POST-NATAL DEVELOPMENT OF THE CEREBELLO-CEREBRAL PROJECTION IN KITTENS

By S. KAWAGUCHI, A. SAMEJIMA* and T. YAMAMOTO*

From the Institute for Brain Research, Faculty of Medicine, Kyoto University, 606 Kyoto, Japan

SUMMARY

1. Post-natal development of the cerebello-cerebral response was investigated in 126 kittens from birth to 142 days of age by analysis of laminar field potentials in the cerebral cortex; the thalamocortical projection mediating the cerebello-cerebral response was examined on four new-born and three one-month-old kittens by means of anterograde axonal transport of horseradish peroxidase.

2. A marked response was evoked in the frontal motor cortex from birth and an appreciable response could be evoked in the parietal association cortex at 2 days after birth. The latency of response in the frontal cortex decreased sharply from birth till 3 weeks of age whereas that in the parietal cortex remained almost unchanged until 2 weeks of age. Maturation of the cerebello-cerebral projection, in every respect, proceeds earlier in the frontal cortex than in the parietal cortex.

3. The cerebello-cerebral response in kittens at any age, like in adult cats, consisted of two types of elementary responses: one which is characterized by a surface positive–depth negative (s.p.–d.n.) wave and the other which is characterized by a surface negative–depth positive (s.n.–d.p.) wave. The response in the frontal cortex was a sequential occurrence of the two waves while the response in the parietal cortex was a pure form of the s.n.–d.p. wave.

4. Two types of thalamocortical projections corresponding to the two types of elementary responses were revealed: one is the projection mainly onto layer I which appears to mediate the s.n.–d.p. wave and the other is the projection mainly onto layer III which appears to mediate the s.p.–d.n. wave.

5. Development of the cerebello-cerebral response and changes in the terminal distribution of the thalamocortical projection during maturation are consistent with the principle of ontogenesis of the mammalian neocortical organization, i.e. ascending sequential maturation.

INTRODUCTION

Ontogenesis of the cerebellum is known to be the latest in the central nervous system. In altricial animals such as the mouse, the rat and the cat, mitosis and migration of the granule cell and synaptogenesis of the neuronal circuitry in the

* Present address: Department of Physiology, Fukui Medical School, 910–11 Fukui, Japan.

cerebellar cortex are for the most part a post-natal occurrence on which a wealth of morphological and electrophysiological studies have been reported (Altman, 1972a, b; Jacobson, 1978; Shimono, Nosaka & Sasaki, 1976). However, the ontogenesis of the deep cerebellar nuclei and cerebellofugal system has not been well studied yet. There are a few morphological studies on the deep cerebellar nuclei (Taber Pierce, 1975; Altman & Bayer, 1978) but no electrophysiological studies on these subjects.

The present paper describes the post-natal development of the cerebello-cerebral projection in kittens from birth, which has been studied mostly electro-physiologically and partly morphologically. The experiments were concerned particularly with ontogenesis of the thalamocortical response. Sasaki, Staunton & Dieckmann (1970) proposed the concept that thalamocortical responses are composed of two elementary responses, i.e. the superficial and deep thalamocortical responses which are characterized by a surface negative–depth positive (s.n.–d.p.) wave and a surface positive–depth negative (s.p.–d.n.) wave respectively. A previous study in adult cats (Sasaki, Kawaguchi, Matsuda & Mizuno, 1972) has shown that stimulation of the cerebellar nuclei induces a pure form of the superficial thalamo-cortical response in the parietal association cortex and a sequential occurrence of both responses in the frontal motor cortex. We simply guessed that the cerebello-cerebral projection, like the neuronal circuitry of the cerebellar cortex, would be formed mostly post-natally and expected that our experiment would shed light on the emergence and development of the two thalamocortical responses. Somewhat surprisingly our results demonstrate that a powerful cerebello-cerebral projection is present in kittens from birth and that two thalamocortical responses are present which are basically the same as those in adult cats.

METHODS

Electrophysiological experiments were carried out on 126 kittens from birth to 142 days of age. Under sodium pentobarbitone anaesthesia (35–40 mg/kg, I.P.) the trachea and the saphenous vein were cannulated. Sodium pentobarbitone was intermittently administered intravenously to maintain anaesthesia during the experiments. The level of anaesthesia was monitored by electro-corticogram. Respiration was always natural. The head was fixed to a stereotaxic apparatus for an adult cat with a minor modification for the small size of the animals. Craniotomy for exposure of the frontal and parietal cortices and the cerebellum was performed with painstaking care to minimize bleeding and to avoid pressure on the cortical surface.

Three sets of concentric electrodes (stainless-steel, outer diameter 0·3 mm, core diameter 0·1 mm, interpolar distance 0·2 mm) for stimulation of the three cerebellar nuclei and a pair of similar electrodes for the thalamic ventral anterior (v.a.) and ventral lateral (v.l.) nuclear complex were placed stereotactically according to scaled down coordinates. Usually a brief single pulse current (0·2–0·5 mA; 0·3 ms) was passed through the electrodes, but at times a train of two or three pulses at 1·0 ms interval was employed to elicit a marked response. A weak stimulation (0·05 mA for 0·1 ms) was occasionally applied to check a current spread. A glass micropipette filled with Fast Green FCF in 2 M Na-acetate was introduced into the cortex perpendicularly to the cortical surface for recording laminar field potentials. A silver-ball electrode was placed on the pia for monitoring the cortical surface response. Recording sites were marked by passing 10 μA of cathodal current through the glass micropipette for 10 min. After the experiment the animal was deeply anaesthetized and the brain was perfused with 10 % formalin solution via the aorta. The marked recording sites and the tracks of the stimulating electrodes were subsequently examined histologically.

Morphological experiments were performed in four kittens of 0–5 days of age and three kittens of 28–36 days of age. Each animal was injected with horseradish peroxidase (HRP) into the thalamic

v.a.–v.l. nuclear complex for anterograde labelling of the thalamocortical projection which mediates the cerebello-cerebral response. The injections were carried out ionophoretically by passing 5 μA of anodal current for 30 min through a glass micropipette filled with a 5 % solution of HRP-conjugated wheat germ agglutinin (Sigma) dissolved in 0·1 M-Tris buffer. Kittens were anaesthetized with sodium pentobarbitone (40 mg/kg, I.P.) and potentials were recorded from the thalamic v.a.–v.l. nuclear complex by the glass micropipette on stimulation of the lateral or the interpositus nucleus of the cerebellum. Then, HRP was injected at the site where a large negative potential with unit discharges had been recorded. After a survival period of 24–30 h the animals were anaesthetized deeply and were perfused through the ascending aorta with 200 ml of 7 % formalin in 0·1 M phosphate buffer (pH 7·6). The brains were removed rapidly, soaked in 0·1 M phosphate buffer containing 30 % sucrose and kept for 2–4 days in a refrigerator. Then, the thalamus, frontal (areas 4 and 6) and parietal cortices (areas 5 and 7) were cut serially at 60 μm in the frontal plane on a freezing microtome. Sections were treated with benzidine dihydrochloride and hydrogen peroxide according to the method of DeOlmos & Heimer (1977).

