A Precision Surgical Approach for Complete or partial Callosotomy in the Mouse

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Abstract:
The corpus callosum (CC) of mice was completely transsected with a thin tungsten knife using a three-cut approach through the dorsal cerebrum just lateral to midline. This method results in almost total transsection of the CC throughout its entire rostrocaudal extent. Advantages of this approach include minimal bleeding and extracallosal damage as well as the possibility of selective transsection of only anterior, middle, or posterior parts of the CC. The technique can be readily adapted to any other rodent species.
Keywords: Corpus callosum, Split brain mouse, Splenium, Stereotaxic surgery, Interhemispheric connections

Article:

Investigation of human and animal behaviour following transsection of the major cerebral commissure, the corpus callosum (CC), provides valuable insights into various aspects of cerebral lateralization arid intracerebral transfer of information (2,17). In humans, this body of research has been contrasted with behavioural deficits following congenital abnormality or absence of the CC, a defect called agenesis of the corpus callosum (10,11,14). This genetic defect has been described only in humans and mice, permitting the potential comparison of behavioural consequences of congenital CC defects with those of CC transsection only in these: two species. To date, however, surgical techniques for CC transsection have been developed for humans (2,17), monkeys (6), cats (15), rabbits (18), and, with varied success, for rats (4,8,9,16), but never for mice. The technical difficulties inherent in transsecting this relatively small structure in the mouse have thus far prevented attempts at developing a surgical technique for callosotomy in the mouse. In one strain of mice (CF-1), it has been found that not only can there be more than one bregma point, but due to individual differences, there are significant errors for locating structures in the frontal plane (20). In addition, there are significant strain differences in the relative locations of lambda, bregma, and the midbrain commissures in mice (21).

We have developed a high precision surgical approach for transsection of the CC in mice. This technique allows transsection of parts of or the entire CC, while avoiding extracallosal damage to the colliculi, cerebellum, hippocampus, and hippocampal commissure. This will permit future behavioural comparisons between CC transsected mice and a variety of acallosal mouse strains and thus an experimental investigation of the differential effects of prenatal CC malformation with those of surgical destruction of the CC in the juvenile or adult mouse. As part of this technique, we introduce a number of improvements of apparatus and methodology which address the general issue of surgical precision. We also point out limitations of the technique which result from strain and age differences.

METHOD

Animals and anesthesia

The subjects used were four male B6D2F2 mice, aged 44 days at the time of surgery, and weighing between 22.7 and 23.9 g. These were obtained by mating B6D2F1/J mice (the hybrid off-spring of a C57BL/6J female mated with a DBA/2J male) purchased from the Jackson Laboratory.

Subjects were injected intraperitoneally (IP) with 0.1 cc of atropine sulfate (0.4 mg/cc) 30 min prior to anesthesia and were then anesthetized by IP injections of 1.3 cc/kg of pentobarbital (65 mg/cc). For local anesthesia, 0.04 cc of Lidocaine (2.86 mg/ cc) was injected under the scalp before incision. When the subjects regained consciousness, they received IP injections of 0.5 cc/kg of buprenorphine (0.04 mg/cc) for postoperative analgesia. A major problem with this technique was mortality due to complications with anesthesia, because hybrid mice have large individual differences in sensitivity to drugs. We have since overcome this drawback by using an anesthesia machine with anesthetic gas (isoflurane, administered at flow rates of 300 ml/ min and concentrations of 2.7 to 3.5% with a Fluotec II vaporizer), which enables the surgeon to adjust the dose according to the subjects' reactions, thereby reducing mortality due to anesthesia from 5 of 19 subjects (using pentobarbital) to 0 of 20 subjects (using isoflurane).

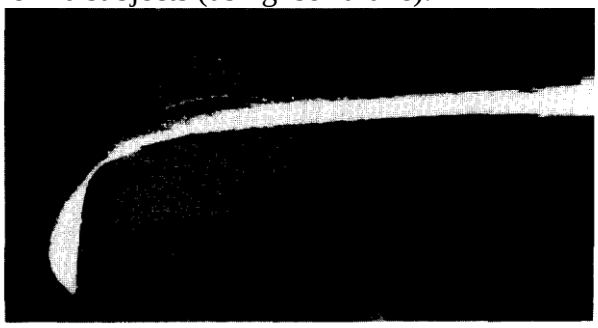

FIG. 1. Anterior end of tungsten knife used during surgery. Scale bar = 1 mm.

