duced by males of *A. hebraeum* is as yet unknown and it is impossible at this stage to measure its concentration in the fed males. However, it appears that even if the amount of pheromone released by the males is affected by the light: dark regimes, the males release sufficient amounts of pheromone to attract unfed males and females irrespective of the time of day.

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Focal brain hyperthermia. I. The cerebellar cortex

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Summary. Focal brain hyperthermic methodology has been described and data presented on the cerebellum which show that enhancement of electrical activity of cerebellar cortex occurs when this method is used with careful monitoring of temperature. The duration of electrically induced cerebral after-discharges is shortened when cerebellar warming reaches 39.5-42.0 °C. Since these effects are repeatable over many hours, there appears to be little, if any, resultant damage. Such induced changes in the cerebrum resemble those previously reported in which electrical stimuli were applied to the cerebellar cortex.

The present study utilizes the well recognized physiological concept that intrinsic metabolic processes of cells can be elevated by increasing the temperature within a vital range. Unlike so very many studies on changes in the brain related to increase in body temperatures, we have limited the warming procedure to small areas which are interconnected to distant centers by known anatomical pathways and studied the resultant changes on these centers.

Methods. The equipment is easy to assemble and use, when compared with conventional electronic stimulators, and at least some of the physiological responses appear to be similar. The 3 essential components are: 1. heat probe, 2. thermistor or thermocouple, 3. constant current source (figure 1).

1. A heat probe for larger areas is prepared by tightly winding 36 or 40 gauge nichrome wire, total resistance 200-600 Ω, around a removable core 1 mm in diameter to a coil length of 1-2 cm. The coil is then encased in a piece of teflon (medical) or polyethylene tubing of the same length with the ends of the wire attached to a connector plug. For surface stimulation this flexible loop is inserted subdurally or extradurally through a 3-5 mm trephine opening in the skull. A heat probe for smaller areas, 3 mm or less in diameter, consists of a glass bead thermistor 1.5 mm in diameter with a resistance of 1000 Ω or more. It is heated with a constant current and the temperature is read as resistance from a conversion tabel furnished with the thermistor.

2. An accurate temperature measuring device consists of a 1-mm standard glass bead or tube (1-5 kΩ), which is attached to the heating coil with an inert adhesive. The 2 insulated wires are lead through a trephine opening to an ohmmeter. The relation between temperature and resistance is established by measuring the resistance of the thermistor as the temperature of a normal saline solution is raised from 30 to 50 °C.

3. Several reliable constant current sources are available. The simplest is a nickel-cadmium rechargeable battery, 6, 9 or 12 V. In chronic experiments it is attached to a harness on the animal's back. The current rate is controlled with a manually adjustable resistor in series with the coil. Greater accuracy and battery life can be obtained by inserting an automatic current limiting device between the battery and the coil. It can be assembled by connecting a micro-integrative voltage regulator to a power transistor and variable micropotentiometer[2].

Greater stability of temperature can be obtained by attaching the heat coil to a physiological stimulator with variable direct current output of 1 mA or more. This increases the accuracy to ±0.25 °C in the range of 32-44 °C and permits attachment of more than 1 coil. For long-term experiments in which the brain of a free-moving animal is warmed several h each day, a cable with swivel joint extends to a wire attachment cemented to the skull. This has not proven useful for monkeys unless severe limitations are imposed

Effect of unilateral cerebellar warming on duration of electrically-induced seizure in contralateral sensorimotor cortex

	Duration of seizure (sec.)	
Temperature of coil:	34-37 °C	39.5-41 °C
Cat. No.		
1	12.5 ± 3.3	10.0 ± 2.4*
8	13.3 ± 2.2	7.2 ± 1.5**
17	12.0 ± 3.7	8.0 ± 3.2**
28	15.5 ± 4.1	11.0 ± 2.2
Average	13.3 ± 1.5	9.0 ± 1.8**

Shown is the mean ± SD duration of seizure in the contralateral (right) cerebral cortex. Each value represents 4-7 pairs of determinations for each of 4 cats. The average duration of seizure in the ipsilateral (left) cortex of the same animals was not altered significantly by warming (10.9 ± 1.9 and 9.7 ± 2.1 seconds, baseline and 39.5-41 °C, respectively). The changes were not due to direct spread of thermal effects to caudal cerebrum since thermistor readings in occipital areas remain unchanged during these recordings.