RESULTS

Post-natal development of the cerebello-cerebral response in the frontal motor cortex

In kittens, as in adult cats (Sasaki *et al.* 1972), stimulation of the fastigial nucleus could not induce any detectable response but stimulation of either the lateral or the interpositus nucleus induced a marked response in the frontal motor and parietal association cortices. The responses induced by stimulation of the lateral or the interpositus nucleus were similar and could hardly be distinguished. Even when the stimulating electrode was placed in the midst of one nucleus it was difficult to exclude the possibility that there was current spread to the other nucleus because the cerebellum in neonatal animals is so small. Therefore, we use the term 'cerebellar nucleus' instead of specifying the lateral or the interpositus nucleus in the following description of responses in the kitten.

The response in the frontal motor cortex in adult cats induced by stimulation of the lateral or the interpositus nucleus is a positive–negative diphasic wave in the superficial cortical layers and a negative–positive diphasic wave in the deep cortical layers (Sasaki *et al.* 1972). The positive and the negative wave in the superficial cortical layers correspond respectively to the negative and the positive wave in the deep cortical layers. Therefore, the response consists of two electrical dipoles of the opposite direction, i.e. the surface positive–depth negative (s.p.–d.n.) wave and the surface negative–depth positive (s.n.–d.p.) wave. These two wave components were observed in kittens from birth onwards.

Fig. 1*A* illustrates field potentials recorded from the medial portion of the precruciate gyrus in a kitten 5–10 h after birth. They are: a small positive wave followed by a large negative wave in the superficial cortical layers and a large negative wave followed by a small positive wave in the deep cortical layers. Unit discharges superimposed on the rising phase of the depth-negative wave are seen at depths of 800 and 1000 μm. Similar responses were evoked in all four other kittens examined on the first day post-partum (0 day old). Fig. 1*B* illustrates a response recorded from a 3-day-old kitten which is a pair of negative waves in the superficial cortical layers and a negative wave followed by a pair of positive waves in the deep cortical layers. The early and the late component of this pair of waves show the same time course and the same time-to-peak in the superficial and deep cortical layers (compare the potentials at depths above 1000 μm with those below 800 μm). The potential recorded

from the cortical surface by a silver-ball electrode (the bottom row) shows an initial positive wave which corresponds to the initial negative wave in the deep cortical layers (i.e. at depths of 800 and 1500 μm). Therefore, the s.p.–d.n. wave is considered to be present despite the lack of a positive wave in the superficial cortical layers which appears to be hidden behind the large negative wave. Thus, the response is considered to be composed of an s.p.–d.n. wave followed by two s.n.–d.p. waves. The occurrence of the late s.n.–d.p. wave is characteristic of animals younger than 10 days of age
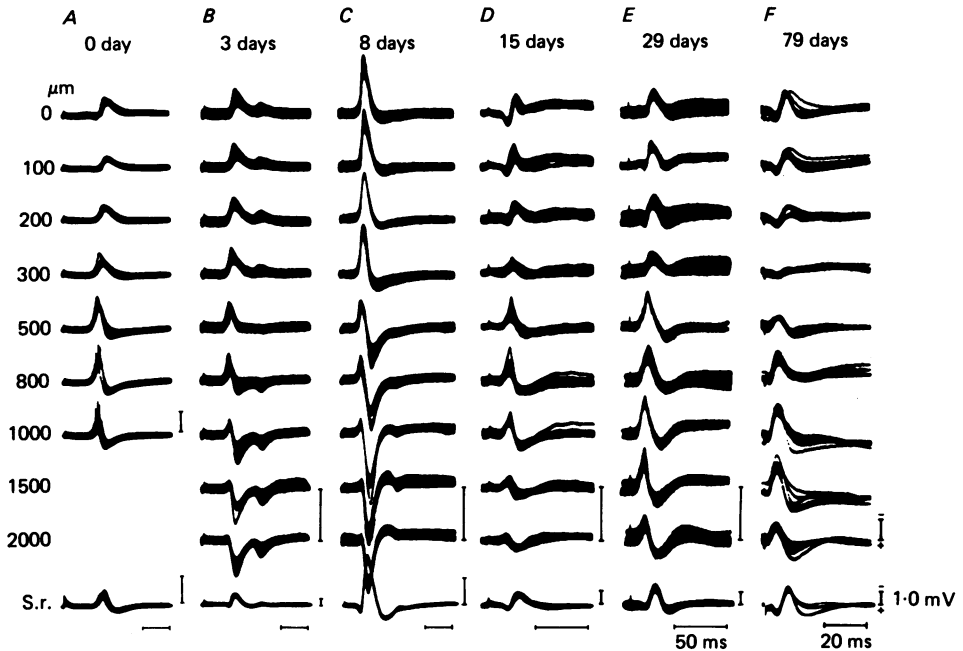

Fig. 1. Post-natal development of the cerebello-cerebral response in the frontal motor cortex. Laminar field potentials recorded from the precruciate gyrus of six kittens ranging from birth to 79 days of age. In this and other Figures, numerals to the left of specimen records are depths (μm) from the cortical surface; the bottom surface record (s.r.) was recorded from the cortical surface by a silver-ball electrode near to respective micro-electrode tracks; six traces were superimposed in each record; negativity is upward. Voltage calibrations are 1·0 mV for all records and time calibrations are 50 ms for A–E and 20 ms for F.

(Fig. 1B and C) and was observed not only in the frontal motor cortex but also in the parietal association cortex (Fig. 2B and C). In such animals the earlier of the two s.n.–d.p. waves is much larger than the s.p.–d.n. wave which precedes it.

Responses recorded from kittens of 15, 29, and 79 days of age are shown in Fig. 1D–F respectively. The basic configuration of the response in these older kittens is similar to that of the younger animals. However, as the animals grow older, the s.p.–d.n. wave becomes larger and the s.n.–d.p. wave becomes smaller. Therefore, at cortical depths below 1000 μm, a negative potential is dominant in the older kittens (E and F) whereas a positive potential is dominant in the younger animals (B and C).

