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Immunocytochemical Study on the Distribution Pattern of Corticotropin Releasing Factor and Norepinephrine in the Middle Lobe of Monkey Cerebellum

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= Abstract = **Immunocytochemical methods, employing a specific antiserum against human corticotropin releasing factor (CRF) and dopamine beta hydroxylase, were applied to investigate the distribution pattern of CRF and norepinephrine fibers in the cerebellar cortex of squirrel monkey. CRF fibers were present mainly in the molecular layer throughout the major regions of cerebellar cortex. However, the most intensely labeled axons were strikingly clustered within particular regions and parasagittal domains. In the vermis and intermediate zone, intensely labeled axons were present only within parasagittal zones similar in location to those defined by climbing fiber innervation from the medial accessory olive. Intensely labeled axons were also densely but uniformly distributed within the uvula, the medial region of the dorsal paraflocculus, and the dorsal region of the pyramis, areas that receive their climbing input primarily from the medial accessory olive. Labeled fibers were much less dense and were not clustered in the lateral hemispheres. Norepinephrine fibers were found throughout the cerebellar cortex, and the prominent population of norepinephrine fibers in cerebellar cortex was localized within the granular layer and Purkinje cell layer. In the vermis, the great density is seen in posterior lobules, especially lobules VII-X. In the hemispheric region, a dense plexus of norepinephrine fibers was present throughout the granule cell layer, and the immunoreactive density in this region was greater than the density in the vermis. These results indicate that (1) CRF is the main neurotransmitter in the molecular layer and norepinephrine is the important transmitter in the granular layer (2) there were significant differences in the laminar distribution in different lobules of the cerebellum between CRF and norepinephrine.**

Key Words : *Immunocytochemistry, CRF, Norepinephrine, Cerebellum*

INTRODUCTION

Classically the cerebellum has served as a model system for synaptic interaction because of the rigorous homogeneity evident in the geometry of its neuronal networks. However, the chemical correlates of these well-defined networks remain largely undefined. Only recently has a profile of the neurochemical organization of the cerebellum begun to emerge. That profile, in contrast to the homogeneity of the morphological relationships, appears to be heterogenous.

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Corticotropin releasing factor (CRF) is a 41-amino acid peptide that is known to act as a hypothalamic releasing factor, stimulating the secretion of adrenocorticotropic hormone and beta-endorphin from the anterior pituitary (Vale et al., 1983). In addition, several lines of evidence (biochemical, histochemical, and electrophysiological) indicate that CRF may function as a neurotransmitter in extrahypophyseal neuronal pathways. For example, there have been several immunohistochemical studies characterizing the anatomic distribution of CRF-like immunoreactivity (CRFLI) in rat brain (Bloom et al., 1982; Merchenthaler et al., 1982; Olschowka, 1982; Cummings et al., 1983; Joseph and Knigge, 1983; Swanson et al., 1983; Fellman et al., 1984; Merchenthaler, 1984; Skofitsch and Jacobwitz, 1985; Sakanaka et al., 1987). These reports have described extensitve, widely distributed systems of CRFLI in extrahypophyseal neuronal perikarya and fibers. However, limited information is available concerning the distribution of CRF in primate brain.

Recently, certain observations have suggested that CRF is contained in neuronal perikarya in the inferior olive and in the axons projecting from this nucleus into the cerebellum. These axons constitute the olivocerebellar pathway which provides climbing-fiber input throughout cerebellar cortex, as well as collateral innervation of deep cerebellar nuclei. In most early immunohistochemical studies, CRFLI was either not observed in the olivocerebellar system or only weak immunoreactivity was evident (Merchenthaler et al., 1982, 1984; Cummings et al., 1983; Schipper et al., 1983). However, recent lignt-microscopic observations in rat (Palkovits et al., 1987; Sakanaka et al., 1987), cat(Cummings et al., 1988; Kitahama et al., 1988), and sheep (Cummings et al., 1988) have demonstrated substantial CRFLI in inferior olive perikarya and in axons in cerebellum. This is compatible with reports of high levels of CRF in inferior olive as measured by radioimmunoassay (Palkovits et al., 1983, 1985) and with demonstrations of CRF mRNA in these neurons by in situ hybridization in rat (Young et al.,1986; Palkovits et al., 1987), baboon, and human (Young et al., 1986). One electron-microscopic study has shown CRFLI in axons terminating on rat Purkinje cell dendritic spines (Palkovits et al., 1987), and one immunohistochemical study has reported CRFLI in human olivary neurons and in axons in cerebellar cortex (Powers et al., 1987). Taken together, these observations suggest that CRF may be a neurotransmitter in the olivocerebellar system of at least several species.

The cerebellar cortex receives not only the classical afferents systems terminating as mossy fibers and climbing fibers, but also a third afferent system originating from norepinephrine containing neurons located particularly in the locus coeruleus (Bloom et al., 1971; Olson and Fuxe, 1971; Chu and Bloom, 1974; Pickel et al., 1974; Pasquier et al., 1980; Foote et al., 1983). This coeruleo-cerebellar projection, which is mainly ipsilateral, reaches the whole area of the cerebellar cortex including the cerebellar vermis (cf. Dietrichs, 1988). The early observation that the noradrenergic afferents to the cerebellar cortex terminate on Purkinje neurons (Fuxe, 1965; Hökfelt and Fuxe, 1969) has been confirmed by later studies showing that these fibers make synaptic contacts primarily on Purkinje cell dendrites in the molecular layer and, to a lesser extent, on the Purkinje cell body and superficial granules cell layers (Bloom et al., 1971; Olson and Fuxe, 1971; Chu and Bloom, 1974; Pickel et al., 1974; Landis and Bloom, 1975; Yamamoto et al., 1977; Kimoto et al, 1981; cf. Powers et al., 1989, in humans).

At present, there are no detailed description of the lobular and laminar distribution of CRF and norepinephrine in the cerebellar cortex of monkey. It is important to investigate the distribution of CRF in the cerebellar cortex and compare this with the distribution of norepinephrine because CRF is the main neurotransmitter in the molecular layer and norepinephrine is the important neurotransmitter in the granular layer of cerebellar cortex.

MATERIAL AND METHODS

Immunohistochemical material was obtained from five adult New World squirrel monkeys.

