

A Reversible Preparation for Observing the Behavior of Fetal Rats In Utero: Spinal Anesthesia With Lidocaine

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Received 21 May 1985

SMOTHERMAN, W P, S R ROBINSON AND B J MILLER *A reversible preparation for observing the behavior of fetal rats in utero Spinal anesthesia with lidocaine* *PHYSIOL BEHAV* 37(1) 57-60, 1986 —A technique is described whereby lidocaine with epinephrine is injected into the spinal cord of the pregnant rat to produce reversible spinal anesthesia This technique is useful for preparing pregnant females to study the spontaneous behavior of their fetuses in utero Comparison of this procedure with an alternative, but irreversible, spinal preparation (chemomyelotomy) indicates that reversible lidocaine anesthesia does not differentially influence the activity of rat fetuses on Day 19 of gestation This procedure will enable repeated observation of the same fetus at various stages of gestation and provide a means for the longitudinal study of behavioral development across the transition from prenatal to postnatal life

Spinal anesthesia Fetal behavior Rat fetus Chemomyelotomy Lidocaine

INTEREST in tracing the origins of behavior into the embryonic period has waxed and waned since the early days of embryology and comparative psychology Much of the early research was motivated by the controversy over the role of experience in behavioral development and the search for continuities in behavior across the transition from prenatal to postnatal life Owing to technical difficulties with observing prenatal behavior, however, much of the original work took advantage of the relative simplicity of observing and manipulating avian embryos in ovo [5,8], the ontogeny of behavior in mammalian fetuses has, until recently, been relatively unexplored (cf [18])

By adapting techniques that were pioneered in the 1930's [1,3], the intrauterine environment of the developing fetus has become accessible for observation and systematic experimentation To observe the spontaneous behavior of fetuses in utero, it is necessary to intervene in the maternal/fetal environment Unfortunately, maternal preparations that are ostensibly similar can produce different effects on the frequency and patterning of spontaneous fetal behavior [13] To date, the investigator has been faced with a trade-off between observation quality and duration the behavior of non-anesthetized fetuses may be assessed indirectly through external monitoring devices, or directly through surgical preparation of the pregnant female Using external monitoring techniques, longitudinal study of the same fetus during both prenatal and postnatal life would be possible, although current monitoring technology could discriminate among relatively few patterns of fetal behavior [4,9] Alternatively, short-duration surgical preparation of animal subjects can enable direct visualization of fetuses within the externalized

uterus, with a concomitant increase in the potential for detailed behavioral description [14,16] What has been lacking until now is a procedure combining the advantages of detailed behavioral analysis with the capability for repeated application

We now report a spinal anesthetic using lidocaine that provides such a balance between observation quality and repetition This reversible technique enables direct, detailed observation of the developing rat fetus over successive days during late gestation, facilitating the study of prenatal-postnatal behavioral continuities

METHOD

Subjects

Adult subjects in this study were female Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) To produce subject fetuses for behavioral observation, 42 females were bred to Long-Evans male rats Vaginal smears were taken daily to identify the day of conception (first detectable sperm = Day 0 of gestation) Females were housed in groups of three in polycarbonate cages (32.7×37.8×9.5 cm) until the day of observation, when they were rehoused individually Cages remained in a temperature- and humidity-controlled colony room on a 12-12 hr light/dark cycle (lights on at 0700) Throughout the experiment, food and water were continuously available

Spinal Preparation

Two different procedures were used to prepare females for observation of fetuses 22 females were prepared by

chemomyelotomy and 20 by lidocaine spinal anesthesia. Spinal preparation was performed on Day 19 with females under ether anesthesia. A dorsal approach was used to insert a 25 ga needle into the spinal cord between the first and second lumbar vertebrae. In chemomyelotomy, 100 μ l of 100% ethyl alcohol was injected into the spinal cord, producing an irreversible form of chemical spinal transection [2,13].

