１．研究課題名：

ラットのバレル・コルテックスにおいて血流、電気的および内因性光信号を同時に計測するための閉鎖開窓法

２．研究機関：

秋田県立脳血管研究センター

３．研究者名と所属　：

藤田英明（科学技術振興事業団秋田研究室）

４．研究協力者名と所属：

松浦哲也（科学技術振興事業団秋田研究室）
山田勝也（秋田大学生理学第一講座）
稲垣暢也（秋田大学生理学第一講座）
菅野巌（秋田県立脳血管研究センター）

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６．要約

脳活動に伴う脳局所の血流調節機構を明らかにするためには、局所の神経活動と血流を同時に評価できる実験系が必要である。神経活動は電気的信号、血流に関する信号は、Laser-Doppler flowmetry や光計測によりもたらされる。これらの計測を同時に行うべく、Arieri A. et al. (1995)のチャンバーを基に、Kawamura S. et al. (1990)の漏斗法を導入した閉鎖開窓法を開発した。同法に用いられるチャンバーは、脳浮腫防止のための調圧機構を有し、O リングを挟んでカバーグラスにより封入される。カバーグラスは、X-Y-Z マニピュレーターを有するフレームに固定され、フレームがマニピュレーターに固定された電極やプローブとともに移動することによって計測部位を選択することができる。カバーガラスは中心を避けた位置に穴があり、穴はゴムキャップで塞がれている。電極とプローブは、ゴムキャップを斜めに通過して固定されるため光計測の同時計測が可能となった。チャンバー内圧は、開頭手術から計測に至るまで 4 mm Hg に管理され、生理的条件下の閉鎖空間で多種の同時計測が可能となった。

７．研究目的

脳活動に伴い脳局所の血流が変化することが知られている(Roy and Sherrington, 1890)が、その調節機序は未だ明らかではない。このような調節機構を明らかにするためには、局所の神経活動と血流を同時に評価できる実験系が必要である。そこで、頭蓋内圧を正常に維持した閉鎖空間の中で脳局所の神経活動と血流を同時に計測可能な閉鎖開窓法を開発した。

８．材料と方法

ラットの顎ヒゲに対応する体性感覚野（以下、バレル・コルテックス）を対象とした。ラットの頭皮を切除し、左側の側頭および頭頂骨を露出した。頭蓋骨に記録用のチャンバーをレジンで固定し、チャンバーの上部に漏斗を設置した。開頭時の脳圧変動を避けるため、チャンバーと漏斗内に深さ 6 cm で流動パラフィンを満たし、流動パラフィンの中で側頭および頭頂骨の一部を除去した。流動パラフィンの中で硬膜を切開し、バレル・コルテックスを露出した。チャンバーの内圧を 4 mm Hg に保ったまま、流動パラフィンを人工髄液に置換した後、チャンバー内には常に新鮮な人工髄液を 8 ml/hr で供給した。チャンバーは、 人工髄液中で O リングを挟んでカバーグラスにより封入されると、チャンバー上部の漏斗と余剰の人工髄液を除去した。カバーガラスは中心を避けた位置二箇所に穴があり、穴はそれぞれゴムキャップで塞いだ。バレル・コルテックスの集合電位を記録するためのガラス微小電極と血流を計測するための Laser-Doppler flowmetry のプローブはゴムキャップを斜めに通過してマイクロマニプレーターを用いて記録部へと設置された。ラット右側の顎ヒゲを屈曲刺激（3 Hz、2 sec）を行い、刺激前後での電位変化、血流変化と同時に、ビデオ画像装置を用いて内因性光計測によるバレル・コルテックスの血液量変化を計測した。

９．結果

新しい閉鎖開窓法により、脳浮腫やヘルニアは認めることなく脳局所の活動と血行動態を同時に計測することが可能となった。

バレル・コルテックスでは、ヒゲ刺激開始 8 msec 後から 1.7 sec までの間神経活動を反映する電位変化が観察された。バレル・コルテックスの血流と血液量は、ヒゲ刺激開始 0.5 sec より増加し、1.7 sec で最大となり、神経活動が休止すると漸減した。

10.考察

新しい閉鎖開窓法により、生理的条件において脳局所の活動と血行動態を同時に計測することが可能となった。同法では漏斗と流動パラフィンにより静水圧をかけるために、観察する大脳皮質は水平に設置しなければならず、頭位の固定装置に回転や屈伸機構が要求される。

11.今後の展開

新しい閉鎖開窓法は、電気的記録や血流計測に留まらず。最大 3 種のプローブに

よる計測が光計測と同時に施行可能である。この開窓法は、電気化学的手法やマイクロアプリケーション法を導入し化学伝達物質の動向が把握できるなら、脳活動に伴う血流調節機構の解明に大いに役立つことであろう。

