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Duration of hornet sleep induced by ether anesthesia is curtailed by exposure to sun or UV irradiation

J. S. Ishay, V. Pertsis and E. Levtov

Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Ramat Aviv 69978 (Israel)
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Abstract. Irradiation of hornets anesthetized by diethyl ether curtails their sleep duration from the ordinary 20–30 min to a mere 2–3 min. This effect on sleep is dependent on the intensity of the sun irradiation or, when exposed to monochromatic UV at 366 nm, on the duration of the irradiation. Of the various hornet cuticular areas of the body, the yellow cuticular areas of the abdominal segments are the most sensitive to the treatment. We assume that the cuticles of both live and dead hornets act as a converter of light to electric energy.
Key words. Ether anesthesia; sleep in insects; hornets and wasps; UV irradiation.

In the course of the past 15 years, a concerted effort has been made to elucidate the electric properties of the cuticle of various social insects. In terms of experiments, the Oriental hornet *Vespa orientalis* (Hymenoptera, Vespinae) in particular was investigated. One area of great interest was the photoelectric effect of hornet cuticle in anesthetized or killed specimens that had been kept in deep refrigeration for prolonged periods[1]. From the studies it became clear that all parts of the cuticle respond to light, and that their electrical sensitivity diminishes upon exposure to illumination. Another very interesting finding is the photovoltaic effect of the hornet cuticle[2]. Hornet cuticle acts as a diode and an energy source[3], with the voltage gradient depending non-linearly on the current flowing through the cuticle and behaving as would be expected for a semiconductor[4]. The 'brown' cuticle behaves as a semiconductor of the *n* type, and the 'yellow' stripes of the cuticle as one of *p* type[5]. Thus far, results were obtained from dead insects. The question remains as to whether the above-mentioned results in some way reflect similar properties or activities in the daily life of hornets. An ancillary and rather surprising finding was that light irradiation can also exert an effect on living hornets, in particular on the duration of sleep under ether anesthesia. The present report describes the curtailing effect on the sleep duration of ether-anesthetized hornets exerted by sun or UV irradiation applied to the entire cuticle or only to cuticular yellow stripes. The possible mechanism for such an effect is discussed.

Materials and methods

Test hornets. These were workers of the Oriental hornet collected daily from the progeny of brood combs maintained in the laboratory under optimal conditions. Originally, these combs were collected from the natural nests in the field, as previously described[6]. Groups of 5–15 workers were kept in special hornet breeding boxes with which we have long been experimenting[7]. Each artificial breeding box (ABB) had a volume of approx. 13,600 cm^3 and contained hornets of a uniform age. In all, experiments were carried out on 21 ABBs containing hornets aged 3–14 days. All hornets received water, sugar cubes and meat morsels in abundance, as customary. Our previous experience with workers kept in similar ABBs has been that after a few days they start building a comb which is suspended from the roof of the box and directed toward the gravitational force[8,9]. This criterion served also in the present study as proof that all was well and normal with the test hornets. The experiments were carried out during the summer season, which is optimal for hornet activity.

Anesthetizing of hornets. The anesthetic was diethyl ether (Bio-Lab Laboratories, Ltd., Jerusalem) which is comprised of 99% absolute ether with 1% water and practically no impurities. The ABB was placed on its largest (rear) side and ether injected into it via a 5 ml syringe using a 15-cm-long polyethylene cannula. The ether injection was aimed at the glass wall facing the ply-wood floor of the ABB, ensuring that distribution of the injected ether throughout the volume of the ABB was as uniform as possible. The duration of ether injection was 3 s and the time reckoned for anesthetizing and subsequent awakening was computed from the moment the ether injection ended.