A series of preliminary investigations were conducted to measure the effectiveness of different lidocaine solutions in producing reversible spinal anesthesia. Following the same general procedures used in preparing females by chemomyelotomy, non-pregnant females received spinal injections of 70 μ l 1.0% lidocaine (Duracaine, Burns Veterinary Supply, Oakland, CA) in an isotonic saline carrier, 70 μ l 2.0% lidocaine, 70 μ l 2.0% lidocaine containing 0.001% epinephrine (10 μ g epinephrine/ml solution, Elkins-Sinn Inc., Cherry Hill, NJ), 100 μ l 2.0% lidocaine containing 0.001% epinephrine, or a saline solution containing epinephrine alone. All lidocaine injections resulted in temporary paralysis of the posterior half of the body followed by complete recovery (no sign of impaired limb movement) upon checking 24 hr later. None of the injections of epinephrine alone produced paralysis. In general, the duration of effective spinal blockade increased across the four dosage groups. Spinal anesthesia was shortest in the 1.0% lidocaine group (mean = 15 \pm 2 min), intermediate with 2.0% lidocaine without epinephrine (mean = 38 \pm 5 min), and longest with 2.0% lidocaine with epinephrine (mean = 55 \pm 8 min at 70 μ l, 51 \pm 2 min at 100 μ l).

In a follow-up study, ten pregnant female rats received spinal injection of 100 μ l 2.0% lidocaine with epinephrine on Day 19 of gestation. Following recovery from spinal anesthesia, each female was housed individually until parturition. All ten females delivered healthy litters at term. There was no evidence that lidocaine spinal anesthesia in any way reduced the number or viability of pups in these litters (mean number of pups/litter = 13.4 \pm 0.6, consistent with the population of normal litters produced in our laboratory). Further, tests on two days subsequent to parturition revealed that treated mothers retrieved pups dispersed from the nest, suggesting that maternal behavior was not affected by lidocaine spinal anesthesia during pregnancy. Based on the findings of these preliminary studies, a solution of 2.0% lidocaine with 0.001% epinephrine in a physiological saline carrier, injected at a volume of 100 μ l, was judged to be most effective in producing (a) complete abdominal and hindlimb paralysis, (b) consistently long periods of spinal anesthesia (in excess of 50 min), and (c) complete recovery after anesthesia. This lidocaine solution was used to prepare pregnant females for observation of fetuses.

Behavioral Observation of Fetuses

Following spinal preparation, the abdomen of the pregnant female was shaved and a midline laparotomy performed. The female was restrained in a plastic holding apparatus (Lothar Products, Corvallis, OR) and her hindlegs and lower abdomen immersed in a warm bath (37 $^{\circ}$ C) containing isotonic saline (Locke's solution). Both horns of the uterus were exteriorized through the abdominal incision and allowed to float freely in the bath. At least 15 min elapsed before onset of observation to allow the female and fetuses to fully recover from ether anesthesia [6].

One subject fetus was observed in each mother from near the middle of one uterine horn. In preparation for observation, the subject fetus, still inside its amniotic sac, was deliv-

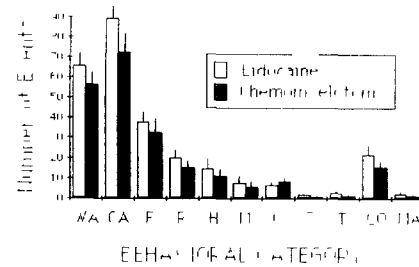


FIG 1 Mean frequency of behavioral events (\pm SEM) for ten categories of fetal movement and one category of maternal movement. WA (Whole Activity), CA (Component Activity), F (Foreleg), R (Hindleg), H (Head), M (Mouth), C (Body Curl), S (Body Stretch), T (Body Twitch), CO (Complex Movement), and MA (Mother Active).

ered through a small incision in the uterus into the water bath, taking care to preserve the connection between placenta and uterus. One person observed the fetus for a period of 10 min, assigned each occurrence of spontaneous fetal movement to a discrete category, and verbally called out the observed events to a second person, the same individual was involved in all fetal observations reported here. A second person entered behavioral data into a real-time event recorder and monitored bath temperature. In previous experiments we have found this observation protocol to have high reliability (with scores in excess of 0.90) while preserving a continuous, sequential record of fetal behavior over the observation period [14].