The animals were deeply anesthetised with ketamine (25 mg/kg, IM) and sodium pentobarbital(15 mg/kg, IP). They were then perfused transcardialy with ice-cold 1% paraformaldehyde in phosphate buffer (0.15 M) for 1.0 minute followed by perfusion with ice-cole 4% paraformaldehyde in phosphate buffer for 9 minutes at a flow rate of 250 ~ 500 ml/minute (depending on body size). The brain was removed immediately and cut into blocks 3 ~ 5 mm thick. These blocks were immersed in cold fixative for 6-2 hours and then washed in a series of cold sucrose solutions of increasing concentration. They were then stored in 18% sucrose in phosphate buffer for 1-7 days. Forty-micron frozen sections were cut in the coronal plane and incubated, freely floating, for 48-72 hours at 4℃ in primary antiserum. The primary antiserum was visualized with the avidin-biotin-complex (ABC) method by using an ABC kit available from Vector Labs (Burlingame, CA, USA). These kits utilize as a secondary antiserum biotinylated antirabbit IgG that is subsequently bound to biotinylated horseradish peroxidase by use of an avidin bridge. The sections were developed from peroxidase reactivity wity 3, 3-diaminobenzidine (LaVail et al., 1973).

The distribution of perikarya and fibers exhibiting CRF-LI was evaluated by careful comparison of the immunohistochemical sections with adjacent Nissl-stained sections.

The primary antiserum used in these studies was raised in rabbits and was directed against the human form of CRF which is identical to the rat form (Vale et al., 1981; Rivier et al., 1983; Shibihara et al., 1983). The antiserum was genourously furnished by W. Vale and J. Rivier of the Salk Institute. For the antiserum utilized in the present studies, a dilution series of 1:1,000, 1:2,000 and 1:4,000 was evaluated. The staining from the 1:2,000 dilution was found to be optimal. The optimal dilution of the antiserum against dopamine-beta-hydroxylase was the same. As controls for nonspecific immunoreactivity, as sample of sections was incubated without primary antiserum and a different sample was exposed to a 3% hydrogen peroxide solution prior to the HRP reaction. Sections processed without primary antiserum did not exhibit any immunoreactivity, while those exposed to hydrogen peroxide, to destroy endogenous peroxidase activity, exhibited the same immunoreactivity as normally treated sections. Also, 25 sections from different levels throughtout the cerebellum were exposed to 1:2,000 primary antiserum which had been preabsorbed for 24 hours with human CRF (Penninsula Laboratores, Belmont, CA, USA) at a concentration of 0.1 mg/ml (2.1×10^{-5} M). Sections from this sample did not exhibit any immunoreactivity.

RESULTS

1. Distribution of CRF fibers in the cerebellar cortex

The most prominent population of labeled axons in cerebellar cortex was localized within the Purkinje cell and molecular layers and had the general appearance of climbing fibers (Fig. 1, 2). These axons were evident within and adjacent to the Purkinje cell layer as thick, isolated processes that typically bifurcated within the deep portion of the molecular layer and then arborized profusely as they ascended toward the surface of the cerebellum. They were generally contained within the deepest 80% of the molecular layer, with only a few branches extending as far as the cerebellar surface. In frontal sections, they appeared as parallel pairs of labeled processes extending across the molecular layer, often within the plane of section. The processes were of larger caliber in the deep portions of the molecular layer, and often formed thick, ring-like structures in the superficial portion of the Purkinje cell layer.

A much less dense population of immunoreactive processes was observed in the granular layer (Fig. 1, 2). These processes were most often evident as efflorescences at various levels between the white matter and the Purkinje cell layer. Each efflorescence was composed of an extremely compact cluster of axonal varicosities and intervaricose segments, which appeared to arise from an individual labeled axon. Other labeled processes with the appearance of fibers cut in cross section or of small rosettes were

also evident in the granular layer. Within and adjacent to the Purkinje cell layer, there were often small-caliber, varicose axons that surrounded Purkinje cell perikarya. Occasional labeled fibers were observed in white matter.

As noted above, labeled axons in cerebellar cortex were most prominent within the molecular layer. For this population, there were striking variations in the intensity of axon labeling and in the clustering of labeled processes in various regions of cerebellar cortex. Certain cortical areas contained dense collections of intensely labeled axons, while other areas contained sparse populations of moderately of lightly labeled axons. The regions of dense innervation were generally confined to the vermis and intermediate zone. For example, the uvula exhibited a uniform, high density of intensely labeled fibers. The dorsal region of the pyramis and the medial region of the dorsal paraflocculus contained large zones composed of dense collections of intensely labeled axons.

In other portions of the vermis and intermediate zone, labeled axons were organized into parasagittal stripes (Fig. 3). These stripes were evident in frontal sections as alternating regions of dense and sparse populations of immunoreactive axons within the molecuse and sparse populations of immunoreactive axons within the molecular layers of individual folia. Patches for adjacent folia were aligned to constitute paraitute parasagittally oriented stripes acorss multiple folia. Labeled axons were also evident between stripes, but these axons were less densely clustered and not as intensely labeled. In these areas between stripes, clearly labeled processes were often evident within the Purjinje cell layer. These usually appeared to have a similar morphology to the basal portion of labeled fibers within stripes, i.e., they were smoother and of larger caliber than the more highly arborized fibers in the superficial portions of the molecular layer.

The organization of densely labeled zones into parasagittal stripes was clear throughout the mediolateral extent of the vermis in frontal sections through both the anterior and posterior lobes. The stripes of dense labeling were of variable width within a given frontal section, and a given stripe varied in width along its rostral-caudal extent. As noted above, the uvula was densely innervated throughout, and the pyramis contained a large zone of densely labeled axons. In certain portions of the pyramis, stripes were also evident. Usually, one additional stripe of dense staining was evident in the intermediate zone, the parasagittal organization of this stripe being clear only in the anterior lobe. In the intermediate zone of the posterior lobe, a circumscribed area of dense innervation was evident along the medial edge of the dorsal paraflocculus.

In the lateral hemispheres, immunoreactive axons in the molecular layer were usually sparsely distributed and only moderately or lightly labeled. A striking exception was the region of crus I just ventral to the posterior superior fissure, and this area contained a dense collection of heavily labeled axons.

2. Distribution of norepinephrine fibers in the cerebellar cortex

Norepinephrine fibers were found throughout the cerebellar cortex. In general, the most prominent population of norepinephrine fibers in cerebellar cortex was localized within the Purkinje cell and granular layer (Fig. 4, 5). However, there were significant differences in the laminar distribution in different lobules of the cerebellum. In addition, the spacial orientation of the fibers in the molecular layer varied in disparate cortical regions.

Norepinephrine is present in all folia of the vermis, however, the greatest density is seen in posterior lobules, especially lobules VIII-X. The immunoreactive fibers present in the granular layer in the vermis had no special orientation, but norepinephrine fibers in the molecular layer tended to orient in the transverse plane of the cerebellum (Fig. 5, 6).

