ONTOGENESIS OF OLIVOCEREBELLAR RELATIONSHIPS

I. Studies by Intracellular Recordings of the Multiple Innervation of Purkinje Cells by Climbing Fibers in the Developing Rat Cerebellum¹

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Abstract

The establishment of the adult innervation of Purkinje cells (PCs) by climbing fibers (CFs) was studied in the cerebellar vermis of the developing rat. Excitatory postsynaptic potentials (EPSPs) evoked in PCs by activation of the climbing fibers (CF-EPSPs) were recorded intracellularly from a total of 310 cells in young rats aged from 3 to 15 postnatal days. The CF system was activated by electrical stimulation of either the inferior olive (IO) nucleus or the region near the fastigial nucleus (juxtastigial or JF stimulation). A given PC at each age was considered to be innervated by more than one CF when the amplitude of the spontaneous or evoked CF-EPSPs fluctuated in a stepwise manner. On the other hand, innervation of a PC by a single CF was established on the basis of the all-or-none character of CF-EPSPs. Two parameters were followed throughout development, the percentage of multiply innervated PCs and the mean number of steps in the evoked CF-EPSPs. The data presented confirm the transient multiple innervation of PCs by CFs on postnatal days 8 and 9 (Crépel, F., J. Mariani, and N. Delhaye-Bouchaud (1976) *J. Neurobiol.* 7: 567–578) and strongly suggest its existence at earlier stages (from postnatal day 3). Moreover, it is shown that the multiple innervation was maximal on postnatal day 5 and then decreased until the innervation by a single CF was established on day 15.

The establishment of the adult innervation in three peripheral systems, the neuromuscular junction (Redfern, 1979; Bagust et al., 1973; Bennett and Pettigrew, 1974, 1975; Benoit and Changeux, 1975; Brown et al., 1976), the rat submandibular ganglion (Lichtman, 1977), and the sympathetic cervical ganglion (Lichtman and Purves, 1980), shares a striking common feature: the formation of the adult network involves the removal during development of synapses that are already functional (see Changeux, 1972; Changeux et al., 1973; Changeux and Danchin, 1976; Purves and Lichtman, 1980).

In the central nervous system, a similar example of

“transient synaptic redundancy” (Changeux and Danchin, 1976) has been observed in the developing rat cerebellum where the one-to-one relationship between climbing fibers (CFs) and Purkinje cells (PCs) in the normal adult (Ramon y Cajal, 1911; Eccles et al., 1966) is preceded by a transient stage of multiple innervation (Crépel et al., 1976).

This result was established mainly on the basis of unitary extracellular recordings. More than 50% of the PCs recorded on postnatal days 8 and 9 exhibited two types of climbing fiber response (CFR) (i.e., a burst of spikes with or without inactivation slow wave), and interaction experiments between these two types of responses revealed the absence of collision. Only a few intracellular records of excitatory postsynaptic potentials mediated through CF activation (CF-EPSPs) were obtained on day 9; these fluctuated in some cells in a stepwise fashion (Crépel et al., 1976).

Extracellular recordings of high frequency bursts, presumably evoked through CFs, appear insufficient to ascertain multiple innervation and to follow its regression during development since (1) subthreshold EPSPs similar to those described in multiply innervated PCs of the adult *staggerer* mutant mouse are not detected (Mariani

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and Changeux, 1980a); (2) as shown in x-irradiated cerebella, some burst of spikes from PCs are not due to climbing fiber input (Woodward et al., 1974; Puro and Woodward, 1978); (3) CFRs are difficult to recognize from many PCs recorded extracellularly before postnatal day 8. In the present work, intracellular recordings of climbing fiber responses were obtained from PCs in young rats. The data confirm the presence of a multiple innervation at 8 and 9 days, and evidence for its existence at earlier stages of development is presented. Finally, the evolution of the multiple innervation until the adult state is established in detail. A preliminary report of some of these results has been published (Mariani and Changeux, 1980b).

