A special cranial nucleus (CSF-contacting nucleus) in primates

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DOI: 10.21203/rs.2.21682/v1

SUBJECT AREAS
Cellular & Molecular Neuroscience

KEYWORDS
CSF-contacting nucleus, primate, rhesus monkey, cerebrospinal fluid, XIII pair of cranial nucleus
Abstract

Background: In higher-order animal brains, twelve pairs of cranial nuclei are known, each performing specific functions. Past 30 years of our studies on rat brain reveal that there is a unique nucleus (CSF-contacting nucleus) in the brain. The extraordinary feature of this unique nucleus is that it is not connected to any substantial organ but to the CSF. The identification of this special nucleus will provide the structural basis for information transmission and functional modulation between the neurons and the body fluids.

Methods: The present study is based on the past 30 years of our relevant achievements. In this study, we used the primate rhesus monkeys as the model and injected the tracer CB-HRP into the CSF. After 48 h, the monkeys were perfused and the brain was dissected out, and sectioned for CB staining. The CB positive structures were observed under confocal and electron microscopy. The three-dimensional (3D) structure of the CB positive neurons cluster was reconstructed by computer software.

Results: Our results show that (1) CB labelling is confined within the ventricle, but not leakage into the brain parenchyma. (2) From the midbrain inferior colliculus superior border plane ventral to the aqueduct to the upper part of the fourth ventricle floor, a large number of CB positive neurons are consistently located, form a cluster, and are symmetrically located on both sides of the midline. (3) 3D reconstruction shows that the CB positive neurons cluster in the monkey brain occupies certain space. The rostral part is large and caudal part is thin appearing a “rivet”-like shape. (4) Under electron microscopy, the CB positive neurons show different types of synaptic connections with the non-CSF-contacting structures in the brain. Some of the processes stretch directly into the ventricle cavity.

Conclusions: The CSF-contacting nucleus is existed objectively in primates. It is also regarded as the XIII pair of cranial nucleus (CSF-contacting nucleus), which is specialised
in the communications between the nerve and the CSF. Our results are crucial for completing the neural structures and understanding the coordination of the entire body functions.

**Introduction**

With the advancement of neuroscience, it is discovered that in primates (including humans) and other higher-order vertebrates, there are twelve pairs of cranial nerves connected to the brain [1, 2]. The order and the names of these nerves are I. olfactory, II. optic, III. oculomotor, IV. trochlear, V. trigeminal, VI. abducens, VII. facial, VIII. vestibulocochlear, IX. glossopharyngeal, X. vagus, XI. accessory, and XII. hypoglossal. The corresponding cranial nuclei exist in the brain parenchyma. Most of the neuronal somas are located in the brainstem. These nuclei are regularly distributed, function separately, and work in a clear division [1, 2]. Based on their functions, these nuclei can be divided into motor and sensory. The motor nuclei are the site of origin of the cranial motor fibres and include the somatic motor nuclei and the visceral motor nuclei (parasympathetic nuclei). The sensory nuclei are the terminus of the cranial sensory fibres and include the somatic sensory nuclei and the visceral sensory nuclei. Similar to the 31 pairs of the spinal nerves, all of these nuclei connect to the organs (somatic or visceral). They are crucial for controlling the motor and sensory functions of the organs and provide a definite anatomical foundation of the central nervous system.

The animal body consists not only of solid organs, but also of fluids (such as extracellular fluid, plasma, and CSF). Physiology studies suggest that the levels and composition of the body fluids are also coordinated under the instructions from the brain. The components of the plasma, CSF, and the brain extracellular fluid are different and these fluids are physically separated from each other. How does the central nervous system relay the information to these fluids and functionally modulate their levels and composition remains
to be answered.

In the past 30 years [3], our studies revealed that after injecting peripheral nerve tracer CB-HRP into the rat ventricular system, the tracer is confined to the ventricular wall because of the existence of the brain barriers. The CB positive neuron cluster is always present at the ventral grey of the lower part of the aqueduct and the upper part of the fourth ventricle floor [3–6]. Only the neural processes that stretch into the CSF can be labelled. These neurons have a consistent location, form an independent cluster, occupy a certain space, and have a clear boundary with the nearby structures. The CSF-contacting neurons cluster is in accordance with the definition of the cranial nucleus. Therefore, we name it as the “cerebrospinal fluid-contacting nucleus” or “CSF-contacting nucleus” [6, 7]. Studies have confirmed that the CSF-contacting nucleus has broad synaptic and non-synaptic connections with the other neurons and the blood vessels, and has the morphology for sensing and modulating the body fluids [4, 8].

