

Signals that go with the flow

Cerebrospinal fluid (CSF) is to the brain what the Great Lakes and waterways are to North America: a system of interconnected fluid spaces that influence the local climate and the environment of the territory that encloses them, while providing a conduit for traffic in goods and services. It is this latter aspect (signaling from the CSF to the brain and vice versa) that was the topic of a one-day meeting organized by Rae Silver (New York, NY, USA) and Mike Lehman (Cincinnati, OH, USA)*.

Fluid spaces of the brain

To appreciate the potential of the CSF as a medium for carrying information, one must know the anatomy and flow patterns of the fluid spaces. Mike Bradbury (London, UK) described how the CSF is contained in a system of ventricles and subarachnoid spaces over the major brain surfaces. The 3D representation of these spaces is complicated, but Fig. 1 shows a section through the whole system¹. In humans, the total cranial volume of CSF is about 140 ml with some 115 ml located in the subarachnoid space. An additional 75 ml surrounds the spinal cord. The major site of CSF production is the choroid plexus, a vascular expansion found in the third, fourth and lateral ventricles. In living animals, this highly vascularized choroidal epithelium resembles bright-red seaweed! Total production of CSF in humans is about 500 ml in 24 h so the total volume of CSF turns over two to three times per day, which could be important when considering the termination of the actions of signals².

The CSF circulates in a regular manner that enables it to carry chemical information in a predictable way. From the lateral ventricles, CSF passes into the third ventricle and then moves on to the fourth ventricle. The small volume of the latter two ventricles ensures a relatively fast flow. Cerebrospinal fluid leaves the ventricular system at the level of the medulla oblongata and enters the subarachnoid space, where it ascends the lateral convex and medial surfaces of the cerebral hemispheres, and eventually flows downward into the space that surrounds the spinal cord. Ultimately, CSF drains into venous channels of the subarachnoid space via arachnoid villi. This circulation is caused by pressure waves that are generated by pulsatile arterial blood flow and brain expansion, and by pressure gradients that result

*Workshop on the CSF as a communication pathway of the brain. Held in Los Angeles, USA; 7 November 1998.

from the production and absorption of CSF. Fast magnetic-resonance imaging has visualized these flow patterns³. Cilia on the ependymal cells that line some of the ventricles could help to move the CSF.

Are there sources of CSF other than that produced by the choroid epithelia? Joan Abbott (London, UK) explained that if such sources existed, they were likely to be the blood vessels of the brain, which might produce 10% of total CSF. However, such production implies that fluid must flow through the brain parenchyma where the interstitial fluid is distinct from the CSF. The likely conduits for such flow are the Virchow–Robbins spaces around major vessels. This confined flow could have a relatively high velocity in a particular direction and could carry signals from one region to another. The late Helen Cserr and her colleagues provided evidence of this by showing that markers of very different molecular weights were removed from the brain with similar rate constants⁴.

Fluid–brain interfaces

Just as the shores of a lake are the sites of crucial interchange between the body of water and the land, so the interfaces of the CSF system and the brain have a crucial role in any discussion of signaling. The ventricles are lined with a layer of non-neuronal cells, the ependyma. Milton Brightman (Bethesda, MD, USA) noted that horseradish peroxidase (43 kDa) and ferritin (560 kDa), could enter brain tissue from the ventricles, which indicates that the spaces between ependyma were generally not closed by tight junctions. Many ependyma are coupled through gap junctions and so have potential for intracellular signaling.

Although some receptors for chemical signals probably are found on cells located in the walls of the ventricles, many signals will have to penetrate further into the brain to reach their targets and signals that originate within the brain parenchyma must make their way to the CSF. Charles Nicholson (New York, NY, USA) discussed the constraints encountered by molecules within the brain. Although the interstitial flow discussed by Abbott could have a role, the most ubiquitous transport process is diffusion. In water, diffusion of a molecule obeys Fick's Laws and is governed by the diffusion coefficient, D . In brain tissue many molecules are confined to move in the extracellular space (ECS), which has a foam-like geometry. Surprisingly, within this space, Fick's Laws are still obeyed, but D is replaced by an

apparent diffusion coefficient, D^* , the value of which is about two-fifths that of D , so that it takes longer for molecules to move between locations in brain than in water. Owing to the reduced volume of the ECS, now known to constitute about 20% of total brain volume, released substances become more concentrated in this confined space. Molecules up to several hundred kiloDaltons can diffuse through the ECS (Ref. 5).

