Exp Brain Res (1982) 46: 209–214

Experimental Brain Research

Mesencephalic and Bulbar Reticular Control of Skin Potential Responses in Kittens

B. Delerm, M. Delsaut, and J.C. Roy

Laboratoire de Psychophysiologie, C.N.R.S. – L.A. 308, Université de Lille I, F–59655 Villeneuve d'Ascq Cedex, France

Summary. Reticular command of Skin Potential Responses (SPRs) was investigated in 30 kittens between 1 and 30 days of age which had been acutely implanted under chloralose anaesthesia (40 mg/kg). The results show that (a) SPRs can be elicited through electrical stimulation of the mesencephalic reticular formation (MRF) in kittens as early as a few hours after birth. As in the adult cat, these SPRs consist of a monophasic negative wave. Up until the age of 10 days SPRs recorded from the forepaws are significantly larger than those from the hindpaws. There is no difference in amplitude between ipsilateral and contralateral responses, relative to the stimulation site. (b) The first of a pair of MRF stimuli is followed by a subnormal period of 50 s, during which a second MRF stimulus of the same intensity evokes SPR of lower amplitude. (c) Stimulation of certain parts of the bulbar reticular formation (BRF) inhibits the SPRs evoked through MRF stimulation. The average percentage of inhibition was 65% in 10 kittens aged from 1 to 15 days.

These results suggest that the reticular centers which control electrodermal activity are functional at birth and that certain characteristics of electrodermal activity are subject to post-natal maturation.

Key words: Skin Potential Responses – Kitten – SPRs reticular control – Bulbar inhibition

Skin Potential Responses (SPRs) which are associated with the activity of sweat glands (Edelberg 1972; Venables and Christie 1980) are an index of reticular formation activation (Bloch 1965). Electrical stimulation of the reticular formation, from the medulla to the mesencephalon, evokes SPRs with low thresholds on all four paws, even after precollicular section (Bloch et Bonvallet 1960; Bloch 1965). The SPR is regulated centrally by the hypothalamus (Wang and Richter 1928; Langworthy and Richter 1930; Bloch 1965) as well as by the limbic cortex (Isamat 1961) and the sensori-motor cortex (Wang and Lu 1930; Wilcott 1969). Furthermore, in the anaesthetized cat, electrical stimulation of some areas of the ventromedial bulbar reticular formation inhibits SPRs evoked either by stimulation of a sensory nerve (Wang and Brown 1956) or of other parts of the reticular formation (Bloch 1965; Roy et al. 1974). In the absence of anaesthesia, this bulbar inhibition of SPRs is either reduced or disappears altogether (Roy et al. 1974).

The ontogenetic evolution of the central control of SPRs has not been investigated to date, and it is not yet known whether this system matures after birth or not. Yamazaki et al. (1969, 1970) have described spontaneous electrodermal activity in the kitten during sleep and waking. They found that the amplitude of spontaneous SPRs diminishes between the age of 8 and 55 days and attributed this decrease to the maturation of an inhibitory, perhaps cortical mechanism, not yet functional at birth.

The purpose of the present study was to examine whether the control mechanisms of electrodermal activity, especially those originating in the reticular formation, do mature during the first month of life. For this purpose the effects of mesencephalic and bulbar stimulations on SPRs were studied in kittens, from birth to the age of 30 days.

Methods

Subjects

Thirty kittens between 1 and 30 days of age were used (100–360 g body weight). They were anaesthetized with alpha chloralose

Offprint requests to: B. Delerm, MD (address see above)

0014–4819/82/0046/0209/$ 1.20

(40 mg/kg i.p.) and additional doses were given as required. They were then fixed in a standard stereotaxic apparatus with small tapered ear bars. Since the ear canals do not usually open before 12 days of age (Olmstead and Villablanca 1980), they were opened surgically in younger kittens. Coordinates for stereotaxic surgery were calculated with reference to the ear bar zero, according to the atlas of Rose and Goodfellow (1973). It should be noted that perfect adjustment into the stereotaxic apparatus is difficult since the skull is fragile in very young animals. A certain error was therefore unavoidable. The experimental room was kept at 25° C, and the animals' central temperature was maintained around 37–38° C by means of an electric heating pad.