Under no circumstances was the ether injected directly upon the hornets, because our previous experience has been that hornets directly sprayed by liquid ether die shortly after. The ABB in which the hornets were anesthetized had several holes. A thermocouple was inserted through one of these as far as the center of the box, which then served to monitor the internal temperature. The injected ether vaporized to gaseous form within 5 s. As soon as all the hornets had succumbed to the

anesthesia (see details in 'Results'), as evidenced by their dropping to the floor of the ABB, the glass wall of the ABB was removed, so that the ether vapors could escape. The glass wall of the ABB was replaced immediately upon awakening of the first hornet, as evidenced by its straightening its body and beginning to walk. The observations on the influence of the ether were undertaken by two people simultaneously and on two different groups of hornets, one of which was kept in the shade while the other was exposed to either sun light or UV irradiation. To ascertain which body segments were photosensitive, white stain was applied in bands onto the brown stripes or yellow stripes of the gaster only, and irradiation then effected by a UV lamp placed within a narrow cardboard tube, which prevented lateral diffusion of this incident light. The white bands were produced by several coatings of UHU paint (actually white correction fluid in trichloroethane-Gmbh 7580, Bühl, Germany). These coatings were applied one or more days prior to the anesthesia. Our previous experience convinced us that UHU correction fluid is not harmful to hornets. We used it to mark queens (60 specimens) at the beginning of autumn (1992–1993). These queens subsequently hibernated through the winter months and most of them emerged from hibernation without any significant change in behavior or longevity as compared to control queens that were not marked with the white paint (Ishay, unpubl. observ.).

In order to assess possible importance of the hornet's head to the course of duration of the anesthesia, 10 hornets were decapitated immediately following the ether anesthesia, and the same observations performed on the headless hornets as on the intact ones.

The experiments were performed during all hours of the day so as to preclude the possibility that hornet circadian rhythmicity might exert an effect on the durations of anesthesia. The UV intensity of the sunlight irradiation at a wavelength range of 325–400 nm was measured via a Model 1350 Radiometer/Photometer series 965 IL (International Light, Newburyport, Massachusetts, USA). The UV lamp was a product of Desaga Co., Heidelberg, set to operate at 366 nm. Observation on hornets anesthetized in the dark were made via a red-light flashlight which did not significantly raise the ambient temperature. As is known, wasps and hornets are blind to red light[10,11]. The temperature in the ABBs was recorded via thermocouples in order to ascertain both the drop in temperature immediately following the introduction of the ether as well as the rise in temperature upon exposure of the ABBs to irradiation. Preparatory procedure for scanning electron microscopy observation was applied to the yellow stripes of 6 hornets under anesthesia as well as to those of another 6 hornets that were not anesthetized but killed by freezing as described elsewhere[12]. The electron microscopy was done via a jeol T300 SEM equipped with a link 10.000 Energy-Dispersive System (EDS).

Statistical analysis of the results on each hornet exposed either to sun irradiation or to UV irradiation was made by the linear regression method, while the results of the comparative study of the effect of irradiation on various parts of the body or on the yellow vs the brown abdominal stripes, were made by analysis of variance (ANOVA).

Results and discussion

The table gives details on the temperature measurements within the ABB before, during and after the ether anesthesia. One complete and typical measurement session is shown. Hornets subjected to ether anesthesia within ABBs either in the dark or in a shaded room underwent a sequence of stereotypical events as follows:

1) a latent period lasting about 20 s;
2) unrest followed by excitation (about 10 s) during which the hornets released their hold on the walls of the ABB (usually underneath the roof where a comb was built) and dropped to the floor;
3) vomiting coupled to a loss of the 'uprighting' reflex, the hornets ending up on their back (about 10 s);
4) contraction of the abdomen into a quasi-fetal position with the tip of the abdomen brought in close proximity to the head next to the ventral side of the thorax and mouthparts, whereupon the stinger was extruded to spray the ventral thorax and the mouthparts with a film or an 'aerosol' of venom (30–40 s);
5) hornets assumed a resting position on the floor with the head lying on its side, i.e. the sleep state (about 25–30 min);
6) ultimate awakening of the hornets whereupon they climbed back to the original resting place, or resumed their preanesthesia activities.