In some cases the degradation or uptake processes dominate the transport of molecules in brain tissue. Margaret Rice (New York, NY, USA) described dopamine movement as a model of an uptake-limited transport process. Experiments in the rat striatum show that uptake into presynaptic terminals limits the spread of dopamine unless the amount released saturates the uptake system when overflow of dopamine into the ECS occurs. By contrast, in regions such as the substantia nigra and ventral tegmental area, where dopamine is released at soma–dendritic, extrasynaptic sites, the spread was controlled by diffusion. Rice mentioned experiments from Ralph Adams' laboratory⁶ showing that, after electrical stimulation of the substantia

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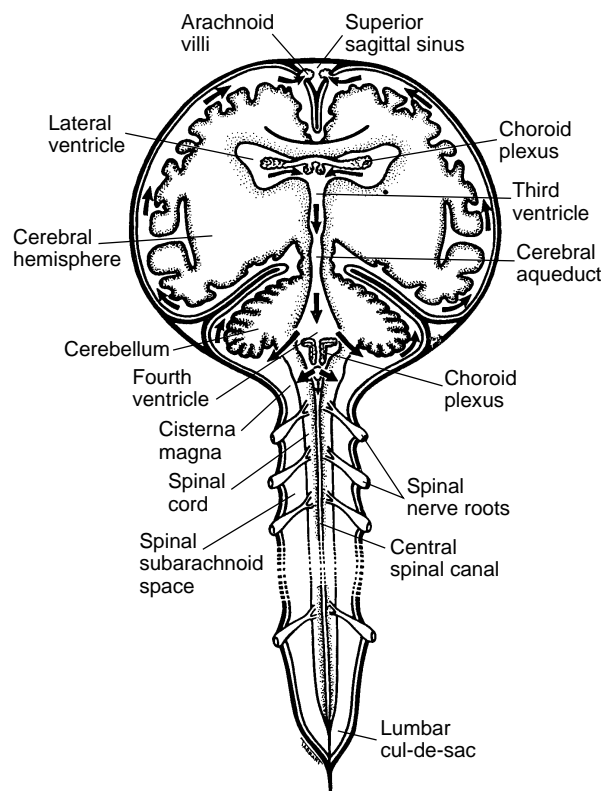


Fig. 1. The circulatory pathway of cerebrospinal fluid (CSF; arrows) in the brain and spinal cord. Most of the CSF is produced in the ventricles and enters the subarachnoid space at the base of the brain. Cerebrospinal fluid drains away mainly through the arachnoid villi into the dural venous system. Adapted, with permission, from Ref. 1.

nigra, homovanillic acid (HVA), a dopamine metabolite, appeared abruptly in the CSF, but with a delay of between 15 and 60 min.

Much of our knowledge about the way in which substances move from ventricles to brain has been obtained by Joe Fenstermacher (Detroit, MI, USA) and colleagues. These researchers have shown that there are four classes of material, which are identified according to the way they behave in the brain after leaving the CSF. Some substances, such as EDTA, do not cross the blood–brain barrier (BBB) and remain confined to the ECS, where they obey the diffusion laws outlined by Nicholson. Other substances, such as mannitol and methotrexate, are mainly confined to the ECS but show moderate cellular uptake, while substances such as hydroxyurea enter cells readily, but cross the BBB slowly and might behave in the same way as dopamine in the striatum. Finally, substances such as cycloleucine and thiopeta both enter cells and are then removed across the BBB (Ref. 7).

Fenstermacher also revealed that after infusion of radiolabeled sucrose into one lateral ventricle, the sucrose flowed throughout the ventricular system and into various subarachnoid spaces within a matter of minutes, but moved surprisingly slowly over the cerebral and cerebellar cortices. The sucrose also diffused freely into the brain over many interfaces but was restricted at others, such as the circumventricular and subfornical organs. These results indicate the importance of understanding regional differences in transport characteristics.

Targets at the interface looking for signals

The hypothalamus is adjacent to the ventricles, which also contain specialized structures such as the circumventricular organs. Other areas at the interface have cells with a specialized morphology. These structures all seem to be potential receptors for signals or donors of substances.

