Detailed Description of a Cranial Window Technique for Acute and Chronic Experiments

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Abstract: Methods for implantation of cranial windows for the direct observation of the pial microcirculation in experimental animals are described in detail. These techniques are suitable for both acute experiments in anesthetized animals and chronic implantation permitting several months of observation in awake animals. Experience over several years shows that these techniques have an acceptably low rate of failure, are low in cost and can easily be mastered in most laboratories. They make possible observation of the microcirculation and accurate measurement of the diameter of pial vessels, and permit study of the effects on the microcirculation of a variety of maneuvers and vasoactive agents which can be studied by direct application as well as by intravascular administration. Because they preserve the integrity of the skull, the techniques permit study of the cerebral microcirculation under conditions closely approximating the normal environment of these vessels.

Additional Key Words: intracranial pressure, microcirculation, localized perfusion, pial diameter, long-term implants

The purpose of this paper is to present a detailed description of the cranial window technique for the direct observation and study of the pial microcirculation in acute and chronic experiments. Although several investigators have used variations of the cranial window technique to study the cerebral microcirculation, the first detailed description of the method was published by Forbes in 1928. In the succeeding years, Forbes and his colleagues, as well as other investigators such as Fog, carried out a series of important investigations of the microcirculation of the brain using this technique. Much of our present knowledge of the physiology of the pial microcirculation is based to a large extent on this work, which is still widely quoted. Despite the demonstrated value of the technique, it has gained only limited use. This limited application of the cranial window technique has persisted even to recent years, despite a marked resurgence of interest in the microcirculation during recent years. Several investigators working in the microcirculation of the brain have used instead methods which incorporate open skull preparations. Although these methods have produced considerable new information, they also introduced departures from the physiological state which may have uncertain effects on the responsiveness of the vessels. They are clearly unsuitable for chronic experiments.

Discussions with other investigators have convinced us that the lack of popularity of the cranial window technique is due to a high rate of failure, resulting in unusable preparations in the hands of many investigators. In our laboratory, the application of this technique has a very low and quite acceptable rate of failure. The difference, we believe, is due to meticulous attention to certain details in the experimental preparation. In order to promote more widespread use of this technique, we therefore present a detailed description which should readily enable others to apply successfully this method. The cranial window technique for chronic preparations has not been previously published. The technique for acute preparations has been published before, but in a less detailed fashion.

Methods

TECHNICAL DESCRIPTIONS

The metallic cranial window suitable for implantation in acute experiments is shown diagrammatically in figure 1. The complete window consists of only two parts, a stainless steel ring and a circular glass cover slip. The junction between the ring and the glass is sealed with melted beeswax. A new cover slip is used for each experiment. At the end of each experiment, the glass cover slip is removed and discarded and the metallic ring is cleaned and readied for a subsequent experiment. The metallic ring has several holes. The two larger holes (denoted by W) are insertion points for a wrench key used to thread the ring into the skull. Two smaller holes (oriented 180° apart and denoted by f) serve as inlet and outlet for flushing the surface area of the brain.
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under the window with artificial cerebrospinal fluid (CSF). The artificial CSF has the following composition: Na⁺ 150 mEq per liter, K⁺ 3 mEq per liter, Ca²⁺ 2.5 mEq per liter, Mg²⁺ 1.2 mEq per liter, Cl⁻ 132 mEq per liter, glucose 3.7 mM, urea 6 mM, and the balance N₂ at 37°C. The f holes have a diameter just sufficient to press-fit a bent pre-cut stainless steel (#17-gauge) hypodermic needle. The third hole (denoted by p), having the same dimensions as those denoted by f and fitted with another #17-gauge hypodermic needle, is connected to a strain-gauge for monitoring intracranial pressure during the experiment. This window can be easily constructed in any machine shop. The cost in our institution is approximately $35.

The acrylic cranial window used for chronic implantation is shown diagrammatically in figure 2. The body of the window is made from a one-inch diameter rod of high optical quality casted plexiglas and the valve stems are made from a half-inch diameter rod of hard machining nylon (white or black). The metal tubing connectors, which are press-fitted in the valve stems, are cut from #19-gauge stainless steel disposable needles. The window is machined in its entirety in our laboratory by means of a Unimat lathe.* The window is sterilized by gas sterilization.

