention interval implies a simple form of motor memory that is established by yoke training.

SPINAL MEDIATION OF INTERLIMB YOKE LEARNING

In adults, motor learning is commonly assumed to be governed by brain regions such as the cerebellum or motor cortex, which have functional connections with spinal motor networks in human infants and rabbit kittens at the ages tested by Thelen (1994) and Viala et al. (1986), but are poorly developed in the newborn rat (Clancy, Darlington & Finlay, 2001; Lakke, 1997). To determine whether supraspinal sources are necessary for perinatal yoke learning, rat fetuses were prepared for in vivo study on E20 following complete midthoracic spinal transection (T6–9) or a sham spinal treatment (Figure 9.4). In a first experiment, spinally transected fetuses showed a 50% reduction in overall hindlimb activity relative to sham-treated controls. They nevertheless exhibited a significant increase in conjugate hindlimb movements during a 30-min period of conjugate yoke training. In a second experiment, the increase in conjugate hindlimb movement during training was replicated and conjugate movements continued to be expressed at high levels after the yoke was bisected (Figure 9.4A). In both experiments, although the incidence of conjugate hindlimb movement was reduced after spinal transection, spinal subjects showed no differences in performance relative to sham controls by the end of the 30-min training period when conjugate movements were expressed as a percentage of overall hindlimb activity. These results suggest that spinal cord circuitry alone is sufficient to support both acquisition and persistence of adaptive changes in interlimb coordination induced by yoke training.

Adaptive changes in interlimb coordination after spinal transection imply that the mechanisms for acquisition of yoke motor learning are localized in the spinal cord in the perinatal rat. But is the motor memory implicated by savings that occurs 30 min to 24 h after an initial training session also mediated by local networks in the spinal cord? To address this question, E20 fetal subjects were prepared for behavioral testing in a 95-min session. Following a 5-min baseline, subjects were exposed to conjugate hindlimb yoke training (yoked) or an unyoked control condition for 30 min. Half of
the subjects in each condition received a midthoracic spinal transection 15 min after training; the other half received a sham spinal treatment. After a 10-min delay, all subjects were exposed to a second training period consisting of a 5-min baseline and 30-min exposure to the interlimb yoke (Figure 9.4B). Conjugate hindlimb movements increased during the first training period in the yoked group and among all fetuses during the second training period. However, fetuses that were yoked during both periods exhibited a more rapid increase in conjugate activity than those yoked only in the second training period. Savings was evident in spinal as well as sham subjects (Marcano-Reik & Robinson, 2004). This result confirmed that motor learning acquired during prior experience with the yoke, when the CNS was intact, could be retained and retrieved by the fetus after midthoracic spinal transection. Thus, the savings in motor learning expressed by fetuses after spinal transection implies a form of motor memory localized in the lumbosacral spinal cord.

Enhancement of conjugate limb coordination runs contrary to the bias toward alternated coordination reported for perinatal rats both in vitro (as measured by VR activity in spinal cord explants...
stimulated with 5-HT or excitatory amino acids) and in vivo (as measured by 5-HT or L-DOPA induced air-stepping). Yet both motor learning promoted by interlimb yoke training and locomotor-like behavior evoked pharmacologically can be supported by neural circuitry in the lumbosacral spinal cord alone. If motor experience influences the development of species-typical action systems, such as locomotion, one may expect motor learning to modulate, and perhaps shape the development of spinal circuits involved in generating basic patterns of locomotor coordination.

To evaluate whether yoke training could influence interlimb coordination during species-typical behavior, stepping was evoked in P1 rats immediately after 30 min of yoke training in either an in-phase (conjugate) or antiphase (alternated) pattern (Marcano-Reik et al. 2005). The two training regimes produced expected results: in-phase yoke training increased conjugate hindlimb movements, while antiphase training promoted alternated hindlimb movements. As previously discussed, administration of quipazine, a serotoninergic agonist, ordinarily is effective in evoking alternated stepping behavior in perinatal rats (Brunley & Robinson, 2005; McEwen et al. 1997). Therefore, quipazine was administered immediately after yoke training, and video recordings of hindlimb activity evoked by the 5-HT agonist were examined during reduced-speed playback to characterize interlimb coordination (Figure 9.5). Quipazine still promoted alternated stepping after yoke training in both training conditions. However, the 5-HT agonist evoked significantly more alternated steps in subjects exposed to antiphase training than subjects exposed to in-phase training (Figure 9.5A). Among subjects who received in-phase training, quipazine also promoted a significant increase in conjugate hindlimb movements, which were absent from subjects exposed to antiphase training (Figure 9.5B) (Marcano-Reik et al. 2005). Because in-phase training reduced quipazine-evoked alternated stepping and increased conjugate movements relative to both unyoked subjects and subjects that experienced antiphase training, the results of this experiment strongly suggest that kinesthetic feedback during hindlimb activity may help shape patterns of interlimb coordination in fetal and neonatal rats. More generally, the ability of a brief period of hindlimb yoke training to alter 5-HT-induced stepping implies experience-dependent plasticity in the prenatal development of central spinal networks that support basic patterns of locomotion.

