

Table 2. Number of beetles trapped in response to synthetic pheromones, to male beetles boring in fresh logs, and to fresh logs only (10 June)

Material tested	Mean response of 5 runs (0.5 h)	Range	Sex ratio ♂:♀
Methylbutenol + <i>cis</i> -verbenol + ipsdienol	77.8	169-22	1:2.1
Methylbutenol + <i>cis</i> -verbenol	56.2	127-7	1:1.7
Infested bolt 40 ♂♂ in 2 days	6.6	10-4	1:1.2
Fresh bolt	3.8	8-2	1:2.1
Control	1.8	5-0	1:0.9

in standing trees and fighting the resin flow; hence, it suggests that they are the primary aggregation pheromones of *Ips typographus*, which attract the large population necessary to overcome the resistance of standing, healthy trees.

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Electrophysiological Recordings from the Lateral Ocelli of *Drosophila*

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Quite a number of mutations affecting receptive or neural structures of the compound eye of *Drosophila melanogaster* have been isolated and the effect of these mutations on anatomy and physiology of the compound eye and on behavior has been examined [1-3]. However, the effect of these mutations on the fly's frontal eyes, the ocelli, has not yet been investigated. This paper describes a method for electrophysiological recording from the ocelli of *Drosophila* and presents some preliminary findings.

Drosophila melanogaster (white-eyed, if not specified otherwise) were immobilized by chilling on ice and fixed in an upright position by waxing the tips of the laterally extended legs to a cover slip. The head was waxed to the cover slip with its caudal surface in an approximately horizontal position. Additional mechanical stabilization of the head and thorax was achieved by means of lateral wax bridges. An oscillating razor blade guided by a micromanipulator was used to remove a small piece of cuticle (ca. 100 µm diameter) from the

caudal head surface. The dorsal edge of the cut just touched the lateral ocelli. Hoyle's saline [4] was applied to the head immediately while a wax barrier kept the abdomen and thorax free of saline. The tips of the micropipettes (filled with 2M KCl, 100-200 MΩ) were aimed just behind the ocellar lens, the site of the retinal cell layer [5, 6]. The electrode was advanced vertically in 2-5-µm steps. The penetration of cells was aided by inducing electronic oscillations at the tip of the electrode

through overcompensation of the input capacitance of the electrometer.

The responses to rectangular flashes of white light (tungsten lamp) most frequently observed in ca. 40 animals are presented in Figure 1 and Table 1. Sometimes transitions from one response type to another during recording were found.

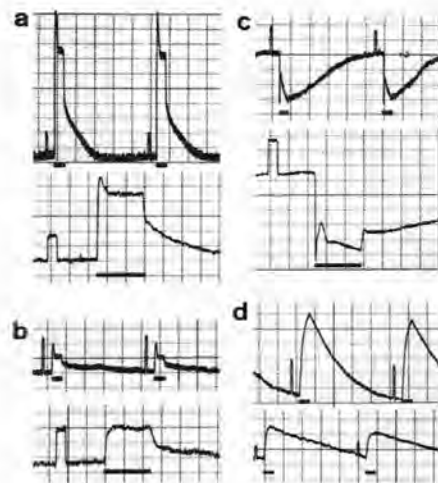


Fig. 1. Responses obtained in the lateral ocelli of *Drosophila*. (a) Type-A response (receptor potential), (b) type-B response, (c) type-C response, (d) type-D response. Bars indicate light stimulus. Calibration pulse: 0.1 s, 5 mV positive-going

The shape, polarity, amplitude, and preceding drop of baseline of the type-A response suggest that it may be an intracellularly recorded receptor potential of the ocellar photoreceptor. This is supported by the fact that the input resistance measured by applying 0.25-nA current pulses and observing the imbalance of the bridge

Table 1. Electrical responses to light flashes observed in the ocelli of *Drosophila*

Response type	Shape	Max. amplitude observed [mV]	Preceding shift of baseline*
A	Fast; positive-going; on-transient and plateau; at low intensities rectangular	30	negative
B	Fast; positive-going; rectangular, often on-transient	10	?
C	Negative-going; often spike-like on-transient	15 usually smaller	usually positive
D	Slow; positive-going; sometimes negative spike-like on-transient	20	usually negative

* DC shift of baseline on advancing the electrode before the described response could be observed

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[7] decreased by 20–34 M Ω during illumination (4 cells tested). The intensity-response curve of the type-A response exhibited the same slope as that of the compound-eye receptors [8]. Type-B responses may result from incomplete penetrations of receptors since they also show a small resistance change and are similar to type-A responses in shape. The origins of type-C and -D responses are not yet clear.

