be obtained if the fibre stimulated belonged to the static type (Figure 1 D). Simultaneous activation of 2 static fusimotor fibres or of 1 dynamic and 1 static fibre converging onto the same spindle ending, gave similar results. On the other hand, at the rates of change of muscle length and fusimotor stimulation frequency used, stimulation of a single dynamic fusimotor fibre, or of 2 converging fibres for that matter, could never increase or even maintain the impulse frequency during muscle shortening (Figure 2 D).

The findings thus suggest that the increase in impulse frequency of the primary ending seen during contraction in natural movements requires static fusimotor activation[12].

Zusammenfassung. Statische oder dynamische γ-Fasern primärer Muskelspindelendigungen in den Fussextensoren der Katze wurden mit sich ändernden Frequenzen gereizt: Nur Stimulation statischer γ-Fasern mit steigender Frequenz bei gleichzeitiger Muskelverkürzung erhöht die Entladungsgeschwindigkeit primärer Endigungen, wie sie in physiologisch induzierten Bewegungen gesehen wird.

G. LENNERSTRAND and U. THODEN

Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm 60 (Sweden), 27th December 1966.

[12] This work was supported by the Swedish Medical Research Council (Project No. 14X-544-02), and by Karolinska Institutet (Reservations anslaget för främjande av ograduerade forskares vetenskapliga verksamhet).

Primary Afferent Depolarization of Trigeminal Fibres Induced by Stimulation of Brain Stem and Peripheral Nerves

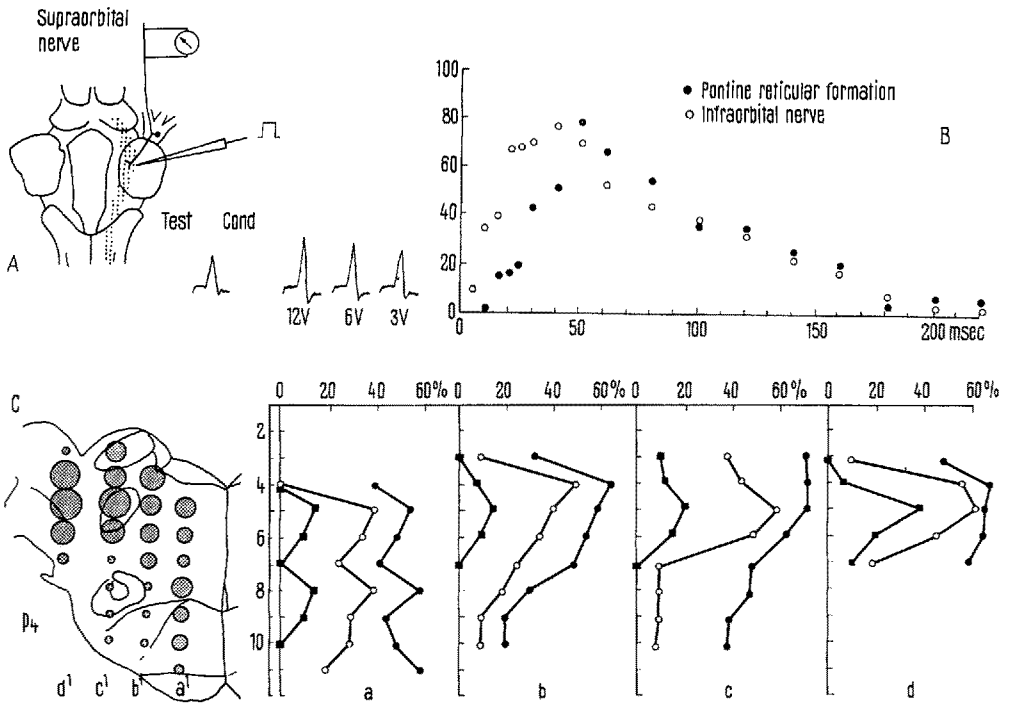
It has recently been shown that in chronic cats the excitability of trigeminal afferent fibres increases synchronously with the rapid eye movements (REM) of desynchronized sleep and at the moment of arousal[1]. Since synchronous potentials have been recorded during REM from brain stem regions[2], the hypothesis might be advanced that the latter structures are actively responsible for other phenomena temporally related to REM, as, for example, for primary afferent depolarization (PAD) of trigeminal afferents. Evidence for PAD of trigeminal fibres have already been reported in acute experiments following stimulation of other trigeminal afferents[3] and cortical areas[4].