The valves work in the following manner (fig. 3). When closed, the indicator dot on the valve stem is in the twelve o’clock position. Turning the valve stem 90° in the lateral direction with respect to the window will line up its lumen with the outside port hold. This external circuit enables one to pre-flush that part of the connecting conduit which is exposed to the external environment. The valve stem is then turned 180° in a central direction with respect to the window, whereby the stem conduit will meet its counterpart in the window body for communication with the space under the window.

Polishing to produce a perfectly clear, scratch-free surface is the most tedious step in the construction of the window. This can be done by mastering the following procedure. The surface is first checked for flatness by lightly stroking it once or twice on a fine stone† and then looking at it under the low power of a dissecting microscope to see if small scratches made by the stone are uniformly distributed across the entire surface. If they are not so distributed, then the surface is not flat and stroking is repeated until this condition prevails. The surface of the window is then manually honed on still another stone‡ of much finer grain, using water to increase the effectiveness of the stone. Honing is complete when the surface of the window appears to have a dull finish to the naked eye and only tiny microscopic grooves are seen

*Edmund Scientific Co, 300 Edscorp Building, Barrington, New Jersey 08007.
†Degussit, 6 Frankfurt-Main, POB 3993, West Germany. Fine grinding tools of oxide ceramics. Stone type fine 44.
‡Sears, Roebuck and Co, combination sharpening stone, #9-64403.
under the microscope. It is very important that both the win-
dow and stone be frequently rinsed under tap water to
remove accumulations of acrylic debris and any other loose
materials which can of themselves produce scratches in the
acrylic surface.

A small felt polishing wheel§ is then mounted on the
lathe. A clean cotton ball is unrolled into a flat band of cot-
tton which is wrapped smoothly over the felt. The cotton is
then wetted with water and four or five more cotton balls are
added on in like manner. The polishing wheel now should be
soft enough to assure a uniform pressure distribution over
the entire surface of the window when the latter is lightly
pressed against it. A pasty mixture of water and White
rouge|| is applied over the surface of the cotton with a clean
tongue blade. White rouge is a fine polishing abrasive and,
unlike Red rouge, can be easily washed away. As polishing
progresses, the window is frequently rinsed under tap water
and its surface checked under the microscope using ap-
proximately 25X magnification. Fresh cotton is added onto

§National Camera, Inc, Englewood, Colorado 80110. Moto-tool
Kit and Accessories NC No. G-6530.

#6775-F75 White Rouge.

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Photograph of a rabbit implanted with a three-valve acrylic cranial window taken six months after implantation. The conduit leading out of each valve stem is in the same plane and directional orientation as the dot of its stem base.

The fixation of the silastic collars onto the acrylic window is the last step in its construction. A piece of silastic tubing** is cut to the precise length of the circular groove in the inner surface of the acrylic window (fig. 2). Note that the groove recessed into the acrylic surface has a depth of approximately one radius of the silastic tubing. Silastic adhesive compound†† is squeezed directly from the tube into the plunger end of a disposable tuberculin syringe and the plunger is returned into the barrel of the syringe. Aspirating the adhesive into the syringe can introduce undesired air bubbles. A 25-gauge disposable needle, cut down to 5 mm in length, is mounted on the syringe and a small amount of adhesive is slowly pressed out and carefully deposited in the base of the groove in an even fashion. The pre-cut silastic tubing is then placed in the groove and retained by an appropriate support while the adhesive is curing. Overnight curing is recommended to assure good fixation. Next, a ring 1.5 mm in width is stamped from silastic sheeting† 0.127-mm thick and affixed with silastic adhesive to the angulated peripheral edge circumscribing the tubular collar. The flat collar serves to seal the surface interface between bone flange and window edge and the tubular collar serves primarily to block tissue growth from the periphery.