Apparatus

The knife (shown in Fig. 1) was constructed of a 0.25 mm diameter tungsten wire of approximately 4 cm length. One end of the wire, about 1 mm in length, was bent at a right angle and the outside edge of the hook was sharpened on 600 grain emery paper. All but this hook and the proximal 8 mm of wire were inserted into a sufficient length of stainless steel tubing (from a hypodermic needle with 0.28 mm i.d.) to decrease the flexibility of the knife. The end of the wire, encased by the tubing, was held firmly against a stereotaxic holder in a horizontal position and aligned with the anteroposterior axis of the stereotaxic apparatus. It is important that the knife is fixed to the extreme distal end of the holder, to permit the assembly to pass over the mouse's body during surgery.

A modified Kopf professional model stereotaxic instrument with 0.01 mm graduations and a palate clamp holder (described in detail elsewhere (12)) was used. During surgery, the subject's body lay on a plastic bed heated to 37°C to prevent hypothermia. This bed could be moved relative to all three axes of the stereotaxic apparatus at any angle. Using a potentiometer connected to a voltmeter, the angle of the anteroposterior axis of the subject's body relative to the apparatus could be measured to the closest 0.1° angle.

Because the palate clamp holder did not fix the head of the mouse in a predetermined flat skull position, the skull of subjects had to be carefully levelled after placement in the sterotaxic instrument. For this purpose a pressure sensitive, battery operated device was attached to a sterotaxic holder. This device gave auditory feedback when it was lowered onto the skull, at which point the ventral position was read on the stereotaxic instrument. Using this method, the skull was levelled to within 0.1 mm precision between lambda and bregma and between two points 2 mm lateral on either side of a point on midline halfway between lambda and bregma.

Because the skull of mice is relatively flexible, it was important to use a high speed drill that would cut through the bone without depressing it. The drill used was a Foredom high speed lapidary drill (Series S, max. 18000 rpm) with a high speed hand-piece (Foredom No. 35), which multiplies motor speed 2.5 times for a maximum drill bit speed of 45000 RPM, and a cylindrical drill bit of 0.6 mm diameter.

Surgery

To minimize sources of surgical complication and distress to the animal, surgery was conducted under sterile conditions. The anesthetized mouse was scissor shaved and placed in the stereotaxic apparatus. Eyes were

covered with Polysporine sterile ophthalmic ointment and the scalp disinfected with Betadine surgical scrub. The scalp was cut with a #15 scalpel blade and the skull cleaned and dried with sterilized cotton tipped swabs before the skull was levelled using the head levelling device described above. To achieve this, the entire body and head of the mouse were moved relative to the apparatus until the skull was level (as defined above).

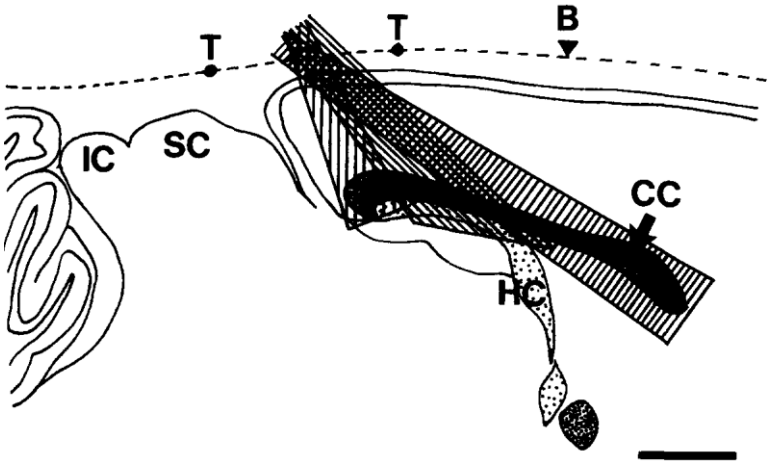

FIG. 2. Sagittal view of mouse brain at 0.4 mm lateral to midline, illustrating effective course of knife cuts. Abbreviations: T—anterior and posterior extent of trephine hole, B—bregma, HC—hippocampal commissure, IC—inferior colliculus, SC—superior colliculus. Scale bar = 1 mm.