Materials and Methods

Methods used in the present study were the same as those previously described (Crépel et al., 1976; Mariani and Changeux, 1980a). Animals were Wistar rats ranging in age from 3 to 15 postnatal days (day of birth = day 0). Under continuous ether anesthesia, the animals were paralyzed (Flaxedil, 80 mg/kg, i.p.), artificially respired with a Palmer pump at a rate of 100/min, and infiltrated with tetracaine at all wounded margins, and the cerebellar vermis was exposed surgically. Intracellular recordings were obtained from PCs in animals unanesthetized but under local analgesia with glass micropipettes filled with 3 M potassium acetate. For 3- to 10-day-old animals, electrodes whose DC resistance measured *in situ* ranged between 60 and 120 megohms were used. In older rats, 40- to 50-megohm pipettes were convenient. A concentric bipolar stimulating electrode (SNE 100, Rhodes Instrument) was inserted in the region of the fastigial nucleus (juxtafastigial or JF stimulation) and/or in the inferior olivary nucleus (IO stimulation). Stimulation sites were histologically confirmed as previously reported by Crépel (1971). DC current and rectangular current pulses were injected intracellularly through the recording microelectrode using the active bridge circuit of the electrometer (W-P Instruments, M 707). Movements of the tissues were minimized by covering the structures with 4% agar in 10% saccharose solution. The data were photographed on a camera, and AC and DC records were stored on a FM magnetic tape (bandwidth, 0 to 5000 Hz) for further analysis and measurements.

Results

The intracellular recording of the responses evoked in PCs by JF or IO stimulation appeared more difficult to obtain in young rats than in adult animals. In the cells retained for analysis, the initial resting potential was larger than -40 mV and the initial spike height was no lower than 40 mV. In most of the cells, the spike potentials disappeared more or less rapidly after impalement, but the underlying excitatory postsynaptic potentials evoked through CF activation (CF-EPSPs) were recorded during several minutes.

Identification of PCs innervated by several CFs. The criteria used to recognize the multiple innervation of a given PC by several CFs were the same at all of the ages studied and the same as those already used with several mutant mice (Crépel and Mariani, 1976; Mariani et al.,

1977; Mariani and Changeux, 1980a). These criteria are illustrated in Figure 1, *A* and *B*, for a singly innervated cell and in Figure 1, *C*₁ to *C*₆, for a multiply innervated one.

The typical full spike climbing fiber response (CFR) was evoked through JF stimulation (Fig. 1*A*). After disappearance of the spike-generating mechanism, the underlying typical CF-EPSP was recorded. Only cells in which the amplitude of the maximal step reached at least 6 to 8 mV were retained. As expected for a chemical synapse, the EPSP reversed when a depolarizing DC current was applied through the microelectrode (Fig. 1*B*₂) (Eccles, 1964; Llinas and Nicholson, 1976). Since full reversal of the maximal response was obtained, the persistence of some abortive regenerative response seemed unlikely in these cells. Basically, a PC was considered as innervated by a single CF collateral when the CF-EPSP evoked through JF (or IO) stimulation was all-or-none in amplitude (Fig. 1, *B*₁ and *B*₂). In the same cell, the CF-EPSPs occurring in the background discharge were also all-or-none in amplitude (Fig. 1*B*₃). On the other hand, a PC was considered as innervated by several CF collaterals when the amplitude of the CF-EPSP was graded in a stepwise manner (two to five steps depending on cells); this stepwise variation was disclosed either when the intensity of the stimulation (JF or IO) was slightly increased (Fig. 1, *C*₁ to *C*₅) or during the background spontaneous discharge (Fig. 1*C*₆). Moreover, the spontaneous steps occurred randomly and the frequency of each

