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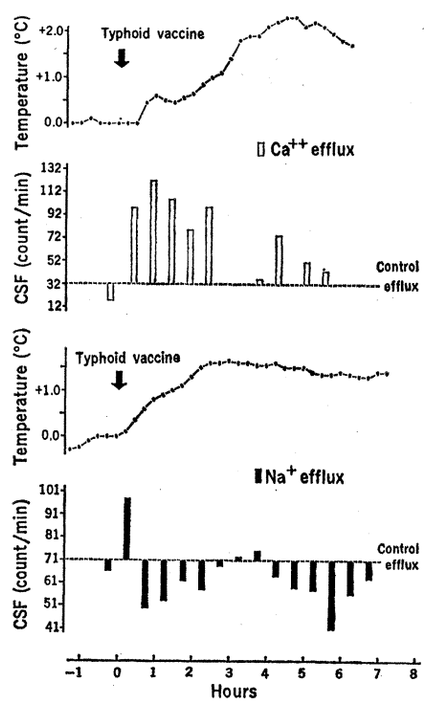
Fever: Reciprocal Shift in Brain Sodium to Calcium Ratio as the Set-Point Temperature Rises

Abstract. *A bacterial pyrogen acts on the brain by disturbing the natural balance between two essential cations in the cerebral region involved in thermoregulation. After typhoid vaccine is administered to the unanesthetized cat, $^{45}Ca^{2+}$ efflux into the third cerebral ventricle increases while $^{22}Na^{+}$ is retained in hypothalamic tissue at the same time that the set-point temperature begins to rise. The subsequent rates of $^{22}Na^{+}$ and $^{45}Ca^{2+}$ efflux parallel the course of the bacterial fever but in a reciprocal fashion. This supports the theory that a change in the set-point temperature is determined by an alteration in the inherent ratio of Na^{+} to Ca^{2+} ions in the hypothalamus.*

An upward shift in the set point for body temperature is a general part of the sequelae associated with a fever of bacterial origin (*1*). Although the cerebral mechanism by which the response to a pathogen is mobilized is unknown, it has been postulated that a pyrogen acts on the posterior hypothalamus to alter the inborn ratio of sodium to calcium ions (*2*). This theory was based on the finding that an intense hyperthermia develops if the endogenous Na^{+} is artificially increased or Ca^{2+} is lowered by a chelating agent in this hypothalamic region. If the theory is correct, either in vivo Ca^{2+} would decline or Na^{+} would increase during a bacterial fever, or both events should occur simultaneously within hypothalamic tissue.

In an anesthetized animal, there is some evidence that a pyrogen may briefly reduce the concentration of Ca^{2+} at some sites in the hypothalamus (*3*) just as systemically administered endotoxin does in serum (*4*). We now report that a reciprocal change in the ratio of the concentrations of endogenous Na^{+} to Ca^{2+} occurs within the brainstem of the conscious cat as a fever develops. Moreover, this ionic ratio parallels the characteristics of the rise in the body temperature of the animal.

In each of three cats, a stainless steel guide tube was implanted aseptically 2 mm above each lateral ventricle according to procedures in (*2*, *5*). After 7 to 10 days, the brain tissue and fluid in the region of the hypothalamus was labeled with either 6 to 8 μc of $^{45}Ca^{2+}$ or of $^{22}Na^{+}$ (New England Nuclear) by injecting the nuclide into the lateral cerebral ventricle. To do this, we connected a length of polyethylene tubing (PE-50) to a 23-gauge, thin-walled injection cannula filled with pyrogen-free, artificial cerebrospinal fluid (CSF) (*6*) containing 0.1 ml of the particular radionuclide. The cannula was then passed through the right guide tube into the ventricle, and after the radioactive solution flowed in, 0.1 ml of additional artificial CSF was infused.



To perfuse the third ventricle of the unanesthetized cat, we modified a procedure used for the monkey (*7*). A 23-gauge, thin-walled, stainless steel cannula was inserted through the guide tube into each lateral ventricle. The right cannula was connected by means of PE-50 tubing to a Harvard variable-speed infusion pump and was used as the inflow, whereas the contralateral cannula served as the outflow (*7*). To collect a sample of CSF, the infusion pump was turned on at a rate of 0.1 ml/min, and the tubing, connected to the left ventricular cannula, was held 5 to 10 cm below the level of the ventricle allowing the CSF to flow out.

