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CHAPTER 1.1

# Mapping the oculomotor system

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**Abstract:** Over the last three decades and together with Bernard Cohen, Volker Henn, Ulrich Büttner, and Anja Horn, it has been possible to morphologically identify several functional cell groups in the oculomotor system: the medium-sized horizontal excitatory and inhibitory burst neurons (EBNs, IBNs) in the paramedian pontine reticular formation (PPRF), the more sparsely scattered vertical EBNs in the rostral interstitial nucleus of the MLF (RIMLF), and the typically elongated omnipause neurons (OPNs) in nucleus raphé interpositus — all essential for the generation of saccades. In contrast, the role of the central mesencephalic reticular formation (cMRF) in saccades is more complex, as is the morphological outlining of its borders. A detailed study of the extraocular motoneurons showed that they can be divided into two separate types: those for singly innervated (twitch) muscle fibres (SIFs) and those for multiply innervated (non-twitch) muscle fibres (MIFs). The two motoneuron types receive different premotor afferents, proving that MIF and SIF motoneurons have different functions. The cell groups were outlined by different tract tracing methods including rabies virus. The localization and histochemical characterization of all these functional cell groups in monkey formed the basis for the identification of the homologous groups in the human brainstem. Taken together these studies provide a neuroanatomical background for understanding clinical eye movement disorders.

**Keywords:** horizontal burst neurons; vertical burst neurons; omnipause neurons; rostral interstitial nucleus of the MLF; interstitial nucleus of Cajal; twitch motoneurons; central mesencephalic reticular formation; rabies virus; non-twitch motoneurons

## Introduction

In the early 1970s the introduction of two new techniques made a great impact on the understanding of the central nervous system. First, was the development of stable single-unit recordings in awake mammals, a technique pioneered by K.-P. Schaefer many years ago. Second, was the development of sensitive and reliable tract tracing techniques, based on retrograde and anterograde axonal transport of substances like horseradish peroxidase and radioactive leucine, that replaced the inaccurate degeneration techniques. At this time eye movements were generally considered to be a subfeature of the vestibular system rather than a field of their own. Clinical observations had shown that the paramedian pontine reticular formation (PPRF) was associated with the generation of horizontal conjugate eye movements but the reason for this, the functional cells groups or anatomical pathways involved, were all unknown. At Mount Sinai Hospital New York, Morris Bender and later Bernie Cohen started stimulation experiments in monkeys to locate the horizontal

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eye movement area more exactly (Bender and Shanzer, 1964; Goebel et al., 1971; Cohen and Komatsuzaki, 1972). With the advent of chronic unit recordings it became clear from several parallel studies of the PPRF that the pontine neurons encoded precisely the parameters of the subsequent eye movement, and from their activity one could predict the subsequent saccade (Cohen and Henn, 1972; Luschei and Fuchs, 1972; Keller, 1974). From this point on the analysis of the oculomotor system exploded into one of the most popular fields of investigation, in which physiologists, like Bernard Cohen, and system-modellers like David Robinson, worked together with clinicians and neuroanatomists to understand how the brain moved the eye. In this article I will describe some of the functional cell groups of the oculomotor system, which we have outlined over the last 30 years, in both monkey and man. These studies were only possible because of the long-standing support of Bernard Cohen, Volker Henn, Ulrich Buettner, and Anja Horn.

## Neuroanatomical methods

The recent development of highly specific and sensitive immunochemical stains, and new tract tracing techniques offer unique possibilities to study the functional connectivity of neuronal networks (Horn et al. 2008; Wickersham et al., 2007). Neurotropic viruses are particularly effective due to their ability to function as self-amplifying markers, and they produce exceptionally intense labelling. In collaboration with Gabriella Ugolini and Werner Graf, we have injected rabies virus (CVS fixed strain 11) into the lateral rectus muscle of monkey and have been able to visualize many of the premotor cell groups of the oculomotor system which we originally discovered using very different techniques (Ugolini et al., 2006). Rabies virus is only taken up at motor endplates and not by sensory or sympathetic endings, furthermore the virus remains in neuronal systems and is not accompanied by spurious uptake, e.g. in glial systems. After survival times of 3–3.5 days the monkeys were perfused with 4% paraformaldehyde. This period is long enough for transsynaptic transport back to the extraocular motoneuron and further retrograde into premotor networks over at least 2–3 synapses; this was not long enough to produce any rabies symptoms. In this review cases LR2 and LR4 are used to illustrate premotor neuronal populations and not demonstrate their connectivity, which has to be worked out using other techniques.