The aim of this investigation has been to test the excitability of trigeminal afferents when conditioned by electrical stimulation of brain stem structures at bulbar, pontine and mesencephalic levels. In addition the conditioning effect of peripheral nerve stimulation was tested.

Method. The experiments were carried out in curarized acute cats under nembutal anaesthesia (25–30 mg/kg). The excitability of central terminals of trigeminal fibres was tested using the WALL technique[5] with the aid of a stainless steel microelectrode (about 100,000 Ω) stereotaxically introduced into the nucleus trigemini sensibilis principalis (NV snpr)[6] at 6–8 mm rostrally to the obex. The antidromic test response evoked by monopolar microelectrode stimulation was monophasically recorded from the killed end of the ipsilateral supraorbital nerve after the eye was enucleated (Figure A). Mineral oil was used to cover the nerve into the orbital cavity. Conditioning stimuli were applied, through stainless steel microelectrodes (about 100,000 Ω) stereotaxically introduced into the brain stem at various planes (from A6 to P11 and from midline to lateral 6, contralaterally to the trigeminal electrode) after the tentorium was removed. Bipolar silver electrodes were used for peripheral nerve – infraorbital (IO), superficial radial (SR), dorsal interosseous (DI), superficial peroneal (SP), deep peroneal (DP) – stimulation. The position of both test and conditioning microelectrodes in the brain stem was carefully controlled at the end of each experiment with Nissl stain technique and the map constructed on this histological evidence.

Results. (A) Single shock stimulation (1/sec, 0.01–0.5 msec, 30–40 V) of the main sensory trigeminal nucleus evoked in the ipsilateral supraorbital nerve an antidromic response with 0.6–0.8 msec latency (Figure A test). Conditioning electrical stimuli (4 impulses at 300/sec, 50–100 μsec, 3–12 V) applied to the controlateral half of the brain stem (medulla, pons and mesencephalon) increased the amplitude of the antidromic test response recorded from the supraorbital nerve (Figure A conditioning). Figure B shows the time course of the curve obtained by conditioning stimulation of pontine reticular formation (n. reticularis pontis caudalis) as compared with stimulation of the infraorbital nerve. It can be seen that PAD evoked by pontine stimulation starts with a longer latency and reaches a maximum (at 50 msec interval) later than that induced by ipsilateral infraorbital nerve stimulation. Figure C shows the conditioning effect induced by stimulation at 3 intensities (12, 6 and 3 V) of a pontine region (P4) at various depths and at different medio-lateral levels. It can be seen that a PAD was present when both medial and lateral regions were stimulated. However, a higher effect was obtained by stimulation of the contralateral trigeminal tract (as shown in c, c1, d, d1) as compared with stimulation of more medially situated regions. Usually 1–2 impulses were enough to give maximal PAD from the trigeminal tract, while at least 4 impulses were required to induce maximal reticular conditioning. A PAD was always present when the central core of the brain stem reticular system was stimulated, a maximal

[1] F. BALDISSERA, G. BROGGI and M. MANCIA, Experientia *22*, 754 (1966).
[2] D. C. BROOKS and E. BIZZI, Archs ital. Biol. *101*, 648 (1963).
[3] I. DARIAN-SMITH, J. Neurophysiol. *28*, 695 (1965).
[4] I. DARIAN-SMITH and T. YOKOTA, J. Neurophysiol. *29*, 185 (1966).
[5] P. D. WALL, J. Physiol., Lond. *142*, 1 (1958).
[6] J. EISENMANN, S. LANDGREN and D. NOVIN, Acta physiol. scand. *59*, suppl. 214 (1963).



