Moth Visual Potentials in Response to Middle UV-Radiation[1]

The physiological basis of UV-vision in insects is incompletely understood. In the insect compound eye, UV-sensitivity peaks ranging from 3400–3700 Å have been recorded using electrophysiological methods[2]. While the response to UV-wave-lengths shorter than 3000 Å has not been investigated, potentials have been obtained from ocelli and compound eyes in response to radiation of 3020 Å and longer wave-lengths[3,4]. Ionizing radiation has also been reported to initiate retinographic potentials[5]. Retinal action potentials in response to irradiation at the shorter UV-wave-lengths are of low magnitude because: (1) the probability of quantal absorptions by photopigment molecules is reduced as UV-wave-length decreases if intensity does not increase[6]; (2) corneal density to UV increases sharply at 3000 Å[7] and (3) the possibility of absorption by shielding pigments and/or aqueous media. However, it has been shown that the 2537 Å band is transmitted through the corneal lens of *Drosophila*, *Apis* and *Sarcophaga*[8] while this waveband serves to attract *Musca* and *Ostrina*[9,10]. The purpose of our study was to stimulate the eye of *Manduca sexta* with middle UV[11] energy (of high spectral purity and of constant and defined intensity) to determine whether this would be a stimulus to the retinulae and/or postsynaptic structures.

Method. Tobacco hornworm moths (20–24 h after emergence) were dark-adapted 1 h before being tested in midafternoon. To record ERGs a test platinum electrode was placed by a micromanipulator into the dorsal aspect of the ocular suture to a point about 600 μ beneath the dioptric apparatus so as to be near the retinulae to avoid damage of corneal lens facets. The reference electrode was similarly inserted in the opposite eye which was obscured by black-lacquer and on the non-irradiated side. A ground electrode was placed in the abdomen. Extracellular records from the contralateral II N 1 nerve[12] were obtained with the insect in the same position and a stainless steel electrode made surface contact with the nerve. In some cases this nerve was severed distally to eliminate afferent components although this did not appreciably alter the response. The electrodes were connected to a cathode follower circuit leading successively to a DC preamplifier and an oscilloscope with camera.

Radiant energy from a tungsten-quartz-iodine lamp passed through 2 high intensity grating monochromators in series. Energy bands (50 Å nominal band width) from the exit slit of the second monochromator were focused by a converging lens into a beam that covered the moth's eye. A solenoid-operated shutter energized by an interval timer provided the stimulus duration. A portion of the beam was reflected by a quartz beam splitter to a calibrated 1P28 photomultiplier tube transducer for a microphotometer and this monitored the radiation intensity. Irradiations of equal intensity at various wave-lengths were controlled by varying the voltage on the lamp with an adjustable autotransformer. Intensity level measurements had previously been made with a calibrated thermopile connected to a nanovoltmeter and a millivolt recorder.

Results. Through interpretation of electroretinographic evidence we observed that subretinular elements of a nocturnal moth appear to be sensitive to a low energy flux of middle UV (2700–3100 Å) radiation. Oscilloscope traces did not reveal a detectable retinal action potential (*c*-wave) but evoked small 'on-off' (*a*-, *b*-, and *d*-waves) responses which rarely exceeded 0.5 mV when the intensity was 7×10^{-3} μ watts/cm^2 (Figure). These 'on-off' components were attributed to underlying neuropiles in the optic tract[13] (no homologies to the vertebrate ERG wave form nomenclature are implied). As radiation increased in wave-length (constant intensity) the visual threshold abruptly decreased at 3100 Å and a *c*-wave was observed. This component prevailed and generally increased as the stimulating radiation increased in wavelength to 5500 Å at this intensity. Radiation that only elicited 'on-off' responses (but no *c*-wave) as recorded under the cornea also initiated spike discharges in flight muscle nerves.

