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Short Communications

The effect of unilateral cerebellar pedunculotomy on the vascular development of the neonatal rat cerebellum

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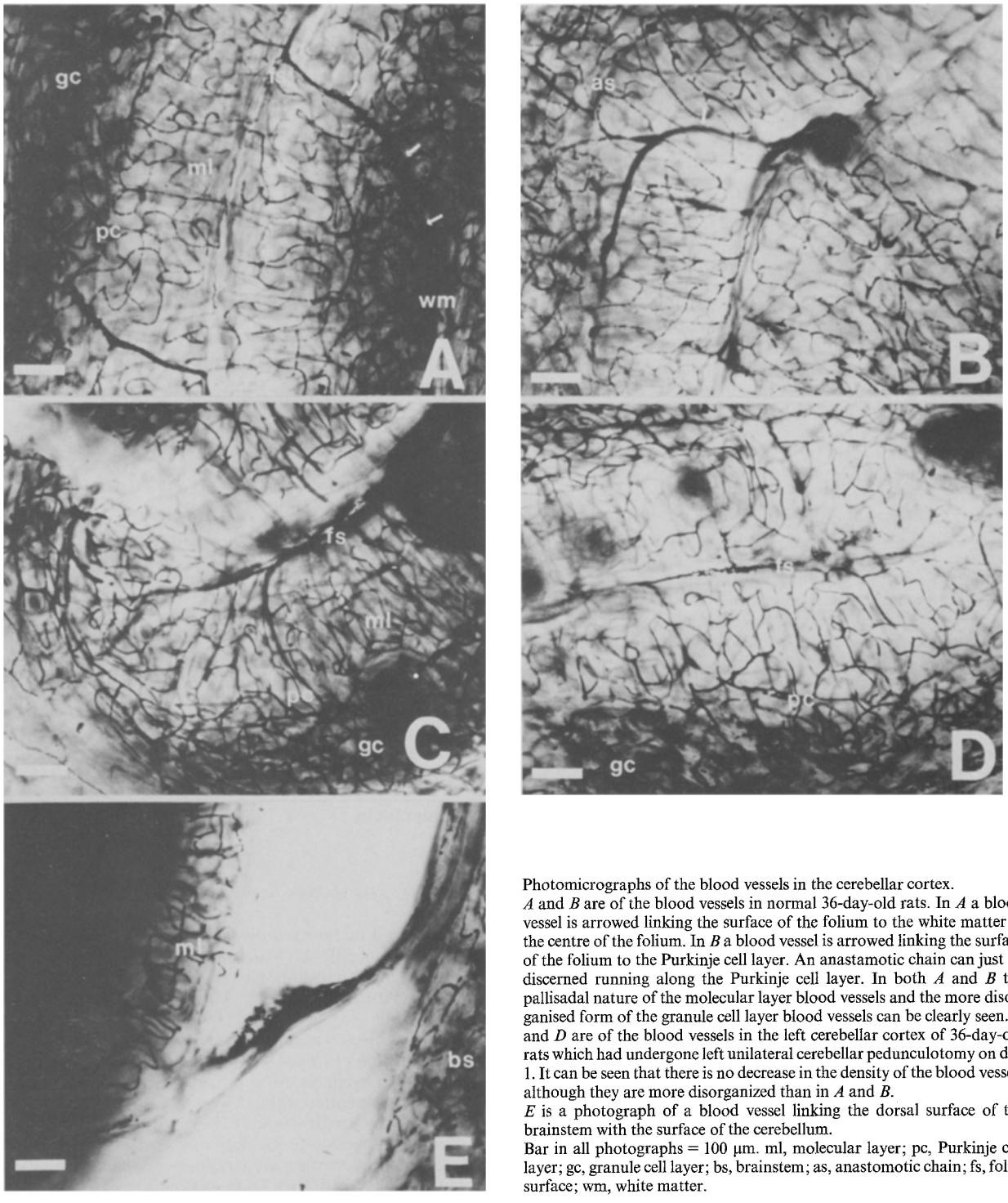
Summary. After cerebellar pedunculotomy the density of the blood vessel network in the cerebellar cortex was not different from that in the control animals. But the pattern of the blood vessels was different, being less organized in the operated animals.
Key words. Cerebellum; pedunculotomy; blood supply; development.

There have been a number of investigations recently that have used the operation of unilateral cerebellar pedunculotomy in the study of the postnatal development of the rat cerebellum[1–8]. One of the consistent findings is that the cerebellar hemisphere on the side of the operation is smaller than the unoperated side. A possible explanation of this finding is that the operation has compromized the normal blood supply to the cerebellar hemisphere thus affecting the normal growth of the neural tissue. Obviously, this would limit the usefulness of pedunculotomy as a tool for investigating the effect of the removal of afferent fibres on the subsequent growth of the cerebellar hemisphere and its constituents.

