

# Fluorescein-Enhanced Characterization of Additional Anatomical Landmarks in Cerebral Ventricular Endoscopy

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**BACKGROUND:** Fluorescein enhancement to detect retinal disorder or differentiate cancer tissue in situ is a well-defined diagnostic procedure. It is a visible marker of where the blood-brain barrier is absent or disrupted. Little is reported in the contemporary literature on endoscopic fluorescein-enhanced visualization of the circumventricular organs, and the relevance of these structures as additional markers for safe ventricular endoscopic navigation remains an unexplored field.

**OBJECTIVE:** To describe fluorescein sodium-enhanced visualization of circumventricular organs as additional anatomic landmarks during endoscopic ventricular surgery procedures.

**METHODS:** We prospectively administered intravenously 500 mg fluorescein sodium in 12 consecutive endoscopic surgery patients. A flexible endoscope equipped with dual observation modes for both white light and fluorescence was used. During navigation from the lateral to the fourth ventricle, the endoscopic anatomic landmarks were first inspected under white light and then under the fluorescent mode.

**RESULTS:** After a mean of 20 seconds in the fluorescent mode, the fluorescein enhanced visualization of the choroid plexus of the lateral ventricle, median eminence-tuber cinereum complex, organum vasculosum of the lamina terminalis, choroid plexus of the third and fourth ventricles, and area postrema.

**CONCLUSION:** Fluorescein-enhanced visualization is a useful tool for helping neuroendoscopists recognize endoscopic anatomic landmarks. It could be adopted to guide orientation when the surgeon deems an endoscopic procedure unsafe or contraindicated because of unclear or subverted anatomic landmarks. Visualization of the circumventricular organs could add new insight into the functional anatomy of these structures, with possible implications for the site and safety of third ventriculostomy.

**KEY WORDS:** Area postrema, Blood-brain barrier, Circumventricular organs, Fluorescein, Median eminence, Neuroendoscopy, Third ventriculostomy

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The circumventricular organs (CVOs) are highly vascularized structures located around the third and fourth ventricles and characterized by the lack of an intact blood-brain barrier (BBB).<sup>1-3</sup> These specialized areas are points of communication between the blood, brain parenchyma, and cerebrospinal fluid.

**ABBREVIATIONS:** **AP**, area postrema; **BBB**, blood-brain barrier; **CVO**, circumventricular organ; **ETV**, endoscopic third ventriculostomy; **ME**, median eminence; **OVLT**, organum vasculosum lamina terminalis

Although the international anatomic nomenclature committee identifies about 70 structures, in our experience only 13 of these are identified by neurosurgeons for safely navigating within the cerebral ventricles during the most common neuroendoscopy procedures. In most cases of hydrocephalus, the anatomic landmarks of the third ventricle floor are well defined and immediately recognizable. However, in complex hydrocephalus caused by hemorrhaging or meningitis, ependymal reactions may affect normal anatomy.<sup>4,5</sup> In the lateral ventricles, chronic and repeated episodes of ependymitis or ventricular

tumors can sometimes conceal the choroid plexus during endoscopy because the underlying membrane layers have become totally opaque. Accordingly, additional anatomic landmarks such as the CVOs would be more than desirable. Since the early 1960s, fluorescein angiography has become one of the main investigation techniques in ophthalmology for the diagnosis and treatment of retinal disorders.<sup>6</sup> It is a well-established diagnostic procedure and, when coupled with neuroendoscopy, could add valuable support to the surgeon. Because of its specific properties, fluorescein sodium has been used as a tracer of BBB disruption in animal models<sup>3</sup> and adopted as a tentative guidance tool in the surgical removal of high-grade gliomas.<sup>7-10</sup> Recently, Kubo et al<sup>11</sup> observed these anatomic landmarks (the median eminence [ME] and corpus vasculosum of the lamina terminalis) in the third ventricle in a single case of endoscopic exploration under fluorescein-sodium enhancement. Because these structures overlap with the site where ventriculostomy is performed, either microsurgically to open the lamina terminalis or endoscopically in obstructive hydrocephalus, we decided to better define these functionally active areas, unfamiliar to neurosurgeons, by means of a fluorescent navigation system and by characterization of all the CVOs described in standard anatomy.

