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A Magnetic Device to Stimulate Selected Whiskers of Freely Moving or Restrained Small Rodents: its Application in a Deoxyglucose Study

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After receiving an intraperitoneal injection of [^{14}C]2-deoxy-D-glucose (2-DG), a total of 28 mice which had pieces of metal wire glued to certain whiskers (all others were clipped) were exposed to magnetic field bursts. The stimulated whiskers were B1 (freely moving mice, set I) or whiskers C1–3 and E1 (restrained mice, set II) on the left side. In set I, stimulated mice were compared with animals of various control groups. Autoradiography demonstrated an activation of columnar shape overlying the presumed corresponding barrel contralateral to stimulation; in a part of the ipsilateral barrelfield, 2-DG uptake was depressed significantly. In the subnuclei caudalis and interpolaris of the trigeminal brainstem complex a spot of activation was observed ipsilaterally but there was no depression contralaterally. Whereas several animals of the control groups showed some aspects of these responses, they were consistent only in stimulated mice. In set II, animals received stimulation with different intensities. 2-DG uptake was higher in barrels C1–3 than in E1. It increased with increasing intensity. The same observations were made in two nuclei of termination. The device we describe here can be used to study stimulus-specific responses at various levels of the somatosensory pathway.

INTRODUCTION

This study was undertaken to test a device for the selective stimulation, over an extended period of time (45 min), of single or multiple vibrissae of freely moving or restrained mice, with stimuli of which certain parameters can be controlled. The stimulus is a whisker displacement whose repetition rate and average amplitude are variable. It is generated by bursts of a sinusoidal magnetic field which acts upon one or more metal pieces glued onto one or more whiskers. Stimulus-evoked responses in the central somatosensory pathway were studied with deoxyglucose (2-DG) autoradiography (Sokoloff et al.[25]). This pathway runs from the whiskerpad through the trigeminal nerve into its principal sensory nucleus and into the subnuclei of its spinal tract; their projections cross the midline and terminate in the ventrobasal complex of the thalamus, which in turn is reciprocally connected with the primary somatosensory cortex. In all these regions, a representation of the whiskerpad had been demonstrated morphologically and by electrophysiological recordings (for literature see Jeanmonod et al.[11]). In layer IV of the primary somatosensory cortex, each whisker is represented by a distinct morphological structure (a 'barrel') which is composed of a 'side' of high neuronal density surrounding a neuropil region scarce in somata, the 'hollow'. Each barrel is separated from its neighbors by 'septa', poor in somata. The big barrels correspond to the larger, caudal whiskers, and the complete set of big barrels in one hemisphere forms the posteromedial barrel subfield[31] (PMBSF, Fig. 1). The topologically equivalent relationship between the cortical barrels and the mystacial vibrissae in mice was demonstrated by the absence of barrels cor-

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responding to follicles lesioned early in postnatal life[27] and by electrophysiological mapping[24]. Responses in the barrelfield to vibrissa stimulation with brushes have been shown with 2-DG autoradiography[8,13,14], but these investigators did not demonstrate unequivocally the correspondence of stimulated whiskers and activated barrels. The first part of the present study served to test the magnetic stimulating procedure against a number of control set-ups at a fixed stimulus intensity. In the second part, stimulus intensity was varied. The changes of the 2-DG uptake and of the correspondence of labeled areas and stimulated barrels under the varied stimulus conditions were studied. For these purposes, the barrelfields were reconstructed from sections cut tangentially to the cortical surface. The recontructions were superimposed onto autoradiograms and this permitted us to determine whether the activated zones in the PMBSF matched the stimulated whiskers. The technique of matching morphology and 2-DG uptake has been applied by others[3–5]. However, in those studies 'complete rows' of whiskers were stimulated by the exploratory activity of freely moving mice whose other whiskers had been plucked. We judged our results to be of sufficient clarity to render a quantitative analysis unnecessary for the conclusions drawn. Four previous descriptions of mechanically coupled whisker stimulators exist: Shipley[22], Armstrong-James[1], Ito[10] and Simons[23]. These devices — the first 3 electromagnetic, the latter piezoelectric — were capable of delivering defined and variable whisker displacements by attaching a moving arm to one or more whiskers. In contrast to our set-up, theirs required anesthesia or paralysis of the animals.

MATERIALS AND METHODS

The stimulator was an upright electromagnetic coil: 201 mm long, 159 mm i.d., with 880 turns of enamel-insulated, 1.7 mm diameter copper wire wound in 8 layers on a thermoresistant plastic cylinder. The device was fed by the alternating current (50 Hz) of the mains (220 V). The voltage could be regulated continuously with a transformer (Variac). Magnetic field bursts were produced by a burst generator which consists of a triac triggered by low power rectangular pulses via an optocoupler. The pulses, of variable length and interpulse distance, were generated by a digital timer. The pulses were imparted to one or more vibrissae by metal pieces glued onto them with cyanoacrylate cement. We used mu-metal, an alloy of iron and 75–80% nickel. The pieces, 0.2 mm in diameter, 2 mm long and weighing about 0.6 mg, were glued with their center about 5 mm away from the skin surface. During stimulation, the animal was housed in a transparent methyl-methacrylic cylinder (126 mm in diameter) in which bedding and food can be put and that contained a water bottle. The cylinder was placed inside the coil and could be cooled by a small ventilator placed underneath. The set-up is shown in Fig. 2. In experiments with unrestrained animals, the bottom of the cylinder was packed with a layer of sawdust to bring the heads of the animals to the level where the magnetic field was at maximum strength (mid-height of the cylinder).

The strength of the magnetic field was directly proportional to the voltage applied to the coil. It was measured with a Gaussmeter (F.W. Bell, Orlando, FL, U.S.A.); 1.0 Gauss equals 79.59 A/m. For stimulation, 4 magnetic field strengths were used: 2.7, 6.6, 9.2, and 13.0×10^3 A/m. The lower two and the higher two values were measured on different scales. For the lower two the error of measurement was $\pm 0.2 \times 10^3$ A/m; for the higher two, $\pm 0.5 \times 10^3$ A/m.

