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# Conjugate Limb Coordination After Experience With an Interlimb Yoke: Evidence for Motor Learning in the Rat Fetus

**ABSTRACT:** This study investigated the capacity of the E20 rat fetus to adaptively alter patterns of interlimb coordination in a prenatal model of motor learning. Fetal limb movement was manipulated with an interlimb yoke, consisting of a fine thread attached at the ankles, which created a physical linkage between two limbs. Exposure to the yoke resulted in a gradual increase in conjugate movements of the yoked limbs during a 30-min training period, which persisted after removal of the yoke. Training effects were evident when the yoke was applied to two hindlimbs, two forelimbs, or a homolateral forelimb–hindlimb pair. A savings in the rate of acquisition also was observed when fetuses experienced yoke training in a second session. These data argue that the rat fetus can respond to kinesthetic feedback resulting from variation in motor performance, which suggests that experience contributes to the development of coordinated motor behavior before birth.  
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**Keywords:** rat fetus; fetal movement; prenatal behavior; motor learning; interlimb coordination; kinesthesia

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## INTRODUCTION

A long-standing debate in developmental neuroscience concerns the role of sensory feedback in the early ontogeny of organized motor behavior. Concepts such as motor learning, motor training, practice effects, and skill development are routinely invoked to account for plasticity in motor control and coordination during childhood, adolescence, and adulthood. But it is considerably less clear to what degree, if at all, experience can influence fundamental patterns of motor behavior or the neural systems that govern motor activity during prenatal development.

Spontaneous movement by the avian or mammalian embryo has been demonstrated to produce beneficial effects on the development of mechanical elements of the motor system, such as stimulating the growth of bones, muscles, connective tissue and integument (Drachman & Sokoloff, 1966; Moessinger, 1983; Muller, 2003). Available evidence also suggests that sensory development can be profoundly influenced by exteroceptive stimulation during the prenatal period. For instance, the type, amount, temporal patterning, and developmental timing of auditory stimulation to which an avian embryo is exposed during the last days of incubation can markedly influence auditory, visual, and intersensory perception after hatching (Gottlieb, 1997; Lickliter, 1995). Ultrasound studies of human fetuses and experimental stimulation of sheep and rodent fetuses similarly have demonstrated that mammals possess functional sensory systems before birth, are responsive to auditory and chemical stimuli in utero, and can retain information from prenatal sensory experiences to modify taste, odor, and sound preferences, feeding and affiliative behavior after birth (Kisilevsky &

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Low, 1998; Lecanuet, Fifer, Krasnegor, & Smotherman, 1995). But is the fetus equally sensitive to proprioceptive and kinesthetic information, and can sensory feedback arising from the consequences of motor performance alter the coordination of fetal motor behavior?

Although the role of conventional sensory stimulation in prenatal perceptual development has been firmly established, it remains less clear what role experience may play in the prenatal development of motor systems. A number of early researchers argued that even the simplest motor acts of the embryo are shaped by the physical environment and by the sensory consequences of motor performance (Kuo, 1967). On the other hand, classic experiments in which amphibian embryos were pharmacologically immobilized without evident long-term effects on motor performance seemed to suggest that sensory feedback from motor activity was unimportant in embryonic motor development (Carmichael, 1926; Matthews & Detwiler, 1926). More recent experiments have substantially replicated these early studies and have confirmed that peripheral blockade of muscular activity with chloroform or lidocaine, which does not suppress central neural activity, apparently does not produce lasting deficits in coordinated swimming or the fine anatomy of the motor system in amphibians (Haverkamp, 1986; Haverkamp & Oppenheim, 1986). These findings generally accord well with neural transection and transplantation experiments with avian embryos, which have shown that the neural circuitry within the brachial or lumbosacral segments of the spinal cord are sufficient to generate spontaneous motor activity, even after bilateral deafferentation. Moreover, recordings of burst discharges in ventral roots of the isolated lumbosacral spinal cord—so-called fictive activity—exhibit patterning that is similar to spontaneous motor activity, which seems to preclude any involvement of sensory feedback generated by motor performance in the control of embryonic motility (Hamburger, Wenger, & Oppenheim, 1966; Narayanan & Hamburger, 1971; Narayanan & Malloy, 1974; Provine, 1972). Classic studies such as these have been instrumental in establishing a prevalent view that sensory feedback from motor performance plays little role in the earliest development of the motor system.

In contrast to the experimental attempts to deprive embryos of motor activity during a period of early development, a growing number of studies have shown that avian embryos and mammalian fetuses can detect and respond to proprioceptive stimulation. Critical features of the proprioceptive system develop in advance of birth, including cutaneous mechanoreceptors and muscle spindles (Fitzgerald, 1987; Kucera, Walro, & Reichler, 1989). Experimental reduction of buoyancy within the egg alters the form and quantity of embryonic movements in the chick (Bradley, 1997), as does external restraint of

movement at a single leg joint (Bradley & Sebelki, 2000). Fetal rats and other rodents show a similar reduction of motor activity when observed within the uterus, compared to activity expressed when fetuses are externalized into an unrestrained fluid medium (Robinson & Kleven, 2005a; Smotherman & Robinson, 1986). These experimental findings are consistent in suggesting that prenatal motor behavior can be influenced by proprioceptive stimuli, raising the theoretical possibility that motor learning may contribute to motor development before birth (Robinson & Kleven, 2005b).

The present study reports a simple experimental paradigm to study motor learning in the rat fetus. Motor learning may be defined as an improvement in the coordination of a motor pattern that occurs as a result of repeated motor performance. Motor learning has not been investigated extensively in very young animals, probably owing to the inadequacy of most motor learning paradigms when applied to the relatively simple movement patterns of infants and fetuses. However, two studies have independently suggested the effectiveness of a simple biomechanical manipulation to promote interlimb coordination during spontaneous leg movements. Using a conjugate reinforcement paradigm, Thelen (1994) attached a soft elastic strap to the ankles of 3-month-old human infants during spontaneous kicking. Kicking was reinforced by a length of ribbon that connected one leg to a mobile suspended overhead, so that every vigorous movement of the infant's leg resulted in a corresponding movement of the mobile. Infants in which the two legs were physically yoked together showed a significant increase in the coordination of the left and right legs in the X dimension (the rostral-caudal axis), which persisted after the elastic yoke between the legs was removed. Robinson and Smotherman (1994) similarly reported effects of a mechanical tether connecting the left and right hindlimbs in the E20 rat fetus. Fetuses that experienced a brief period of hindlimb coupling showed a significant increase in the expression of coordinated motor responses to sensory stimulation. The results of these studies suggest that human infants or rat fetuses may alter patterns of interlimb coordination to adjust to the modified biomechanical context created by the interlimb yoke.

In this study, a series of experiments was designed to assess the ability of the rat fetus to modify interlimb coordination in response to a biomechanical constraint of limb movement. Fetal motor behavior was manipulated during a period of training with an interlimb yoke, which physically coupled two limbs in varying combinations. Changes in interlimb coordination during and after yoke training provided evidence that fetuses were able to detect perturbations in motor performance, implying a role for functional kinesthesia during fetal motor activity. Moreover, changes in fetal limb coordination may represent

adaptive motor responses because they resulted in effective, albeit simple, movement strategies that compensated for the effects of the interlimb yoke.

## GENERAL METHODS

### Subjects

Sprague–Dawley rats (Harlan Labs) were time-mated in the animal care facilities at the University of Iowa to provide fetal subjects for behavioral study. Owing to the length of experimental sessions, only a single fetal subject was used from each pregnancy. A total of 78 fetuses served as subjects in the five experiments reported below. Female rats were housed in groups of three with a single male during a 4-day breeding period. Vaginal smears were collected daily and examined for the presence of sperm; the date of conception (E0) was defined as the first day in which sperm were detected. During housing, breeding, and gestation, rats were exposed to a 12 hr light:12 hr dark photoperiod and provided with food and water ad libitum. All animals were treated in accordance with guidelines established by the NIH (National Institutes of Health, 1986) and the International Society for Developmental Psychobiology; experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Iowa.

### Prenatal Preparation

On day 20 of gestation (E20), pregnant rats were anesthetized by placement in an animal jar suffused with ethyl ether. During general anesthesia, the spinal cord was prepared by chemomyelotomy, in which 100  $\mu$ L of 100% ethanol was injected into the spinal cord between vertebrae L1 and L2. This procedure results in irreversible chemical blockade of transmission within the spinal cord at a low thoracic level. The prepared rat then was placed in a holding device that elevated her body at a 45° angle and the uterus was exteriorized through a midline laparotomy into a warm (37.5°C) bath containing buffered physiological saline (Locke's solution). The rat and constituent fetuses were permitted to recover from ether anesthesia and acclimate to the bath environment for at least 20 min before fetal testing.

A single fetus from one uterine horn was identified as the test subject and was externalized through a small incision in the uterus into the saline bath. The embryonic membranes (chorion and amnion) were gently removed to provide experimental access to the subject, taking care to preserve the integrity of the umbilical cord and placental connection to the uterus. The condition of subject fetuses was continuously monitored during the experimental session by noting general coloration of the fetus and umbilical cord (pink indicating good oxygenation), the presence of motor activity, and the absence of stereotypic movements indicative of hypoxia. All fetuses reported in these experiments remained in good physiological condition throughout the experimental session. These methods for preparing fetal subjects for behavioral study, which have become standardized

over the past two decades, permit direct observation and experimental manipulation of fetal subjects and creation of high-quality video recordings for subsequent analysis of fetal motor behavior (Smotherman, Richards, & Robinson, 1984; Smotherman & Robinson, 1991).

