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Parturition in the Rat

A Physiological Pain Model

Gwénaëlle Catheline, Ph.D., Bastien Touquet, Sc.B.,† Jean-Marie Besson, Ph.D.,‡ Marie-Christine Lombard, Ph.D.‡*

Background: **Pain during labor is a common and severe phenomenon, but its clinical management remains haphazard because its neurophysiology is poorly understood. In the current study, the authors evaluate the parturient rat as a relevant model to study the pharmacology of labor pain.**

Methods: **Control of birth timing in term pregnant rats was achieved by gavage with RU 486 (5 mg/kg) the day before the expected day of parturition. The behavioral events preceding the expulsion of the first pup were analyzed, and immunodetection of the c-Fos protein was used to evaluate the spinal neuronal activity at the lumbosacral level where genital and perineal inputs terminate.**

Results: **Hind limb and abdominal stretches occurred during labor (mean number, 57 ± 10), arbitrarily defined as the time elapsed between the first stretch and the expulsion of the first pup (mean duration, 62 ± 5 min). Subcutaneous oxytocin increased the frequency of stretches, accounting for the fact that these manifestations are linked to uterine contractions. Finally, epidural morphine (30 μg/10 μl) in oxytocin-treated rats, although resulting in no change of labor duration, significantly decreased the number of stretches (8 ± 2 *vs.* 57 ± 12 for epidural saline) and the number of c-Fos–positive neurons in the lumbosacral spinal segments (80 ± 25 *vs.* 165 ± 17 for epidural saline).**

Conclusions: **These results indicate that stretches during labor in the rat correspond to a behavioral response to nociception associated with uterine contractions and suggest that parturition in the rat could be a relevant model to investigate nociceptive mechanisms associated with parturition in women.**

PHYSIOLOGIC functions are generally painless. There is an exception for women regarding the reproductive tract; menstrual pain is common, but the most painful event occurs during labor at parturition. According to Bonica's survey of 2,700 parturient women,[1] 15% reported no or little pain, 35% reported moderate pain, 30% reported intense pain, and 20% reported very intense pain. In women, labor has been divided into three stages. The first one starts with the appearance of weak but regular uterine contractions; as the intensity of contractions increases, distension, stretching, and tearing of the lower uterine segment and the cervix becoming stronger and produce visceral pain with afferent informations traveling by the hypogastric and pelvic nerves. The second stage of labor, the expulsion phase, is described as the most painful stage and results from distension of the cervix, added to pressure applied on the pelvis and perineum, innervated by the pudendal nerve. The third stage of labor, the delivery, consists of the expulsion of the placenta and is not painful. Labor pain can be reduced by the use of local anesthetic and opioids.[2,3] An anesthetic method that could provide excellent analgesia with no adverse effects on the course of labor is an ideal goal that has not been achieved.[4,5]

As in other areas of pain research,[6] the use of an appropriate animal model is a necessary step to achieve this goal and to elucidate the underlying neurophysiologic and neuropharmacologic mechanisms of pain during parturition. Most studies using animals concern endocrine modifications associated with gestation and parturition,[7–10] but studies of pain associated with this process are rare.[11,12] The aim of the current study was to evaluate the parturient rat as a model of physiologic pain. We first made behavioral observations during the period preceding the expulsion of the first pup. In the same rats, we then studied the parturition-induced activity of spinal neurons receiving afferents of female reproductive tract and perineal sphere, using immunodetection of c-Fos protein, which is an indirect indicator of noxiously activated spinal neurons.[13] Because labor pain is associated with uterine contractions in women,[1] we analyzed the effects of the well-known uterotonic substance oxytocin[14,15] on behavioral manifestations observed during labor. Finally, we evaluated the analgesic potency of epidural morphine to assess the nociceptive component of the behavioral manifestation and spinal c-Fos expression observed at parturition.

Materials and Methods

This study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Helsinki Declaration. Therefore, these experiments were conducted in an authorized laboratory and under the supervision of authorized researchers (G.C., M.-C.L., and J.-M.B.).

Animals

Experiments used primipara (310–400 g) and virgin (235–270 g) albino Sprague-Dawley female rats (Charles River, France; n = 62), purchased at gestation day 15 and arriving 1 week before the beginning of the exper-

* Senior Lecturer, † Technician, ‡ Professor.

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Address correspondence to Dr. Catheline: Institut François Magendie, Université Victor Segalen Bordeaux II, 146 Rue Léo-Saignat 33 077 Bordeaux Cedex, France. g.catheline@imf.u-bordeaux2.fr.

iments. They were housed 6 per cage in a room with a controlled temperature (22° ± 1°C) and a 12-h alternating light–dark cycle (lights on at 7:00 to 19:00 h). Food and water were made available *ad libitum*.