The magnetic field was homogeneous at any horizontal level within the coil, but its strength decreased considerably with increasing vertical distance from the center (Fig. 3). Thus, during the stimulation of freely moving animals, which can stand up on their hindlegs and dig in the sawdust, the energy delivered during stimulation was somewhere between the extreme values possible which are maximal strength and 20% less. In contrast, as the mobility of the restrained animals is quite limited, the energy the whiskers in question are exposed to can be determined more precisely; in our experiments, it was close to 90% of the maximal strength. As the animals could turn their heads (they were permitted to do so even when restrained), the amplitude of the displacement of the whiskers was not constant.

At the onset of a pulse, the metal wire pieces were moved either up or down, dependent on the position of the animal's head at that moment. The displacement of the metal is a function of two forces: one pulling it to a level at mid-height of the coil (where the magnetic field is strongest), the other leading to its

alignment with the magnetic field. During the pulse the metal piece moved following the frequency of the mains.

Set I: stimulation of freely moving mice

In a first set of experiments (termed I), albino mice of ICR stock origin[28] bred for a standard whisker pattern, aged 8 weeks and weighing from 25 to 30 g were deprived of food but not of water for a total of 19–24 h. They were anesthetized with pentobarbital (60 mg/kg i.p.) 15–19 h before stimulation. All vibrissae were clipped at their emergence from the follicle, except B1 (Fig. 1) on the left side, on which the metal piece was glued. Control animals underwent the same treatment (but see below). All animals were injected i.p. with 2-[1-^{14}C]deoxy-D-glucose (New England Nuclear) in saline (16.5 μCi/100 g b.wt.) and immediately placed in the stimulator. Four groups of 3 unrestrained mice were each treated as follows: (a) animals with the metal piece were stimulated with magnetic bursts (intensity of the magnetic field 6.6×10^3 A/m, rms), repetition rate 7.35/s, duration 46 ms, pause 90 ms, rise and fall time 5 ms, power 1.9 W (Fig. 4); (b) animals provided with the metal were not stimulated while in the coil; (c) animals without metal pieces were exposed to the same conditions as in a; and (d) mice without the metal were left in the stimulator, again without power supply. By comparing the 2-DG-uptake in experiment a with that in control experiments b, c and d, we could judge the effectiveness of the pulsating magnetic field, mediated via the movements of the mu-metal piece, on the vibrissa and, after transduction and transmission across 3 synaptic relays, on the appropriate barrel in the PMBSF (Fig. 3).

Set II: stimultion of restrained mice

A second set of experiments (termed II) with restrained animals was carried out in order to locate the responses in the PMBSF to stimulation of single and multiple whiskers and to determine the dependence, if any, of the intensity of the response on the intensity of the magnetic field. The pre-experimental manipulations were basically the same as in the experiments with freely moving mice, except that now *several* adjacent whiskers of one row, i.e. whiskers C1, C2, C3 and — separated by one row — a solitary E1, were equipped with mu-metal wire. In order to reduce self-stimulation, the animals were restrained in casts carved into polystyrene foam sheets with adhesive tape before the anesthesia wore off. Limited head movements were possible. Within the stimulator cage, the head end of the cast was lifted from the horizontal bringing the snout of the animal up so that the displaced whiskers touched neither walls nor bottom. Not only mice of the ICR stock, but also two of an NMRI strain[28] were used. The stimulus parameters applied were the same as those described above (group a in I); however, the intensities of the magnetic field bursts were: 0 (two animals), 2.7×10^3 A/m, rms (4 animals); 9.2×10^3 A/m, rms (4 animals); and 1.3×10^4 A/m, rms (6 animals); which produced mean powers of 0.5, 3.5 and 6.6 W, respectively.

Forty-five min after the 2-DG injection, i.e. immediately after stimulation or mock-stimulation, all mice of both sets (I and II) of experiments were anesthetized (pentobarbital i.p.) and perfused intracardially with 3.3% formalin in 0.12 M phosphate buffer at pH 7.4 for 10 min. Their brains were removed and frozen in isopentane at −70 °C. The frozen tissue was mounted onto the object holder of a cryostat with chilled No. M-1 embedding matrix (Lipshaw Manufacturing Co.).

Twenty micrometer thick sections were cut coronally, or tangentially to the surface of the somatosensory cortex, in a Frigocut 2700 cryostat (Jung, Heidelberg, F.R.G.) at about −16 °C. All sections through the region of interest were picked up on chilled glass slides and dried on a hot plate at 60 °C. They were exposed to a single-coated blue base Röntgen film (Cronex MRF 31, Dupont) with the vacuum-contact-method[12] at 5 °C for 10–14 days. After equilibration with room temperature, films were developed in rapid X-ray film developer (TD, Dupont) for 2 min, rinsed in deionized water for 1 min, fixed in X-ray film fixative (TF, Dupont) for 4 min, and finally rinsed and hardened in running cold tap water for 20 min. All solutions were at room temperature. The sections were stained with cresyl violet. Their contours were superimposed on those of the corresponding autoradiograms so that areas of interest in the latter could be accurately correlated with barrels or other cytoarchitectonic details in the sections. The drawings were made with the help of a compound microscope fitted with a low power lens and a drawing tube.

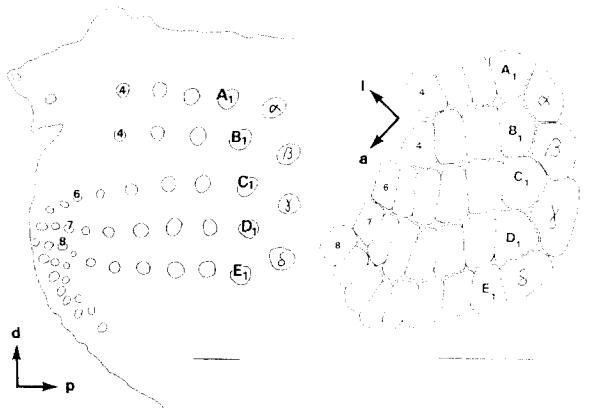

Fig. 1. Line drawings to help with orientation in the description of the experiments. Left: left whiskerpad, tangentially cut, with letters and numbers drawn in (compass: d, dorsal; p, posterior; bar, 1 mm). Right: part of right barrelfield which corresponds to the whiskerpad illustrated to the left (compass: l, lateral; a, anterior; bar, 0.5 mm). Compasses indicate that the whiskerpad is shown as if attached to a mouse in the 'normal' position, but that the barrelfield is placed roughly upside down so as to highlight topologic equivalence between the pattern of barrels and whiskers.