Stimulating and Recording Procedures

Following craniotomy two bipolar concentric electrodes (0.4 mm in diameter; 0.5 mm distance between tip and core) were lowered into the brain-stem to allow stimulation of the Mesencephalic Reticular Formation (MRF) and Bulbar ventromedial Reticular Formation (BRF). Stimuli were rectangular pulses with the following parameters: (a) for MRF: pulse duration 0.2 ms, 300 per s, total train duration 250 ms; (b) for BRF: pulse 0.5 ms, 100 per s, total train duration 5–8 s. The intensity range was 0.1 mA to 1 mA. SPRs were recorded from the central pad of the paws by means of Beckman miniature electrodes filled with electrolytic paste. The paws were cleaned with ether-alcohol and dried before fixing the electrodes. A hypodermic needle was inserted under the skin a few mm above the pad and used as reference electrode. SPRs were recorded from one paw in eight kittens and from two or more paws in 22 kittens (SPRs from one forepaw and one hindpaw were recorded in 18 animals). They were displayed on a polygraph with either DC coupling or with a time constant set at 3.5 s. The position of the tip of the stimulating electrodes was histologically verified in seven kittens. The brain was perfused with physiological saline followed by 10% formaldehyde and removed. After embedding the brain-stem in paraffin, 15 μm serial sections were cut and stained by cresyl-violet.

Results

SPRs Elicited Through Mesencephalic Stimulation

Age. SPRs could be evoked through short train stimulation of the mesencephalic tegmentum (see Methods) as early as a few hours after birth. Figure 1 shows SPRs recorded from the forepaws and hindpaws in a newborn kitten. Stereotaxic exploration of the brain-stem showed that the most effective coordinates for evoking SPR with low intensity stimulation (about 0.1 mA) corresponded to the mesencephalic reticular formation (L2–L3, H0 to H-4, A2 to A3.5). The histological controls showed this location to be correct (see Fig. 2A from a one-day-old kitten).

Morphology and Latency. When recorded with DC amplification, SPRs appear as a negative monophasic deflexion; their morphology is thus identical to that in the adult cat. Latencies were calculated using stimulation evoking SPRs of maximal amplitude (stimulation intensity ranging from 0.3 to 0.7 mA).

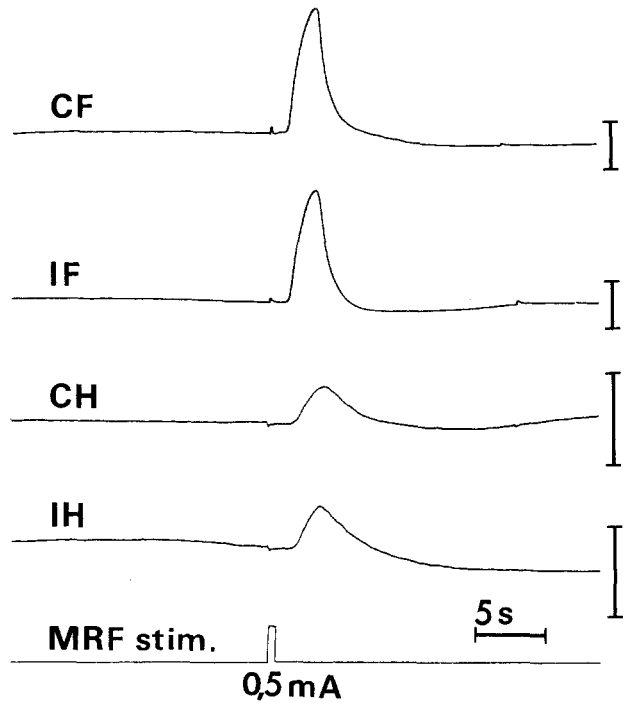

Fig. 1. SPRs evoked on the four paws by MRF stimulation (300/s, 0.2 ms, 250 ms, 0.5 mA) in a 2-day-old kitten. (I = Ipsilateral; C = Contralateral; F = Forepaw; H = Hindpaw). Note the difference in amplitude in SPRs evoked on forepaws and hindpaws. (Responses recorded with DC coupling). Calibration: 1 mV

They were measured from the beginning of stimulation to the beginning of the ascending limb. No significant difference could be found between latencies of SPR recorded from the forepaws ipsilateral ($\overline{m}$ = 1.59 s) and contralateral ($\overline{m}$ = 1.65 s) to the site of stimulation; the same is true for both hindpaws ($\overline{m}$ = 1.82 s for ipsilateral hindpaws; $\overline{m}$ = 2.3 s for contralateral hindpaws). Consequently, the data obtained from both forepaws were pooled, as were those obtained from the hindpaws. The mean latencies were 1.6 s (σ = 0.54) for SPRs recorded from forepaws (N = 24 kittens) and 2.06 s (σ = 0.72) for SPRs from hindpaws (N = 14 kittens). The difference is statistically significant (t = 4.45, $p < 0.01$). However, no correlation between age and latencies could be established.