Control of Birth Timing

With the standard light regimen used in this study, parturition in the rat occurs between day 22 and day 23 of gestation (day 1 being the day of mating). Because it proved difficult and time-consuming watching the rats 24 h a day around the time of parturition, control of birth timing was achieved by treating the animals with the antiprogestative synthetic steroid RU 486. Because of its strong antagonist activity, RU 486 given on the day before the expected day of parturition reduces the activity of progesterone and thus mimics the normal decrease of progesterone, which constitutes the first step of parturition in the rat.[16]

On gestational day 21, all of the rats (primipara and control virgin) received 5 mg/kg RU 486 by gavage (1 ml RU 486 solution/300 g body weight). After treatment, the rats were placed alone in a topless homemade transparent plastic chamber with bedding, food, and water in a 12-h alternating light–dark cycle observation room to get accustomed to their environment.

In a pilot preliminary study (M.-C. Lombard, Ph.D., unpublished results, 1999, poster presentation, 9th World Congress of the International Association for the Study of Pain, Vienna, Austria, abstract No. 64), we have observed that 5 mg/kg oral treatment of term pregnant rats (gestational day 21) with RU 486 results in birth time distribution between 9:30 and 15:00 h on day 22 (n = 20/20) as compared with non treated term pregnant rats giving birth from 10:25 to 18:00 h on day 22 (n = 14/20) and on day 23 (n = 6/20). This birth timing has no incidence either on its progress (mean duration for litter delivery, 117 ± 6.7 *vs.* 106.9 ± 7. 9 min), on the litter size (13.9 *vs.* 14.1 pups), or on the number of living pups (98.9% *vs.* 100%).

Experimental Groups

In a first set of experiments, the behavior of gestating rats (n = 12) on the day of parturition (day 22) was followed, with a focus on the labor period, arbitrarily defined as the time elapsed between the first stretching behavior (see Results) and the expulsion of the first pup. Animals were free of any handling or injection during the period of observation. In parallel, virgin rats (n = 4) submitted to the same experimental procedure were observed.

In a second set of experiments, the effects of subcutaneous administration of oxytocin were analyzed in parturient rats (n = 15). Oxytocin was injected subcutaneously at the level of the nape neck at a dose of 10 $\mu g \cdot kg^{-1} \cdot ml^{-1}$ just at the beginning of labor,[15] *i.e.*, appearance of the first stretches. For this series, control parturient rats (n = 9) received subcutaneous saline instead of oxytocin. Virgin rats received the subcutaneous injection of oxytocin 90 min before they were killed (n = 7).

In a third set of experiments, we analyzed the effects of epidural administration of morphine in parturient rats receiving subcutaneous oxytocin (n = 8).

Because in our first series of experiments, we observed that labor duration in parturient rats could vary from 30 to 90 min, whereas in the second series oxytocin reduced the duration, we decided to test out the effects of morphine on labor "calibrated" by subcutaneous oxytocin injection. The epidural mode of administration was chosen because it is less traumatic for the animal and it is the classic way used in obstetric practice.[3] A single injection of 30 μg in a volume of 10 μl sterile saline[17] was made concomitantly to the administration of subcutaneous oxytocin, when the first behavioral manifestation of labor was observed. The dose was chosen according to reported results of previous studies concerned with the effects of intrathecal and epidural morphine on various experimental pain models. It noteworthy that there are very few studies dealing with the epidural route and that a great discrepancy is found between the reported ED_{50} for analgesia in the different pain models. A control group of parturient rats (n = 7) received subcutaneous oxytocin and epidural saline after the same procedure.

Surgical Implantation of Epidural Catheter

Chronic surgical implantation of the epidural catheter was made during halothane anesthesia (2%) on day 20 of gestation. Preparation and placement of the catheter were slightly modified from the methods previously described by Durant and Yaksh.[17] We used a polyurethane microcatheter (28 gauge, 0.20 mm ID, 0.40 mm OD, 14 cm long, L-CATH® Catheter System; Harvard Apparatus, Edenbridge, United Kingdom) marked every 1 cm to allow precise measurement and placement. After removing of the internal stylet, a loose overhand knot was made 2 cm from the tip and fixed with a small amount of dental acrylic cement (lens shape, 2 mm diameter). The other end of the catheter, already equipped with a Luer hub, was prepared for injection. The inside hub space was maximally reduced by filling it with two small pieces of polyethylene catheters, fitting tightly together so as to leave only a central canal open (0.8 mm OD, 2 mm long). A rubber membrane (0.5 mm thick) was then placed inside the Luer hub on top of the catheter, and a plastic cap with a 1-mm-diameter central hole was finally screwed over. This construction allowed injections with a 21-gauge needle connected to a Hamilton syringe through the rubber membrane into the catheter with a mean dead space of 5 μl. The crests of the ilium were palpated to locate the spinal process of the sixth lumbar vertebra, lying between them. A midline skin incision (2 cm long) was then made over the L4–L6 spinal pro-

cesses, and another one (1 cm long) was made at the neck level. The prepared catheter was tunneled subcutaneously from the neck skin incision back to the lumbar incision. At this level, the L5 dorsal spinal process was cut off to allow a parallel insertion of the catheter to the spinal cord. While the L4 spinal process was held with teeth forceps, the yellow ligament was pierced between vertebras L4 and L5 with the aid of a hook made of a 21-gauge needle with a bent tip. The catheter was then gently pulled into the epidural space to a length of 2 cm so that the tip came to the L2 vertebra, *i.e.*, at the level of L6–S1 spinal cord segments. The dental cement piece was then positioned in place of the removed L5 spinous process. Two stitches were then placed between the paravertebral muscles on both sides of the piece of dental cement to avoid displacement of the catheter. The cephalic portion of the catheter was externalized through the skin above the skull area where it was relatively inaccessible to the paws. All animals seemed to be free of infection upon gross inspection. Using this nontraumatic implantation, no animals exhibited motor impairment after surgery.