The blood supply of the cerebellar cortex arises from the vascular network that covers the pia meter[9]. Arterioles supplying the cortex penetrate the surface at 90° and ramify to form a dense plexus in the substance of the cerebellum. The Purkinje cells (PC) have a special network of vessels around them[9] and the molecular layer is the most poorly supplied[10]. The granular layer is supplied from both the pial network and vessels that are derived from the white matter. The white matter and deep nuclei are supplied from blood vessels which arrive via the inferior cerebellar peduncle[11].

The major postnatal growth of the cerebellum occurs in the first 30 postnatal days[12] and is closely paralleled by the developing vasculature[13]. In all cerebellar components there is a general increase in vascularity until the adult pattern is reached at about day 21. An exception to this is in the molecular layer where the density of blood vessels declines between days 5 and 10 and with the adult pattern not being reached until day 90[13]. Recently, Koppel et al.[14] have confirmed Craigie's 1924 result and have also shown that the density of the pial capillary network decreases during the first three postnatal weeks.

The present investigation was undertaken to see what effect, if any, the operation of unilateral cerebellar pedunculotomy had on subsequent cerebellar development. Neonatal inbred Wistar rats aged 1, 3, 5, 7, 10, 15 and 20 days were used. The day of birth was counted as day 0. One litter of 10 pups was used for each of the ages investigated. Under open ether anesthesia half of each litter had a left or right unilateral cerebellar pedunculotomy using a technique previously described[15]. The other half had a sham operation in which the knife was inserted into the 4th ventricle but the peduncles were not cut. After the operation the animals were allowed to recover and survive for 35 days. At the end of this time, using a modification of the technique described by Koppel et al.[14], the animals were perfused with a regime which demonstrates the blood vessels. Under deep ether anesthesia the animals were perfused transcardially with 60 ml each of the following solutions at 37 °C: 0.9% saline, 10% phosphate buffered formalin, 0.9% saline and finally with India ink in 3% aqueous gelatin. The perfusion with buffered formalin meant that the brains did not have to be stored in fixative for several weeks as described by Koppel et al.[14]. The second wash with 0.9% saline ensured that the gelatin was not fixed by the intravascular formalin before the fixation was complete. The India ink solution contained of 3 g of gelatin dissolved at 50 °C in 40 ml of water and 60 ml of India ink. This is a thicker solution than the



Photomicrographs of the blood vessels in the cerebellar cortex.
A and *B* are of the blood vessels in normal 36-day-old rats. In *A* a blood vessel is arrowed linking the surface of the folium to the white matter in the centre of the folium. In *B* a blood vessel is arrowed linking the surface of the folium to the Purkinje cell layer. An anastamotic chain can just be discerned running along the Purkinje cell layer. In both *A* and *B* the pallisadal nature of the molecular layer blood vessels and the more disorganised form of the granule cell layer blood vessels can be clearly seen. *C* and *D* are of the blood vessels in the left cerebellar cortex of 36-day-old rats which had undergone left unilateral cerebellar pedunculotomy on day 1. It can be seen that there is no decrease in the density of the blood vessels although they are more disorganized than in *A* and *B*.
E is a photograph of a blood vessel linking the dorsal surface of the brainstem with the surface of the cerebellum.
Bar in all photographs = 100 µm. ml, molecular layer; pc, Purkinje cell layer; gc, granule cell layer; bs, brainstem; as, anastomotic chain; fs, folial surface; wm, white matter.

one described by Koppel et al.[14] and in our hands gave better results. After perfusion the brains were stored in buffered formalin for 3 weeks to fix the gelatin at the end of which they were bisected and treated as described by Koppel et al.[14]. Thick slices between 0.25 and 0.5 mm were cut free hand and viewed with a Vickers M17 microscope. At this thickness the molecular and granular layers were easily distinguished so counter-staining was not carried out.

Results and discussion. The results from the control animals confirmed the previous findings[10, 11, 13, 14] that the major source of cerebellar cortical blood vessels arises from a vascular plexus on the pial surface. There tend to be fewer vessels in the molecular layer than in the granular layer and they are arranged in a palisade formation. Side branches linking those running vertically were seen. Large vessels were occasionally seen leaving the pial layer at 90° to run through the molecular layer to the Purkinje cell layer where they turn to run along the molecular/granular layer interface. Even if there was not such a large vessel an anastamotic chain was formed along the interface from the blood vessels of the molecular and granular layers. The density of the vessels in the granular layer appeared to be greater than those in the molecular layer but with a less regular arrangement.

