Some in vivo electrophysiological properties of locus coeruleus neurones in fetal rats

T. Sakaguchi\(^1\) and S. Nakamura\(^2\)

\(^1\) Department of Neurophysiology, Institute of Higher Nervous Activity, Osaka University Medical School, Osaka 530, Japan
\(^2\) Department of Physiology, Faculty of Medicine, Kanazawa University, Kanazawa 920, Japan

Summary. The electrical activity of locus coeruleus (LC) neurones was recorded extracellularly in fetal rats still in contact with their dams by an intact umbilical cord. Pregnant rats, at gestation days 18 to 22, were anesthetized with urethane. The head of a fetal rat was exposed from the uterus and fixed to a conventional stereotaxic apparatus by means of a simple device. The location of the LC in the fetal rats was determined by the appearance of field responses evoked by stimulation of the dorsal noradrenergic bundle. Antidromic spikes of single LC neurones were evoked superimposed upon the field responses. The mean conduction velocity of LC axons was calculated to be 0.25 m/s. Some fetal LC units were activated antidromically by stimulation of the frontal cortex (FC) with latencies ranging from 21 to 67 ms, values nearly the same as those obtained in neonates and adults. Although the majority of fetal LC neurones recorded were not spontaneously active, a small number of them revealed epochs of sporadic firing, which appeared to occur synchronously in many or all of the LC neurones. Sensory stimuli (e.g., air puffs to the skin) were effective in activating LC neurones. These results indicate that LC neurones have already developed projections to the FC, and are functionally active in prenatal periods.

Key words: Electrophysiology - Fetal rat - Frontal cortex - Locus coeruleus - Unit activity

Introduction

The locus coeruleus (LC), a brain stem nucleus composed mainly of noradrenaline (NA)-containing neurones (Amaral and Sinnamon 1977; Foote et al. 1983; Ungerstedt 1971), appears early in embryonic development. In the rat, NA fluorescence-positive cells are seen in the LC between embryonic days (E)12 and 14 (Lauder and Bloom 1974; Olson and Seiger 1972; Sievers et al. 1981; Specht et al. 1981a), and NA terminals in the cerebral cortex are detectable by E16-17 (Levitt and Moore 1979; Schlumpf et al. 1980; Specht et al. 1981b). Since the appearance of NA terminals originating in the LC precedes the development of their target neurones (Lauder and Bloom 1974; Sievers et al. 1981; Yamamoto et al. 1977), a regulatory role of the LC in brain development of the neocortex has been proposed (Blue and Parnavelas 1982; Felton et al. 1982; Maeda et al. 1974; Parnavelas and Blue 1982; Wendlandt et al. 1977).

Based on this view, using morphological and biochemical methods (Coyle 1977; Jonsson 1976; Levitt and Moore 1979; Sievers et al. 1981), the ontogeny of the LC has been intensively studied. Knowledge of the electrophysiology of LC neurones in the developing brain is also essential for understanding the role of the LC in brain development. The electrophysiological properties of developing LC neurones have been studied in vitro preparations (Finlayson and Marshall 1985, 1986; Marshall and Williams 1986; Masuko et al. 1986). However, direct in vivo recordings have not been undertaken due mainly to technical difficulties. Recently, we succeeded in recording the single-unit activity of LC neurones in newborn rats (Kimura and Nakamura 1985; Nakamura et al. in press). Although the majority of LC neurones in neonatal rats were not spontaneously active, most responded to natural somatosensory stimuli such as air puffs to the skin and tail pinches. In addition, a considerable number of LC neurones in neonatal rats were activated antidromically by electrical stimulation of the cerebral cortex. These results indicated that LC neurones are already functionally active in neonatal periods.
In the present experiments, we further extend our studies to encompass their embryonic stages. The electrical activity of single LC neurones was recorded extracellularly in fetal rats which were kept physiologically connected with their dams by an intact umbilical cord. We now report on the electrophysiology of in vivo central noradrenergic neurones in prenatal periods. Part of the present study has been published previously in abstract form (Nakamura and Sakaguchi 1986).