Behavioral Analyses

On day 22, each rat was directly observed from 8:00 AM until the delivery of the first pup. Thereafter, using videotape recordings, we focused our analysis on the 90 min preceding the expulsion of the first pup, including the labor period. Two types of stretching behavior were observed: One consisted of inward turning of one hind paw, and the second one consisted of straining and squashing the lower abdomen on the floor. These two components were quantified by counting their occurrence during succeeding 5-min periods. Sequences of exploration and self-licking were also noticed during this period.

Spinal Neuronal Activation Evaluated by Immunohistochemical Detection of c-Fos Protein

In a preliminary study (M.-C. Lombard, Ph.D., unpublished results, 1999, poster presentation, 9th World Congress of the International Association for the Study of Pain, Vienna, Austria, abstract No. 64), bilateral c-Fos expression was observed throughout segments T12–S2 of the spinal cord 1 h after the birth of the first pup, clearly peaking in L5–S1 segments (fig. 1). These segments correspond to the main projection site of the hypogastric nerve (T12–L1) and of the pudendal and perineal nerves (L5–S1). The prominent labeling in L5–S1 suggests that uterine contractions during labor and pup expulsion mainly activate neurons receiving pelvic inputs from the cervix region of the uterus. Because our pharmacologic study aimed at studying the pain of labor, which is mainly the result of tearing and stretching of the lower uterine segment and cervix, the current c-Fos study focused on the lumbosacral segments.

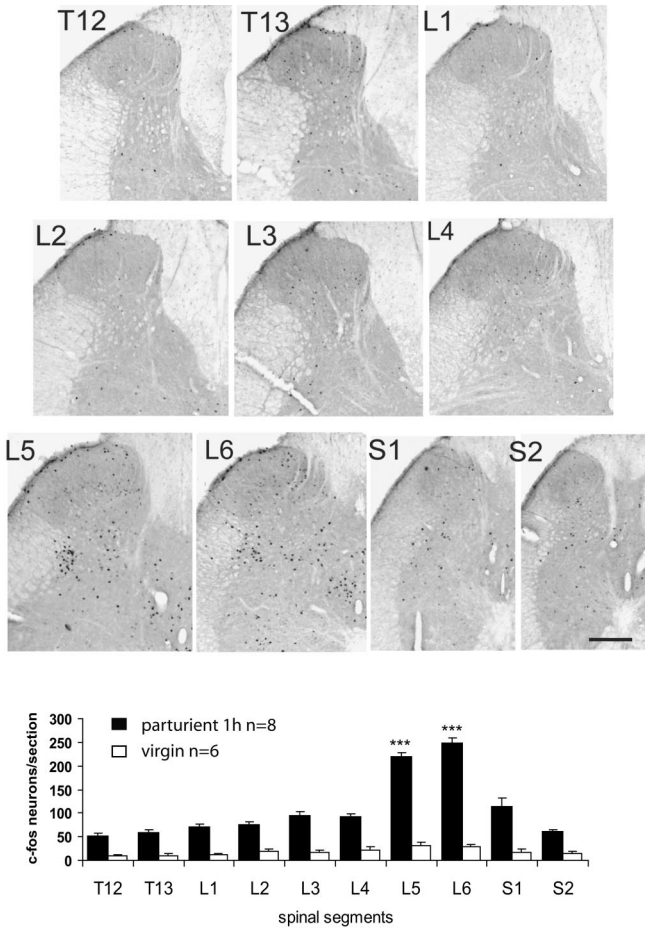

Fig. 1. c-Fos expression along the thoracic (T12–T13), lumbar (L1–L6), and sacral (S1–S2) segments of the spinal cord in parturient rats, 1 h after the birth of the first pup. (*Upper part*) Individual representative microphotographs of spinal cord 40-μm sections. c-Fos immunohistochemistry was processed as described in Materials and Methods (paragraph 6). *Scale bar* represents 200 μm. (*Lower part*) Histogram showing the quantification of c-Fos expression in each spinal cord segment from T12 to S2 for eight parturients as compared with six virgin rats. All the rats (parturient and virgin) received the antiprogestative treatment (see Materials and Methods, paragraph 2). For each animal and each segment, the total number (bilateral) of c-Fos neurons was counted in 8–10 nonserial 40-μm sections and averaged. For both groups, the mean numbers for each segment were averaged according to the number of rat per group. Data are expressed as mean ± SEM. Note (1) the significant increase in c-Fos expression in all the spinal cord segments of the parturient rats as compared with the virgin rats ($P < 0.001$ for all segments) and (2) the peaking numbers of c-Fos neurons in L5, L6, and to a lesser extent S1 segments in the parturient rats.* $P < 0.001$ when compared with all other segments for parturient rats. From M.-C. Lombard, Ph.D., 1999, poster presentation, 9th World Congress of the International Association for the Study of Pain, Vienna, Austria, abstract No. 64; used with permission.**