IMPLANTATION OF CRANIAL WINDOW

For acute experiments, we use cats anesthetized with sodium pentobarbital; to a limited extent, we also have utilized rabbits and dogs. The stages of the technique are shown as actual photographs (fig. 4) and in diagram form (fig. 1, left). The window, of course, can be used easily in any other species of comparable size to the cat or larger without modification. Following anesthesia and tracheostomy, the animal’s head is mounted in a standard stereotaxic apparatus. A mid-sagittal incision (10 cm long) is made over the frontal and parietal portions of the scalp. The soft tissues are retracted laterally and the bone surface is cleaned and dried with gauze (fig. 4, upper left). Two methods are used for opening the skull. The first method is preferred by most investigators in our laboratory because it has virtually no chance of damaging the surface of the brain. A bone rongeur is used to make a circular hole in the calvarium 17.7 mm in diameter just caudal to the transverse suture line connecting the frontal and parietal bones (fig. 4, upper right). The bone is removed piece by piece with the instrument. Bleeding from the bone is controlled with bone wax. A second method utilizes a lightweight electric drill equipped with a specially constructed borer which makes a smooth circular groove in the skull (fig. 5). The borer is so designed that a tapered plate of bone is left at the base of the hole. The circularly cut bone is then gently lifted and carefully freed from the underlying adhering dura. The technique utilizing the electric drill is considerably faster, but in some experiments, particularly in the hands of relatively inexperienced investigators, it may generate sufficient heat to damage the vessels underneath and render them temporarily unresponsive in acute experiments. We have not experienced this difficulty in chronic experiments. For this reason, this technique is used mostly for chronic implantation which necessitates a precision fit between bone and window. We generally prefer to remove only one semicircle of the dura for two reasons (fig. 4, lower left). This avoids bleeding from the sagittal sinus, which may result if attempts are made to remove the dura from both hemispheres. Also, removing only one semicircle exposes a limited portion of the brain to

**Matheson, Coleman and Bell, East Rutherford, New Jersey. Castile soap #C540 L283.
††Dow Corning Corporation, Medical Products Division, Midland, Michigan 48640. Silastic tubing Cat. #602-131, 0.020" ID by 0.037" OD.
††Dow Corning Corporation, Medical Products Division, Midland, Michigan 48640. Silastic medical adhesive Cat. #891. Silastic sheeting, non-reinforced, Cat. # HH0815.
Four stages in the implantation of cranial window in a cat. Left upper: view of the skull after removal of the soft tissues and the periosteum. Right upper: position of the opening in the skull. The intact dura is seen at the bottom of the opening. Left lower: appearance of the opening in the skull following the removal of a semicircle of the dura. The surface of the brain is seen. Right lower: the window has been put in place and is sealed with dental acrylic. Note the grooves in the acrylic anterior to the window for securing the head on the microscope stage.

Any agents that are applied topically through the CSF and limits absorption from the brain's surface. To remove the dura, a fine hypodermic needle or microforceps is used to raise it off the brain's surface and a cut is made with ophthalmic scissors. The dura is then cut first sagittally along the edge of the sagittal sinus and then radially in apple-pie fashion. The flaps of dura are reflected over the bone so that when the window is placed into the skull, they are caught between the bone and the window. The brain surface is kept moist with artificial CSF until the window is threaded into position. Once the window is in position, bone wax is used to seal it (fig. 1, upper left). The window and the adjacent skull are then cleaned and dried meticulously, otherwise the dental acrylic will not adhere to the bone surface. Liberal amounts of dental acrylic are then poured over the exposed bone area and along the edge of the window to rigidly secure the window in position (fig. 4, lower right).

Two or three grooves are made with a blunt instrument anterior to the window and directed laterally before the acrylic solidifies (fig. 1, lower left). These are used to hold the head of the animal with a clamp on the microscope stage. Three-way stopcocks are then attached to each of the three hypodermic needles in the window ring, and the area under the window is flushed with artificial CSF to remove any air bubbles.