Amplitude. The variations in SPR amplitude were examined as a function of the stimulation intensity (other parameters being kept constant). It was observed that low amplitude SPRs appeared at a certain threshold, and that their amplitude then increased with increasing stimulus intensity. Above a certain intensity, the amplitude reached a maximum and no longer increased (see Fig. 3, taken from a 7-day-old kitten). The intersubject dispersion was

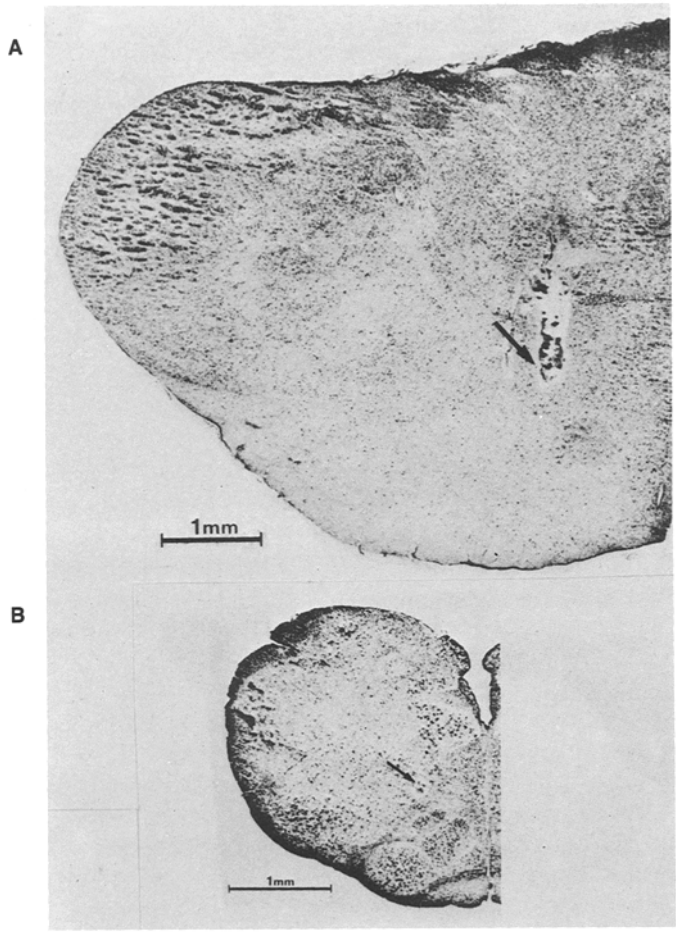

Fig. 2. **A** Location of the electrode tip (*arrow*) in MRF. Coordinates: A = 3; L = 1.7; H = –2 (1-day-old kitten). **B** Location of the electrode tip in BRF. Coordinates: P = 8; L = 0.5; H = –7. Stimulation at this point resulted in inhibition of SPRs evoked by MRF stimulation (4-day-old kitten)

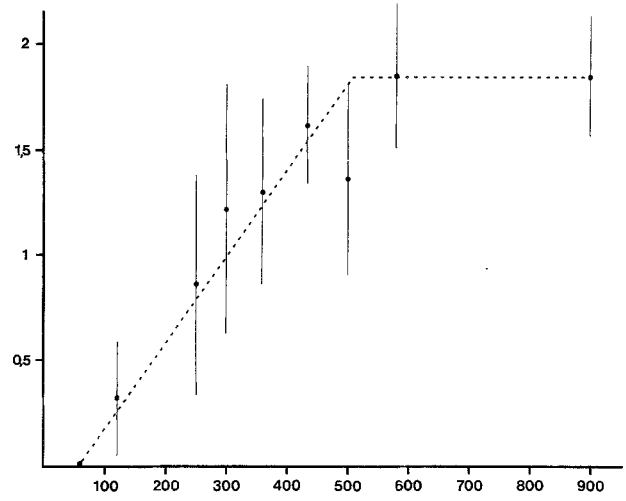

Fig. 3. Mean amplitudes and standard deviations of SPRs evoked by MRF stimulation as a function of stimulation intensity in a 7-day-old kitten. Abscissae: intensity of mesencephalic stimulation in μA; ordinates: SPR amplitude in mV

