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POST-NATAL DEVELOPMENT OF PYRAMIDAL TRACT NEURONES IN KITTENS

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SUMMARY

1. The post-natal development of pyramidal tract neurones (p.t.n.s) was investigated in twenty-one barbiturate-anaesthetized kittens from birth to 28 days of age using a combination of electrophysiological and anatomical techniques.

2. P.t.n. responses were recorded intracellularly as well as extracellularly with glass micropipettes filled with horseradish peroxidase (HRP) on stimulation of the medullary pyramid and cerebellar nuclei.

3. Latency histograms of antidromic responses of p.t.n.s were compared at various ages. In the neonate, p.t.n.s were divided into two groups which were presumed to be analogous with fast and slow p.t.n.s in adult animals. During the first post-natal week, latency shortening was not conspicuous, but by the end of the second post-natal week, the faster group showed a marked decrease of latencies (up to around 10 ms at 14 days of age), while those of the slower group did not change so much. The slower group increased their conduction velocity during the third post-natal week (latencies up to around 18 ms). At the end of the fourth post-natal week, the distribution of antidromic latencies was in a narrower range, but the values were still longer than those reported in adult animals.

4. Intracellular HRP staining revealed that apical dendrites of p.t.n.s spread fully to the pial surface even at birth. The somata of these neurones were characteristically covered with somatic appendages and development of the basal dendritic tree was immature in 0–1-day-old kittens. Basal dendrites developed nearly completely by 7 days, but somata were still covered with appendages. During the fourth post-natal week, these appendages disappeared almost completely. The sizes of the dendritic field, especially of apical dendrites, became larger in parallel with the development of cortical layers.

5. From the morphological point of view, differentiation of fast and slow p.t.n.s was not clear until 28 days of age except in somatic volumes, which were already different in the first post-natal week. At the end of the fourth post-natal week, p.t.n.s with short antidromic latencies had a tendency to bear spines more sparsely over the secondary and tertiary dendritic surface in comparison with p.t.n.s with longer latencies.

6. Intracortical axonal trajectories developed fairly well in the immature cerebral cortex, and the general pattern of ramification changed little during the first month

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after birth. In a few cases, collateral ramification was observed in the upper limit of layer II and in layer I.

7. Synaptic activation by stimulation of the cerebellar nuclei or the pyramidal tract was also investigated. The mode of cerebellar activation in the immature cortex mainly consisted of excitatory post-synaptic potentials (e.p.s.p.s), but after 3 days of age e.p.s.p.s were often curtailed by inhibitory post-synaptic potentials. Some neurones received presumed recurrent facilitation upon stimulation of the pyramidal tract even at the earliest stage.

8. Post-natal development of the efferent system in the motor cortex appeared to occur in parallel with the development of the afferent system.

INTRODUCTION

Many anatomical studies have been reported on the post-natal development of the precruciate cortex in cats (Ramon y Cajal, 1960; Noback & Purpura, 1961; Scheibel & Scheibel, 1963; Purpura, Shofer, Housepian & Noback, 1964; Batuev & Demianenko, 1983), and rats (Wise, Fleshman & Jones, 1979), using the Golgi method. The intracellular horseradish peroxidase (HRP) staining method has several advantages for investigating the morphological features of neurones: it is concurrently possible to characterize neurones electrophysiologically and to examine axonal trajectories as well as soma-dendritic morphology.

In the sensorimotor cortex, pyramidal tract neurones (p.t.n.s) are classified into slow and fast p.t.n.s by their different conduction velocities in adult cats (Takahashi, 1965), and some morphological differences were reported between these two groups: i.e. slow p.t.n.s bear many spines on the apical as well as basal dendrites, while fast p.t.n.s have fewer spines on the apical dendrites and are nearly devoid of spines on dendrites in layer I (Deschênes, Labelle & Landry, 1979*b*). Concerning the post-natal development of the pyramidal tract, there have been reports of electrophysiological studies on myelination of the pyramidal tract in cats (Huttenlocher, 1970; Purpura *et al.* 1964) and rabbits (Conway, Wright & Bradley, 1969), but no reports on morphological and functional aspects of differentiation between fast and slow p.t.n.s using intracellular HRP staining.

Kawaguchi, Samejima & Yamamoto (1983) have described the post-natal development of the cerebello-cerebral projection in kittens. The afferent systems into the motor cortex through the thalamic ventral anterior and lateral nuclei (v.a. and v.l., respectively) are almost completely organized even at birth.

Our present study indicates that p.t.n.s are separable into fast and slow conduction types at early stages after birth, although the differentiation of soma-dendritic morphology is complete only by the end of the first post-natal month. A preliminary report has appeared elsewhere (Yamamoto & Samejima, 1985).

METHODS

Twenty-one kittens, ranging in ages from 0 (at birth) to 28 days old, were used in the present study. They were anaesthetized with sodium pentobarbitone (35–40 mg/kg, intraperitoneal injection) and a tracheal tube was inserted. The animals were fixed in a stereotaxic apparatus with modified ear bars and head holder for the small size of the animals. After craniotomy over the

pericruciate cortex and cerebellar cortices bilaterally, stimulating electrodes (outer diameter of 0·3 mm, core diameter of 0·1 mm and interpolar distance of 0·3 mm) were inserted into the medullary pyramid (three electrodes) at the level of the trapezoid body and the cerebellar nuclei contralateral to the recording side (three electrodes).

The animals were paralysed with gallamine triethiodide and artificially respirated. Additional doses of anaesthetic were injected intraperitoneally every 2 h (5–10 mg/kg). Body temperature was kept between 36 and 38 °C by a heating pad. A single pulse current of 0·3–0·5 mA strength and 0·1–0·2 ms duration was passed through the stimulating electrodes. Antidromic responses and cerebellar-evoked responses were recorded with a gross silver-ball electrode on the surface of the cerebral cortex to check the position of stimulating electrodes. Exposed brain areas were covered with paraffin (melting point at 40 °C) to minimize pulsation. Single cortical neurones were recorded with a glass micropipette filled with 5 % HRP (w/v) dissolved in 0·5 M-K acetate or 0·5 M-KCl (d.c. resistance of 30–50 MΩ). Responses were amplified with low-gain d.c. and high-gain a.c. amplifiers, and photographed on the oscilloscope. As has been reported (Conway *et al.* 1969; Huttenlocher, 1970), antidromic responses of immature p.t.n.s are easily fatigued with repetitive stimulation in comparison with those in adult cats. In field potential recording, we used faithfully following at a fixed latency over 30 Hz repetitive stimulation as a criterion for antidromic responses in young kittens. In single cell recording, we used (1) responsiveness to double shocks at a short interval (less than 10 ms); (2) a collision test with spontaneous discharges at a longer interval than the refractoriness; (3) absence of prepotential due to e.p.s.p.s, as criteria for antidromic responses (see Fig. 1*A*).

236 neurones were identified as p.t.n.s, and out of 103 intracellularly recorded neurones, 91 neurones were stained by HRP injection. HRP was injected through recording micropipettes by anodal current pulses (2–5 nA, 300–400 ms duration, 2 Hz for 2–5 min) after checking the electrophysiological characteristics.

At the end of experiments, animals were deeply anaesthetized and perfused with 7 % formalin (v/v) dissolved in 0·1 M-phosphate buffer (pH 7·4). Brains were immediately dissected and stored in 30 % sucrose (w/v) in the same buffer solution until they sank. Brains were cut serially at 100 μm on a freezing microtome. Parasagittal sections from the motor cortex were treated for the histochemical reaction of HRP with cobalt intensification (Itoh, Konishi, Nomura, Mizuno, Nakamura & Sugimoto, 1979). The position of the stimulating electrodes was checked by the blue reaction of iron ions with 0·2 % potassium ferrocyanide solution. Sections were stained with neutral red and HRP-injected neurones were examined under a light microscope and photographed.

Out of ninety-one HRP-injected neurones, thirty-two well-stained neurones were used for detailed morphological analysis. Dendritic arborizations were traced by camera lucida drawing at a magnification of ×200, somata and dendritic spines were traced at a magnification of ×1000 with oil immersion. Axonal trajectories were traced at a magnification of ×400. Somatic volumes of ninety-one neurones were calculated from a formula after Haug (1958) by measuring the longer and shorter diameters.