Figure 1. Intracellular recordings of Purkinje cell responses to the activation of climbing fibers in developing rats. IO stimulation was used in *A*, *B*, and *D* and JF stimulation was used in *C*. Several sweeps are superimposed in all traces. *A*, Full spike climbing fiber response evoked in a 10-day-old rat. The response was all-or-none in character. *B*₁ to *B*₃, All-or-none CF-EPSP evoked from another PC in a 9-day-old animal. Reversal of the EPSP was obtained (*B*₂) with a DC depolarizing current of 11 nA. Resting potential of the cell was -42 mV. *B*₃, All-or-none CF-EPSP in the spontaneous discharge of the same PC as in *B*₁ and *B*₂. *C*₁ to *C*₆, CF-EPSP recorded in a PC of a 5-day-old rat. The intensity of stimulation was increased smoothly from *C*₅ to *C*₁ and several steps appeared in the amplitude of the response. *D*, Reversal properties of CF-EPSP in a 4-day-old rat; The maximal response (0 nA) almost fully reversed with a depolarizing current of 6.5 nA. Resting potential of the cell was -37 mV.

of these steps was in the range of the spontaneous discharge of inferior olivary neurons at the same age (see Mariani and Changeux, 1981).

Concerning these criteria, the following remarks have to be made: (a) Since spikes have disappeared, the cells studied were obviously damaged; the percentage of cells retained versus cells impaled was lower at early stages than later in development. Nevertheless, the quality of the recordings from retained cells did not vary significantly between the ages studied at least up till 10 days. (b) No systematic study of the reversal properties of the CF-EPSPs (Llinas and Nicholson, 1976) was made in the present work. However, it was obvious (Fig. 1D) that, in young animals, until days 8 and 9, reversal was obtained with amounts of current (3 to 6 nA) strikingly smaller than those necessary later on (Fig. 1B) or than reported in adult animals (Llinas and Nicholson, 1976; Crpel and Delhay-Bouchaud, 1979) even when comparing cells whose resting potential was in the same range.

Characteristics of CF-EPSPs throughout development. A total of 310 cells was retained for analysis from postnatal days 3 to 15. An additional sample of 20 cells was recorded in two young adult rats.

The different steps evoked through JF (or IO) stimulation in a given cell occurred in most of the cells at

Figure 2. Synaptic potentials recorded intracellularly from PCs in developing rats. Several sweeps are superimposed in all traces except D_2 . IO stimulation was used in A and B and JF stimulation was used in C to F. A_1 and A_2 , EPSPs evoked in a 3-day-old rat by IO stimulation that was increased progressively from A_1 to A_2 . B_1 to B_3 , Compound EPSP recorded on postnatal day 7. The latency of the second component of the EPSP decreased when the stimulation was increased (B_1 to B_2) until a typical CF-EPSP was recorded (B_3). C_1 and C_2 , IPSP evoked by JF stimulation in an 11-day-old animal (C_1). A CF-EPSP appeared at a slightly higher threshold (C_2). D_1 and D_2 , Presumed "dual" responses occurring after stimulation (D_1) or spontaneously (D_2) in a 9-day-old rat. E , Example of graded CF-EPSP recorded from postnatal day 11 in which the two steps exhibited very different amplitudes. F_1 to F_5 , Example of the few graded CF-EPSPs recorded on day 13. The JF stimulation was increased progressively from bottom to top and evoked direct (F_1 to F_3) and reflex (F_4 to F_5) EPSPs that exhibited both a graded and compound character (see text for details).

almost the same latency (Fig. 1C) which was identical to the latency of the responses recorded prior to spike deterioration and closely agrees with the values reported with extracellular recordings of presumed CFRs during development (Crpel, 1971; Crpel et al., 1976); it varied from 28 ± 5.1 msec on day 3 to 7.1 ± 1.8 msec on day 21 with IO stimulation. In some cells, however, the response to maximum stimulation could be resolved by fine gradation of the stimulus into components of different latencies (Fig. 2, B_1 and B_3) as already reported in "compound" EPSPs recorded at the developing neuromuscular junction (Benoit and Changeux, 1978).