Seven brainstems from animals of set I, and 4 brainstems from set II were cut transversely and treated as the cortical sections, except that no drawings were made. In experimental set II, alternate sections from the brainstems were thaw-mounted on chilled glass slides and freeze-dried at −20 °C overnight. After conventional exposure to X-ray film, they were processed for cytochrome oxidase histochemistry[30]: they were incubated at 37 °C for 4–5 h with 0.1 M phosphate buffer at pH 7.4, containing 15% (w/v) cytochrome C (Sigma, No. C-2506) and 50% (w/v) diaminobenzidine (Sigma, No. D-5637). Thereafter, they were dehydrated and coverslipped.

RESULTS

The animals were observed during the entire 45 min period after tracer injection. Unrestrained mice roamed through the cage within the coil, palpating the cage walls, from time to time digging in the sawdust. All freely moving as well as restrained animals showed spontaneous bursts of whisking. These active bursts were so intense that they appeared to override the artificially induced whisker movements. No mouse lost its metal piece or its remaining vibrissa(e) during the time of stimulation, although, in the period between placement of the metal and onset of stim-

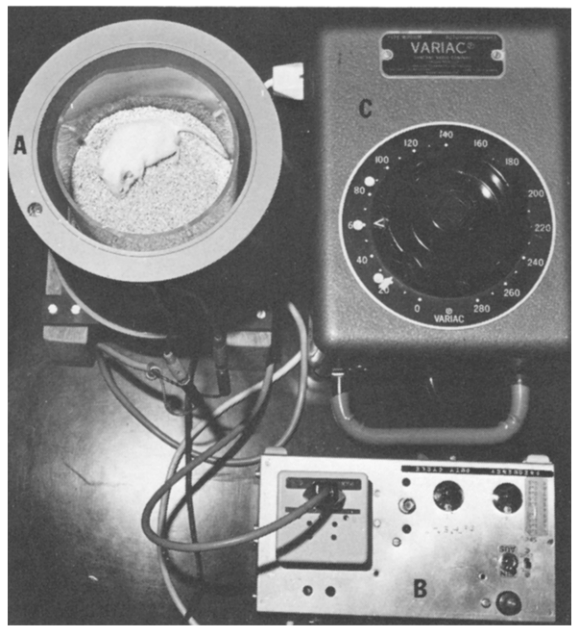

Fig. 2. View of the stimulator showing the electromagnetic coil (A) containing a freely moving mouse. The coil is connected to the burst generator (B) which is essentially composed of a timer controlling the power circuit fed by the variable transformer (C) via a triac. A ventilator (not visible) is mounted under the coil.

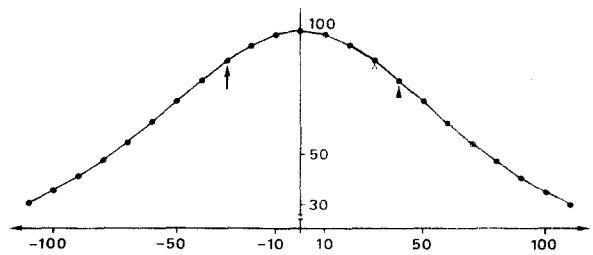

Fig. 3. Graph showing the empirically determined magnetic field strength at various horizontal levels in the coil which contained the cylindrical cage in which the whisker-stimulated mice were placed (abscissa: distance in mm under and above the vertical center of the electromagnetic coil; ordinate: relative strength of the magnetic field in % compared with the maximal strength). The maximal strength of the magnetic field is in a horizontal plane at the vertical center of the electromagnetic coil. Its magnitude is proportional to the voltage supplied. The strength is uniform at a specific horizontal level, but drops off with increasing distance from the vertical center. In experimental set I, the bottom of the animal container (arrow) is packed with a layer of sawdust so as to bring the animal's head close to the vertical center. Unrestrained animals can move their heads in a magnetic field whose strength ranges between maximum and 80% (closed arrowhead); restrained animals were placed so that their muzzles were at a level of about 90% of maximal strength (open arrowhead).

ulation, a metal piece with or without its whisker occasionally disappeared. In these few animals, either metal pieces were re-glued or the bare vibrissae were clipped. 2-DG autoradiography revealed clear stimulus-evoked tracer uptake in the contralateral PMBSF under both sets of experimental conditions.

Set I: experiments on freely moving animals

In all experiments of type Ia there was a region of discrete, high DG uptake presumably associated with barrel B1, covering its hollow as well as its side (Fig. 5a). This 'hot spot' was confined to layer IV. A column-like zone of increased grain density (GD) but less intense than the hot spot, surrounded and extended above and below it through layers II–VI. Regions medial and lateral to the active site were considerably less labeled in comparison with the active site, thus accentuating its columnar shape. Ipsilateral to the stimulated whisker there was a zone, roughly coinciding with the PMBSF, with conspicuously diminished GD. In control group b, one animal had a well-defined hot spot (Fig. 5b, top), a second mouse had a less well-defined one but with spread to inferior layers while, on the other side, there was an area of diminished GD, as described for the animals of set Ia (Fig. 5b, center). A third animal showed no increased GD at all in the corresponding region. Increased GD at the same contralateral location could also be observed in mice of control group c: clearly in two animals (Fig. 5c, center and bottom) and less so in the third (Fig. 5c, top). The animal shown in Fig. 5c, center, again exhibited the ipsilateral area of diminished GD as described for the animals of set Ia. In control group d, two animals had the area of high GD: one (Fig. 5d, center) with the spread of activity to layer VI; the other (Fig. 5d, bottom), without. Thus, the majority of control animals of groups b, c and d (7 out of 9) had an increased GD over what we presumed to be B1. However, only 4 of these 7 gave an image comparable to that in animals of group Ia.