(A) Experimental scheme of microelectrode stimulation of trigeminal afferent fibres into the main trigeminal sensory nucleus, and recording from ipsilateral supraorbital nerve. Test: antidromic response of supraorbital nerve to single shock stimulation of its presynaptic terminals. Conditioning: same response preceded at 50 msec by 4 stimuli at 300/sec, 50 μsec to medial pontine reticular formation at 3 different intensities (12, 6 and 3 V). (B) Time course of excitability changes of supraorbital presynaptic terminals produced by 4 impulses at 300/sec delivered to medial pontine reticular formation (filled circles) and by 1 impulse to ipsilateral infraorbital nerve (open circles). In ordinates the % increase of antidromic test response amplitude. (C) On the left: map indicating the loci in pontine regions (P4) whose stimulation (4 impulses, 300/sec, 6 V) induced PAD of trigeminal fibres. The diameter of stippled circles is proportional to the % increase of test response amplitude. The curves a, b, c and d in the remaining part of the Figure show the % increase of test response (abscissa) obtained by conditioning stimulation of brain stem points at different depths in mm below zero of Horsley-Clark coordinates (ordinata) along the tracks of the map (a1, b1, c1 and d1) at 3 stimulus intensities (12 V = filled circles, 6 V = open circles, 3 V = filled squares).

effect being observed from n. reticularis magnocellularis, n. reticularis pontis caudalis and pontis oralis. A PAD was also induced when the conditioning electrode reached the pyramidal pathways at all brain stem levels.

Electrical stimulation of ipsilateral and contralateral cutaneous (SR, SP) and muscular (DI, DP) nerves could induce an increase of antidromic test response. The conditioning curve obtained with a single shock to low threshold SR fibres had a time course with maximal facilitation at about 60 msec for the ipsilateral and 70 msec for the contralateral SR. Stimulation of group I muscular fibres had no effect on antidromic test response. Single shock stimulation of group II muscular fibres induced a PAD which was maximal at about 60 msec for the ipsilateral and at 70 msec for the contralateral DI fibres. An additional effect was seen after stimulation of both SR and DI group III fibres.

All the described effects induced by central and peripheral stimulations were still present after acute anaemic decerebration[7] or in animals surgically decerebrated and cerebellectomized.

(B) In decerebrated cats, a negative slow potential change (100–150 msec) has been recorded from the isolated trigeminal sensory root following stimulation of brain stem, contralateral infraorbital nerve and common radial trunk. Because of its analogy with a dorsal root potential (DRP) of the spinal cord[8] we named it trigeminal dorsal root potential (TDRP).

(C) By recording with micropipettes, exploring in a frontal plane the region of the trigeminal complex in the pons, potential fields (P-wave) similar to those recorded in the spinal cord[9] and cuneate nucleus[10] have been found following stimulation of brain stem and infraorbital nerve. These slow potential changes lasted for about 200 msec, had maximal positivity in the trigeminal tract, maximal negativity in the nucleus and reversal line between nucleus and tract perpendicularly to the course of primary fibres entering the nucleus.

Conclusions. The results show that brain stem reticular regions (particularly n. reticularis gigantocellularis, n. reticularis pontis oralis and pontis caudalis) are able to induce a PAD of trigeminal afferents, thereby presynaptically controlling[11] the trigeminal input. This effect is present also in the absence of diencephalic, telencephalic and cerebellar structures. A presynaptic control of trigeminal afferents is also exerted by cutaneous and muscular group II and III fibres. The hypothesis may be

[7] L. J. Pollock and L. Davis, Archs Neurol. Psychiat., Chicago *10*, 391 (1923).