Categories of Fetal Behavior

Seven specific categories of fetal behavior were distinguished. Head movement comprised any discernible motion of the head, involving flexion and/or rotation of the head and neck. Mouth movement involved one cycle of opening and closing the mouth, without regard to movements of the tongue. Foreleg involved flexion or extension of one or both forelimbs, including movements that originate at the shoulder, elbow, wrist or digits. Hindleg included flexion or extension of one or both hindlimbs, originating at or distal to the pelvis. Twitch was a momentary, spasmodic contraction of muscles of the lateral and ventral portions of the trunk in the general vicinity of the diaphragm. Curl consisted of ventral or lateral flexion or torsion of the entire body trunk, causing the posterior end of the body to move to one side of the medial sagittal plane. Stretch involved dorsal extension of the body trunk, causing the body to straighten or curve backward with the pelvic region moving above the horizontal plane of the body. These seven categories were sufficient to incorporate virtually all movements of the Day 19 rat fetus, only rare, local movements (such as eye-blinking and ear-wiggling) were ignored [16].

Because the seven basic categories of behavior were defined independently, it was possible for them to occur individually or in any combination. Several summary categories were developed to reflect overall fetal activity and the synchronous movement of different body parts. Whole Activity was the total number of times a fetus was recorded as active during the entire 10-min observation session, in this index each Complex Movement was scored as a single Whole Act

Component Activity was the total number of individual body-part movements of a fetus, with each behavioral component of a Complex Movement scored as a separate Component Act. Complex Movement was the number of instances when two or more individual movement patterns occurred simultaneously (e.g., Foreleg and Mouth). The frequency of Complex Movement was also converted to a percentage of total Whole Activity as a measure of Synchronicity of fetal behavior. An index of behavioral diversity was calculated from the Shannon-Weaver measure of entropy [12]

$$D = \sum_1^n (p_i \times -\log_2 p_i),$$

where n is the number of behavioral categories, P_i is the probability that behavior i will occur as the next act, and D is the overall entropy or Diversity of behavior expressed in bits per act. D will reflect variations in the distribution of fetal activity among the seven basic categories of behavior and will range from a value of 0 (when one category has a probability of 1.0) to a maximum of $\log_2 n$ (when all categories are equally probable). In addition to recording fetal behavior, movements of the pregnant female that caused the free-floating uterus and fetus under observation to be passively moved were noted (Mother Activity).

Post-Observation Procedures

Following the fetal observation, ten lidocaine-prepared females received treatment to facilitate their recovery and subsequent parturition. The unmanipulated uterine horn was rinsed with isotonic saline and replaced in the female's body cavity. The observed fetus and its placenta were removed from the manipulated uterine horn and the incision in the uterus sealed using one of three different closures: 6-0 silk sutures, stainless-steel wound clips, or cyanoacrylate adhesive [17]. After the manipulated uterine horn also was replaced in the body cavity, the laparotomy was closed with sutures and wound clips, and the female towel-dried and placed under a heat lamp to facilitate recovery. These females were returned to the colony room and allowed to deliver litters. The pups in each litter were weighed, counted, and their condition noted on the day of birth.

RESULTS

All 20 females prepared by lidocaine spinal anesthesia showed complete paralysis of the abdomen and hindlimbs after immersion in the warm water bath and throughout the period of fetal observation. Movements by the mother were infrequent and did not differ between chemomyelotomy (mean \pm SEM = 1.1 ± 0.5 movements per 10 min) and lidocaine preparation (2.0 ± 0.6).