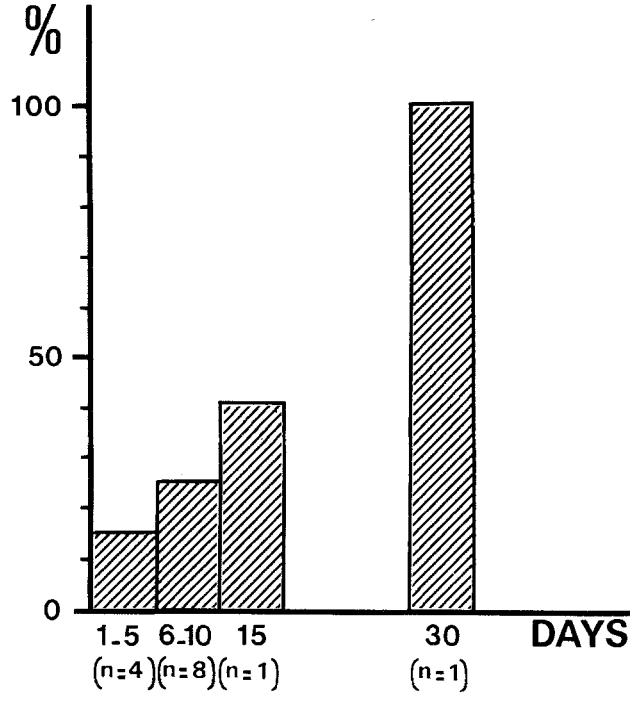

Fig. 4. Evolution of the SPRs amplitude on the hindpaws compared with SPRs amplitude on the forepaws. The hindpaw SPRs amplitude is expressed in the ordinates as the percentage of the forepaw SPRs amplitude in each kitten (n = number of kittens for each age interval)

high: in our anaesthetized kittens, the mean SPR amplitude, for a maximal stimulation, ranged from 0.15 mV to 5.4 mV with no significant difference between paws ipsi- or contralateral to the site of stimulation ($t = 0.60$, N = 14 kittens for forepaws; $t = 0.80$, N = six kittens for hindpaws). The amplitude of SPRs recorded from forepaws was also compared to that recorded on hindpaws. In four of the youngest kittens (less than one week old) mesencephalic stimulation never evoked any SPR on the hindpaws, although it did so on the forepaws. When SPRs existed on all pads, the maximal amplitude of forepaw SPRs was usually higher than the maximal amplitude of hindpaw SPRs (Fig. 1). In 14 kittens, SPRs were thus recorded from both hindpaws and forepaws. For the intervals of 1–5 days (n = 4) and of 6–10 days of age (n = 8), the difference between forepaws and hindpaws was significant (respectively, $t = 20.80$ and $t = 11.34$, $p < 0.01$). Only one animal was tested at the age of 15 days and one at the age of 30 days. Although data from these animals suggest an amplitude increase with age, no conclusion can be drawn (see Fig. 4).