### Procedures for Yoke Training

The motor learning paradigm in this study involved attachment of an interlimb yoke to two limbs to constrain limb movement during a training period. Different experiments utilized interlimb yokes that were constructed in slightly different ways, and hence differed in their properties of restraining fetal movement. The basic *thread yoke* consisted of a length of 3-0 silk suture that was tied to both feet at the level of the ankle; the suture thread prevented lengthening (tension), but did not interfere with shortening (compression) or bending (torsion). An *elastic yoke* was fabricated from elastic sewing thread in which loops at each end were slipped over the feet of the subject fetus; the elastic yoke resisted but did not prevent lengthening, and offered little resistance to shortening or bending. Finally, a *rigid yoke* also was fashioned from elastic thread, but the connecting segment was stiffened by application of cyanoacrylate adhesive. The rigid yoke thus resisted forces of tension, compression and torsion. Because all subjects were tested at the same developmental age, the length of the connecting segment of the yoke was adjusted to 8 mm, which approximated the mean distance between the ankles of an E20 fetus at rest.

The purpose of the interlimb yoke was to constrain spontaneous movements of the yoked limbs. With the yoke in place, an active movement by one limb was hindered by its connection to the passive limb. Conversely, the passive limb often was dragged to a new position by movement of the active limb. I expected that both of these consequences—hindrance of active movement and occurrence of passive movement—would alter proprioceptive feedback produced during spontaneous motor activity. In all experiments, the yoke remained attached to both limbs during a 30-min period (designated the Training period). Changes in fetal behavior were recorded continuously during the period of yoke training. Persistent effects of yoke training were assessed by dividing the connecting segment of thread at the conclusion of Training and recording fetal behavior during a subsequent 30-min period (designated the Testing period). Subjects in control conditions (Unyoked groups) were exposed to the same handling required to attach the yoke to both limbs, but the yoke was cut immediately, at the beginning of the Training period, so subjects in unyoked conditions experienced restraint of limb movement for only a few seconds.

### Analysis of Behavioral Data

To facilitate clear observation of limb movements and creation of videotape records, fetuses were maintained in a supine posture throughout the experimental session. Fetal behavior was quantified by scoring the movement of each limb, treating each movement as a point event entered into a computer using real-time event recording software. This data acquisition system preserved information about the limb involved in movement (left

vs. right, fore vs. hind), the time the movement occurred ( $\pm 0.1$  s), and whether movements involving two limbs were conjugate. Limb movements usually were expressed as independent events involving a single limb. Occasionally, however, two limbs initiated movements at the same time. Such movements were considered *conjugate* if the two limbs moved as one, following parallel trajectories with similar velocity. In practice, such conjugate limb movements (CLM) were readily distinguished from passive dragging of one limb during yoke training, because passive movement involved one limb leading and the other trailing through a movement trajectory. Further, CLM involved movement of both limbs at an angle orthogonal to the line of the interlimb yoke, whereas passive movement involved one limb moving in the same direction as the line of the yoke. In such cases, the active movement of the leading limb was scored as an individual limb movement, but the passive movement of the trailing limb was not.

The frequencies of individual and CLM were summarized in 5-min intervals across the Training and Testing periods. Forelimb activity was computed as the sum of both left and right forelimb movements. Hindlimb activity similarly was computed as the sum of both left and right hindlimb movements. In addition to absolute movement counts, the relative frequency of CLM was calculated as twice the number of conjugate movement events (because two limbs are involved in each event), divided by the total number of all hindlimb movements. Changes in limb activity were assessed by mixed model Analyses of Variance (ANOVA), with the time factor (5-min intervals) treated as a repeated measure. Following significant interaction effects, one-factor ANOVAs were performed to test for simple main effects, and post hoc comparisons of means were conducted by the method of Fisher's Protected Least Significant Difference (PLSD). The alpha level was set at  $p < .05$  for all tests of statistical significance.

## EXPERIMENT 1: YOKE TRAINING OF HINDLIMB MOVEMENTS

Previous studies of motor behavior in developing animals and human infants have suggested that physical constraint of limb movement may influence patterns of interlimb coordination. In rat fetuses, attachment of a length of thread to both hindlimbs facilitated expression of the stretch response, an action pattern that depends on coordinated extension of the hindlimbs (Robinson & Smotherman, 1994). Exposure to an elastic cord connecting the ankles of 3-month-old human infants promoted interlimb coordination when spontaneous kicking was reinforced by movement of an overhead mobile (Thelen, 1994). Repeated enforcement of in-phase or alternated limb activity also resulted in lasting changes in the pattern of interlimb coordination in spinalized rabbits during the first 3 weeks after birth (Viala, Viala, & Fayein, 1986). These studies imply that experience with a physical linkage between the limbs may have immediate and

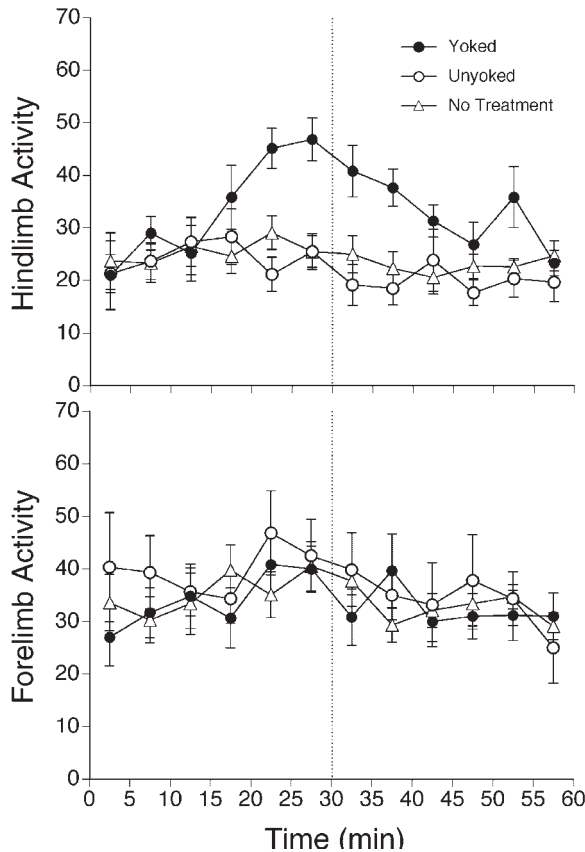
persistent effects on the patterning of interlimb coordination in fetuses and neonates. The aim of Experiment 1 was to explicitly assess the effects of an interlimb yoke on the amount of limb movement and frequency of CLM during and after a 30-min period of yoke training.

## Methods

Subjects were assigned to one of three experimental conditions ( $N = 6$  fetuses per condition). The experimental session consisted of a 30-min Training Period followed by a 30-min Testing Period. For subjects in the Yoked group, a thread yoke was attached to both hindlimbs during Training, which was cut at the beginning of Testing. The thread yoke also was tied to both hindlimbs in the Unyoked group ( $N = 6$ ), but was immediately cut (before Training). A third group received no treatment (NT) throughout the Training and Testing periods. Limb movements were scored to provide summary measures of forelimb and hindlimb activity, CLM, and relative frequency of CLM. Only active movements of a limb were scored as motor activity; passive movements resulting from a limb pulled by the interlimb yoke or swaying with the movement of water in the bath were not scored as movement events.