## PATIENTS AND METHODS

We prospectively used fluorescent endoscopy in our latest 12 endoscopic third ventriculostomy procedures. We involved our ophthalmology colleagues to share their expertise and common protocols on retinal fluorescein angiography. The protocol was submitted to the local ethics committee, and each patient provided a signed informed consent. We used a fiberscope equipped with dual observation modes for both white light and fluorescence under excitation of blue light (D-Light AF system; Karl Storz Endoscopy). The steerable endoscope had an external diameter of 2.5 mm (Karl Storz) and an operative length of 53 cm. The internal diameter of the working channel was 1.2 mm. Access was precoronal through a 12-mm burr hole. Lateral ventricular cannulation was achieved with a semirigid peel-away cannula, and systolic blood pressure kept at 120 mm Hg. Throughout the procedure, instruments were managed with a free-hand technique. Before performing the ventriculostomy, all accessible ventricular cavities were inspected (Figures 1A and 1B). After this initial inspection with the standard white light mode,

a solution of 500 mg fluorescein sodium was administered intravenously by the anesthesiologist to the patient while the interior of the ventricles was observed with the endoscope in the fluorescent mode switched to white light. Each change in endoscope direction was made in white light to avoid damaging the ventricular walls. The procedures were recorded in their entirety and reviewed to define the precise pattern of fluorescence of any functionally active area.

## RESULTS

### Choroid Plexus

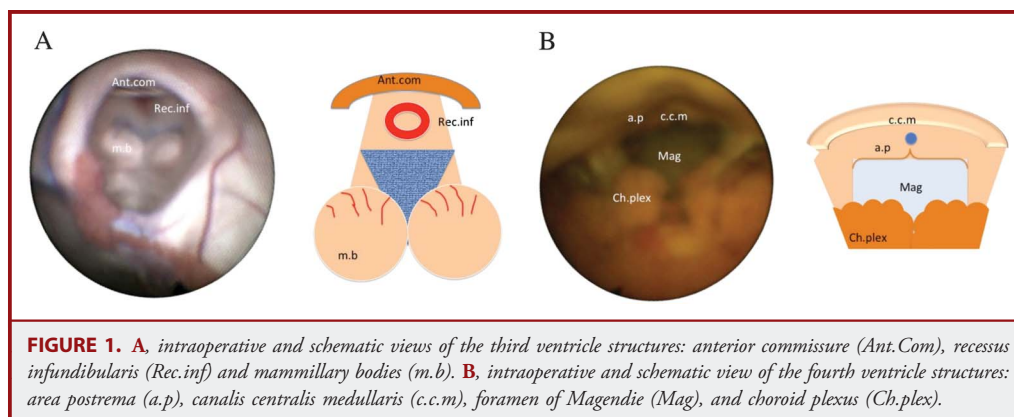
Approximately 20 seconds after intravenous injection of fluorescein sodium, the choroid plexus began to glow green when visualized in the fluorescent mode. Plexus fluorescence was very high and uniform along its entire structure. A few minutes later, the dye permeated through the plexus barrier and active secretion of fluorescein into the cerebrospinal fluid was clearly visible.