A unilateral trephine hole was drilled as a fine slot centered 0.5 mm lateral to bregma between points 1.4 mm to 3.6 nun posterior to bregma, which generally avoided damage to the superior sagittal sinus. Bone shards were cleared from the hole and dura was broken using a pair of fine forceps. Through the single trephine hole, three separate consecutive cuts at different angles and aimed at the posterior, middle, and anterior parts of the CC were made (see Fig. 2). For each cut, the tip of the knife was positioned on bregma with the head at the appropriate entry angle for that cut and lateral, ventral and anteroposterior positions noted for calculation of coordinates for entry. These coordinates were determined such that the knife would be positioned at 3.95 mm posterior to bregma at skull level. Because the head was not level at this time, the knife had to be moved posterior from bregma and then ventrally to be positioned at the entry point (see coordinates below). After the knife was inserted at an angle which would position it just dorsal to the CC, the head was moved to a steeper angle with the knife in place. In effect, this procedure resulted in lifting the CC up tightly against the under-side of the knife, with the hook extending down over the genu. During withdrawal of the knife, the posterior end of the hook thus cut through the remaining CC fibres. Coordinates for the three cuts were, respectively: Cut 1-Entry angle 43°, withdrawal angle 70°, at -2.72 mm ventral and -2.82 mm posterior to bregma, length from entry point 3.60 mm; cut 2-entry angle 32°, withdrawal angle 46°, at -1.87 mm ventral and -2.92 mm posterior to bregma, length 5.0 mm; cut 3-entry angle 27°, withdrawal angle 37°, at -1.59 mm ventral and -3.08 mm posterior to bregma, length 6.3 mm. All cuts were made at 0.4 mm lateral to bregma.

If bleeding occurred during the knife cuts, this was stopped by application of pressure after withdrawal of the knife. After completion of all three knife cuts, the site was cleaned with sterile saline and the incision closed with sterile cyanoacrylate (Nexa-band). Subjects were kept at 37°C until they regained consciousness. Since developing this technique, we have also performed this surgery on 14 mice which were used for behavioural testing and we have found excellent recovery from surgery and no problems with long term survival.

Histology

Two days after surgery, subjects were deeply anesthetized with 1 cc/kg of pentobarbital (Euthanyl, 12 mg/cc) and perfused intracardially with isotonic saline followed by buffered 4% paraformaldehyde. Brains were removed immediately and stored in buffered 4% paraformaldehyde for four days before blocking the brains to a standard configuration. Brains were cut in coronal sections of 60 μm throughout the extent of the CC, and every fourth section was mounted on gelatin coated slides. Sections were stained with the Schmued gold chloride

stain (19) and permanently coverslipped. Serial coronal sections illustrating the ex-tent of knife cuts are shown in Fig. 3.