Even at the earliest ages studied (Fig. 2, A_1 and A_2), the shape of the CF-EPSPs was similar to that recorded in adult mammals (Eccles et al., 1966; Mariani and Delhay-Bouchaud, 1978). In particular, when JF stimulation was used and slightly varied, typical "direct" and "reflex" CF-EPSPs were recorded (Eccles et al., 1966) (Fig. 2, F_1 to F_5). The CF-EPSPs evoked through IO stimulation, as well as each step of the spontaneous discharge, very often exhibited the multiwave shape typical of CF-mediated EPSPs (Figs. 1, B_1 , B_3 , and C_6 , and $2B_3$). These synaptic potentials differed, however, from the adult ones by their longer duration during the first postnatal week. In particular, the time to peaks of EPSPs evoked by supramaximal stimulation ranged between 2 and 3 msec on days 3 to 5 (mean = 2.3 ± 0.54 msec on day 4, $n = 11$) and reached 1.10 ± 0.22 msec ($n = 23$) on day 10 (i.e., values similar to the adult ones of 0.91 ± 0.12 msec, $n = 10$).

Although the CF-EPSPs recorded throughout the development had many similarities, a few comments should be added depending on the age considered. (a) JF stimulation frequently evoked inhibitory postsynaptic potentials (IPSPs) that followed the CF-EPSPs or occurred even with a stimulus subthreshold for the EPSP (Fig. 2, C_1 and C_2). They were observed in multiply as well as in singly innervated PCs; the earliest one was recorded on day 7, but they were seen more consistently on day 9 and the following days, in agreement with the intracerebellar inhibition disclosed by extracellular recordings on day 10 in normal rats (Crpel, 1974). (b) In a few PCs recorded from postnatal days 7 to 10, the stimulation (IO or JF) evoked two or three closely spaced CF-EPSPs with generally the same threshold and that also occurred spontaneously, usually separated by less than 50 msec (Fig. 2, D_1 and D_2). These "dual" CF-EPSPs could be the intracellular counterpart of the dual burst responses described by Puro and Woodward (1977) with extracellular recordings. (c) Two features were observed for multiply innervated PCs recorded on postnatal days 10 to 13, i.e., at the end of the regression: (1) For 6 cells, the amplitude of the two steps was very different since one of them had a very reduced size (Fig. 2E). (2) For 4 cells, it was impossible to separate within compound EPSPs the components that exhibited different latencies (Fig. 2F); however, when the intensity of the stimulus was progressively increased, the latency of the second step decreased until summation with the first one was complete in both the direct and reflex EPSPs (Fig. 2, F_1 to F_5). A plausible explanation for this observation is that the corresponding PCs were innervated by several branches of the same inferior olivary neuron.

Evolution of the multiple innervation with age. To define more precisely the regression of the multiple innervation, two parameters were followed throughout development (Fig. 3 and Table I), the percentage of multi-

ply innervated PCs and the mean number of steps in the evoked CF-EPSPs. This last criterion was taken as a minimal estimate of the mean number of CF collaterals impinging on each PC. The evolution of the percentage (Fig. 3A) was characterized by a plateau from postnatal days 3 to 7 (approximately 90 to 95%), followed by a regular decrease until the innervation by one CF was established on postnatal day 15 (or 14).

Cells in which CF-EPSPs displayed more than two steps were recorded only until day 8 with a maximum of five steps apparent in some PCs. From day 9 to day 13, the responses of all of the multiply innervated cells exhibited only two steps. The mean number of observed steps increased from day 3 ($m = 2.43 \pm 0.51$, $n = 20$) to day 5 ($m = 3.33 \pm 0.85$, $n = 20$) and then decreased regularly until only one step persisted on postnatal day 15. Thus, the multiple innervation of PCs by CFs seems to be maximum on postnatal day 5. However, some underestimation of the multiple innervation at very young stages cannot be excluded because of the difficulty of intracellular recordings at this age.