The discovery of this special structure not only brings new insights to the knowledge of the brain but also describes the “source” regulating the physicochemical property and biological composition of the CSF under different physiological or pathological conditions. Moreover, it provides the morphological evidence of systemic effects after interventions via body fluids. Compared with the already known twelve pairs of cranial nuclei, the CSF-contacting nucleus exclusively functions as a regulator of communication and functional modulation of the body fluids. The identification of this special nucleus will help to understand the structural foundation of the central nervous system-mediated control of the entire body (including organs and body fluids).

Based on the previous studies on the rat brain, the present study employed the primate rhesus monkey as the animal model. Our study is the first to describe the CSF-contacting neurons distribution, their nearby structures, 3D spatial morphology, and the synaptic and
non-synaptic transmissions between the CSF and the brain parenchyma in primates. The present study will provide scientific evidence and theoretical basis of the CSF-contacting nucleus in the primate’s brain.

Materials And Methods

1. Experimental animals
Adult male rhesus monkeys (average weight of 5.64 ± 2.10 kg) were acquired from the Kunming Primate Research Center of the Chinese Academy of Sciences. All the animal procedures were performed in accordance with the Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of the Kunming Institute of Zoology, Chinese Academy of Sciences. The animals were singly housed (0.80 × 0.80 × 0.80 m) in a controlled environment (temperature: 18–26 °C; humidity: 40–70%), with 12 h light/12 h dark cycle.

2. Tracer administration
Monkeys were anaesthetised with ketamine (10 mg/kg, i.m.), and the head was fixed on a stereotaxic instrument. 50 µl 30% CB-HRP (Sigma, United States), a specific tracer of the CSF-contacting nucleus through the ventricular system, was injected into the lateral ventricle according to the stereotaxic coordinates provided by Paxinos [9]. The successful injection was confirmed by extracting the CSF using a microsyringe. After 48 h, monkeys were perfused and sacrificed.

3. Perfusion and fixation
The animals were anaesthetised with ketamine (10 mg/kg, i.m.), the thoracic cavity was opened, and the pericardium was cut to expose the heart. A perfusion needle was inserted into the heart lateral ventricle. The animals were transcardially perfused with 2000 mL of 0.9% saline to wash out the blood. After, 4000 mL of 4% paraformaldehyde solution was
used for fixation. After perfusion, the brain and the spinal cord were dissected out and placed in a 4% paraformaldehyde solution for post-fixation for 48 h at 4 °C.

4. Tissue sectioning and immunofluorescence

After fixation, the brain and the spinal cord were immersed in a 30% sucrose solution until it sank to the bottom. Serial sections (50 µm thick) of the brain and the spinal cord were prepared using a freezing microtome (Leica, Germany). The sections were collected in PBS for immunofluorescence staining. The sections were incubated with anti-CB primary antibody (1:600 dilution, Abcam) at 4 °C for overnight, followed by incubation with donkey anti-goat Alexa Fluor 594 secondary antibody (1:200 dilution, Life Technologies). Some of the sections were retained for CB-HRP/NeuN double immunofluorescence staining. The CB stained sections were incubated with anti-NeuN primary antibody (1:50 dilution, CST) at 4 °C overnight, followed by incubation with donkey anti-rabbit Alexa Fluor 488 secondary antibody (1:200 dilution, Life Technologies). The sections were then mounted in sequence on the slides and counterstained with DAPI before covering with coverslips. The sections were observed and images were captured on a confocal microscope (Zeiss, Germany) using uniform parameters. After capturing the images, some sections were reused for Nissl staining to observe the neural morphology and Nissl body distributions.

5. CSF-contacting nucleus mapping and 3D reconstruction

The neurons in the CSF-contacting nucleus were mapped using the Adobe Illustrator software. The outline of the brain sections and the landmarks were illustrated and the neurons in the CSF-contacting nucleus were plotted. The brain serial sections and the CSF-contacting nucleus were 3D reconstructed using the Imaris software version 8.4.1 (Bitplane, USA).