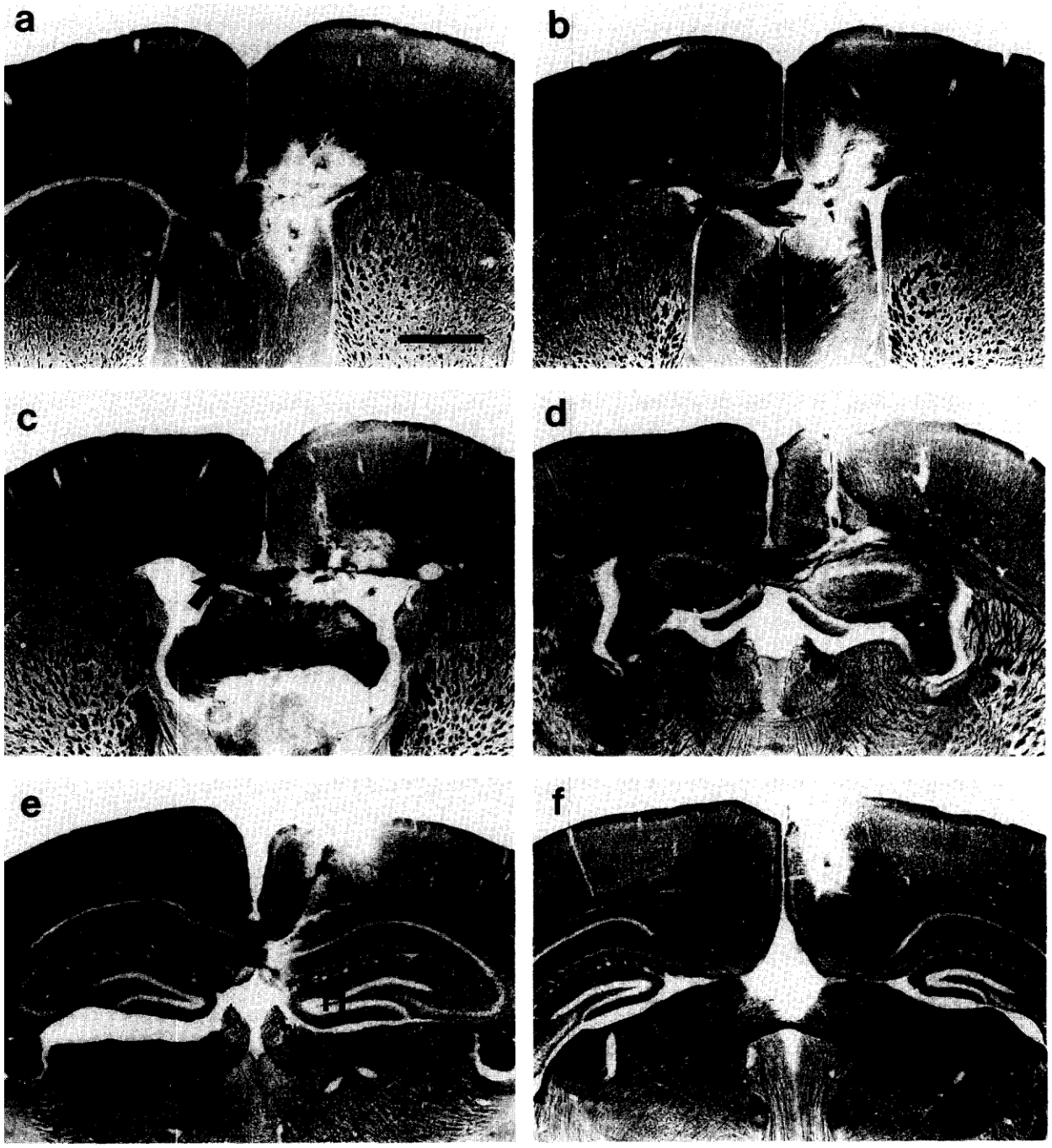

FIG. 3. (a–f) Coronal sections illustrating extent of knife cuts and associated damage from most anterior extent (a) to posterior end (f) of cuts. Abbreviations: F—fornix, HC—hippocampal commissure, H—hippocampus. Scale bar = 1 mm.

DISCUSSION

This three-cut precision approach for callosotomy in the adult mouse reliably results in transsection of the CC with minimal extracallosal damage. Examination of all mounted sections from the brains of surgical subjects indicated that the extent of callosal transsection was approximately 89% to 100%. This lies well within the range considered to constitute a total transsection in the literature on rats (1,5).

In the past, a variety of techniques has been employed to transsect the CC in rats, all of which resulted in an extent of extracallosal damage which, in our opinion, would make the validity of later behavioural data questionable. In two of these techniques, an unsharpened wire knife was used to make single cuts, resulting in

tearing of the surrounding tissue as well as a significant risk of cerebellar damage (4,8). Another technique involved the use of a modified dental hook, which was inserted underneath the superior sagittal sinus and used to tear the CC in a nonstereotaxic procedure, resulting in significant cortical and hippocampal damage and the loss of tissue fragments (16). A third procedure utilized a piece of surgical silk which was stretched taut between two needles inserted anterior and posterior to the CC lateral to midline. This method resulted in visible cortical damage and degeneration along the entire line of cutting, as well as in hippocampal damage.

By comparison, our technique results in no perceptible dam-age to the cerebellum and colliculi, and damage to the hippocampal commissure and hippocampus proper is negligible (see Fig. 3). However, the most dorsal portion of the fornix, which is adjacent to the ventral side of the CC, is generally lesioned. Cortical damage is unilateral and is restricted to the cingulate cortex be-low the trephine hole, referred to as the cytoarchitectonic field 29c (22). This area of damage receives minimal interhemispheric callosal projections (22) and lies posterior and medial to the hind-limb area and medial to the trunk area of the primary somatosensory cortex (3). Its role in somatosensory processing is un-known (13). The mouse cingulate cortex has been implicated in neural transmission between visual processing areas, though its function has not been clearly specified (7).

This technique can easily be modified for use in partial callosotomy. Because three separate cuts were necessary to transsect the entire CC, one or two cuts could section specific parts of the CC. However, the coordinates presented here are highly strain and even age-specific. As reported previously (21), there is both significant between- and within-strain variability in the structure of the skull of mice. Consequently, new coordinates must be developed when using different strains of mice or older (above 10 weeks of age) subjects with thicker skull bones. However, we are confident that the principles of our precision technique could easily be adapted to achieve higher accuracy and minimal collateral damage in callosotomies not only in mice but a variety of other species.

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