Essentially the same behavior was displayed by hornets that had undergone decapitation. These, too, turned

Time following ether anesthesia	0	2 s	10 s	20 s	30 s	40 s	50 s	60 s	$1\frac{1}{2}$ min	2 min	3 min	4 min	5 min	6 min
Temperature in °C	26.66	26.33	26.00	25.66	25.66	25.33	25.33	25.33	25.33	25.66	25.99	26.33	26.48	26.66
Change in temp. (ΔT)	0.00	−0.33	−0.66	−1.00	−1.00	−1.33	−1.33	−1.33	−1.33	−1.00	−0.67	−0.33	−0.18	0.00

Usually by the end of a minute all the hornets were already anesthetized, whereupon the glass wall of the ABB was removed. Within a temperature range of 24–30°C no differences were discernible in the rates of anesthesia and awakening. Once the temperature in the ABB reverted to starting level, it remained fixed.

over onto their backs, extruded their stingers and sprayed venom. Anesthesia duration of headless hornets was about the same as that of the intact ones and upon termination of anesthesia they stood up on their legs and may have remained so for hours (or even days if a high humidity was retained in the ABB). This sequence of events was observed on innumerous occasions over many years. We found that a group of hornets can be anesthetized up to 6 times daily and the sequence of events described above will repeat itself unfailingly at the stated time intervals for each phase. However, when the ABB containing the anesthetized hornets was exposed either to sunlight or UV irradiation, an acceleration of the awakening process was immediately noticeable with the speed of awakening directly proportional to the intensity of the light. Thus the irradiated hornets awoke within 1.7–2.0 min as opposed to 25–30 min for hornets in the shade or dark. Details on the curtailing effect of sunlight on 'sleep' duration of anesthetized hornets are given in figure 1A; the temperature range here was 26–30°C, which is optimal for hornets in all instances. As can be seen from figure 1A, at an illumination intensity of 2.12 mW/cm^2 and taking into account only the UV irradiation component of the sunlight at wavelength of 325–400 nm, the time interval till awakening is 1.75 min ($\pm$0.17 SE) while at zero UV irradiation, the time interval till awakening is 29.62 min ($\pm$1.45 SE). The sample size at each measuring point was 10–20 hornets. It is noteworthy that the hornets awakening under sun or UV irradiation commenced flying immediately upon awakening, unlike those awakening in the shade or dark. Those decapitated never flew. Processing of the data obtained by log t versus log sunlight intensity yielded a calculated regression line of $Y = 0.51 - 1.16\times$. The computed regression line for sunlight is shown in figure 1B. The dots above and below the lines represent the experimental results and one can see that these dots are usually quite close to the regression line. Thus, so far as the effort of sun irradiation on the sleep duration is concerned, the correlation amounts to 83%, $p < 0.0001$. To ascertain the influence of UV rays at a constant intensity (of 0.15 mW/cm^2) on

