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1 Nigrovic, V., Int. J. Rad. Biol. *7* (1963) 307.
2 Nigrovic, V., Bohne, F., and Madshus, K., Strahlentherapie *130* (1966) 413.
3 Havlicek, F., Kleisner, I., Dvorak, P., and Pospisil, J., Strahlentherapie *134* (1968) 123.
4 Madshus, K., Stroemme, A., Nigrivic, V., and Bohne, F., Int. J. Rad. Biol. *10* (1966) 519.
5 Nigrovic, V. Phys. Med. Biol. *10* (1965) 81.
6 Bozorgzadeh, A., Strahlentherapie *142* (1971), 734.
7 Dvorak, P., Arzneimittel-Forsch. *12* (1970), 1886.
8 Seletskaya, L. I., and Borisov, V. P., Radiobiologija *13* (1973) 313.
9 Bozorgzadeh, A., and Catsch, A., Archs int. Pharmacodyn. *197* (1972) 175.
10 Müller, W. H., Ducousso, R., Causse, A., and Walter, C., Strahlentherapie *147* (1974) 319.
11 Nielsen, P., Dresow, B. and Heinrich, H. C., Z. Naturf. *42 B* (1987) 1451.
12 Heinrich, H. C, Gabbe, E. E., and Whang, D. H., Atompraxis *11* (1965) 430, 660.
13 Pfau, A. A., Rudolphi, K., Heinrich, H. C, and Gabbe, E. E., in: Nuclear Techniques in Animal Production and Health, p. 127. International Atomic energy Agency, Vienna 1976.
14 Ekman, L., Acta vet. scand. Suppl. *4* (1961) 48.
15 Riedel de Haen, Produktinformation – Giese-Salz Ammoniumeisen(III–)-hexacyanoferrat(II); 1987.
16 Giese, W., and Hantzsch, D., Zentrbl. VetMed., Beiheft *11* (1970) 185.

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The nature of posterior hypothalamic projections to cardiorespiratory centers in the brainstem

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Summary. Focal electrical stimulation of the midlateral posterior hypothalamus in the rat produces rapid shallow respiration accompanied by a rise in arterial blood pressure. Stimulation of presumably intrinsic neurons only by glutamate induces slower deeper respiration associated with a fall in blood pressure.
Key words. Posterior hypothalamus; blood pressure; respiration.

Stimulation of points throughout the length of the hypothalamus in the rat evokes a variety of behavioral functions such as attack, flight, defence, copulation and feeding[1]. There is some separation of response depending upon whether sites medial or lateral to the fornix are stimulated. Nevertheless, anatomically many nuclear groups such as the posterior hypothalamic nucleus, dorsal premammillary nucleus, submammillary nucleus and nucleus geminus as well as the subthalamic nucleus and zona inserts have been stimulated when producing these behaviors.

Focal electrical stimulation of a small locus of the midlateral posterior hypothalamus (MLPH)[2] in chloral hydrate anesthesized rats produces a rise in arterial blood pressure accompanied by rapid shallow respiration[3]. MLPH is, however, a conduit for caudally directed axons originating from centers which, when stimulated also result in alterations in cardio-respiratory function. These areas include the hippocampus[4], septum[4,5], habenula[6], medial hypothalamus[7], lateral hypothalamus[8–10], anterior hypothalamus[7,11], thalamus[12], cingulate cortex[13,14], and prefrontal cortex[13,15]. In this study gluatmate was used by both pressure and iontophoretic injection to stimulate neurons intrinsic to MLPH avoiding stimulation of axons of passage[16].

Materials and methods. Twenty 300-g Wistar rats were used in this study. The common carotid artery on the side opposite to that used for brain stimulation was cannulated, flushed with heparinized saline, and arterial blood pressure was monitored with a tham P23AA pressure transducer. Respiration was monitored with an impedance converter from electrodes inserted in intercostal muscles in the midaxillary line. Body temperature was maintained at 37 °C by a heating pad activated by a rectal thermistor probe. The site stimulated is immediately lateral to the mamillothalamic tract and above the fornix lateral to the posterior hypothalamic nucleus (AP – 3.8 mm, lat. 1.1 mm, vertical 8.2 mm in the atlas of Paxinos and Watson 17).