![Figure 9.5 Quipazine-induced hindlimb coordination in neonatal rats after interlimb yoke training. P1 subjects received 30-min exposure to in-phase (conjugate) or antiphase (alternated) yoke hindlimb training (yoked groups) or unconnected thread loops (unyoked) immediately before IP injection of Quipazine or saline control. (A) Number of alternated hindlimb movements by pups in 15-min test session after conjugate or alternated yoke training and administration of Quipazine or saline. (B) Number of conjugate hindlimb movements in test session after conjugate or alternated yoke training and injection of Quipazine or saline. All bars depict mean hindlimb movements (alternated or conjugate) per 5 min; vertical lines show S.E.M. Note that Quipazine induced more conjugate hindlimb movements and fewer alternated movements after in-phase training.](image)

**Biomechanical Influences and Embodied Development**

Most developmental neuroscientists are accustomed to think of the primacy of the nervous system in the control and regulation of behavior. But behavior does not occur in a vacuum; it is produced by animals with a physical body moving in a physical environment. For the nervous system to produce coordinated movement, it must activate specific muscles with specific timing, which exert specific amounts of force on limb segments and other
skeletal elements in a multijointed biomechanical system. In rapidly growing animals, the mass and dimensions of body segments undergo nonlinear change, necessitating different amounts and timing of muscular force to produce the same pattern of coordinated movement. The nervous system thus must continually adjust to maintain, let alone improve, functional motor performance in the face of changing physical dimensions. This "calibration problem" is recognized by a growing number of investigators interested in motor control and development (Adolph & Avolio, 2000; Adolph & Berger, 2006; Adolph, Vereijken, & Shout, 2003; Carrier, 1996; Lam, Wolstenholme, & Yang, 2003; Myowa-Yamakoshi & Takeshita, 2006; Rieser, Pick, Ashmead, & Garing, 1995; Robinson & Kleven, 2005; Thelen, Corbetta, Kamrn, Spencer, Schneider, & Zernicke, 1993; Thelen, Fisher, & Ridley-Johnson, 1984; Van Heijst, Vos, & Bullock, 1998). But it is difficult to imagine a solution to the calibration problem without neutral plasticity and sensory feedback from actual motor performance.

The calibration problem may be most evident in the effects of changing body mass on motor development. Behavior occurs in a gravitational environment. In the womb, the fetus is immersed in amniotic fluid that provides buoyant support, which counters the effects of gravity on the motor system. But in the newborn, gravity constrains and alters motor performance. The effects of gravity on motor control and development are well illustrated by Thelen's reexamination of the stepping response of human newborns. Minutes after birth, human infants who are held upright can perform organized, alternated stepping movements that look remarkably like mature locomotion (Thelen et al., 1984). However, within a few weeks to months, infants no longer express the stepping response, and alternated stepping does not reappear until later in the first postnatal year with the emergence of cruising and walking. Neonatal stepping was well known to classic child developmentalists (e.g., McGraw, 1940) and its disappearance was traditionally explained as the suppression or "unlearning" of a primitive reflex with the maturation of cortical inhibitory mechanisms (Illingworth, 1966; Peiper, 1963). However, stepping disappears more quickly in infants with a higher body fat:mass ratio (Thelen et al., 1984), and daily stepping practice extends the time that infants continue to show the stepping response (Zelazo, Zelazo, & Kolb, 1980). Finally, Thelen et al. (1984) demonstrated that applying external weights to the legs suppressed the stepping response in 2–6-week-old infants, whereas immersing them to waist depth in water promoted the expression of stepping. Their conclusion was that early infant growth results in body mass increasing faster than muscle strength, eventually suppressing the performance of stepping until later ages. But reducing the gravitational load on the legs by providing buoyant support in water allows stepping to be expressed.