Bob Moore (Pittsburgh, PA, USA) showed that the way in which molecules crossed the interfaces in predictable patterns suggested specificity of CSF-mediated signals. When cholera toxin, β subunit (11.5 kDa) and FluoroGold (0.5 kDa) were injected into the lateral or third ventricle of the rat, neurons were labeled in the medial hypothalamic regions while ependymal cells were labeled in the circumventricular organs. Neurons of the dorsal raphe were also labeled, which probably reflected uptake by axons in the supraependymal plexus. Moore also drew attention to the presence of dopaminergic and serotonergic nerve terminals located in the CSF, which must surely release their

contents there, although the consequences of such release would depend on whether the local CSF moved with a systematic flow or random eddies.

These data raised the issue of whether the cells at the interface might show specializations that indicate a role in the sampling of the ventricular environment. Béla Vigh (Budapest, Hungary) explained that the system of CSF-contacting neurons represented a very ancient system. In the seawelling proto-chordate, *Branchiostoma lanceolatum*, the central canal is open to the seawater. Later in evolution, as the canal and ventricles became sealed off from the outside environment, the CSF-contacting cells were retained and sampled the internal environment, even in humans. Many CSF-contacting cells have ciliated dendrites that resemble the mechanoreceptors of the lateral line system. Maria Manzano e Silva (Lisbon, Portugal) noted that some CSF-contacting neurons of the hypothalamus contain opsin and can be classified as 'deep encephalic photoreceptors'. Indeed, retinal and pineal photoreceptors possess features in common with CSF-contacting neurons⁸.

In addition to specialized cells embedded in the interface, Patrick Card (Pittsburgh, PA, USA) said that there were entire intraventricular neuronal networks. They are found on the floor of the third ventricle where these supraependymal cells form dense clusters. Some axonal processes ramify and terminate within the ventricle but others proceed through the ependymal layer to unknown targets within the brain.

Signals at the interface looking for targets

There is much evidence for significant levels of neuroactive substances in the CSF as well as targets for these substances. Jim Krueger (Pullman, WA, USA) addressed the search for some of the most tantalizing putative CSF signaling molecules, namely the substances responsible for sleep. In classic experiments, Pappenheimer had transferred CSF from sleep-deprived goats to rats, which became sleepy as a consequence, but the attempt to isolate a single factor responsible for sleep⁹ has been unsuccessful. It seems that many compounds are involved in causing sleep. One well-established candidate is interleukin 1 β (IL-1 β), a compound that increases non-rapid eye movement (non-REM) sleep. Furthermore, IL-1 β levels increase with sleep deprivation. IL-1-receptor knockout animals do not respond to IL-1 β , however they do respond to tumor necrosis factor α , which shows that substances other than IL-1 are involved in sleep¹⁰.

To demonstrate the controversies of current sleep research, Miles Herkenham

(Bethesda, MD, USA) questioned the data on IL-1-receptor localization in brain. He then discussed his own work showing that intravenous injection of IL-1 β produces an increase of *Fos* expression in non-neuronal cells in the arachnoid plexus, blood vessels and choroid plexus. After 1 h this pattern disappeared but at 3 h another pattern of activation appeared in regions more deeply inside the brain. The circumventricular organs also showed a characteristic temporal and spatial reaction pattern. Thus, barrier cells can transduce IL-1 signals in widespread brain areas by means of a secondary diffusing signal¹¹.

These examples highlighted the relationship between CSF signaling and volume transmission¹². Anders Jansson (Stockholm, Sweden) described current concepts of volume transmission and mentioned experiments¹³ showing that β -endorphin could spread far from its release site and could enter the CSF where it was transported to its targets.

Do the contents of the CSF alter behavior?

The final approach to the issue of signaling from the CSF took a more global perspective, which sought to correlate the levels of neuroactive substances in CSF with changes in behavior. A fundamental behavior is feeding. Satya Kalra (Gainesville, FL, USA) reported that an increase in levels of neuropeptide Y (NPY) in the paraventricular nucleus was associated with an increased appetite for food. An enhanced appetite was also apparent when NPY was administered to the lateral or third ventricles. These data support a role for peptides in the ventricles in the mediation of behavior.

The theme of diffusible factors in juxtaventricular locations and their role in reproduction was taken up by Donal Skinner (Nouzilly, France). He believes that understanding what controls the release of gonadotropin releasing hormone (GnRH) is crucial to understanding reproduction itself. Recent work by Skinner and colleagues had found that GnRH could be detected in the CSF of the ewe. The appearance was pulsatile and coincided with the estradiol-induced luteinizing-hormone surge, which accurately reflects the changes measured in hypophyseal portal blood¹⁴. The consequences of the presence of GnRH in CSF are unknown.