In adult cats both the s.p.–d.n. and s.n.–d.p. waves reverse polarity at a depth of about 200 μm though the reversal depth is always slightly deeper for the s.p.–d.n. wave than for the s.n.–d.p. wave (cf. Sasaki *et al.* 1972). The level of such potential reversal is much deeper in kittens as compared with adult cats. In the response recorded from a 79-day-old kitten (Fig. 1 *F*), the reversal depth is between 300 and 500 μm for the s.p.–d.n. wave and 300 μm for the s.n.–d.p. wave. In younger kittens, it is rather difficult to determine the level of potential reversal because the time-to-peak of the negative wave shifts in the mid layers. For example, as shown in Fig. 1 *B* and *C*, the time-to-peak of the negative wave at depths below 800 μm is fixed and is the same as that of the positive wave on the cortical surface. However, the time-to-peak of the negative wave at depths above 500 μm is gradually delayed as the depth becomes more superficial and it finally coincides with the time-to-peak of the s.n.–d.p. wave. When the depth of potential reversal is assumed to be the level at which the shift of time-to-peak of the negative wave occurs, it is very deep in neonatal kittens and becomes more superficial with the advance of age. The late s.n.–d.p. wave in Fig. 1 *C* apparently reverses polarity at a depth of 500 μm where the time-to-peak of the negative wave begins to delay.

Post-natal development of the cerebello-cerebral response in the parietal association cortex

The cerebello-cerebral response in the parietal association cortex (areas 5 and 7) in kittens, like that in adult cats (Sasaki *et al.*, 1972), is negative in the superficial cortical layers and positive in the deep cortical layers.

In two kittens at birth, stimulation of the cerebellar nucleus induced a very small potential which could be recorded only from the cortical surface by a silver-ball electrode (Fig. 2 *A*) whereas a marked response was evoked in the frontal motor cortex in the same animal (Fig. 1 *A*). Illustrated in Fig. 2 *B* and *C* are responses recorded from the 3- and 8-day-old kittens whose frontal cortical responses are shown in Fig. 1 *B* and *C* respectively. The responses are composed of two s.n.–d.p. waves. Both waves change polarity at a depth between 300 and 800 μm. The parietal cortical response consists of a pure form of the s.n.–d.p. wave irrespective of the animal's age. However, the cortical depth at which potential reverses polarity differs remarkably between new-born and older kittens. In young animals, particularly before 2 weeks of age, the potential reverses polarity gradually over a wide range of cortical depth. The depth of potential reversal in Fig. 2 *B* may be assumed to be 500 μm but the potential at this depth differs little from those at 400 and 600 μm (not illustrated). In contrast to the responses of the young kittens, those of the older animals (Fig. 2 *E* and *F*) reverse polarity over a narrow range of cortical depths. For example, in the 79-day-old kitten (Fig. 2 *F*), a marked negative or positive potential was recorded when a micro-electrode was moved up or down by as little as 100 μm (not illustrated) from a depth of 300 μm which was the level of zero potential. The level of potential reversal for the parietal cortical response, as for the frontal cortical response, was deep in the young kittens (Fig. 2 *B* and *C*) and became more superficial in older animals (Fig. 2 *E* and *F*). In animals younger than 2 weeks of age, coexistence of positive and negative waves was frequently observed in the mid layers (e.g. Fig. 2 *C*, at a depth of 500 μm).
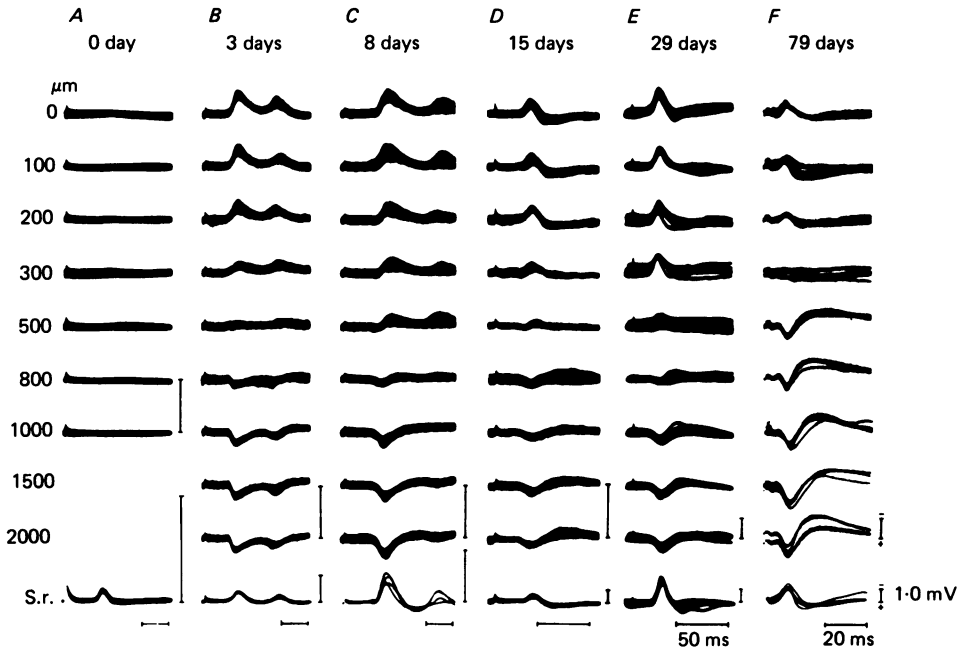
Fig. 2. Post-natal development of the cerebello-cerebral response in the parietal association cortex. Laminar field potentials recorded from the rostral portion of the middle suprasylvian gyrus of the same six kittens shown in Fig. 1. Voltage calibrations are 1·0 mV for all records and time calibrations are 50 ms for A–E and 20 ms for F.
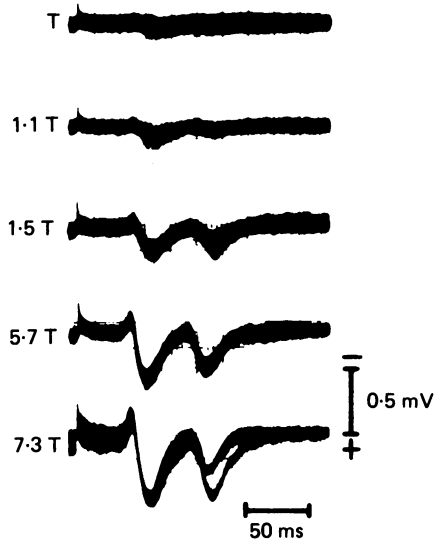

Fig. 3. The early and late s.n.–d.p. wave tested by graded stimulation. Responses were recorded from the precruciate cortex at a depth of 1500 μm by graded stimulation of the cerebellar nucleus in an 8-day-old kitten. Intensity of stimulation relative to threshold (T) is given to the left of specimen records. Threshold was almost the same for both the early and late s.n.–d.p. waves and the two waves increased their magnitude equally with increase in the intensity of stimulation.

The late s.n.–d.p. wave in the frontal and parietal cortices

The late s.n.–d.p. wave was observed in fifteen out of seventeen kittens before 10 days of age in both the frontal and parietal cortices but could seldom be evoked after 12 days of age. To elucidate the neuronal connexions responsible for this wave, graded electrical stimulation was employed.