In the neonatal rat, which is born in a far less mature condition than humans, gravity severely restricts motor performance, and new action systems that function to maintain or modify body posture emerge, such as contact and vestibular righting responses (Pellis, Pellis, & Teitelbaum, 1991; Ronca & Alberts, 2000). Because behavior is expressed by whole animals, not disembodied nervous systems, behavioral performance is affected as the infant rat changes postures and encounters different effects of gravity on the motor system. Many investigators attempt to minimize the pervasive effects of gravity on motor performance by testing immature animals under conditions that free the limbs from contact with a surface. Newborn rats and mice, for instance, can perform facial wiping and grooming behavior if provided with external postural support or immersed in water (Fentress, 1978; Robinson & Smotherman, 1992d; Smotherman & Robinson, 1989). Like Thelen's case of neonatal stepping, facial wiping behavior disappears a few days after birth and then reappears late in the second postnatal week, when young rats begin to discover alternative strategies for maintaining postural support while freeing the forelimbs for paw–face contact (Smotherman & Robinson, 1989).

Stepping behavior also is expressed with much less constraint when rat pups are tested in a supine position (Bruinley & Robinson, 2005), or when suspended in a harness in a prone posture (Fady et al., 1998; Van Harreveld et al., 1991). However, the effects of gravity on limb extension and flexion are not equivalent in different postures. In a suspended, prone position, gravitational loading promotes a resting posture in which the limbs are extended away from the body; in a supine posture, the limbs assume a more flexed position near the body; and in a lateral posture, the limbs on the side facing up are drawn toward the midline of the body, whereas the limbs on the side facing down are pulled away from the midline. We have examined the possibility that spontaneous motor activity in neonatal rats varies under different conditions of gravitational load by recording spontaneous
limb movements of P0 or P1 rat pups in a prone, supine, or lateral posture (Brumley, Gregory & Robinson, 2002). Effects of posture on limb activity appeared to change during the first 24 h after birth. P0 subjects appeared insensitive to the three postural conditions. P1 subjects, however, showed almost twice as many hindlimb movements in the supine posture as in the other two postures and expressed fewer synchronized movements of both forelimbs or both hindlimbs when placed in a lateral posture (where gravity exerts differential effects on left and right limbs). Effects of initial posture also affect the specific motor patterns and efficiency of performing contact righting responses in newborn rats (Pellis et al., 1991). These findings provide evidence that limb movements during spontaneous and evoked activity in newborn rats are influenced by variations in vestibular or proprioceptive stimulation (cf., Clarac, Vinay, Cazalets, Fady & Jamon, 1998; Eilam & Smotherman, 1998; Ronca & Alberts, 2000). Furthermore, they suggest that experience with a gravitational environment affects the responsiveness of spontaneous motor activity to sensory feedback during perinatal development.

Could variation in physical forces, such as gravity, exert similar effects on behavior of fetuses in utero? Studies of chick embryos provide clues that gravitational loading may affect spontaneous motor activity. Reduction of amniotic fluid volume, which reduces buoyant support of the embryo in the egg, results in altered movement kinematics in the E9 chick embryo (Bradley, 1997). Limb activity increased after fluid reduction, as did intralimb coordination, particularly within the wings. But interlimb coordination decreased as patterns of movement expressed by wings and legs diverged: chicks ultimately showed synchronous, in-phase activity of the wings, and alternated, antiphase coordination of the legs. All three behavioral effects of diminished buoyancy, experimentally induced on E9 of incubation, resemble normal development trends correlated with reduced amniotic fluid volume and growth of the embryo from E9 to E13 (Hamburger, Balaban, Oppenheim, & Wenger, 1965; Provine, 1980; Sharp, Ma, & Bekoff, 1999).

Physical constraint of movement, associated with body growth within the egg or uterus, also creates biomechanical limitations for embryonic and fetal movement. As noted above, the chick embryo experiences sharply reduced free space within the egg during incubation, which likely contributes to increased motor activity and altered intralimb coordination from E9 to E13 and diminished motor activity from E13 to hatching (E21) (Bradley, 2001). During the last few days of incubation, physical crowding within the egg is extreme as the embryo assumes a contorted body posture, with the head and neck tucked under one wing. Activity is sharply reduced at this stage and proprioceptive signals generated by the bending of the neck in this prehatching posture trigger the onset of hatching movements (Bekoff & Sabich, 1987).