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13. 研究業績

13-1. 原著論文

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13-2. 総説など：なし

13-3. 国際学会発表

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14. A sealed cranial window system for simultaneous recording of blood flow, and electrical and optical signals in the rat barrel cortex
15. Akita Research Institute of Brain and Blood Vessels
16. Hideaki Fujita (JST Akita)
17. Tetsuya Matsuura (JST Akita)
    Katsuya Yamada (Department of Physiology, Akita University)
    Nobuya Inagaki (Department of Physiology, Akita University)
    Iwao Kanno (Akita Research Institute of Brain and Blood Vessels)
18. 1996-2000

19. Abstract

We developed a sealed cranial window (from Arieli A, Grinvald A etal, 1995) coupled to a manipulable electrode and Laser Doppler Flpwmetry probe for simaltaneous optical recording. The cranial window consists of three chambers. The first chamber is basal part of the window which fixed above the skull. The second chamber is funnel (Kawamura S etal, 1990) which temporarily attaches on the first chamber during operation. Thierd chamber is pressure controllable open chamber which is connected to the first chamber and pressure transducer. The chambers are filling with liquid pareffin. This exerted a pressure of 4mmHg on the brain surface. Under liquid pareffin, craniectomy is perfomed. After incision of the dura, liquid pareffin is replaced by artificial CSF as soon as possible. The cover glass is pressed against an O-ring sealing the first chamber. The second chamber is removed with artificial CSF above the cover glass. The cover glass is held by a metal frame attached to an X and Y- manipulators. Thus the cover glass can be moved relative to the brain. Two or tree holes in the cover glass are fitted with natural rubber gasket, flexible enough to be easily penetrated by syring needles.

A sealed cranial window system for simultaneous recording of blood flow, and electrical and optical signals in the rat barrel cortex

Hideaki Fujita[a], Tetsuya Matsuura[a], Katsuya Yamada[b], Nobuya Inagaki[b], Iwao Kanno

[a]Akita Laboratory, Japan Science and Technology Corporation, 6-10 Senshu-Kubota machi, Akita 010-0874, Japan
[b]Department of Physiology, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan
[c]Department of Radiology and Nuclear Medicine, Akita Research Institute of Brain and Blood Vessels, 6-10 Senshu-Kubota machi, Akita 010-0874, Japan

Abstract

There has been no report previously of any methods to manipulate multiple sensor probes, such as a glass microelectrode and a laser-Doppler probe, simultaneously and independently through a sealed cranial window. We have developed a new cranial window technique which makes this possible. Furthermore, normal intracranial pressure (4 mm Hg) can be maintained throughout the craniectomy and the experiment. Using this technique, we measured the neuronal activity and local cerebral blood flow together with the intrinsic optical properties in the rat barrel cortex during mechanical stimulation of whiskers. The onset of field response recorded by an extracellular electrode in the principal barrel columns was about 8 msec from the beginning of stimulation. The field responses were well correlated with the displacements (3 Hz, 2 sec) of the whiskers. The local cerebral blood flow measured by laser-Doppler flowmetry started to increase about 0.5 sec after the first field response, peaked at about 1.7 sec, and then gradually waned. A similar time-course of changes of the local blood volume was observed by intrinsic optical imaging at the hemoglobin-isosbestic wavelength (570 nm). These results suggest that our technique would be useful for assessment of the mechanism underlying neurovascular coupling under physiological conditions *in vivo*.

1. Introduction

Coupling between hemodynamic changes in the brain and neuronal activity was first proposed by Roy and Sherrington (1890). However, the physiological basis underlying the coupling is not yet fully understood. Laser-Doppler flowmetry (LDF) (Fabricius et al., 1997; Barfod et al. 1997; Malonek et al., 1997) and intrinsic optical imaging (Frostig et al., 1990; Dowling et al., 1996; Woolsey et al., 1996; Malonek et al., 1997) have been used by a number of investigators for the measurement of cerebral hemodynamics. LDF is a noninvasive technique and is considered to reflect relative blood flow changes in

the vascular bed (Stern et al., 1977; Nilsson et al., 1980). The ease with which the procedure is handled and its continuous recording features are of great advantage. On the other hand, intrinsic optical imaging at a hemoglobin-isosbestic wavelength (570 nm) offers a two-dimensional profile that is correlated with changes in the local cerebral blood volume (Frostig et al., 1990). Thus, a combination of such measurements with the recordings of the neuronal activities may provide helpful information for the understanding of the changes in activity-dependent hemodynamics. In addition, to explore the spatial and temporal relationship between the activated neuron and vascular responses, it is essential to obtain fine vascular images around the sites where electrical recordings and LDF are targeted. Opening of the dura matter and use of an adequate sealed cranial window technique are essential for this purpose (Kawamura et al., 1990). However, it has been difficult to manipulate microelectrodes and LDF probes freely when they are inserted through the cranial window. In the present study, we report a novel multiprobe-manipulable sealed cranial window system. This system is considered to be useful for the simultaneous assessment of neuronal activity and hemodynamic changes, not only for the combination of methods we used, but also for other measurement methods such as hydrogen clearance method (Skarphedinsson et al. 1988) or polarographic recording of oxygen (Leniger-Follert et al. 1976), in the same cortical region.