*$p < 0.05$; **$p < 0.01$ vs. 34-37 °C value (statistics by 2-tailed t-test on matched pairs.

on mobility. Small areas within the brain stem can be warmed by stereotaxic placement of a bipolar electrode, insulated except at tip, and locally heated by a two-megacycle current source (Grass Instruments LM3). The current output is monitored to prevent irreversible damage to the tissue. However, mechanical damage occurs as a result of electrode placement and such a method is not advised without accurate histological monitoring.

Results and discussion. All records were obtained in acute experiments on cats by warming the cerebellar vermis while the animals were anesthetized with ether and immobilized with gallomine triethiodide. Barbiturate anesthesia was not used because of depression of activity. Under baseline conditions the rectal temperature ranged between 35 and 37 °C and the cerebellar surface was usually within 0.5 °C of this. Bone removal was kept at a minimum through the use of dental drill openings in the skull.

The present study indicates that the level of cerebellar warming required to suppress electrically induced cerebral paroxysms can change less than 0.5 °C over an 18-h period with as many as 16 warmings of the cortex to 41 °C. These data are given as evidence that such procedures do little damage to cerebellar neurons. Histological studies are in progress to obtain additional information on this critical point.

Figure 2 and the table show distant and local effects of cerebellar warming. The temperature of the left vermal cortex was raised from baseline (34–37 °C) to 39.5 °C, then to 41 °C over a 10–20-min interval. Electrical recordings were made (figure 2) then the vermis was allowed to return to baseline temperature (during 10 min) and a 2nd record was taken. Seizures were induced electrically in left or right cerebral cortex by the method of Snider and Maiti[3]. Parameters of induction were kept constant and the duration of the seizure was measured as a function of cerebellar temperature while keeping cerebral and rectal temperatures unchanged.

It is generally recognized that an increased temperature,

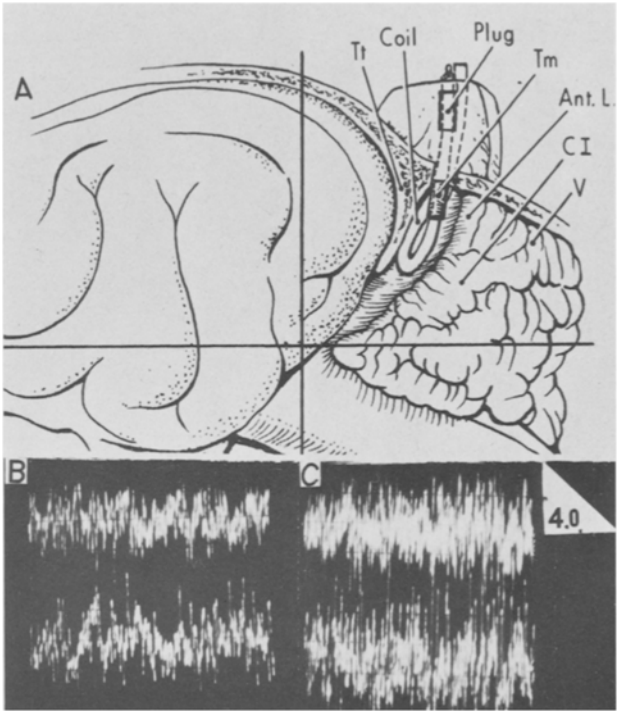

Fig. 1. *A* Schematic brain drawing showing heat coil (Coil), and thermistor (Tm), on anterior lobe (Ant. L) between tentorium (Tt) and crus I (C I) anterior vermis (V). Cerebral gyri are shown anterior to tentorium and the medulla below the cerebellum. The large horizontal line and vertical line indicate stereotaxic zero in that plane. *B* Cathode ray tracings of spontaneous activity in white matter between cerebellar nuclei and cortex (upper trace) and in nucleus ruber (lower trace) when cerebellar surface was (36 °C) body temperature. *C* As *B* except cerebellar lobule IV and V was warmed to 41 °C by means of heat coil. Calibration: Vertical 4 × 100 μV, horizontal 4 × 10 msec.