One hour after the birth of the first pup, mothers were killed, and spinal cords were removed to process immunohistochemical investigation. Virgin females were killed 1:30 h after the beginning of the observation. The animals were deeply anesthetized with 55 mg/kg intra-

peritoneal pentobarbital and underwent intracardiac perfusion with 0.1 M phosphate-buffered saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The spinal cord was then removed, postfixed for 4 h in the same fixative, and cryoprotected overnight in 30% sucrose in phosphate buffer. Frontal frozen sections of the lumbar spinal cord, L5–S1 segment, 40 μm thick, were cut, collected as free-floating sections, and immunostained for Fos-like protein according to the avidin-biotin-peroxidase method.[18] They were incubated in a rabbit polyclonal c-Fos antibody (Tebu, Le Perray en Yvelines, France; sc-52, 0.1 mg/ml diluted at 1:30,000). The avidin-biotin-peroxidase complex (Vectastain; Vector Laboratories, Burlingame, CA) revealed using a VIP kit (Vectastain) was used as immunolabels. Labeled nuclei were counted under light field microscopy at $\times$125, using a camera lucida attachment. The distribution was evaluated by counting the number of Fos-like immunoreactive (Fos-LI) neurons per specific laminar region of the spinal gray matter[19] in five nonserial sections of L5, L6, and S1 segments on both sides of the spinal cord. All Fos-LI neurons were analyzed without considering the intensity of the staining. The investigator responsible for plotting and counting the Fos-LI neurons was blind to the experimental situation of each animal.

Drugs

Drugs used and their source were synthetic steroid RU 486 (17β-hydroxy-11β-(4-dimethylaminophenyl-1)-17α(prop-1-ynyl)oestra-4,9 dien-3one; provided by Roussel-Uclaf Laboratories, Romainville, France), oxytocin (Neosystem, Strasbourg, France), morphine hydrochloride (Cooper, Melun, France), and pentobarbital (Ceva Santé Animale, Libourne, France). RU 486, because of its low solubility in water, was first dissolved in ethanol (0.05% of final solution) and then added to a 1% solution of carboxyl-methyl-cellulose; oxytocin and morphine were diluted in 0.9% saline.

Statistical Analysis

To compare the duration of labor, the total number of stretches and the total number of spinal Fos-LI neurons statistical analysis was performed using one-way analysis of variance for the different groups of animals, followed by a protected least significant difference test of Fisher; $P < 0.05$ was considered as significant. All data are presented as mean $\pm$ SEM.

Results

Description of Parturition in the Rat

Behavioral Analyses. All of the observations were made during the light period *i.e.*, the resting period for the rats. First, we characterized the spontaneous behavior of the animals before the expulsion of the first pup.