There was no evidence that the two methods of spinal preparation produced differential effects on spontaneous fetal behavior. Although there was a tendency for fetuses from lidocaine-prepared mothers to be more active, the frequency of Whole Activity, Component Activity and Complex Movement did not differ significantly between lidocaine spinal anesthesia and chemomyelotomy (Fig. 1). Similarly, none of the seven specific categories of fetal movement differed between preparations (all p 's > 0.05). Overall, about 29% of all bouts of fetal activity consisted of simultaneous movement of two or more body parts. The Synchronicity of

fetal movements did not differ between preparations, however (lidocaine 30.8%, chemomyelotomy 26.7%). Behavioral diversity was nearly identical in the two preparations (lidocaine 1.83 bits per act, chemomyelotomy 1.88 bits per act, maximum diversity 2.81 bits per act). Subjectively, the behavior of fetuses in the lidocaine group appeared comparable in every respect to fetal behavior observed in previous research [13,14].

Two to three days after observation, nine of the ten lidocaine-prepared females that received post-observation treatment delivered live litters; one female was sacrificed owing to poor condition. The mean (\pm SEM) number of pups in these litters was 7.1 ± 1.1 (range 1 to 12), which was 58% of all fetuses remaining in the uterus on the day of observation. Delivered pups did not differ in birth weight from nonexperimental pups, and 96% of the pups alive at birth survived to the time of the final litter check two days postpartum. With the exception of one female, all mothers exhibited normal pup retrieval responses on both test days. Two days after birth, each litter and mother was sacrificed and the female necropsied. While no attempt was made at the time of observation to individually mark fetuses, it was evident from initial counts of fetuses in utero and counts after birth that (a) some fetuses from the manipulated uterine horn survived and were delivered normally, and (b) some fetuses from the unmanipulated horn failed to survive until birth. The necropsies revealed several fetuses that had not been delivered. In all cases, the undelivered fetuses were in the manipulated uterine horn on the ovarian side of the uterine incision. To conclude, it is apparent that none of the three methods of uterine closure employed in this study (suture, wound clip, and adhesive) was entirely successful in facilitating normal delivery from incised uterine horns. This may present difficulties for repeated observation of the same subject fetus ex utero. However, because the complications arise as a result of surgical incision of the uterus, we see no impediment for the repeated use of reversible lidocaine spinal anesthesia when the subject fetus is viewed in utero, through the wall of the uterus.

DISCUSSION

With current technology, direct and detailed observation of fetal behavior in utero is possible only by externalization of the uterus without the use of general maternal anesthesia [15]. Currently, two alternative procedures are being used to produce spinal blockade in pregnant animals: chemomyelotomy and spinal transection [7, 10, 11, 14]. Unfortunately, these two maternal preparations are irreversible and result in different levels of fetal activity and different patterns of behavioral development [13]. We have reported that chemomyelotomy may be the preferable technique owing to the simplicity of its administration, consistency of effect on mother and fetuses, reduced surgical trauma, and infrequency of mortality (about 2%).

The lidocaine spinal anesthetic procedure described in this study provides a tool comparable to chemomyelotomy for the direct study of fetal behavior. Lidocaine injection into the spinal cord at the L1-L2 level produces consistent spinal anesthesia for an hour or more. The distribution of fetal behavior among different categories of movement is identical in the two preparations, and the overall level of fetal activity is similar (if anything, activity is slightly higher in lidocaine spinal anesthesia). The principal distinction of lidocaine spinal anesthesia is its reversibility. Pregnant females prepared

with lidocaine not only recover in the short term, but survive to deliver live litters and exhibit normal maternal behavior. With the caveat that fetuses be observed through the uterine

wall, this reversible preparation can provide the first means for repeated, detailed observation of the fetus in utero, bridging the transition from prenatal to postnatal life.

ACKNOWLEDGEMENTS

This research was supported by Grant HD 16102-03 from the National Institute of Child Health and Human Development (NIH) and a grant from Oregon State University Laboratory Animal Resources (Project 153) to WPS. The authors would like to acknowledge Ted Trask for his assistance.

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