The rabbit has been used exclusively for long-term cranial window implantation. Although other species might be used, rabbits are preferable because of their docility and the ease with which they can be handled for experimentation in the awake state. The important steps in implantation are illustrated in figure 6. The animals are first anesthetized with intravenous sodium pentobarbital (45 mg per kilogram body weight) in the following way, thereby significantly reducing the mortality rate due to barbiturate anesthesia. Three-
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Diagram of the borer used in preparing rabbits for chronic implantation of the acrylic window. The contour and dimension of the hole in the skull fit precisely the base of the window.

fourths of the dose is given in one injection via an ear vein. While the rabbit is in a state of semiconsciousness, a 7.5 mm diameter plexiglas rod, 8 cm long with a center hole, is placed behind the incisors to protect the tube from damage and is held in position by means of a string. The rabbit is then intubated with a Foregger§§ endotracheal tube bent in a semicircular shape to facilitate blind entry into the glottis. The remaining dose of sodium pentobarbital is given and the animal is placed on control ventilation using a Phipps and Bird animal respirator || || set at a rate of 35 and a tidal volume of 32 to 35 ml (for 3 kg rabbits).

Surgery is carried out under aseptic conditions. A 6-cm mid-sagittal incision is made over the frontal and parietal portions of the scalp. All soft tissues are retracted laterally and the bone surface is cauterized only where major bleeding occurs. The exposed bone surface is then cleaned and dried with alcohol sponges for better fixation of the dental acrylic. A low-powered dissecting microscope is then brought into position over the animal's head, and the dural membrane is carefully incised along both left and right lateral borders of the sagittal sinus. The dura is then cut in radial fashion (fig. 6, top left) and each flap is folded over the bony flange at the base of the trephine hole (fig. 6, top right). The pial surface is kept moist at all times by using artificial CSF. Blood from the incised dural vessels and excessive CSF is carefully removed from the bone edge with cotton applicators. It is appropriate here to note that the thin arachnoid membrane generally is removed with the dura. However, torn segments of arachnoid are sometimes left over the pia mater and their removal should be verified with the aid of the microscope. If present, the pial surface will have a net-like appearance due to numerous thin connective tissue trabeculae connecting the pia mater with the transparent avascular arachnoid membrane. Otherwise, the brain surface appears smooth and glossy, although wavy, due to the highly vascular surface of the cerebral hemispheres.

The acrylic window, with all of its conduits filled with artificial CSF and the valves set in the closed position, is then gently placed in the bone opening and firmly pressed in position on the bone flange. The silastic tubular collar of the window should be partly compressed by the edge of the bone flange (fig. 6, lower right). The external bone edge is checked for dryness and fresh dental acrylic, prepared to a consistency sufficient to flow in the interface between the bone and window, is applied around the window and over the already embedded stainless steel screws. The window is held firmly pressed in position for about ten minutes, during which time the dental acrylic solidifies to an extent sufficient to rigidly fix the window. The scalp tissues are then re-approximated with 3-0 traumatic silk sutures. The space under the window is flushed with CSF to complete the implantation of the window.

The primary source of bleeding is the cut bone edge. Flushing of the space under the window must be carried out on a daily basis if the fluid is not clear. If the bone edge was properly cauterized and the meningeal flaps were effectively wedged between the window and bone flange, no blood should pool within the suprapial space under the window. Dural vessels, however, are sometimes cut in the process of incising the lateral borders of the mid-sagittal sinus. These borders remain free under the window and, on occasion, have been a source of minor bleeding. If no bleeding occurs, and the suprapial space remains clear, we have found it best not to disturb the intracranial area under the window for two weeks after surgical implantation. By then, the tissue reaction to surgical trauma has largely subsided and tissue adaptation to the implant appears to be virtually complete. Thereafter, the animal is ready for experimental study.

Each valve stem should be thoroughly purged before flushing. A 10-ml syringe filled with sterile saline and fitted with a PE-100 tubing is connected successively to the hollow connecting pin in each valve. A screwdriver type key (fig. 2) is used to turn each valve open to its outside port hole. After all valve stem conduits have been purged, a 2 to 3-ml syringe filled with sterile artificial CSF is connected to one of the valves, for example, the right valve (fig. 3). With the valve still being open to the outside, 1 ml is flushed through the

§Foregger, P. O. Box 538, Allentown, Pennsylvania 18105. Foregger 10 FE BIG 2 mm.
|| || Phipps & Bird, Inc. Box 2-V, Sixth and Byrd Streets, Richmond, Virginia 23298. Animal respirator pump, Cat. #7088-700.
***Small Parts Inc, 6901 Third Avenue, NE, Miami, Florida 33138. Machining screws, stainless steel, 1/72 X 1/8", flat heads, #MX 172-2B.