In contrast to birds, mammalian fetuses develop within an environment that expands as growth proceeds. Nevertheless, amniotic fluid volume reaches a peak in the rat fetus on E19 and diminishes sharply thereafter (Marsh, King, & Becker, 1963; Robinson & Smotherman, 1992b). Coupled with a nearly exponential rate of body growth (doubling mass every 1.4 days), the rat fetus is faced with a dramatic reduction in free space in utero late in gestation (Figure 9.6) (Robinson & Brumley, 2005). Spontaneous motor activity in utero increases steadily until the peak of amniotic fluid volume on E19, then levels off or diminishes over the last 2–3 days of gestation (Narayan et al., 1971; Smotherman & Robinson, 1986), suggesting a suppressive effect of physical constraint similar to that seen in avian embryos. Indeed, one of the most robust effects reported on spontaneous motor activity in rodents is the difference in movement patterns when fetuses are observed in utero versus ex utero (externalized from the uterus and amniotic sac into the surrounding bath medium). Many studies agree that E20–21 rat fetuses show increased motor activity when released from intrauterine restraint (Bekoff & Lau, 1980; Narayan et al., 1971; Robinson, 1989; Ronca, Kamm, Thelen, & Alberts, 1994; Smotherman, Richards, & Robinson, 1984; Smotherman & Robinson, 1986). Movements also become more temporally clustered in bouts, with briefer intervals between successive movement events (Robinson & Smotherman, 1988; Ronca et al., 1994), and movement synchrony and overall movement diversity increases ex utero (Robinson, 1989; Ronca et al., 1994; Smotherman & Robinson, 1987). Paradoxically, some forms of behavioral organization are facilitated when fetuses remain within the amniotic sac. Facial wiping behavior, for instance, involves forelimb and head coordination that is expressed in amnion and ex utero on E20, but occurs only when fetuses remain in amnion on E19. Video motion analysis suggests that the facilitative effect of the amniotic sac is a consequence of dampening lateral head movement, which promotes paw–face contact.
during sensory-evoked wiping responses in younger fetuses. The amniotic sac thus appears to serve as scaffolding, structuring the coordination of behavior (Robinson & Smotherman, 1991a).

Several explanations have been offered for the behavioral differences between fetuses in utero and ex utero. Early observers speculated that removal from the uterus might interfere with placental function and fetal oxygenation, resulting in hypoxia ex utero (Narayan et al., 1971; Windle, Minear, Austin, & Orr, 1935). However, the behavior expressed by fetuses after externalization from the uterus does not resemble the effects of hypoxia induced by umbilical cord occlusion (Robinson & Smotherman, 1992b), and fetuses maintain stable levels of motor activity and sensory responsiveness for extended observation sessions (up to 2 h), suggesting that the enhanced behavioral organization evident ex utero is not due to general physiological impairment. Removal from the amniotic sac also eliminates fetal access to amniotic fluid and its complex assortment of chemical constituents (Ronca et al., 1994), some of which exert an influence on fetal behavior (e.g., Korthank & Robinson, 1998). But an alternative explanation is that fetuses either cannot move or inhibit movement under conditions of biomechanical restraint. It is possible that low-amplitude movements may be dampened by the restraining effects of the embryonic membranes and uterine wall, in effect filtering the expression of motor activity and preventing occurrence of fine movements. Or fetuses may detect the difference in physical restraint in utero and ex utero by cutaneous or proprioceptive sensation, and actively alter their motor behavior in the two conditions.

Additional evidence for the influence of the physical environment in utero has emerged from studies of posture and movement in human perinates. Free space in utero also diminishes sharply during the third trimester in humans (Almli, Ball, & Wheeler, 2001), which is associated with decreasing rates of fetal body movements in general (de Vries, Visser, & Prechtl, 1988; Edwards & Edwards, 1970; Roberts, Griffin, Mooney, Cooper, & Campbell, 1980), and leg movements in particular (Almli et al., 2001). Premature rupture of the amniotic sac and its concomitant reduction of amniotic fluid volume has been reported to decrease the amplitude and velocity of fetal movement (Sival, Visser, & Prechtl, 1990). Newborns exhibit dominance of flexor muscles and tightly flexed postures for weeks after birth, which has been interpreted as persistence of fetal accommodation to physical restraint during the last few months of gestation (Thelen et al., 1984).

Ultrasound assessment of human fetal posture and orientation in utero has confirmed a developmental trend toward increased limb flexion and lateraliized head position during gestation (Ververs, de Vries, van Geijn, & Hopkins, 1994; Ververs, Van Gelder-Hasker, de Vries, Hopkins, & van Geijn, 1998). Some aspects of arm flexion begin to develop as early as 12 weeks of gestation, when intrauterine space offers little restriction for fetal movement. But other aspects of arm posture (Ververs et al., 1998) and lateraliized head orientation (Hopkins, Lems, Janssen, & Butterworth, 1987) are associated with diminishing free space in utero and may be less pronounced in breech body orientation, which provides more freedom for head and hand movement than typical cephalic/vertex (head
Environmental Contingencies That Shape Movement and Task Demands