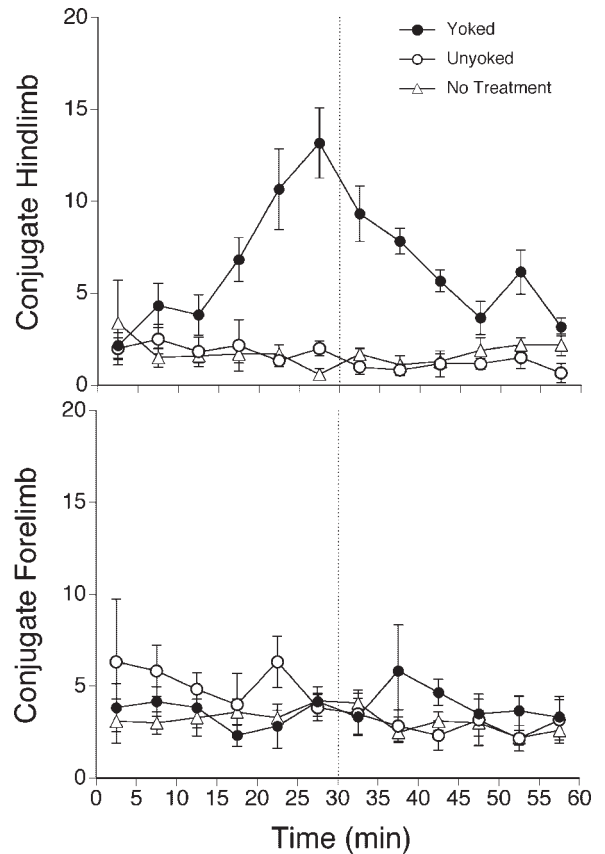
## Results and Discussion

The overall frequency of hindlimb activity was compared in a two-factor ANOVA (Conditions  $\times$  5-min Intervals), with the Intervals factor treated as a repeated measure. This analysis indicated the significant main effects of Condition,  $F(2,15) = 10.4$ ,  $p < .005$ , and Intervals,  $F(11,165) = 3.1$ ,  $p < .001$ , and the significant interaction of Conditions  $\times$  Intervals,  $F(22,165) = 1.8$ ,  $p < .05$  (Fig. 1, top). To examine the nature of this interaction, a series of one-way ANOVAs was conducted to assess the simple main effect of Conditions at each 5-min Interval. These tests revealed significant differences among the three Yoked conditions at 25 ( $p < .001$ ) and 30 min ( $p < .001$ ) during the Training period, and at 35 ( $p < .001$ ) and 40 min ( $p < .005$ ) during the Testing period. At all four intervals, subjects in the Yoked group showed elevated hindlimb activity relative to the Unyoked and NT conditions (Fisher's PLSD,  $p < .05$ ). Forelimb activity also was compared in a two-factor repeated measures ANOVA (Conditions  $\times$  5-min Intervals), which indicated no significant main or interaction effects ( $p$  values  $> .05$ ) (Fig. 1, bottom). These findings suggest that the hindlimb yoke produced a selective effect on the yoked limbs, resulting in increased hindlimb activity in the last 10 min of the Training period and the first 10 min of the Testing period, but did not alter activity of forelimbs.



**FIGURE 1** Overall limb activity of E20 fetuses exposed to interlimb yoke training in Experiment 1. In this and subsequent graphs, the vertical dotted line divides the experimental session into an initial 30-min period of yoke training and a subsequent 30-min test period. The three experimental conditions involved fetuses that were exposed to the interlimb yoke created by attaching a length of flexible suture thread to both hindlimbs during training (Yoked), fetuses exposed to a control procedure (Unyoked), and fetuses that received no yoke manipulation (no treatment). Symbols represent mean number of limb movements of hindlimbs (top panel) or forelimbs (bottom panel) in successive 5-min blocks during the training and test periods; error bars depict *SEM*.

The frequencies of CLM were compared in a two-factor ANOVA (Conditions  $\times$  5-min intervals), which indicated the significant main effects of Condition,  $F(2,15) = 95.3$ ,  $p < .001$ , and Intervals,  $F(11,165) = 5.2$ ,  $p < .001$ , and the significant interaction of Conditions  $\times$  Intervals,  $F(22,165) = 5.9$ ,  $p < .001$  (Fig. 2, top). A series of one-way ANOVAs conducted to assess simple main effects of Conditions at each 5-min Interval revealed significant differences among the three Yoked conditions at 20 min and all subsequent 5-min intervals ( $p < .05$ ). During the last half of the Training period (20–30 min), Yoked subjects exhibited more hindlimb CLM than either



**FIGURE 2** Number of conjugate limb movements (CLM) of hindlimbs (top) and forelimbs (bottom) expressed by fetuses during and after hindlimb yoke training in Experiment 1. Points show the mean number of CLM in 5-min blocks during the training and test periods for fetuses in which hindlimbs were yoked, exposed to an unyoked control procedure, or not treated during the training period; error bars depict *SEM*.

Unyoked or NT controls. Hindlimb CLM continued to be elevated in Yoked subjects relative to both control groups through 55 min, and were marginally higher than Unyoked subjects during the last 5-min of the Testing period. No differences were found between Unyoked and NT control subjects at any time. In contrast to these effects on hindlimb behavior, analysis of CLM of forelimbs indicated no significant main or interaction effects (all  $p$  values  $> .35$ ; Fig. 2, bottom).

Because overall hindlimb activity increased during yoke training, it was not clear whether the interlimb yoke promoted a disproportionate increase in CLM. To adjust for differences in hindlimb activity, hindlimb CLM also was expressed as a percentage of overall hindlimb activity. Analysis of percent CLM varied significantly as a function of Condition,  $F(2,15) = 45.2$ ,  $p < .001$ , and Intervals,  $F(11,165) = 2.0$ ,  $p < .05$ . The interaction of Condition  $\times$  Intervals also was significant,  $F(22,165) = 2.9$ ,

$p < .001$ . Tests for simple main effects of Condition at each interval indicated that the percentage of hindlimb movements that were conjugate was greater in Yoked fetuses than Unyoked or NT fetuses at 15 min ( $p < .05$ ), 20 and 25 min ( $p < .01$ ), 30, 35, 40, and 45 min ( $p < .001$ ), 50, 55, and 60 min ( $p < .05$ ). In the Yoked group, percentages increased from 18.2% in the first 5-min interval to 56.8% in the last interval of the Training period, then declined to 26.8% by the end of the Testing period (Fig. 3). Finally, a parallel analysis of the relative frequency of forelimb CLM, expressed as a percentage of overall forelimb activity, indicated no significant main or interaction effects (all  $p$  values  $> .15$ ).

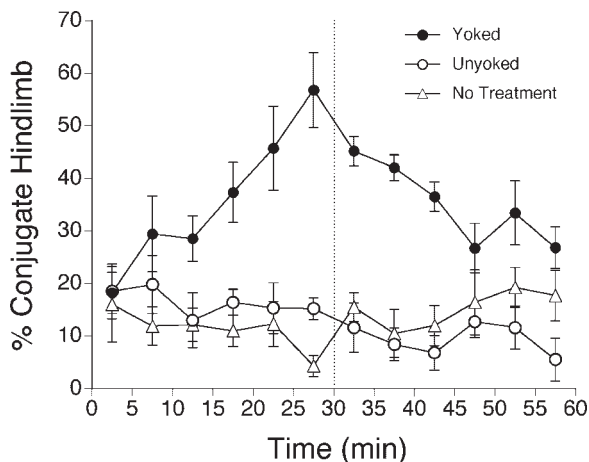
The analyses reported above demonstrated that exposure to the interlimb yoke resulted in an increase in hindlimb activity during the period of yoke training, an increase in the absolute frequency of hindlimb CLM, and an increase in CLM expressed as a percentage of overall hindlimb activity. These analyses do not resolve, however, whether nonconjugate hindlimb movements also increased as a consequence of exposure to the interlimb yoke. To evaluate this possibility, hindlimb CLM was subtracted from overall hindlimb activity in each 5-min interval of the experimental session, and the remainder compared in a 2-factor ANOVA (Conditions  $\times$  5-min intervals). This comparison indicated no significant main or interaction effects ( $p$  values  $> .15$ ), suggesting that there was no systematic change in nonconjugate hindlimb movements during or after yoke training.

The pattern of results from Experiment 1 confirmed that a 30-min exposure to an interlimb yoke that

physically linked the two hindlimbs produced an increase in conjugate hindlimb movements—even when expressed as a percentage of overall hindlimb activity—during the last 15–20 min of the Training period. Hindlimb CLM declined after Training, but continued to be expressed at elevated levels for 30 min after the interlimb yoke was cut. However, the effects of the interlimb yoke appeared to be restricted to the limbs that were connected during training; the yoke did not result in any change in general forelimb activity or forelimb CLM. Moreover, the increase in hindlimb activity appeared to be due to a selective increase in hindlimb CLM, and not to a general activation of the hindlimbs.

## EXPERIMENT 2: DIFFERENTIAL EFFECTS OF YOKE ELASTICITY

Experiment 1 demonstrated that a 30-min period of training with an interlimb yoke altered the pattern of interlimb coordination expressed by E20 rat fetuses. Specifically, CLM of the hindlimbs increased in frequency during Training and persisted at elevated levels during the subsequent 30-min Testing period. This experimental finding implies that the fetus can detect the biomechanical constraint of limb movement and express an adaptive behavioral response in the form of CLM. The interlimb yoke used in Experiment 1 consisted of ordinary suture thread, which is nonelastic and therefore would prevent movement in diverging vectors of limb motion. However, the thread is compliant to compression or torsion forces, so should permit a variety of limb movements with parallel or converging vectors. If fetuses indeed alter the coordination of their limb activity in response to constraint, then changes in the characteristics of the interlimb yoke should result in corresponding changes in the pattern of adaptive response by the fetus. The aim of Experiment 2 was to determine whether fetuses could detect and alter the pattern of behavioral response to interlimb yokes that differed in elasticity or stiffness, and which therefore varied in their resistance to forces of tension, compression or torsion. I expected that an elastic yoke, which results in less constraint on fetal movement, would be less effective in promoting CLM than a rigid yoke, which would resist both diverging and converging vectors of hindlimb movement.



**FIGURE 3** CLM of hindlimbs expressed as a percentage of overall hindlimb activity during and after hindlimb yoke training in Experiment 1. Points show the mean percentage of hindlimb movements that were conjugate in 5-min blocks during the training and test periods for fetuses in which hindlimbs were yoked, exposed to an unyoked control procedure, or not treated during the training period; error bars depict SEM.