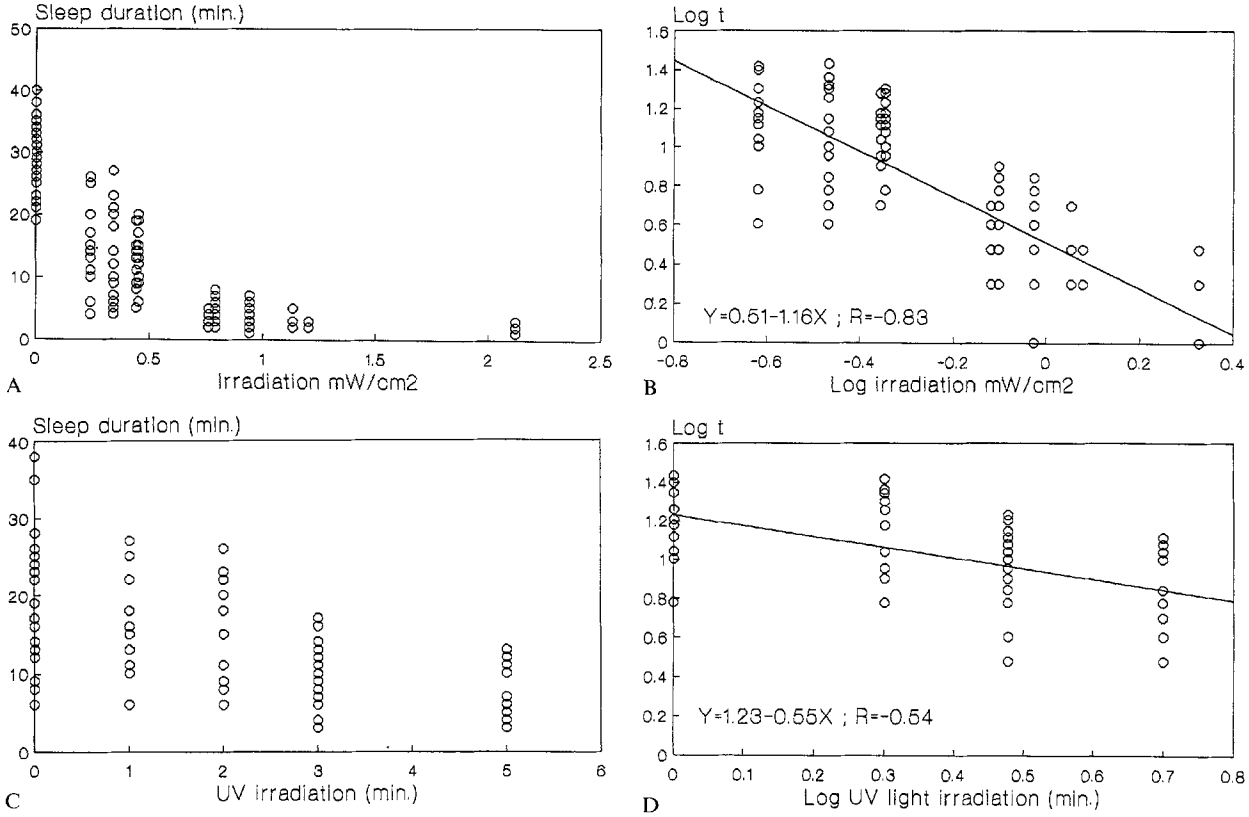

Figure 1. *A* Sleep duration of hornets as dependent on solar irradiation. As can be seen, under increased direct sun irradiation the sleep duration is shortened by about one order of magnitude or more than in the shade or dark (0 UV irradiation).
B Transformation of the results obtained in figure 2A to log t vs log irradiation yields a regression line with a high degree of correlation between the sun irradiation and the duration of sleep. Open circles = measured results. Correlation level = 83%, $p < 0.0001$. Wavelengths measured 325–400 nm.
C Sleep duration vs duration of UV irradiation. Irradiation with this wavelength of a constant intensity of 0.15 mW/cm^2 shortened the duration of sleep in accordance with the duration of exposure. Open circles = measured results.
D Transformation log t sleep duration vs log time of UV irradiation of the experimental results obtained under UV irradiation (fig. 1C). Open circles = measured data. Correlation level R = 54%, $p < 0.0001$.

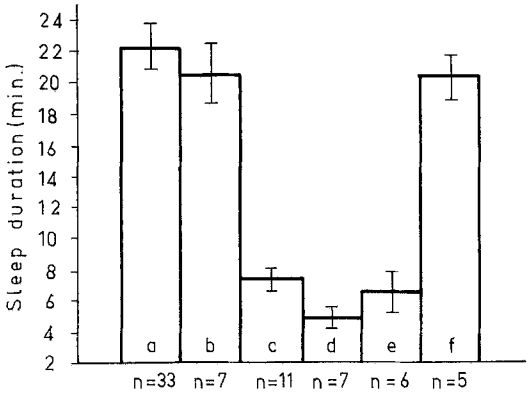

Figure 2. Sleep duration under UV irradiation of various body segments. UV irradiation: 3 min; 366 nM; 0.35 mW/cm^2. As can be seen irradiation of the head (*b*) or the abdominal brown stripes (*f*) does not abbreviate the duration of sleep compared to the non irradiated hornets (*a*), while irradiation of the whole body (*c*), the whole abdomen (*d*) or only the yellow stripes on the abdomen (*e*) yields rather shorter sleeping time. Bar = standard error, $p < 0.0001$.