The initial portion of the effluent, determined by the volume of nonradioactive CSF in the tubing, was discarded and approximately 0.2 to 0.3 ml of the subsequent perfusate was collected for the determination of radioactivity. A 100-μl portion of each CSF sample was added to a counting vial filled with 15 ml of a scintillation solution containing reagent grade toluene and 2-ethoxyethanol in a 1 to 1 ratio plus 0.8 percent (weight per volume) of scintillation grade 2,5-diphenyloxazole. The samples were then counted for 10 minutes in a Packard 3320 Tri-Carb liquid scintillation spectrometer with automatic external standardization.

During the first 2 hours, two to four samples were collected at 30-minute intervals until the level of radioactivity had stabilized. Then, the inflow cannula was removed and replaced by a similar cannula that was connected to PE-50 tubing filled with a 1 : 4 dilution of *Salmonella typhosa* containing 10^{9} organisms per milliliter (*8*). A volume of 0.2 ml of the diluted pyrogen solution was injected; this amount produces a profound hyperpyrexia in the cat when administered by this route (*9*). Immediately thereafter, the inflow perfusion cannula was inserted again into the right ventricle and CSF samples were again collected at 30-minute intervals throughout the development of the fever.

Fig. 1. Fevers produced by 0.2 ml of diluted typhoid vaccine injected into the lateral cerebral ventricles of two cats at zero hour. Each bar represents a 3- to 4-minute perfusion of the third cerebral ventricle. The efflux of $^{45}Ca^{2+}$ (top) and $^{22}Na^{+}$ (bottom) into CSF is expressed in counts per minute. The value of the sample 1 hour before the typhoid injection served as the baseline (control) after the rate of efflux in each experiment had become stabilized.

Table 1. Mean percentage change in radioactivity (± the standard error) in CSF samples collected at 30-minute intervals beginning 15 minutes after the injection of the pyrogen. Nothing was injected into the controls. The increase in Ca^{2+} or the decrease in Na^{+} for the animals that received the pyrogen was based on an average of three or four determinations for each sample and compared to the baseline concentration for each ion. For changes of the ions in control animals, one determination was made for each sample and compared to the baseline. Temperature change during each interval (ΔT) was also determined.

Time (min)	$^{45}Ca^{2+}$		$^{22}Na^{+}$	
	Increase (%)	ΔT (°C)	Decrease (%)	ΔT (°C)
		Pyrogen injected		
15	91.2 ± 52.8*	−0.09 ± .06	7.7 ± 24.0†	0.08 ± .07
45	120.4 ± 63.7	0.28 ± .13	−28.5 ± 10.9	0.23 ± .13
75	96.5 ± 54.5	0.21 ± .18	−37.1 ± 11.5	0.30 ± .13
105	54.1 ± 40.4	0.44 ± .24	−36.0 ± 11.3	0.20 ± .05
135	83.3 ± 64.0	0.48 ± .13	−27.0 ± 10.9	0.26 ± .04
Mean ± S.E.	89.1 ± 10.71		−32.2 ± 3.0	
		Control		
15	16.0‡	−0.03	24.6‡	−0.10
45	16.0	0.23	− 1.6	0.00
75	0.0	0.08	8.2	+0.10
105	0.0	0.02	−16.4	−0.15
135	−36.0	0.00	6.6	+0.15
Mean ± S.E.	− 0.8 ± 9.5		4.3 ± 6.7	

* $N = 4$. † $N = 3$. ‡ $N = 2$.

After the intraventricular injection of the typhoid solution, the temperature of the cat began to rise, usually within 20 to 40 minutes (Fig. 1). In the first sample of CSF collected after the pyrogen was given, $^{45}Ca^{2+}$ and sometimes $^{22}Na^{+}$ increased sharply, independent either of a change in body temperature or of any signs of a febrile response including shivering. Thereafter, however, the efflux of both $^{45}Ca^{2+}$ and Na^{+} into the ventricle closely paralleled the changes in the set-point temperature but in an opposite fashion. The rate of $^{45}Ca^{2+}$ efflux remained relatively constant until the fever began to reach an asymptote or the temperature declined at the start of defervescence (Fig. 1). On the other hand, $^{22}Na^{+}$ was retained in the tissue during the sharp, initial rise in the temperature of the cat. Then, the rate of efflux of $^{22}Na^{+}$ into the CSF increased above the baseline as the pyrexic response stabilized (Fig. 1). This was followed again by a reduction in the efflux of $^{22}Na^{+}$, which fell below the baseline as the set point was maintained from the fourth through seventh hours of the fever. In the control perfusions, when the pyrogen was not given, the release of $^{22}Na^{+}$ and $^{45}Ca^{2+}$ varied around a relatively constant baseline; however, no consistent alterations of cation efflux were observed.