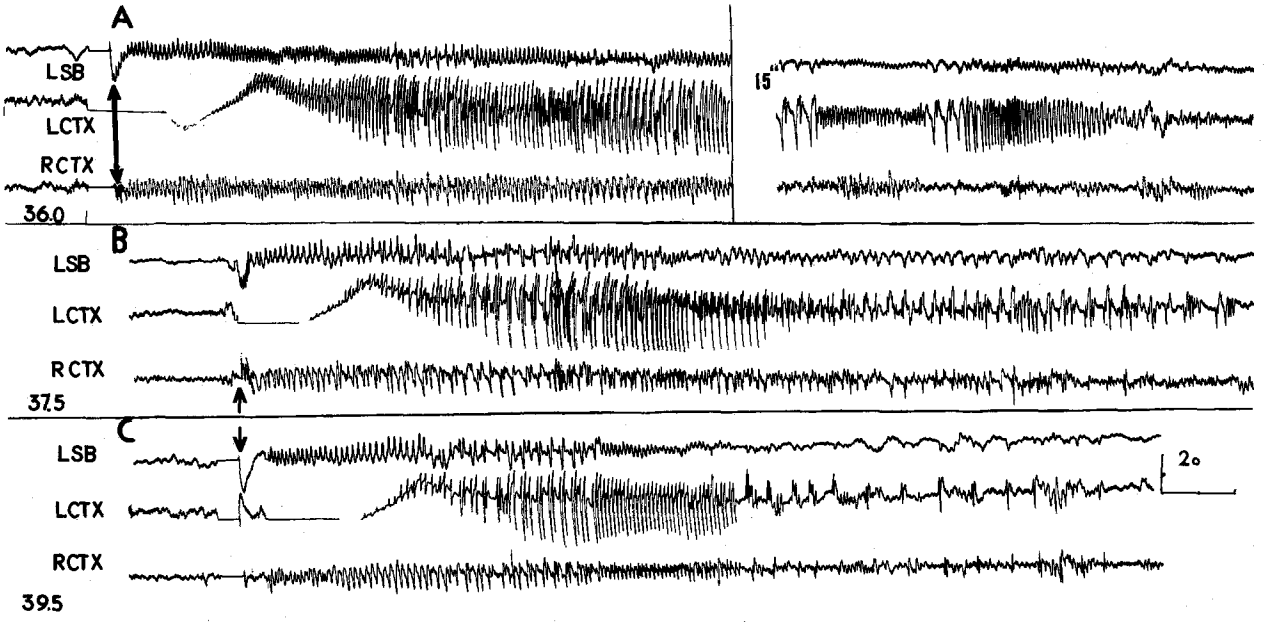

Fig. 2. EEG tracings taken from left superior cerebellar brachium (LSB) left and right cerebral sensori-motor cortex (L CTX; R CTX). At arrow application of 15 V (40 pulses per sec, and 2 msec duration) to left sensori-motor cortex for 3.5 sec induced post stimulatory paroxysmal discharges which were recorded to spontaneous stoppage. The unstimulated EEG tracing is shown in front of the arrow. Identical conditions were maintained except in *A* the cerebellar cortical temperature was 36 °C (rectal temperature). The paroxysmal discharges continued for 29 sec (15 sec removed at 15″). *B* shows paroxysmal discharges of 13.5 sec and *C* shows them shortened to 11.0 sec as a result of warming the cerebellar cortex (lobules IV and V – anterior lobe) to 37.5 °C in *B* and 39.5 °C in *C*. Calibration: Vertical 2 × 100 μV; horizontal 2 sec.

within physiological range, can result in increased activity in a biological system[4]. Thus the results of the present experiments are not unexpected. What is surprising is that so many neurobiologists have failed to use this technique to stimulate physiological activity and interactions between anatomically related centers such as the cerebellum and cerebrum. A search of the literature indicates that the hypothalamus is one of the few areas of the brain to be studied in this manner. Nakayama et al.[5] have reported increased activity of single units in the preoptic area with warming to 41 °C and indicate this is directly related to maintenance of body temperature. Additionally, the authors write 'most neurons, even thalamic units, increased their discharge frequency at temperature higher than 41-42 °C'. We have not done single unit studies but the upper traces in figure 1, B and C clearly demonstrate that warming increased cerebellar activity as observed from records obtained from cerebellar white matter deep and adjacent to the warmed cortex. A common finding was an enhancement of activity between 39.5 and 42 °C. The effects of higher temperatures have not been studied, but work in progress indicates that warming to 41 °C enhances activity in the sensori-motor cerebral cortex.