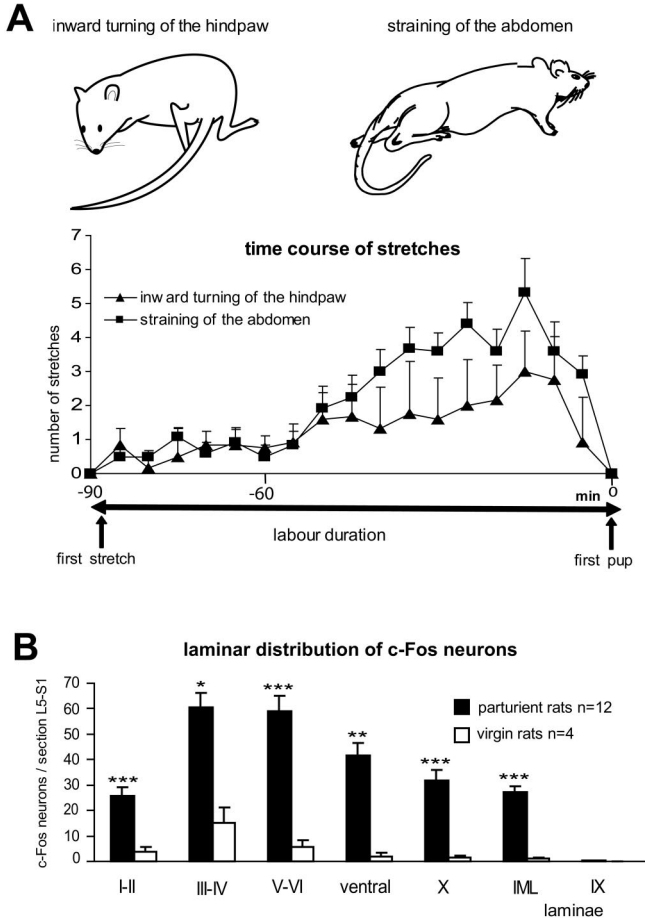

Fig. 2. (*A*) Characteristic stretches observed during parturition in rat: inward turning of the hind paw, straining of the abdomen, and time course of these behavioral manifestations during the 90 min preceding expulsion of the first pup (time 0). (*B*) Laminar distribution of Fos-like immunoreactive neurons at the lumbosacral level of the spinal cord in parturient rats 1 h after the expulsion of the first pup as compared to the distribution of Fos-like immunoreactive neurons in the same spinal level of virgin rats. Data are expressed as mean ± SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Superimposed to inactive phases and active phases of normal behavior, abnormal postures were observed. During inactive phases, the rats either lay on their side or adopted a curling posture with their head hidden. During active phases, rats displayed licking or grooming, explored, or prepared their nest. Abnormal postures consisted of two main stretching behaviors: (1) inward turning of the hind limb (fig. 2A) and (2) straining and squashing of the lower abdomen and the hind limb on the floor (fig. 2A). More rarely, rats jumped, adopted humpbacked positions, lay on their back, or bit their tail. Delivery of the first pup was characterized by a semi-crouched position and licking of the vulva.

According to the criteria, we chose to characterize the labor period, *i.e.*, the time elapsed between the first stretch and the expulsion of the first pup; the mean labor duration was 62 $\pm$ 5 min. During this period, parturient rats displayed an average of 23 $\pm$ 4 inward turnings of

the hind limb and 34 ± 8 squashings of the abdomen (mean total number of stretches, 57 ± 10). The time courses of these two behavioral manifestations were similar because their frequency progressively increased and reached a maximum just before the expulsion of the first pup (fig. 2A); squashings being more frequent as labor proceeded. In virgin rats, these stretching behaviors were not observed.

Spinal c-Fos Expression Analyses. One hour after the expulsion of the first pup, the mean total number of Fos-LI neurons in the lumbosacral level of the spinal cord was significantly increased (214 ± 27 Fos-LI neurons/section) compared with that observed in the spinal cord of virgin rats (29 ± 10 Fos-LI neurons/section; $P < 0.001$; fig. 2B). The number of Fos-LI neurons increased in all laminae of the spinal cord (fig. 2B).

Systemic Oxytocin Increases Frequency of Stretches

To assess the link between the observed stretching manifestations and the uterine contractions, we analyzed the effects of systemic administration of oxytocin and as a control the effect of systemic administration of saline (fig. 3).

Oxytocin-parturient rats had a mean labor duration of 30 ± 4 min, which was significantly shorter than the labor duration of the saline-parturient rats (127 ± 31 min; $P < 0.001$). In contrast, the mean number of stretching manifestations was 42 ± 6, not statistically different from the mean number measured in saline-parturient rats (50 ± 13). This result reveals that subcutaneous oxytocin increases the frequency of stretches. We also noticed that for oxytocin-parturient rats, the number of born pups 1 h after the first delivery was significantly lower than that observed for saline-parturient or parturient rats (table 1; $P < 0.001$).

It is noteworthy that saline-parturient rats presented a significantly longer mean labor duration than the parturient ones (127 ± 31 *vs.* 62 ± 5 min; $P < 0.01$).

Although subcutaneous oxytocin produced changes of the time course of behavioral parameters, it did not modify spinal c-Fos expression. The mean numbers of c-Fos–positive neurons per section were 216 ± 29, 198 ± 33, and 214 ± 27 in oxytocin-parturient, saline-parturient, and parturient rats, respectively (fig. 3C; $P > 0.05$).

In virgin rats, the injection of oxytocin did not induce any stretching behavior and had no significant effects on the number of spinal Fos-LI neurons (40 ± 4 neurons/section) in comparison with the number observed in virgin nontreated rats (29 ± 10 neurons/section).

Epidural Morphine Decreases the Number of Stretches

The nociceptive component of the stretches observed during parturition was assessed by looking at the effects of epidural morphine (30 μg/10 μl) administered concomitantly with subcutaneous oxytocin at the beginning of labor.