2 Methods

2.1. Animal preparation

Twelve male Sprague-Dawley rats (360-455 g) were anesthetized using a gas mixture of halothane (4 % for induction and 1.5 % for maintenance during surgery), O_2 (30 %) and N_2O (remainder). Body temperature was maintained at 37.0±0.5°C using a homeothermic heating pad (ATC-201, Unique Medical, Tokyo, Japan). Polyethylene catheters were introduced into the caudal artery and the left femoral vein for monitoring of blood pressure, arterial blood gas sampling and intravenous drug administration. Following tracheotomy, α-chloralose (125 mg/kg, slow bolus i.v.) was administered, after which halothane and N_2O were discontinued. Anesthesia was then maintained with α-chloralose (38 mg/kg/hr, i.v.). Each rat was artificially ventilated with a small animal respirator (SN480-7, Sinano, Tokyo, Japan) using room air enriched with O_2 using pancuronium bromide (0.5 mg/kg/hr, i.v.) for muscle relaxation. Following infiltration of both external auditory meati with xylocaine, the head of the rat was tilted in a stereotaxic apparatus to 36° towards the left superior oblique position and a sealed cranial window was attached. Arterial blood (85 l) was anaerobically collected, for blood gas analysis (ABL330, Copenhagen, Denmark), at the beginning and end of the recordings. Hematocrit was measured only at the beginning of the recordings. Mean arterial blood pressure (MABP) was continuously monitored throughout the experimental period (TP-400T, AP-641G, Nihon Kohden, Tokyo, Japan).

This study followed the guidelines of the Physiological Society of Japan and was approved by the Animal Care and Use Committee of the Akita Research Institute of Brain and Blood Vessels.

2.2. Sealed cranial window system

The sealed cranial window system consists of three major parts, a recording chamber unit, a funnel, and a pressure-controllable subchamber unit.

2.2.1. The recording chamber unit and funnel
The recording chamber (Fig.1, A, a) fits tightly into a hole in the base plate (Fig.1, A, b). The gap between the chamber and the base plate is sealed with an O-ring (Fig.1, A, c). The lower edge of the recording chamber is positioned 1 mm above the skull with the aid of a supporting rod (Fig.1, A, d) attached to the stereotaxic apparatus. The gap between the recording chamber and the skull is completely filled with dental acrylic (Fig.1, A, e). During the operation, the funnel (Fig.1, A, f) is attached to the base plate. Craniectomy and dural incision (over an area of 7 x 7