Fig. 3 illustrates the potentials evoked in the precruciate cortex at a depth of 1500 μm by graded stimulation of the cerebellar nucleus. The threshold (T) for the late s.n.–d.p. wave is almost identical to that of the early wave complex and the magnitude of the two responses increases *pari passu* with increase in the stimulus intensity from threshold to supramaximal levels (7·3 T). Thus, the neuronal connexion responsible for the late s.n.–d.p. wave is unlikely to be slowly conducting cerebello-thalamic fibres but more likely to be a reverberating circuit in the thalamus or the cerebral cortex, or both. The presence of such a reverberating circuit in the thalamic relay mechanism is presumed to be the most plausible explanation since direct electrical stimulation of the thalamic nucleus could seldom elicit the late s.n.–d.p. wave, i.e. in only one out of sixteen kittens examined.

Post-natal changes in the latency of the cerebello-cerebral response

Fig. 4 shows the latencies for the cerebello-cerebral response in the frontal and parietal cortices in seventy-four kittens ranging from 10 h after birth to 142 days of age. The method of measuring latencies is shown in the inset responses. In the frontal cortex the latency to onset of the initial s.p.–d.n. response was measured together with the times-to-peak of the two s.n.–d.p. waves. In the parietal cortex onset latency and time-to-peak were measured for the first s.n.–d.p. response and time-to-peak was also measured for the late s.n.–d.p. response. The latency of the first (i.e. s.p.–d.n.) response in the frontal cortex (filled circles) decreases sharply from 40 ms at birth to 10 ms by 3 weeks and then decreases more gradually until at 6 weeks it reaches 2–3 ms which is comparable to the latency of the adult response (Sasaki *et al.* 1972). On the other hand, the latency of the first (s.n.–d.p.) response in the parietal cortex (open circles) remains almost unchanged up to 2 weeks of age, then decreases sharply until one month after which it continues to decrease slowly until a latency comparable with the adult response is attained by 2 months. The rate of decrease in latency in the two cortices is about 1·5 ms per day during the period of greatest change. This rate of decrease is all the more remarkable because the brain becomes larger as animals grow older: the length of the lateral fissure is 20 mm at birth and 30 mm by one month of age.

Times-to-peak of the early and late s.n.–d.p. waves are plotted between 0 and 10 days in the right-hand part of Fig. 4. In the frontal cortex, the time-to-peak for the late s.n.–d.p. wave (filled squares) decreases *pari passu* with that for the early s.n.–d.p. wave as animals grow older. On the other hand, in the parietal cortex times-to-peak of both the early (open triangles) and the late s.n.–d.p. wave (open squares) do not change significantly until the 10th post-natal day when the late s.n.–d.p. wave comes to occur seldom. Therefore, the delay between the times-to-peak of the early and late s.n.–d.p. wave remains nearly constant in both cortices during maturation, though the delay is less in the frontal cortex than in the parietal cortex.
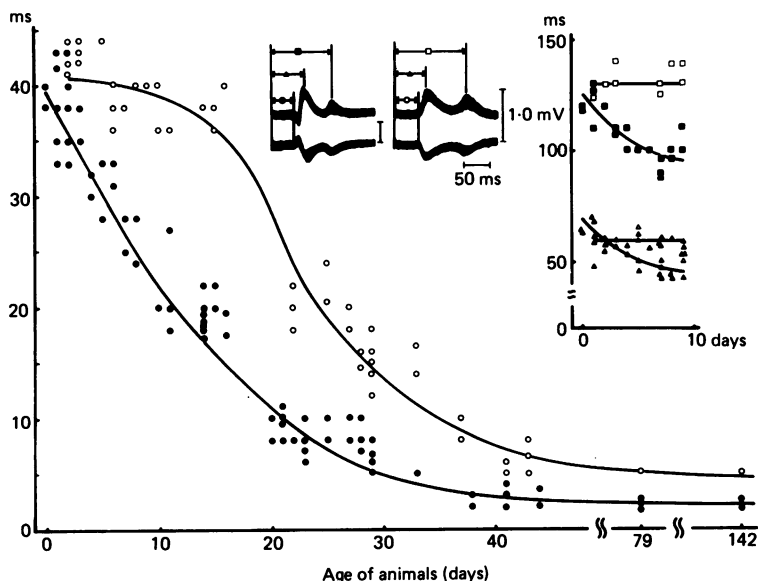
Fig. 4. Post-natal changes in the latency and time-to-peak of the cerebello-cerebral response. Ordinates: latencies of response (●) and times-to-peak of the early s.n.–d.p. wave (▲) and the late s.n.–d.p. wave (■) in the frontal motor cortex; latencies of response (○) and times-to-peak of the early s.n.–d.p. wave (△) and the late s.n.–d.p. wave (□) in the parietal association cortex. Latencies and times-to-peak were measured in a manner shown in the inset illustrations. The two responses were recorded from the precruciate gyrus (left hand) and the middle suprasylvian gyrus (right hand) at depths of 0 (upper row) and 1500 μm (lower row) in a 2-day-old kitten. Voltage calibration of 1·0 mV is for both responses and time calibration of 50 ms is for all records.

Developmental changes in the distribution and magnitude of responses in the frontal and parietal cortices

A large potential recorded from the cortical surface does not always mean the presence of an actual response in that cortical area. A 'false' response, which merely reflected a response occurring far distant from the recording site was occasionally observed. Fig. 5F shows an example of such a false response in which the configuration of potential does not change throughout the cortical layers. This response was recorded from the post-cruciate gyrus. However, when the micro-electrode was advanced further into the brain, an actual response similar to that in the precruciate cortex (i.e. similar to that in Fig. 5E) was recorded from the underlying roof cortex of the folded cruciate sulcus. Examples of an actual response and a false response recorded in the parietal cortex of a 2-day-old kitten are shown in Fig. 5G and I respectively. In the actual response, the negative potential on the cortical surface changes polarity at a depth of 600 μm and becomes positive in the deep cortical layers whereas the false response shows no reversal as depth increases. The cerebellar-induced cerebral cortical response was occasionally an admixture of an actual and a false response as shown in Fig 5H. In the superficial cortical layers (see 0 μm and surface record (s.r.)), there is a positive–negative–negative potential sequence which is similar to the motor cortical response in Fig. 5E. The double