## Method

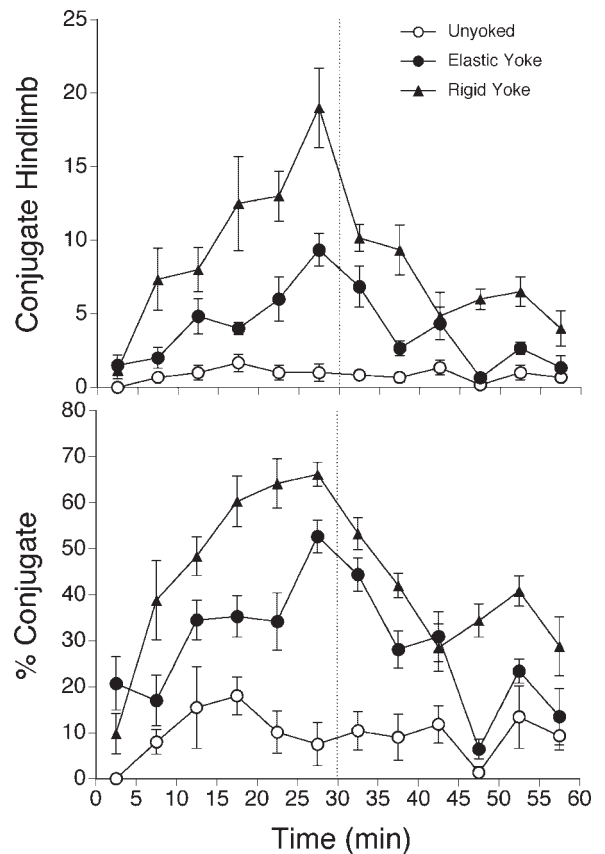
Subjects were assigned to one of three experimental Conditions ( $N = 6$  fetuses per condition). As in Experiment 1, the experimental session consisted of a 30-min Training period followed by a 30-min Testing period. An

interlimb yoke was attached to both hindlimbs during the Training period in the first two conditions. In the Elastic group, the yoke was constructed by forming a loop at both ends of a length of elastic sewing thread. In the Rigid group, the span of thread between the two elastic loops was stiffened by application of cyanoacrylate adhesive. Both the elastic and rigid yokes were attached to the subject fetus by slipping the elastic loops of the yoke over each hind foot to the level of the ankle. The resulting interlimb yokes created a gap of approximately 8 mm between the left and right hindlimbs. In the third group (Unyoked controls), the elastic loops were attached to both hindlimbs, but the connecting span of thread was immediately cut prior to the onset of Training. In the Elastic and Rigid groups, the interlimb yoke was cut after Training, at the beginning of the Testing period. Hindlimb CLM, as well as overall hindlimb activity, was scored and summed in 5-min intervals for analysis by ANOVA.

## Results and Discussion

Hindlimb activity was compared in a two-factor ANOVA (Conditions  $\times$  5-min intervals), with the Intervals factor treated as a repeated measure. This analysis indicated the significant main effect of Condition,  $F(2,15) = 24.5$ ,  $p < .001$ , and Intervals,  $F(11,165) = 7.6$ ,  $p < .001$ . The interaction of Conditions  $\times$  Intervals also was significant,  $F(22,165) = 1.6$ ,  $p < .05$ . One-way ANOVAs were conducted at each 5-min interval to test for simple main effects of Condition. The pattern of these tests revealed that hindlimb activity was elevated from 10 to 55 min (except at 45 min) in the Rigid group compared to Unyoked controls ( $p < .05$ ). Among subjects in the Elastic group, hindlimb activity increased relative to Unyoked controls only at 15 and 25 min.

Hindlimb CLM also was compared in a two-factor ANOVA, which indicated the significant main effects of Condition,  $F(2,15) = 56.9$ ,  $p < .001$ , and Intervals,  $F(11,165) = 14.7$ ,  $p < .001$ , and the significant interaction of Conditions  $\times$  Intervals,  $F(22,165) = 4.9$ ,  $p < .001$ . A series of one-way ANOVAs conducted at each 5-min interval revealed significant differences among the three conditions at 10–40 min and 50–60 min. In general, CLM by subjects exposed to the Rigid yoke condition increased earlier in the Training period, increased to higher levels during Training, and persisted later into the Testing period than among fetuses in the Elastic Yoke group (Fig. 4, top). In contrast, fetuses in the Unyoked group showed low levels of CLM throughout the Training and Testing periods. CLM in the Rigid group exceeded Unyoked controls at 10 min and all subsequent 5-min intervals (except 45 min) (Fisher PLSD,  $p < .05$ ). CLM in the Elastic group increased relative to Unyoked controls at 15 min and 25–35 min, but not thereafter. In addition,



**FIGURE 4** CLM of hindlimbs during and after hindlimb yoke training in Experiment 2. Fetuses were exposed to an interlimb yoke made of elastic thread (Elastic), thread stiffened with cyanoacrylate (Rigid), or a control procedure (Unyoked) during the 30-min training period. The graphs present the frequency of hindlimb CLM (top panel) and relative CLM expressed as a percentage of overall hindlimb activity (bottom panel). Points show the means in each 5-min block; error bars depict SEM.

more CLM was expressed by subjects in the Rigid group than the Elastic group at 10, 20–40, and 50–55 min.

To compensate for differences in hindlimb activity, CLM also was analyzed as a percentage of overall hindlimb activity. The two-factor ANOVA comparing percent CLM indicated the significant main effects of Condition,  $F(2,15) = 74.7$ ,  $p < .001$ , and Intervals,  $F(11,165) = 15.1$ ,  $p < .001$ , and the significant interaction of Conditions  $\times$  Intervals,  $F(22,165) = 4.0$ ,  $p < .001$  (Fig. 4, bottom). A series of one-way ANOVAs conducted at each 5-min interval revealed the simple main effects of Condition at all intervals except 55–60 min ( $p < .05$ ). Posthoc comparison of group means indicated that fetuses in the Rigid yoke group showed a higher percentage of CLM than Unyoked controls from 5 to 55 min, and subjects in the Elastic group exceeded Unyoked controls from 0–5 min and 10–45 min. Moreover, percent CLM

was greater in Rigid than Elastic at 10, 20–30, 40, and 50–55 min, suggesting that CLM was elevated for a greater portion of the Training period and persisted later in the Testing period in the Rigid group.

A parallel series of two-factor ANOVAs comparing the occurrence of forelimb activity, forelimb CLM or percent forelimb CLM across the three yoke conditions and 5-min intervals revealed no significant main or interaction effects involving the Condition factor (all  $p$  values  $>.10$ ). Neither the Elastic nor the Rigid interlimb yoke, when attached to the hindlimbs, appeared to influence the expression of forelimb behavior during Training or Testing.

The pattern of results from Experiment 2 confirmed that both the Rigid and Elastic yoke conditions were effective in promoting a change in hindlimb coordination during the Training period, and that high levels of CLM continued to be expressed in the Testing period, after the yoke was cut. The duration of elevated CLM differed between the two yoke conditions, persisting only 5 min in the Elastic group relative to Unyoked controls, and 30 min in the Rigid group relative to controls. Overall, the Rigid yoke was more effective than the Elastic yoke used in Experiment 2, and at least as effective as the Thread yoke used in Experiment 1, in promoting CLM. For this reason, the rigid yoke was used in all subsequent experiments of this study.

### EXPERIMENT 3: YOKE TRAINING OF FORELIMB MOVEMENTS

The first two experiments focused on CLM following attachment of an interlimb yoke to the two hindlimbs. Following a 30-min period of hindlimb yoking, hindlimb CLM increased significantly, whereas forelimb activity was not affected. These results imply that the effect of the interlimb yoke is restricted to the specific limbs that are subjected to biomechanical constraint of motion. The objective of Experiment 3 was to test the generality of this motor learning paradigm by applying an interlimb yoke to the two forelimbs. I expected that yoke training would result in an increase in the conjugate movement of forelimbs without an associated change in hindlimb activity.

#### Method

Subjects were assigned to one of two experimental conditions ( $N=6$  fetuses per Condition). An interlimb yoke was attached to both forelimbs during the Training period in the first group (Yoked). The interlimb yoke consisted of an 8 mm length of elastic thread stiffened with cyanoacrylate, as in the Rigid condition of Experiment 2. The second group consisted of Unyoked controls,

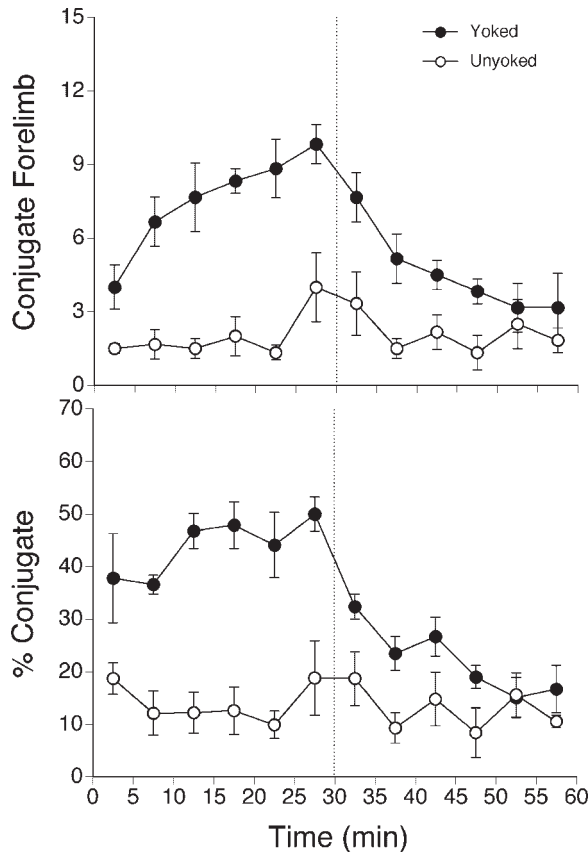
in which the elastic loops of the interlimb yoke were attached to both forelimbs at the level of the wrists, but the connecting length of thread was cut before the onset of the Training period. Subjects in both groups were observed during a 60-min session, consisting of a 30-min Training period followed by 30-min Testing period. In the Yoked group, the interlimb yoke was cut at the beginning of the Testing period. Overall activity and conjugate movements of the forelimbs as well as the hindlimbs were scored and summed in 5-min intervals for analysis by ANOVA.