As discussed in the preceding section, the fetus develops in a physical environment surrounded by the concentric envelopes of the embryonic membranes (amnion and chorion), uterus (vascularized endometrium and muscular myometrium), and abdomen of the pregnant mother. For much of the early history of research in behavioral embryology, this physical environment was widely believed to be buffered from the continual bombardment of sensory stimuli available in the outside world. In recent decades, however, the intrauterine environment has been explored more thoroughly with recording instruments and experimental methods that have revealed a diverse sensory environment with stimuli penetrating from the outside world, which varies between pregnancies and across time within pregnancies. Foundational research on the embryology of sensory receptors and sensory regions of the brain (e.g., Bradley & Mistretta, 1975) revealed that all of the principal sensory systems begin to develop when the fetus resides in this environment, and functional sensation emerges in a lawful sequence across many species, beginning with the somatic senses and proprioception, proceeding with the chemical senses (main olfaction, vomeronasal olfaction, and gustation) and vestibular sensation, and culminating with audition and vision (Alberts, 1984; Gottlieb, 1971). In precocial species, such as sheep, primates and Guinea pigs, all of these systems begin to transduce environmental stimuli and evoke fetal responses before birth; in less mature, altricial species, including rats and mice, only for audition and vision is the onset of function postponed until after birth. Investigators concerned with motor systems have long emphasized the nonreflexogenic nature of centrally generated motor patterns in the embryo and fetus, yet it is clear that the fetus is exposed to a rich array of sensory stimuli in multiple modalities that has the potential to evoke, modulate, and shape the development of motor responses. The abilities of the fetus to detect and express organized responses to sensory stimulation, and to modify its responses as a consequence of sensory experience, have served as the major focus of experimental work in the field of prenatal behavioral research. Many of the distinctive, species-typical action patterns that can be expressed by the mammalian fetus or avian embryo were discovered during efforts to measure responsiveness to sensory stimulation (Robinson & Smotherman, 1992a; Smotherman & Robinson, 1987). This research also has revealed that fetal exposure to sensory stimuli under controlled experimental conditions can support nonassociative and associative learning, including habituation (Kisilevsky & Muir, 1991; Smotherman & Robinson, 1992a; van Heteren, Boekkooi, Jongma, & Nijhuis, 2000), familiarity, or preference learning through mere exposure (DeCasper & Spence, 1986; Hepper, 1988; Kisilevsky et al., 2003; Mennella, Jagnow, & Beauchamp, 2001; Mickley, Remmers-Roeben, Crouse, Walker, & Dengler, 2000; Smotherman, 1982a), and classical conditioning of general motor activity (Smotherman & Robinson, 1991a), as well as specific appetitive (Robinson, Arnold, Spear, & Smotherman, 1993), aversive (Kawai, Morokuma, Tomonaga, Horimoto, & Tanaka, 2004; Mickley et al., 2001; Smotherman, 1982b; Smotherman & Robinson, 1985), and physiological responses (Robinson et al., 1993). Indeed, some forms of prenatal learning can extend beyond the cesura of birth to influence sensory-mediated behavior into adulthood (Hepper, 1993; Smotherman, 1982a). Reviews are available that provide more complete summaries of research over the past 25 years on fetal sensation and learning capacities (Alberts & Ronca, 1993; Kisilevsky & Low, 1998; Lecanuet, Fifer, Krasnegor, & Smotherman, 1995; Lecanuet & Schaal, 1996; Lickliter, 2005; Robinson & Smotherman, 1991b; Schaal, 2005; Smotherman & Robinson, 1998).
The relevance of exteroceptive sensory function to the prenatal development of action systems is twofold. Methodologically, controlled sensory stimulation has made the study of coordinated action patterns possible in an experimental setting. Although descriptive studies have provided anecdotal information about species-typical action patterns in the fetus (e.g., Carmichael, 1954), detailed analysis of the causal mechanisms and development of action patterns has been greatly enhanced by the ability to evoke specific responses under controlled conditions. This is well illustrated by examples of fetal responses to appetitive and aversive chemosensory stimuli, such as the rat fetus's stretch response to milk (Robinson & Smotherman, 1992a) and facial wiping to lemon (Brumley & Robinson, 2004; Robinson & Smotherman, 1991a), respectively. The second source of relevance of prenatal sensory function is conceptual. Functional patterns of postnatal behavior are elicited and oriented with respect to sensory cues available in the environment. Although the fetus would seem to face few contingencies within the womb (cf. discussion in next section), the ability to distinguish sensory stimuli of biological relevance, express organized behavioral responses, orient motor responses relative to the spatial and temporal distribution of cues in the environment, modulate the expression, duration, and intensity of responses, and alter subsequent behavior as a result of sensory experience, all are defining characteristics of an integrated action system (Gallistel, 1980).