In the hemispheric region, a dense plexus of norepinephrine fibers was present throughout the granule cell layer, and the immunoreactive density in this region was greater than the density in the vermis. In the paraflocculus norepinephrine fibers are present predominantly in the upper part of granule cell layer, immediately sub-

jacent to the Purkinje cell layer. In the crus I and crus II the distribution of norepinephrine fibers is similar to that just described for the paraflocculus. Characteristically the norepinephrine fibers in the molecular layer of hemispheric region, especially in the crus II, appear to be oriented perpendicular to the pial surface in the coronal plane. In the flocculus few fibers were observed in the granular and molecular layer.

DISCUSSION

In the cerebellum, immunoreactivity is present in the molecular layer in axons with the same morphology as that previously reported for climbing fibers (Scheibel and Scheibel, 1954; Palay and Chan, 1974). In the sagittal plane, for example, individual axonal arbors originate from isolated, thick processes just superficial to the Purkinje cell layer and arborize over a wide area within the sagittal plane in a pattern similar to the abrorization of climbing fibers. In coronal sections, radially oriented, parallel pairs of labeled axons are evident. Also, examination of the present material in several planes of section indicates that there is a large-diameter process that completely excapsulates the base of the Purkinje cell apical dendrite. Immunoreactive axons are also evident as efflorescences in the granular layer and as apparent terminal arbors in cerebellar nuclei. It has previously been reported that collaterals of climbing fibers project into both of these areas and exhibit the types of terminal morphology observed in the present study (Scheibel and Scheibel, 1954; Palay and Chan, 1974). Although morphology alone does not allow an unambiguous classification of the variety of labeled processes observed in the granular layer, at least a subset of them most likely comprises those formed by collaterals of climbing fibers.

The interpretation that CRFLI is contained within the olivocerebellar system is also supported by the observation in the present study of a distinct regional and parasagittal organization of labeled molecular layer axons. The present observations indicate that CRF is contained in immunohistochemically detectable levels in all inferior olive neurons and their projections in these species. This is evidenced by the presence of clear immunoreactivity in all inferior olive perikarya examined in counterstained sections and by the fact that labeled axons are evident in all those regions of cerebellar cortex examined. However, the present observations also suggest that there are substantial differences in the intracellular levels of CRF in different subdivisions of the olivocerebellar projection system. In the monkey, the perikarya of the medial accessory olive are more densely labeled than those of other olivary subdivisions. This congruent with the presence of parasagittal zones of intensely labeled terminal axons in the vermis and portions of the intermediate zone, with the observation of areas of dense innervation in the pyramis and dorsal paraflocculus, and with the existence of a uniform, dense innervation of the uvula, since these terminal fields match the known projections of the medial accessory olive in these species (Brodal and Brodal, 1981, 1982; Whitworth and Hains, 1986). In monkey, the medial accessory olive is known to project to parasagittal zones A and C2 (Brodal and Brodal, 1981, 1982), and these are presumably the zones exhibiting CRFLI stripes in the present study. Dense labeling of presumed climbing fibers was also observed in a limited portion of crus I. Although much of crus I receives its climbing fiber innervation from the principal olive, there is evidence that parts of this area are innervated by the medial accessory olive (Courville and Faraco-Cantin, 1980).

Several lines of evidence suggest that parasagittal zonation is an essential element of cerebellar cortical organization. In addition to the previous demonstrations of parasagittal zonation of olivocerebellar afferents in monkey, there have been similar demonstrations in cat (Groenewegen and Voogd, 1977; Groenewegen et al., 1979) and rat(Campbell and Armstrong, 1983a, b; see also Brodal and Kawamura, 1980, for review). Previous studies have also provided immunohistochemical evidence for parasagittal zonation of antigens intrinsic to Purkinje cells (Chan-Palay et al., 1981, 1982; Hawkes and Leclerc, 1987). It is not clear how the parasagit-

tal zones defined by CRFLI are spatially or functionally related to the zones defined by other methods.

The present observations reinforce many previous reports indicating that CRF is localized in extrahypophyseal circuits, where it may serve as a neurotransmitter (see Vale et al., 1983; Emeric Sauval, 1986, for reviews). There is also evidence that aspartate and/or glutamate may be a neurotransmitter in the olivocerebellar pathway (Wiklund et al., 1982, 1984; Toggenburger et al., 1983; Matute et al., 1987). Thus, it is possible that CRF is a cotransmitter with one of these substances in the olivocerebellar pathway, with the peptide playing a relatively greater role in those projections that originate in the medial accessory olive. There have been numerous speculations (see Ito, 1986) about the role of climbing -fiber input in the overall functioning of cerebellar cortex, but for the present the functional physiological effects of this pathway remain unclear, as do the possible contributions of CRF to these actions.

Several lines of evidence suggest that CRF-containing circuits in many brain regions may serve to coordinate the centrally mediated autonomic and behavioral aspects of stress responses (see Valentino and Foote, 1986, for review). In this regard, it is of interest that CRF within the cerebellum is concentrated in the vermis and the associated fastigial nucleus, areas previously implicated in arousal and autonomic and affective functions (e.g., Dempesy et al., 1983; Haines et al., 1984; Albert et al., 1985; Arneric et al., 1987). Thus, these cerebellar circuits may constitute one component of a larger CRF-containing network that becomes activated in response to stress-inducing stimuli.

Over a period of many years a fascilitating effect of norepinephrine on neuronal responsiveness to afferent synaptic inputs and putative transmitter substances has been demonstrated in the rat cerebellum, cerebral cortex, facial motor nucleus, hippocampus, lateral geniculate nucleus and lateral hypothalamus. The results of these studies suggest that the syof these studies suggest that the synaptic release of endogenous norepinephrine may facilitate the transfer of information through central circuits by enhancing excitatory and inhibitory components of cellular responby enhancing excitatory and inhibitory components of cellular responses to nonmonoamine synaptic inputs.

Noradrenergic innervation of rodent cerebellum has been demonstrated with immunohistochemical and histofluorescence techniques, but similar studies have not reported in monkeys.

The present immunocytochemical study, using DBH antibody, demonstrates abundant plexus of noradrenergic axons mainly in the granular layer and, to a lesser extent, Purkine and molecular layers of monkey cerebellar cortex. Norepinephrine fibers distributed in the molecular layer were slightly thicker but smaller number of fibers were observed compared to the climbing fibers stained with antisera to CRF. Very high density of norepinephrine fibers were distributed in the granular layer, and this is the different finding with the previous reports of rodents (Kimoto et al., 1981) in which norepinephrine fibers were present predominantly in the molecular layer. So these findings suggest the species difference in the distribution of norepinephrine in the cerebellar cortex.