This experiment depends on the cerebellar peduncles having been cut so each experimental brain was examined very carefully to ensure that the pedunculotomy was total. Any brains in which there was the slightest doubt were discarded. The overwhelming impression from the experimental results is that regardless of the age at operation the overall density of the blood vessels in the cerebellar cortex was the same as in the control animals. However, the arrangement of blood vessels was much more disorganized and the clear palisadal appearance in the molecular layer had been lost. The vessels in the granular layer were also more irregularly arranged. The Purkinje cell layer was always seen to have a good blood supply in all the experimental animals. Large vessels were seen to run either from the pial surface or the white matter core towards the PC layer, and an anastamotic network was clearly visible. Sometimes vessels were seen to link the white matter in the core of the folium to the pial surface, although with this type of experiment it is impossible to say in which direction the blood was flowing. Reinforcement of the blood supply to the cerebellum from unusual sources was also sometimes seen, with vessels linking the tectum or the dorsal surface of the brainstem to the cerebellar cortex. These were never seen in the control animals. Examples of the appearances seen in both control and experimental animals are shown in the figure.

The conclusion that can be drawn from the experiment is that whatever the effect of the operation on the subsequent growth of the cerebellum and its constituents it is unlikely to be because of a reduced vascularity within the cerebellar cortex. In particular the changes observed in the Purkinje cells[5] are probably not caused by a compromised blood supply. This means that the operation of pedunculotomy is a useful, specific method of deafferenting the cerebellar cortex and therefore can be used to study further the effect of removal of afferents in the subsequent development of neuronal tissue. If the operation is carried out bilaterally it gives an in vivo tissue culture which has its own blood supply for the study of the effect of different substances such as drugs or hormones on the development of neonatal neural tissue.

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Excitation-contraction coupling in the myocardium of hibernating chipmunks

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Summary. In the myocardium of nonhibernating chipmunks, replacing external Ca by Sr markedly prolongs the action potential plateau with an increase in contraction, while in preparations from hibernating animals this procedure inhibits both responses. Pretreatment with 4-aminopyridine causes a prolongation of the action potential plateau by Sr in hibernating animals.
Key words. Hibernating chipmunks; myocardium; plateau potential; slow inward current; strontium; 4-aminopyridine.

It has recently been demonstrated that some characteristics of cardiac excitation-contraction coupling are markedly changed during hibernation[1]. Namely, the shape of the myocardial action potential of a hibernating animal is different from that of a nonhibernating animal. In hibernating animals the action potential and the slow action potential show a reduced amplitude of the plateau phase[1]. Such characteristics of the action potential of the myocardium of hibernating animals may be explained by either a smaller contribution of the slow inward current or a greater contribution of the transient outward current which masks the slow inward current[2-4]. In the present experiments, therefore, the effects of replacing external Ca by Sr and the application of 4-aminopyridine (4-AP) were examined by using microelectrode techniques, because Sr permeates the slow calcium channels but strongly reduces the rate of inactivation of the slow inward current and the potassium outward current, and 4-AP inhibits the transient outward current.

Materials and methods. Asian chipmunks *(Tamias sibiricus)* of either sex were trapped in September and transferred to individual wire mesh cages. Some of them were kept in a room controlled at 25 °C and used for experiments on nonhibernating preparations. Others were introduced to a darkened cold room (4 ± 1 °C) with food (a standard diet of pelleted laboratory rat chow) and water available. Most of them had exhibited preliminary bouts of hibernation within 3 weeks and subsequently they showed several consecutive bouts of hibernation of more than 1 week duration. Animals during deep hibernation were used for experiments on hibernating preparations. Animals were killed by a blow on the head. The heart was quickly excised, and a papillary muscle, 2–3 mm in length and less than 1 mm in diameter, was isolated from the right ventricle. The preparation was mounted with the ends impaled on two hooks, one of which was attached to a force displacement transducer, and equilibrated for 2 h in a tissue bath containing Krebs-Ringer solution aerated with 95% O_2 and 5% CO_2[1]. The composition of the Krebs-Ringer solution in millimoles per liter was: NaCl, 120; KCl, 4.8; $CaCl_2$, 1.2; $MgSO_4 \cdot 7\ H_2O$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 24.2; and glucose, 5.5 (pH 7.4). When external Ca^{2+} was replaced by strontium, 1.2 mM $CaCl_2$ was replaced by 2 or 4 mM strontium chloride. The temperature of the superfusate was maintained at 30 °C. The preparations were stimulated at 0.2 Hz with pulses 1 ms in duration and twice the diastolic threshold.