### Median Eminence

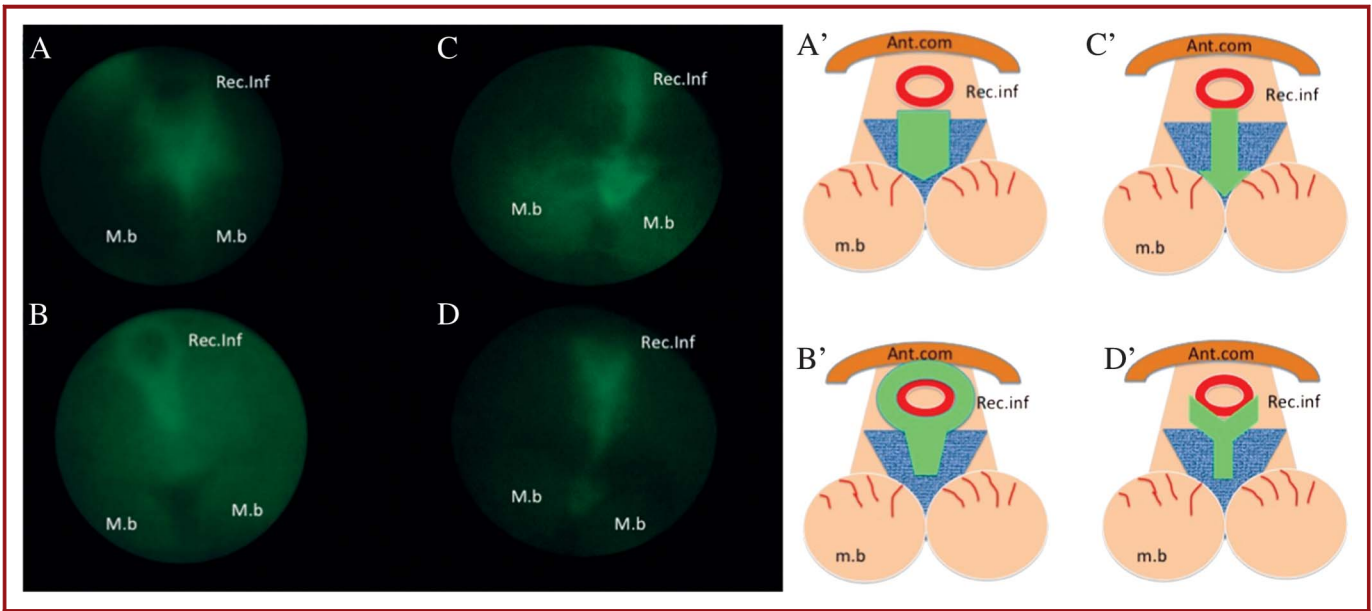
The ME started to glow green approximately 30 seconds after the choroid plexus. We were able to identify 4 different patterns of fluorescence, suggesting unexpected anatomic variability: pentagon (Figure 2A-A'), keyhole (Figure 2B-B'), intermammillary arrowhead (Figure 2C-C'), and epsilon (Figure 2D-D'). The ME is part of the tuber cinereum but circumferential to the infundibulum. The appearance of almost 4 different patterns of fluorescence poses some relevant questions of interpretation. We ascertained that each pattern outlined the labrum eminentiae medianae separating the infundibular recess.

### Organum Vasculosum Lamina Terminalis and Subfornical Organs

The endoscope was directed through the foramen of Monro toward the anterior third ventricle, and the classic anatomic landmarks were recognizable as the anterior commissure, optic recess, mammillary bodies, and tuber cinereum. The third ventricle seemed to harbor most of these receptive areas. Of the described CVOs, only the ME and organum vasculosum lamina terminalis (OVLTL) captured fluorescein and were clearly visible



**FIGURE 1.** A, intraoperative and schematic views of the third ventricle structures: anterior commissure (Ant.Com), recessus infundibularis (Rec.inf) and mammillary bodies (m.b). B, intraoperative and schematic view of the fourth ventricle structures: area postrema (a.p), canalis centralis medullaris (c.c.m), foramen of Magendie (Mag), and choroid plexus (Ch.plex).