These pyrogen-induced fluctuations in cation efflux occurred in each animal but varied in magnitude and duration. Table 1 presents the average percentage rise in $^{45}Ca^{2+}$ and decline in $^{22}Na^{+}$ effluxes during the period after intraventricular injection of the pyrogen. For each successive interval, the mean change of the colonic temperature of the cats is given. The efflux of $^{22}Na^{+}$ often increased only in the sample collected 15 minutes after the typhoid solution was given (Table 1). This could reflect a drastic change in the permeability of the ependymal wall to both ions or perhaps an interference with the membrane binding or mitochondrial accumulation of the cations (*10*). When compared to control values, the differences were significant for the $^{45}Ca^{2+}$ ($t = 6.28$, d.f. $= 8$, $P < .005$) and the $^{22}Na^{+}$ changes ($t = 4.60$, d.f. $= 7$, $P < .005$) with sample 1, $^{22}Na^{+}$, excluded from the analysis.

These results provide direct evidence that a bacterial organism acts in the brain to disturb the ratio of Na^{+} to Ca^{2+}, which is hypothesized to be the inborn mechanism that maintains a stable set-point temperature of 37°C in the mammal (*2, 11*). Further, they correspond with earlier experiments in which Na^{+} or Ca^{2+} were increased in the ventricle of the cat (*12*) and other species (*11*). Since the third ventricle was perfused, the origin of this ionic shift is presumably in the hypothalamus. Moreover, morphological mapping data obtained by artificially altering the ratio of Na^{+} to Ca^{2+} by push-pull perfusion of these cations in the cat and the monkey (*2*) clearly indicate that the site of maximum sensitivity to an ionic imbalance, in terms of a temperature response, is the posterior hypothalamus.

The pathological disturbance to the neurons of the hypothalamus caused by a foreign lipopolysaccharide must be profound in order to evoke such an alteration in the relatively stable set-point temperature. Although the cellular mechanisms are unknown, it is likely that the changes in cation concentration observed in the CSF may reflect at least two different kinds of activity in the neuronal elements involved in the set-point function. (i) The concentration of each ion could be inversely related to that of the hypothalamus so that the pyrogen could act to uncouple tissue-bound Ca^{2+} and cause its active transport into the ventricle at the same time that the movement of Na^{+} is restricted to the hypothalamus. (ii) Further, a pyrogen could exert a pathological action on the ependymal wall and interfere with the normal exchange of Na^{+} and Ca^{2+} between neuronal tissue and CSF. There is now evidence that leukocyte pyrogen is itself extruded from brain tissue into the cerebral ventricle; if the egress of pyrogen is prevented, hyperpyrexia persists (*13*).

A protein-like factor capable of producing a profound decline in serum Ca^{2+} has been isolated from hypothalamic tissue (*14*). In addition, prostaglandin E_1, a compound which possesses thermogenic effects when injected into the hypothalamus (*15*), has the capacity to facilitate mitochondrial uptake of Ca^{2+} (*16*). These findings lend additional support to the postulated function of hypothalamic Ca^{2+} in regulating the set-point mechanism.

Finally, we do not yet know how the endogenous shift in the Na^{+} to Ca^{2+} ratio is related to the hyperthermia produced by serotonin, prostaglandins, and cholinergic substances injected into the hypothalamus (*15, 17*). Although monoamine activity may not be related to a bacterial fever (*18*), each of these naturally occurring substances may play a prominent role in the thermosensitivity of those neurons mediating the regulatory changes that necessarily accompany a pathological elevation in the temperature of an animal. Additional research is essential to quantitate the possible long-term shifts in the ratio of Na^{+} to Ca^{2+}, particularly during defervescence, as well as the changes in the millimolar values of each cation.

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6. The constituents of the artifical CSF in millimoles per liter were: NaCl, 127.7; KCl, 2.6; $CaCl_2$, 1.3; $MgCl_2 \cdot 6H_2O$, 0.9; $NaHCO_3$, 21.0; Na_2HPO_4, 1.3; and glucose, 3.4. The solution was prepared in pyrogen-free glassware and passed through a sterilized 0.22-μm Millipore filter.
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Inactivation of Horizontal Cells in Turtle Retina by Glutamate and Aspartate

Abstract. *Glutamate and aspartate completely suppress the activity of horizontal cells but only partially affect the response of receptor cells to light. The changes observed in the receptor responses are consistent with the interruption of a synaptically mediated process rather than with a direct action on the receptor membrane.*

In vertebrates the electroretinogram consists of three major components: the a wave, attributed primarily to the activity of photoreceptors; the b wave, related to postsynaptic activities; and the c wave, presumably generated in the pigment epithelium (*1*). Acidic amino acids such as glutamate and aspartate modify the electroretinogram by eliminating the b wave and leaving a vitreous-negative component called PIII (*2*). The PIII component is believed to reflect the electrical activity of photoreceptors not complicated by the activity of other retinal elements.