## Premotor cell groups of the oculomotor system

Already in 1982 the combined effort of scientists had worked out the basic scaffolding of oculomotor pathways essential for the generation of horizontal and vertical saccades (Fig. 1A, B). A discrete group of medium-sized neurons in PPRF, called excitatory burst neurons (EBNs), lie below the medial longitudinal fasciculus (MLF) rostral to the abducens nucleus (VI) in part of nucleus reticularis pontis caudalis (NRPC) (Fig. 2A). The EBNs relay a premotor saccadic burst signal, from areas such as the superior colliculus (SC), to the lateral rectus motoneurons and to the internuclear neurons (INT) in the ipsilateral VI. The medial rectus motoneurons receive their saccadic burst signal via the crossed axons of INT in the MLF (Fig. 1B). However the EBNs are under a continual inhibition from omnipause neurons (OPNs), whose activity pauses only before and during horizontal or vertical saccades (Optican, Chapter 2.5 this volume). Just before a saccade, the OPNs are inhibited from 'higher centres,' which releases the EBN activity, activates motoneurons and INTs, and generates a coordinated horizontal saccade. There is a second group of burst neurons in the reticular formation ventromedial to VI in a region of the pontomedullary reticular formation called nucleus paragigantocellularis dorsalis (PGD) (Figs. 1A, B, and 2C). These are the inhibitory burst neurons (IBNs), which project to the contralateral VI as well as to the OPNs (Rucker, this volume).

The vertical and torsional components of saccades are elaborated in burst neurons near to the vertical moving motoneurons in the mesencephalon. They lie rostral to the oculomotor and trochlear nuclei in the most rostral tip of the

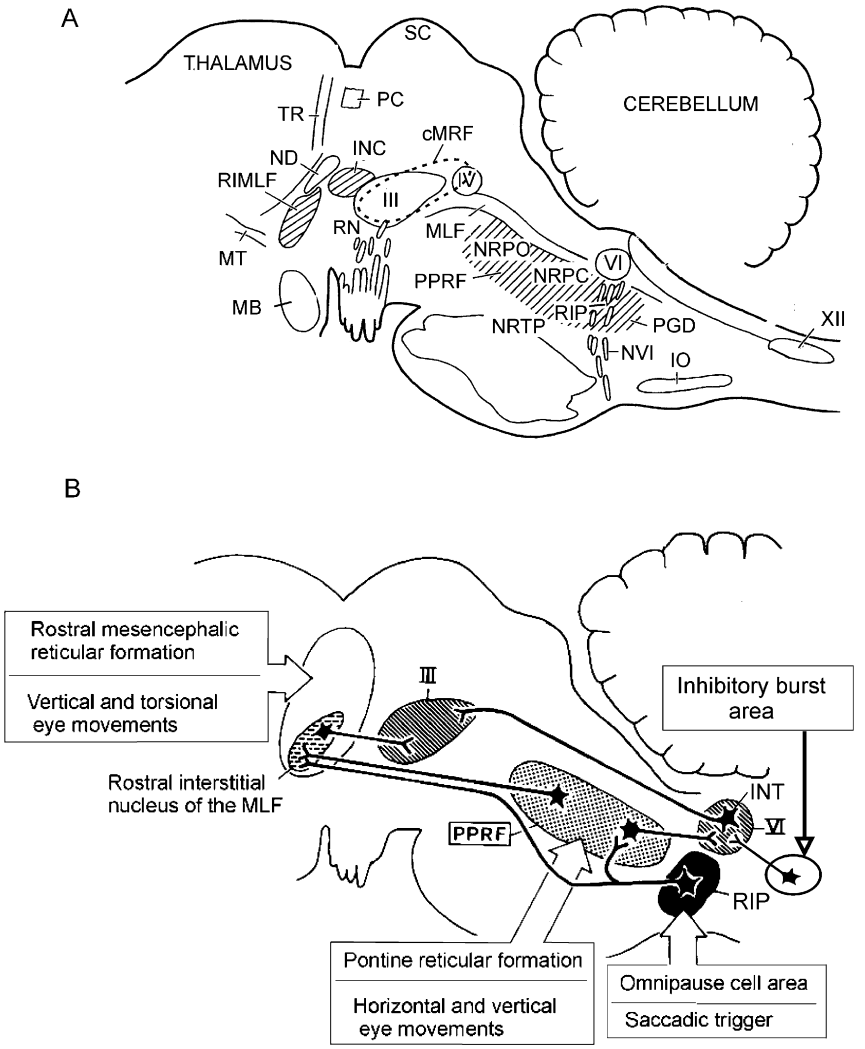


Fig. 1. (A) Drawing of a sagittal view of the brain to show some brainstem areas involved in the generation of eye movements. The dotted line indicates the lateral position of cMRF with relation to III. (B) Drawing of a sagittal view of the brain with some interconnections of premotor cell groups in the brainstem essential for the generation of saccades.