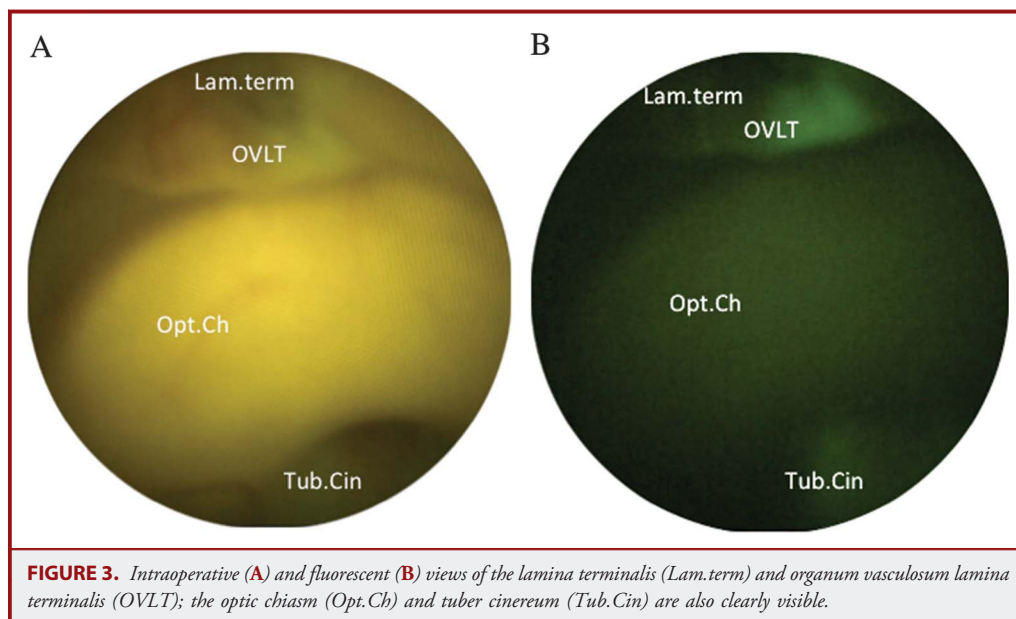


**FIGURE 2.** Intraoperative and schematic views of the third ventricle structures in the fluorescent mode: anterior commissure (Ant.com), recessus infundibularis (Rec.inf) and mammillary bodies (m.b); 4 patterns of fluorescence of the median eminence complex were recognizable: pentagon (A-A'), keyhole (B-B'), intermammillary arrowhead (C-C'), and epsilon (D-D').

during endoscopy (Figure 3). The subforminal organ was not reachable even when the flexible endoscope was positioned ventrally to the fornix, corresponding to the anterior triangle formed by the columnae fornicis and anterior commissure. The OVLT presented constant bright central fluorescence because of the specific type of vascularization. At higher magnifications, small capillaries around the area of the tuber and lamina terminalis could be detected in the fluorescent mode.

**Pineal Gland and Subcommissural Organ**

After exploring the anterior third ventricle, the endoscope was flexed backward toward the posterior third ventricle, permitting inspection of the aditus aquaeducti, posterior commissure, and pineal and habenular recesses. In the fluorescent mode, only the choroid plexus exhibited fluorescence; the pineal gland and subcommissural organ probably did not enhance in this mode because of their intrinsic anatomic location outside the blood-brain barrier.



**FIGURE 3.** Intraoperative (A) and fluorescent (B) views of the lamina terminalis (Lam.term) and organum vasculosum lamina terminalis (OVLT); the optic chiasm (Opt.Ch) and tuber cinereum (Tub.Cin) are also clearly visible.