### Results and Discussion

Forelimb activity was compared in a two-factor ANOVA (Conditions  $\times$  5-min Intervals), with the Intervals factor treated as a repeated measure. This analysis indicated the significant main effect of Intervals,  $F(11,110)=4.2$ ,  $p<.001$ . However, neither the main effect of Condition nor the interaction of Conditions  $\times$  Intervals was significant ( $p>.05$ ). A second ANOVA compared hindlimb activity, in which no significant main or interaction effects were found ( $p>.05$ ). The interlimb yoke thus appeared to have no influence on overall levels of forelimb or hindlimb activity.

The two-factor ANOVA comparing forelimb CLM indicated the significant main effects of Condition,  $F(1,10)=48.7$ ,  $p<.001$ , and Intervals,  $F(11,110)=5.8$ ,  $p<.001$ , and the significant interaction of Conditions  $\times$  Intervals,  $F(11,110)=3.5$ ,  $p<.001$  (Fig. 5, top). Comparison of Yoked and Unyoked groups at each 5-min Interval revealed that Yoked subjects expressed significantly more forelimb CLM than Unyoked subjects at every interval except 50–60 min ( $p<.05$ ). In contrast, the two-factor ANOVA comparing hindlimb CLM revealed no significant main or interaction effects ( $p>.05$ ). Hindlimb CLM did not differ between Yoked and Unyoked subjects at any interval.

Although there appeared to be no systematic changes in overall forelimb activity associated with yoke training, forelimb CLM also was analyzed as a percentage of overall forelimb activity. The two-factor ANOVA comparing percent conjugate indicated the significant main effects of Condition,  $F(1,10)=46.8$ ,  $p<.001$ , and Intervals,  $F(11,110)=6.5$ ,  $p<.001$ , and the significant interaction of Conditions  $\times$  intervals,  $F(11,110)=4.8$ ,  $p<.001$  (Fig. 5, bottom). A series of unpaired  $t$ -tests conducted at each 5-min interval revealed the simple main effects of Condition at all intervals from 10–40 min ( $p<.05$ ), confirming that fetuses in the Yoked group showed a higher percentage of forelimb CLM than Unyoked controls. A parallel analysis of hindlimb CLM expressed as a percentage of hindlimb activity revealed no significant main or interaction effects ( $p>.05$ ).



**FIGURE 5** CLM of forelimbs during and after forelimb yoke training in Experiment 3. Fetuses were exposed to a rigid interlimb yoke or unyoked control condition during the 30-min training period. The graphs present the frequency of forelimb CLM (top panel) and relative CLM expressed as a percentage of overall forelimb activity (bottom panel). Points show the means in each 5-min block; error bars depict *SEM*.

The results of Experiment 3 confirm the prediction that application of an interlimb yoke results in a selective increase in CLM of the yoked limbs. Forelimb CLM did not increase among Unyoked subjects, nor did hindlimb CLM increase in either yoke condition. These findings are consistent with the results of the first two experiments, in which yoke training of the hindlimbs resulted in an increase in hindlimb CLM. However, the hindlimbs in rats are more specialized for locomotion and possess fewer degrees of freedom of movement than the forelimbs, which are also used in exploration, grasping and manipulation of objects. The results of Experiment 3 are important in suggesting that the conjugate movements expressed in response to yoke training are not the result of biomechanical or neural specialization of the limbs. Experiment 3 therefore reinforces the conclusion of Experiment 2 that the increase in CLM is restricted to the two limbs that are connected by the interlimb yoke,

and is not the result of a generalized increase in motor activity evoked by biomechanical constraint of limb motion.

#### EXPERIMENT 4: YOKE TRAINING OF HOMOLATERAL LIMBS

Experiments 1–3 involved manipulation of activity of homologous limbs, within a single girdle. When the movement of both limbs within either the pectoral or pelvic girdle was constrained by an interlimb yoke, the yoked limbs exhibited a pronounced increase in conjugate movement. These results indicate that interlimb yoke training can affect intrasegmental coordination of limb activity. Many behavioral patterns (postural maintenance, locomotion, object manipulation) require interlimb coordination between homologous limbs, but not necessarily coordination between limbs in different girdles. During normal walking, for example, an alternated pattern of coordination is maintained between the forelimbs and between the hindlimbs, as well as between homolateral limbs. In other gaits, such as the half-bound, the forelimbs step alternately while the hindlimbs move synchronously in a hopping pattern. And in other patterns of behavior, the hindlimbs may provide postural support while the forelimbs engage in entirely independent movement. These basic observations of motor behavior may suggest that coordination between girdles is less rigidly controlled than within a girdle, which might limit the effects of yoke training of homolateral limbs. The purpose of Experiment 4 was to explore the limits of the yoke training paradigm by determining whether biomechanical manipulation between a homolateral forelimb-hindlimb pair could produce a similar change in intersegmental coordination as shown for homologous limbs in the first three experiments. Because only one homolateral limb pair was exposed to yoke training in each fetus, this experiment also provided a within-subject control condition to further evaluate the specificity of effects of yoke training on interlimb coordination.

#### Method

Subjects were assigned to one of two experimental conditions ( $N = 6$  fetuses per condition). An interlimb yoke was attached to one forelimb and the homolateral hindlimb during the Training period in the first condition (Yoked). The interlimb yoke was lengthened from previous experiments to accommodate the greater distance (20 mm) between forelimb and hindlimb. The second condition consisted of Unyoked controls, in which the interlimb yoke was attached to a homolateral forelimb–hindlimb pair, but the connecting length of

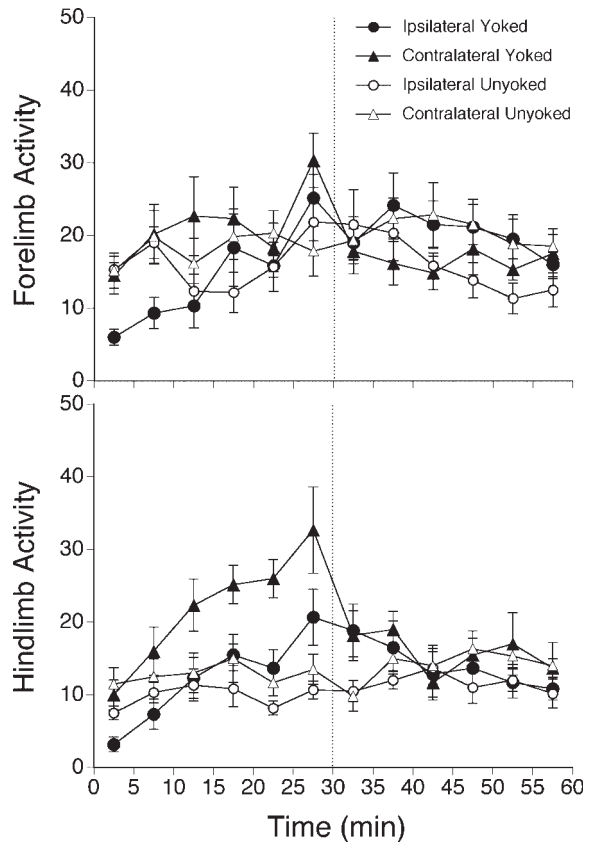
thread was cut before the onset of the Training period. The limb pair manipulated with the Yoked or Unyoked control treatment (referred to as the Ipsilateral pair) was counter-balanced across subjects between left and right sides of the body; in each group, three subjects were yoked on the left and three on the right. The limb pair on the opposite side from the Yoked or Unyoked treatment remained unmanipulated throughout the Training and Testing periods and was referred to as the Contralateral pair.

Subjects in both Yoked and Unyoked conditions were observed during a 60-min session, consisting of a 30-min Training period followed by a 30-min Testing period, with the yoke cut at the beginning of the Testing period in the Yoked condition. Overall activity and forelimb-hindlimb CLM were scored. Ipsilateral limb pairs, which were manipulated directly with the Yoked or Unyoked treatment, provided a measure of the experimental effect of the Yoked condition. Contralateral limb pairs provided a within-subject control measurement for assessing the selectivity of the yoke effect. Limb activity and CLM of both forelimb-hindlimb pairs were summed in 5-min intervals and analyzed by ANOVA.