A spot-like zone of high 2-DG uptake was found in the subnuclei caudalis and interpolaris of the nucleus of the spinal tract of the trigeminal nerve ipsilateral

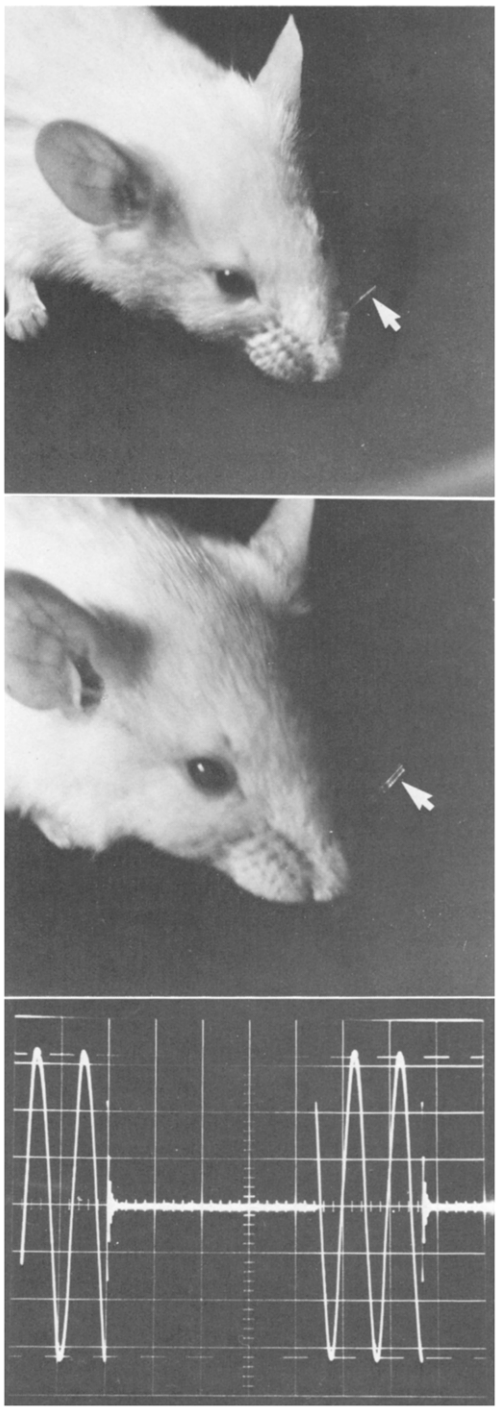

Fig. 4. Top: photograph of an unrestrained mouse whose whisker B1 on the left is provided with a metal wire (arrow). Center: same animal, but stimulated with magnetic field bursts; note movement of whisker (arrow) photographed with an exposure time of 1/60 s. Bottom: an oscilloscope display of a burst sequence as used in the present study. The burst is determined by the alternating mains current (50 Hz). Its peak voltage is 68 V, its duration of 46 ms includes rise plus fall times (about 5 ms). The interpulse pause is 90 ms (abscissa 20 ms/major division; ordinate 20 V/major division).