Kimoto et al. (1981) have clearly shown that the noradrenalin-containing terminals of the external surface of glomeruli were in close contact with granule all dendrites from serial thin sections of electron microscopy. Thus it is probable that noradrenalin-containing terminals in the granular layer participitate in the function of the cerebellar glomerulus.

It is well known that noradrenalin agents may modify the discharge of Purkinje-cells. Experiments performed in situ have shown that microiontophoretic application of norepinephrine (Hoffer et al., 1971, 1974; Freedman et al., 1975, 1977; Moises et al., 1979b), as well as LC stimulation (Hoffer et al., 1973a; Moises and Woodward, 1980), decreased the spontaneous firing rate of Purkinje-cells. This effect was associated with hyperpolarization of the Purkinje-cell membrane (cf. Waterhouse et al., 1982), which was coupled with an increase in their input resistance, suggesting that these effects were probably mediated through cAMP (Siggins et al.,

1969, 1971a, b, 1973; Hoffer et al., 1973a). On the other hand, depletion of norepinephrine in the cerebellum was associated with slight increases in Purkinje-cell background activity (Hoffer et al., 1973b; McElligott et al., 1986).

Observations made in the in vivo preparations have shown that local application of norepinephrine (Siggins et al., 1971b; Freedman et al., 1976, 1977; Woodward et al., 1979; Moises et al., 1979a, 1990) as well as LC stimulation (Hoffer et al., 1973a; Moises et al., 1979a, 1981, 1983; Moises and Woodward, 1980), while depressing the spontaneous activity of the Purkinje cells, enhanced the responses of these cells to both excitatory(mossy fiber and climbing fiber) as well as inhibitory (basket and stellate cells) inputs. Similarly, iontophoretically applied norepinephrine (Freedman et al., 1975; Moises et al., 1979b; Woodward, et al., 1979, Waterhouse et al., 1982; Yeh et al., 1981; Marshall and Tsai, 1988) or LC stimulation (Moises and Woodward, 1980; Moises et al., 1983) enhanced the responsiveness of Purkinje cells to excitatory (glutamate and aspartate) and inhibitory (GABA) neurotransmitters of the cerebellar cortex. These effects have been reported to be mediated by β-adrenergic receptors (Moises et al., 1981, 1983, 1990; Waterhouse et al., 1982; Yeh and Woodward, 1983) however, it cannot be ruled out that α-adrenoceptors also contributed to these effects.

As a result of these findings it has been postulated that one of the main functions of the NE-containing input in cerebellar operation is to augment target neuron responsiveness to conventional afferent systems which are directly concerned with detailed information transfer, thus increasing the signal-to-noise ratio of the evoked versus spontaneous activity (cf. Woodward et al., 1979; Waterhouse et al., 1988). The same input could also act to gate the efficacy of subliminal synaptic inputs conveyed by classical afferent systems (Moises et al., 1990).

Chemical heterogeneity in anatomically defined fiber populations is increasingly becoming evident in cerebellar circuitry, though the functional relevance of such organization remains to be determined. Electron microscopic analyses of chemically coded synaptic relationships with in cerebellar cortex, as well as iontophoretic studies, are needed in order to understand interactions within this circuitry.

REFERENCES

Albert TJ, Dempesy CW, Sorenson CA. Anterior cerebellar vermal stimulation: Effect on behavior and basal forebrain neurochemistry in rat. Biol Psychiatry 1985; 20:1267-1276

Arneric SP, Iadecola C, Underwood MD, Reis DJ. Local cholinergic mechanisms participate in the increase in cortical cerebral blood flow elicited by electrical stimulation of the fastigial nucleus in rat. Brain Res 1987; 411:212-225

Bloom FE, Battenberg ELF, Rivier J, Vale W. Corticotropin releasing factor (CRF): Immunoreactive neurons and fibers in rat hypothalamus. Regulat Peptides 1982; 4:43-48

Bloom FE, Hoffer BJ, Siggins GR. Studies on norepinephrine-containing afferents to Purkinje cells of rat cerebellum. I. Localization of the fibers and their synapses. Brain Res 1971; 25:501-521

Brodal A, Kawamura K. Olivocerebellar projection: A review. Adv Anat Embryol Cell Biol 1980; 64:1-140

Brodal P, Brodal A. Further observations on the olivocerebellar projection in the monkey. Exp Brain Res 1982; 45:71-83

Brodal P, Brodal A. The olivocerebellar projection in the monkey. Experimental studies with the method of retrograde tracing of horseradish peroxidase. J Comp Neurol 1981; 201:375-393

Campbell NC Armstrong DM. Topographical localization in the olivocerebellar projection in the rat: An autoradiographic study. Brain Res 1983b; 275:235-249

Campbell NC, Armstrong DM. The olivocerebellar projection in the rat: An autoradiographic study. Brain Res 1983a; 275:215-233

Chan-Palay V, Nilaver G, Palay SL, Beinfeld MC, Zimmerman EA, Wu JY, O'Donohue TL. Chemical heterogeneity in cerebellar Purkinje cells: Existence and co-existence of glutamic acid decarboxylase-like and motilin-like immu-

noreactivities. Proc Natl Acad Sci USA 1981; 78:7787-7791

Chan-Palay V, Palay SL. Wu JY. Sagittal cerebellar microbands of taurine neurons: Immunocytochemical demonstration by using antibodies against the taurine synthesizing enzyme cysteine sulfinic acid decarboxylase. Proc Natl Acad USA 1982; 79:4221-4225

Chu NS, Bloom FE. The cathecholamine-containing neurons in the cat dorsolateral pontine tegmentum: Distribution of the cell bodies and some axonal projections. Brain Res 1974; 66: 1-21

Courville J, Faraco-Cantin F. Topography of the olivocerebellar projection: An experimental study in the cat with an autoradiographic tracing method. In the Inferior Olivary Nucleus: Anatomy and Physiology. 1980; J Courville et al., eds., p.235-277, Raven, New York

Cummings S, Elde R, Ells J, Lindall A. Corticotropin-releasing factor immunoreactivity is widely distributed within the central nervous system of the rat: An immunohistochemical study. J Neurosci 1983; 3:1355-1368