reticular formation, rostral interstitial nucleus of the MLF (RIMLF) (Figs. 1 and 3B–D) (Büttner-Ennever and Büttner, 1978). The long name of RIMLF arose from cumulative attempts to distinguish it from the interstitial nucleus of Cajal (INC), which lies immediately caudal to RIMLF (Fig.3B), and which over time has been given many different names. Bender emphasized the principle that bilateral lesions of the mesencephalon were necessary to produce vertical gaze paralysis. While this is generally correct, some unilateral lesions around the posterior commissure (PC) can give rise to upward or up and downgaze paralysis. Although the medium-sized vertical burst neurons are unobtrusive the RIMLF is clearly outlined by the posterior thalamo-subthalamic paramedian artery (also called the intermediate interpeduncular artery) (Fig. 3D asterisk). The artery arises from the posterior cerebral artery from a single origin: it divides and supplies each side of the brain, thus an

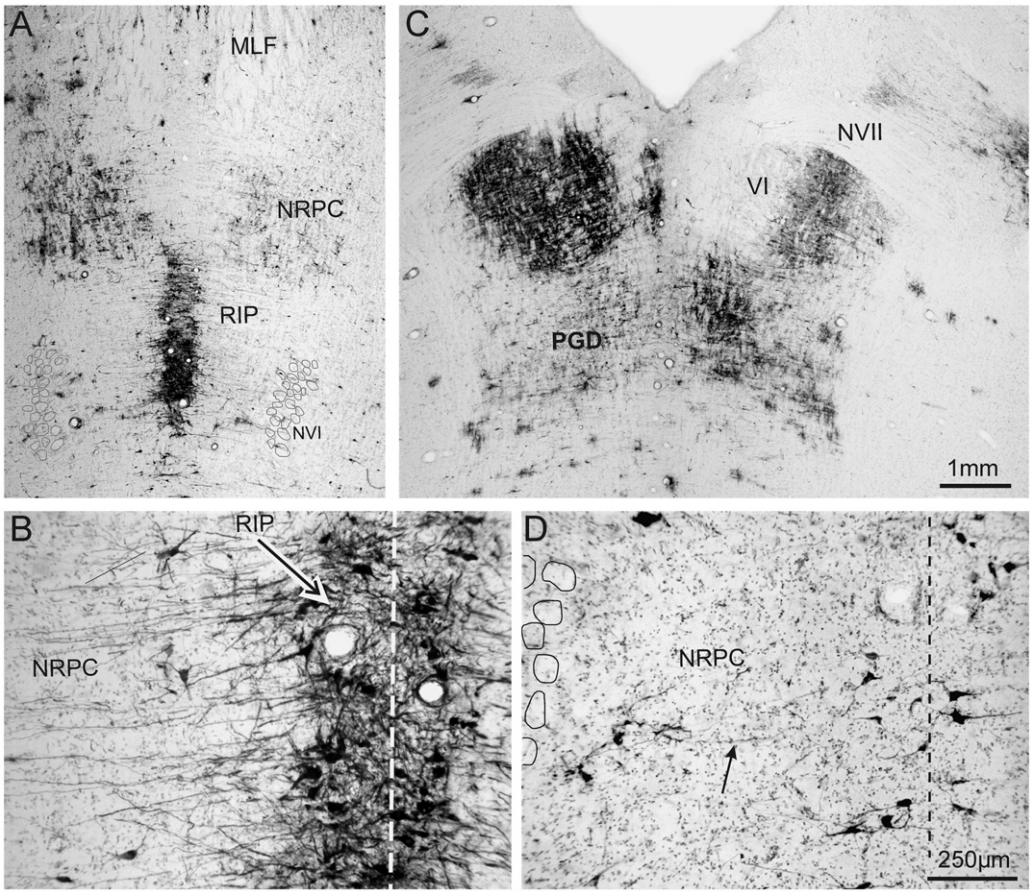


Fig. 2. (A) Labelling in the PPRF with rabies virus after injection into lateral rectus and a survival time which allows retrograde transsynaptic transport over 2–3 synapses (LR4). Note the strong labelling of the OPNs in RIP, and the relatively compact EBN regions in NRPC where the labelled is stronger ipsilaterally (left side). The abducens rootlets serve as a reliable landmark. (B) An enlargement of RIP in (A) to show the morphology of OPNs. Note the labelled neurons scattered laterally. (C) Abducens nucleus of the same experiment is completely filled on the ipsilateral (left) side, but contralaterally only the ABI area is labelled in VI. Ventrally the IBN areas in PGD are labelled, and the contralateral side being stronger. In (C) and (D) the midline is indicated by a dashed line. (D) OPN area of experiment LR2 with a shorter survival time than LR4. It shows the first OPN cells labelled in RIP and the scattered labelled neurons lateral to them with fine projections (arrow) onto OPNs. Note the NVI rootlets as landmark.

infarct of the artery could lead to *bilateral* lesions of RIMLF. Soon after the anatomical and physiological identification of RIMLF (Büttner et al., 1977; Büttner-Ennever and Büttner, 1978) a clinical case of vertical gaze paralysis with increasing drowsiness presented. Later the autopsy verified a bilateral RIMLF lesion which accounted for the vertical gaze paralysis (Büttner-Ennever et al., 1982). The concomitant bilateral loss of ascending brainstem pathways feeding into the medial forebrain bundle, the H-fields and ansa lenticularis accounted for the changes in wakefulness (Saper, 2006).

Before the discovery of these premotor cell groups one lively argument involving Ed Keller, Albert Fuchs, Mike King, and Craig Evinger at the Royaumont Meeting in 1977 stands out in my memory (Baker and Berthoz, 1977). It concerned how stimulation of the OPN area could arrest vertical and horizontal saccades. In independent