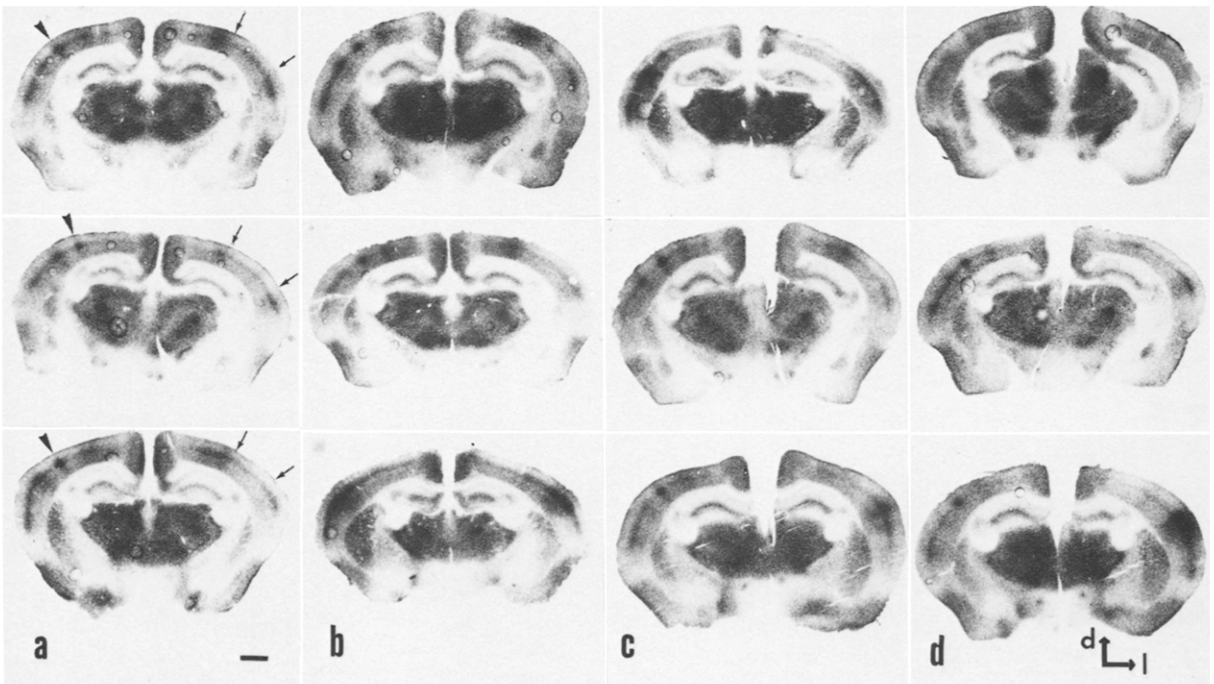

Fig. 5. Enlarged autoradiograms of coronal brain sections. Each picture illustrates the cortical response to the stimulus of one animal. The contralateral response is in the right hemisphere, depicted on the left hand side, (see arrowheads); it is accompanied by a depression of 2-DG uptake in the ipsilateral hemisphere (areas between small arrows). The images are grouped in columns according to the animals' stimulation conditions. Note that all whiskers except the left B1 were clipped. From left to right: (a) animals with metal piece glued to whisker B1 and stimulated by magnetic bursts; (b) animals which had a metal piece and were placed in the unenergized coil; (c) animals which had no metal piece and were exposed to the same magnetic field as in a; and (d) mice which had neither metal piece nor stimulation. In a, the region of increased grain density has a columnar shape and stretches from layers II to VI with a maximum in layer IV. Note that some animals in the control groups (b, c and d) show a cortical activation pattern presumably due to self-stimulation (see text). The areas of depressed activity cover several barrels but are always restricted to the barrelfield. Bar in column a, bottom, represents 1 mm; compass in d, bottom: d, dorsal: l, lateral, animal's left; compass and bar hold for all illustrations.

to the stimulated whisker in all mice whose brainstems were analyzed and which displayed cortical activation (7 cases out of a total of 12; see Fig. 8). No depressed 2-DG uptake was apparent in the contralateral subnuclei, and no responses could be seen in the subnucleus oralis of the spinal tract and in the principal sensory nucleus of the trigeminal nerve. The same holds for that part of the ventrobasal thalamus where the vibrissae are represented[26].

Set II: experiments on restrained animals

An increase in stimulus intensity, i.e. strength of the magnetic field, caused an increase of GD in the corresponding barrels (Figs. 6 and 7). No stimulation gave no responses. Whereas at 2.7×10^3 A/m, rms, stimulus-evoked responses were visible in barrels C1–3; this was also the lowest intensity at which a whisker displacement could be discerned by the naked eye. At 9.2×10^3 A/m, rms, GD reached the same level as at the highest burst intensity, i.e. 1.3×10^4 A/m, rms. The GD was related to the intensity of the stimulus also for the single barrels in row E, but here the activity did not seem to be saturated at 9.2×10^3 A/m, rms; at 1.3×10^4 A/m, rms, a still higher GD was obtained. In animals with a single C whisker (the others had been lost), the GD over the C barrel was similar to that over the E barrel. At all intensities their GDs were less than those obtained by stimulation of 3 neighboring vibrissae. When single vibrissae were stimulated, the activated regions always stayed within the appropriate barrels and their immediate surrounding. A center region of high GD over the barrel(s) was surrounded by a periphery of less GD that appeared to overlie the septa bordering the stimulated barrel(s). Both center and surrounding zone had a GD significantly higher than that of adjacent

non-activated barrels (Fig. 7). In one of two animals tested at the lowest intensity, the area of high GD did not cover all cytoarchitectonically defined barrels whose whiskers were stimulated. In one out of 4 cases in which vibrissae C1, C2 and C3 were stimulated with the highest intensity, barrels C4 and C5 were activated as strongly as C1–3, while 2-DG uptake of barrel C6 was increased but less so.

In the subnuclei interpolaris and caudalis of the nucleus of the spinal tract of the trigeminal nerve, a spot of increased 2-DG uptake corresponding to the representation of the posterior row C vibrissae was observed with the lowest stimulus intensity (Fig. 8). With the higher intensities (9.2×10^3 and 1.3×10^4 A/m, rms), 2-DG uptake in the representation of vibrissa E1 became visible, while the activation in the C row was more pronounced. Here, as in the cortex, single whisker stimulation gave rise to less GD than stimulation of the 3 adjacent whiskers in a row. No stimulus-evoked responses could be observed in the subnucleus oralis and in the principal sensory nucleus of the trigeminal nerve.

No differences were noticed between the animals of the ICR and NMRI strains.

DISCUSSION

We present a device capable of stimulating one or more individual whiskers in restrained and freely moving mice (and other small rodents). With appropriate stimulus parameters, the effects of whisker stimulation as judged by 2-DG uptake of the corresponding barrel or barrels were consistent, and notably more so than the effects of exposing the whiskers to various control conditions. This could be observed not only in the barrelfield, but also in subnuclei interpolaris and caudalis of the trigeminal nerve. The positive results of the control conditions (experiments I, b, c and d) presumably stem from the fact that the mouse itself stimulated the follicular receptors of the one remaining whisker through active exploration of the cage (see also Durham and Woolsey[3]). This is also borne out by a number of preliminary observations in animals in which no whisker was clipped; although one whisker was provided with metal and, thus, actively stimulated, there was a continuous band of intense 2-DG uptake spanning the entire PMBSF and obscuring the stimulus dependent 2-DG uptake, if any, of the corresponding barrel.

How did we know that the 'hot spots' obtained were indeed over the barrels that corresponded to the stimulated vibrissae? For coronally cut brains this question is difficult to answer; a serial section reconstruction of the barrelfield from such material allowing for unequivocal identification of individual barrels is next to impossible. However, we could extrapolate from our findings in the tangentially cut barrelfields of the experiments of set II; there, careful reconstructions of the barrelfields were made from serial sections and the autoradiograms brought in register with those sections. With the limitation that [^{14}C]-2-DG-autoradiography has a spatial resolution of about 100 μm Full-Width-Half-Maximum[6] (for a discussion and critical application of this concept see