[8] D. H. Barron and B. H. C. Matthews, J. Physiol. *92*, 276 (1938).

[9] J. C. Eccles, F. Magni and W. D. Willis, J. Physiol. *160*, 62 (1962).

[10] P. Andersen, J. C. Eccles, R. F. Schmidt and T. Yokota, J. Neurophysiol. *27*, 78 (1964).

[11] J. C. Eccles, *The Physiology of Synapses* (Springer Verlag, Berlin 1964).

advanced that the latter control is mediated through brain stem reticular neurones. However, the activation of a more specific pathway in such a control cannot be excluded.

Riassunto. L'eccitabilità delle terminazioni afferenti del trigemino, saggiata con il metodo di WALL[5] aumenta quando stimoli condizionanti sono dati a strutture reticolari del tronco (in particolare ai nuclei reticularis gigantocellularis, pontis caudalis e pontis oralis) ed a fibre di nervi periferici cutanei e muscolari di gruppo II, III.

F. BALDISSERA, G. BROGGI and M. MANCIA

Istituto di Fisiologia Umana dell'Università di Milano and Impresa di Elettrofisiologia del CNR, sezione di Milano (Italy), 31st October 1966.

STUDIORUM PROGRESSUS

Zur Frage des Vorkommens von N-Nitroso-Verbindungen im Tabakrauch

Als DRUCKREY und PREUSSMANN[1] im Jahre 1962 darauf hinwiesen, dass die Bildung von Nitrosaminen aus Stickoxiden und sekundären Aminen auch im Tabakrauch möglich sein müsste, fehlte es an geeigneten Methoden, diese Verbindungsklasse im Zigarettenrauch nachzuweisen. Inzwischen sind jedoch einige Arbeiten erschienen, die den qualitativen Nachweis und mindestens eine semiquantitative Bestimmung dieser Stoffe zulassen[2–5]. Mit einigen dieser Methoden ist der Nachweis ausserordentlich geringer Mengen von Nitrosaminen im Tabakrauch geführt worden[6–8]. Bei den Arbeiten hat es sich jedoch gezeigt, dass die Untersuchungen experimentell aufwendig und schwierig sind und die Beachtung einer ganzen Reihe von Besonderheiten erfordern. Ausserdem sind in der Zwischenzeit eine Reihe von Grundlagen-Untersuchungen, so über das Vorkommen von Flüchtigen Basen – insbesondere sekundären Aminen – in Tabak und Rauch[9,10], über den Nitratgehalt verschiedener Tabaktypen[11–13] und über geeignete Bestimmungsmethoden[14,15] für diese Ausgangskomponenten zur Bildung von N-Nitroso-Verbindungen erschienen.

Es ist daher sicher an der Zeit, eine zusammenfassende Darstellung dieses Arbeitsgebietes und seiner Problematik zu geben.

Analytik der Nitrosamine. Die Nitrosamine haben sich auf zwei Wegen für Nachweis- und Identifizierungsreaktionen zugänglich erwiesen. Der erste ist die photolytische Spaltung der N-NO-Bindung, bei der, nach Abspaltung von NO, Stickoxide entstehen, die dann alkalisch abgefangen und mit den üblichen Nitritreagenzien, z.B. dem Griess'schen Reagenz, nachgewiesen werden können[2,3]. Der zweite Weg ist die Reduktion der N-Nitroso-Verbindungen zu asymmetrischen Hydrazinen, die wiederum als 5-Nitro-2-hydroxybenzal-Derivate[4] oder als 4'-Nitroazobenzolcarbonsäure-(4)-hydrazide[4,5] in Form von kristallisierten, gefärbten Derivaten zum Nachweis der Nitroso-Verbindungen dienen können.

Nach den beiden letztgenannten Methoden ist der Nachweis von Spuren Nitrosaminen im Tabakrauch geführt worden[6–8].