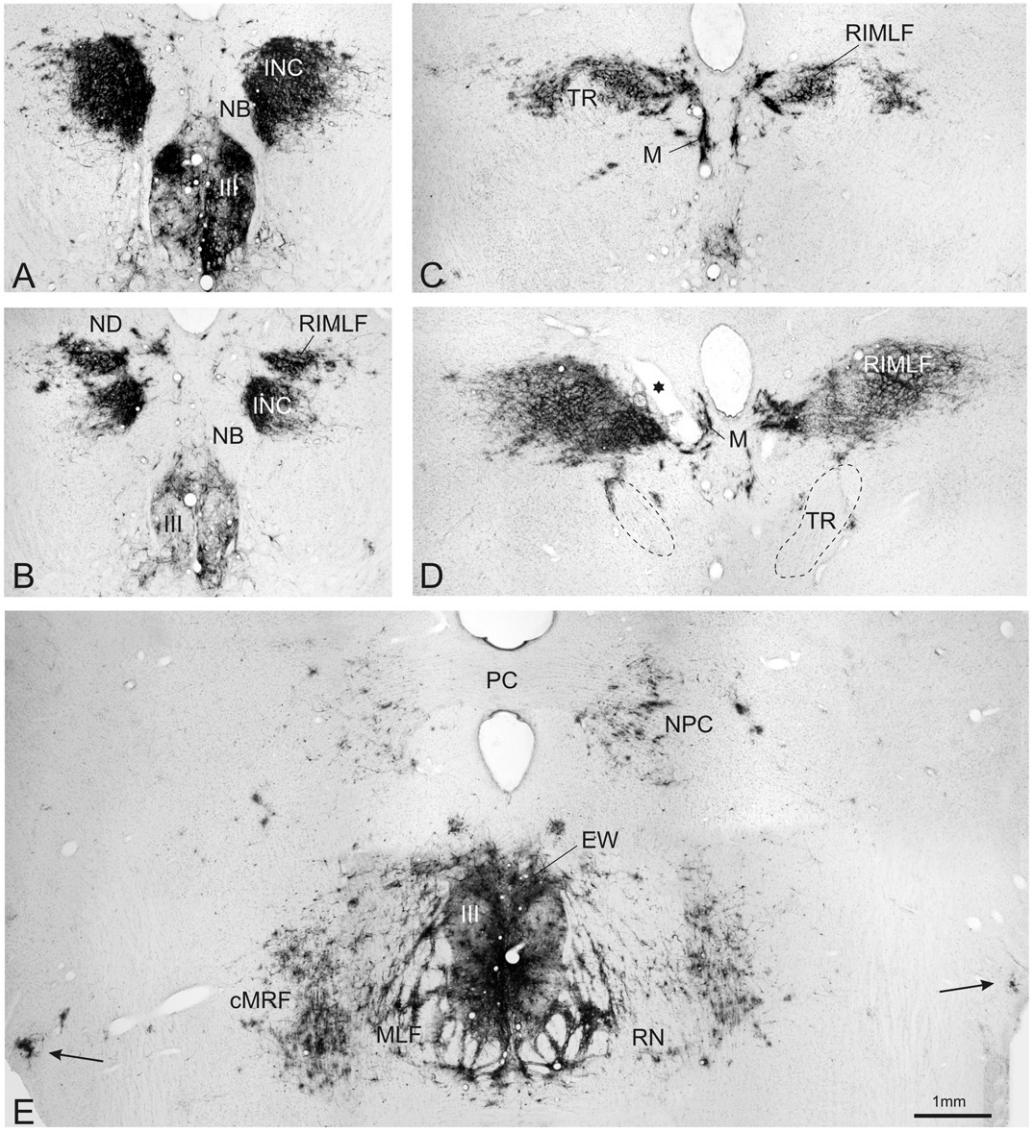


Fig. 3. Labelling in the rostral mesencephalon with rabies virus after injection into lateral rectus and a survival time which allows retrograde transsynaptic transport over 2–3 synapses (LR4). The virus labels vertical burst regions probably as a result of horizontal and vertical cross connections in the vestibular nuclei (Ugolini et al., 2006). Notice the strong bilateral labelling of INC and III in (A). Further rostrally in (B) rostral III, INC, and the caudal most tip of RIMLF is labelled. (C) RIMLF area is crossed by TR, and medially the M group is strongly labelled. In (D) the posterior thalamic subthalamic paramedian artery (asterisk) marks the dorsal border of mid RIMLF. (E) At the level of the PC (caudal to A–D) the III, EW, NPC, and the pretectum are labelled. The cMRF lies lateral to III but only the medial part is labelled here. At the lateral edge of the mesencephalon the accessory optic nuclei (arrows) are also visible.

experiments on monkey and cat, they had all found physiological evidence for a slender column of pause neurons near the midline in the pons, which paused for all saccades, and was thought to provide a high frequency (presumably inhibitory) control of burst neurons. The results were so clear and dramatic that it seemed to me that one must be able to see which cells were the OPNs on brain

sections (morphologically), even though up to then no one had been able to find them. I decided then that I would try. We know now that the 'slender column of OPNs' do have a distinctive morphology, with their horizontally oriented dendrites (Fig. 2A, B and D). We first highlighted them by staining PPRF with cytochrome oxidase (Büttner-Ennever et al., 1988), then identified them through their inhibitory transmitter, glycine (Horn et al., 1994). In all mammals so far inspected the OPNs always lie either side of