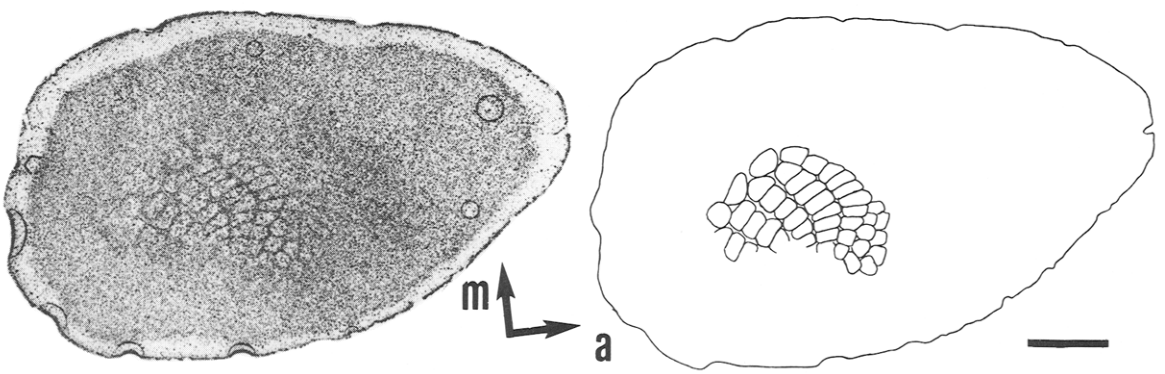

Fig. 6. Left: low power photomicrograph of a Nissl-stained section cut tangentially through the right posteromedial barrel subfield (PMBSF). Right: camera lucida drawing of the barrel pattern in the same section. Such drawings were used to reconstruct the entire PMBSF which allowed the identification of the activated barrels, as demonstrated in Fig. 7. The autoradiogram derived from this section is displayed in Fig. 7d. Compass: a, anterior, m, medial; bar, 1 mm; compass and bar hold for both illustrations.

Melzer[17]), there was perfect correspondence between the stimulated whisker and the activated barrel (but see next paragraph). Because of that limitation, it could not be determined whether the sole sites of activity were hollows, or also sides and adjacent septa. In no case were we able to confirm (or deny) the claim by Durham and Woolsey[4] that 2-DG uptake in activated barrels is particularly marked over the walls. In coronally cut brains, the radial distribution of stimulus-related 2-DG uptake observed agrees with those shown in the mouse[4] and in the rat[7] except that we did not see an increased activation in layer I. To return to the question asked at the beginning of this paragraph, we are confident that what was true for tangential sections holds for the coronally cut brains as well. This supports the claims by other authors of the one-to-one relationship between the periphery and the cortex based on 2-DG studies[3,7,13,14].

In the experiments of set II the animals were restrained; thus the chance of an animal removing whisker and/or metal during the period before stimulation was diminished, and vertical displacement of the whiskerpad was restricted, keeping variations of magnetic field strength at minimum. These experiments were done with two purposes in mind. One was to see whether a relationship exists between stimulus intensity and GD of the evoked responses; the other,

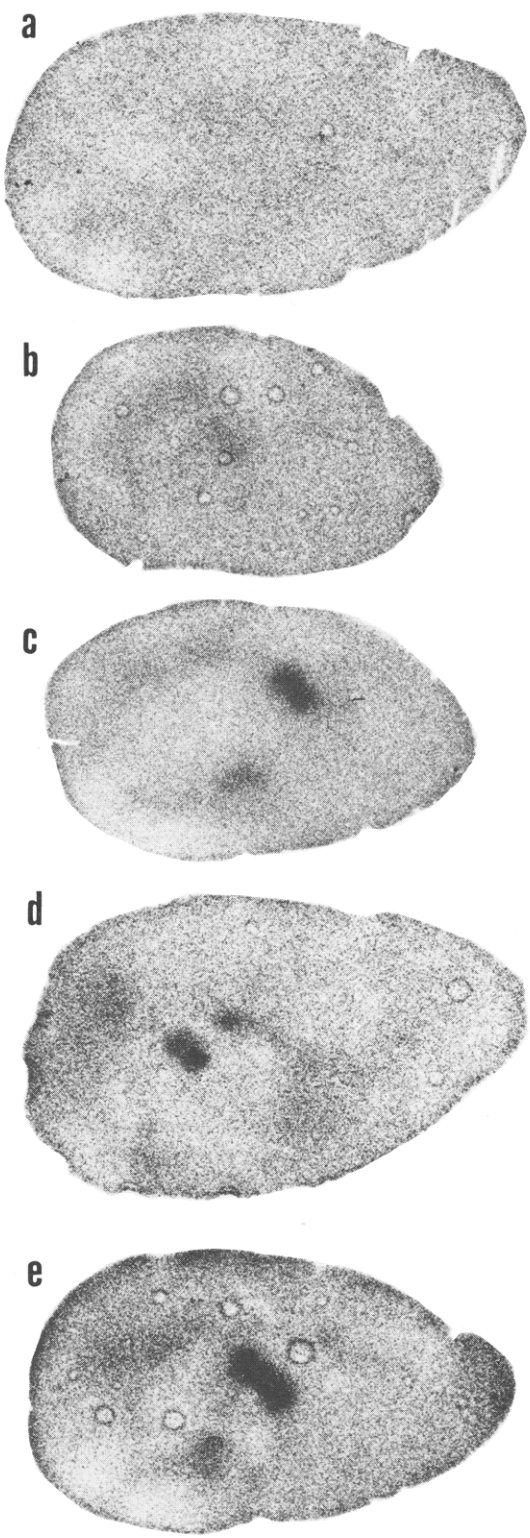

Fig. 7. Enlargements of autoradiograms of 5 sections cut tangentially through the right posteromedial barrel subfield. The animals were restrained (set II, see text), and whiskers C1–C3 and E1, on the left side, had received pieces of metal wire. The stimulation intensities were: 0 (row a), 2.7×10^3 (row b), 9.2×10^3 (row c) and 1.3×10^4 A/m, rms (rows d and e). Left column: photographic reproductions of autoradiograms. Center column: photographic reproductions of the same autoradiograms. The camera lucida drawings of the barrels present in the corresponding Nissl-stained sections (Fig. 6) were superimposed. Right column: photographic reproductions of the same autoradiograms. Here, reconstructions of the entire posteromedial barrel subfield, made from camera lucida drawings of a number of serial sections, were superimposed. Note the perfect correspondence between the stimulated whiskers and the barrels which show increased 2-DG uptake (arrows in right column; refer to Fig. 1 for topography), the intensity-dependent uptake by correspondings barrels and the higher uptake by the three neighboring barrels compared with the single one (visible in d only). In e, stimulus-specific 2-DG uptake spread into neighboring barrels. Compass: m, medial; a, anterior; l, lateral; p, posterior. Bar = 1 mm. Compass and bar hold for all illustrations.