RESULTS

Electrophysiological studies of the maturation and differentiation of p.t.n.s

We stimulated the medullary pyramid at the level of the trapezoid body to identify p.t.n.s. Evoked cortical surface responses explored by a silver-ball electrode were composed of an initial positive and succeeding negative potentials. The early positive potential could follow faithfully at more than 30 Hz repetitive stimulation even in new-born kittens and was composed of two positive peaks. In adult cats, the evoked cortical responses to stimulation of the medullary pyramid consist of two positive potentials (a- and b-waves), which are ascribed to excitation of the fast pyramidal tract axons and the slow pyramidal tract ones with recurrent synaptic events, respectively (Takahashi, 1965; Humphrey, 1968). In our experiments we could not exclude the possibility that stimulating currents spread out of the medullary pyramid because of the small size of the brain stem. We could, however, record most

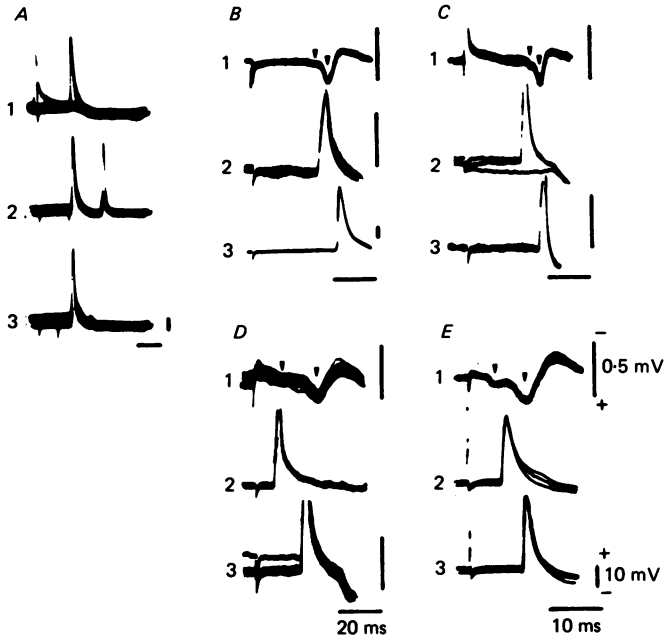


Fig. 1. Intracellularly recorded antidromic responses at various post-natal ages. *A*, identification of antidromic responses from a 3-day-old kitten. *A*1: constant and fixed latency (30 ms) of pyramidally evoked impulses which collide with a spontaneous impulse (at an interval of 33 ms). *A*2: double shocks to medullary pyramid at an interval of 30 ms. Note that the second responses mainly consist of initial segment (i.s.) spikes. *A*3: double shocks at an interval of 18 ms produce only M spikes after the second stimulus. *B*, pyramidally evoked cortical responses (1) and intracellular action potentials (2 and 3) from a 0-day-old kitten. Arrow heads in 1 indicate the two peaks of positivity. Note that impulses shown in 2 and 3 are at the latencies of the two positive deflexions respectively. *C*, specimen records from a 7-day-old kitten. Arrangement of this and following Figures are the same as that in *B*. *D*, records from a 14-day-old kitten. *E*, records from a 28-day-old kitten. Note that in *C*2 and *D*3, i.p.s.p.s follow antidromic action potentials. Voltage calibrations of 0·5 mV and 10 mV are applicable for surface recordings (*B*1, *C*1, *D*1 and *E*1) and for intracellular recordings, respectively. Time calibrations are 20 ms for *A*–*D* and 10 ms for *E*.

antidromic unitary activity in phase with the two positive surface potentials, so it can be assumed that these positive potentials are equivalent to those observed in adult cats as reported previously in post-natal rabbits (Conway *et al.* 1969) and kittens (Huttenlocher, 1970).

Criteria for the identification of antidromic activation of cortical neurones are shown in Fig. 1*A*. The latency was very long (30 ms) in young animals, but impulses had a fixed latency. The neurone of Fig. 1*A* had a refractory period of 18 ms (*A*2 and *A*3). Moreover, a collision occurred with a spontaneous spike at a longer interval (33 ms) than the refractory period (*A*1).

Fig. 1*B*–*E*, shows cortical surface responses (*B*1, *C*1, *D*1 and *E*1) and antidromic responses with short and long latencies at the various ages. *B* shows specimen records from a 0-day-old kitten. The cortical surface response was composed of small and large positive potentials as previously described (Conway *et al.* 1969) (shown by arrow heads

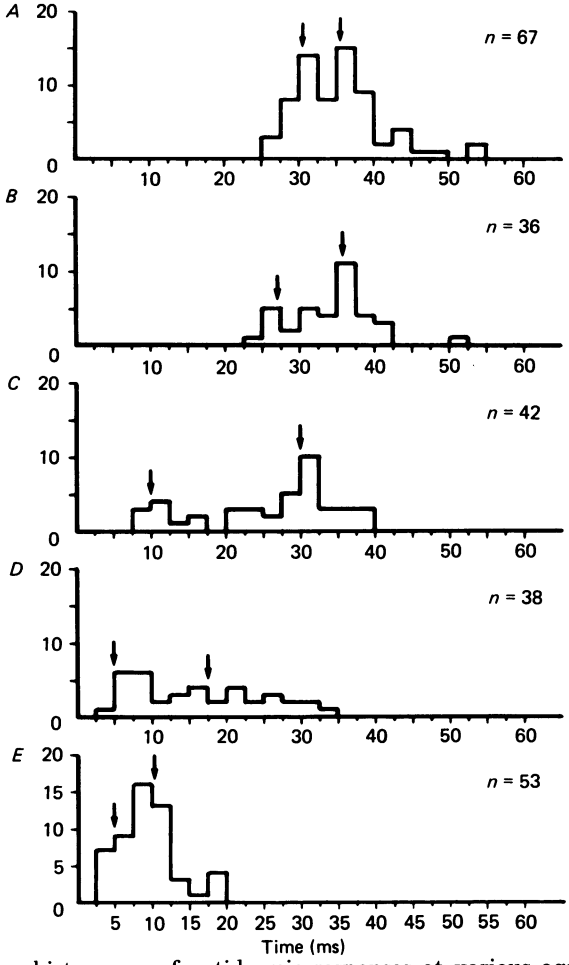


Fig. 2. Latency histograms of antidromic responses at various ages during post-natal development. *A*, histograms obtained from sixty-seven neurones of 0–3-day-old kittens. Arrows correspond to the peak latencies of the two positive waves of the pyramidally evoked cortical responses in this and subsequent graphs. *B–E*, histograms obtained from 7-, 14-, 17–21- and 28-day-old kittens respectively. Number of neurones are shown at the right upper corner in the respective graphs.

at respective peaks in *B*1). *B*2 and *B*3 show antidromic responses at latencies of 30 and 38 ms respectively, which correspond with the two components of the surface potential. The general features of the cortical surface responses were almost unchanged during the first post-natal week (*C*1). At the end of the second post-natal week, the latency of the first surface positivity as well as that of the corresponding unitary responses decreased rapidly to around 10 ms, whereas that of the second surface and unitary responses changed little (*D*). The second component decreased its peak latency during the third post-natal week. At 28 days (*E*), these two components decreased their peak latencies to 5 and 10 ms respectively.