Full spike climbing fiber response in singly and multiply innervated cells. In some intracellular penetrations of the present study, the spike-generating mechanism was conserved during almost the whole recording session. These cells ranged from -40 to -60 mV (mean = 51 ± 7.5 , $n = 30$). Such stable recording of spikes was obtained from 35 cells, and the height of the spikes varied from 45 up to 80 mV (Fig. 4). The response evoked in PCs by JF or IO stimulation closely resembled the typical climbing fiber response (CFR) found in adult mammals (Eccles et al., 1966; Martinez et al., 1971; Mariani and Changeux, 1980a), a full spike followed by a depolarizing potential on which partial spikes were superimposed sometimes (Fig. 4, A, E, and F) even on postnatal day 3 (Fig. 4D). Typical "direct" and "reflex" CFRs (Eccles et al., 1966) were recorded after day 7 (Fig. 4A) and, with JF stimulation, were preceded sometimes by an antidromic response, generally in animals older than 9 days, confirming the identification of impaled neurons as PCs (Fig. 4B). Often, the response was followed by a prolonged hyperpolarization (more than 100 msec) that began to appear on 7-day-old animals (Fig. 4C). In 25 of these cells (21 from 10- to 15-day-old and 4 from 5- to 9-day-old animals), the response was all-or-none in character (Fig. 4A). However, in 10 cells (from 4- to 9-day-old rats), the burst-like response was graded when the intensity of stimulation was increased as illustrated in Figure 4E. A slightly suprathreshold IO stimulus evoked a CF-EPSP whose size fluctuated in a stepwise manner (Fig. 4, E₁ and E₂); when the intensity of the IO stimulus was increased further, the summation of the steps reached the firing level (Fig. 4, E₂ and E₃) and a response resembling a typical CFR was finally obtained (Fig. 4E₄). The spontaneous discharge of the same cells consisted of bursts of spikes exhibiting a variable degree of inactivation (Fig. 4, G₁ and G₂). These bursts coexisted with subthreshold CF-EPSPs while the resting potential remained constant (not illustrated). Finally, in PCs recorded from young rats, the spike appeared after the onset of a visible EPSP (Fig. 4, D, E, and F) as already described in multiply innervated PCs of the *staggerer* mutant mouse (Mariani and Changeux, 1980a), whereas

Figure 3. Evolution with age of the multiple innervation of PCs by CFs in developing rats. *A*, Evolution of the observed percentage of PCs multiply innervated by CFs. *B*, Evolution of the mean number of steps in CF-EPSPs recorded from singly and multiply innervated PCs. Vertical bars represent the confidence limit interval of the mean (at 95%).

TABLE I
Evolution with age of the percentage of multiply innervated PCs and the mean number of steps in CF-EPSPs

	Age in Days									
	3	4	5	7	8	9	10	11	13	15
PCs: multiply innervated/total number	18/21	18/20	19/20	24/29	17/26	13/28	12/42	4/24	2/50	0/50
Multiply innervated PCs (%)	86	90	95	83	65	46	29	17	4	0
Number of steps in CF-EPSPs										
Mean	2.43	2.80	3.33	2.28	1.77	1.46	1.29	1.17	1.04	1
(SEM)	(0.131)	(0.194)	(0.189)	(0.136)	(0.124)	(0.094)	(0.070)	(0.075)	(0.028)