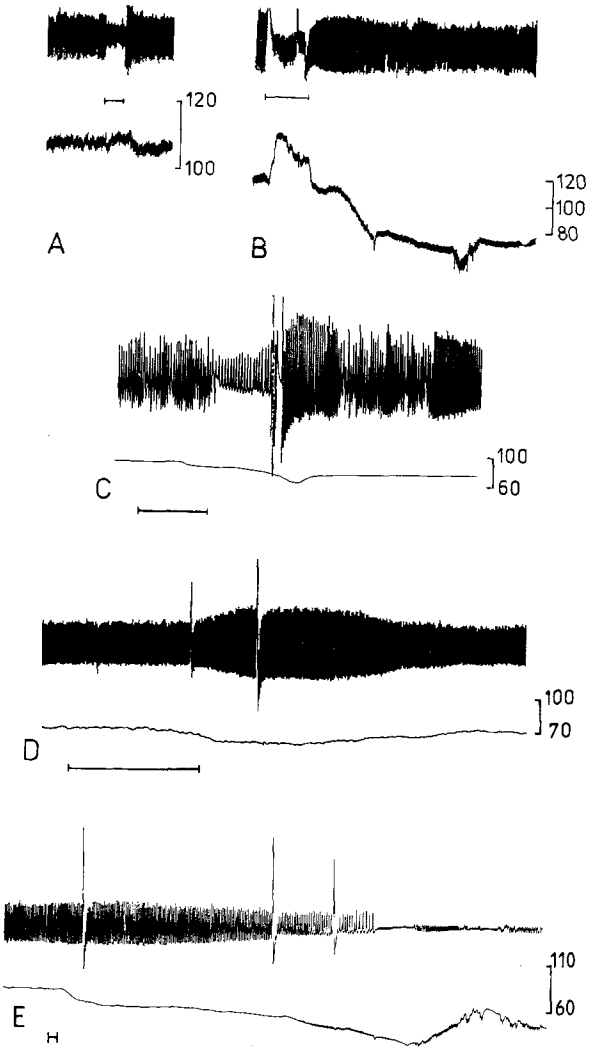
In 10 rats, MLPH was stimulated by a pressure injection of 50 or 100 nl of M sodium glutamate pH 7.5 –8 over 30–60 s through a glass electrode (tip 40 μm) using a nanoliter pump (WPI) or 0.5 μl over 10 s using a Hamilton syringe. The site of injection was verified histologically after substituting the electrode with one containing Methyl Blue in potassium acetate and making a 100-nl injection.

In a further series of 10 rats, one side of a double glass electrode was filled with 2 M NaCl (used to verify electrode location by electrical stimulation) and the other with 3 M sodium glutamate which was injected by a constant current of 12 μA of 15–60 s duration. Controls for this procedure comprised observing respiration or arterial blood pressure while passing the same current through the saline electrode or the same current of opposite polarity through the glutamate electrode. The site of injection was verified histologically after substituting the electrode with one containing Methyl Blue in potassium acetate and marking the site by passing negative current through the electrode[18,19].

In some cases the carotid bodies were denervated by the method of Favier and Lacaisse[20].

Results. Electrical stimulation of MLPH produced rapid shallow respiration for the duration of the stimulus in which both inspiration and expiration were equally shortened (fig., A). Arterial blood pressure showed a rapid rise and began to fall before cessation of the stimulus. A similar response could be elicited from points following the medial forebrain bundle forward to the anterior hypothalamic and preoptic areas. Following Carotid Body denervation, stimulation of MLPH produced a rise in blood pressure followed by a prolonged period of post stimulus recovery. Respiration also showed some post stimulus augmentation (fig., B).