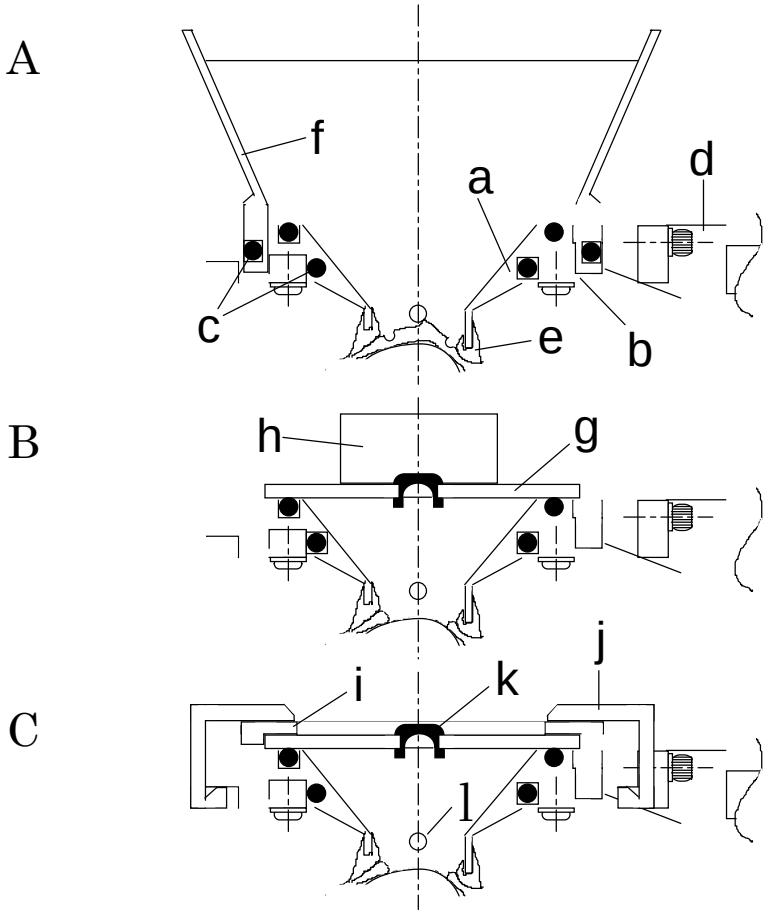


Fig.1 Illustration of the recording chamber unit and its preparation
A. The recording chamber (a) of the sealed cranial window system is fitted into a hole in the base plate (b). The gap between the chamber and the base plate is sealed with an O-ring (c). The recording chamber is positioned above the skull with the aid of a supporting rod (d). The gap between the recording chamber and the skull is filled with dental acrylic (e). During the operation, a funnel (f) is attached to the base plate. The gap between the funnel and the base plate is sealed with an O-ring (c). The recording chamber and the funnel are then filled with liquid paraffin. Craniectomy is performed under liquid paraffin. After dural incision, the liquid paraffin is replaced with artificial CSF.
B. A quartz cover glass (g) and a 110-g lead block (h) are immersed into the artificial CSF and placed over the O-ring, sealing the recording chamber. Then the funnel is removed.
C. The cover glass is held by an inner plate (i) and outer frame (j). It has holes molded

with rubber gaskets (k). The pressure of the recording chamber is maintained at a level (4 mm Hg) equal to that in a pressure-controllable subchamber (see section 2.2.3.), with the aid of plastic tubing (l).

mm, centered 5mm caudal and 5mm lateral to the bregma) are performed at 34°C under a 6-cm column of liquid paraffin filled into the recording chamber and funnel. After controling any bleeding, the liquid paraffin was replaced with artificial cerebrospinal fluid (CSF) at 34°C. The artificial CSF (composition in mmol/l: Na^+, 158.0; K^+, 3.2; Cl^-, 142.4; Ca^{2+}, 1.5; Mg^{2+}, 1.33; HCO_3^-, 24.5; and glucose, 3.3) is aerated with a mixture of CO_2 (6 %), O_2 (10 %) and N_2 (remainder). The artificial CSF in the recording chamber is supplied continuously through the inlet and drained through the outlet of the recording chamber at a rate of 8 ml/hr using a push-pull syringe pump fabricated by us. In the funnel, a quartz cover glass (Fig.1, B, g) is immersed into the artificial CSF and placed over the O-ring, which seals the recording chamber. A 110-g lead block (Fig.1, B, h) is placed on the cover glass to prevent leakage of the artificial CSF. The artificial CSF above the cover glass is drained, and the funnel carefully removed. The cover glass is held by an inner plate (Fig.1, C, i) and an outer frame (Fig.1, C, j).
Detailed engineering drawings are available on request from the corresponding author or from the European Editorial Office of Journal Neuroscience Methods.

2.2.2. Manipulation of the recording electrode and LDF probe
The cover glass has two holes molded with natural rubber gaskets (Fig.1, C, k). The glass microelectrode for electrical recording is introduced into the recording chamber through a syringe needle which penetrates the gasket. The gap between the microelectrode and the syringe needle is immediately filled with alkyl-α-cyanoacrylate (Aron alpha A, Sankyo, Tokyo, Japan) to prevent any leakage of the artificial CSF. The syringe needle and the electrode are driven by a water-driven Z-micromanipulator (MW-10N, Narishige, Tokyo, Japan) (Fig.2). The angle between the horizontal plane of the cover glass and the axis of the electrode can be adjusted within the range of 45-70° with the assistance of a swing arm connected to the inner plate.
The inner plate can be slid into place with the aid of an X-micromanipulator (MHK-15, Mitutoyo, Kanagawa, Japan) against the outer frame, allowing fine mediolateral motion of the electrode and cover glass relative to the brain. The outer frame can also be slid into place with the aid of a Y-micromanipulator (MHK-15, Mitutoyo, Kanagawa, Japan) against the base plate (Fig.1, A, b), allowing fine rostrocaudal motion of the electrode, cover glass and inner plate relative to the brain.
A LDF probe was obliquely inserted into the recording chamber through another gasket using micromanipulators (MW10 and SM11, Narishige, Tokyo, Japan) fixed on the stereotaxic apparatus (Fig.2).