From sensitive oscillographic records a very low threshold intensity could be ascertained (eliciting a 50 μ volt *c*-wave) for radiation of 2700–3000 Å. This barely perceptible event was usually obtained with a corneal flux of approximately 7×10^{-4} μ watts/cm^2. At 3100 Å, threshold for the *c*-wave dropped abruptly to about 9×10^{-5} μ watts/cm^2. These intensity levels also approximate those required to elicit from one to several spikes from the II N 1 nerve.

The response of the II N 1 nerve to irradiation of the contralateral eye did not indicate a correlation between spike type or frequency, and wave-length within the middle or near UV-region. The UV-area in general evoked a considerably more prolonged response than did the longer wave-lengths at a comparable intensity. On occasion a short burst of spikes was observed (Figure) at 300–400 msec after cessation of light.

Discussion. The relative lack of retinal action potentials following exposure to radiation of 2700–3000 Å does not necessarily indicate that these wave-lengths at the given intensity were subliminal to the photoreceptor cells since there is no exact correlation between ERG threshold and that of single receptor cells[14]. In other studies[15] the spectral sensitivity curve for this compound eye indicated that a threshold *c* wave response in the near UV required more than a 2 log unit increase in radiation intensity above that required to produce a constant effect at 5000–5500 Å. An absolute sensitivity peak occurred at 5500 Å with lesser maxima recorded for 5000 and 3800 Å respectively.

To ascertain the reflex motor response of middle UV-radiation, the contralateral II N 1 nerve was selected for 2 reasons: (a) this mixed nerve supplied a considerable bulk of flight muscle (pleurodorsals and dorsolongitudinals) which could, under natural conditions, effect flight

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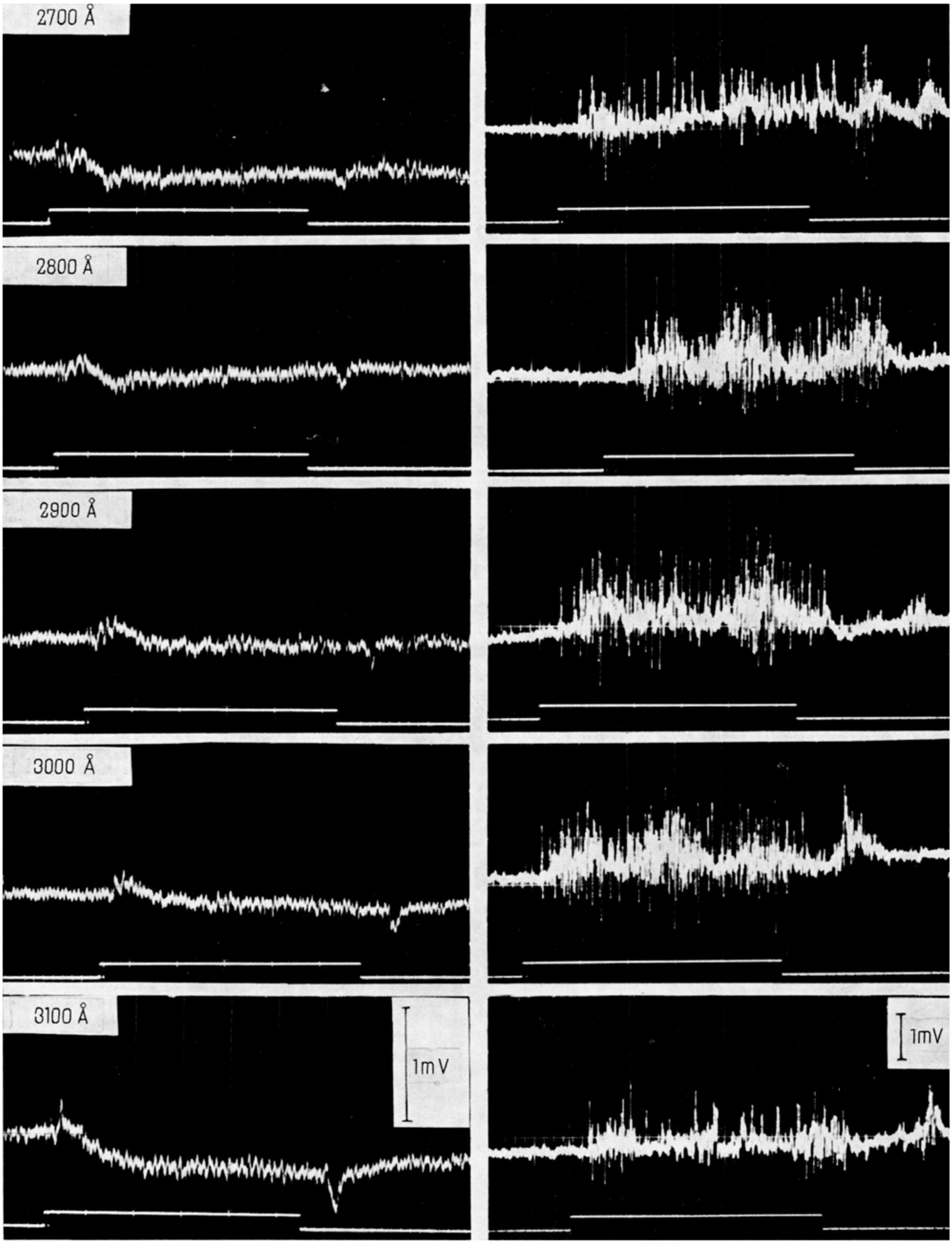