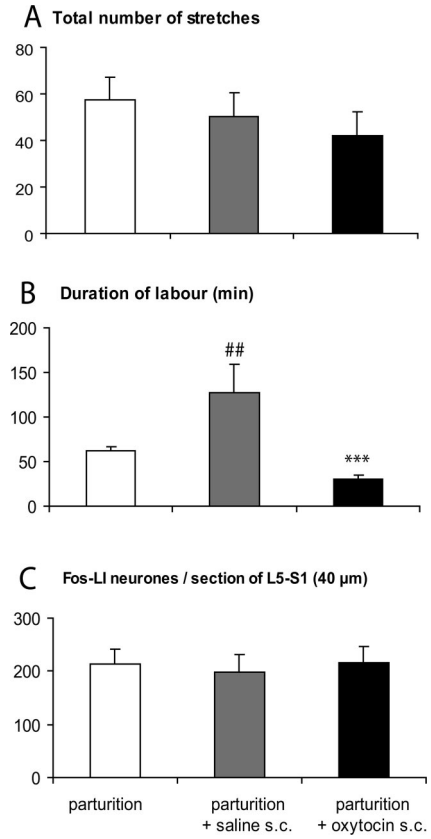

Fig. 3. Effects of subcutaneous treatment (saline or oxytocin) on behavioral manifestations of parturition and on induced spinal Fos-like immunoreactive (Fos-LI) neurons. (*A*) Number of stretches, (*B*) labor duration (min), and (*C*) number of Fos-LI neurons at the level of L5–S1 of the spinal cord in parturient rats (*white bars*), in saline-parturient rats (*gray bars*), and in oxytocin-parturient rats (*black bars*). Data are expressed as mean ± SEM. *** $P < 0.001$ when compared with saline-parturient group. ## $P < 0.01$ when compared with parturient group. s.c. = subcutaneous.

Morphine significantly decreased the number of stretching manifestations from 57 ± 12 to 8 ± 2 in epidural saline– and epidural morphine–treated groups, respectively ($P < 0.01$). By contrast, it did not significantly modify the mean labor duration (47 ± 7 *vs.* 35 ± 8 min for morphine- and saline-treated groups, respectively; figs. 4A and B). Moreover, it significantly decreased the number of spinal c-Fos–positive neurons ($P < 0.01$). The mean total numbers of c-Fos positive neurons per section were 80 ± 21 *versus* 165 ± 17 in oxytocin-parturient rats receiving epidural morphine and epidural saline, respectively (fig. 4C). This decrease concerned all laminae of L5, L6, and S1 segments of the spinal cord (fig. 5).

Discussion

The current study reveals that parturient rats present behavioral stretches related to uterine contractions dur-

Table 1. Effects of the Treatments on the Number of Pups Born during the First Hour of Delivery

Groups	Litter Size	Pups Born
Parturition	13	8
Parturition + subcutaneous saline	13	9
Parturition + subcutaneous oxytocin	13	3*
Parturition + subcutaneous oxytocin + epidural saline	11	3*
Parturition + subcutaneous oxytocin + epidural morphine	12	3*

All the rats of the five groups were treated with RU 486.

* $P < 0.001$ with the other groups.

ing the phase preceding the expulsion of the first pup. These behavioral manifestations are sensitive to the epidural administration of morphine because their number decreased after its injection. Concomitantly, we also observed that parturition results in a huge increase in neuronal c-Fos protein expression at the lumbosacral level of the spinal cord, which could be reduced by epidural morphine. It is interesting that, compared with the intrathecal approach, the epidural placement of the catheter is safer because none of the operated rats presented neurologic impairments or infections. Moreover, this procedure has no incidence on (1) the time course of parturition, because this time course was comparable in operated and unoperated rats; (2) the state of the tissue for immunodetection; or (3) c-Fos expression itself.

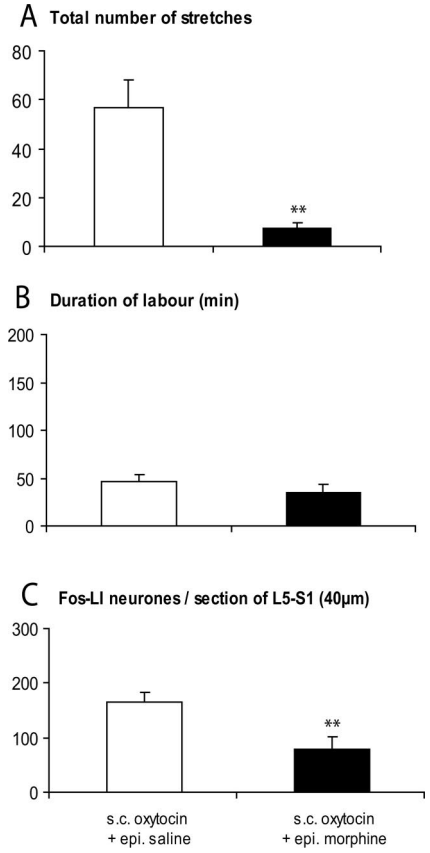

Fig. 4. Effects of epidural morphine on behavioral manifestations of parturition and on induced spinal Fos-like immunoreactive (Fos-LI) neurons. (*A*) Number of stretches, (*B*) labor duration (min), and (*C*) number of Fos-LI neurons at the level of L5–S1 of the spinal cord in oxytocin-parturient rats receiving either epidural saline (*white bars*) or epidural morphine (*black bars*). Data are expressed as mean ± SEM. ** $P < 0.01$ when compared with epidural saline group. epi. = epidural; s.c. = subcutaneous.