the anesthesia duration, experiments were run in the dark, with the anesthetized hornets exposed to UV irradiation of 366 nm. The results of this experiment are shown in figure 1C.

Dots represent the actual measured results. Statistical processing and conversion to log t against the log exposure time to UV irradiation yielded a regression line whose equation is $Y = 1.23 - 0.55\times$, $R = 54\%$, $p < 0.0001$ (fig. 1D).

Next, we were interested in finding out whether there is any significance in the site of the irradiation. Figure 2 provides an answer to this question. As can be seen, UV irradiation (at a wavelength of 366 nm) for 3 minutes at a constant intensity (0.35 mW/cm^2) of the head alone did not alter the anesthesia duration of test hornets compared to the control. Regrettably we found no way of irradiating the thorax alone, for to do this would have necessitated removing the wings and also fastening the legs to the body – a procedure which is not really practical. Irradiation of the brown abdominal stripes alone was feasible but proved of no significance. In contrast, irradiation of the whole abdomen or irradiation of the yellow stripes only curtailed the sleep duration to the same extent as irradiation of the entire body of the hornet.

From the ANOVA evaluation, we learned that group *d* (abdomen irradiated) varied from groups *a*, *b*, and *f* (control, head irradiated and abdominal brown stripes irradiated, respectively), and so did group *e* (abdominal yellow stripes only irradiated) and group *c* (whole body irradiated). There was no significant difference between groups *a*, *b* and *f*, nor between groups *c*, *d* and *e* ($p < 0.0001$). It seems fairly clear, therefore, that irradiation of the yellow stripes only suffices to wake the anesthetized hornets within a period of time similar to that required by whole-body-irradiated hornets.

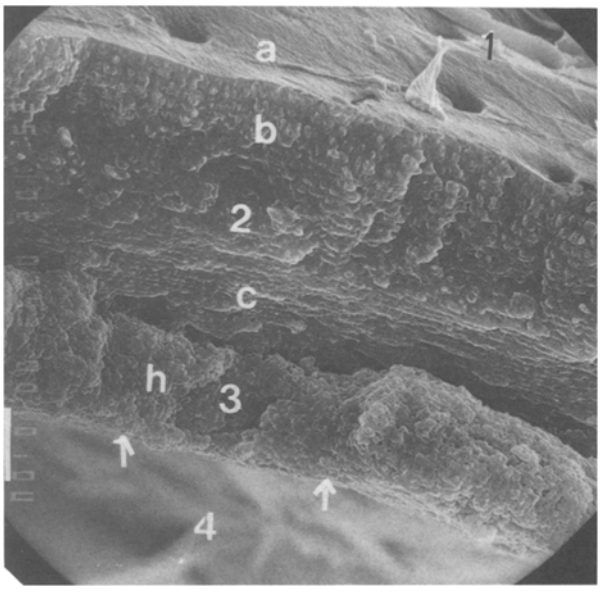

Figure 3. Scanning electron microscope (SEM) view of a cross section through a yellow stripe on a hornet abdominal segment. The cuticle taken in figure 3 is from a live hornet previously light-irradiated. The tissue for SEM examination was prepared shortly after irradiation (a matter of minutes). No sign of the irradiation treatment undergone by the hornet could be traced. One can see from top to bottom: 1) hairs (setae), 2) the transparent cuticular layers, 3) the bulk of yellow pigment, 4) the basement membrane. No difference could be detected between yellow pigment of the previously UV irradiated hornets and that of the control. Bar = 10 µm; a, epicuticle; b, exocuticle; c, endocuticle; h, hypodermis; arrows = the beginning of the basement membrane.

