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Ultrasonographic observations of the maturation of basic movements in guinea pig fetuses

Communication

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Abstract: Ultrasonography has not previously been used for studying fetal movements in precocial rodents. The objective of this study was to ultrasonographically determine the sequence of the appearance of basic movements in a guinea pig fetus. The research included eight guinea pig females carrying one fetus each. Fetal movements were observed for 10 minutes each day, from the 25th to 38th day of gestation. The time and sequence of the appearance of movements was observed as follows: whole body flexion (mean 27.6 SD $\pm$ 1.68), whole body extension (mean 28.1 SD $\pm$ 1.12), head flexion (mean 28.1 SD $\pm$ 1.80), head extension (mean 30.5 SD $\pm$ 2.67) forelimbs flexion (mean 30.5 SD $\pm$ 2.32), forelimbs extension (mean 30.7 SD $\pm$ 1.84), trunk rotation (mean 31.9 SD $\pm$ 2.23), forelimbs alternating flexion and extension (mean 32.1 SD $\pm$ 2.1), hind limbs extension (mean 32.2 SD $\pm$ 3.2), hind limbs flexion (mean 32.4 SD $\pm$ 3.16), and hind limbs alternating flexion and extension (mean 33.5 SD $\pm$ 2.39). The identical sequences of basic movement appearances in guinea pigs, sheep, and rats suggest that the rostrocaudal gradient of basic movement appearance could be a general developmental pattern in mammalian species.

Keywords: *Fetus • Ultrasound • Movement • Development*

1. Introduction

Using the criteria of a newborn's motor maturity at the time of birth, mammals can be divided into immature (*i.e.,* altricial species) and mature (*i.e.,* precocial species). Precocial species are capable of independent locomotion immediately following birth [1]. It is known that in precocial species, motor development takes place before birth, yet so far, it has not been studied ultrasonographically. As far as guinea pigs are concerned, different techniques of externalization of fetuses have been employed, all of which involved cesarean delivery at any time of gestation without detaching the placenta from the umbilical cord. In the externalization studies, offspring are placed in isotonic NaCl solution immediately after birth and observed for movements [2]. The significance of ultrasonographic study is that it represents a noninvasive technique for observing the fetus in its natural environment. Ultrasonographic examination may provide information about the degree to which data obtained in studies with externalized fetuses differ from the physiological intrauterine development. In addition, data obtained in ultrasonographic studies about prenatal motor development in precocial species

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could be compared with data on postnatal maturation in altricial species, the purpose of which is to determine general principles of motor development or potential differences.

The objective of this study was to ultrasonographically determine the sequence in the appearance of basic movements in a guinea pig fetus.

2. Experimental Procedures

2.1 Animals

The organisms studied were four months old albino guinea pigs (*Cavia porcellus*) obtained from the Department of Biochemistry of the Novi Sad School of Medicine. The Ethics Committee of the Clinic of Neurology in Novi Sad approved these animal experiments. The guinea pigs were kept in 400 mm width by 1000 mm depth by 300 mm height plastic containers, organized in a "harem system": *i.e.*, 2 females and 1 male. The animals were fed a standard commercial pellet diet (Veterinarski Zavod Subotica, Srbija) and *ad libitum* water enriched with vitamin C (30 mg/100 ml water). Artificial daylight cycles were provided with 12 hours of light (08:00-20:00) and 12 hours of darkness. The room temperature was maintained at 24 ± 2°C. There were 10 to 13 air changes per hour. Individual guinea pigs were identified by observing yellow patterning on their backs.