Stickoxide im Tabakrauch. Die gleichzeitige quantitative Erfassung von Stickstoffmonoxid und Stickstoffdioxid im Tabakrauch ist ein noch ungelöstes Problem. Stickstoffmonoxid ist ein analytisch aus Gemischen wie Tabakrauch schwierig absorbierbares Gas (Kp = – 151,8 °C), das nur unter gleichzeitiger Oxydation zu Stickstoffdioxid gefasst werden kann, während das Stickstoffdioxid (Kp = 22,4 °C) bei der Rauchtemperatur weitgehend dimer vorliegt und bei der Absorption in wässerigem Alkali sofort zu Nitrit und Nitrat disproportioniert. Mit Wasser wird aus Stickstoffdioxid Salpetersäure und NO gebildet.

Die gemeinsame Oxydation beider Oxide zu Nitrat ist für den Tabakrauch wegen der gleichzeitigen Anwesenheit von Ammoniak und Aminen, die ebenfalls oxydiert würden[16], nicht anwendbar. Mit den zur Verfügung stehenden bekannten Methoden sind immerhin Bestimmungen der Stickoxide im Tabakrauch durchgeführt worden[17,18], deren Ergebnisse in Tabelle I zusammengefasst sind.

Tabelle I. Stickoxidgehalt im Zigarettenrauch ppm (v/v)

Autoren	NO	NO_2
HAAGEN-SMIT et al.[17]	145– 655	
BOKHOVEN und NIESSEN[18]	170– 210	
TADA[19]	72– 271	19–118
NORMAN und KEITH[20]	442–1008	0– 25
WESTCOTT[21]	96–1120	

[1] H. DRUCKREY und R. PREUSSMANN, Naturwissenschaften *49*, 498 (1962).
[2] R. PREUSSMANN, D. DAIBER und H. HENGY, Nature, Lond. *201*, 502 (1964).
[3] R. PREUSSMANN, G. NEURATH, G. WULF-LORENTZEN, D. DAIBER und H. HENGY, Z. analyt. Chem. *202*, 187 (1964).
[4] G. NEURATH, B. PIRMANN und M. DÜNGER, Chem. Ber. *97*, 1631 (1964).
[5] W. J. SERFONTEIN und P. HURTER, Nature, Lond. *209*, 1238 (1966).
[6] G. NEURATH, B. PIRMANN und H. WICHERN, Beiträge zur Tabakforschung *2*, 311 (1964).
[7] G. NEURATH, B. PIRMANN, W. LÜTTICH und H. WICHERN, Beiträge zur Tabakforschung *3*, 251 (1965).
[8] W. J. SERFONTEIN und P. HURTER, Cancer Res. *26*, 575 (1966).
[9] G. NEURATH, A. KRULL, B. PIRMANN und K. WANDREY, Beiträge zur Tabakforschung *3*, 571 (1966).
[10] G. NEURATH, M. DÜNGER, J. GEWE, W. LÜTTICH und H. WICHERN, Beiträge zur Tabakforschung *3*, 563 (1966).
[11] G. NEURATH und H. EHMKE, Beiträge zur Tabakforschung *2*, 333 (1964).
[12] G. LIPP und U. DÖLBERG, Beiträge zur Tabakforschung *2*, 345 (1964).
[13] G. M. BROADDUS, J. E. YORK und J. M. MOSELEY, Tob. Sci. *9*, 149 (1965).
[14] G. NEURATH, H. EHMKE und K.-H. MÜLLER, Beiträge zur Tabakforschung *2*, 321 (1964).
[15] G. NEURATH und E. DOERK, Chem. Ber. *97*, 172 (1964).
[16] F. WÖHLER, Justus Liebigs Annln Chem. *136*, 256 (1865).
[17] A. J. HAAGEN-SMIT, M. F. BRUNELLE und J. HARA, A.M.A. Archs ind. Hlth *20*, 399 (1959).
[18] C. BOKHOVEN und H. J. NIESSEN, Nature, Lond. *192*, 458 (1961).