Changes of the latency distribution of 236 unitary antidromic responses are shown in Fig. 2. From the top to the bottom latency histograms at increasing ages during

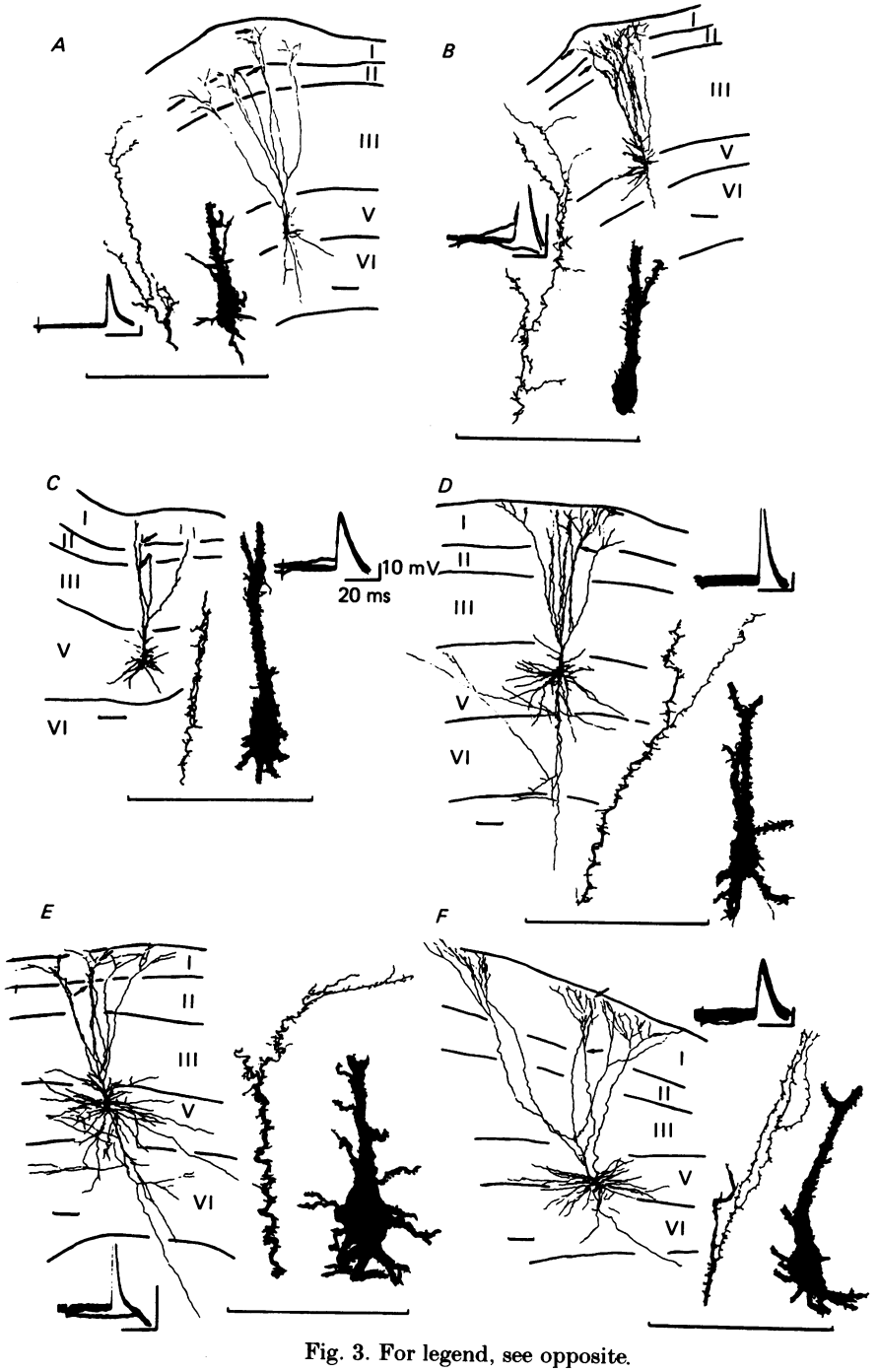


Fig. 3. For legend, see opposite.

maturation are shown. Fig. 2*A* is a latency histogram obtained from 0–3-day-old kittens and antidromic latencies were distributed from 25 to 54 ms. Arrows show the two peak latencies of the surface positive potential. It is clear that the latency distribution of the antidromic responses was not so different during the first post-natal week (*B*), and we could not detect statistical difference in the latency distribution during the first post-natal week. At 14 days (*C*) the latencies of the early responding group decreased significantly in comparison with those of the late responding group. Shortening of the latency of the late component mainly occurred after the third post-natal week. At 28 days the latency distribution showed a fall to a much narrower range, but latencies were still longer than those reported in adult cats. The completion of myelination of the pyramidal tract would occur after the age of 1 month as reported by several authors (Purpura *et al.* 1964; Huttenlocher, 1970).

Morphological investigation of p.t.n.s

Of ninety-one neurones identified electrophysiologically and stained with HRP intracellularly, thirty-two were used for the morphological analysis at various ages. In this section, we examined the post-natal maturation of p.t.n.s, i.e. the differentiation of the fast and slow p.t.n.s from the morphological point of view.

Fig. 3 shows camera lucida drawings of intracellularly stained p.t.n.s in the first post-natal week. In Fig. 3*A–D*, neurones from 0–3-day-old kittens are displayed, each part consisting of a low-power drawing of the dendritic arborization, high-power ones of the soma and apical dendrites in layers I and II (indicated by two arrows in the low-power drawing) and an antidromic response. It should be noted that apical dendrites spread nearly up to the pial surface at 0 (*A*) and 1 day (*B*). Somata were covered with somatic appendages. Fig. 3*A* shows a neurone recorded from a kitten 10 h after birth, and it had immature development of basal dendrites. Fig. 3*B* and *C* are from 1-day-old kittens, and basal dendrites seemed to be still immature when compared with *D*, which was from a 3-day-old kitten. The somata was slightly larger in *C* and *D* than in *A* and *B*, and this difference was related to the difference in conduction velocities, i.e. latencies of antidromic responses were 31 ms in *C*, 28 ms in *D*, 38 ms in *A*, and 31 ms in *B*. The apical dendrites were covered with spines

Fig. 3. Camera lucida drawings of intracellularly HRP-stained p.t.n.s with their antidromic responses at various ages during the first post-natal week. Each Figure consists of a low magnified soma-dendritic morphology, a high magnified soma and an apical dendrite which is indicated by two arrows in the low-power drawing. Roman numerals indicate the cortical layers. *A*, a neurone from a 0-day-old kitten. Staining is not good, and basal dendrites are poorly elaborated, but note that apical dendrites spread into layer I. Latency of antidromic response: 38 ms. *B* and *C*, neurones from 1-day-old kittens. Latencies of antidromic responses of these neurones are both 31 ms. *D*, a neurone at 3 days. Note the full expansion of apical dendrites in layer I and well-developed basal dendrites. Latency of antidromic response: 28 ms. *E* and *F*: neurones at 7 days. General features of the neurones are similar to those reported in adult cats. A neurone drawn in *F* spreads apical dendrites widely due to its location at the convexity of the precruciate cortex. Latencies of antidromic responses are 25 and 31 ms, respectively. Note that all neurones are covered with filamentous appendages over their somata and proximal dendrites. Apical dendrites bear fine spines which increase at 7 days old. Voltage and time calibrations are all 10 mV and 20 ms. Length calibrations are all 100 μm.