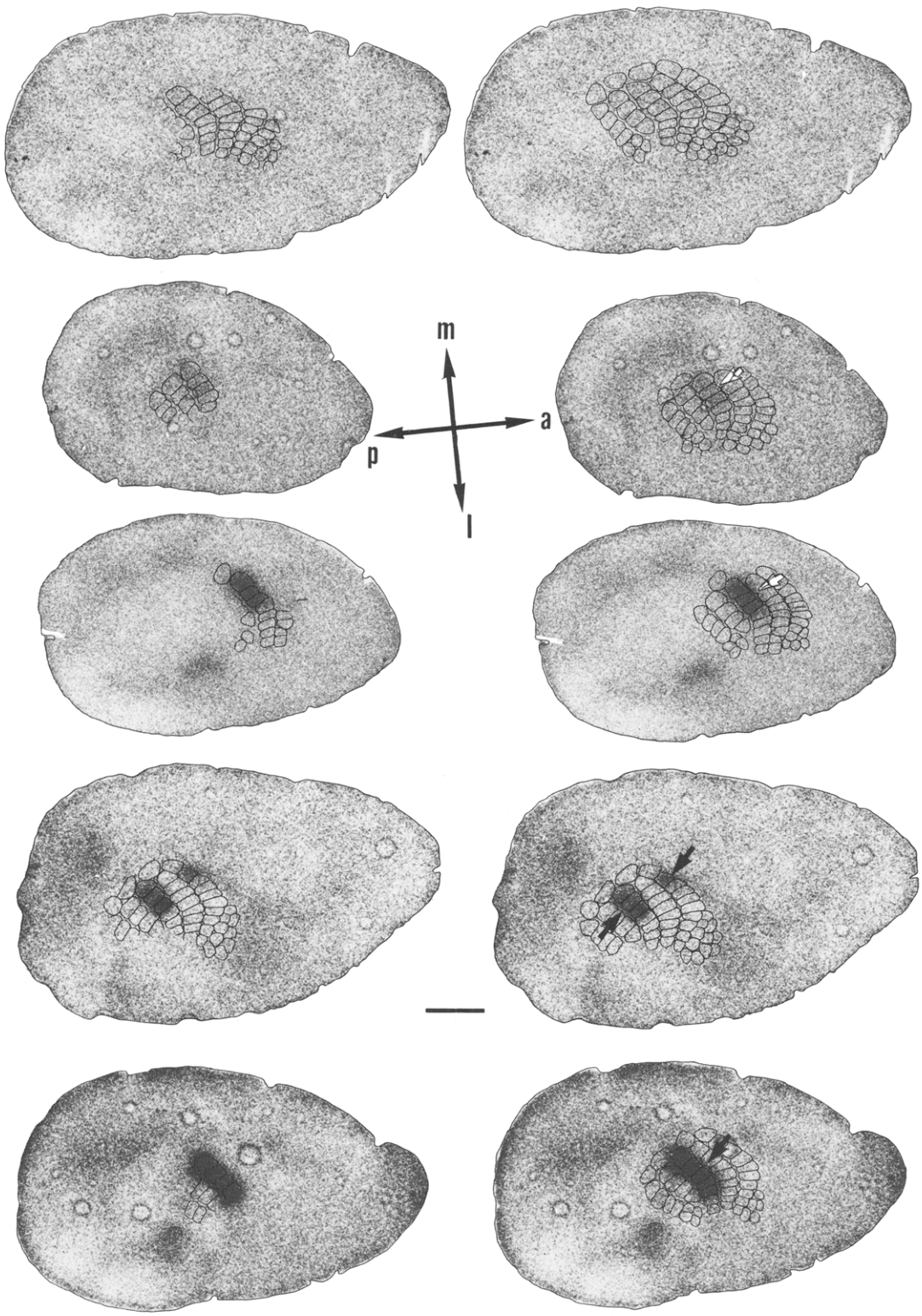

Fig. 7 (middle and right columns).