negative potential in the superficial cortical layers changes polarity to become a double positive potential in the cortical depth. By contrast, the initial positive potential indicated by an arrow in the surface record (s.r.) shows no reversal of potential throughout the cortical layers. This potential which is also seen at depths of 0 and 600 μm (arrows) remains unchanged at a depth of 1500 μm although it is rather difficult to distinguish it from the immediately following positive potential
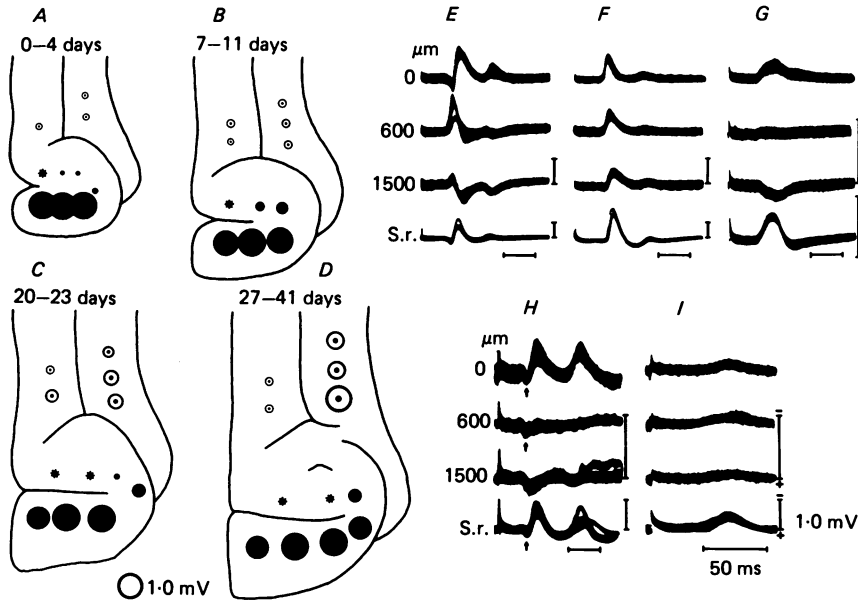

Fig. 5. Developmental changes in the distribution and magnitude of the cerebello-cerebral response. Results from twenty-eight kittens were summarized in four groups of different ages: *A*, eleven kittens of 0–4 days of age; *B*, six kittens of 7–11 days of age; *C*, five kittens of 20–23 days of age; *D*, six kittens of 27–41 days of age. The magnitude of response in the frontal and parietal cortices was measured by the amplitude of the largest negative potential in the deep and superficial cortical layers respectively (see text). The diameter of circles signifies the relative magnitude of response, e.g. the smallest and largest filled circles are 0·18 and 1·18 mV respectively for the frontal cortical response, and the smallest and largest dotted circles are 0·29 and 1·10 mV for the parietal cortical response. False responses are indicated by asterisks. Examples of actual responses (*E* and *G*); false responses (*F* and *I*) and an admixture of a false and an actual response (*H*). In *E–I* (s.r.) trace is the response recorded from cortical surface using a ball electrode. Voltage calibration is 1·0 mV and time calibration is 50 ms for all records.

belonging to the early s.n.–d.p. response. These examples emphasize that recording of laminar field potentials by a micro-electrode is absolutely necessary to detect the responsive area and to measure the latency of response accurately.

Fig. 5*A–D* illustrate the distribution and magnitude of the cerebello-cerebral responses in twenty-eight kittens which were assigned to the following four groups according to the animal's age: *A*, 0–4 days of age ($n = 11$); *B*, 7–11 days of age ($n = 6$); *C*, 20–23 days of age ($n = 5$); *D*, 27–41 days of age ($n = 6$). The s.p.–d.n. responses in the frontal cortex are represented by the filled circles whilst the early s.n.–d.p.

responses in the parietal cortex are shown by the open circles. The size of each symbol is proportional to the average response amplitude in the animals making up the group. The responses in the frontal cortex were measured using the negative waves deep in the cortex (i.e. d.n. responses) while those in the parietal cortex were measured using the negative waves superficial in the cortex (i.e. s.n.). This procedure was followed because these waves appear to indicate the presence of active current sinks due to excitatory post-synaptic potentials generated by nearby thalamocortical synapses. In Fig. 5 *A–D* 'false' responses (see above) are indicated by asterisks.

In the animals of group A whose eyes are not open yet, the frontal cortical response in the area rostral to the cruciate sulcus and its lateral extension is remarkable and the magnitude of responses in this area is comparable to that of adult cats. At these ages, the cruciate sulcus is actually not a sulcus in the frontal view but a shallow groove on the mesial surface of the cortex and the frontal cortex is transitional from isocortex to agranular cortex in ontogenesis. In contrast to the marked response in the frontal cortex, the response evoked in the parietal cortex is weak. The parietal cortex is known to be more vulnerable than the frontal cortex under experimental conditions (Sasaki *et al.* 1972). The poor performance of the cerebello-cerebral response in the parietal cortex in these kittens is, however, not due to deterioration of that cortical area but rather due to inactivity of the cerebellothalamic projection to the thalamic v.a. nucleus. Thus, supramaximal stimulation of the cerebellar nucleus in a 0-day-old kitten could hardly elicit a response in the parietal cortex, but direct stimulation of the thalamic v.a. nucleus nevertheless induced a marked response at the same recording site (Fig. 6 *C*). In the post-cruciate gyrus, there was only a false response similar to that shown in Fig. 5 *F*.

The cruciate sulcus in the animals of group B extends laterally in the frontal view. The frontal motor cortex becomes a typical agranular cortex. At these ages kittens crawl with eyes open and can temporarily lift their belly from the floor. The distribution and magnitude of response in the frontal cortex are similar to those in group A except for a slight enhancement of response in the area caudal to the lateral extension of the cruciate sulcus. In the parietal cortex, there is a marked enhancement of response. The cruciate sulcus in the animals of group C extends further laterally in the frontal view. At these ages kittens can walk quite well, although still awkwardly as compared to the older animals. The distribution and magnitude of response in the frontal cortex differ little from those in the younger kittens whereas a further enhancement of response is evident in the parietal cortex. The cruciate sulcus in the animals of group D has a width comparable to that in adult cats. The distribution and magnitude of response in the frontal and parietal cortices in group D are much the same as in adult cats.

In the frontal cortex, the responsive areas caudolateral to the cruciate sulcus decrease gradually with advance of age and finally disappear by 4 weeks after birth. These areas are perhaps incorporated into the folded part of the cortex as the cruciate sulcus extends laterally.

In summary, the magnitude of the frontal cortical response in neonates, as judged from the peak amplitude of the depth-negative wave, is as large as in adults despite the enhancement of the s.p.–d.n. wave and regression of the s.n.–d.p. wave which occur during maturation and are accompanied by remarkable morphological develop-

ment in the cortical architecture. In the parietal cortex, the magnitude of response is small in neonates and becomes larger as animals grow older, particularly after 2 weeks of age when the latency of response begins to decrease sharply. The location and extent of the responsive areas remains unchanged through the post-natal development.