2.2 Ultrasonographic examination

Inspection of the vaginal introitus of the females was performed each day, and the first day of gestation was determined based on the microscopically established presence of spermatozoids in a vaginal smear. The abdominal regions were shaved after the animals were put into a brief state of narcosis after inhaling ether on the 23rd day of gestation. An ultrasonographic examination was performed using the Toshiba Nemio SSA-550A apparatus with a 6-11 Hz linear probe. Examinations were performed each day from the 25th to the 38th day of gestation. Only females with one fetus were included. Pregnant animals were held with their bellies facing upward by an assistant during the examinations. Fetal movements were observed for 10 minutes in the coronal and axial plane (Figure 1). For each fetus, the occurrence of the following movements was recorded: whole body flexion, whole body extension, head flexion, head extension, forelimbs flexion (moving leg backward and/or toward the trunk), forelimb extension (moving leg forward and/or toward the head), hind limb flexion (moving leg forward and/or toward the trunk), hind limb extension (moving leg backward), trunk rotation, alternating flexion and extension of forelimbs, and alternating flexion and extension of hind limbs.

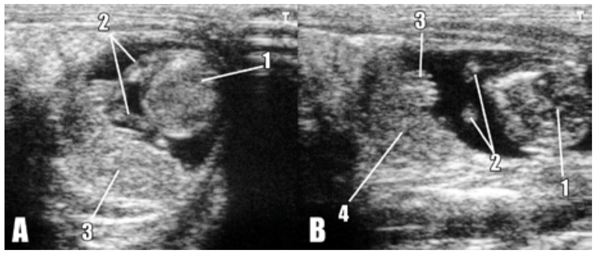

Figure 1. Axial (A) and coronal (B) image of a guinea pig fetus on day 35 of gestation.(A) 1-trunk, 2-forelimbs, 3-placenta (B) 1-head,2-forelimbs, 3-hind limbs, 4-placenta.

3. Results

The animals were calm during ultrasound examination. The various types of movements were easily identified. Movements that appeared first were whole body flexion (mean 27.6 gestation day, SD ± 1.68), then whole body extension (mean 28.1 gestation day, SD ± 1.12), and head flexion (mean 28.1 gestation day, SD ± 1.80). These movements were followed by head extension (mean 30.5 gestation day, SD ± 2.67), forelimbs flexion (mean 30.5 gestation day, SD ± 2.32), forelimbs extension (mean 30.7 gestation day, SD ± 1.84), trunk rotation (mean 31.9 gestation day, SD ± 2.23), alternating flexion and extension of forelimbs (mean 32.1 gestation day, SD ± 2.1), hind limbs extension (mean 32.2 gestation day, SD ± 3.2); hind limbs flexion (mean 32.4 gestation day, SD ± 3.16), and finally alternating flexion and extension of hind limbs (mean 33.5 gestation day, SD ± 2.39).

4. Discussion

The first appearance of fetal movements were observed on the 25th day of gestation, and on average, fetal motor activity started on the 28th day of gestation. This finding corresponds with two previous studies that reported the first fetal movements on the 26th to the 28th day of gestation [3,4]. Published data indicates that the first movements of a guinea pig fetus cannot be associated with the function of a mature neuromuscular synapse. Administering curare to fetuses did not inhibit fetal motor activity in the period from the 26th to 30th day of gestation. However, inhibition of the neuromuscular synapse from cessation of motor activity was observed on the 31st day of gestation [3]. In rat fetuses, the first movements occur on the 15th day of gestation [5], when the muscles are in the myotubular stage [6]. On the 15th day of gestation, there is first contact of the axons with muscles, forming a rudimentary neuromuscular synapse [6]. Data suggests that the occurrence of the first movements can be attributed to the initial contact of axons with muscles.