6. Visualisation of the synaptic and non-synaptic structures of the CSF-contacting
nucleus in the monkey brain

The brain segment of the CSF-contacting nucleus was sliced using a vibratome (Leica, Germany) at 300 μm. CB-HRP staining was performed using the TMB-ST method [5, 10]. CSF-contacting nucleus and the ventricle wall were isolated. Transmission and scanning electron microscopy were used to observe the synaptic and non-synaptic connections. (1) Transmission electron microscopy: The samples were immersed in a 2.5% glutaraldehyde solution for overnight at 4 °C and then immersed into a 1% OsO4 solution for post-fixation for 2 h at 4 °C. After, the samples were dehydrated using serial ethanol and embedded in Epon 812 resin. Ultra-thin sections (60 nm thick) were cut using the ultramicrotome (Leica EM UC7), stained with 2% uranyl acetate and lead citrate, and observed under the electron microscope (JEM 1400 Plus, Japan). (2) Scanning electron microscopy: The samples were post-fixed in 1% osmic acid for 2 h. After, the sections were washed with distilled water three times (2 min each) and dehydrated using serial ethanol. The samples were then placed in isoamyl acetate and CO2 for critical point drying. The samples were placed on the stage using the conducting resin for spraying. The ultrastructures of the ventricle wall were observed using a scanning electron microscope (Teneo VS, USA).

Results

1. CB-HRP flow within the ventricular system and brain parenchyma labelling

(1) CB-HRP flow within the ventricular system

In Fig. 1, CB-HRP is represented as red fluorescence. The red-fluorescence was confined to the ventricular system and formed a clear outline of the lateral ventricle (LV), the third ventricle (3V), the aqueduct (Aq), the fourth ventricle (4V) of the brain and the central canal (CC) of the spinal cord. The nearby structures are not labelled suggesting that there
is no leakage of the tracer (Fig. 1).

(2) CSF-contacting nucleus labelling in the rhesus monkey brain

In the lower part of the ventral grey of the aqueduct and the upper part of the 4V floor of the monkey brain, a large number of CB positive neurons are located at consistent regions in the serial sections and form an independent cluster. The somas of these neurons appear to be fusiform or polygonal in shape and are easily distinguished from the nearby, unlabelled structures (Fig. 2). Thus, these CB positive neurons are cerebrospinal fluid-contacting neurons.

(3) Morphology of the CSF-contacting nucleus in the rhesus monkey brain under light microscopy

Immunofluorescence double labelling shows that the somas of the CSF-contacting neurons are co-labelled with NeuN, a neuron marker. Further, Nissl staining reveals that the neurons in the nucleus have abundant Nissl bodies. The staining is clear and the CSF-contacting neurons are brightly stained. The somas are slightly larger and the nucleolus is clear and obvious (Fig. 3).

2. Position and adjacency of the CSF-contacting nucleus in the rhesus monkey brain

Similar to the distribution of the CSF-contacting nucleus in rat, the nucleus in the monkey brain is located at the ventral grey of the lower part of the aqueduct and the upper part of the fourth ventricle floor. The rostral part of the CSF-contacting nucleus is deep in the brain parenchyma and begins ventrally to the decussation of the superior cerebellar peduncle (xscp). The nearby structures are the median raphe nucleus (MnR), medial longitudinal fasciculus (mlf), periaqueductal grey (PAG), and dorsal raphe nucleus (DR). The core of the CSF-contacting nucleus is symmetrical and ventral to the aqueduct. In this plane, the nucleus is near to mlf, DR, and PAG. The caudal part of the nucleus is located in
the ventral grey of the 4V floor, near to the dorsal tegmental nucleus (DTg) and central grey (CG) (Fig. 4)

3. Three-dimensional reconstruction of the CSF-contacting nucleus in the rhesus monkey brain

The three-dimensional morphology of the CSF-contacting nucleus in the monkey brain was reconstructed using Imaris software. It shows a clear spatial morphology within the brain with definite spatial boundaries and specific location. The nucleus exists independently and consistently between the inferior segment of the midbrain and the superior segment of the pons. The spatial morphology appears as a rivet-like shape (Fig. 5).

4. Ultrastructure of the CSF-contacting nucleus axons, terminals, non-synaptic and synaptic connections in the rhesus monkey brain

(1) The axon and the terminal of the CSF-contacting nucleus under electron microscopy

The injected CB-HRP it is transported along the axons of the CSF-contacting neurons. The CB-HRP electron-dense areas can be detected under electron microscopy and the CB positive axons are covered by a thick myelin sheath. The CB-HRP negative axons show no electron-dense areas. The CSF-contacting neuron terminal contains synaptic vesicles, which are round and flattened. Some of the vesicles have a dense core (Fig. 6).