Figure 4. Full spike responses evoked in PCs by activation of the climbing fibers in developing rats. Superimposed sweeps are used in A, E, F₁, and F₃. A, Direct and reflex all-or-none climbing fiber responses (CFRs) in a 10-day-old rat. B, Response of another PC to JF stimulation on postnatal day 13. The CFR is preceded by an antidromic response. C, CFR recorded at a lower sweep speed to illustrate the hyperpolarization following the CFR. D, Response evoked by IO stimulation on postnatal day 3. Note the onset of the spike on a clearly visible EPSP (arrow). E, Response evoked in a 5-day-old rat. The IO stimulus was increased progressively from E₁ to E₄; subthreshold EPSPs were evoked before the onset of one or two spikes (E₂ and E₃). The maximal response resembled a typical CFR (E₄), but the spike arose on a visible EPSP (arrow). F₁ to F₄, Same sequence as in E₁ to E₄ from the same PC but with a hyperpolarization of 20 mV. The resting potential of the cell was 60 mV. G₁ to G₂, Full spike burst in the spontaneous discharge of the same PC as in E and F, exhibiting a variable degree of inactivation. The sweeps were triggered by the rising edge of the responses and photographed with a hyperpolarization of about 20 mV.

after day 8, the spike arose abruptly from the base line as in the adult (Fig. 4, A, B, and C).

The subthreshold character of CF-EPSPs persisted even if the cells studied were hyperpolarized artificially with DC current up to a resting potential of 100 to 120 mV (as measured from the output of the bridge) (Fig. 4, F₁ to F₄). This result makes unlikely the possibility that this feature of some steps could be a consequence of a depolarization due to electrode injury.

Moreover, the input membrane resistance was determined in a sample of PCs. Hyperpolarizing current pulses of 50 msec duration were applied through the recording microelectrode with a bridge circuit and resistance was

Figure 5. Input resistance of Purkinje cells in developing rats. A₁ and A₂, Transmembrane voltage changes (A₁) induced by various hyperpolarizing currents (A₂) injected into the cell through the recording microelectrode. A₃ and A₄, As in A₁ and A₂ but with the electrode outside of the cell. B, Relationship between current and voltage for the cell illustrated in A and obtained from a 9-day-old rat.

tested before penetration and after withdrawal of the micropipette (Fig. 5, A₁ to A₄). For each cell studied, the transmembrane voltage change at the plateau of polarization was plotted against applied current (Fig. 5B), with the slope of the curve corresponding to the input resistance, Rin. Rin was measured in 4 PCs from which spikes were still recorded and in 15 cells when only CF-EPSPs persisted.

The mean value measured was 7.3 ± 0.90 megohms ($n = 8$) on 13- to 15-day-old animals and 10.7 ± 1.46 megohms on 5- to 11-day-old animals ($n = 10$). These values of input resistance were in the range of those published for multiply innervated PCs of the adult agranular rat and were slightly higher than in control adult rat (Crépel and Delhaye-Bouchaud, 1979). This would suggest that no marked decrease of membrane resistance occurred that could explain the subthreshold character of some steps.

Discussion

The present work is a systematic study of the multiple innervation of PCs by CFs by intracellular recordings performed during development in the normal rat.

Criterion used to establish multiple innervation. The

criterion used in the present study was the stepwise graded character of the CF-EPSPs; a very similar one has been used already to demonstrate a multiple innervation of PCs by CFs in various agranular cerebella of adult animals (Woodward et al., 1974; Crépel and Mariani, 1976; Mariani et al., 1977; Puro and Woodward, 1978; Mariani and Changeux, 1980a; Crépel et al., 1980) as well as to reveal a transient multiple innervation at the neuromuscular junction (Redfern, 1970; Bagust et al., 1973; Bennett and Pettigrew, 1974, 1975; Benoit and Changeux, 1975, 1978) and in the rat submandibular ganglion (Lichtman, 1977).