Electroretinograms (ERGs) and the contralateral flight muscle-nerve (II N 1) responses of *Manduca sexta* to a 1-sec flash of middle UV-radiation at the indicated 100 Å increments. Both types of response were recorded extracellularly. Stimulus energy was constant at all wavebands and the corneal flux was 7×10^{-3} μ watts/cm^2. These afferent and efferent discharges were obtained in separate experiments.

toward a radiation source and (b) there is evidence[16,17] that the visual impulse is predominantly conducted via this pathway to the opposite lateral thoracic connectives of the ventral nerve cord in certain insect species.

The implications of irritability to middle UV energy by postsynaptic units are intriguing. There is no evidence to the contrary that second and higher order neurons (responsible for the *a*-, *b*-, and *d*-wave) could not be at least partly responsible in initiating phototactic behavior in response to middle UV and thus obviate or depreciate contributions from neurons leading from the functional rhabdomere. There are precedents[18] for ganglionic sensitivity to light in the visible spectrum. The concurrent discharge in a major contralateral flight muscle nerve in response to middle UV suggests further that positive phototaxis might be a behavioral outcome following such stimulation, i.e. increased contralateral flight activity would veer the moth toward radiation received in the eye of the opposite side. However, this experiment cannot indicate if the efferent activity noted represents enough information to the moth so that it would orient to radiant energy of this type. The earth's ozone layer absorbs much solar radiation of the 2000–3000 Å band[19]. It would be of interest to know if host plants or other environmental objects emit or reflect this scant radiation in the far and middle UV-band and so provide meaningful stimuli to which the moth might orient.

Zusammenfassung. Das dunkeladaptierte Facettenauge vom Nachtschmetterling *Manduca sexta* wurde mit UV-Licht mittlerer Wellenlänge (2700–3100 Å) bei niedriger Intensität bestrahlt. Der photorezeptorische Anteil des ERGs war vermindert oder verschwunden, aber nachsynaptische Wirkungen wurden beobachtet. Impulsaktivitäten wurden bei einem hauptsächlich efferenten Flugmuskelnerv ausgelöst, wenn das kontralaterale Auge beleuchtet wurde.

S. D. CARLSON[20], J. S. SMITH JR.[21] and J. M. STANLEY

Department of Entomology, Virginia Polytechnic Institute and Agricultural Engineering Research Division, Agricultural Research Service, U.S. Department of Agriculture Blacksburg (Virginia 24061, USA), 21 August 1967.