The first observed fetal movements in this study were head flexion and trunk flexion, which corresponds with previous findings among guinea pigs [3,4]. In this study, the appearance of isolated movements of body parts had a craniocaudal direction. The first were movements of the head, then of the forelimbs, and finally of the hind limbs. Also, the alternating movements of the forelimbs occurred before the alternating movements of the hind limbs. The craniocaudal direction of the appearance of movement in guinea pig fetuses were also observed in two previous studies [3,4]. Among rat fetuses, cutaneous reflexes as well as spontaneous movements also has a craniocaudal sequence of appearance [7,8]. Alternating movements occur first in forelimbs, then in hind limbs during prenatal development of rat fetuses [2]. In a sheep fetus, the first movements are movements of the forelimbs, neck, and trunk on the 35th day of gestation [9]. The craniocaudal sequence of movements was observed as well in sheep fetuses [9]. Identical sequences of basic movements were observed in guinea pigs, sheep, and rats, suggesting that the craniocaudal gradient of basic movements is a general developmental pattern in mammalian species. The rostrocaudal sequence of movements is caused by the rostrocaudal gradient during the maturation of key components of the nervous and muscular systems. Neurogenesis of motoneurons in rat spinal cords is organized in a rostrocaudal direction [10]. Maturation of spinal neuronal circuitry in rats is also oriented in a rostrocaudal direction [8]. In addition, the muscles of the head mature before the caudal muscles in fetuses of sheep [11].

The results of our study show that isolated movements of the limbs precede the alternating flexion and extension of the limbs. The appearance of the first movements of the limbs corresponds with the initial contact of axons with muscles. For the alternating movements of the limbs, functional maturity of the interneurons and neural circuits is required. This explains the later development of these movements compared with basic movements [1].

This research has shown that ultrasonography can be a practical and successful method for observing basic movements of guinea pig fetuses, along with the existing technique of fetal externalization. Our results show no difference in the occurrence and sequence of the appearance of fetal movements observed with ultrasonography compared with data obtained by fetal externalization techniques. The advantages of ultrasonographic study over externalization are its noninvasiveness, the *in vivo* observation of motor development of the fetus, and the possibility of repeated examinations of experimental animals. Its disadvantages lie in the two-dimensional nature of ultrasound. Advancements in the field of 4D ultrasonography will overcome this shortcoming.

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References

[1] Muir G.D., Early ontogeny of locomotor behaviour: a comparison between altricial and precocila animals, Brain. Res. Bull., 2000, 53, 719-726

[2] Robinson S.R., Kleven G.A., Learning to move before birth, In: Hopkins B., Johnson S. (Eds.), Prenatal development of postnatal functions (Advances in Infancy research Series), New York, Greenwood Press, 2005

[3] Cerebelle J.C., Appearance of motoricity in the guinea pig embryo, C. R. Seances. Soc. Biol. Fil., 1964, 158, 510-514

[4] Carmichael L., An experimental study in the prenatal guinea-pig of the origin and development of reflexes and patterns of behavior in relation to the stimulation of specific receptor areas during the period of active fetal life, Genet. Psychol. Monogr., 1934, 16, 337-491

[5] Geisler H.C., Westerga J., Gramsbergen A., Development of posture in the rat, Acta. Neurobiol. Exp., 1993, 53, 517-523

[6] Rubinstein N.A., Kelly A.M., Development of muscle fiber specialization in the rat hindlimb, J. Cell. Biol., 1981, 90, 128-144

[7] Narayanan C.H., Fox M.W., Hamburger V., Prenatal development of spontaneous and evoked activity in the rat (Rattus norvegicus albinus), Behaviour, 1971, 40, 100-134
[8] Nakayama K., Nishimaru H., Iizuka M., Ozaki S., Kudo N., Rostrocaudal progression in the development of periodic spontaneous activity in fetal rat spinal motor circuits in vitro, J. Neurophysiol., 1999, 81, 2592-2595
[9] Goto S., Kato T.K., Early fetal movements are useful for estimating the gestational weeks in the first trimester of pregnancy, Ultrasound. Med. Biol., 1983, (suppl 2), 577-582
[10] Nornes H.O., Das G.D., Temporal pattern of neurogenesis in spinal cord of rat. I. An autoradiographic study--time and sites of origin and migration and settling patterns of neuroblasts, Brain. Res., 1974, 73, 121-138
[11] Boethius J., Resting membrane potential in neck and leg muscles of young rats, Acta. Physiol. Scand., 1969, 75, 253-254