Electroretinograms from retinas treated with glutamate and aspartate have been used for studying the photoreceptor response when intracellular measurements are too difficult or impossible (*3*). Consequently, many conclusions about the functional properties of photoreceptors are based on extracellularly recorded responses from retinas treated with one of these amino acids. These conclusions depend on the assumption that glutamate and aspartate selectively block the response of the more proximal retinal cells without altering the receptor response.

To test the validity of this assumption, we studied the effect of glutamate and aspartate on the intracellularly recorded response of receptors and horizontal cells in the perfused retina of the turtle (*Pseudemys scripta elegans*). This research is a continuation of our studies on skate retina (*4*).

The turtle eye was removed and cut along a medial lateral axis. After the vitreous chamber was drained, the eyecup was mounted in a chamber where oxygenated and buffered Ringer solution continuously flowed over the vitreous side at 4 to 5 cm^3/min. The ionic composition of the Ringer solution used was similar to that of cerebrospinal fluid of the turtle (*5*). Test solutions contained glutamate or aspartate substituted for equimolar amounts of sodium chloride. Intracellular recordings were made by conventional methods with high-impedance (200- to 500-megohm) glass microelectrodes filled with 4*M* potassium acetate.

The retina was stimulated by white light from a tungsten quartz iodine lamp (*6*) run from a regulated 7-amp d-c supply. The reduced image of a circular diaphragm was focused on the retina with a diameter that could be varied between 60 and 2000 μm. The light intensity was attenuated by a pair of

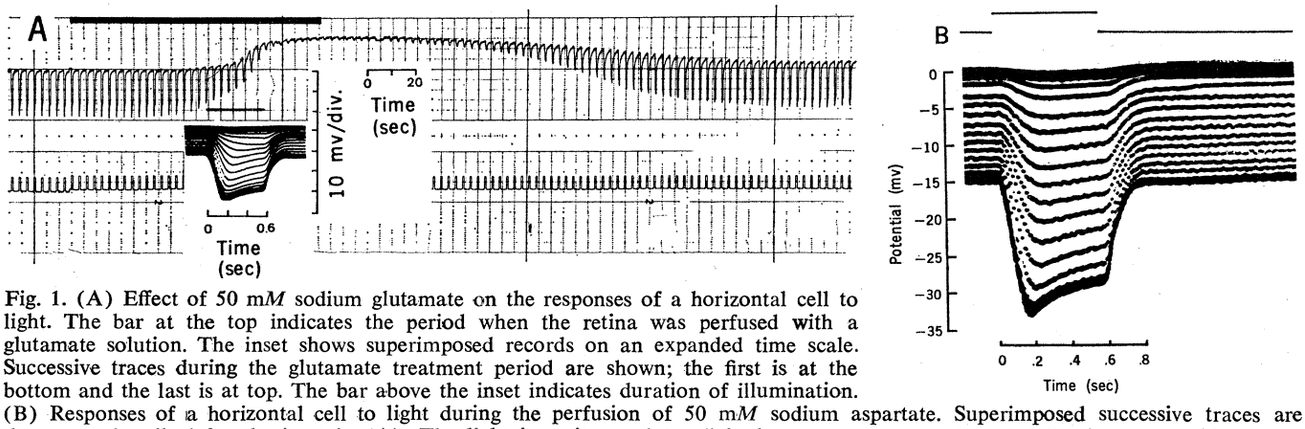

Fig. 1. (A) Effect of 50 m*M* sodium glutamate on the responses of a horizontal cell to light. The bar at the top indicates the period when the retina was perfused with a glutamate solution. The inset shows superimposed records on an expanded time scale. Successive traces during the glutamate treatment period are shown; the first is at the bottom and the last is at top. The bar above the inset indicates duration of illumination. (B) Responses of a horizontal cell to light during the perfusion of 50 m*M* sodium aspartate. Superimposed successive traces are shown, as described for the inset in (A). The light intensity used to elicit the responses was attenuated 2.4 log units with respect to the total available energy. The area of retina illuminated by the spot was 500 μm. The zero level of membrane potential is arbitrary and indicates the final depolarization reached by the membrane during perfusion of aspartate. The upper bar at the top indicates the illumination period.