Lack of daily rhythm in the release of assembly pheromones by the tick *Amblyomma hebraeum* (Koch)

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Summary. Tests conducted under natural light: dark regimes showed that the release of assembly pheromones by fed males of *Amblyomma hebraeum* as well as the response of unfed adult ticks to the pheromones were not affected by daily rhythms.

Studies of ticks have shown that males of *Amblyomma maculatum* (Koch) and *A. hebraeum* (Koch) produce assembly pheromones[1-3]. It has also been demonstrated that some behavioural patterns such as detachment of *A. hebraeum* from its host, are regulated by circadian rhythms (Rechav, unpublished data). Other studies have shown that the release of sex pheromones by the moth *Dioryctria abietella* (Denis & Schiffermüller) was regulated by circadian rhythms[4]. No information has been published on the release of tick pheromones. The release of assembly pheromones by males of *A. hebraeum* and the response of unfed males and females to the pheromones were investigated.

Petri dishes (15 cm diameter) were marked into 8 sectors as has been described previously[5]. A disc of filter paper (Whatman No. 1; 1.8 cm in diameter) was placed in each sector. At the beginning of an experiment 10 ticks, males or females, were placed in the center of the petri dish. The pheromone was introduced in one of 2 ways: a) 0.2 ml of concentrated extract of whole male ticks (2 ml = 100 males) was placed on 1 of the discs. The method of extraction has been described preivously[3]. b) 1 or 3 males which had been previously allowed to feed on cattle for 8 days were enclosed in a gauze bag, which was then placed on a disc in 1 of the 8 sectors. In both cases the distribution of ticks in the various sectors was determined 3 min after introduction of the pheromone sources. Tests were conducted under natural light: dark regimes in constant temperature of 26±1 °C. A torch which emitted red light was used in experiments that were carried out during the dark periods of the day, as it has been established that most arthropods are insensitive to red light[6].

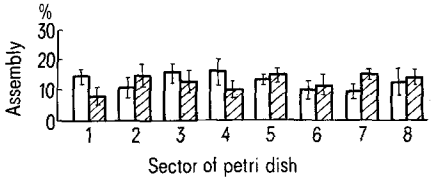

Fig. 1. Distribution of *A. hebraeum* males and females when no pheromones were present in any of the sectors of the petri dish. ▨ males ($\chi^2 = 3.72$, $p < 0.9$, 7 df), □ females ($\chi^2 = 4.01$, $p < 0.9$, 7 df), vertical lines represent SEM.

1-month-old unfed males and females showed no significant preference for any of the sectors (figure 1) when pheromones were not present. The fed males which had been used as a source for such pheromones had been placed in the petri dishes 10 min after being pulled off their hosts. The response of unfed females to fed males at various times of the day was not different (figure 2). Furthermore, when extracts of fed males (0.2 ml = 10 males) served as a source of pheromone, the assembly of unfed females inside the sector in which the extract had been placed at 06.00, 10.00, 14.00, 18.00, 22.00 and 02.00 h was very similar (figure 2). Similar results were obtained with unfed males which aggregated inside the sector to which the extract (0.2 ml) had been applied, or that in which the fed male or 3 males, had previously been placed (figure 3). Although the males response to the pheromone was weaker than that of the females, the assembly to the sector in which the pheromone had been placed was always highly significant ($p < 0.001$, χ^2-test). The lowest value obtained was $\chi^2 = 69.31$ 7 df. The data presented in figures 2 and 3 indicate that the same amount of extract attracted the adult ticks irrespective of the time of day. i.e. the response of *A. hebraeum* males and females was not affected by daily oscillations.

It has been shown[7] that some American ticks such as *Dermacentor variabilis* (Say) responded positively even to low concentrations of 0.00005 ng of 2,6 dichlorophenol, the common sex pheromone of hard ticks. It is also known[8] that a female of *Rhipicephalus sanguineus* (Latrielle) produced 9 ng of 2,6 dichlorophenol, but no information is available on the exact amount of sex pheromone released by the fed females of this species.

The chemical structure of the assembly pheromone(s) pro-

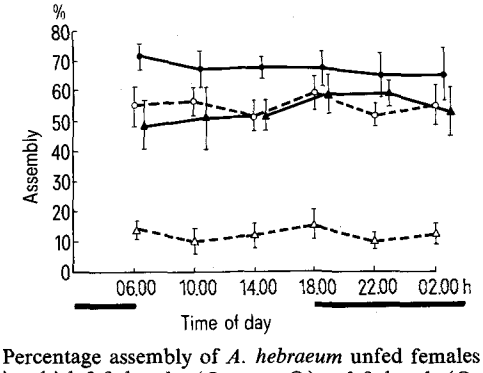

Fig. 2. Percentage assembly of *A. hebraeum* unfed females to secsectors in which 3 fed males (O———O) or 1 fed male (O---O), or extract of fed males (▲———▲) or no pheromone (control) (△---△) had been previously placed at various times of the day. Vertical lines represent SEM. Black areas represent duration of darkness during the day.

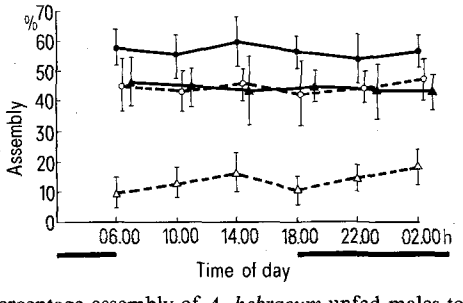

Fig. 3. Percentage assembly of *A. hebraeum* unfed males to sectors in which 3 fed males (O———O), or 1 fed male (O---O), or extract of fed males (▲———▲) or no pheromone (control) (△---△) had been previously placed at various times of the day. Vertical lines represent SEM. Black areas represent duration of darkness during the day.

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.