Slow pressure injection of M glutamate produced a different response (fig., C). After a delay, blood pressure fell and respiration slowed and became shallow. This was followed by rebound of deeper and more rapid respiration during which blood pressure returned toward preinjection levels. Blood pressure was allowed to recover completely before subsequent stimulations and no more than two injections were made at one site.



Effects on respiration (above) and arterial blood pressure (below) of stimulation of MLPHA by different methods indicated. Vertical bars show blood pressure in mms Hg and horizontal bars the duration and timing of stimulation or injection. *A* Electrical stimulation (8-s train, 1 ms pulses, 100 Hz, 5 V,100 μA). *B* Electrical stimulation (16-s train, 1 ms pulses, 100 Hz, 5 Volts, 100 μA) following carotid body denervation. *C* Pressure injection by nanoliter pump of 50 nl of M glutamate over 32-s period. *D* Iontophoretic injection of 3 M glutamate over 1-min period. (Constant current 12 μA, electrode +ve relative to an electrode inserted in dorsal neck musculature). *E* Pressure injection by Hamilton syringe of 0.5 μl of M glutamate over 5-s period.

Iontophoretic injection of 3 M glutamate affected respiration after a delay and comprised a period of slower and deeper movements outlasting the period of glutamate application. Blood pressure slowly declined with respiratory changes and slowly returned to pre-injection levels (fig., D). No changes in respiration or blood pressure were produced by application of current of opposite polarity or by application of the same current to the saline electrode. Injection of larger volumes of glutamate (0.5 μl of M glutamate), less than volumes used to produce cardiorespiratory responses in rat to other chemicals[21], in the presence of carotid body denervation produced intense depression that could be irreversible (fig. E).

Discussion. The experiments described suggest that the response elicited by electrical stimulation of MLPH presumably excites fibers of passage in addition to intrinsic neurons resulting in a net rapid shallow respiration and rise in blood pressure. In contrast, the glutamate-induced response differs significantly with depression of respiratory rate and blood pressure predominant. Since there is evidence that glutamate does not stimulate axons of passage[16] the depression may be attributed to stimulation of intrinsic neurons alone. This finding is supported by recent neuroanatomical studies where microiontophoretic injections of peroxidase and 3H leucine into MLPH apart from a weak afferent projection from the parabrachial nuclei, failed to show direct connections with medullary cardiorespiratory centers[3]. These studies did, however, show connections with centers such as periaqueductal grey and raphe which do project to cardiorespiratory centers.

The results of this study are at variance with those of Tan et al.[22] who recorded poor or no response to glutamate stimulation of the lateral hypothalamic area in the rabbit. This may however be attributed to differences in anesthesia used in location of injection site and differences between species particularly in density of intrinsic neurons and their exposure to glutamate.

The pathway between MLPH and cardiorespiratory centers is indirect and could involve an opioid. This has been suggested by experiments modulating analgesia by lateral hypothalamic stimulation[23]. Enkephalinergic neurons form dense arborizations around cells of the nucleus of the parabrachial nuclei, solitary tract and nucleus ambiguus[24]. In addition enkephalin is co-localized with serotonin in the raphe[25] which projects to the solitary tract as well as the phrenic nucleus[26, 27], and stimulation of the raphe has been shown to inhibit cardiorespiratory neurons[28]. MLPH may represent a relay through which limbic inputs gain access to cardiorespiratory neurons resulting in their depression, e.g., in the depression which follows stress particularly noticed in neonates[29].