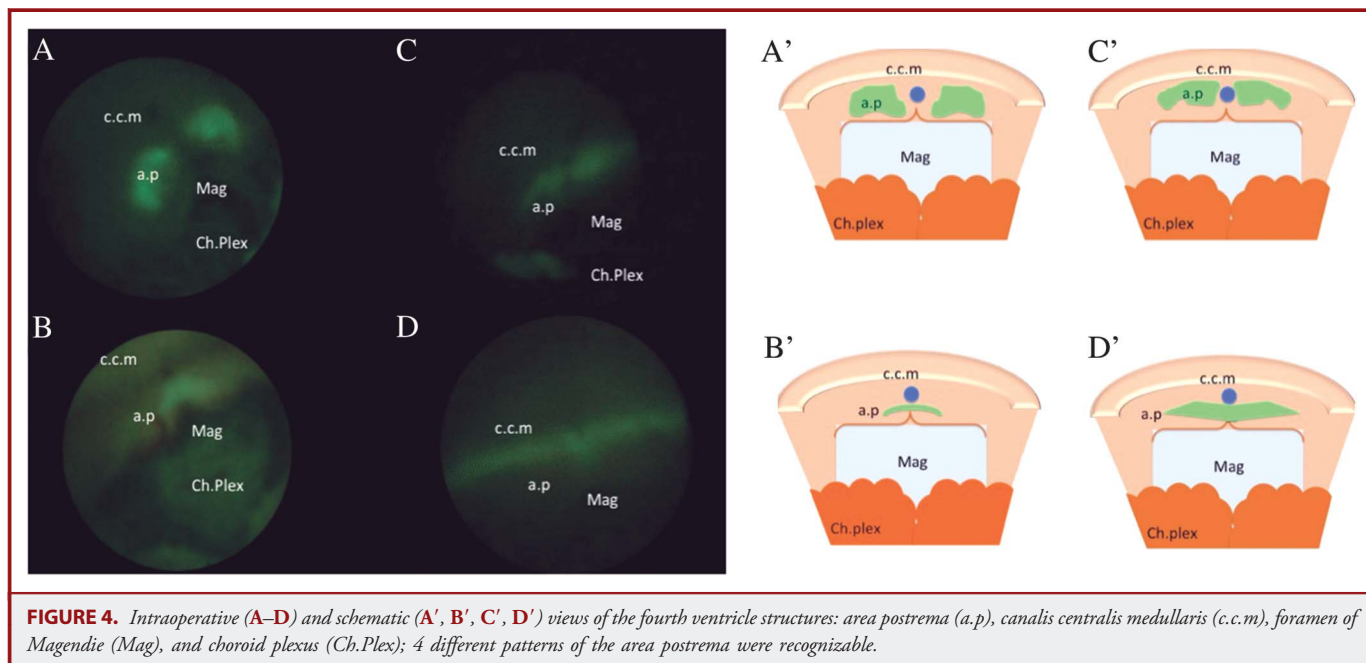
### Area Postrema

In 4 cases we inspected the fourth ventricle to detect outlet obstruction. The endoscope was directed anteriorly and inferiorly toward the aditus ad aqueductum cerebri and advanced through the ampulla into the fourth ventricle. The sulcus medianus of the fourth ventricle is the guiding anatomic landmark leading to the posterior triangle of the rhomboid fossa, with clear evidence of the calamus scriptorius and foramen of Magendie. In the fourth ventricle, the sulcus medianus acts as a railroad to the calamus scriptorius, but as the instrument advances, it tends to physiologically deflect toward the contralateral left lateral recess and then toward the left foramen of Luschka. Although anatomically delimited by the striae medullares, the posterior triangle of the rhomboid fossa can be navigated by rotating the endoscope over the midline toward the calamus scriptorius and the obex overhanging the canalis centralis medullae spinalis. Tonsillae and posterior inferior cerebellar arteries surround and delineate the dorsal part of the foramen of Magendie, which, when patent and wide, permits visualization of the posterior oblongata and the posterior part of the foramen magnum outside the fourth ventricle. The area postrema (AP) is a small protuberance found at the inferoposterior limit of the fourth ventricle. It is formed by 2 small strips at the inferior apex of the caudal ventricular floor at an equidistance between the canalis centralis medullaris and the foramen of Magendie. This breakdown in the BBB, exhibiting different patterns of fluorescence, is clearly visible in the fluorescent mode. We were able to identify a variable appearance pattern of fluorescence (Figure 4).

### DISCUSSION

Since 1923, when Mixer<sup>12</sup> first attempted an endoscopic third ventriculostomy (ETV) by passing a urethroscope through the floor of the third ventricle, technical and instrumental improvements have considerably improved the safety and feasibility of endoscopic surgery. The effectiveness of endoscopic procedures such as ETV as an alternative to shunting has been demonstrated in the treatment of obstructive hydrocephalus.<sup>13</sup> Despite technical refinements, ETV is not without risks. Complications such as basilar and perforating vessel injury<sup>14</sup> are still the nightmare of any experienced neuroendoscopist. Despite the birth of neuronavigation and advances in magnetic resonance imaging, there remains a lack of methods to predict the anatomy and configuration of the third ventricle floor in cases of complex hydrocephalus.<sup>4</sup>

Although the international anatomic nomenclature committee has identified approximately 70 structures, neurosurgeons should master a few landmarks by which to safely navigate the cerebral ventricles in the most common neuroendoscopy procedures. Other landmarks such as CVOs do, however, exist and can be easily enhanced using intravenous fluorescein and proper light filtering.<sup>11</sup> This report of a single patient,<sup>11</sup> almost disregarded by the neuroscientist community, showed for the first time in living humans the 2 main CVOs of the third ventricle, ie, the organum vasculosum of the lamina terminalis and the ME. Intravenous fluorescein, as described by Kubo et al,<sup>11</sup> penetrates sites of disrupted or absent BBB, a property that enables the CVOs to be enhanced via a simple procedure borrowed from