## Results and Discussion

Separate analyses were conducted to assess responses of forelimb-hindlimb pairs that were ipsilateral to the yoke or contralateral to the yoke. Overall activity of forelimbs ipsilateral to the yoke was analyzed in a two-factor ANOVA (Condition  $\times$  5-min intervals), with the Intervals factors treated as a repeated measure, which indicated the significant main effect of Intervals,  $F(11,110) = 4.6$ ,  $p < .001$ , and the 2-way interaction of condition  $\times$  intervals,  $F(11,110) = 2.6$ ,  $p < .005$ . To simplify this result, a series of  $t$ -tests were conducted at each interval to compare forelimb activity in yoked and unyoked subjects. These posthoc comparisons revealed that Yoked subjects showed less activity in ipsilateral forelimbs than Unyoked subjects during the first interval of Training, but Unyoked subjects were less active than Yoked subjects during min 55 of the testing period ( $p < .05$ ). The analysis of activity in contralateral forelimbs also found the significant interaction of Condition  $\times$  Intervals,  $F(11,110) = 2.3$ ,  $p < .05$ . Post hoc  $t$ -tests indicated that Yoked subjects showed more activity in contralateral forelimbs than Unyoked subjects during the last interval of the Training period ( $p < .05$ ). Together, these analyses documented that the overall effect of yoke training was to suppress activity in the Ipsilateral forelimb during the first half of the Training period, and to promote greater activity in the Contralateral forelimb through the end of Training (Fig. 6, top).

Hindlimb activity also was compared in separate analyses for ipsilateral and contralateral limbs. As in the



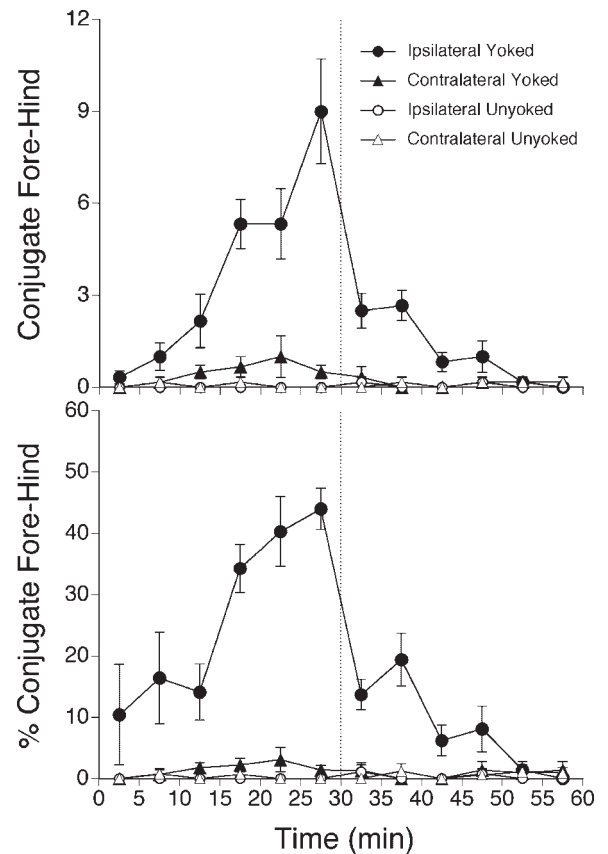
**FIGURE 6** Overall limb activity of homolateral limb pairs (forelimb and hindlimb) during and after yoke training in Experiment 4. Fetuses were exposed to a rigid interlimb yoke or unyoked control condition applied to one homolateral limb pair during the 30-min training period. The top graph presents the frequency of movements of the forelimb that was exposed to the interlimb yoke or unyoked condition during training (Ipsilateral) and the opposite forelimb that was not manipulated (Contralateral). The bottom graph shows the frequency of movements of hindlimbs ipsilateral or contralateral to the yoke during training. Points show the mean number of movements of each limb across 5-min blocks; error bars depict SEM.

analysis of ipsilateral forelimbs, the two-way ANOVA comparing activity in ipsilateral hindlimbs indicated the significant main effect of intervals,  $F(11,110) = 4.7$ ,  $p < .001$ , and the two-way interaction of Condition  $\times$  intervals,  $F(11,110) = 2.9$ ,  $p < .005$ . Post hoc  $t$  tests revealed that ipsilateral hindlimbs were less active in Yoked subjects than Unyoked subjects during the first Interval of Training, but more active in Yoked subjects during the last interval of Training ( $p < .05$ ). The analysis of contralateral hindlimb activity also showed the significant main effect of Intervals,  $F(11,110) = 4.2$ ,  $p < .001$ , and the interaction of Condition  $\times$  intervals,  $F(11,110) = 4.3$ ,  $p < .001$ . Post hoc comparisons indicated that Yoked subjects exhibited significantly more

contralateral hindlimb movements than Unyoked subjects during the last half of the Training period (min 20–30) ( $p < .05$ ), but no differences between Yoked and Unyoked were evident during Testing. The pattern of results thus indicated that yoke training initially suppressed activity of the Ipsilateral hindlimb at the outset of Training, but through most of the Training period resulted in elevated activity of the Contralateral hindlimb (Fig. 6, bottom). By the end of Training, both the Ipsilateral and Contralateral hindlimb were more active in Yoked subjects than in Unyoked controls.

CLM of forelimb–hindlimb pairs was compared in separate two-way ANOVAs for ipsilateral and contralateral limbs. The analysis of CLM in ipsilateral limbs confirmed that both main effects and the interaction effect were significant; Condition:  $F(1,10) = 51.7$ ,  $p < .001$ ; Intervals:  $F(11,110) = 15.0$ ,  $p < .001$ ; Condition  $\times$  Intervals:  $F(11,110) = 15.2$ ,  $p < .001$ . Post hoc comparisons indicated that CLM was more frequent in Yoked subjects than Unyoked during min 10–30 of Training and min 35–45 of Testing. However, documentation of these effects was complicated by the fact that conjugate fore-hind movements were absent during many intervals among Unyoked control subjects. Therefore, the number of subjects that exhibited one or more CLM in a homolateral forelimb–hindlimb pair also was compared in a series of Chi-square tests of independence, which confirmed that the incidence of CLM of the Ipsilateral limb pair was significantly different between Yoked and Unyoked subjects across the 12 5-min intervals,  $\chi^2(33, N = 12) = 49.6$ ,  $p < .05$ . CLM of the contralateral limb pair was examined in a parallel series of analyses. The overall two-way ANOVA indicated no significant main or interaction effects across the Training and Testing periods. The Chi-square analysis also suggested that CLM of the Contralateral limb pair did not differ between Yoked and Unyoked conditions,  $\chi^2(33, N = 12) = 29.2$ ,  $p > .50$ . Overall, the effect of yoke training of a forelimb–hindlimb pair was to promote the expression of conjugate fore-hind movements (Fig. 7, top), but this effect was restricted to the limb pair that directly experienced the interlimb yoke.

Because yoke training influenced the overall activity of forelimbs and hindlimbs during the experimental session, CLM of each forelimb–hindlimb pair also was expressed as a percentage of overall limb activity. Owing to the generally reduced activity of hindlimbs relative to forelimbs during spontaneous fetal activity, hindlimb activity provided an upper limit to the potential for fore-hind CLM. Therefore, percent conjugate was calculated as the frequency of fore-hind CLM divided by the frequency of hindlimb movements. The two-way ANOVA that compared percent conjugate activity of ipsilateral limbs indicated the significant main effect of Condition,



**FIGURE 7** CLM of homolateral limb pairs (forelimb and hindlimb) during and after yoke training in Experiment 4. The graphs present the frequency of CLM (top panel) and relative CLM expressed as a percentage of hindlimb activity (bottom panel) for limbs that were exposed to the interlimb yoke or unyoked condition (Ipsilateral) or unmanipulated (Contralateral). Points show the means in each 5-min block; error bars depict *SEM*.

$F(1,110) = 49.2$ ,  $p < .001$ , the main effect of intervals,  $F(11,110) = 13.2$ ,  $p < .001$ , and the interaction of Condition  $\times$  Intervals,  $F(11,110) = 13.4$ ,  $p < .001$  (Fig. 7, bottom). As in the analysis of CLM frequency, post hoc *t*-tests that compared percent conjugate revealed that Yoked subjects showed a significantly higher percentage of CLM than Unyoked subjects during the last four 5-min intervals of Training and the first three intervals of Testing ( $p < .05$ ). However, analysis of percent conjugate in the contralateral limb pair indicated no significant main or interaction effects, confirming that the effect of yoke training was restricted to the ipsilateral forelimb–hindlimb pair.

Overall, the results of Experiment 4 were consistent with previous experiments: application of the interlimb yoke between a forelimb and hindlimb increased the occurrence of conjugate movements of the yoked limbs. However, the interlimb yoke also produced unexpected

behavioral effects. The presence of the yoke on the Ipsilateral limbs increased the activity of the Contralateral limbs, which was evident during the Training period in both forelimb and hindlimb activity. But the interlimb yoke increased the occurrence of fore-hind CLM only in Ipsilateral limb pairs, an effect that was evident during the last two-thirds of Training and persisted during the first half of Testing. Because CLM did not significantly increase in frequency as overall limb activity increased in Contralateral limbs, this experiment provides further support for the conclusion that CLM is not the simple consequence of increased limb activity.