Cummings S, Sharp B, Elde R. Corticotropin-releasing factor in cerebellar afferent systems: A combined immunohistochemistry and retrograde transport study. J Neurosci 1988; 8:543-554

Dempesy CW, Tootle DM, Fontana CJ, Fitziarrell AT, Garey RE, Heath RG. Stimulation of the paleocerebellar cortex of the cat: Increased rate of synthesis and release of catecholamines at limbic sites. Biol Psychiatry 1983; 18: 127-132

Dietrichs E. Cerebellar cortical and nuclear afferents from the feline locus coeruleus complex. Neuroscience 1988; 27:77-91

Emeric-Sauval E. Corticotropin-releasing factor (CRF)-A review. Psychoneuro-endocrinology 1986; 11:277-294

Fellman D, Bugnon C, Bresson JL, Gouget A, Cardot J, Clavequin MC, Hadjiyiassemis M. The CRF neuron: Immunocytochemical study. Peptides (Suppl. 1) 1984; 5:19-33

Foote SL, Bloom FE, Aston-Jones G. Nucleus locus coeruleus: New evidence of anatomical and physiological specificity. Physiol Rec 1983; 63:844-914

Freedman R, Hoffer BJ, Woodward DJ, Puro D. Interaction of norepinephrine with cerebellar activity evoked by mossy and climbing fibers. Exp Neurol 1977; 55:269-288

Freedman R, Hoffer BJ, Woodward DJ. A quantitative microiontophoretic analysis of the responses of cerebral neurons to norepinephrine: Interactions with cobalt, manganes, verapamil and dichloroisoproterenol. Br J Pharmacol 1975; 54:529-539

Fuxe K. Evidence for the existence of monoamine neurons in the central nervous system. IV. The distribution of monoamine terminals in the central nervous system. Acta Physiol Scand, Suppl, 1965; 247:37-85

Groenewegen HJ, Vogd J. The parasagittal zonation within the olivocerebellar porjection. J Comp Neurol 1977; 174:417-488

Groenewegen HJ, Voogd J, Freedman SL. The parasagittal zonation within the olivocerebellar projection. J Comp Neurol 1979; 183:551-602

Haines DE, Dietrichs E, Sowa TE. Hypothalamocerebellar and cerebello-hypothalamic pathways: A review and hypothesis concerning cerebellar circuits which may influence autonomic centers and affective behavior. Brain Behav Evol 1984; 24:198-220

Hawkes R, Leclerc N. Antigenic map of the rat cerebellar cortex: The distribution of parasagittal bands as revealed by monoclonal anti-Purkinje cell antibody mabQ113. J Comp Neurol 1987; 256:29-41

Hoffer BJ, Seiger A, Ljungberg T, Olson L. Electrophysiological and cytological studies of brain homogenate in the anterior chamber of the eye: Maturation of cerebellar cortex in oculo. Brain Res 1974; 79:165-184

Hoffer BJ, Siggins GR, Bloom FE. Studies on norepineprhine-containing afferents to Purkinje cells of rat cerebellum. IV. Sensitivity of Purkinje cells to norepinephrine and related substances administered by microiontophoresis. Brain Res 1971; 25:523-534

Hoffer BJ, Siggins GR, Oliver AP, Bloom FE. Activation of the pathway from locus coeruleus to rat cerebellar Purkinje neurons: Pharmaco-

logical evidence of noradrenergic central inhibition. J Pharmacol Exp Ther 1973a; 184:553-569

Hoffer BJ, Siggins GR, Woodward DJ, Blood FE. Spontaneous discharge of Purkinje neurons after destruction of catecholamine-containing afferents by 6-hydroxydopamine. Brain Res 1973b; 30:425-430

Hökfelt T, Fuxe K. Cerebellar monoamine nerve terminals, a new type of afferent fibers to the cortex cerebelli. Exp Brain Res 1969; 9: 63-73

Ito M. Long-term depression as a memory process in the cerebellum. Neurosci Res 1986; 3: 531-539

Joseph S, Knigge KM. Corticotropin releasing factor: Immunocytochemical localization in rat brain. Neurosci Lett 1983; 35:135-141

Kimoto Y, Tohyama M, Satch K, Sakumoto T, Takahashi Y, Shimizu N. Fine structure of rat cerebellar noradrenaline terminals as visualized by potassium permenganate in situ perfusion fixation method. Neuroscience 1981; 6: 47-58

Kitahama K, Luppi PH, Tramu G, Sastre JP, Buda C, Jouvet M. Localization of CRF-immunoreactive neurons in the cat medulla oblongata: Their presence in the inferior olive. Cell Tissue Res 1988; 251:137-143

Landis SC, Bloom FE. Ultrastructure identification of noradrenergic boutons in mutant and normal mouse cerebellar cortex. Brain Res 1975; 96:299-305

Marshall KC, Tsai WH. Noradrenaline induces short and long duration potentiation of glutamate excitations of cultured Purkinje neurons. Can J Physiol Pharmacol 1988; 66:848-853

Matute C, Wiklune L, Streit P, Cuenod M. Selective retrograde labeling with d-[3H]-aspartate in the monkey olivocerebellar projection. Exp Brain Res 1987; 66:445-447

McElligott JG, Ebner TJ, Bloedel JR. Reduction of cerebellar norepinephrine alters climbing fibers enhancement of mossy fiber input to the Purkinje cell. Brain Res 1986; 397:245-252

Merchenthaler I, Vigh IS, Schally AV, Stumpf WE, Arimura A. Immunocytochemical localization of corticotropin releasing factor (CRF)-like immunoreactivity in the thalamus of the rat. Brain Res 1984; 323:119-122

Merchenthaler I. Corticotropin releasing factor (CRF)-like immunoreactivity in the rat central nervous system. Extrahypothalamic distribution. Peptides (Suppl.) 1984; 5:53-69

Moises HC, Burne RA, Woodward DJ. Modification of the visual response properties of cerebellar neurons by norepinephrine. Brain Res 1990; 514:259-275

Moises HC, Waterhouse BD, Woodward DJ. Locus coeruleus stimulation potentiates Purkinje cell responses to afferent input: The climbing fibers system. Brain Res 1981; 222:43-64

Moises HC, Waterhouse BD, Woodward DJ. Locus coeruleus stimulation potentiates local inhibitory processes in rat cerebellum. Brain Res Bull 1983; 10:795-804

Moises HC, Waterhouse BD, Woodward DJ. Potentiation of monosynaptic Purkinje cell excitation and inhibition following locus coeruleus activation. Soc Neurosci Abstr 1979a; 5:345