During the period preceding the first pup expulsion, we observed two main types of stretches: inward turning of the hind limb and squashing on the floor of the lower abdomen. These postures were partially described in previous studies related to parturition, but not quantified.[7,9,20] More rarely, we observed humpbacked and supine positions and jumping. None of these behaviors were manifested in virgin rats. It is noteworthy that neither audible vocalization nor ultrasounds (personal observation) were uttered by the rats along labor, but parturition is only seldom accompanied by vocalizations because they would make a female more easily detectable by predators.[21] The subcutaneous oxytocin injection immediately induced an increase in the frequency of the stretches, suggesting that they are related to uterine contractions. However, it has been demonstrated that, in

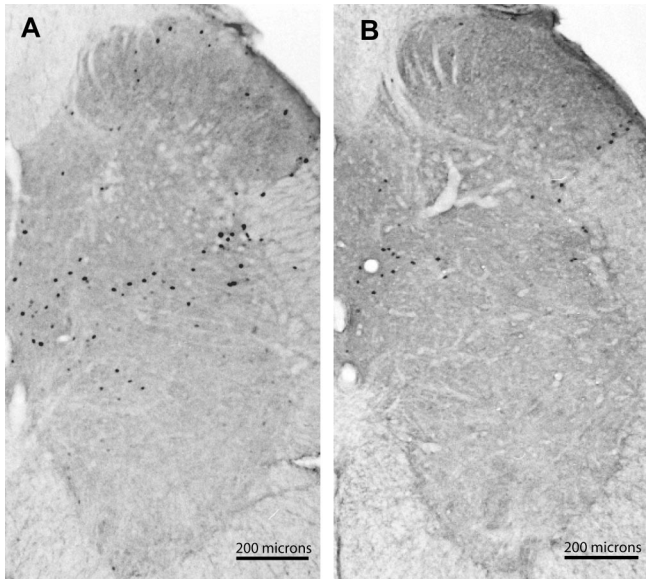

Fig. 5. Photomicrographs illustrating the effects of epidural morphine on c-Fos expression in the spinal cord of parturient rats. Individual examples of hemi–spinal cord sections (thickness 40 µm) at the L5–S1 lumbosacral level of the spinal cord, (*A*) in oxytocin-parturient rats receiving epidural saline and (*B*) in oxytocin-parturient rats receiving epidural morphine. *Scale bar* represents 200 µm.

pregnant rats, uterine contractions can start 24 h before expulsion[22]; this implies that, in our study, the first stretch is only observed when a certain intensity of uterine contraction is reached. For these reasons, we think that the stretches are related to uterine cervical distension resulting from pressure applied by the fetus after intense uterine contractions rather than to uterine contractions themselves. Moreover, it has been demonstrated that uterine cervical distension produced a contraction of abdominal muscles, which could be at the origin of the stretches observed in our study.[11] On the one hand, the increased frequency of contraction induced by oxytocin accelerates the birth of the first pup, *i.e.*, it reduces labor duration. On the other hand, in these same rats, the whole process of delivery is prolonged. Indeed, in saline-parturient rats as in parturient rats, nearly all of the pups (80% of the litter) were born during the hour after the expulsion of the first one. In contrast, in oxytocin-parturient rats, only part of the litter (25%) is delivered during this hour, indicating that the single injection of oxytocin induces a transitory increase in frequency of uterine contractions that is not sufficient to cover the whole process of delivery. This is in accordance with the short half-life of oxytocin[23] and suggests that in these oxytocin-parturient rats, the endogenous liberation of oxytocin did not take place or became inefficient. The stress induced by the handling associated with the subcutaneous injection could block the spontaneous release of oxytocin. Indeed, the rats receiving subcutaneous administration of saline did present an increase in the duration of the labor in our study. It has been demonstrated that stressful situations activate opioid pathways, which delay parturition by acting on opioid receptors of the pituitary, which directly inhibit blood oxytocin release.[24] Opioids may restrain oxytocin secretion either by μ- or κ-opioid receptors on oxytocin cell bodies[25] or by preterminal κ-opioid receptors located at the neuronal lobe of the pituitary.[26] Here, labor pain is under consideration, but future pain studies on whole parturition should be cautious with the route and sequence of administration of oxytocin.[27]