(2) Non-synaptic connections of CSF-contacting nucleus with CSF

The surface of the brain ventricle was observed under the scanning electron microscope. Under the flagellum of the ependymal cells surface, numerous nerve fibres are visible. These nerve fibres have many inflated structures on the segments or terminals. Using transmission electron microscopy, we observed that the nerve fibres are the CB-HRP positive terminals of the CSF-contacting nucleus, which stretch into the ventricular system.
and contact the CSF forming the non-synaptic connections. The terminals contain many vesicles (Fig. 7).

(3) Synaptic connections of the CSF-contacting neurons with the brain parenchyma

The terminal of the CSF-contacting neurons can be a pre-synaptic structure and form Gray I type excitatory (asymmetrical) or Gray II type inhibitory (symmetrical) synapses. Meanwhile, the terminals can also be post-synaptic structures and receive input from other neurons by Gray I or Gray II synaptic forms (Fig. 8).

Discussion

1. Identification of the CSF-contacting nucleus in primates

Our previous studies [4, 6] in rodents confirmed that after CB-HRP injection into the CSF of the unilateral ventricular system, it can only flow to the contra-lateral LV, 3V, Aq, 4V, CC of the spinal cord and subarachnoid space. The tracer is localised on the walls of these structures and forms a clear outline. The present study in rhesus monkey confirms that the CB-HRP can only flow within the ventricular system and cannot pass through the CSF-brain barrier and leak into the brain parenchyma. Thus, the neurons whose processes stretch into the CSF can be labelled by CB-HRP. Therefore, the CB positive neurons observed in our study can only be the CSF-contacting neurons.

The criteria for naming cranial nuclei are as follows [11-13]: (1) Similar morphology and functions: In the present study, all of the CB positive neurons are CSF-contacting neurons whose functions are specified for information connection between the brain and the CSF. (2) Similar location and occupy certain space: The CB positive neurons are located in the ventral grey of the aqueduct and 4V floor, form an independent cluster and are significantly different from the nearby structures. The three-dimensional reconstruction
results show that the entire morphology of the nucleus presents “rivet-like” shape and occupies a certain spatial volume. (3) The distribution of these nuclei has an evolutionary similarity: In both, rodents as well as in primates, the CSF-contacting nucleus always initiates ventral of the xscp and at the ventral grey of the 4V floor. The core of the nucleus is always located at the ventral grey of the Aq and the nearby structures are always DR, PAG, and mlf. The CSF-contacting nucleus in the monkey brain is larger than that in the rats, however, the inner structures are similar. The morphology of the nucleus is highly homologous between the two species [9, 14]. Therefore, we have sufficient evidence suggesting that the CSF-contacting nucleus is existed objectively in primates.

2. **The CSF-contacting nucleus is regarded as the XIII pair of cranial nucleus**

The ancient Greek scientist Gelen (129-200 AD) was the first to describe cranial nerves and summarised seven pairs of cranial nerves [1]. Soemmerring (1755-1830, Germany) supplemented the concept and numbered the cranial nerves from I to XII, which is still in use [1, 2]. With the development of imaging techniques and microscopy, the distribution and location of the cranial nuclei in the brain parenchyma corresponding to each pair of the cranial nerves have been confirmed, and the “concept” of XII pairs of cranial nuclei is accepted. Most of these nuclei are located in the brainstem. The nerve fibres go in or out through the outer brain surface and have definite functions and clear division. For example, the midbrain oculomotor nucleus sends out the third pair of oculomotor nerves to control the extraocular muscle movement. The hypoglossal nucleus in the medulla oblongata sends out hypoglossal nerves to control the tongue movement. The trigeminal nucleus in pons receives sensory inputs from the head and the face. The common features of the XII pairs of cranial nuclei are that the neural somas are located in the brain (mainly in the brainstem), and the processes connect with the organs (somatic or visceral). They perform specific functions and are the anatomical foundations of the central nervous
system modulating the activity of the organs.

The animal body is composed of not only organs, but also includes fluids such as extracellular fluid, plasma, and CSF. It is believed that the body fluids are controlled by the instructions from the brain; however, presently there is no description of any structure in the central nervous system that directly modulates the body fluids. Due to the lack of information regarding this structure, it is difficult to address why the type and content of the substance in the CSF changes, and the "lumbar anaesthesia" via CSF has higher effects such as nausea and vomiting.