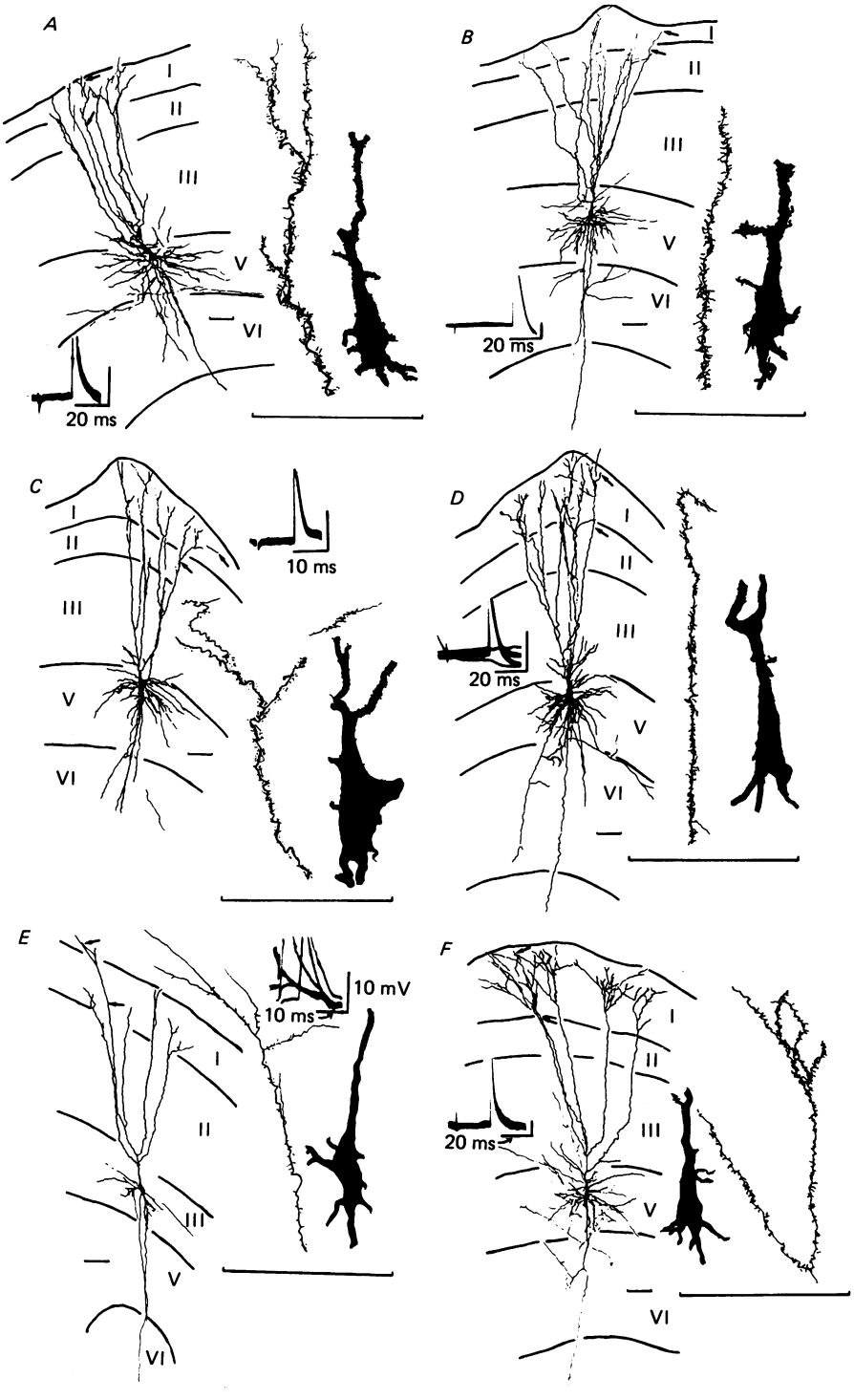
although their density was less than in older kittens (Pl. 2). Fig. 3*E* and *F* shows neurones from 7-day-old kittens. By this time the fundamental morphological features of p.t.n.s were fairly well established. However, the somata were still covered with appendages. The number of spines on the surface of apical dendrites and the soma size of p.t.n.s increased especially during the second post-natal week. We could not detect morphological differences between neurones with short and long latencies of antidromic responses except the size of somata. As described in the previous section, the latency distribution of antidromic responses did not change during the first post-natal week, and we examined for correlation between soma size and antidromic latency of seventeen p.t.n.s recorded in the first post-natal week. A negative correlation was detected between them; the correlation coefficient was $-0{\cdot}53$ ($P < 0{\cdot}05$; Student's t test), i.e. a faster conducting neurone had a larger somatic volume. When neurones were separated into two groups by their antidromic

TABLE 1. Comparison of antidromic latencies and cell volumes of p.t.n.s during the first post-natal week

Antidromic latency (ms)	Somatic volume (μm^3)	Age of kittens (days)
37	251·2→502·4	0
38	284·2→569·4	0
28	1780·4→3560·8	1
30	1899·4→3799·4	1
31	871·9→1743·7	1
36	753·6→1507·2	2
27	640·6→1281·1	2
39	1025·7→2051·4	3
28	1128·3→2256·6	3
36	392·5→785·0	3
33	1339·7→2679·5	3
34	287·8→575·7	3
25	3891·5→7783·0	7
36	1695·6→3391·2	7
27	1025·7→2051·4	7
30·5	392·5→785·0	7
31	871·5→1743·7	7

The correlation coefficient is $-0{\cdot}53$ ($P < 0{\cdot}05$; Student's t test). A statistical difference in somatic volumes of p.t.n.s with shorter and longer latencies than 30 ms is clear ($P < 0{\cdot}05$; Mann–Whitney U test).

Fig. 4. Camera lucida drawings of intracellularly HRP-stained p.t.n.s with their antidromic responses at various ages from 14 to 28 days. Each Figure consists of the same arrangement as in Fig. 3. *A* and *B*, neurones from 14-day-old kittens. Somatic appendages are fewer and spines on the apical dendrites more numerous. Latencies of antidromic responses are 20 and 32 ms, respectively. *C* and *D*, neurones at 17 days. Note that the somata are nearly smooth and spines on the apical dendrites in *C* are finer compared with those in *D*. Latencies of antidromic responses are 8·5 and 26 ms respectively. *E* and *F*, neurones at 28 days. Note that the somata are completely smooth, and spines of apical dendrites in *E* are much sparser than those in *F*. Latencies of antidromic responses are 4·6 and 20 ms respectively. Voltage calibrations are all 10 mV and length calibrations are all 100 μm.



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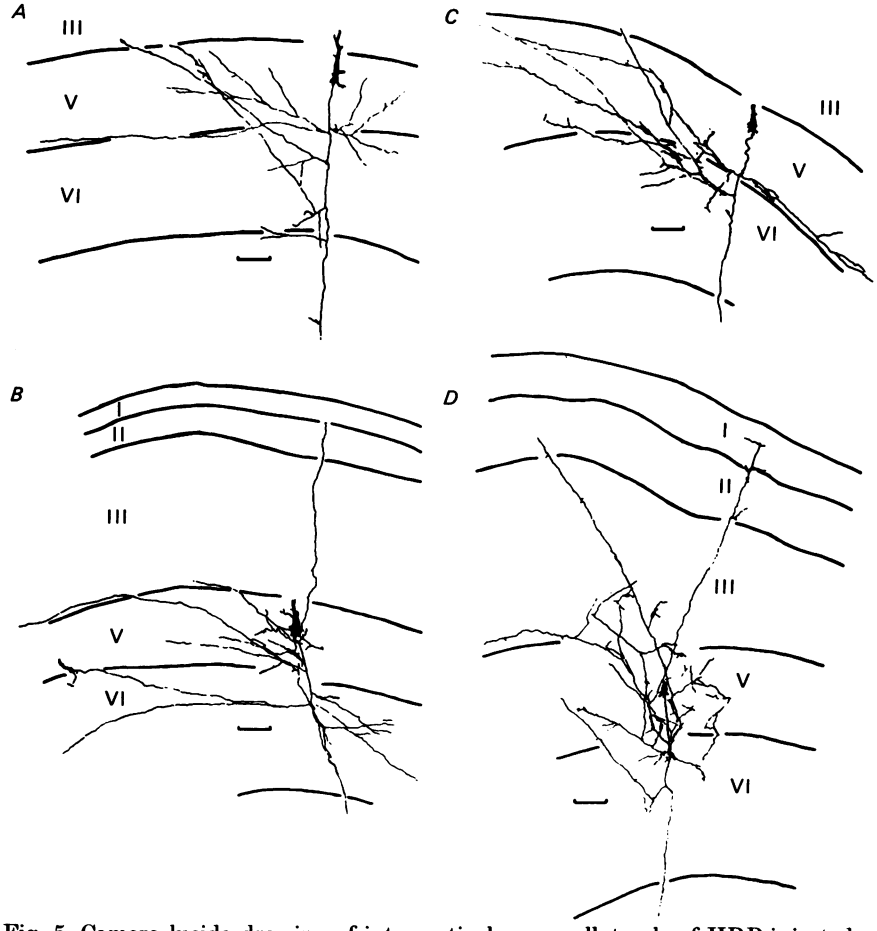

Fig. 5. Camera lucida drawing of intracortical axon collaterals of HRP-injected p.t.n.s at various ages. Roman numerals indicate cortical layers. *A*, a neurone from a 3-day-old kitten. Note the abundant collateralization in layers V and VI. One axon can be traced over a distance of about 1 mm along the border of layer V and VI. *B*, a neurone at 7 days. One ascending collateral enters the border of layer I and II. *C*, a neurone at 14 days. *D*, a neurone at 28 days. Note that one of two ascending collaterals enters into layer I. Note that the general features of the axonal collateralization are the same from 3 to 28 days of age. Length calibrations are all 100 μm.

latencies, i.e. shorter and longer than 30 ms, a statistically significant difference in cell volumes was detected ($P < 0{\cdot}05$; Mann–Whitney U test) (Table 1).