Moises HC, Woodward DJ, Hoffer BJ, Freedman R. Interactions of norepinephrine with Purkinje cell responses to putative amino acid neurotransmitters applied by microiontophoresis. Exp Neurol 1979b; 64:493-515

Moises HC, Woodward DJ. Potentiation of GABA inhibitory action in cerebellum by locus coeruleus stimulation. Brain Res 1980; 182:327-344

Olschowka JA, O'Donohue TL, Mueller GP, Jacobowitz DM. Hypothalamic and extrahypothalamic distribution of CRF-like immunoreactive neurons in the rat brain. Neuroendocrinology 35:305-308

Olson L, Fuxe K. On the projections from the locus coeruleus noradrenaline neurons: The cerebellar innervation. Brain Res 1971; 28:165-171

Palay SL, Chan V. Cerebellar Cortex, Springer-Verlag, New York 1974

Palvovits M, Brownstein MJ, Vale W. Corticotropin releasing factor (CRF)-like immunoreactivity in hypothalamic and extrahypothalamic nuclei of sheep brain. Neuroendocrinology 1983; 37:302-305

Palvovits M, Brownstein MJ, Vale W. Distribution

of corticotropin releasing factor in rat brain. Fed Proc Fed Am Soc Exp Biol 1985; 44:215-219

Palvovits, M, Leranth C, Gorcs T, Young III WS. Corticotropin releasing factor in the olivocerebellar tract of rats: Demonstration by light- and electron-microscopic immunohistochemistry and in situ hybridization histochemistry. Proc Natl Acad Sci USA 1987; 84:3911-3915

Pasquier DA, Gold MA, Jacobowits DM. Noradrenergic perikarya (A5-A7, subcoeruleus) projections to the rat cerebellum. Brain Res 1980; 196:270-7275

Pickel VM, Segal M, Bloom FE. A radioautographic study of the efferent pathways of the nucleus locus coeruleus. J Comp Neurol 1974; 155:15-42

Powers RE, De Souza EB, Walker LC, Price DL, Vale WW, Young III WS. Corticotropin releasing factor as a transmitter in the human olivocerebellar pathway. Brain Res 1987; 415:347-352

Powers RE, O'Connors DE, Price DL. Noradrenergic systems in human cerebellum. Brain Res 1989; 481:194-199

Sakanaka M, Shibasaki T, Lederis K. Corticotropin releasing factor (CRF)-like immunoreactivity in the rat brain as revealed by a modified cabalt-glucose oxidase-diaminobenzidine method. J Comp Neurol 1987; 260:256-298

Scheibel ME, Scheibel AB. Observations on the intracortical relations of the climbing fibers of the cerebellum. Golgi study. J Comp Neurol 1954; 101:733-764

Schipper J, Steinbusch JWM, Vermes I, Tilders FJH. Mapping of CRF-immunoreactive nerve fibers in the medulla oblongata and spinal cord of the rat. Brain Res 1983; 267:145-150

Siggins GR, Battenberg EF, Hoffer BJ, Bloom FE, Steiner AL. Noradrenergic stimulation of cyclic adenosine monophosphate in rat Purkinje neurons: An immunocytochemical study. Science 1973; 179:585-588

Siggins GR, Hoffer BJ, Blood FE. Cyclic adenosine monophosphate: Possible mediator for norepinephrine effects of cerebellar Purkinje cells. Science 1969; 165:1018-1020

Siggins GR, Hoffer BJ, Bloom FE. Studies on norepinephrine-containing afferents to Purkinje cells of rat cerebellum. III. Evidence for mediation of norepinephrine effects by cyclic 3'-5'-adenosine monophosphate. Brain Res 1971a; 25:535-553

Skofitsch G, Jacobowitz DM. Distribution of corticotropin releasing factor-like immunoreactivity in the rat brain by immunohistochemistry and radioimmunoassay: Comparison and characterization of ovine and rat/human CRF antisera. Peptides 1985; 6:319-336

Swanson LW, Sawchenko PE, Rivier J, Vale WW. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: An immunohisto-chemical An immunohisto-chemical study. Neuroendocrinology 1983; 36:165-186

Toggenburger G, Wiklund L, Henke H, Cuenod M. Release of endogenous and accumulated exogenous amino acids from slices of normal and climbing fiber-deprived rat cerebellar slices. J Neurochem 1983; 41:1606-1613

Vale W, Rivier C, Brown MR, Spiess J, Koob G, Swansong L, Bilezikjian L, Bloom F, Rivier J. Chemical and biological characterization of corticotropin-releasing factor. Rec Prog Horm Res 1983; 39:245-269

Valentino RJ, Foote SL. Brain noradrenergic neurons, corticotropin-releasing factor, and stress. In Neural and Endocrine Peptides and Receptors. T Moody, ed., pp.101-120. 1986; Plenum, New York

Waterhouse BD, Moises HC, Yeh HH, Woodward DJ. Norepinephrine enhancement of inhibitory synaptic mechanisms in cerebellum and cerebral cortex: Mediation by beta adrenergic receptors. J Pharmacol Exp Ther 1982; 221:495-506

Waterhouse DB, Sessler FM, Cheng JT, Woodward JD, Azizi SA, Moises HD. New evidence for a gating action of norepinephrine in central neuronal circuits of mammalian brain. Brain Res Bull 1988, 21:425-432

Whitworth RH JR, Haines DE. The inferior olive of Saimiri Sciureus: Olivocerebellar projections to the anterior love. Brain Res 1986; 372:55-71

Wiklund L, Toggenburger G, Cuenod M. Aspar-

tate: Possible neurotransmitter in cerebellar climbing fibers. Science 1982; 216:78-79

Wiklund L, Toggenburger G, Cuenod M. Selective retrograde labelling of the rat olivocerebellar climbing fiber system with D-[3H]-aspartate. Neuroscience 1984; 13:441-468

Woodward DJ, Moises HC, Waterhouse BD, Hoffer BJ, Freedman R. Modulatory actions of norepinephrine in the central nervous system. Fed Proc 1979; 38:2109-2116

Yamamoto T, Ishikawa M, Tanaka, C. Catecholaminergic terminals in the developing and adult rat cerebellum. Brain Res 1977; 132:355-361

Yeh HH, Moises HC, Waterhouse BD, Woodward DJ. Modulatory interactions between norepinephrine and taurine, beta-alanine, gamma-aminobutyric and muscimol, applied iontophoretically to cerebellar Purkinje cells. Neuropharmacology 1981; 20:549-560