Much of the emphasis of research on fetal sensory competence has been devoted to understanding how stimuli in the world outside the mother may penetrate to the intrauterine environment, or how sensory stimuli may be mediated or generated by mother's own behavior and physiology. The best known examples include human fetal exposure to maternal speech sounds (DeCasper & Fifer, 1980; DeCasper & Spence, 1986; Fifer & Moon, 1995), and the parallel case of avian embryos hearing parental vocalizations in the egg (Gortlieb, 1997; Lickliter, 2005), and fetal exposure to complex olfactory and gustatory cues derived from the maternal diet (Hepper, 1988; Mennella et al., 2001; Mennella, Johnson & Beauchamp, 1995; Schaal, 2005; Schaal, Marlier, & Soussignan, 2000). Creative experimental techniques by Ronca and Alberts also have documented the range of maternal activities that may give rise to fetal cutaneous and vestibular stimulation in utero and the responsiveness of fetal rats to experimental stimuli that mimic those sensory qualities (Ronca & Alberts, 1994; Ronca, Lamkin, & Alberts, 1993). For example, pregnant rats produce rhythmic vibrational stimulation as they lick and groom their abdomen, and fetal rats are responsive to rhythmic tactile stimulation that simulates the frequency of maternal licking. Fetuses also are exposed to sources of sensory stimulation that are unique to the prenatal environment. However, the potential impact of these forms of prenatal sensory stimulation on early motor development has received comparatively little attention.

One example is the fetal response to occlusion of the umbilical cord, which results in a brief period of hypoxia. Gradual reduction of O₂ levels in experimental preparations of large animal fetuses, such as fetal sheep, or in newborn rodents results in diminished motor activity until normoxic conditions are restored (Eden & Hanson, 1987). However, acute occlusion of the umbilical cord, which can be produced experimentally by applying a microvascular clamp, evokes a dramatic but transient increase in motor activity (Smotherman & Robinson, 1988). The form and developmental context of this motor activity suggest that fetal responses to hypoxia may be functional to remove the source of accidental cord compression. In rats and other rodents, the cord compression response can be elicited only during the last few days of gestation, as fetal growth begins to reduce free space in utero, perhaps making accidental occlusion of the cord more likely (Robinson & Smotherman, 1992b). Moreover, components of the motor response are vigorous and directed away from the body, including sudden lateral flexions of the trunk, dorsiflexion of the head and neck, and repeated kicking of the hindlimbs; in some experimental cases, these movements are sufficient to dislodge the clamp from the cord. If this interpretation is correct, then the fetal response to cord occlusion may represent an ontogenic adaptation involving fetal behavior (Smotherman & Robinson, 1988). But it also is possible that the hypoxic reaction is the product of associative learning in utero. Odor cues associated with the onset of umbilical cord occlusion are effective in producing conditioned aversion to the odor (Hepper, 1991), whereas cues presented at the time of removal of the clamp and release from hypoxia result in conditioned preference for the odor (Hepper, 1993). These findings offer the intriguing possibility that fetal movements may be modified during normal development in response to naturally occurring contingencies in utero, such as...
Transient fluctuations in oxygen availability resulting from torsion or compression of the umbilical cord.

Transient episodes of hypoxia and greatly increased pressure within the uterus occur in the form of nonlabor uterine contractions, also called contractures (Nathanielsz, 1995). Coordinated epochs of myometrial activity are a normal feature of the uterine environment throughout gestation, recurring 1-4 times per hour in sheep (Jenkin & Nathanielsz, 1994) and much more frequently in rats (Fuchs, 1969). During a contracature, fetal blood pressure rises as oxygen content decreases and the fetal thorax is significantly compressed (Harding & Poore, 1984). In the fetal sheep, contractures result in changes in behavioral state variables, such as eye movements, breathing activity, and high-amplitude electrocortical activity. The rhythmic occurrence of such myometrial activity during the last trimester may contribute to the development of normal sleep states (Nathanielsz, Bailey, Poore, Thoburn, & Harding, 1980; Robertson et al., 1996). In the rat fetus, stimulation of moderate pressure changes during uterine contractions consistently evokes heart rate deceleration and increased motor activity (Ronca & Alberts, 1994). Because contractions are expressed in segments and not simultaneously throughout the uterus (Fuchs, 1969), siblings in different parts of the uterus are likely to be exposed to stimulation at different times, perhaps with different patterns or amounts of exposure that coincide with other sources of exteroceptive stimulation (e.g., from maternal activity). Yet the potential contribution of this ubiquitous feature of the prenatal sensory environment to individual differences in behavior, even among genetically similar siblings derived from the same pregnancy, has received scant attention.