Reproduction is an immensely complex behavior and it can be expected that a wealth of chemical mediators will be involved. Benoit Malpoux (Nouzilly, France) stated that melatonin was involved in reproductive behavior via its actions in the hypothalamus in the pre-mammillary area. Melatonin could reach the hypothalamus

through a circuitous vascular pathway, but of more interest to the meeting was the idea that melatonin could act more directly through the third ventricle. These two pathways are not exclusive. Melatonin concentrations are 20-times higher in the third ventricle than in the jugular plasma and 100-times higher than in the carotid-artery plasma. The melatonin concentration in the CSF of the lateral ventricles is less than half of that in the third ventricle, which suggests that melatonin enters at the third ventricle. The simplest explanation of these facts is that melatonin is secreted and diffuses from the pineal gland into the third ventricle, creating a large concentration gradient between it and the surrounding neural tissue. There is evidence that melatonin receptors are widely distributed around the ventricles¹⁵.

In a final example of an important behavior where CSF signals are implicated, Lehman and Silver described their studies on 24 h circadian rhythm. Numerous experiments on the golden hamster, which involved ablation and transplantation, established that the clock is located in the suprachiasmatic nucleus (SCN). Moreover, the transplantation of an SCN from a mutant donor that has a different clock period can change the circadian rhythm in the host. Lehman and Silver asked whether SCN transplants communicated with host via a diffusible signal. In the hamster, the addition of encapsulated SCN cells¹⁶ was sufficient to restore the circadian rhythm. When the implanted capsule or graft was in the ventricle, the target had to be downstream of the source and efficacy varied depending on the separation between source and target. These results all pointed to a diffusible factor being responsible. Questions that remained were whether an endogenous factor in the ventricles was involved in the circadian rhythm and where the sites of action of the factor were in the brain.

The correlation of CSF composition and behavior has long been a mainstay of clinical practice when a lumbar puncture is used to extract CSF to diagnose an illness, particularly psychiatric disorders. Thomas Geraciotti (Cincinnati, OH, USA) warned that many conclusions were invalid because the sampling procedure perturbs the system. When performed correctly,

however, interesting new correlations of importance for psychiatric diagnosis emerge, for example the levels of 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT, and HVA co-vary with time.

Concluding remarks

The presentations at this meeting left no doubt that many neuroactive substances could be detected in the CSF and that many receptors for substances were located in the vicinity of the CSF system. But are these substances just by-products that spill over into the ventricles after accomplishing their function in the brain? Are they a useful means to eavesdrop on activities elsewhere but of no functional consequence themselves? Do the hydrodynamics of CSF flow disrupt the movement of these signals or are they real signals that make use of the characteristics and flow patterns of the ventricular-subarachnoid spaces to distribute information in a unique pattern? An underlying theme of the meeting was how to distinguish between a signal and a by-product. This discussion continued after the meeting over the Internet. Two opinions emerged: those in the neuroendocrine field favored a comprehensive but complex set of criteria, while those working with neurotransmitters favored simplicity. The criteria listed below lean towards simplicity and reflect input from Kruger, Skinner, Moore, Bradbury and Silver. Silver divided the issue into two parts:

(1) Necessary and sufficient criteria must be met to confirm the existence of a signal in a large fluid volume. It should be shown that the removal or replacement of the signaling substance results in a change in the response being controlled and an assay of the substance should indicate that it is present or increases, or both, in a well-defined temporal relationship to the response (and similarly declines when the response disappears).

(2) Evidence must be obtained that a fluid compartment is the conduit for a diffusible or transported signal. The signal must have access to and enter the compartment and the fluid dynamics and turnover in the compartment should allow appropriate movement of the signal.

In this meeting these criteria were applied to the CSF but they would equally

apply to the interstitial fluid of the brain, in the context of volume transmission, and to the blood. Indeed, these latter compartments could compete with the CSF as communication channels. In all cases the way in which the fluid moves is an important variable.

Silver contributed a final summary. For a number of physiological systems, there is excellent evidence for the existence of a diffusible signal in a large fluid volume (for example, sleep signals, GnRH, melatonin, circadian signals). In each of these instances the data meet the criteria for the first part above. For the criteria of the second part, much, but not all, of the necessary evidence has been gathered for these systems. Overall, it seems that there is already evidence of a signaling, rather than an eavesdropping, function for CSF in several systems. It is still necessary to meet all the above criteria within a single system. The CSF system seems appropriate for further study with new techniques and seems likely to provide a model for diffuse chemical signaling in the whole nervous system.

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