Materials and methods

Nine female Sprague-Dawley rats, at least 3 months old, were housed in separate cages during gestation beginning on the first day in which they were sperm positive (E1). At E18–22, dams were anesthetized with urethane (1.3 g/kg, i.p.), and supplemented as necessary during experiments. The anesthetic was injected directly to the fetuses if required, as judged by increased movement. Body temperature of mother rats was maintained at 37 ± 1° C by a heating pad. For fetal rats, warm (about 37° C) saline was frequently poured on the surface of the skin and uterus to maintain an appropriate body temperature and humid environment. Only one fetus was used from each pregnant rat. Artificial respiration (70–80 strokes/min) was performed for some of the pregnant rats, in which recording was not stable. Details on the head fixation of fetal rats are given in Fig. 1.

Stimulation

Bipolar electrodes consisting of two insulated stainless steel wires (200 μm diameter) with bared tips approximately 0.2–0.5 mm apart were used for electrical stimulation of the dorsal noradrenergic bundle (DNB) and frontal cortex (FC). The coordinates for the DNB were 0.5 mm posterior to lambda, 0.5 mm lateral to the midline and 2.8–3.0 mm deep from the cortical surface. Stimuli applied to the DNB and FC were monophasic square wave pulses of 0.5–1 ms duration with currents ranging from 0.1 to 5 mA. At the end of each experiment, the stimulating site in the DNB was marked by passing DC current of 200–300 μA for 1 to 2 s for histological verification of the position of the stimulating electrodes.

For sensory stimulation, air puffs were given to the skin of fetal rats. Since in most fetal rats only a small part of the skin was exposed from the uterus, the total skin area available for air puff stimulation was very limited. The effects of air puffs upon LC activity were only examined in rats with a relatively large amount of exposed skin.

Recording

Single-unit discharges were recorded extracellularly in the left LC by means of glass micropipettes filled with 0.5 M sodium acetate containing 2% pontamine sky blue. The dye was iontophoretically ejected at the recording site for histological verification at the completion of the experiment. The electrodes for recording LC units were inserted vertically into the brain from a point 2.0 mm posterior to lambda and 0.7 mm lateral to the midline. As previously described, the location of the LC during recordings was determined by the appearance of a field response evoked by DNB stimulation (Nakamura 1977; Nakamura and Iwama 1975). In addition, the presence of a short train of multiple units with small amplitudes following FC stimulation was useful for confirming that the recording electrode was in the LC (see Fig. 2) (Nakamura et al. in press). Responses of LC neurones to DNB and FC stimulation were considered to be antidromic in nature provided that the following criteria were satisfied: 1) a fixed latency, 2) ability to follow high frequency stimulation (> 100 Hz) and 3) collision with spontaneous spikes. The last criterion was not examined in all experiments because the majority of fetal LC neurones had no spontaneous activity.

Fig. 1A–C. A method for head fixation of fetal rats. Pregnant dams were laid in an open box made of acrylic boards with a heating pad on the bottom. The uterus was exposed by a Caesarean section and partially cut for exposure of the fetal head (A, B). The head was supported by placing an acrylic board below the fetus’ jaw, and the head was attached to a conventional stereotaxic apparatus using a simple device made with a small stainless steel tube and a “U-shaped” piece of stainless steel wire attached to both edges of the tube (C). The device was fixed to a stereotaxic apparatus by inserting slightly modified ear bars into the tube (arrow). After the skin over the cranium was removed, the rostral part of the skull was glued directly to the wire with dental cement and cyanoacrylate glue. Care was taken to assure that the skull was horizontal between bregma and lambda and that the head was not tilted to one side.

After the experiments, animals were killed by injecting an overdose of the anesthetic. The fetal brain was removed and fixed in 10% formalin. The recording site was verified histologically in 60 μm frozen sections stained with neutral red in each experiment. In 4 of 9 animals, the distance between the LC and stimulation site in the DNB was carefully measured to estimate the conduction velocities of LC axons.

Results

Electrophysiological identification of LC neurones

The location of the LC in the fetal rat was determined electrophysiologically in the same way as in the adult (Nakamura 1977; Nakamura and Iwama 1975) and neonatal rats (Kimura and Nakamura 1985; Nakamura et al. in press). A recording elec-