the midline at the same level as the abducens rootlets, as they pass through the dorsal tegmentum (Fig. 2A, B and D). Now after 20 years of studying the OPNs we are beginning to think that the group may not be a homogeneous (Horn, this volume), and that cells lying outside the EBN cluster, lateral to the OPNs may play a role in smooth pursuit (Fig. 2A, B and D) (Keller and Missal, 2003). Whether or not these cells are involved in the 'saccadic latch' is not clear.

The central mesencephalic reticular formation (cMRF) has been defined as an oculomotor structure by lesions, stimulation experiments, single-unit recordings, and tract tracing its neural connections (Waitzman et al., 2002). Its function is complex and currently three different hypotheses of cMRF function are being investigated (Cromer and Waitzman, 2006): (1) it interacts with OPNs to control saccadic triggering. Certainly we have seen monosynaptic projections from cMRF to the OPNs in nucleus raphe interpositus (RIP) using tritiated leucine tract tracer, which is only taken up by neurons in the injection site and not passing axons (personal observation). (2) It participates in the transformation of spatiotemporal coding of SC signals onto EBNs in the pons (feed-forward). (3) It provides an efference copy of saccade velocity, a feedback loop, to higher structures. The cMRF forms the rostral part of nucleus subcuneiformis in monkeys: Chen and May (2000) define it with respect to the tightly coupled reciprocal projections to SC; whereas the results of single unit recording suggest that it may extend rostrally to the Fields of Forel (Waitzman et al., 2000a, b). The rabies experiments labelled cMRF mainly bilaterally after 3 days, but only the medial part of the cMRF region reciprocally connected to SC was labelled in this experiment, implying that a subpopulation of cMRF cells is highlighted here (Fig. 3E).