Fig. 4 shows camera lucida drawings during the third and fourth post-natal weeks. The dendritic patterns of apical as well as basal dendrites were nearly the same as those at 7 days. A difference was that the somatic appendages were fewer at 17 days (*C* and *D*, Pls. 1*B*3 and 2*C*), and disappeared by 28 days (*E* and *F*). It was also noted that the number of spines on apical dendrites of the fast-conducting neurones (*E*, 4·6 ms of antidromic latency) was much sparser than that with slow conducting ones (*F*, 20 ms, antidromic latency) (also see Pl. 2*D* and *E*). As shown in Pl. 1*A*, the thickness of the cortex increases enormously during maturation (Pl. 1*A*1–*A*4). At the

age of 28 days (Pl. 1 *A*3), the cortical laminar organization appeared to be complete, when compared with that in adult cats (Pl. 1 *A*4). We could not find any growth cones at the tips of the basal and apical dendrites even in 1-day-old kittens, although some of them were very fine as shown in the picture of Pl. 1 *B*1. Dendritic spines in immature neurones seemed to be fine and 'drum stick' in type with longer stalks in comparison with those observed in the slow p.t.n.s in adult cats (Deschênes *et al.* 1979*b*) (Figs. 3, 4 and Pl. 2).

Intracortical axonal trajectories of p.t.n.s

Fig. 5 shows the intracortical axonal ramification drawn from the intracellularly stained neurones at various ages. Fig. 5*A* shows a neurone from a 3-day-old kitten. The fundamental features were the same as those reported in adult cats (Landry, Labelle & Deschênes, 1980); there were many collaterals in layers V and VI, and some of them were traced over a distance of up to 1 mm. Thus, intracortical axonal trajectories appeared to change little during maturation from the neonate to 28 days. In two neurones, one from a 7-day-old kitten and the other from a 28-day-old kitten, collaterals were traced up to the border of layers I and II (*B*) or into layer I (*D*). Most of the axons which extended into layer III could not be detected as terminals, and some of these collaterals might have penetrated into layer II or even into layer I as reported by Donoghue & Kitai (1979) in rats or by Martin & Whitteridge (1984) in the striate cortex of cats.

The mode of synaptic activation of p.t.n.s

Stimulation of the pyramidal tract, the thalamic v.l. nucleus and the cerebellar nuclei have different synaptic effects on fast and slow p.t.n.s (Yoshida, Yajima & Uno, 1966; Takahashi, Kubota & Uno, 1967; Deschênes, Landry & Clercq, 1982; Noda, Yamamoto, Miyata & Nishimura, 1983; Noda & Yamamoto, 1984). We tried to check whether such differences in synaptic activation are present in the immature cerebral cortex, where the general feature of dendrites and axon collaterals of p.t.n.s as well as thalamic afferents are already established (Purpura, Shofer & Scarff, 1965; Wise, Hendry & Jones, 1977; Kawaguchi *et al.* 1983).

Fig. 6 shows specimen records produced by pyramidal and cerebellar nuclear stimulation. Cerebellar stimulation induced mainly e.p.s.p.s in p.t.n.s at 0 (*A*2) and 1 day (*B*2), and the time courses of these e.p.s.p.s were similar to those reported by Purpura *et al.* (1965). After 3 days, e.p.s.p.s were often curtailed by i.p.s.p.s (*C*2 and *D*2). At 14 days (*E*2), a slow p.t.n. received mainly e.p.s.p.s upon cerebellar nuclear stimulation, and after the third post-natal week, fast p.t.n.s showed e.p.s.p.–i.p.s.p. sequences (*F*2 and *G*2) with nearly the same time course as in adult fast p.t.n.s (Yoshida *et al.* 1966; Noda *et al.* 1983; Noda & Yamamoto, 1984). This electrophysiological finding corresponded to the morphological one that differentiation of dendritic characteristics in slow and fast p.t.n.s started at around the fourth post-natal week.

Pyramidal stimulation was known to induce synaptic effects on p.t.n.s with or without antidromic impulses (Phillips, 1959; Stefanis & Jasper, 1964; Brooks & Asanuma, 1965; Takahashi *et al.* 1967; Deschênes, Labelle & Landry, 1979*a*). As shown in Fig. 6, *A*1, a p.t.n. from a 0-day-old kitten received conspicuous e.p.s.p.s at a latency of about 65 ms. In some neurones, as shown in *D*1, i.p.s.p.s preceded