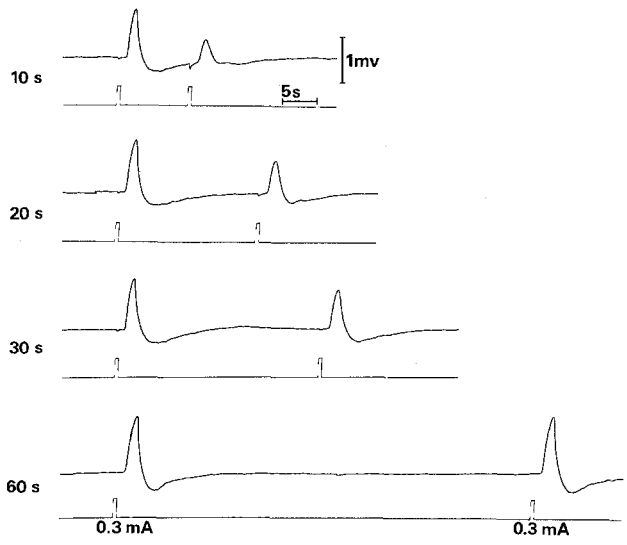

Fig. 5. Subnormal period of SPRs evoked by MRF stimulations in a 6-day-old kitten. The two identical stimulus trains are separated by intervals of 10, 20, 30, and 60 s; the amplitude of the SPR evoked by the second stimulation is 47%, 60%, 77%, and 100% of the control, respectively. (Responses recorded with AC coupling: time-constant = 3.5 s)

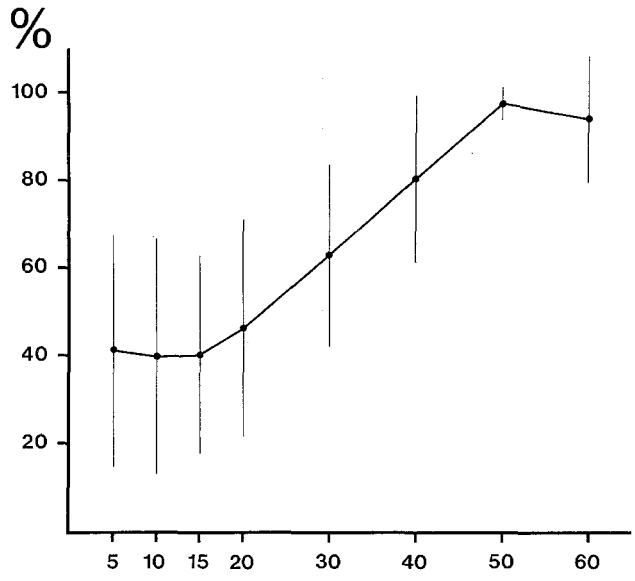

Fig. 6. Mean amplitudes and standard deviations of SPRs evoked by a second MRF stimulus train, as a function of the interval between the two trains. Abscissae: intertrain interval in s. Ordinates: amplitude of the second SPR expressed in % of the control SPR amplitude. (n = 15 kittens; age = 1–14 days)

Paired Mesencephalic Stimulations. The influence of the interval between two successive trains of MRF stimuli of the same intensity on the amplitude of the response to the second train was investigated. The interstimulus (i.e. "intertrain") interval varied from 5 to 60 s and the stimulus pairs were separated by an interval of several minutes. The results obtained in a 6-day-old kitten are shown in Fig. 5: Following the first MRF stimulus, it was only after about 50 s that a second shock would elicit an SPR of equal amplitude to that evoked by the first one. Figure 6 shows the mean curve plotted from the results on 15 kittens aged between 1 and 14 days. As a whole, it was similar to that established for the anaesthetized adult cat (Bloch 1965); however, it was different for the shorter intervals (5, 10, and 15 s), where the second SPR was less depressed than in the adult.

Inhibition of SPRs Through Bulbar Stimulation

In this series of experiments, control SPRs were first evoked by MRF stimulation. The bulbar reticular formation was then stimulated repetitively (see Methods). The intensity of the bulbar stimulation was usually lower than the intensity of the MRF stimulation. The amplitude of the control SPR was compared to that of an SPR evoked by a similar stimulus delivered 1 s after the beginning of the bulbar stimulation. All stimulations were separated

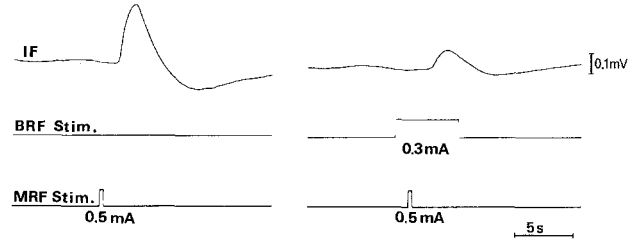

Fig. 7. Bulbar inhibition of SPR in a 4-day-old kitten. MRF stimulation (*left*) elicits a control SPR; the same stimulation applied during BRF repetitive stimulation (50/s, 0.5 ms, 5 s) elicits a SPR with an amplitude of only 30% of the control SPR (*right*). IF = Ipsilateral Forepaw. (AC coupling: time-constant = 3.5 s)