One of the more recent cell groups that we have described are the non-twitch motoneurons, or, more strictly termed, the motoneurons of multiply innervated muscle fibres (MIFs) of the extraocular muscles. These have become the centre of our attention partly because we do not know to which part of the oculomotor system they belong. Many investigations have shown that extraocular motoneurons in all vertebrates lie in subgroups within the oculomotor, trochlear, and abducens nucleus (III, IV, and VI) (Büttner-Ennever, 2006). They have a phasic and a tonic component of activity, which varies widely in extent, but are considered as a continuum. Only one type of extraocular motoneuron has been recognized in physiological recordings from III, IV, and VI in awake monkeys, and they responded during all types of eye movement. This led to the concept of a 'final common pathway.' However, morphologically at least six different morphological types of striated muscle fibres have been described in extraocular muscles, and these can be divided into two main categories: those that are singly innervated by a central motor endplate (SIFs), and those that are MIFs with endplates along the whole length of the muscle fibre. The MIFs are a very unusual type of muscle fibre, unique to eye muscles, that is they do not occur in the skeletal muscles of mammals: they respond to activation with a relatively slow, graded contraction, not an all-or-nothing twitch like the other fibre types innervated by a single motor endplate (SIFs).

We have found the location of the motoneurons innervating the MIF muscle fibres of the global layer using tract tracers (Büttner-Ennever et al., 2001). They lie around the periphery of the classical III, IV, and VI boundaries and do not intermingle with the SIF motoneurons (Fig. 4). In VI the LR MIFs surrounded the medial aspect of the nucleus; the SO MIFs lay in a dorsal cap over IV; and in III the MIFs of MR and IR are gathered into a small group on the dorsomedial boarder of III (C-group), while those of SR and inferior olive (IO) lay around the midline between the two halves of the III (S-group). In terms of neuroanatomy, when neuronal cell groups lie

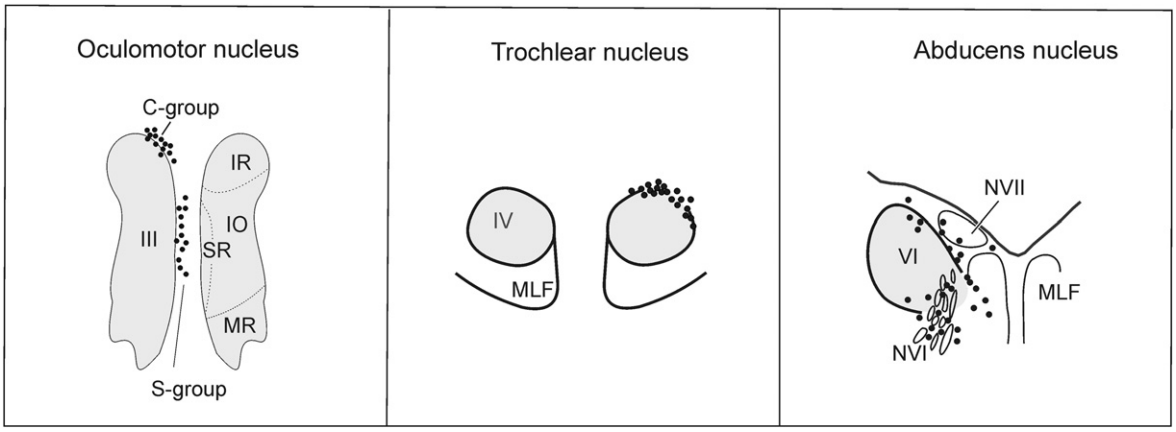


Fig. 4. Drawings of the oculomotor, trochlear, and abducens nuclei to show the location of non-twitch (MIF) motoneurons (black dots) around the periphery of the classical motor nuclei. The position of the motoneuron subgroups innervating medial, superior, and inferior rectus (MR, SR, IR) and inferior oblique muscles (IO) are indicated in III.

separately it is often a sign that they receive different afferent inputs and have different functions. This is indeed the case with SIFs and MIFs, they receive different afferents (Büttner-Ennever et al., 2002; Wasicky et al., 2004; Ugolini et al., 2006). The classical SIF motoneurons have premotor inputs from PPRF and the magnocellular regions of the vetibular nuclei, but these do not directly innervate the MIF motoneurons. The MIFs and their dendrites in III have a close relationship to the Edinger–Westphal complex (EW, near response neurons). The function of MIFs is not clear. Whereas the SIFs are mainly responsible for the rapid eye movements our current hypothesis is that MIFs are involved in more tonic functions such as eye alignment, and may participate in feedback networks which regulate vision and proprioception.