The structure of sectioned hornet cuticle in the region of the yellow stripes is shown in figure 3 where one can discern numerous strata of transparent cuticle (more than 30) and underneath them, within a cavity between the cuticle and the basement membrane, numerous spherical bodies containing yellow pigment comprised of purines and pteridines[13–15]. It should be pointed out that in other parts of the body, like the so-called brown cuticle, the strata contain the pigment melanin.

The exact mechanism and site of action of volatile anesthetics are unknown[16]. When applied as vapors in clinical concentrations they produce variable effects on isolated crayfish neurons and induce EEG seizure activity in man. This observation supports the concept that general anesthetic activity is selective and may involve multiple sites of action both in vertebrates as well as in invertebrates[17].

It seems that light of high energetic value and shorter wavelength, like UV and possibly also blue light, when irradiated upon yellow hornet cuticle (and most probably also on the cuticle of various other wasp species) converts to an electric current (in this respect acting like a solar cell), which induces depolarization of the membranes from a state of hyperpolarization, or can affect the membrane stabilization brought about by the ether anesthesia. Interestingly, hornets that had undergone decapitation also 'woke' early upon sun or UV irradia-

tion which means the anesthesia in such cases affects only the ventral nerve cord and involves inhibition of synaptic transmission. Normally the head is actively involved in sleep. Sleep in hymenopterans has been investigated by Kaiser and Steiner-Kaiser[18] and has neuronal correlates involving the optic lobes and brain. The manner whereby light energy converts to electric energy has yet to be elucidated, nor do we understand why there is need for a minimal time interval of about 2 min before awakening can take place. Apparently, there is a requisite for cumulative irradiation energy which demands a minimum of 2 min for awakening. What we do know for sure is that hornets deprived of sun on UV irradiation waken much more slowly from ether anesthetic than do non-irradiated hornets. If we accept the premise that the anesthetic produces anesthesia through a reversible inhibition of synaptic transmission[18] then it is conceivable that the energy obtained from the UV or sun irradiation counteracts this delay. What is clearly observed following the wakening of illuminated hornets is that they immediately take wing and fly, much the same as they do upon excitation, unlike non-illuminated hornets which, after wakening from anesthesia, are seen to slowly crawl back to their original resting site.

Upon repeating anesthesia and repeating UV or sun irradiation no change was observed in the mortality of test hornets nor did we discern any sort of damage which might have occurred from these repeated procedures. As a matter of fact, there does not seem to be any significant change in hornet behavior following repeated ether treatments and light-induced wakening of the hornets. Borisevitch et al.[19] have demonstrated that light activation of photosynthetic membranes leads to formation of an electric field. The membrane polarization manifests itself by electrochromism of intrinsic pigments and an electric effect of the macromolecular components. In the cuticle of (dead) hornets an electric field appears in the light[20] as well as in the dark[21] where, at optimal temperature for vespine biological activity, voltages of several hundred mV and currents of up to several hundreds nA have been recorded and measured[22]. It seems to us that the same energy conversion, from sunlight or UV light to electric energy, takes place in the cuticle of living hornets. In summarizing the described effect, it is reasonable to assume that in every case the wakening of the hornets when kept in the same temperature is the result of the total energy uptake from the immediate environment during the anesthesia. In the case of UV energy, as we have shown, about 2 min are needed as compared to about 20 min for hornets kept in the dark. With laser light energy or light sources of shorter wavelength[1] the sleep duration is apparently even briefer (Ishay, unpubl. observ.), but such highly energetic wavelengths cause irreversible damage to the hornet or its cuticle and are rather uncommon in natural sunlight which reaches the earth, so we have opted not to utilize them.

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