**FIGURE 4.** Intraoperative (A–D) and schematic (A', B', C', D') views of the fourth ventricle structures: area postrema (a.p), canalis centralis medullaris (c.c.m), foramen of Magendie (Mag), and choroid plexus (Ch.Plex); 4 different patterns of the area postrema were recognizable.

current ophthalmology and pulmonary diagnostics.<sup>6,15</sup> BBB impairment is known to occur not only in pathological conditions but is also physiological in some intraventricular ependymal organs considered the sensory sites of cerebrospinal fluid circulation<sup>1-3</sup> (the so-called CVOs). These are midline anatomic structures strategically positioned at the entrance and exit of cerebral ventricles and characterized by their incomplete BBB. Common features described by Duvernoy and Risold<sup>3</sup> are the presence of fenestrated capillaries and loosely apposed astrocytic processes that give rise to relatively large perivascular spaces allowing the passage from the blood of large molecules such as peptides and polar substances without the need for specialized transport. Neurons located in CVOs are directly exposed to peripheral signals and can sense the concentrations of different compounds.<sup>3</sup> They play a critical role in sodium-water balance,<sup>1,3</sup> cardiovascular regulation,<sup>3</sup> and energy metabolism.<sup>3</sup> They are also involved in complex mechanisms and reflexes such as vomiting<sup>16</sup> and fever<sup>3</sup> and other responses to potentially noxious stimuli.<sup>3</sup> CVOs include the pineal gland, ME, subfornical organ, AP, subcommissural organ, and organum vasculosum of the lamina terminalis (OVLT). The choroid plexuses of the lateral, third, and fourth ventricles share the same properties but are considered secretive rather than sensory organs. In our experience with a flexible endoscope, only the OVLT, ME, and AP were detectable using intravenous fluorescein. In all our cases, careful exploration of the pineal recess and posterior commissure showed no fluorescence according to the observations of Duvernoy and Risold, who maintained that these CVOs had an intact BBB.<sup>3</sup> We attempted to systematize these data, particularly relating to the ME, which notoriously coincides with the site where neurosurgeons perform ETV, and found some variability in the shape and extension of this anatomic structure. In our opinion, these observations could have important operative implications. Neurosurgeons may occasionally face difficult anatomic situations when positioning a stoma or performing a septostomy in a safe place.<sup>4</sup> Many anatomic variations, such as thick opaque floors, occlusion of the foramen of Monro by dense membranes, and a hidden choroid plexus, can occur in complex hydrocephalus, hindering surgeon orientation and making endoscopic navigation unsafe.

Last, but not least, little is known about the functions of the anatomic structures coagulated during ETV,<sup>17</sup> which should be further investigated in view of our observations on the complex fluorescent anatomy of the ME. After publishing our experience in the transaqueductal navigation,<sup>18-20</sup> especially in cases of fourth ventricle outlet obstructions, we were able to show for the first time in live humans the fluorescent anatomy of the bulbar AP, a complex CVO only identifiable with some uncertainty by the naked eye in standard anatomic specimens. This latter observation could also prove important in the microsurgical approach to the anterior triangle of the posterior fossa. For now, however, this is mere speculation and further studies are needed. The revival of fluorescein could give

neurosurgeons additional insights into the functional anatomy of ventricular cavities and further may be an additional guide to tailor endoscopic navigation when unclear or subverted anatomic landmarks suggest that a procedure might be unsafe or contraindicated.

## CONCLUSION

Besides visualizing for the first time in humans the exact position and extension of some of these functional receptor areas, including the bulbar AP, and demonstrating that the site of third ventriculostomy is a functionally active anatomic structure, these observations are important in that they confirm fluorescence as a new additional method that may help neurosurgeons with endoscopic orientation and may be an additional tool for safer navigation in complex cases. However, despite these initial anatomic observations, the clinical relevance of the technique for safer navigation in complex hydrocephalus has yet to be proved in larger series.

## Disclosure

The authors have no personal financial or institutional interest in any of the drugs, materials, or devices described in this article.

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