Yeh HH, Woodward DJ. Beta-1 adrenergic receptors mediate noradrenergic facilitation of Purkinje cell responses to gamma-amino-butyric acid in cerebellum of rat. Neuropharmacology 1983; 22:629-639

Young WS III, Walker LC, Powers RE, De Souza EB, Price DL. Corticotropin-releasing factor mRNA is expressed in the inferior olives of rodents and primates. Mol Brain Res 1986; 1: 189-192

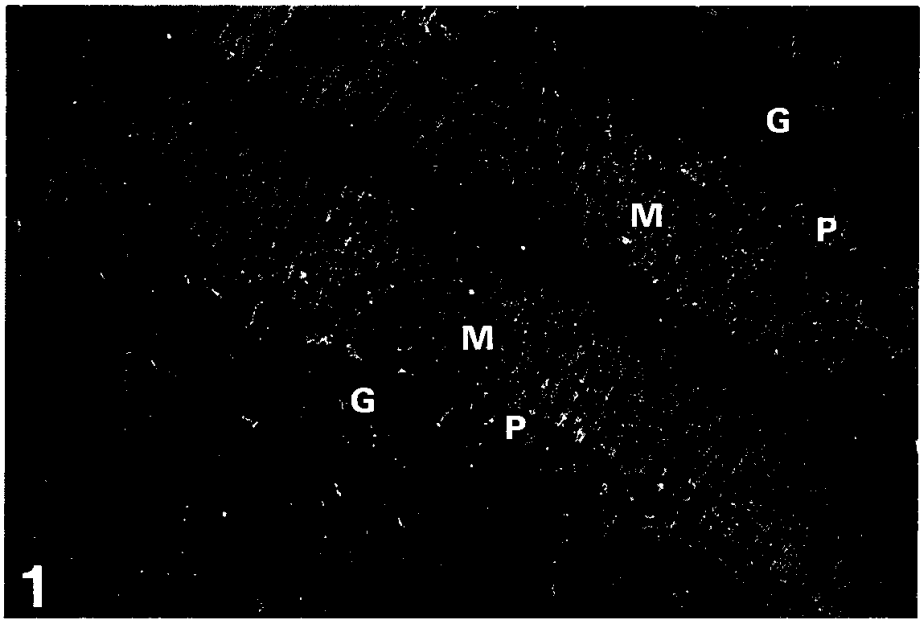

Fig. 1. CRF fibers in the cerebellar cortex of monkey. Immunoreactive fibers are predominantly located in the molecular layer (M). P: Purkinje cell layer. G: Granular layer. ×100

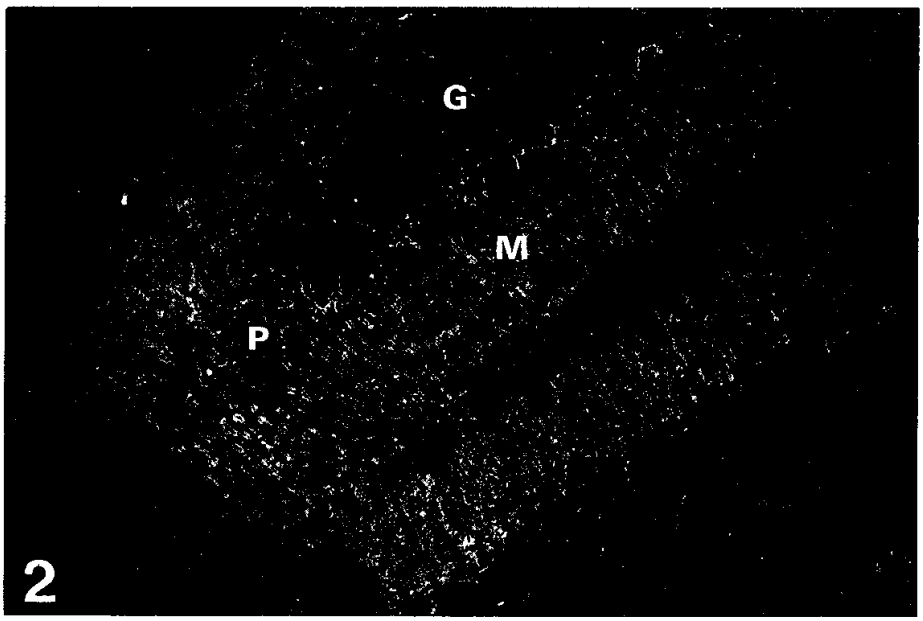

Fig. 2. CRF fibers in the intermediate lobe of cerebellar cortex of monkey. CRF fibers are mainly distributed in the molecular layer (M) and Purkinje cell layer (P). G: Granular layer. ×100

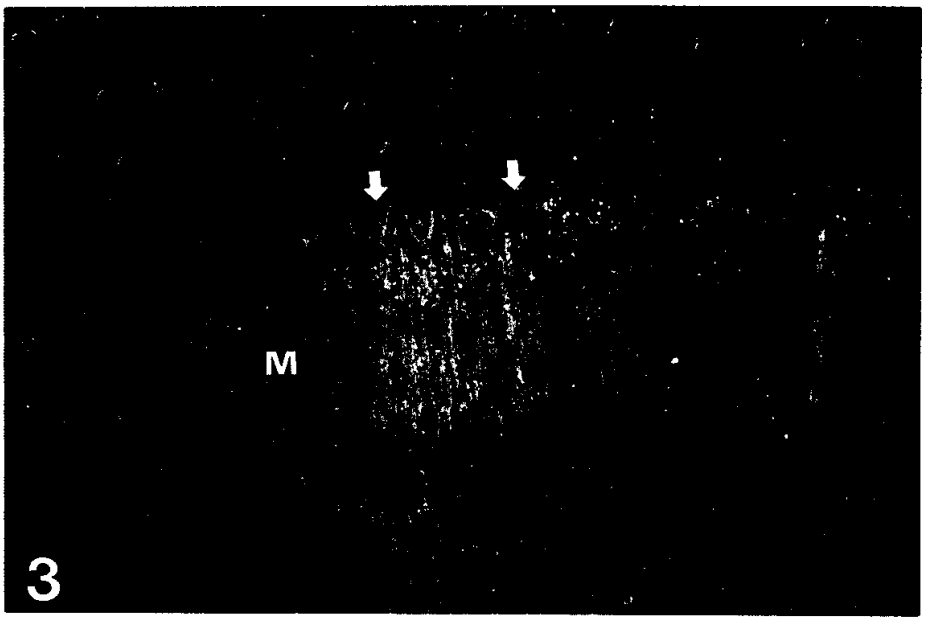

Fig. 3. CRF fibers in the vermis of monkey cerebellum. Intensely labeling CRF fibers are characteristically located in the parasagittal zones (between the arrows). M: Molecular layer. ×100