A comparison between stimulation of the cerebellar nucleus and the thalamus

The thalamic relay nuclei for the cerebello-cerebral responses in the frontal and parietal cortices are mainly the v.l. and v.a. part of the nuclear complex, respectively (Sasaki *et al.* 1972; Sasaki, Matsuda, Kawaguchi & Mizuno, 1972; Mizuno, Konishi, Sato, Kawaguchi, Yamamoto, Kawamura & Yamawaki, 1975).
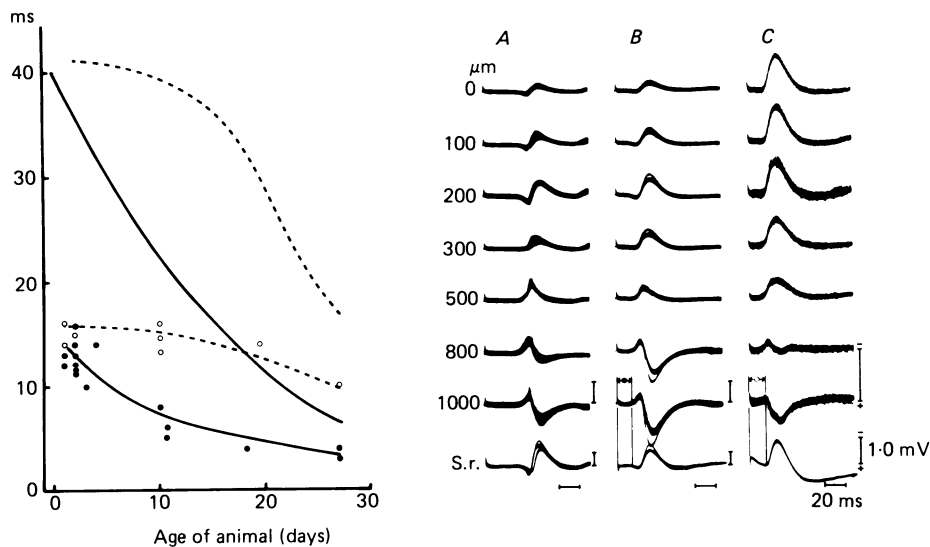

Fig. 6. Comparison between stimulations of the cerebellar nucleus and the thalamus. The frontal cortical response induced by stimulation of the cerebellar nucleus (*A*) and the thalamic v.l. nucleus (*B*), recorded from a 1-day-old kitten and the parietal cortical response induced by stimulation of the thalamic v.a. nucleus (*C*), recorded from a 0-day-old kitten. On the left-hand side, latencies of response in the frontal (●) and parietal cortices (○) by stimulation of the thalamic v.l. and v.a. nucleus respectively are plotted on the ordinate and animal's age on the abscissa. The upper two curves show changes in latencies of the cerebello-cerebral response which are transferred from Fig. 4 for convenience of comparison. Continuous line, frontal; dashed line, parietal.

Laminar field potentials recorded from the precruciate cortex in a 1-day-old kitten on stimulation of the cerebellar nucleus and the v.l. nucleus of the thalamus are illustrated in Fig. 6*A* and *B* respectively. The latency of response is 35 ms for the former stimulation and 13 ms for the latter. The two responses are similar in form except that stimulation of the cerebellar nucleus but not the thalamus induced a late s.n.–d.p. wave (of which only the initial deflexion is seen in Fig. 6*A*). Fig. 6*C* shows a well-developed parietal cortical response induced by stimulation of the v.a. nucleus of the thalamus in a 0-day-old kitten. In this animal, as in another 0-day-old kitten shown in Fig. 2, stimulation of the cerebellar nucleus induced barely any response in

the parietal cortex although a marked response was evoked in the frontal cortex at the same time. Similar findings were obtained in three other kittens of the same age. Occurrence of a marked response in the parietal cortex on stimulation of the thalamic v.a. nucleus at birth contrasts markedly with poor effectiveness of cerebellar nuclear stimulation and indicates that the thalamic v.a.–parietal cortical projection matures earlier than the cerebello-thalamic v.a. projection.

Latencies of response in the frontal cortex on stimulation of the thalamic v.l. nucleus and those in the parietal cortex on stimulation of the thalamic v.a. nucleus are plotted in Fig. 6 with filled and open circles respectively. The upper two curves indicate the change in latency of the cerebello-cerebral response in the frontal (continuous line) and parietal cortices (dashed line) which are transferred from Fig. 4 for convenience of comparison. In kittens, unlike in adult cats (Sasaki *et al.* 1972), the latency from the cerebellar nucleus to the thalamus is longer than that from the thalamus to the cerebral cortex. The post-natal decreases in latency is therefore more conspicuous in the cerebello-cerebral response than in the thalamocortical response. Thus, maturation of the thalamocortical projection appears to precede to that of the cerebellothalamic projection.

Double shock stimulation of the cerebellar nucleus and the thalamus

The cerebello-cerebral response in young kittens could not follow repetitive stimulation at low frequency (4–5/s). There may be a powerful, long-lasting, inhibitory process in the thalamus or the cerebral cortex, or both. To elucidate it, double shock stimulation of the cerebellar nucleus and the thalamus was tested at various time-intervals.

In Fig. 7, the magnitude of response to a second stimulus (as a percentage of that to the first stimulus) is plotted on the ordinates while the time-interval between the first and second stimuli is shown on the abscissae. In the cerebello-cerebral response of a 0-day-old kitten (filled circles), the second stimulus could not induce any response for 250 ms after which a 50% response was elicited by 300 ms and full recovery was attained by 320–350 ms. A prominent rebound facilitation then appeared and lasted up to intervals over 500 ms (not shown). Similar results were obtained in four other kittens examined at 0–4 days of age. On the other hand, in five kittens ranging 10–28 days of age, complete suppression of the response lasted only for 100–120 ms and rebound facilitation was not so conspicuous. In a 28-day-old kitten (filled squares) complete suppression lasted for 100 ms and full recovery was attained by 160 ms. It was followed by a partial suppression at around 200 ms and then by a period of facilitation.