A third source of cutaneous stimulation in utero (apart from maternal activity and uterine contractions) is available in animals that give birth to multiple offspring. In rats, mice, and other polytocous species, many fetuses develop within the two deeply divided horns of the duplex uterus. Because all of these fetuses express spontaneous motor activity, their movements serve as a potential source of stimulation for nearby siblings. The developmental consequences of hormonal and other chemical exchange between fetuses in utero (the vom Saal effect) has been well documented (Meisel & Ward, 1981; Ryan and Vandenberg, 2002; vom Saal & Bronson, 1978), but the potential for direct behavioral interactions between fetuses has only recently been confirmed (Brumley & Robinson, 2002). In an initial analysis, we found that fetuses occupying contiguous positions in utero expressed synchronized movements more often than fetuses in noncontiguous positions or fetuses from different pregnancies (a control for coincidence). To assess sibling interactions experimentally, E20 fetal rats adjacent to focal subjects were immobilized by intraperitoneal injection of curare (10 mg/kg). Focal fetuses (not injected) that lay between two curarized siblings showed less activity than noninjected fetuses in other uterine positions. Conversely, noninjected focal subjects between two siblings that were injected with the kappa opioid agonist USO,488, which produces a four- to five-fold increase in fetal motor activity (Andersen, Robinson, & Smotherman, 1993), showed selective increases in motor behavior. In both pharmacological manipulations, drug-induced changes in the activity of adjacent siblings resulted in disproportionate changes in hindlimb movements of noninjected focal subjects (decreases when siblings were immobile; increases when siblings were activated). In some cases, subjects altered orientation within the uterus while expressing hindlimb movements that resembled alternated stepping. It is noteworthy in this context that chick embryos also exhibit alternated stepping movements of the legs as they rotate within the egg during hatching, between episodes of simultaneous leg extension as the eggshell is cracked (Bakhus, 1974; Hamburger & Oppenheim, 1967). Real-time ultrasound recordings suggest that human fetuses may similarly employ step-like leg movements to turn to a vertex (head down) position within the uterus (Suzuki & Yamamura, 1985). The implication of these comparative observations is that coordinated hindlimb behavior, perhaps stimulated by movements or changes in orientation by adjacent fetuses, may be functional before birth as a means of adjusting orientation in utero. More generally, these findings suggest that motor activity of nearby siblings may contribute to the prenatal sensory environment and thus to the motor and sensory development of the fetus.

Significance of Experience during Perinatal Development of Action Systems

The approach to understanding perinatal behavioral development that we are advocating attempts to relate the origin and development of behavioral competencies to mechanisms of perception and action and environmental resources that may
functionally shape action systems. In the foregoing sections, we have presented evidence of (1) developmental continuity between prenatal and postnatal behavior, (2) overlapping mechanisms for different modes of action, (3) use of sensory feedback by perinatal animals to alter and learn new patterns of motor coordination, and (4) responsiveness of perinatal animals to features of the environment and the biomechanical context in which movement occurs. It is our view that consideration of all of these determinants of behavior is necessary to explain the development of action systems. Much of our discussion has focused on spontaneous and locomotor activity. In this final section, we speculate about a possible relationship between the development of these two types of behavior during perinatal ontogeny, while adopting a multifactorial approach whereby motor experience and environmental context both play significant roles in the shaping of these action systems.

In the few days before birth, spontaneous movement by the fetus changes in frequency and organization (de Vries et al., 1982, 1988; Kleven et al., 2004; Smotherman & Robinson, 1986) as the intrauterine environment changes markedly in terms of biomechanical constraints on movement (Almli et al., 2001; Robinson & Brumley, 2005). During this same period of time in which the fetus is essentially coming into more direct contact with the surrounding uterus, the neural mechanisms supporting locomotion are forming and changing in their pattern of evoked activity (Clarac et al., 2004; Kudo et al., 2004; Ozaki et al., 1996). Because there appears to be some sharing of neural resources between the mechanisms of spontaneous activity and locomotion (recall the experiment in which evoked stepping behavior was influenced by previous interlimb yoke training) (cf. Bradley et al., 2005; Bekoff, 1992), it is possible that contingencies in the fetal environment help to shape these neural resources and their behavioral expression.