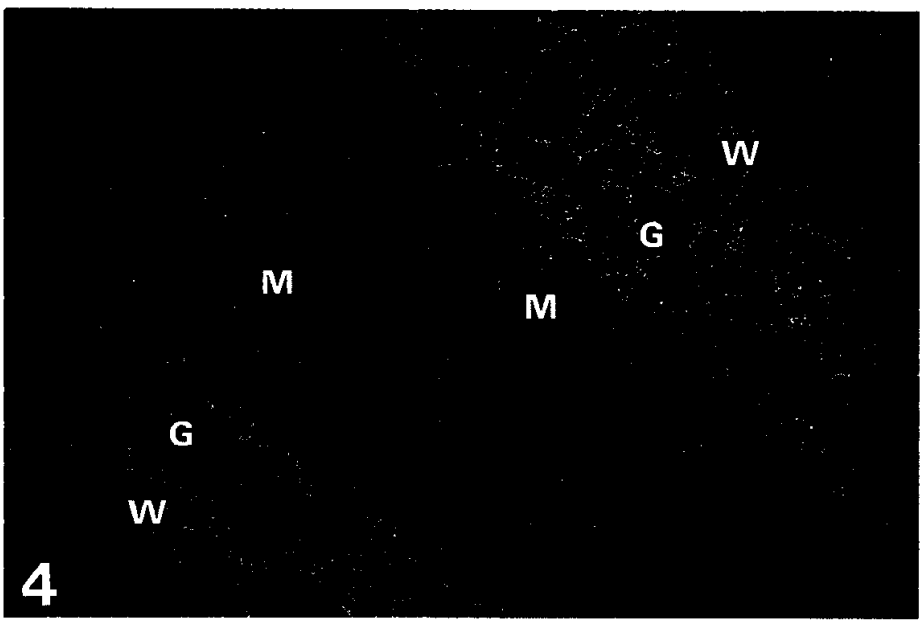

Fig. 4. Norepinephrine fibers in the cerebellar cortex of monkey. Immunoreactive fibers are predominantly located in the granular layer (G) and white matter (W). M: Molecular layer. ×100

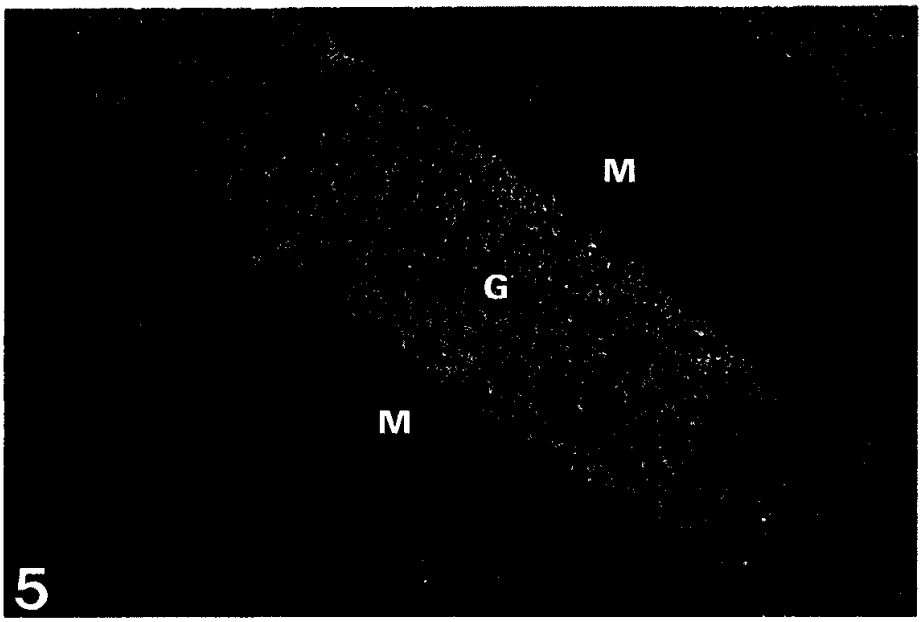

Fig. 5. Norepinephrine fibers in the intermediate lobe of cerebellar cortex of monkey. CRF fibers are mainly distributed in the granular layer (G). M: Molecular layer. ×100

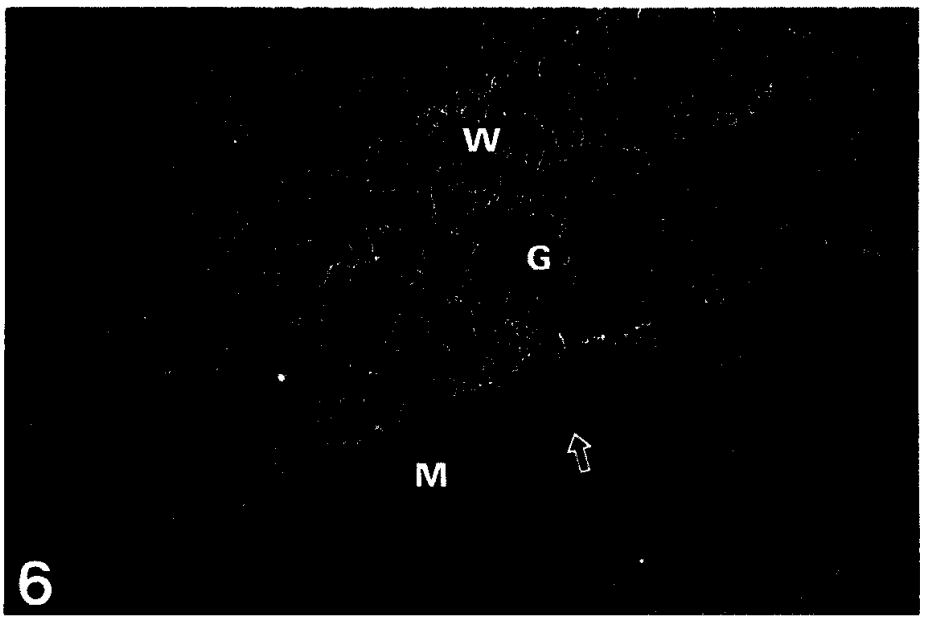

Fig. 6. Norepinephrine fibers in the vermis of cerebellar cortex of monkey. Immunoreactive fibers are mainly located in the granular layer (G) and white matter (W). Note the fine fibers (arrow) located in horizontal plane in the molecular layer (M). ×100.