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FIGURE 6

Left: drawing illustrating the position of the trephine hole in the calvarium of the rabbit and the method for incising the meningeal membranes. Left lower half: drawing to show the orientation of the four stainless steel screws in the bone. Right upper half: drawing to show the folding of the dura (and arachnoid) over the bone flange. Right lower half: drawing to show the proper placement of the window in the skull bone to effectively seal the interface between bone and window and block dural growth into the window area. The screws are essential for permanent fixation of the window.

valve to remove air which might be trapped therein and the valve is then turned counterclockwise 180°, i.e., until the indicator dot points toward the center of the window. The other two valves are then turned until each conduit is open to the suprapial space under the window. The artificial CSF is slowly infused through the right valve, thereby flushing the area under the window. The fluid should exit from all three valves; if not, the valves through which fluid is not flowing should also be flushed to verify patency through their conduit. flushing is always done slowly so as not to raise the intracranial pressure. This is of extreme importance, since even moderately rigorous flushing may raise the intracranial pressure to very high levels, inducing vasodilation and vessel unresponsiveness and, at times, acute pulmonary edema.

EXPERIMENTAL PROCEDURE
In acute experiments, observation of the pial microcirculation is carried out using a Leitz compound microscope. The stage of the microscope is modified and equipped with a clamp so that the animal’s head can be held rigidly on the stage. One outlet of the cranial window is connected to a plastic tubing which is filled with artificial CSF and opened to the atmosphere. The distal end of the tubing is placed at a predetermined height to set the intracranial pressure at any
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desired level. Illumination is provided by a Xenon lamp after filtering with heat filters and a green filter, which enhances the contrast of the blood vessels by virtue of the absorption characteristics of hemoglobin. A mercury lamp or halogen lamp also may be used, but incandescent lamps usually do not provide sufficient light intensity for use with a TV camera. We are using either a 6.5X or an 11X dry objective lens. We do not recommend the use of zoom lenses, because the magnification with these lenses is critically dependent on the zoom setting. As a result of this, serious errors might arise from unsuspected changes in magnification. The advantage of the zoom, of course, is the rapid location of any desired microvessel without interruption of visual sighting. Diameter is measured with an image-splitting device and closed-circuit TV camera and monitor as described by Baez.5 The standard deviation of repeated measurements of vessel diameter with this method under seemingly steady state conditions every 10 to 15 seconds for five-minute periods is 1.5 μ for vessels between 25 and 100 μ; it is independent of vessel size.

The vascular diameter may be measured by another method; it is one which we have used to a limited extent. A Nikon 35 mm camera capable of recording up to four frames per second and of being operated automatically or by a pushbutton is substituted for the image-splitting device and TV camera. The moment of each exposure is recorded electrically on paper. Kodak Plus-X black and white film is used. The developed filmstrips are projected at a distance of ten feet using a Leitz Prado projector equipped with a filmstrip attachment and a 50 mm lens. Arterial diameter is measured on the projected image using a blind technique, in which the scale markings are invisible during the measurement. Calibration is carried out with a stage micrometer photographed and projected in the same manner as the vessel images during the experiment. Calibration is reproducible and linear in all parts of the image. The variation of repeated measurements of the same vessel is of the same order and magnitude as with the other method described above.

For chronic experiments, we use basically the same microscope system equipped with the image-splitting device and TV camera and monitor, but without a stage. The microscope stem is attached to a heavy support which allows it to be brought on top of the animal’s head. The rabbit is wrapped in a towel and placed in a plaster of Paris cast form-fitted to the animal in resting position. Rabbits generally stay quiet under the microscope for as long as several hours.

Vessel images are usually sharp and of high quality in most instances in both acute and chronic preparations (fig. 7). However, faint images may occur in anemic animals and indistinct images may rarely occur in acute animals in which, because of the shape of the skull, the window is far removed from the brain. Because of vertical movement with respiration, the vessel images may also become indistinct during certain phases of respiration. In chronic animals, the images may remain sharp and clear for periods in excess of a year when the pial surface is left undisturbed; however, prolonged and repeated irrigations tend in some way to irritate the pial surface, invoking a progressive deterioration in its normal, healthy appearance and ultimately resulting in an unusable preparation.