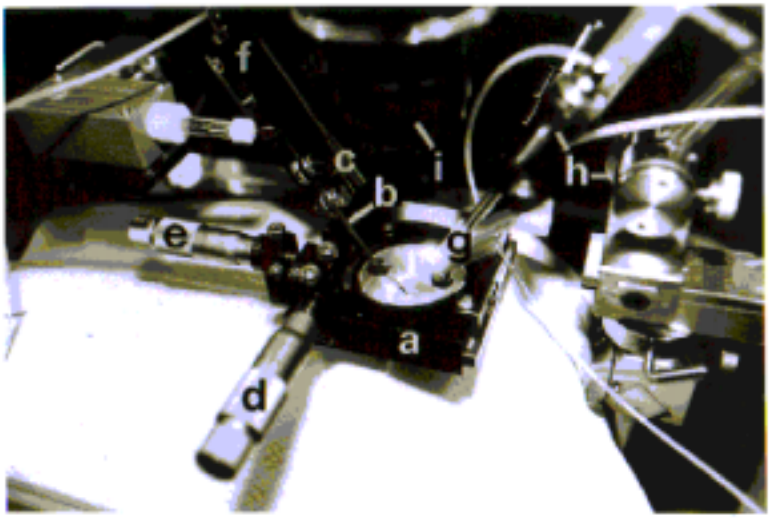


Fig. 2

Fig.2 Photograph of the experimental setup
The experimental animal was laid such that the rostrum is on the right side of the photograph. (a) The outer frame of the cranial window, (b) stainless steel sheath (syringe needle) of the glass microelectrode, (c) electrode holder, (d, e, f) X-, Y- and Z-micromanipulators of the electrode, respectively, (g) LDF probe holder, (h) micromanipulators of the LDF probe and (i) camera lens for optical imaging.

2.2.3. Maintenance of the intracranial pressure
The intracranial pressure is maintained at 4 mm Hg relative to the atmospheric pressure from the beginning of craniectomy until the end of the examination, by changing the levels of the liquid in the funnel and a pressure-controllable subchamber unit. The pressure-controllable subchamber unit consists of a subchamber diverted from a disposable 20-ml syringe, a pressure transducer (TP-400T, Nihon Kohden, Tokyo, Japan) and a three-way valve: One horizontal end is connected to a plastic tubing (Fig.1, C, l) inserted into the recording chamber, the opposite end to the transducer and the vertical end to an upright syringe without the piston. The horizontal level of the central axis of the transducer is adjusted to the same level as that of the brain surface of the animal. During the measurements, the recording chamber is completely closed by the three-way valve.

2.3. Whisker stimulation
Whisker nomenclature was in accordance with that proposed by Van der Loos and Woolsey (1973). The C1, C2, D1 and D2 whiskers on the right side were cut to a length of 10 mm from the base of the whisker. All the other whiskers were cut at the base. A fine rod (recording pen SJ-120AM, Nihon Kohden, Tokyo, Japan) attached to a galvanometer (OA-115, Nihon Kohden, Tokyo, Japan) was placed just on the anterior aspect of each whisker, 5 mm away from its base. The four whiskers were mechanically deflected in the rostrocaudal direction (about a 5-mm displacement of 167 msec duration) synchronously by four galvanometers. Following triggering of the optical imaging system by a master pulse (see 2.6. Acquisition of intrinsic optical signals), deflections of the whiskers at the rate of 3 Hz (duration 2 sec) were generated by a pulse generator (Master-8, A.M.P.I.,

Jerusalem, Israel). Whisker stimulation was delivered 40 times at intervals of 80 sec.

2.4. Acquisition of electrical signals

The glass microelectrode (4 M□) was filled with 1M potassium acetate containing 2.5 % methylene blue. The angle between the electrode and the tangential axis of the barrel hollow was 30°. The tip of the glass microelectrode was positioned at the upper border of the principal barrel hollow (500 m below the surface of the cortex). The electrical signals were amplified (Axoclamp-2B, Axon Instruments Inc., Foster, U.S.A.), digitized at 400 Hz (Mac Lab 8e, AD Instruments Pty. Ltd., Castle Hill, Australia), averaged 40 times for each rat and analyzed using a personal computer (Fig.3, A). For presice determination of the onset latency, electrical recordings were obtained at a higher sampling rate (20 kHz) after the simultaneous measurements of local cerebral blood flow (CBF), and electrical and optical signals. At the end of the experiment, the recording site was marked with methylene blue by applying an anodal current (10 A, 5 min) between the glass microelectrode and the reference electrode.