## EXPERIMENT 5: EFFECTS OF REPEATED YOKE TRAINING

Experiments 1–4 provide a consistent account of the effects of yoke training on fetal motor behavior. During a 30-min period of yoke training, two limbs that are physically linked with an interlimb yoke gradually come to express more CLM. CLM also persists for 10–30 min after the connecting link is severed. The gradual increase during training and decrease during testing suggests that CLM is not a simple unconditioned reflexive response to limb restraint. Moreover, the effects of yoke training are evident only in the limbs that experience the yoke, they vary with the characteristics of the yoke, and they are not a simple consequence of changes in overall limb activity. Interlimb yoke training thus appears to satisfy many of the characteristics one would expect of motor learning.

Experiment 5 was designed to further evaluate whether yoke training in the rat fetus represents a form of motor learning. In traditional learning paradigms, subjects can show evidence of lasting effects of previous experience, which may be manifested as re-expression of the behavioral modification when the learning context is encountered again or as a savings in the rate of re-acquisition upon subsequent training. This experiment sought to evaluate these possibilities by exposing fetal subjects to a second Training period 30 min after the end of the first Training period.

### Method

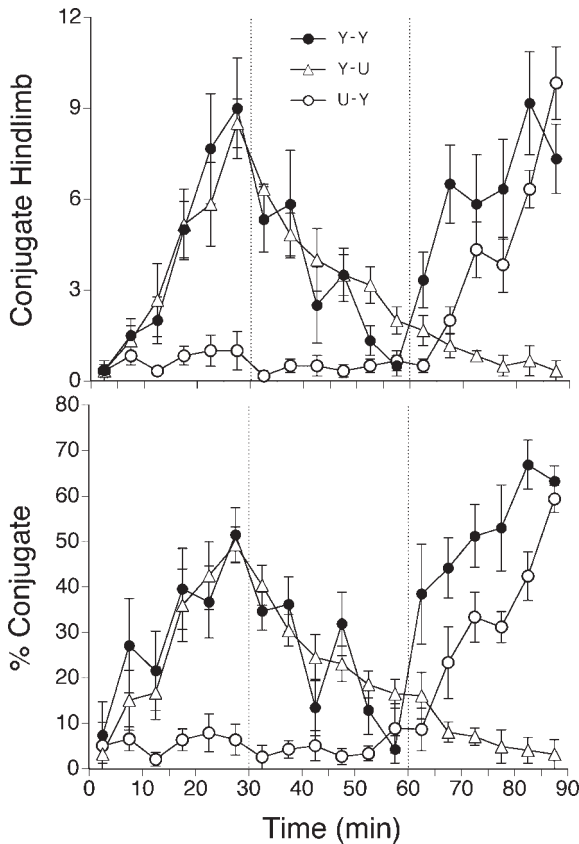
A single fetus was selected as an experimental subject from each pregnancy and assigned to one of three experimental Conditions ( $N=6$  fetuses per condition). The 90-min experimental session was divided into three equal periods, which consisted of a 1st Training Period, a Testing Period, and a 2nd Training Period. Subjects in all three groups were exposed to interlimb yoke training of the hindlimbs during one or both Training Periods. In the Y–U group, subjects were exposed to the interlimb yoke

only during the 1st Training. Subjects in the U–Y group were exposed to the interlimb yoke only during the 2nd Training. Finally, subjects in the Y–Y group were exposed to the interlimb yoke twice, during both Training periods. For subjects that experienced the interlimb yoke in the 1st Training, the yoke was cut immediately at the end of Training, at the beginning of the Testing period. For subjects in unyoked conditions during either Training period, an interlimb yoke was initially attached to both hindlimbs and immediately cut, as in previous experiments. Limb movements were scored to provide summary measures of forelimb and hindlimb activity, CLM, and relative frequency of CLM.

## Results and Discussion

An initial series of two-factor ANOVAs (Condition  $\times$  Intervals, with the Intervals factor treated as a repeated measure) was conducted to compare limb activity and CLM across the entire 90-min experimental session. The analysis of hindlimb activity revealed significant main effects of both Condition,  $F(2,255) = 12.3, p < .001$ , and Intervals,  $F(17,255) = 12.8, p < .001$ , and the interaction of these factors,  $F(34,255) = 13.8, p < .001$ . To simplify this interaction, a series of one-way ANOVAs assessed the simple main effect of Condition in each 5-min interval. These comparisons indicated that during the first twelve intervals, comprising the 1st Training and Testing periods, hindlimb activity differed only in the fifth interval, in which the Y–Y group showed more activity than either the U–Y or Y–U groups ( $p < .05$ ). However, during the last six intervals, comprising the entire 2nd Training period, subjects in the U–Y group showed significantly more hindlimb movements than subjects in either other group. A similar two-way ANOVA comparing forelimb activity during the experimental session indicated the significant main effect of Intervals,  $F(17,255) = 2.5, p < .005$ , with neither the main effect of Condition nor the Condition  $\times$  Intervals interaction reaching significance. Post hoc comparison of Interval means (collapsed across conditions), indicated that forelimb activity increased midway through the 1st Training period in all conditions, and declined modestly during the 2nd Training.

A two-way ANOVA also compared the incidence of CLM across the 90-min experimental session. This analysis indicated the significant main effect of Condition,  $F(2,255) = 7.3, p < .001$ , and intervals,  $F(17,255) = 15.9, p < .001$ , and the significant interaction of Condition  $\times$  Intervals,  $F(34,255) = 11.0, p < .001$  (Fig. 8, top). Tests for simple main effect of Condition at each Interval revealed that CLM increased during min 20–30 of the 1st Training period in both the Y–U and Y–Y groups, relative to unyoked subjects ( $p < .05$ ). CLM persisted at elevated



**FIGURE 8** Changes in CLM of hindlimbs during a 90-min experimental session in Experiment 5. Graphs present the frequency of CLM (top panel) and relative CLM expressed as a percentage of overall hindlimb activity (bottom panel) during the 1st training period, a test period, and a 2nd training period. Fetuses were exposed to interlimb yoke training of the hindlimbs in the 1st period only (Y-U group), during the 2nd period only (U-Y group), or during both training periods (Y-Y group). Points show the means in each 5-min block; error bars depict SEM. Note that fetuses that experienced yoke training for the second time exhibited a more rapid increase in CLM during a 2nd training period.

levels after the yoke was cut, at min 35–40 and min 50, in both the Y-U and Y-Y groups, and in the Y-U group alone at min 55–60 ( $p < .05$ ). During the 2nd Training period, hindlimb CLM was elevated in the Y-Y group relative to both other groups during the first two intervals of the 2nd Training period, and in both the U-Y and Y-Y groups relative to unyoked (Y-U) subjects during the last four intervals of the 2nd Training ( $p < .05$ ). This pattern of results confirmed that yoke training resulted in increased hindlimb CLM during interlimb yoke training, regardless of whether the training was the first or second time the fetus experienced the interlimb yoke.

To compensate for variation in hindlimb activity during the experimental session, which was particularly

evident in the U-Y condition, changes in hindlimb CLM also were expressed as a percentage of overall hindlimb activity and compared in a separate two-way ANOVA. This analysis indicated the significant main effects of Condition,  $F(2,255) = 22.7$ ,  $p < .001$ , and Intervals,  $F(17,255) = 10.1$ ,  $p < .001$ , and the interaction of Condition  $\times$  Intervals,  $F(34,255) = 9.8$ ,  $p < .001$ . Tests for the simple main effect of condition at each interval confirmed that hindlimb CLM increased in both yoked groups during the second half of the 1st Training period relative to unyoked controls, persisted above unyoked subjects in both groups for 20 min during the Test period, and increased in response to yoke training in the U-Y and Y-Y groups during the 2nd Training. However, percent conjugate was elevated during the first 15 min of the 2nd Training only in the Y-Y group (compared to both other groups), and was elevated in both Y-Y and U-Y groups during the last 15 min of training. Overall, CLM increased to a peak of about 50% of all hindlimb movements in both yoked groups during the 1st Training period and to about 65% of all hindlimb movements during the 2nd Training (Fig. 8, bottom).

The difference in the timing of the increase in percent conjugate during the 2nd Training was consistent with the hypothesis that prior experience with the interlimb yoke would accelerate the rate of acquisition during a second exposure to yoke training. This prediction was evident in a comparison of the U-Y and Y-Y groups during the 2nd Training period. To further evaluate this hypothesis, the expression of hindlimb CLM during both training periods was explicitly examined in a planned comparison within the Y-Y group in a two-factor ANOVA (Training  $\times$  Intervals), with both factors treated as repeated measures (Intervals nested within Training). This analysis confirmed the significant interaction of Training  $\times$  Intervals,  $F(5,25) = 4.1$ ,  $p < .01$ . Post hoc comparisons revealed that Y-Y subjects showed significantly more CLM during the first three 5-min intervals of the 2nd Training ( $p < .05$  for the first interval;  $p < .01$  for the second and third intervals) (Fig. 8). This pattern of results agreed with the predicted effect of repeated yoke training, namely, that a savings in the rate of acquisition of a conjugate hindlimb response would be evident upon re-exposure to yoke training after prior experience with the interlimb yoke.