Throughout the search for premotor cell groups of the oculomotor system a major aim has always been to identify the homologous regions in humans. Usually this has been achieved by using additional immunohistochemical techniques. That is, in monkey the motor or premotor groups would be first labelled by tract tracers, and then the histochemical properties of these same labelled neurons would be determined by double-labelling techniques. Next, sections from a likely homologous region in the human brain would be taken, and the material investigated for neurons with the same histochemical properties as the labelled cells in the monkey. These human projects have been led by Anja Horn and have provided a solid scientific basis for the analysis of clinical cases with eye movement disorders (Horn et al., 1994, 1995, 2000; Horn and Büttner-Ennever, 1998). A clear example of this is the recent re-definition of the EW nucleus in man (Horn et al., 2008; May et al., this volume).

## Abbreviations

| | |
|---|---|
| III | oculomotor nucleus |
| IV | trochlear nucleus |
| VI | abducens nucleus |
| ABI | abducens internuclear neurons |
| cMRF | central mesencephalic reticular formation |
| EBNs | excitatory burst neurons |
| EW | Edinger–Westphal complex |
| IBNs | inhibitory burst neurons |
| INC | interstitial nucleus of Cajal |
| IO | inferior olive |
| M | M-group |
| MB | mammillary body |
| MIF | multiply innervated fibre |
| MT | mammillothalamic tract |
| MLF | medial longitudinal fasciculus |
| NVI | abducens nerve |

| | |
|---|---|
| NVII | facialis nerve |
| NB | nucleus of Bechterew |
| ND | nucleus Darkschewitsch |
| NPC | nucleus of the posterior commissure |
| NRPC | nucleus reticularis pontis caudalis |
| NRPO | nucleus reticularis pontis oralis |
| NRTP | nucleus reticularis tegmenti pontis |
| OPN | omnipause neuron |
| PC | posterior commissure |
| PGD | nucleus paragigantocellularis dorsalis |
| PPRF | paramedian pontine reticular formation |
| RIMLF | rostral interstitial nucleus of the medial longitudinal fasciculus |
| RIP | nucleus raphe interpositus |
| RN | red nucleus |
| SC | superior colliculus |
| SIF | singly innervated fibre |
| TR | tractus retroflexus |

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## References

Baker, R. and Berthoz, A. (1977) Control of gaze by brain stem neurons. Elsevier/North-Holland Biomedical Press, Amsterdam/New York.

Bender, M.B. and Shanzer, S. (1964) Oculomotor pathways defined by electrical stimulation and lesions in the brain stem of the monkey. In: Bender M.B. (Ed.), The Oculomotor System. Harper and Row, New York, pp. 81–140.

Büttner, U., Büttner-Ennever, J.A. and Henn, V. (1977) Vertical eye movement related activity in the rostral mesencephalic reticular formation of the alert monkey. Brain Res., 130: 239–252.

Büttner-Ennever, J.A. (2006) The extraocular motor nuclei: organization and functional neuroanatomy. Prog. Brain Res., 151: 95–125.

Büttner-Ennever, J.A. and Büttner, U. (1978) A cell group associated with vertical eye movements in the rostral mesencephalic reticular formation of the monkey. Brain Res., 151: 31–47.

Büttner-Ennever, J.A., Büttner, U., Cohen, B. and Baumgartner, G. (1982) Vertical gaze paralysis and the rostral interstitial nucleus of the medial longitudinal fasciculus. Brain, 105: 125–149.

Büttner-Ennever, J.A., Cohen, B., Pause, M. and Fries, W. (1988) Raphe nucleus of the pons containing omnipause neurons of the oculomotor system in the monkey, and its homologue in man. J. Comp. Neurol., 267: 307–321.

Büttner-Ennever, J.A., Horn, A.K.E., Graf, W. and Ugolini, G. (2002) Modern concepts of brainstem anatomy. Ann. N.Y. Acad. Sci., 956: 75–84.

Büttner-Ennever, J.A., Horn, A.K.E., Scherberger, H. and D'Ascanio, P. (2001) Motoneurons of twitch and nontwitch extraocular muscle fibers in the abducens, trochlear, and oculomotor nuclei of monkeys. J. Comp. Neurol., 438: 318–335.

Chen, B. and May, P.J. (2000) The feedback circuit connecting the superior colliculus and central mesencephalic reticular formation: a direct morphological demonstration. Exp. Brain Res., 131: 10–21.