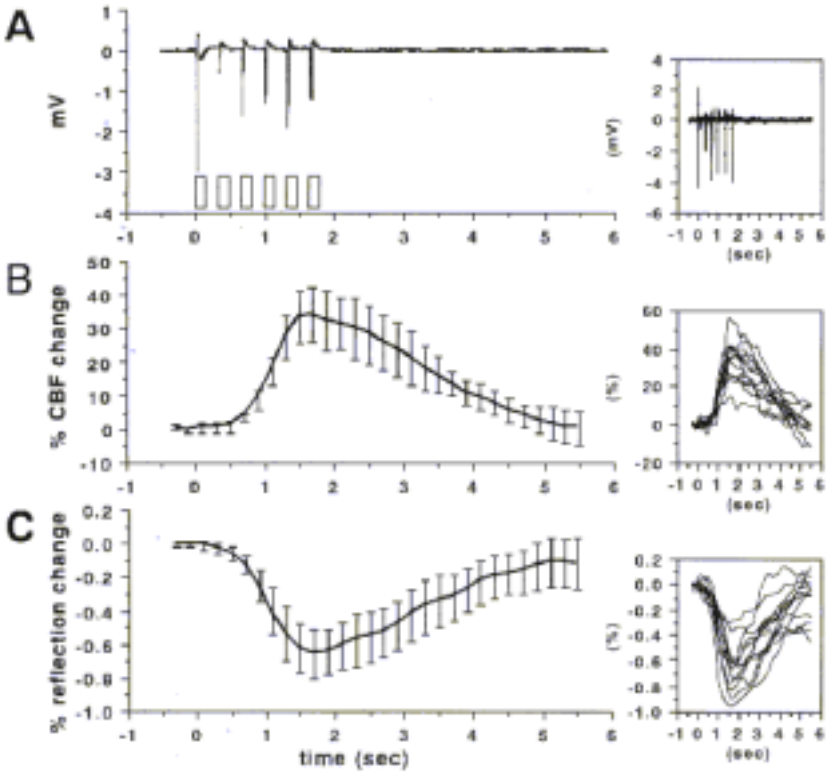
2.5. Measurement of CBF

CBF was continuously monitored with the probe (411 probe, Perimed, Stockholm, Sweden) of the LDF (Periflux 4001 Master, Perimed, Stockholm, Sweden). The time constant was set to 3 msec. The spatial resolution of the probe was lower than 1mm^3 (Nilsson et al., 1980). The maximal depth of the probe *in vivo* was roughly estimated as 500 m (Fabricius et al., 1997). The probe was introduced obliquely into the recording chamber. The angle between the probe and the tangential axis of the barrel hollow was about 30°. The tip of the probe carefully touched the pia above the principal barrel hollow, at the recording site of the field potential. The LDF signal was digitized at 5 Hz (Mac Lab 4e, AD Instruments Pty. Ltd., Castle Hill, Australia), averaged 40 times for each rat and stored for further analysis using a personal computer. The change in CBF evoked by whisker stimulation was defined as the percentage increase in CBF (arbitrary unit, PU) relative to the average CBF 0.4 sec preceding the stimulation (Fig.3, B).

Fig. 3 Time-course of the cortical responses

C1, C2, D1 and D2 whiskers on the right side were mechanically displaced at 3 Hz for 2 sec. t=0 represents onset of stimulation. Averaged data obtained from 12 rats (for each rat, the data of 40 trials were averaged on-line) are depicted in the left of each row. Error bars represent the S.D.. Superimposed records of all the 12 rats are shown in the insets. A. Extracellular field potentials recorded at a depth of 500 m in the principal barrel column on the left side. Whisker displacements are shown above the abscissa by rectangles. B. Percent CBF changes in the principal barrel column. C. Percent changes in intrinsic signals at the hemoglobin-isosbestic wavelength (570 nm) within the region of interest (0.3 mm^2) in the principal barrel column.

Fig. 3



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2.6. Acquisition of intrinsic optical signals

To acquire the intrinsic optical signals, we used an enhanced differential video system (Imager 2001, Germantown, U.S.A.) attached to a tandem macroscope (Ratzlaff and Grinvald, 1991). To avoid interference of optical imaging by the laser light (780 nm) of LDF, a band-pass filter (BG40, Schott, Wiesbaden, Germany) was placed in the tandem macroscope whenever flowmetry was simultaneously performed with optical imaging. The outer ring of a 50-mm camera lens (Nikkor F1.2S, Nikon, Tokyo, Japan) close to the cortex was removed to maximize the working space for manipulation of the electrode and the LDF probe (Fig.2, i). A 105-mm camera lens (Nikkor F1.8S, Nikon, Tokyo, Japan) was used for the top lens of the tandem macroscope. The cortex was illuminated with green light (570 nm, λ 1/2=13 nm). The reflected light from the cortex was captured by a charge-coupled device (CCD) camera at a frame rate of 30 Hz. To identify the position of each barrel column relative to the pial vessels, a pial vessel image was

obtained by summing up 64 video frames (Fig.4, A). The matrix size of the data frames was 648 (horizontal)×480 (vertical). After defocussing by 500 m below the cortical surface, the recording was started. The frame rate was decreased to 5 Hz during the on-line analysis. The matrix size of the data frames was 324 (horizontal)×240 (vertical). The duration of a each single trial was 6 sec. To improve the signal-to-noise ratio, video data frames were averaged 40 times for each rat frame-by-frame on-line. In the case of off-line analysis, the intrinsic optical signal was defined as the percentage increase in optical reflection within a 0.3-mm^2 region of interest in the principal barrel column relative to the average of that in the two frames preceding the stimulation (Fig.3, C, Fig.4, B).