Double shock stimulation of the thalamic v.l. nucleus was tested in two kittens of 1 day old and two kittens of 10 days old. The results were similar in all the animals. The data from one 1-day-old and one 10-day-old kittens are plotted in Fig. 7 with open circles and squares respectively. Complete suppression of response lasted only for 10–15 ms in both animals and recovery to 50% was attained by 30 ms and to 80% by 100 ms. However, full recovery was not attained until 1 s (not shown). These findings indicate that in neonates the thalamic neurones are evidently influenced by a powerful, long-lasting inhibition which explains why the cerebello-cerebral response could not follow repetitive stimulation at low frequency.
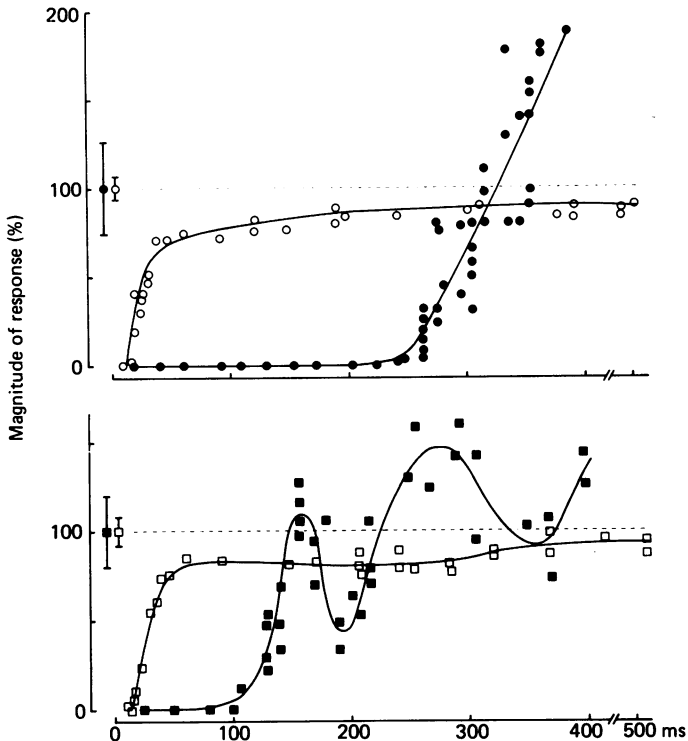
Fig. 7. Double shock stimulation of the cerebellar nucleus and the thalamus. The magnitude of response to the 2nd stimulation is plotted on the ordinates as a percentage of the first response and time intervals between the 1st and 2nd stimulations are shown on the abscissae. ●, the cerebello-cerebral response in a 1-day-old kitten, ○, the thalamic v.l.–frontal cortical response in the same animal; ■, the cerebello-cerebral response in a 28-day-old kitten; □, the thalamic v.l.–frontal cortical response in a 10-day-old kitten. Bars next to ordinates indicate standard deviation of control response.

Morphological correlates of the s.n.–d.p. and s.p.–d.n. waves

A morphological study using the anterograde axonal transport of HRP revealed two distinct thalamocortical (t.c.) projections which are consistent with the physiological findings. Plate 1 shows sections of the frontal motor and parietal association cortices after injection of HRP into the thalamic v.a.–v.l. nuclear complex which relays the cerebello-cerebral projection. Terminals of t.c. projection fibres labelled anterogradely and corticothalamic projection neurones labelled retrogradely are seen under dark field illumination. Findings on the corticothalamic neurones are omitted from the following description.

In the sections of the motor cortex taken from a 4-day-old (*A*) and a 35-day-old kitten (*C*), terminals are densely labelled in both the superficial (layer I) and deep layers (layers III). On the other hand, in the sections of the parietal cortex taken from the same 4-day-old (*B*) and the same 35-day-old kitten (*D*), terminals are densely labelled in the superficial layer but not in the deep layer. Labelled grains are also present densely in layer VI and less densely in layer V of the frontal cortex, and sparsely in layers V and VI of the parietal cortex. However, with the present method,

it is difficult to determine whether these grains represent afferent terminals or fine dendritic branches labelled retrogradely. A degeneration study by Strick & Sterling (1974) indicates that the labelled grains in layer VI of the frontal cortex, at least partly, are afferent terminals of the t.c. projection from the v.l. nucleus.

The superficial layer in which labelled terminals are distributed is thicker in the 4-day-old kitten than in the 35-day-old kitten, whereas the deep layer with labelled terminals is thicker in the 35-day-old kitten than in the 4-day-old kitten. Labelled terminals in the superficial layer in the 4-day-old kitten and three other neonates were distributed in the whole thickness of layer I although their density was much higher in the outer than the inner half of this layer. By contrast, in the 35-day-old kitten and two other 1-month-old kittens labelled terminals in layer I were confined to the outer half (note that layer I is relatively thicker in neonates).

Labelled terminals in the deep layers of the frontal cortex in neonates were in the inner portion of layer III whereas those in 1-month-old kittens were distributed in both the inner and outer portions of layer III.

A comparison of electrophysiological and morphological findings strongly suggests that the t.c. projection terminating in layer I mediates the s.n.–d.p. wave and the t.c. projection terminating in layer III mediates the s.p.–d.n. wave.

DISCUSSION

The temporal sequence of formation of synapses in the cerebellar cortex is retrograde to the direction of synaptic transmission: the Purkinje cells send axons to the deep cerebellar nuclei before developing dendritic branches and receiving parallel fibre terminals; migrating granule cells make synaptic contact with Purkinje cell dendrites before reaching internal granular layers and receiving mossy fibre terminals; feed-back and feed-forward inhibition on the Purkinje cell by the Golgi, basket, and stellate cells have been established prior to emergence of excitation on the Purkinje cell via the mossy fibre–granule cell–parallel fibre synapses (Larramendi, 1969; Altman, 1972a, b; Sidman & Rakic, 1973; Shimono et al. 1976). Exceptional to this retrograde order of formation is the functional development of the climbing fibre which begins sustained activity earlier than any other element (Woodward, Hoffer & Lapham, 1969). However, the functional significance of the climbing fibre in early development appears to be an inductive or trophic action on the Purkinje cell rather than an action related to information processing. Thus, the dendrites of the Purkinje cell are markedly stunted and devoid of elaborate dendritic branches when the climbing fibres are removed in the early post-natal period (Kawaguchi, Yamamoto, Mizuno & Iwahori, 1975; Bradley & Berry, 1976; Sotelo & Arsenio-Nunes, 1976). Formation of neuronal circuitry in the cerebellar cortex in kittens has been reported to occur mostly post-natally (Anderson & Stromberg, 1977). By contrast, stimulation of the cerebellar nucleus induced a response in the frontal cortex at birth which had a magnitude comparable to that in adult cats. In the parietal cortex, a detectable response was evoked at 2 days of age but not at birth. However, stimulation of the thalamic v.a. nucleus induced a marked response in the parietal cortex from birth. This is consistent with the morphological finding that thalamo-cortical afferents arrive in the primordial cortex very early in ontogenesis (Marin-

Padilla, 1971). Thus, the principle of retrograde order of formation applies to both the cerebellar cortex and the cerebello-thalamo-cerebral projection.