We measure the interior diameter of arterial vessels. The arterial wall is visible in only about 50% of the arteries as a clear zone around the red cell column. Its outer margin is usually indistinct and difficult to determine precisely. Arteries are easily distinguishable from veins by several criteria: first, the most reliable criterion is that the direction of flow in the arteries is from larger caliber vessels to smaller caliber vessels, while the reverse is true in veins. Second, movement of individual red blood cells in arteries usually cannot be distinguished due to rapid blood flow. This usually can be seen in veins, but if flow is rapid, such as under conditions of CO₂ breathing, this may not be the case. Third, arteries are normally of a brighter red color than that of veins but this is not always a dependable guideline. And fourth, arterial flow is usually pulsatile, even down to the arterial end of the capillary, whereas venous blood flow is normally steady.

Results

In the past five years, we have carried out 534 acute experiments in cats using the technique described above. Of these, 33 experiments were considered failures because of defects in the preparation. The most common cause of failure was hemorrhage under the window. This occurred mostly in the course of experiments in which increases in blood pressure or marked vasodilation were induced by various means, such as inhalation of CO₂. Other less common causes of failure were ill-fitting windows resulting in leakage of fluid, damage to the surface of the brain in the process of removing the dura, swelling of the brain causing the window to be too close to the surface of the brain so that fluid could not reach certain areas of the brain surface under the window, complete unresponsiveness of the vessels to all stimuli for unknown reasons, and blockage of the outlets of the window with debris.

We carried out chronic experiments in 50 rabbits over a period of three years. During this period, the chronic window technique underwent repeated modification, so that only the last 12 experiments have been carried out with the final version of the window described in the preceding section. The first prototype acrylic window preparation, which consisted of just a window without valves, was often first covered with blood and subsequently covered, within one to three weeks, by regrowth of the dura. The addition of valves enabled flushing the space under the window and maintaining it free of blood, thereby extending the period of usefulness of the preparation to approximately one month, at which time the visual field became significantly obstructed with meningeal tissue regrowth. Several versions of collar-equipped windows were subsequently constructed and tested in rabbits. The present design offers maximum effectiveness both in retarding regrowth of dura and in sealing the intracranial cavity. We now feel confident that the primary cause of rapid visual obstruction of a chronic window implant is an imperfect seal between bone and window. Unfortunately, there is no sure way of telling if the seal is defective at the time of surgery; however, if this be the case, it usually becomes apparent within
A typical photomicrograph of the surface vessel of the cerebral hemispheres in the rabbit as viewed through the chronic window. Note the clarity and sharpness of the image.

the next few days. A whitish amorphous precipitate appears at the periphery of the window and progressively spreads with time over the surface of the pia. The nature of this precipitate is not entirely clear. Whatever its composition, however, it does not appear in "water-tight" implantations. We have had no instances of infection as a result of contamination of the space under the window.

Discussion

The cranial window technique for acute experiments described above has several advantages. By restoring the integrity of the skull, it maintains an environment for the pial vessels as close to the normal one as possible with respect to intracranial pressure, composition of the surrounding fluid, and prevailing gas tensions. The technique makes possible the measurement of caliber of vessels over a considerable range of size. In addition, it has considerable flexibility. For example, one can alter intracranial pressure at will. The effects of various changes in the composition of the fluid surrounding the vessels can be studied. The effects of vasoactive substances can be studied by injection intravenously or intra-arterially, as well as by local application. The principal difference between the present technique and the one described by Forbes is that our window is much smaller, so that the possibility of infringement on the surface of the brain is minimized considerably.

There are two studies in the literature utilizing preparations involving the chronic implantations of cranial windows and prolonged visualization of the pial microcirculation. These windows were simple and not equipped with valves. Such windows in our experience become unusable within a matter of weeks. The window described above for chronic implantation makes it possible to study animals in the unanesthetized state, and allows the performance of long-range experiments, such as the study of adaptation of the pial vessels to exposure of the animals to abnormal environments for prolonged periods of time.
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References

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