2.7. Staining of the barrel cortex

The rat was sacrificed after the recordings by intravenous injection of a fatal dose of pentobarbital sodium. The left cerebral hemisphere was removed from the skull and 100- m-thick frozen (-20°C) sections were cut in a plane parallel to the barrel cortex, followed by succinic dehydrogenase (SDH) histochemical analysis (Nachlas et al., 1957; Killackey and Belford, 1979; Ito and Seo, 1983; Seo and Ito, 1987). The incubation solution was composed of equal volumes of 0.1 % nitroblue tetrazolium and buffered succinate solution mixed with equal parts of phosphate buffer (pH 7.4) and 0.2 M sodium succinate. The sections were then incubated at room temperature (24°C) for 20 min, fixed in a mixture of 3 % paraformaldehyde and 1 % glutaraldehyde, and rinsed and mounted on glass slides. Penetrating vessels in the sections were used as markings for topographical matching of the outlines of the histochemical images of SDH with those of the optical images (Woolsey and Van der Loos, 1970; Cox et al., 1993). The intrinsic optical images were superimposed on the histochemical images for the analysis of signal distribution in the barrel cortex. (Fig. 4,B).

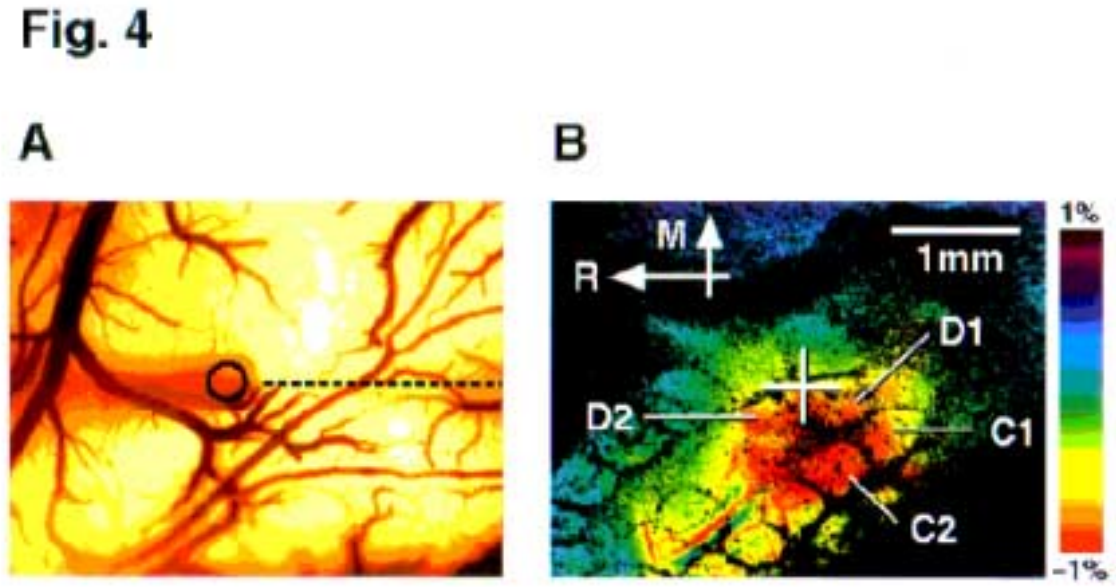

Fig.4 Optical imaging of the rat barrel cortex
A. An image of pial vessels in the left barrel cortex. The top of the image corresponds to the medial side of the brain. The glass microelectrode was inserted along the dotted line. The open circle indicates the LDF probe. B. An intrinsic optical image superimposed on a histochemical image of the barrel cortex. The intrinsic optical image (wavelength 570 nm)

was obtained 1.7 sec after the onset of mechanical stimulation of the C1, C2, D1 and D2 whiskers on the right side. The image was focused at 500 μm below the cortical surface. Red colors indicate greater absorbance. The color scale represents the range of -1 % to 1 %. The histochemical image of SDH was obtained from 750 μm below the cortical surface. C1, C2, D1 and D2 represent the barrel columns. The site of electrical recording, which was verified by methylene blue staining, was localized in the D1 barrel column and is indicated by the plus (+) mark. M: medial. R: rostral. Scale bar=1mm.