The observed stretches could constitute a behavioral response to the nociceptive component of uterine contractions but could also constitute a postural adjustment facilitating the expulsion of the fetus. Strikingly, most of the postures observed in parturient rats have already been described in experimental visceral pain models induced by intraperitoneal injection of acetic acid,[28] by implantation of an artificial stone in one ureter,[29] or by injection of mustard oil in the uterine horns.[30] Moreover, epidural morphine dramatically decreased the number of stretches without impairment of the expulsion of the first pup. This may be accounted for by the fact that these behavioral manifestations may reflect nociceptive events, rather than essential muscular events.[31] According to this, we also observed an increase in the number of Fos-positive spinal neurons in the lumbosacral level 1 h after delivery of the first pup. In the spinal cord, c-Fos protein expression is considered as an indicator of noxiously activated spinal neurons.[13] The distribution of Fos-LI neurons we observed closely mirrored the spinal terminations area of visceral primary afferents coming from lower genital parts (lower uterine segment, cervix, vagina, and perineum).[32] At the lumbosacral level, there is a great genital and perineal convergence which explains the intense neuronal activity during parturition. The pelvic nerve, innervating the lower uterine segments and cervix and the pudendal nerve, innervating the perineal skin, inner thigh, and clitoral sheath, project to L5–S1 segments.[33–36] Some afferents coming from hypogastric nerve, which innervates the uterus and the cervix, also project to this segmental level.[37] Most available clinical data support the concept that this lower genital part constitutes the major source of pain during labor in women.[1] More precisely, pain associated with labor results from distension, stretching, and tearing of the cervix and lower uterine segment after uterine contractions (visceral pain) but also from pressure applied on the pelvis and perineum (somatic pain). In our study, the segmental localization of Fos expression and, at this level, the presence of Fos positive neurons in laminae I, II, and X, which contain visceral nociceptive neurons,[38] support the idea that at least a part of the afferent information coming from the pelvic and the pudendal nerves is nociceptive during parturition in the rat. These results, however, do not exclude the possibility that in addition, nociceptive input could be carried by afferent fibers in the hypogastric nerve to the thoracolumbar segments of the spinal cord. Indeed, uterine cervical distension in the virgin rat induces c-Fos expression in the T12–L2 spinal segments.[39] Moreover, in a previous report,[35] a difference in sensitivity between the afferents fibers of the hypogastric and pelvic nerves was evidenced in the virgin nulliparous rat: The hypogastric fibers were more sensitive to high-threshold mechanical stimulations, and the pelvic nerve fibers were sensitive to a wide range of mechanical and chemical stimulations. During parturition in the rat, nociceptive information is likely to be differentially transmitted *via* both nerves to the thoracolumbar and lumbosacral segments of the spinal cord and reflect different localization, quality, and intensity of nociception (purely visceral and viscerosomatic at the beginning and at the end of labor, respectively). Actually, as illustrated in figure 1, parturition in the rat induced neuronal c-Fos expression throughout segments T12–S2 of the spinal cord, but a clear peak was present in the L5–S1 segments. Such a prominent labeling suggests that this spinal level is the major target of excitatory inputs during parturition. Several nonexclusive hypotheses can be proposed to support this differential neuronal activation of spinal levels, mainly, a reduced input from the hypogastric nerve, a

modified sensitivity of afferent fibers due to the hormonal condition, a sensitization of the pelvic nerve afferent fibers due to the local chemical changes during ripening of the cervix, and the repeated mechanical stimulation of the cervix by pressure from the pups during uterine contractions. In this respect, a reversible profound denervation within all layers of the body of the uterus but not within the cervix is present in term pregnant rats,[40] a variation in the response properties of peripheral afferent fibers supplying the vagina and uterus of the rat takes place in the estrus stage,[41] and inflammatory agents are produced during cervical ripening together with neurogenic inflammation.[42,43] Alternatively, it could be argued that the delay we chose, *i.e.*, 1 h after the birth of the first pup, represents only an arbitrary moment in the dynamics of painful events occurring along the whole process of parturition and thus does not allow differentiation of the successive involvements of hypogastric and pudendal/pelvic nerve inputs. c-Fos protein expression as studied by immunohistochemistry, can be detected in the spinal cord as soon as 30 min after nociceptive peripheral stimulation, and numerous studies agree that maximal expression is present 2 h after the stimulation.[13] We choose to measure c-Fos expression 1 h after the birth of the first pup, because the labor period had a mean duration of approximately 1 h. Summation of these successive periods led to a total delay (2 h) compatible with a measurable expression of c-Fos induced at least by the events occurring at the beginning stage of labor as defined with our behavioral criteria. A more detailed study considering the expression of c-Fos in the different laminae of the different spinal segments at different time periods before, during, and after expulsion of the first pup is needed to discriminate more precisely the differential implication of hypogastric and/or pudendal/pelvic nerve inputs in spinal c-Fos expression along the whole process of parturition. Such a study is under completion in our laboratory.

Finally, our pharmacologic results showing that epidural morphine significantly decreased the number of neurons expressing c-Fos at the lumbosacral spinal level (52% of reduction) further support the assertion that, during parturition, noxious inputs activate spinal neurons in the rat. Moreover, they are in good agreement with electrophysiologic studies performed on visceral pain models[12,44] and with studies showing that intrathecal morphine reduces spinal c-Fos expression evoked by visceral nociceptive stimulation.[13,45] Although we have no indication about the diffusion of morphine in the depth of the spinal tissue after epidural administration, the decrease observed in laminae I and II is likely to result from the activation of the presynaptic opioid receptors, which are abundantly described in immunohistochemical and binding studies.[46] The effects observed in the other laminae may result from polysynaptic pathways.

This is the first report showing that epidural morphine in parturient rats reduces the behavioral manifestations of the labor phase and the related spinal lumbosacral neuronal c-Fos expression. These results suggest that nociceptive events occur during labor in the rat. Moreover, the time course of the stretches (maximum frequency 10 min before the first pup expulsion) is in accordance with clinical studies indicating that, in women, the intensity of pain increases progressively and reaches a maximum at delivery.[1] In conclusion, parturition in the rat seems to be a relevant model allowing for studying the pharmacology of analgesia for pain associated with parturition in women.

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