## CONCLUSIONS

The experiments reported in this study demonstrate that a brief period of biomechanical constraint of movement can induce the rat fetus to alter the coordination of spontaneous limb activity. All five experiments present a consistent pattern of fetal response to the interlimb yoke. Four salient aspects of this pattern of response may be

identified. First, conjugate limb movement (CLM) is infrequently expressed in unmanipulated and unyoked subjects. Second, the increase in CLM that occurs during a 30-min period of yoke training occurs gradually, not abruptly. Third, changes in CLM are expressed only in the two limbs that are physically coupled during yoke training. And fourth, CLM persists after the yoke is removed, but gradually returns to baseline levels during a 30-min test period. This pattern of fetal response to interlimb yoke training is consistent with a simple form of motor learning.

One alternative to the motor learning hypothesis is that the interlimb yoke evokes a general increase in limb activity, resulting in an indirect increase in conjugate movements. However, the increase in CLM represented a 6 to 15 fold increase compared to unyoked controls, and was pronounced even when CLM was expressed as a percentage of overall limb activity. Another potential explanation of the changes in CLM might be that the interlimb yoke evokes an unconditioned struggling reflex that happens to involve synchronized movement of the limbs. But one would expect a reflexive response to be expressed promptly after the limbs are restrained and to disappear rapidly after restraint is removed. Instead, the gradual increase in CLM observed during training and subsequent decline during testing more closely resemble the phases of acquisition and extinction in conventional conditioning paradigms. Moreover, a simple reflex explanation would appear inadequate to account for the specificity of effects on yoked limbs, or for savings in the rate of acquisition of hindlimb CLM observed during a 2nd Training period in Experiment 5. These findings present a consistent pattern of evidence that the rat fetus can facultatively modify motor coordination in response to an interlimb yoke.

The yoke training paradigm described in the present report is similar to training techniques used to study the development of motor coordination in newborn rabbits (Viala, Viala, & Fayein, 1986). During normal locomotor development, newborn rabbits exhibit the typical mammalian quadrupedal gaits of crawling and walking. These gaits involve an alternated pattern of interlimb coordination, which is evident in both the forelimbs and hindlimbs and is expressed both behaviorally and at the level of neural activity recorded in the ventral roots of the lumbosacral spinal cord (Viala et al., 1986). However, between 10 and 20 days of age, this alternated stepping is replaced by the adult-typical gait of the half-bound, which involves alternation of the forelimbs but synchronized hopping by the hindlimbs. The transition from anti-phase to in-phase coordination of the hindlimbs also has been noted in the locomotor development of other species, such as gerbils and jerboas, that exhibit hopping or ricochet locomotion as adults (Blumberg-Feldman & Eilam,

1995; Eilam, 1997; Eilam & Shefer, 1997). But in the experiments reported by Viala and colleagues, the pattern of interlimb coordination expressed by young rabbits could be altered by motor experience. Infant rabbits were spinalized at 2 days of age (to restrict experience to spinal motor systems), then at 10 days were exposed to motor training six times daily. Motor training consisted of securing the young rabbit in a harness and attaching both hind feet to pedals. The pedals were configured to allow either an in-phase pattern of rotation or an alternating pattern (like typical bicycle pedals). After 1 week of limb training, about 75% of rabbits that experienced the in-phase pattern expressed synchronized hindlimb activity in response to tail-pinch, which is consistent with a hopping gait. But all of the rabbits that experienced the anti-phase pattern continued to express alternated hindlimb activity after tail-pinch, which is characteristic of newborn stepping (Viala et al., 1986).

The effects of motor training on locomotor development in rabbits are closely mirrored on a shorter time scale by a number of recent studies of motor learning in human infants. In the first of these reports, Thelen (1994) applied a conjugate reinforcement learning paradigm to investigate the ability of 3-month-old infants to adapt to an interlimb yoke. The design of this experiment was to expose infants to an elastic yoke between the legs (or no yoke in controls) during two consecutive baseline periods, then to provide reinforcement to experimental subjects by attaching a ribbon between one leg and an overhead mobile during five acquisition trials. Infants quickly learned to kick more frequently and more vigorously to cause the mobile to move, an effect that has been extensively explored by Rovee-Collier and other developmental researchers interested in infant memory (Kraebel, Fable, & Gerhardstein, 2004; Rovee & Rovee, 1969; Rovee-Collier, Hayne, & Colombo, 2001; Rovee-Collier, Morrongiello, Aron, & Kupersmidt, 1978). In the Thelen study, infants that experienced the interlimb yoke learned to kick both legs in a conjugate pattern, thereby moving the mobile more vigorously (Thelen, 1994). More recently, other forms of constrained limb motion have been reported to produce comparable modifications of infant motor coordination (Angulo-Kinzler, 2001; Angulo-Kinzler & Horn, 2001; Chen, Fetters, Holt, & Saltzman, 2002). These modifications of limb coordination can be viewed as adaptive in the sense that the infant adjusts its behavior to overcome biomechanical constraint of movement (the interlimb yoke) to achieve a particular goal or outcome (movement of the mobile).

Although the yoke training procedure used in the present study is similar to these previous experiments with rabbit and human neonates, there are important differences as well. First, the study by Viala et al. (1986) employed a motorized device to produce passive movement

of the hindlegs of neonatal rabbits. But the yoke training of the present study represented a manipulation of limb movements actively produced by the fetus itself. Second, a feature that all of the studies of human infant motor learning have in common is the presence of explicit reinforcement provided by the overhead mobile. In the present study, however, rat fetuses responded to yoke training in the absence of explicit reinforcement, which highlights the difficulty of relating yoke-induced changes in motor coordination to well established paradigms of associative learning, such as classical or instrumental conditioning. Thus, only the intrinsic characteristics of the interlimb yoke, expressed in the context of spontaneous motor activity, appear to be necessary to promote changes in fetal motor coordination.

Although the expression of CLM may seem to constitute a primitive form of motor learning, a brief consideration of the task suggests that it is no mean feat. To achieve the changes in motor performance evident during yoke training, the E20 rat fetus must (a) be able to detect the presence of the interlimb yoke, (b) perceive differences in motor performance when hindlimbs are moved independently versus when yoked, and (c) selectively alter the paths and velocities of both limbs to coordinate the three-dimensional positions of the two legs. The first ability suggests that the yoke provides a somatosensory cue, either cutaneous or proprioceptive, that is perceived by the fetus. But the latter two abilities imply that the rat fetus possesses a more sophisticated kinesthetic sense, and that kinesthetic feedback may play a role in regulating movement trajectories during spontaneous motor activity.

The inference that fetal motor activity is modulated by sensory feedback stands in contrast to classic experiments in behavioral embryology, which have suggested that amphibian and avian embryos, and by logical extension mammalian fetuses, are largely unresponsive to proprioceptive stimulation (Hamburger et al., 1966; Oppenheim, 1972), and that motor development is not retarded when embryos are prevented from moving (Haverkamp & Oppenheim, 1986; Matthews & Detwiler, 1926). However, experimental work has provided compelling evidence that spontaneous activity and prenatal action patterns are strongly influenced by proprioceptive cues. For example, hatching behavior in the chick embryo appears to be initiated by proprioceptive stimuli generated by the posture of the neck (Bekoff & Sabichi, 1987). 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; Bradley & Sebelksi, 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 et al., 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 E17 (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 (Robinson & Kleven, 2005a; Smotherman & Robinson, 1986) and interlimb synchrony (Kleven, Lane, & Robinson, 2004), and expression of action patterns such as oral grasping of an artificial nipple (Robinson et al., 1992) and facial wiping (a component of postnatal grooming behavior) (Robinson & Smotherman, 1991). Thus, the concordance between the development of proprioceptive sensory systems and fetal motor behavior implies that kinesthetic feedback may have a structuring effect on the central neural networks controlling interlimb coordination during early development.

If fetuses are responsive to proprioceptive stimuli and can exhibit motor learning, as the experiments of the present study suggest, then kinesthetic experience during spontaneous motor activity may contribute to the prenatal development of interlimb coordination required for organized patterns of behavior. This hypothesis is scarcely new (Kuo, 1967), but has not yet been subjected to empirical test. The past four decades have witnessed important advances in our understanding of the role of experience in prenatal sensory development, including the repeated demonstration that olfactory, gustatory and auditory stimuli can be detected by the avian embryo (Lickliter, 1995) and mammalian fetus (Fifer & Moon, 1995; Kisilevsky & Low, 1998; Lecanuet & Schaal, 1996; Smotherman & Robinson, 1992), and can serve to shape the responsiveness of the newly hatched bird or newborn mammal to natural features of the postnatal sensory environment (Gottlieb, 1997; Robinson & Smotherman, 1995). Direct experimental control over stimulus exposure is one of the principal reasons underlying this progress in the study of prenatal sensory experience. The advent of an experimental paradigm applicable to the study of fetal movement—such as yoke motor training—may provide a comparable tool to promote investigation of the role of experience in prenatal motor development.

## NOTES

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