The past 30 years of our studies in rodents and our present study in the monkey brain confirm that similar to the XII pairs of cranial nuclei, the CSF-contacting nucleus is located in the brainstem; however, its processes cross through the brain-CSF barrier and contact the CSF in the ventricular system. Our concept of the CSF-contacting nucleus (XIII pair of cranial nucleus) which specifically connects with the body fluids will help to bridge the gap in understanding the CNS mediated regulation of the body fluids regulation. The twelve pairs of cranial nuclei are connected to the organs and modulate sensory or motor functions. However, the XIII pair of cranial nucleus (CSF-contacting nucleus) connects and regulates the body fluids. The concept of the CSF-contacting nucleus is important and necessary for understanding the anatomical structures of the CNS as well as for understanding the coordination of the functions in life activities.

3. **Role of XIII pair of cranial nucleus (CSF-contacting nucleus) in body fluids regulation**

In the central nervous system, the synaptic structures are the key for information transmission between the neurons [15]. We used the well-established method of CB-HRP labelling to specifically label CSF-contacting nucleus in combination with electron microscopy [4, 5] and found that the CB-HRP positive axons are covered with a myelin
sheath. The vesicles in the axon terminals are round, flattened, polygonal, and contain dense cores. It has been confirmed that the round vesicles mainly contain excitatory neurotransmitters [16, 17], flattened vesicles mainly contain inhibitory neurotransmitters [18, 19] and the dense core vesicles mainly contain protein or peptide neurotransmitters [20]. Therefore, the neurons in the CSF-contacting nucleus participate in functional modulation via releasing excitatory, inhibitory, or peptide neurotransmitters.

Using scanning and transmission electron microscopy, we confirmed that the CB-HRP positive nerve fibres stretch into the ventricular system and contact the CSF. The nerve fibres not only have local expansions but also contain many vesicles. This unique morphological feature suggests that the CSF-contacting nucleus regulates the release of biological substances into the CSF. The nerve fibres can sense the physicochemical properties and changes in the CSF and transmit the information to the brain via different types of synapses.

According to the classic synaptic transmission theory [15], the information is transmitted from the pre-synaptic component to the post-synaptic component. In the brain parenchyma, we observed that the neurons in the CSF-contacting nucleus form both pre-synaptic and post-synaptic components. Besides, the CSF-contacting nucleus forms both Gray I type excitatory synapse as well as Gray II type inhibitory synapse. According to the diversity of the synapse, complicacy of the neurotransmitters, and bidirectional information transmission of the CSF-contacting nucleus, it is inferred that the nucleus participates in the complex neural circuits and is involved in the fine regulation of life activities. Due to the special structure of the CSF-contacting nucleus, we can further speculate that under certain physiological or pathological situations, the CSF-contacting nucleus nerve fibres can release biological compounds into the CSF to alter its composition. By sampling and detecting the changes in the CSF, the auxiliary diagnosis
can be achieved. On the contrary, when the chemical components of the CSF change, the CSF-contacting nucleus can sense the alterations and send the information to the non-CSF-contacting structures in the brain parenchyma via different types of synapses. Hence, medical interventions via the CSF might be developed as efficient therapeutic methods. The present study provides strong morphological evidence to unveil the existence of a cranial nucleus involved in regulating the CSF.

**Conclusion**

In summary, the CSF-contacting nucleus exists in the higher-order primate brains. It can be regarded as the XIII pair of cranial nucleus, which plays a pivotal role in neural-body fluids communication and regulation. The establishment of the thirteenth cranial nucleus, the CSF-contacting nucleus, provides powerful morphological evidence to complete the concept of cranial nuclei. Furthermore, the study is important as it provides an understanding of the coordinating process that unifies the entire body functions.

**Abbreviations**

CSF: cerebrospinal fluid; CSF-contacting nucleus: cerebrospinal fluid contacting nucleus; CB-HRP: Cholera toxin B subunit conjugated to peroxidase; CB: Cholera toxin B subunit; 3D: three-dimensional; LV: lateral ventricle; 3V: 3rd ventricle; Aq: aqueduct; 4V: 4th ventricle; CC: central canal; xscp: decussation of superior cerebellar peduncle; MnR: median raphe nucleus; mlf: medial longitudinal fasciculus; PAG: periaqueductal grey; DR: dorsal raphe nucleus; DTg: dorsal tegmental nucleus; CG: central grey.