Purkinje cell axons give rise to the major efferent axons of the cortex and the increased activity in figure 1 is a direct result on increased cortical temperature. The enhanced activity (lower trace, figure 1, B and C) in the red nucleus, a known terminus of cerebellar efferent fibres[6], also appears to be related to cerebellar warming since allowing the cerebellar surface temperature to return to 36–37 °C results in decreased activity.

The data shown in figure 2 and the table indicate that increasing the local temperature of the cerebellar anterior lobe reduces the length of experimentally induced paroxysmal discharges. As demonstrated in a number of basic and clinical studies[6–8], electrical stimulation of the cerebellum can suppress and/or stop seizure discharges. The present data show strong compatibility with those obtained by conventional methods for both acute and chronic experiments[7].

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Neuronal responses to extracellularly applied cyclic AMP: Role of the adenosine receptor

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Summary. At low doses, theophylline blocks the neuronal depressant effects of 5′-AMP, but not cyclic AMP. Higher doses (100 mg/kg) block cyclic AMP responses and reduce the effects of noradrenaline and GABA. It is concluded that cyclic AMP does not depress neurones via the adenosine receptor.

Several recent studies have attempted to correlate the response of central neurones to synaptic transmitters and nucleotides by observing changes of firing rate when these substances are applied extracellularly to single neurones by microiontophoresis[4]. Some clear correlations have resulted, particularly for acetylcholine and cyclic GMP, and noradrenaline and cyclic AMP[5–8].

It has now been shown that purines other than cyclic AMP can produce changes of firing rate by acting on an extracellular adenosine receptor[9], raising the possibility that extracellularly applied cyclic AMP might alter neuronal firing via this same receptor. As the adenosine receptor can be blocked by theophylline[9,10], we have used this substance to try to differentiate between neuronal responses to cyclic AMP and related compounds.

Materials and methods. Adult male rats were anaesthetised with urethane, 1.25 g/kg i.p. Recordings were made of the firing rates of neurones in the parietal cerebral cortex, many of which were identified as pyramidal tract cells. Cyclic AMP, adenosine and 5′-AMP were ejected by microiontophoresis from 5- or 7-barrelled micropipettes filled with cyclic AMP sodium salt; adenosine hemisulphate; 5′-AMP sodium salt (all 250 mM); gamma-aminobutyric acid (GABA) 1 M. The net current at the pipette tip was automatically maintained at zero. The centre barrel was filled with 5 M sodium chloride or 1 M sodium acetate for recording unit activity. Aminophylline (theophylline ethylenediamine) was dissolved in saline and injected i.v. at doses of 25–100 mg/kg.

Results and discussion. The 3 nucleotides used in these experiments proved able to depress the firing of many cortical cells. Cyclic AMP was the least effective but it depressed 12 of 26 cells when applied with currents of 40–150 nA[5]. Adenosine and 5′-AMP depressed all but 2 of the cells tested (28 of 30), (figure 1). Gamma-aminobutyric acid (GABA) ejected with currents of 5–80 nA, readily depressed neuronal firing and was used as a control agonist in the examination of the effects of aminophylline.

Aminophylline, 25–100 mg/kg i.v. was injected whilst recording from 16 neurones depressed by both 5′-AMP and cyclic AMP. The drug caused a slowing of firing rate of 10 cells and an acceleration of 4, which lasted for between 30 sec and 4 min, but thereafter firing returned to the pre-injection level. On all the cells, responsiveness to 5′-AMP and adenosine was reduced following the injection, the purine responses being completely blocked after the 50 or 100 mg/kg doses. Responses to cyclic AMP were reduced by no more than 50% on 12 cells following 25 or 50 mg/kg theophylline, (figure 1) although they were substantially reduced in size after doses of 100 mg/kg. Recordings from 8 of the 16 units were sufficiently stable to follow recovery