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[20] Present address: Department of Physiology II, Karolinska Institutet, Stockholm, Sweden.
[21] Present address: USDA-AERD, P.O. Box 167, Kingshill, St. Croix U.S. Virgin Islands.

Die Einlagerung von radioaktivem Schwefel in die Flügel von Acrididen nach Applikation von ^{35}S-Natriumsulfat- und ^{35}S-L-Cystinlösungen

Das Studium über die Markierbarkeit von Pigmenten in den Flügeln des Tagpfauenauges (*Inachis io* L.) nach Applikation von ^{32}Phosphor[1] sowie in den Farbarealen der Vogelfeder nach Applikation von ^{35}Schwefel[2,3] und die Untersuchungen an verschiedenen Lepidopteren nach Behandlung mit ^{35}S-Natriumsulfat- und ^{35}S-DL-Cystinlösungen[4] ergaben deutliche Beziehungen zwischen den Pigmentfeldern und den Ablagerungzonen des Isotops. Bei der Musterbildung in den Flügeln der Orthopteren sind u.a. die Farbstoffklassen: Carotinoide, Melanine und Gallenfarbstoffe beteiligt[5]. Auch hier liessen Markierungsversuche mit radioaktiven Isotopen wichtige Hinweise erwarten.

Als Versuchstiere dienten die Feldheuschrecken *Psophus stridulus* L. und *Oedipoda coerulescens* L. Den kurz vor der Imaginalhäutung stehenden Larven wurde ^{35}S-Natriumsulfat- bzw. ^{35}S-L-Cystinlösung entweder oral verabreicht oder lateral intersegmental in das Abdomen injiziert. Die Flüssigkeitsmengen betrugen 0,005 ml und 0.01 ml entsprechend einer Strahlungsaktivität von etwa 10 μC bzw. 20 μC. Nach der Imaginalhäutung wurden die Flügel abgetrennt und autoradiographiert.

Nach Applikation von ^{35}S-Natriumsulfat waren in den Flügeln von *Psophus* 2 Typen von Strahlungsmustern festzustellen, die in ihren Abgrenzungen streng dem natürlichen Pigmentmuster entsprachen. Wurde den Versuchstieren das Isotop als ^{35}S-Natriumsulfat etwa 8 Tage vor der Imaginalhäutung verabreicht, so konnte die höchste Strahlungsaktivität in den roten Bereichen der Hinterflügel nachgewiesen werden. Erfolgte die Applikation des Isotops weniger als 4 Tage vor der Imaginalhäutung, so konnte in einigen Fällen die höchste Strahlungsaktivität dagegen in dem von den Melaninen gebildeten schwarzen Spitzensaum der Alae beobachtet werden (Figur 1a, mF).

Bei den mit ^{35}S-L-Cystin behandelten Tieren trat die höchste Strahlungsintensität stets im Bereich des roten Farbstoffes auf (Figur 1b, rF). Das schwarzpigmentierte Areal im apikalen Teil des Subcostalfeldes der Alae war sowohl in den Versuchen mit ^{35}S-Natriumsulfat als auch mit ^{35}S-L-Cystin markierbar (vgl. Figur 1a und b, ScF). Eine Markierung der Melaninareale in den Elytren war wegen der geringen Ausdehnung innerhalb des engmaschigen, stark strahlenden Flügelgeäders nicht feststellbar.

Bei *Oedipoda* war eine Markierung der blauen Pigmentzonen in den Alae nach Applikation von ^{35}S-Natriumsulfat möglich, wenn der Applikationszeitpunkt etwa 7 Tage vor der Imaginalhäutung lag (Figur 2a, bF). Wurde das Präparat 1 Tag vor der Imaginalhäutung verabreicht, wiesen die von den Melaninen gebildeten Areale die höchste Strahlungsaktivität auf (Figur 2b, mQ). Diese war sowohl in dem schwarzen Querband der Alae als auch in den braunen Querbändern der Elytren nachweisbar (vgl. auch [6]).

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