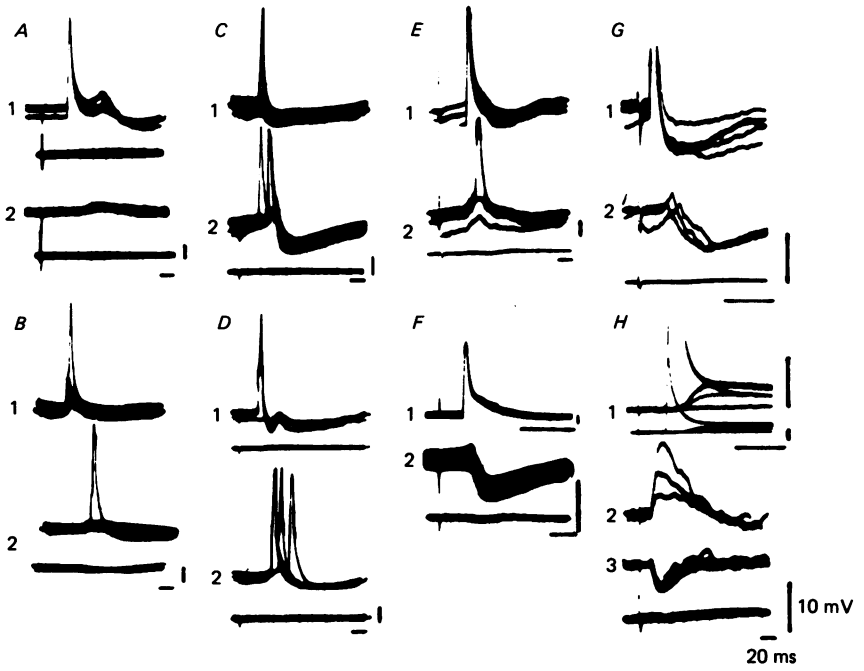

Fig. 6. Intracellular recording from p.t.n.s at various ages after birth on stimulation of the medullary pyramid and cerebellar nuclei. *A*, specimen records from a 0-day-old kitten. *A*1: antidromic responses at a latency of 34 ms are followed by e.p.s.p.s at a latency of 65 ms. *A*2: cerebellar-induced e.p.s.p.s at a latency of 64 ms. *B*, recording from a 1-day-old kitten. *B*1: antidromic action potentials at a latency of 48 ms. Note i.s.–s.d. (soma-dendritic) spikes without any synaptic potentials. *B*2: cerebellar-induced e.p.s.p.s at a latency of 65 ms give rise to full action potentials of 65 mV. *C* and *D*, records from 3-day-old kittens. *C*1 and *D*1 show antidromic impulses at a latency of 28 and 30 ms respectively. In *D*1, i.p.s.p.–e.p.s.p. sequence is observed in a trace in the absence of the impulse. In both recordings, cerebellar e.p.s.p.s are curtailed by i.p.s.p.s *E*, specimen records from a 14-day-old kitten. *E*1: antidromic impulses are induced at a latency of 20 ms. *E*2: cerebellar-induced e.p.s.p.s at a latency of 20 ms are not followed by conspicuous i.p.s.p.s. *F* and *G*, specimen records from 17- and 28-day-old kittens respectively. *F*1: antidromic impulses at a latency of 8·5 ms. *F*2: typical e.p.s.p.–i.p.s.p. sequence is observed by stimulation of the cerebellar nucleus. *G*1: antidromic impulses at a latency of 4·4 ms. *G*2: cerebellar nuclear stimulation evokes an e.p.s.p.–i.p.s.p. sequence in this neurone. *H*, presumed recurrent e.p.s.p.s induced by pyramidal stimulation. *H*1: high-gain a.c. and low-gain d.c. recordings are displayed together. Note the fixed latency of slow time course e.p.s.p.s at 17 ms by changing intensity of stimulation. *H*2: effects of hyperpolarizing d.c. current injection on slow e.p.s.p.s. Three recordings correspond respectively to those of the control, with 1 nA hyperpolarizing current, and 2 nA hyperpolarizing current from the bottom to the top. *H*3: effects of 2 nA depolarizing d.c. current injection on slow e.p.s.p.s. Note that a complete reversal of e.p.s.p.s occurs. The upper and lower records of every pair are intracellular potentials and extracellular ones recorded at just outside of respective neurones. Voltage and time calibrations are all 10 mV and 20 ms, respectively.

e.p.s.p.s. As shown in *B*1, *C*1, *E*1, *F*1 and *G*1, most of the neurones did not show conspicuous synaptic potentials. In *H*, presumed recurrent e.p.s.p.s which were very similar to those reported in adult cats (Takahashi *et al.* 1967; Deschênes *et al.* 1979*a*) were observed. The latency of e.p.s.p.s (17 ms) was fixed to various intensities of stimuli (*H*1), and temporal summation occurred with a 5 ms interval of double shocks (not shown in the Figure), which indicates the monosynaptic nature of this e.p.s.p.

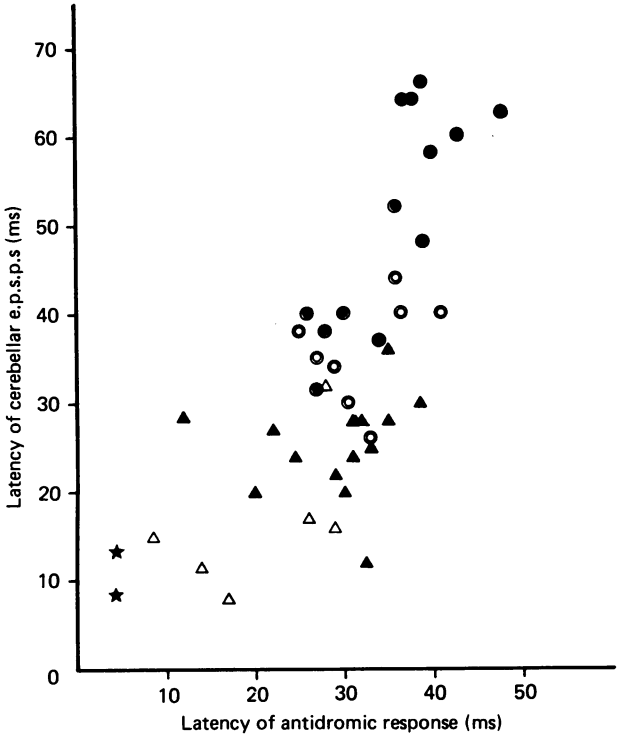

Fig. 7. Relations between the latencies of antidromic responses and those of cerebellar-induced e.p.s.p.s during the first post-natal month. The abscissa shows the latency of antidromic responses and the ordinate shows that of cerebellar e.p.s.p.s. Different symbols correspond to the various ages of animals. ●, 0–1-day-old; ⊙, 2–3-day-old; ◎, 7-day-old; ▲, 14-day-old; △, 17- and 21-day-old and ★, 28-day-old kittens. A clear correlation is observed between them, the correlation coefficient is 0·73 ($P < 0·001$; Student's t test).

This neurone was recorded from a 28-day-old kitten. When hyperpolarizing direct current of 1 and 2 nA was passed through the recording electrode, the amplitude of the e.p.s.p.s became larger than that of the control (the bottom trace) in parallel with the intensity of current (*H*2). When depolarizing current of 2 nA was passed, complete reversal of e.p.s.p.s was observed (*H*3). This phenomenon was in contrast with recurrent e.p.s.p.s observed in adult cats (Takahashi *et al.* 1967). As Deschênes *et al.* (1979*a*) proposed, even if the recurrent e.p.s.p.s were evoked in the distal part of basal dendrites, it is possible to induce such phenomena by current injection depending on the site of penetration. We could not compare the effect of current injection on the cerebellar e.p.s.p.s in this neurone.

The presence of presumed recurrent synaptic action in the immature animals might be supported from the morphological findings of intracortical axon collaterals which were well established even in very young kittens as described in the previous section.

Fig. 7 shows the relation between the change in latency of antidromic and cerebellar-induced responses during the first post-natal month. The abscissa shows the latency of antidromic responses and the ordinate that of cerebellar e.p.s.p.s. The correlation coefficient was 0·73 ($P < 0·001$; Student's t test), which strongly indicates a parallel maturation of afferent and efferent systems in the motor cortex during post-natal development.

DISCUSSION

The present study describes the post-natal development of p.t.n.s from both electrophysiological and morphological aspects. The two groups of p.t.n.s could be detected by comparing their antidromic latencies, and differentiation of fast and slow p.t.n.s on the basis of cell volumes could be detected in the first post-natal week. However, the morphological differentiation of dendrites started at around the fourth post-natal week.

Electrophysiological findings

As Conway *et al.* (1969) reported, we also distinguished two positive peaks in the cortical surface potential in neonatal kittens. These were assumed to be a prototype of the antidromic cortical responses in mature animals. Although we did not perform field potential analysis in detail, these positive potentials could follow a stimulus rate of more than 30 Hz at 0 day, which is compatible with the findings of Conway *et al.* (1969) and Huttenlocher (1970). As is suggested from experiments on adult animals, cortical positive evoked responses are ascribed to the antidromic activation of fast p.t.n. axons (the first positivity: a-wave) and that of slow p.t.n. axons together with intracortical p.s.p.s (the second positivity: b-wave). In the present study the amplitude of the late positive potential was usually larger than that of early one. When the positive evoked potentials were considered as a rough index of the population of p.t.n.s activated antidromically by medullary pyramidal stimulation, it is suggested that the population of slow conducting p.t.n.s is much larger than that of fast conducting p.t.n.s. As expected, most of the unitary antidromic responses corresponded in latency to these two cortical surface potentials. The change in latency of antidromic responses may indicate that the myelination of the pyramidal tract occurs first in the fast conducting group in the second post-natal week and then in the slow conducting one predominantly after the third post-natal week. Purpura *et al.* (1964) estimated that the largest calibre of pyramidal tract fibres was less than 2 μm (conduction velocity less than 1 m/s) at birth and 4–6 μm (conduction velocity of 15–20 m/s) at 1 month. Calculation from the present study indicated that the conduction velocities of axons of p.t.n.s around at birth were 0·7 m/s to 0·3 m/s if the distance from stimulating points to the precruciate cortex was estimated at about 20 mm. By the end of 1 month, these values were 15 m/s to 1·5 m/s if the distance was assumed to be 30 mm. The data are compatible with those reported by Purpura *et al.* (1965).

Several authors have reported that intracellular action potentials could not be

recorded successfully, or not be induced by afferent stimulation, before 3 days (Huttenlocher, 1967; Conway *et al.* 1969). In the present study, we could record antidromic or orthodromic impulses up to 70 mV in amplitude at very early stages after birth (see Figs. 1 *B*3 and 6 *A* and *B*). As concluded by Purpura *et al.* (1965), action and resting potentials in immature neurones do not greatly differ from those in adult ones.