3. Results

We conducted a whisker stimulation study in 12 rats using our new sealed cranial window technique. The MABP during the recordings was 102±11 (Mean±SD; n=12) mm Hg, and did not change significantly during the whisker stimulation. The values of $PaCO_2$, PaO_2 and pH were 35.3±3.5 mm Hg, 133.1±12.7 mm Hg and 7.356±0.050, respectively. The hematocrit was 42.4±1.5 %.

The extracellular field potentials evoked by mechanical displacement (3 Hz, 2 sec) of the C1, C2, D1 and D2 whiskers on the right side were recorded in the principal barrel columns on the left cortex. The pattern of the field potential in response to the stimuli obtained from the 12 rats was essentially similar across animals. As shown in Fig.3, A, the averaged field response (n=12) was cleary correlated with the train of whisker displacements. The onset of field response in the principal barrel column was about 8 msec.

The percent CBF changes in the barrel cortex were simultaneously measured by LDF (Fig.3, B). CBF started to increase about 0.5 sec after the first field response, peaked at about 1.7 sec (33.7±8.2%), and then decreased gradually toward the baseline level.

The time-course of changes of the intrinsic optical signals at the hemoglobin isosbestic wavelength (570 nm) was a mirror image of those of the CBF (Fig.3, C). The percent changes in the reflected signals in the principal barrel columns started to decrease about 0.5 sec after the first field response, and became minimal at about 1.7 sec (-0.658±0.148%). The spatial distribution of the intrinsic optical signals evoked by whisker stimulation corresponded well with that of the principal barrel columns in the cortex as determined by SDH staining (Fig.4, B).

4. Discussion

4.1. The sealed cranial window technique

In the present study, we report on the usefulness of a novel sealed cranial window technique for exploration of the mechanism underlying neurovascular coupling. Various sealed cranial window techniques have been reported previously (Forbes, 1928; Ellis et al., 1983; Smith et al., 1985; Auer et al., 1985; Morii et al., 1986; Kawamura et al., 1990; Grinvald et al., 1991; Irikura et al., 1994; Hadetz et al., 1995). In these studies, cranial windows were seald by a fixed cover glass or acrylic plastic. Although pial vessels have been clearly observed through these windows, the electrode not can be introduced through the windows. A rubber gasket glued into a hole of the cover glass enabled insertion of an electrode through the window (Arieli et al., 1995; Bonhoeffer and Grinvald, 1996). Arieli improved the chamber of the cranial window so that the position of the single electrode could be

manipulated (Shoham et al., 1997). However, it was difficult to access the recording site freely and independently with multiple probes for diverse measurements. Our window system is applicable for up to triple-modal simultaneous recordings in combination with intrinsic optical imaging. Moreover, it works even in small animals such as rat.
Since exposure of the brain under atmospheric pressure induces blood-brain barier dysfunction (Olesen, 1987), it is critical to maintain the intracranial pressure within the normal range during and after craniectomy. By using the funnel (Kawamura et al., 1990) during craniectomy and sealing of the cranial window, possible damage to the cerebral vessels is avoided. The limitation of this technique is that the funnel must be in the upright position relative to the brain surface. For this reason, the animal's head is required to be tilted in the case of recordings in the barrel cortex.

4.2. Simultaneous measurements of neuronal activity and vascular responses
Extracellular field potentials were recorded in the barrel cortex using a glass microelectrode at 500 m below the surface of the cortex. The pattern of the field responses was clearly correlated with the whisker displacements, and was consistent with that reported previously (Di et al., 1990). The amplitude of the initial field potential was larger compared to that of the subsequent ones. Similar results have been reported from extracellular unit recordings (Simons, 1978).
In our study, the CBF as measured by LDF increased by about 34 % of the baseline in response to whisker stimulation, and the value was comparable to that reported in a previous study (Irikura et al., 1994). The CBF changes possibly reflect the alterations of erythrocyte flow in the principal barrel column up to 500 m in depth below the cortical surface (Fabricius et al., 1997).
The spatiotemporal profile of the intrinsic optical signals shown in our study is consistent with that reported in previous studies (Grinvald et al., 1986; Dowling et al., 1996; Woolsey et al., 1996). The wavelength we used was the hemoglobin-isosbestic wavelength in the visible range and primarily reflected blood volume changes (Frostig et al., 1990). The signals from the blood vessels were larger at shorter wavelengths, due to the large hemoglobin absorption at these wavelengths (Frostig et al., 1990). Thus, for fine imaging of blood volume changes, the wavelength of 570 nm was applied instead of one near the infrared region in the present study.

In conclusion, our sealed cranial window system, which enables simultaneous multimodal recordings, is a promising tool for investigating the mechanism underlying neurovascular coupling.

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