Contingencies that are present during the last few days of gestation may provide conditional feedback during spontaneous fetal movement that helps to create an intrinsic bias in movement coordination (Robinson, Marciano-Reik, Brumley, & Kleven, 2006). For the fetus to move in utero, it must overcome resistance created by elasticity of the uterine myometrium. However, the force necessary to deform the uterus depends on the configuration and timing of leg extension during fetal movement. To measure the force necessary for limb extension in utero, a section of the uterus comprising uterine endometrial and myometrial tissue was collected on E20 of gestation, opened along the mesometrial border, and stretched over a 3.5 cm diameter circular frame. One or two blunt probes (with 2-mm spheres at the tips) then were pressed against the center of the myometrial sheet, separated by varying distances, simulating the effects of fetal extension of one or two limbs. As shown in Figure 9.7, when the elastic membrane was deformed simultaneously at two contiguous points (0 mm separation), the total force required was less than twice the force involved in equal deformation at just one point. But when the membrane was deformed simultaneously at two points separated by 10 mm (the approximate distance between the two hindfeet of the rat fetus at term), more than twice the force was required than at a single point (Robinson et al., 2006).

The force relationships found in our deformation experiment are not unique to uterine tissue; they are intrinsic to all elastic membranes. The contingencies thus presented to fetuses surrounded by an elastic environment suggest that fetuses may expend less energy, or achieve greater limb extension for the same work, by extending one limb at a time (independently or by or alternated extension) rather than synchronously (conjugate extension) when pushing against the uterus. If these

Figure 9.7 Contingent force required for deformation of myometrial tissue. Uterine tissue was collected on E20 of gestation. Each curve represents the force (vertical axis) necessary to deform a sample of myometrium to a specified extent (horizontal axis), when deformation occurs at one or two points on the myometrial sheet. Note that the force required when points of deformation are separated by 10 mm is more than twice that required to deform the tissue to the same extent at a single point. See text for further discussion.
contingent force relationships accurately reflect conditions in utero, the physics of moving within an elastic envelope created by the embryonic membranes, uterus, and maternal abdomen may provide an intrinsic form of reinforcement that would favor antiphase limb movements. Kinesthetic feedback gained from spontaneous movements made in such an environment therefore may serve to strengthen the developing bias of spinal circuits to exhibit alternated activity.

The suggestion that sensory feedback gained from spontaneous activity is used to help shape neural circuitry and functional behavior during ontogeny is not completely novel. In one of the best-studied examples, mallard duck embryos emerge from hatching with a predisposition to recognize and approach the source of a mallard maternal call. This predisposition was viewed by classical ethologists, such as Konrad Lorenz, as a definitive example of innate, instinctive behavior. However, Gottlieb showed in an elegant series of experiments (summarized in Gottlieb, 1997) that normal auditory perceptual development and preference for the mallard maternal call is dependent on auditory experience within the egg. Embryos that hear the mother through the shell, or other siblings vocalizing in nearby eggs, develop normally. But Gottlieb also showed that exposure to their own embryonic vocalizations in the egg is sufficient to establish the auditory bias. Thus, spontaneous vocal activity by embryos helps to tune the developing auditory system in ovo, establishing a perceptual bias that can develop when eggs are incubated in complete isolation (Gottlieb, 1997).

In the rat, Schouenborg and colleagues have discovered a similar experience-dependent pattern of development of the tail withdrawal reflex. The nociceptive withdrawal reflex, originally described by Sherrington (1910), is a fundamental mammalian action system that is governed by relatively simple neural circuitry located within the spinal cord (Schouenborg, 2002). In the newborn rat, however, application of a pain stimulus (such as heat) to a localized region of the tail results in erroneous flexion of the tail toward the stimulus in 50%-80% of trials (Waldenström, Thelin, Thimasson, Levinsson, & Schouenborg, 2003). Refinement of the tail withdrawal response occurs over the first few postnatal weeks as spontaneous tail movements, which occur during active sleep, result in localized contact of the tail with surrounding objects. By experimentally providing "incorrect" sensory feedback during tail twitches, Schouenborg and colleagues demonstrated that abnormal withdrawal responses develop, confirming that development of an efficient, functional nociceptive withdrawal response depends on specific cutaneous sensory feedback generated when different tail muscles are active (Petersson et al., 2003; Chapter 12). Examples of self-stimulation during development, such as the duckling auditory preference and nociceptive withdrawal response, highlight the active role of the organism as well as nonobvious sources of behavioral control and regulation in the developmental process of behavior construction.

In conclusion, there are multiple endogenous and exogenous factors that can influence behavioral performance in the fetus and newborn, and hence are likely to contribute to the developmental construction of perinatal action systems. This perspective is consistent with the principles of developmental systems theory (see Chapter 2), but it is not conceptually new. Many of the developmental resources that have been identified in psychobiological research in our laboratories and others were recognized as interacting participants in the developmental process by Zing Yang Kuo 75 years ago (Kuo, 1976). But such an experimental approach nevertheless is still needed to move perinatal research from "demonstration studies" of what the immature animal is capable of to explanations of the developmental process itself (Albers, 2008).

References