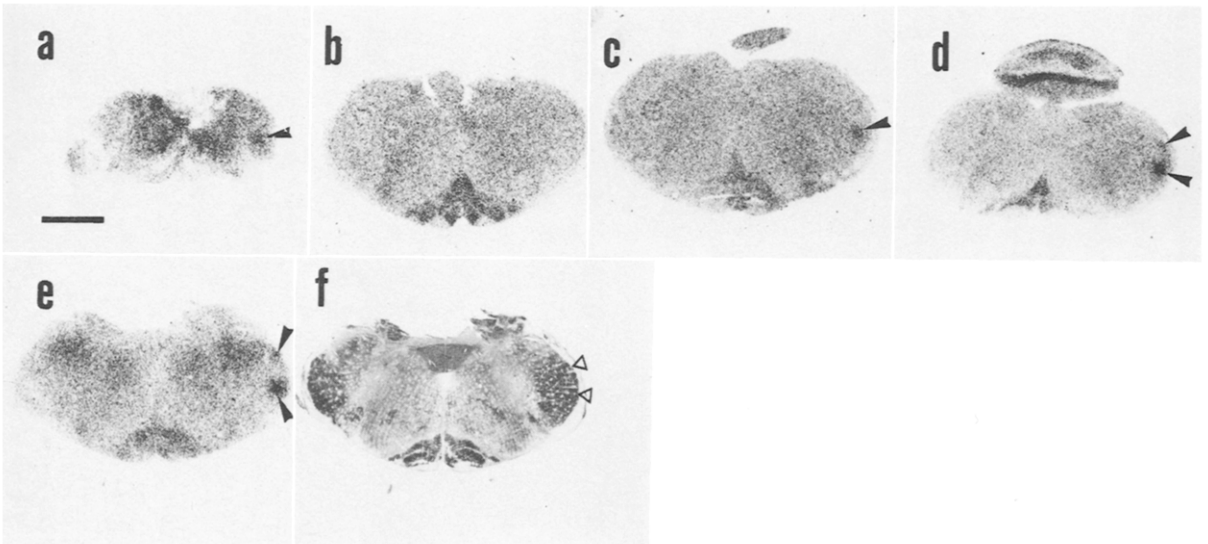

Fig. 8. Enlarged autoradiograms of coronal sections from the brainstems of: (a) a freely moving mouse whose left B1 whisker was stimulated (same animal as Fig. 5a, center); (b) a restrained animal with pieces of metal wire on left whiskers C1–3 and E1 without stimulation; animals manipulated in the same way but stimulated with 2.7×10^3 (c), 9.2×10^3 (d) and 1.3×10^4 A/m, rms (e). All sections were through the subnucleus interpolaris of the trigeminal nerve, except that shown in a, which was taken through the subnucleus caudalis. Stimulus-evoked responses are indicated by solid arrowheads. 2-DG uptake is higher in the 3 adjacent C-whisker representations than in that of E1. The organization of rows confirms Arvidsson[2]. f: micrograph of the section corresponding to the autoradiogram in e, freeze-dried and stained for cytochrome oxidase activity. Densely stained rows of patches show the representation of whiskers. Rows C and E are indicated by open arrowheads. (Bar in a represents 1 mm and pertains also to b–f; dorsal is up, the left of the animals is displayed to the right.)

to determine whether stimulation of several adjacent whiskers in one row would lead to a GD over their representations in the pathway different from that evoked by a single whisker.

There was, indeed, intensity dependence, not only at brainstem but also at cortical levels. Previous 2-DG studies on the rodent visual[18] and auditory system[21] showed that local metabolic rates of glucose and GDs were proportional to stimulus strength in subcortical relays but *not* in the cortex.

With regard to the second purpose of the experiments of set II, we noticed that the GD over the stimulated neighboring barrels was higher than that over a single one. Whereas 2-DG uptake was saturated in 3 barrels in a row at 9.2×10^3 A/m, rms, for a single barrel the GD came to the observed maximum at the highest intensity only. We could rule out that this observation was dependent on different properties of rows; when only C1 was stimulated in combination with E1, the barrels corresponding to these whiskers showed an equal 2-DG uptake. These differences are visible at the nuclei of termination of the trigeminal nerve. We propose that the 'mutual amplification' of responses in one row is a significant mechanism that acts already in the lower stations of the sensory pathway in question.

Our experiments indicate that indeed a one-to-one relationship exists between stimulated follicle and activated barrel. However, in one out of the 4 cases stimulated with the highest intensity there was an anisotropic 'spread of activity', involving barrels corresponding to the whiskers placed more rostrally in the same row with respect to those that were stimulated. The GD over those barrels was as high as that over the ones related to the actively stimulated whiskers, except for the most anterior barrel involved in the observed spread. As far as it could be ascertained, there were no differences in the stimulation procedure which could account for this observation. If it could be repeated, it would be most interesting to see *where* in the pathway from whiskerpad to barrelfield this activity over not-directly activated barrels is generated. Data on the nuclei of termination lack at this point. The thalamus will be difficult to investigate using 2-DG (see following paragraph).

With regard to the absence of stimulus-evoked re-

sponses in the subnucleus oralis and the principal sensory nucleus of the trigeminal nerve, it may be that the GD here was too faint, or its area too small, to be resolved by the 2-DG technique as applied. For the ventrobasal thalamus, demonstrations of stimulus-evoked responses lack. With respect to our own observations, we failed to obtain such responses, for the overall 2-DG uptake in that part of the brain was uniform and high.

As to stimulus parameters other than magnetic field strength, we appear to have used a favorable repetition rate, burst duration and pause between bursts. In this choice we were inspired by data given by Armstrong-James[1] in his paper on the development of the cortical responses to somesthetic stimuli in the rat. We had experimented — with negative results — with only one other repetition rate: 1.8/s where burst duration and pause were of equal length. It is not excluded that yet other parameters would lead to more pronounced results. We shall be investigating this point.

With respect to contralateral effects, we note that there was an area of low GD roughly coinciding with the PMBSF contralateral to the one in which barrel B1 was activated (Fig. 5, column a); a similar configuration is clearly present in two of the controls (Fig. 5, columns b and c, center). At first sight, one might have attributed this low GD area to the fact that on the side of the snout contralateral to that phenomenon (and, thus, contralateral to the stimulus) all whiskers were clipped. But the phenomenon was *consistently* present only in the experiments involving active stimulation (Fig. 5, column a). Thus, we propose that we are dealing here with a stimulus-dependent event. The pathway through which this PMBSF attained its low GD most likely involves the corpus callosum. For the existence of direct, not necessarily one-to-one, inter-PMBSF connections, see White and De Amicis[29] and Olavarria et al.[19]; for interhemispheric connections, perhaps allowing more easily for widespread effects, see Porter and White[20].

In summary, our magnetic stimulation device evokes specific stimulus-related 2-DG uptake in the barrelfield and in the sensory trigeminal brainstem nuclei of freely moving and restrained mice. In restrained animals, a dependence of the 2-DG uptake on stimulus intensity could be demonstrated semiquantitatively. The device promises to be particularly useful in that it allows for variable stimulus conditions. In combination with double radionuclide autoradiography[16], the approach could be used as a powerful tool to monitor the stimulus-specific uptake of potential brain imaging agents[9,15], as compared to 2-DG in a conscious animal model. A special advantage of the device is that the stimulus can be applied for a virtually unlimited time (our largest period was 15 days). Thus, a number of parameters, metabolic, enzymatic and morphological, that we expect to change upon long-term stimulation, may be investigated.

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