Concerning the synaptic activation of p.t.n.s, our previous study (Kawaguchi *et al.* 1983) indicated that cerebellar stimulation could induce impulse discharges superimposed on the evoked field potentials at a depth corresponding to layer V and Purpura *et al.* (1965) demonstrated that thalamic v.l. stimulation in immature cortical neurones could elicit e.p.s.p.s and impulse discharges. The present study confirms these previous reports by intracellular recording from identified p.t.n.s. Purpura *et al.* (1965) also reported that in young kittens less than 1 week old, most synaptic potentials induced by v.l. stimulation were accompanied by i.p.s.p.s. In our study, cerebellar stimulation induced mainly e.p.s.p.s in kittens 0–2 days old, while in kittens older than 3 days, e.p.s.p.–i.p.s.p. sequences were much more often observed. A parallel maturation of cerebello-cerebral projection and the pyramidal tract fibres was suggested from comparison of the latencies of antidromic spikes and cerebellar e.p.s.p.s during post-natal development.

In our experiments, even at 0 day old, some p.t.n.s received e.p.s.p.s after antidromic impulses. In some cases, i.p.s.p.–e.p.s.p. sequences were observed. Most of such synaptic potentials could not easily be isolated from antidromic impulses. In a few cases, e.p.s.p.s were isolated from antidromic spikes and checked in detail as shown in Fig. 6*H*. In this case, the latency of the e.p.s.p.s was quite constant after changing stimulus intensity and the time course was very similar to that reported by Takahashi *et al.* (1967). In contrast to the observation in adult cats (Takahashi *et al.* 1967), this e.p.s.p. behaved as other afferent e.p.s.p.s, i.e. hyperpolarizing current increased its amplitude. This might be due to some difference of membrane properties of p.t.n.s between the adult and this 28-day-old kitten, or difference of current injection methods, i.e. Takahashi *et al.* (1967) used a pulse current (about 100 ms duration) and we used a direct current. Another possibility might be that our electrode penetrated a cell where the current injection affected the distal part of basal dendrites, since recurrent e.p.s.p.s are suggested to be evoked at the distal part of basal dendrites (Deschênes *et al.* 1979*a*).

These presumed recurrent synaptic actions could be explained by the well-established intracortical axonal collaterals even in immature kittens as discussed later, although a possibility of current spread to the adjacent structures could not be excluded completely because of the small size of the brain stem.

Morphological findings on intracellularly stained p.t.n.s

Many anatomical studies using Golgi methods have shown that immature central neurones have many growth cones at the tips of dendrites and axons in rats (Wise *et al.* 1979), kittens (Morest, 1969; Phelps, Adinolfi & Levine, 1983) and monkeys (Lund, Boothe & Lund, 1977). In our material, the number of neurones stained with HRP was much smaller than those observed in Golgi preparations. However, intracellular HRP staining reveals very fine detail in various nervous structures

(Deschênes *et al.* 1979*b*; Kitai & Bishop, 1981). In 0-day-old kittens, we failed to recover enough well-stained cells to allow firm conclusions. However, we recovered well-stained neurones from 1- and 2-day-old kittens, and in these cells, basal dendrites were very fine but no growth cones were detected at the tips of basal or apical dendrites. Wise *et al.* (1979) described several growth cones at the tips of dendritic processes in 3–4-day-old rats. They also reported that pyramidal neurones in layer V were usually free of typical dendritic spines until 4 days after birth except in some large pyramidal neurones. The differences between our results and theirs in soma-dendritic morphology might be due to differences in species or methods. Wise *et al.* (1979) also suggested that the maturation of layer V cells preceded that of cells in the superficial layers (corresponding to the general rules of inside-out neurogenesis in the cerebral cortex, Sidman & Rakic, 1973). In our material, several neurones in the superficial cortical layers were also stained with HRP. It was noted that all neurones of pyramidal form spread their apical dendrites into layer I, but some of them showed immature somata and basal dendrites (i.e. a tendency towards pleomorphic somatic shape etc.). It is possible to conclude that the maturation of cells in the superficial cortical layers seems to be slower than of p.t.n.s (T. Yamamoto, A. Samejima & H. Oka, in preparation).

As is shown in Pl. 1*A*, the thickness of the cerebral cortex increases enormously after birth. In parallel with these changes, the length of apical dendrites also increased, but the basic pattern of arborization changed little. The basic pattern of soma-dendritic morphology was established as early as 7 days. However, the surface of the somata was covered with filamentous appendages until the end of third post-natal week. The spines covering apical dendrites seemed to be different from those in adult cats; spines in immature neurones were fine with longer stalks. Lund *et al.* (1977) reported that all neurones in the monkey visual cortex showed a gradual increase of the number of spines up to 8 weeks after birth and then the number decreased again. In our material we could not detect this, although the number of spines increased gradually during the first post-natal month (except in presumed fast p.t.n.s which were losing spines in the apical dendrites by about the end of the first month).

Intracortical axonal trajectories of immature cortical neurones have not previously been reported by intracellular HRP staining. We have now shown that the intracortical axonal ramification is established fairly well at early post-natal stages. Even at 3 days some axons ran horizontally for 1 mm and showed fairly dense collaterals in layers V and VI, as in adult cats (Landry *et al.* 1980; Noda & Yamamoto, 1984). These observations might explain the electrophysiological findings of presumed recurrent synaptic action in these kittens. Strikingly, several neurones projected axon collaterals into the border between layers I and II or even into layer I. In the motor cortex of the adult cat, such collaterals have not been detected by intracellular HRP staining, although Donoghue & Kitai (1979) and Martin & Whitteridge (1984) reported such collaterals in the rat sensorimotor cortex and the cat visual cortex, respectively. It is known that collateral elimination occurs extensively in callosal and pyramidal tract neurones during post-natal development in kittens, rabbits and rats (see review by Stanfield, 1984). However, this reorganization seems to finish at around the second and third post-natal weeks. On the other hand, intracortical collaterals

seemed not to change significantly during the first post-natal month. So it appears unlikely that some axon collaterals will regress during maturation after the first post-natal month.

Morphological maturation of p.t.n.s and behavioural development

In kittens at about 10 days of age, the eyes are open, and at about 3 weeks of age they start to walk. After this stage kittens acquire more skill in motor activities and at the time of weaning, i.e. about 4 weeks of age, they walk fairly well. Completion of myelination of the pyramidal tract and maturation of afferent systems such as cerebello-cerebral projection and sensory afferent pathways are necessary for the enormously increased motor function. In comparing the development of motor functions and the morphological maturation and differentiation of p.t.n.s, it appears that the time of morphological differentiation of slow and fast p.t.n.s corresponds with the time when animals start to gain motor skills.