The cerebello-cerebral response in kittens at any age, as in adult cats, consists of two distinct types of elementary response. One is characterized by a wave which is negative in the superficial cortical layers and positive in the deep cortical layers (s.n.–d.p. wave). The other is characterized by a wave which is positive in the superficial cortical layers and negative in the deep cortical layers (s.p.–d.n. wave). The cerebello-cerebral response in the frontal motor cortex is a sequential occurrence of the s.p.–d.n. and s.n.–d.p. waves and that in the parietal cortex is a pure form of the s.n.–d.p. wave. Both the s.n.–d.p. and s.p.–d.n. waves are composed mainly of an electrical dipole which is oriented perpendicularly to the cortex (though the polarity is opposite). In considering the cytoarchitecture of the cerebral cortex, the neuronal element which can produce such an electrical dipole is presumed to be an ensemble of pyramidal neurones; the electrical dipole can be ascribed to a longitudinal distribution of source and sink currents in the apical dendrites and the somata of pyramidal neurones. In a study on the cortical incremental response in the cat, Sasaki *et al.* (1970) proposed a concept of superficial and deep thalamocortical (t.c.) responses which correspond to the s.n.–d.p. and s.p.–d.n. waves respectively. They attributed the superficial t.c. response mainly to excitatory post-synaptic potentials (e.p.s.p.s) in the upper part of the apical dendrites of pyramidal neurones and the deep t.c. response mainly to e.p.s.p.s in somata and dendrites near the somata of pyramidal neurones. Accordingly, they named the projections responsible for the superficial and deep t.c. responses as the superficial and deep t.c. projections respectively. On the basis of studies on the excitability change in pyramidal tract neurones tested with antidromic invasion (Sasaki & Prelević, 1972), the contribution of inhibitory post-synaptic potentials and spike potentials to both the s.n.–d.p. and s.p.–d.n. waves was considered to be small if any, which is consistent with later studies on field potentials in the visual cortex by current source density analysis (Mitzdorf & Singer, 1978).

When kittens were injected with HRP into the thalamic v.a.–v.l. nuclear complex which relays the cerebello-cerebral projection, terminals of t.c. afferents were antero-gradely labelled densely in layer I in both the frontal and parietal cortices. In the frontal cortex but not in the parietal cortex, terminals were also labelled in layer III. Thus, the distribution of t.c. afferents is compatible with the concept of the superficial and deep t.c. projections: the projection onto layer I corresponds to the superficial t.c. projection and appears to mediate the s.n.–d.p. wave; the projection onto layer III corresponds to the deep t.c. projection and appears to mediate the s.p.–d.n. wave. A wealth of literature describes the existence of two distinct types of t.c. projections, i.e. 'specific' and 'unspecific' (or 'non-specific' or 'diffuse') projection and the occurrence of two distinct types of t.c. responses, i.e. augmenting and recruiting responses (Jasper, 1960). Morison & Dempsy (1942) postulated that recruiting responses were mediated by 'unspecific' cortical afferents projecting onto layer I described by Lorente de Nó (1938). In fact, repetitive stimulation of the thalamic v.a. nucleus induces a recruiting response in the parietal cortex which is a pure form of the s.n.–d.p. wave (Sasaki *et al.* 1970). However, the v.a. nucleus receives monosynaptic inputs from the cerebellum and projects directly onto the cerebral cortex (Sasaki *et al.* 1972; Mizuno *et al.* 1975). In this sense, the t.c. projection onto

layer I from the v.a. nucleus is neither 'unspecific' nor 'diffuse'. Therefore, the terms 'superficial' and 'deep' t.c. projections are preferred here.

Although the cerebello-cerebral response in kittens at any age consisted of the s.n.–d.p. and s.p.–d.n. waves, the cortical level at which the two waves reversed polarity was deep in neonates and became more superficial as animals grew older. In the frontal cortex the s.n.–d.p. wave was much larger than the s.p.–d.n. wave in young animals and with advance of age, the s.n.–d.p. wave became smaller and the s.p.–d.n. wave became larger. Similar changes were also observed in the auditory cortex (Miyata, Kawaguchi, Samejima & Yamamoto, 1982) and visual cortex (Kato, Kawaguchi, Yamamoto, Samejima & Miyata, 1983). A possible explanation for these developmental changes is that synaptogenesis of the deep t.c. projection and regression of the superficial t.c. projection proceed from the deep layer to the more superficial layer. This explanation is consistent with the principle in ontogenesis of mammalian neocortical organization, i.e. ascending sequential maturation: neurones in migration take positions in the cortex from the deep layer to the superficial layer according to their time of origin; maturation of cortical plates proceeds sequentially in a similar ascending course; dendritic spines appear progressively from the deep layer to the more superficial layer (Marin-Padilla, 1971; Sidman & Rakic, 1973). The difference in terminal distribution of t.c. projection fibres in the neonates and the 1-month-old kittens revealed in the present experiments is in harmony with this explanation.

Developing of t.c. projections may not be uniform: some projections matured earlier may mediate a response of a shorter latency with a level of potential reversal in more superficial cortical layers while some projections matured later may mediate a response of a longer latency with a level of potential reversal in deeper cortical layers. This assumption could explain the co-existence of positive and negative waves in the mid layers of the parietal cortex in young kittens (Fig. 2C, at a depth of 500 μm).

Latencies of the frontal cortical response and the parietal cortical response decreased in maturation in a different manner. Latencies in the frontal cortex decreased sharply from birth, whereas those in the parietal cortex remained almost unchanged up to 2 weeks of age, then decreased sharply. This, and the changes in response amplitude, indicates that maturation of the cerebellothalamic and thalamocerebral projections for the parietal cortex occurs later than for the frontal cortex. This agrees with the well known fact that ontogenesis of the parietal association cortex lags that of the frontal motor cortex (Ariëns Kappers, Huber & Crosby, 1967).

The late s.n.–d.p. wave in both the frontal and parietal cortices was frequently observed in the animals before 10 days of age but could seldom be evoked after 12 days of age. As discussed in Results, the neuronal connexions responsible for the late s.n.–d.p. wave are presumably a reverberating circuit in the thalamic relay mechanism. Neuronal cell death (Arees & Astrom, 1977) and elimination of synapses (Altman, 1972b; Cragg, 1975) are well known to occur during ontogenesis. Disappearance of the late s.n.–d.p. wave may suggest that the relevant neuronal circuit is eliminated during maturation. The period of disappearance of the late s.n.–d.p. wave corresponds to the time when the latency of the parietal cortical response begins to decrease sharply and also to the time when the early s.n.–d.p. wave becomes less significant and the s.p.–d.n. wave becomes more remarkable in the frontal cortex.

Predominance of the s.n.–d.p. wave in various cortical responses is universal in young animals (Marty, 1962; Persson, 1973; Miyata *et al.* 1982; Kato *et al.* 1983), which may imply that the s.n.–d.p. wave (i.e. presumably the dense projection onto layer I) plays an important role in the maturation of the cortical network. In adult cats, the recruiting response which is a pure form of the s.n.–d.p. wave can be evoked in the frontal and parietal association cortices but not in the primary sensory cortices (Matsuda, Sasaki & Mizuno, 1972).

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EXPLANATION OF PLATE

Frontal sections of the cerebral cortex after injection of HRP into the v.a.–v.l. nuclear complex of the thalamus, treated with benzidine dihydrochloride by the method of DeOlmos & Heimer (1977). *A*, area 6 of a 4-day-old kitten; *B*, area 7 of the same animal; *C*, area 6 of a 35-day-old kitten; *D*, area 7 of the same animal. Unstained, dark field. Roman numerals signify cortical layers. Scale bar, 200 μm.