In a few experiments, only the immediate region of the ocelli of wild-type flies (containing screening pigment) was illuminated with a light guide having small tip diameter. Usual activity of the ocelli was observed suggesting that the ocelli are rela-

tively well-insulated electrically from the compound eyes.

A prolonged depolarizing afterpotential (PDA) [8] could not be induced from the ocellar receptors by illumination with blue light (Corning filter 5–59) in 10 independent experiments in which all four types of responses were recorded. Light stimuli of the same intensity, duration, and wavelength readily induced the PDA in the compound eye. However, higher intensities of blue light need to be tested to confirm the absence of PDA in the ocelli.

Preliminary data on ocellar responses of three mutants are summarized in Table 2. The mutation *trp* which influences the

transduction process in the compound-eye receptors [9] does also affect the electrical activity in the ocelli, whereas the mutations *rdgB* and *sev* which eliminate certain receptor types in the compound eye [2] do not. Not all the response types described could be observed in the mutants. This is probably due to the small number of flies tested rather than a significant effect of any given mutation.

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Table 2. Effect of mutations on the electrophysiology of the ocelli of *Drosophila*

Mutation	Effect on the compound eye	Genotype	Age [days]	Responses recorded from the ocelli	Number of flies tested
<i>trp</i> [9]	Receptor potential drops to baseline on constant illumination	<i>w; trp</i>	3–5	Mutant B, C, D	4
<i>rdgB</i> ^{KS222} [2]	Receptors 1–6 degenerate in light	<i>w rdgB</i>	9 kept under 12/12 h LD	Wild B, C, D	3
<i>sev</i> ^{LY3} [2]	Receptor 7 missing	<i>w sev</i>	7	Wild A, B, C	3

Factors of Haploid Production by Isolated Pollen Cultures

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Pollen cultures for the production of haploids provide a system with haploid single cells which are not influenced by the anther walls. Therefore, they are a unique tool for basic as well as applied research [1]. However, in contrast to anther cultures, only a rather limited number of reports on the successful use of pollen cultures has been published [2]. This indicates that pollen culture is still a controversial matter. Obviously the difficulties with this material are caused by different factors.

In investigations on enormous variations in the yield of plantlets by pollen cultures of *Nicotiana tabacum*, var. Badischer Burley (unpublished), we could show now that firstly, in contrast to anther culture, the binucleate stage is best suited and secondly the conditions for the growth of the donor plants are also essential for the induction

and the development of androgenic embryos.

It is known that in anther culture haploid production reaches a maximum at the mitotic stage (stage 4 according to [3]). The highest number of induced anthers, i.e., the induction frequency, occurs later, at the binucleate stage (stage 5), but these anthers will form less plantlets. This discrepancy has been explained by the inhibitory activity of factors produced in the anther walls [3]. In experiments on the yield of haploid production we obtained identical results with anther cultures, namely a maximum of production of plantlets at the mitotic stage. Cultures of isolated pollen, however, behaved differently. In this case only the use of binucleate pollen guaranteed the regular production of plantlets. Plantlet formation at the mitotic stage occurred very rarely with this material

and isolated uninucleate microspores (stage 3) never produced plantlets. This result can be understood, if it is assumed that in the binucleate stage the induction frequency is high compared with earlier stages and the inhibitory effect of the anther wall is eliminated by the use of isolated pollen. Therefore, the induced pollen, liberated from the inhibitory effect of the anther wall, has a better chance to develop into plantlets.

The second point of interest concerns the physiological status of the donor plants. It has been pointed out for instance that a causal relation might exist between the time of flower initiation determined by day-length and anther productivity ([4], see also [3]). In our experiments we have observed that salt nutrition affects the time of flower initiation and consequently success with pollen cultures. Constant addition of salt during growth resulted in a shorter vegetative period of the donor plants, from 15–16 to 12–13 weeks. With binucleate pollen from buds harvested during the first two weeks of flowering, plants with

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