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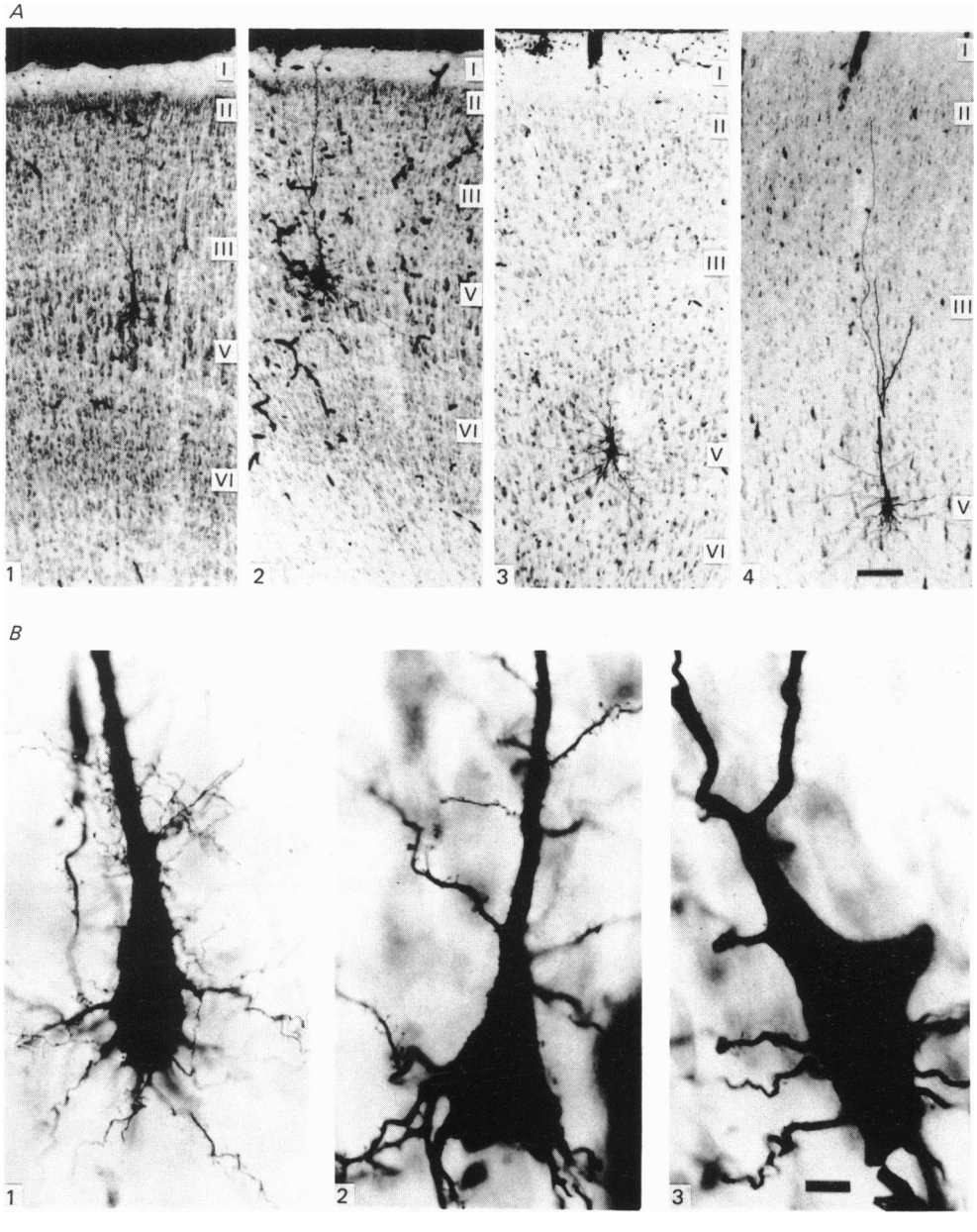
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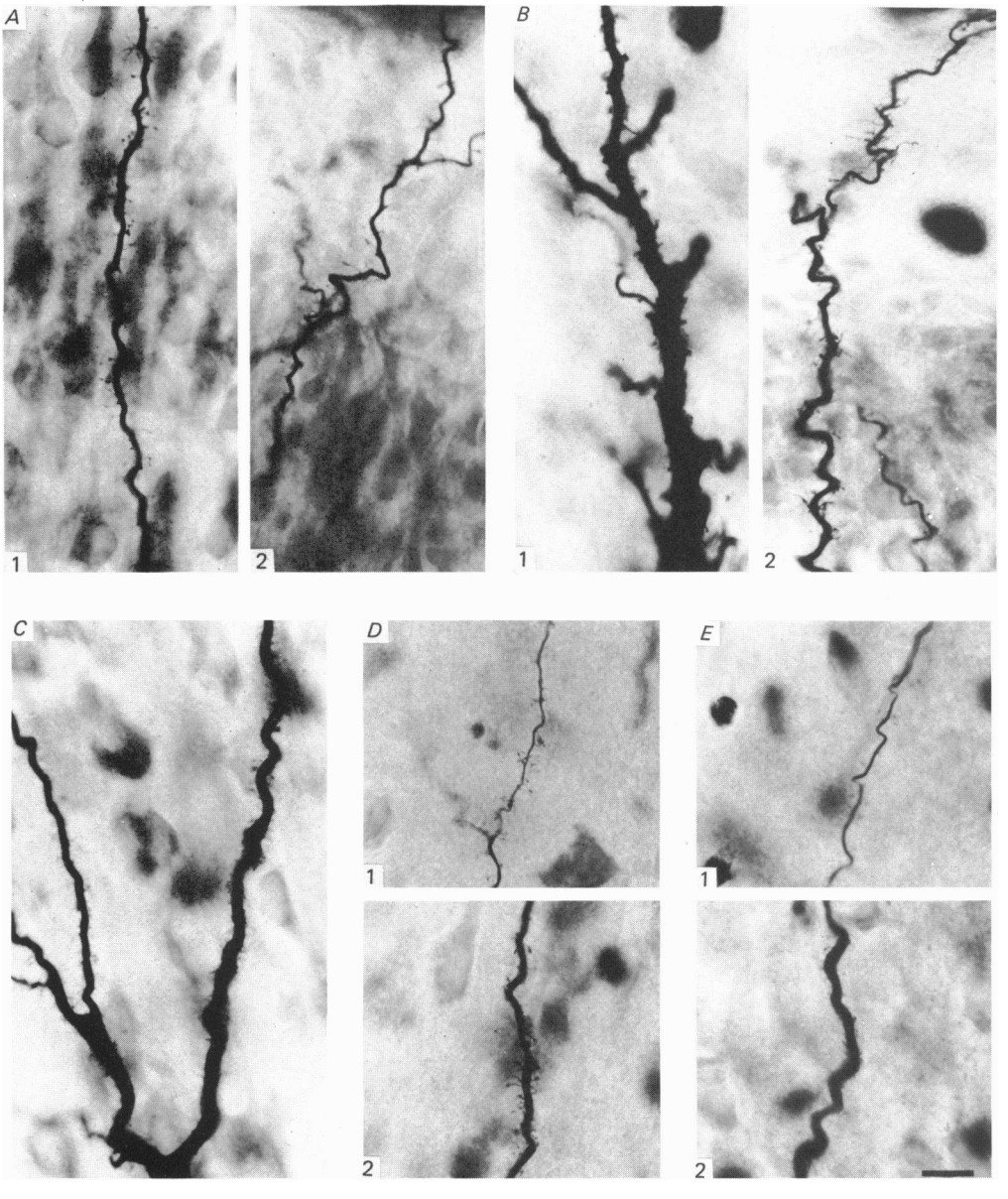
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EXPLANATION OF PLATES

PLATE 1

Photomicrographs of HRP-injected p.t.n.s at various ages. *A*, low-power photomicrographs of p.t.n.s in the area 4γ. Note that the conspicuous increase of thickness of the cortical layers during maturation (*A*1: 3-day-old, *A*2: 14-day-old, and *A*3: 28-day-old kitten; *A*4: adult cat). At 28 days of age, the development of the cortical layers is nearly established. Roman numerals indicate the cortical layers. Scale bar is common and 100 μm. *B*, high-power photomicrographs of the somata of p.t.n.s. *B*1: 1-day-old, *B*2: 14-day-old and *B*3: 17-day-old kittens. Note that very fine filamentous appendages protrude from the soma and the primary dendrite and that basal dendrites are fine at 1 day old (*B*1) in comparison with those in 14- and 17-day-old kittens. At 17 days of age, the soma is nearly smooth. Scale bar is common and 10 μm.

PLATE 2

High-power photomicrographs of dendritic spines on apical dendrites at various ages after birth. *A*, photomicrographs of the secondary (*A*1) and the tertiary dendrites (*A*2) from a 3-day-old kitten. The surface of dendrites is covered with 'drum stick'-like spines. *B*, photomicrographs of the primary (*B*1) and the tertiary dendrites (*B*2) from a 7-day-old kitten. Note that the surface of the primary dendrites have thick spines. On the tertiary dendrites, the number of spines seems to increase in comparison with that at 3 days of age. *C*, photomicrographs of the basal part of the secondary dendrites from a 17-day-old kitten. Note that around the top of the primary dendrite is smooth. *D*, dendritic spines on the tertiary (*D*1) and the secondary dendrites (*D*2) of apical dendrites of a presumed slow p.t.n. from a 28-day-old kitten. *E*, similar sets of photomicrographs as in *D* of a presumed fast p.t.n. from a 28-day-old kitten. Note that the density of spines on the tertiary as well as the secondary dendrites is much sparser than that observed in *D*. Scale bar is common and 10 μm.