On the basis of the features of the responses presented under "Results," it seems very unlikely that any of these graded EPSPs could be due to an activation through the mossy fiber-parallel fiber pathway. The arguments could be summarized as follows: (1) The latencies of graded EPSPs evoked by stimulation were the same as those of CFRs recorded before cell deterioration; they were also similar to the latencies of extracellularly recorded CFRs during development (Crépel, 1971; Crépel et al., 1976). (2) Each component of the graded CF-EPSP occurred randomly during the resting discharge and exhibited a typical multiwave shape frequently; its firing frequency was in the range reported for spontaneous discharge of inferior olivary neurons at the same age (see Mariani and Changeux, 1981). (3) The graded responses disappeared while the bulk of synapses between parallel fibers and PCs was established (Altman, 1972a, b, c). (4) The use of microelectrodes filled with potassium acetate rules out the possibility that some of these steps represented IPSPs reversed by chloride diffusion.

Thus, a plausible explanation of the graded character of these EPSPs is that they are mediated by several CFs impinging on each PC. However, such complex EPSPs could arise from other modes of synaptic organization that have to be discussed: (a) Electrical coupling between PCs at an early stage of development is a possible source, but no transient gap junctions have been described yet between PCs, and moreover, the different steps of the responses were reversed by current injection. (b) Synaptic interactions between PCs during the first postnatal days should be considered also; recurrent collaterals of PC axons have been described as more abundant in young than in adult animals (Ramon y Cajal, 1911), but no conclusive morphological or electrophysiological evidence has been provided yet that these collaterals make synapses; moreover, these synapses should be inhibitory since PC axons are known to exert inhibitory effects of their target cells (Obata et al., 1967). (c) Increased input resistance in young cells could make a constant level of multiple innervation more obvious than later in development; the slight difference of input resistance observed in the sample of PCs that we have studied seems insufficient to explain these graded responses.

To summarize, our results suggest that CF-PC synapses were already functional on postnatal days 3 and 4 in agreement with the extracellular recordings of bursts on the same age (Crépel, 1971) and that, at early stages, almost all of the PCs recorded were innervated by several CFs.

The CF-EPSPs recorded during the first postnatal week have a slow rise time, a feature already noticed

although not really explained yet at other newly formed synapses (Naka, 1964; Purpura et al., 1965; Landmesser and Pilar, 1972).

Evolution of the multiple innervation of Purkinje cells by climbing fibers. The number of steps in the response provides a rough and minimal (two fibers may have the same threshold) estimation of the multiple innervation; its extent, as judged from this index, does not exceed 3.5 fibers per PC, in agreement with the data obtained from the comparison of spontaneous activity of PCs and IO neurons (see Mariani and Changeux, 1981).

The percentage of multiply innervated cells found on postnatal days 8 (65%) and 9 (46%) closely agrees with the value previously found (57%) on the basis of extracellular unitary recordings when cells from 8- to 9-day-old rats were pooled (Crépel et al., 1976). On the contrary, a difference appears between the percentage of multiply innervated cells disclosed in the present study on day 10 (i.e., 29%) and the value previously found (14%; Crépel et al., 1976) using extracellular recordings and collision experiments. Two reasons could explain why the decrease was less abrupt than previously mentioned: (1) Some of the CF collaterals converging on a PC could belong to the same IO neuron with a conduction failure occurring intermittently at the level of the branching as already demonstrated for other axons (Parnas et al., 1969; Van Essen, 1973; Westerfield et al., 1978). Such collaterals would not have been taken into account by the collision experiments. It is conceivable that the last supernumerary collaterals that remain at the end of the regressive process belong to the same CF, as suggested under "Results." (2) The most plausible explanation remains that some of the steps of the CF-EPSPs did not reach the firing level of the cell and therefore were not detected by extracellular recordings. In fact, intracellular recordings with conservation of the spike revealed in the present study that some of the spontaneous or evoked steps remained subthreshold in the young rat until postnatal day 9. Moreover, the spike arose on a clearly visible EPSP before day 9, in agreement with the initial somatic site of CF-PC synapses preceding their dendritic location later on (Altman, 1972b). Similar results were obtained in adult *staggerer* mice where each PC received somatic synapses from several CFs (Mariani and Changeux, 1980a).

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