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1 Jurgens, U., Prog. Brain Res. *41* (1974) 460.
2 Morgane, P. J., Historical and modern concepts of hypothalamic organization and function, in: Handbook of the Hypothalamus, vol. 1, pp. 1–64. Eds P. J. Morgane and J. Panksepp. Marcel Dekker, New York 1979.
3 Hinrichsen, C., Proc. anat. Soc. Aust. NZ (1987) 8.
4 Nauta, W., J. comp. Neurol, *104* (1956) 247.
5 Valenstein, E. S., and Nauta, W. J. H., J. comp. Neurol. *113* (1959) 337.
6 Herkenham, M., and Nauta, W. J. H., J. comp. Neurol. *187* (1979) 19.
7 Conrad, L. C. A., and Pfaff, D. W., J. comp. Neurol. *169* (1976) 221.
8 Swanson, L. W., and Kuypers, H. G. J. M., J. comp. Neurol. *194* (1980) 555.
9 Wolff, G., and Sutin, J., J. comp. Neurol. *127* (1966) 137.
10 Hosoya, Y., and Matsushita, M., Exp. Brain Res. *35* (1979) 312.
11 Chi, C. C., and Flynn, J. P., Brain Res. *35* (1971) 49.
12 Herkenham, M., J. comp. Neurol. *177* (1978) 589.
13 Beckstead, R. M., J. comp. Neurol. *184* (1979) 43.
14 Domesick, V. B., Brain Res. *12* (1969) 296.
15 Leonard, C. H., Brain Res. *12* (1969) 321.
16 Curtis, D. R., and Ryall, R. M., Exp. Brain Res. *1* (1966) 195.
17 Paxinos, G., and Watson, C., The Rat Brain in Stereotaxic. Coordinates. Academic Press, Australia 1982.
18 Wilson, V. J., Talbot, W. H., and Kato, M., J. Neurophysiol. *27* (1964) 1063.
19 Bystrzycka, E. K., Brain Res. *185* (1980) 59.
20 Favier, R., and Lacaisse, A., J. Physiol., Paris *74* (1978) 411.
21 Myers, R. D., Handbook of Drug and Chemical Stimulation of the Brain. Van Nastrand Reinhold, New York 1974.
22 Tan, E., Goodchild, A. K., and Dampney, R. A. L., Clin. exp. Pharmac. Physiol. *10* (1983) 305.
23 Carr, K. D., and Yusal, S., Brain Res. *335* (1985) 55.
24 Finley, J. C. W., Maderdrut, J. L., and Petrusz, P., J. comp. Neurol. *198* (1981) 541.

25 Petrusz, P., Merchenthaler, I., and Maderdrut, J. L., Distribution of enkephalin containing neurons in the central nervous system. in: Handbook of Chemical Neuroanatomy, vol. 4, Part 1, pp. 273–334. Eds A. Björklund, and T. Hökfelt. Elsevier, Amsterdam 1985.
26 Steinbusch, H. W. M., and Nieuwenhuys, R., The Raphe Nuclei of the Rat Brainstem: A cytoarchitectonic and immunohistochemical study, in: Chemical Neuroanatomy, pp. 131–207. Ed. P. C. Emson. Raven Press, New York 1983.
27 Holtman, J. R., Fedn Proc. *42* (1984) 331.
28 Sessle, B. J., and Henry, J. L., Brain Res. *327* (1985) 221.
29 Grunstein, M. M., J. appl. Physiol. *51* (1981) 122.

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Massive striatal dopamine release in acute cerebral ischemia in rats

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Summary. Extracellular dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and cerebral blood flow were simultaneously determined using in vivo brain dialysis and a hydrogen clearance method in the striatum of spontaneously hypertensive rats during ischemia and after recirculation. Massive striatal dopamine release was demonstrated in acutely induced ischemic brain.
Key words. Dopamine release; cerebral ischemia; striatum; in vivo brain dialysis; spontaneously hypertensive rats.

It has been proposed that the neurotransmitter dopamine which escapes from ischemic neurons may exacerbate the tissue damage in the striatum, probably owing to a direct effect on neurons[1], or to a hypoperfusion secondary to the dopamine-induced vasoconstriction[2]. To investigate the effects of ischemia on striatal dopamine release, in vivo brain dialysis[3] was applied to a cerebral ischemic model to determine the changes in striatal extracellular dopamine in acute cerebral ischemia.