by intervals longer than 1 min to avoid the 50 s subnormal period described above. For each animal, the ratio between the amplitude of SPRs evoked during bulbar stimulation and the control amplitude was converted into an inhibition percentage. Only data from kittens whose inhibition percentage was over 50% were retained. Inhibition percentages for each of the ten kittens are reported in Table 1. Figure 7 shows the typical effect of bulbar inhibitory stimulation on SPRs in a 4-day-old kitten. The difference between the mean amplitude of control SPRs ($\bar{m}$ = 1.14 mV) and that of inhibited SPRs ($\bar{m}$ = 0.35 mV) was significant ($t = 4.50$; $p < 0.01$); the mean inhibition percentage was 65%. Further-

Table 1. Individual percentages of SPRs inhibition following bulbar stimulations (see text). MRF train delivered 1 s after initiation of bulbar repetitive stimulation

Kitten n°	Age in days	Percentage of inhibition
1	1	57
2	2	70
3	2	58
4	4	84
5	5	50
6	6	55
7	6	77
8	10	69
9	10	80
10	15	50

more, Table 1 shows that, between 1 and 15 days, there was no relationship between the inhibition ratio and the age of the subjects. The effective stereotaxic coordinates for inhibiting SPRs corresponded to the bulbar reticular formation (L0.4–L1, H-5 to H-8 and P5 to P9). Histological controls showed that the tip of the electrode had been introduced into the caudal part of the nucleus reticularis gigantocellularis or into the nucleus reticularis ventralis (Fig. 2B). This electrode position showed itself to be a critical factor; shifting the electrode tip as little as 0.5 mm often sufficed to make the inhibitory effect disappear completely. When delivered alone, bulbar inhibitory stimulation did not evoke any SPR, neither at its beginning nor its end.

Discussion

Our results show that there are reticular control mechanisms of SPR at birth and that their pathways are functional at this time. In the kitten, SPRs recorded with DC coupling are monophasic negative responses, as is the case in adult cats (Niimi 1968; Yamazaki et al. 1975). This early appearance of SPRs in the kitten can be compared to that in the human infant who is also very immature at birth. In premature infants Curzi-Dascalova et al. (1973) have shown that spontaneous SPRs appear as early as 7 months of conceptional age. It is also known that the innervation of sweat glands of the palm and the sole is present as early as 4,5 months of conceptional age (Ellis 1967) and that myelinisation of reticulospinal pathways is completed at birth (Langworthy 1933; Minkowski 1938).

However, under our experimental conditions, SPRs recorded from forepaws and hindpaws were found to differ in amplitude up to 10 days of age. This difference, and the fact that it is frequently impossible to evoke SPRs from hindpaws in the youngest animals seems to be due to a lag in maturation, the origin of which is, however, unknown. Similar differences have been observed in premature human infants: in the most immature subjects (28 weeks of conceptional age) spontaneous SPRs were more easily recorded and more consistently obtained from the upper extremities (Curzi-Dascalova et al. 1973). More precisely, the authors stated that 31% of spontaneous SPRs were larger in the upper extremities than in the lower, and that only 1.5% had higher amplitudes in the lower extremities.

The results obtained with paired mesencephalic stimulation trains show that the amplitude of SPRs is depressed, in anaesthetized kittens, for about 50 s after the first stimulus. This depression does not seem to be related to exhaustion of the sweat glands, which would have to be refilled after the first response. In the adult cat, we found that two stimuli only 5 s apart, applied to the distal end of the sectioned medial nerve could evoke two SPRs of equal amplitude (unpubl. observ.). Therefore, this depression probably originates at the central level. In the unanaesthetized intact animal, a double mesencephalic stimulation elicits responses of equal amplitude, even with an interval of less than 10 s (Bloch 1965). In the anaesthetized animal, each stimulation train produces a long lasting depression, which remains unchanged after sectioning the brain just below the diencephalon. An inhibition originating from the cortex can therefore be excluded. From his data with sectioning at various levels of the brainstem, Bloch (1965) hypothetized that the post-stimulus depression was the result of an inhibitory bulbar reticular mechanism. However, no direct evidence has yet been found in favour of this hypothesis.