**Declarations**

**Acknowledgements**

Not applicable.

**Authors’ Contributions**
SS and LZ designed the study and prepared the manuscript. SS, YL, XZ, LL, Y-HL, CB, CS, JH, and JC conducted the studies. All authors read and approved the manuscript.

**Funding**

This research is supported by the National Natural Science Foundation of China (Grant Nos. 81371243 and 81901131), the Natural Science Foundation of Jiangsu Province (Grant No. BK20190987) and Chinese Postdoctoral Science Foundation (Grant No. 2018M642328).

**Availability of data and materials**

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

**Ethics approval and consent to participate**

All the animal procedures were performed in accordance with the *Guide for Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee (IACUC) of the Kunming Institute of Zoology, Chinese Academy of Sciences.

**Consent for publication**

Not applicable.

**Conflicts of interest**

The authors declare no competing financial interests.

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Figures
Figure 1

CB-HRP flow in the ventricular system. (A) Schematic of the lateral ventricle injection (B–E) CB-HRP immunofluorescence (red) on the ventricular wall. Bar = 0.5 mm (B–E) and 0.1 mm (F). LV, lateral ventricle; 3V, 3rd ventricle; Aq, aqueduct; 4V, 4th ventricle; CC, central canal.
CB positive neurons of the CSF-contacting nucleus in the parenchyma of the rhesus monkey brain. (A) Schematics of the serial sections, (B) CSF-contacting nucleus in the serial sections. 10× is the lower magnification. 20× and 40× are the higher magnification of the boxed regions. Aq: aqueduct. Bar=200µm in 10×, Bar=50µm in 20×, Bar=20µm in 40×.
Figure 3
Morphological features of the CSF-contacting nucleus in the rhesus monkey brain under light microscopy. (A-D) Neurons in the CSF-contacting nucleus (red), neuronal marker-NeuN (green), and DAPI (blue) are located in the same neuron (yellow). (E-F) The CSF-contacting nucleus (E) and its corresponding Nissl staining (F). Bar=50 μm.
The position and adjacency of the CSF-contacting nucleus in the rhesus monkey brain. (A-L) The neurons in the CSF-contacting nucleus are presented as red dots (arrow). The CSF-contacting nucleus position and adjacent structures are shown. (Aq: aqueduct, PAG: periaqueductal grey, DR: dorsal raphe nucleus, mlf: medial longitudinal fasciculus, xscp: decussation of superior cerebellar peduncle, MnR: median raphe nucleus, 4V: the 4th ventricle, DTg: dorsal tegmental nucleus, CG: central grey)
Three-dimensional morphology of the CSF-contacting nucleus in the rhesus monkey brain from different perspectives. (A) Anterior view; (B) Lateral view; (C) Ventral view; (D) Posterior view.
The ultrastructure of the CSF-contacting neuron's axon and terminal in the rhesus monkey brain. (A, B) CB-HRP positive CSF-contacting neuron axon (A), the non-CSF-contacting neuron does not contain positive electron-dense deposits (B). (C, D) CB-HRP positive CSF-contacting neuron terminal contains abundant neurotransmitter vesicles (C), D is the local higher magnification of C. Bar=200 nm.
Figure 7

Non-synaptic connections of the CSF-contacting neuron with CSF in the rhesus monkey brain. (A) Scanning electron microscopy reveals that a large number of neural fibres (blue) with local varicosity (↑) and bouton (▲) are present on the surface of the ventricle wall. (B) Transmission electron microscopy reveals the CB-HRP positive CSF-contacting neuron terminal (blue) stretch into the ventricle and contacts the CSF. Bar=200 nm in A, Bar=500 nm in B.
Synaptic connections of the CSF-contacting neuron (blue) with the non-CSF-contacting neuron (red) in the rhesus monkey brain. (A) The asymmetrical synapse of the CSF-contacting neuron→non-CSF-contacting neuron (↑); (B) Symmetrical synapse of the CSF-contacting neuron→non-CSF-contacting neuron (▲); (C) Both asymmetrical (↑) and symmetrical (▲) synapse; (D) Asymmetrical (↑) and symmetrical (▲) synapse of the non-CSF-contacting neuron→CSF-contacting neuron. Bar=200 nm.