Methods. Cerebral ischemia was induced in spontaneously hypertensive rats (SHR) by bilateral carotid artery ligation (BCL) as previously described[4, 5]. Extracellular dopamine and regional cerebral blood flow (CBF) were simultaneously determined in the striatum using in vivo brain dialysis[3] and a hydrogen clearance method[5], respectively. Five male SHR aged 5 months, weighing 300–340 g, were anesthetized with amobarbital (100 mg/kg b. wt i.p.). Both femoral arteries were cannulated; one for anaerobic sampling of blood and the other for blood pressure recording with electromanometer. Both common carotid arteries were exposed through a ventral midline incision in the neck, separated from the vagosympathetic trunks carefully, and loosely encircled with sutures for later ligation. The rat's head was fixed in a head holder, and two burr holes were made on the skull for inserting a dialysis probe and CBF electrodes. A dialysis probe, 500 μm in outer diameter (Bioanalytical System, USA) and a teflon-coated platinum electrode for CBF study, 200 μm in diameter, with a 1-mm portion at its tip uncoated, were placed stereotactically in the right striatum, 0.5 mm anterior, 2.5 mm lateral to the bregma and 4.5 mm from the brain surface. Another platinum electrode was inserted in the parietal cortex.

The striatum was perfused with a Ringer's solution of the following composition: Na^+ 147 m mol/l, Ca^{2+} 2.3, K^+ 4 and Cl^- 155.5. The solution was perfused at the rate of 1.97 μl/min with a Harvard pump. Perfusates were collected every 10 min into a plastic tube containing 5 μl of 0.2 N perchloric acid. Each 20 μl of sample was injected directly into high performance liquid chromatography with electrochemical detection (HPLC-ECD). Dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) were quantified by HPLC-ECD. The HPLC system consisted of a L-6000 pump

Arterial acid-base parameters and mean blood pressure before and during ischemia and after 60 min of recirculation

		Before	Ischemia	Recirculation
P_{CO_2}	(mm Hg)	31.8 ± 1.7	15.0 ± 1.6**	23.9 ± 1.7**
P_{O_2}	(mm Hg)	82.3 ± 1.6	95.9 ± 4.8	83.9 ± 2.9
pH		7.45 ± 0.01	7.64 ± 0.02**	7.51 ± 0.02*
MBP	(mm Hg)	200 ± 7	240 ± 6**	198 ± 7

Values are mean ± SEM (n = 5). *, $p < 0.05$; **, $p < 0.01$ vs before ischemia, paired t-test.

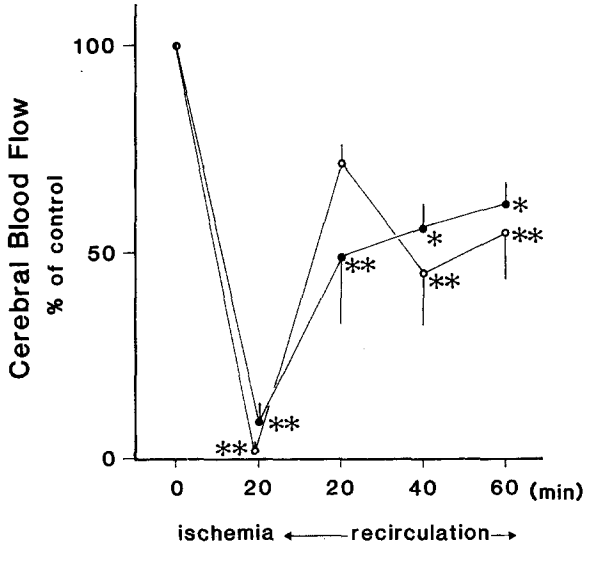

Figure 1. Percent changes in striatal (closed circle) and cortical (open circle) cerebral blood flow during ischemia and after recirculation. Bars represent SEM (n = 5). * $p < 0.05$; ** $p < 0.01$ vs before ischemia, paired t-test.