Cohen, B. and Henn, V. (1972) Unit activity in the pontine reticular formation associated with eye movements. Brain Res., 46: 403–410.

Cohen, B. and Komatsuzaki, A. (1972) Eye movements induced by stimulation of the pontine reticular formation. Evidence for integration in oculomotor pathways. Exp. Neurol., 36: 101–117.

Cromer, J.A. and Waitzman, D.M. (2006) Neurones associated with saccade metrics in the monkey central mesencephalic reticular formation. J. Physiol., 570: 507–523.

Goebel, H.H., Komatsuzaki, A., Bender, M.B. and Cohen, B. (1971) Lesions of the pontine tegmentum and conjugate gaze paralysis. Arch. Neurol. (Chicago), 24: 431–440.

Horn, A., Eberhorn, A., Härtig, W., Ardelenanu, P., Messoudi, A. and Büttner-Ennever, J. (2008) Perioculomotor cell groups in monkey and man defined by their histochemical and functional properties: a reappraisal of the Edinger–Westphal Nucleus. J. Comp. Neurol., 507: 1317–1335.

Horn, A.K.E. and Büttner-Ennever, J.A. (1998) Premotor neurons for vertical eye-movements in the rostral mesencephalon of monkey and man: the histological identification by parvalbumin immunostaining. J. Comp. Neurol., 392: 413–427.

Horn, A.K.E., Büttner-Ennever, J.A., Gayde, M. and Messoudi, A. (2000) Neuroanatomical identification of mesencephalic premotor neurons coordinating eyelid with upgaze in the monkey and man. J. Comp. Neurol., 420: 19–34.

Horn, A.K.E., Büttner-Ennever, J.A., Suzuki, Y. and Henn, V. (1995) Histological identification of premotor neurons for horizontal saccades in monkey and man by parvalbumin immunostaining. J. Comp. Neurol., 359: 350–363.

Horn, A.K.E., Büttner-Ennever, J.A., Wahle, P. and Reichenberger, I. (1994) Neurotransmitter profile of saccadic omnipause neurons in nucleus raphe interpositus. J. Neurosci., 14: 2032–2046.

Keller, E.L. (1974) Participation of medial pontine reticular formation in eye movement generation in monkey. J. Neurophysiol., 37: 316–332.

Keller, E.L. and Missal, M. (2003) Shared brainstem pathways for saccades and smooth-pursuit eye movements. Ann. N.Y. Acad. Sci., 1004: 29–39.

Luschei, E.S. and Fuchs, A.F. (1972) Activity of brain stem neurons during eye movements of alert monkeys. J. Neurophysiol., 35: 445–461.

Saper, C.B. (2006) Staying awake for dinner: hypothalamic integration of sleep, feeding, and circadian rhythms. Prog. Brain Res., 14: 243–252.

Ugolini, G., Klam, F., Doldan Dans, M., Dubayle, D., Brandi, A.-M., Büttner-Ennever, J.A. and Graf, W. (2006) Horizontal eye movement networks in primates as revealed by retrograde transneuronal transfer of rabies virus: differences in monosynaptic input to ''slow'' and ''fast'' abducens motoneurons. J. Comp. Neurol., 498: 762–785.

Waitzman, D.M., Pathmanathan, J., Presnell, R., Ayers, A.S. and DePalma-Bowles, S. (2002) Contribution of the superior colliculus and the mesencephalic reticular formation to gaze control. Ann. N.Y. Acad. Sci., 956: 111–129.

Waitzman, D.M., Silakov, V.L., DePalma-Bowles, S. and Ayers, A.S. (2000a) Effects of reversible inactivation of the primate mesencephalic reticular formation. I. Hypermetric goal-directed saccades. J. Neurophysiol., 83: 2260–2284.

Waitzman, D.M., Silakov, V.L., DePalma-Bowles, S. and Ayers, A.S. (2000b) Effects of reversible inactivation of the primate mesencephalic reticular formation. II. Hypometric vertical saccades. J. Neurophysiol., 83: 2285–2299.

Wasicky, R., Horn, A.K.E. and Büttner-Ennever, J.A. (2004) Twitch and non-twitch motoneuron subgroups of the medial rectus muscle in the oculomotor nucleus of monkeys receive different afferent projections. J. Comp. Neurol., 479: 117–129.

Wickersham, I.R., Lyon, D.C., Barnard, R.J.O., Mori, T., Finke, S., Conzelmann, K.-K., Young, J.A.T. and Callaway, E.M. (2007) Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. Neuron, 53: 639–647.