Our results show that SPR inhibitory mechanisms, localized within the bulbar reticular formation, are functional in new-born kittens. Simultaneous stimulations of MRF and BRF resulted in an average inhibition percentage around 65%. Although this percentage is high, it does not reach the 85–90% level normally observed in the adult cat under deep anaesthesia (Roy et al. 1974). In an attempt to interpret this difference, the question of post-natal maturation of the bulbar inhibitory center is raised. As mentioned above, the second SPR evoked by the double MRF train is less depressed in the kitten when shorter intervals are used, than in the adult. This leads us to suggest that these differences might reflect the incomplete maturation of bulbar structures in the kitten.

References

Bloch V (1965) Le contrôle central de l'activité electrodermale. J Physiol (Paris) [Suppl 13] 57: 132

Bloch V, Bonvallet M (1960) Le déclenchement des réponses électrodermales à partir du système réticulaire facilitateur. J Physiol (Paris) 52: 25–26

Curzi-Dascalova L, Pajot N, Dreyfus-Brisac C (1973) Spontaneous skin potential responses in sleeping infants between 24 and 41 weeks of conceptional age. Psychophysiology 10: 478–487

Edelberg R (1972) Electrical activity of the skin: Its measurement and uses in psychophysiology. In: Greenfield NS, Sternbach RA (eds) Response systems. Holt, Rinehart and Winston, New York (Handbook of psychophysiology, pp 367–418)

Ellis RA (1967) Eccrine sweat glands: Electron microscopy, cytochemistry and anatomy. In: Jadassohn J (Hrsg) Normale und Pathologische Anatomie der Haut. Springer, Berlin Heidelberg New York (Handbuch der Haut- und Geschlechtskrankheiten, Bd I, S 224–266)

Isamat F (1961) Galvanic skin responses from stimulation of limbic cortex. J Neurophysiol 24: 176–181

Langworthy OR (1933) Development of behavior patterns and myelinization of the nervous system in the human fetus and infant. Contrib Embryol Carnegie Inst 24: 3–57

Langworthy OR, Richter CP (1930) The influence of efferent cerebral pathways upon the sympathetic nervous system. Brain 53: 178–193

Minkowski M (1938) Les étapes du développement psychique; organisation somatopsychique. Encyclopédie française, 8

Niimi Y (1968) Spontaneous variations of the skin potential reflexes during natural sleep in the cat. Jpn J Physiol 18: 190–197

Olmstead CE, Villablanca JR (1980) Development of behavioral audition in the kitten. Physiol Behav 24: 705–712

Rose GH, Goodfellow EF (1973) A stereotaxic atlas of the kitten brain. Coordinates of 104 selected structures. Brain Information Service. Brain Research Institute, University of California, Los Angeles, CA, USA

Roy JC, Delerm B, Granger L (1974) L'inhibition bulbaire de l'activité électrodermale chez le chat. Electroencephalogr Clin Neurophysiol 37: 621–632

Venables PH, Christie MJ (1980) Electrodermal activity. In: Martin I, Venables PH (eds) Techniques in psychophysiology. Wiley, Chichester

Wang GH, Brown VW (1956) Suprasegmental inhibition of an autonomic reflex. J Neurophysiol 19: 564–572

Wang GH, Lu TW (1930) Galvanic skin reflex induced in the cat by stimulation of the motor area of the cerebral cortex. Chin J Physiol 4: 303–324

Wang GH, Richter CP (1928) Action currents from the pad of the cat's foot produced by stimulation of the tuber cinereum. Chin J Physiol 2: 279–284

Wilcott RC (1969) Electrical stimulation of the anterior cortex and skin potential responses in the cat. J Comp Physiol Psychol 69: 465–472

Yamazaki K, Tajimi T, Niimi Y (1969) The ontogeny of spontaneous skin potential responses in kittens during awake rest. Jpn Psychol Res 11: 167–173

Yamazaki K, Tajimi T, Niimi Y (1970) The ontogeny of spontaneous skin potential responses during natural sleep in kittens. J Phys Soc Jpn 32: 606–616

Yamazaki K, Tajimi T, Okuda K, Niimi Y (1975) Skin potential activity in rats, cats and primates (including man): A phylogenetic point of view. J Comp Physiol Psychol 89: 364–370

Received June 20, 1981