Functional Group Recognition of Pheromone Molecules by Sensory Cells of *Antheraea polyphemus* and *Antheraea pernyi* (Lepidoptera: Saturniidae)*

H. J. Bestmann, Wu Cai-Hong, B. Döhla, and Li-Kedong

Organisch-Chemisches Institut, Universität Erlangen-Nürnberg, D-8520 Erlangen, Bundesrepublik Deutschland

K. E. Kaissling

Max-Planck-Institut für Verhaltensphysiologie, D-8131 Seewiesen, Bundesrepublik Deutschland

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Dedicated to Professor Helmut Simon on the occasion of his 60th birthday

Antheraea polyphemus, A. pernyi, Pheromone Analogues, Single Cell Recording, Functional Group Recognition

The pheromone components of *A. polyphemus,* (6*E*, 11*Z*)-6,11-hexadecadienyl acetate and (6*E*, 11*Z*)-6,11-hexadecadienal, both effective also in *A. pernyi,* were synthetically varied in their chemical structures and these compounds electrophysiologically tested in single cell recordings. It appeared that for the interaction between the signal molecule and the receptor region at the dendritic membrane of the receptor cell the electronic character of the functional end group (acetate or aldehyde) of the stimulus molecule is important. The excitation of the sensory cell also depends on the chain length.

A dynamic model of the interactions between the signal molecule and the molecular receptor region at the dendritic membrane in the sensilla of Lepidoptera based on structure-activity relationships of pheromones of numerous lepidopteran species has been presented [2]. This model could be confirmed by investigations of chiral derivatives of achiral pheromone molecules [3]. According to this model, the pheromone molecule is imbedded flexibly into the specific receptor site with a defined conformation.

The intersexual transmission of information of insects often takes place *via* molecules, which differ only by their functional end group. The saturniid species *Antheraea polyphemus* uses (*E*)-6,(*Z*)-11-hexadecadienyl acetate (**1**) (abbr. E-6,Z-11-HDAc) and (*E*)-6,(*Z*)-11-hexadecadienal (**2**) (abbr. E-6,Z-11-HDAl) as its pheromone complex, two molecules differing only by the acetate and the aldehyde group. Specific receptor cells [4] for both molecules exist in the olfactory sensilla (s. trichodea) of the antennae. The responses of these receptor cells can be distinguished by the different amplitudes of the action potentials in single cell recording. The acetate cell produces larger spikes than the aldehyde cell [4]. The closely related species *Antheraea pernyi* among others also uses both molecules **1** and **2** in its pheromone complex [5]. However, with this saturniid species, the aldehyde cell shows larger action potential amplitudes than the acetate cell [6].

If structural variations in the chain of molecules **1** and **2** are carried out without changing the functional group, only the acetate cell of *A. polyphemus* or *A. pernyi* responds to acetates, and only the aldehyde cell responds to aldehydes [7]. This suggests that the polar functional part of the molecule may determine whether the signal molecule is imbedded in the receptor site and the cell thus stimulated, or not. The question arises, to what extent the stereoèlectronic character of the aldehyde and acetate group are responsible for the high specificity of recognition of the pheromone by its receptor site and how much is contributed by parts of the molecule chain.

To clarify this problem the following substances have been synthesized: (*E*)-6,(*Z*)-11-hexadecadienyl formate (**3**) (abbrev. E6,Z11-HDFo), (*E*)-5,(*Z*)-10-pentadecadienyl formate (**4**) (E5,Z10-PDFo), (*E*)-4,(*Z*)-9-tetradecadienyl formate (**5**) (E4,Z9-TDFo), (*E*)-5,(*Z*)-10-pentadecadienyl acetate (**6**) (E4,Z10-PDAc), (*E*)-5,(*Z*)-10-pentadecadienal (**7**) (E5,Z10-PDAl), (*E*)-4,(*Z*)-9-tetradecadienyl acetate (**8**) (E4,Z9-TDAc), (*E*)-4,(*Z*)-9-tetradecadienal (**9**) (E4,Z9-TDAl). The formate group of **3**, **4** and **5** is

* Pheromones 56 [1].

Reprint requests to Prof. Dr. H. J. Bestmann.

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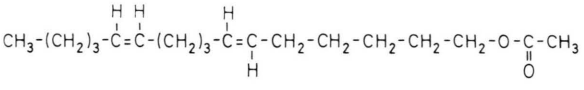
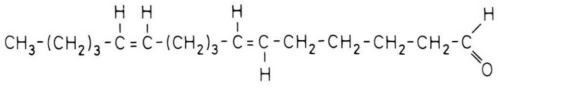


1 *E*-6, *Z*-11-HDAc



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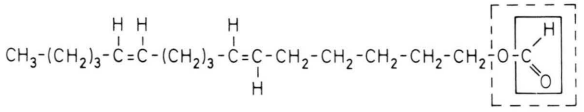

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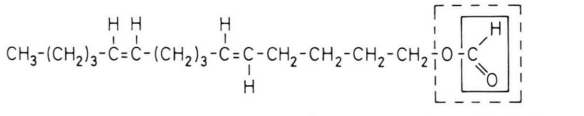
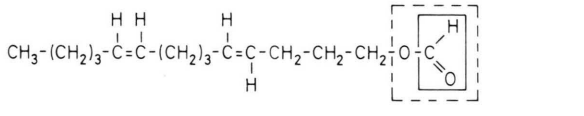
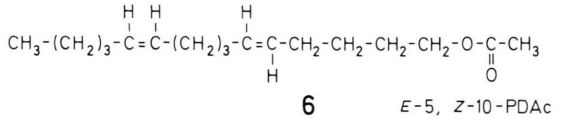


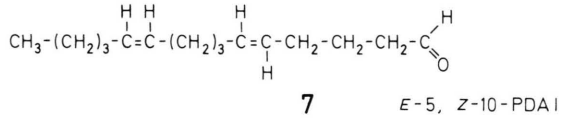
4 *E*-5, *Z*-10-PDFo



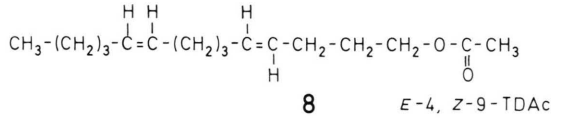
5 *E*-4, *Z*-9-TDFo



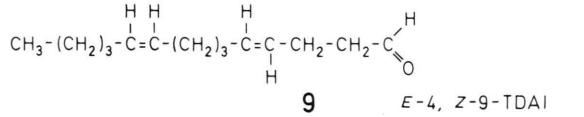
6 *E*-5, *Z*-10-PDAc



7 *E*-5, *Z*-10-PDA l



8 *E*-4, *Z*-9-TDAc



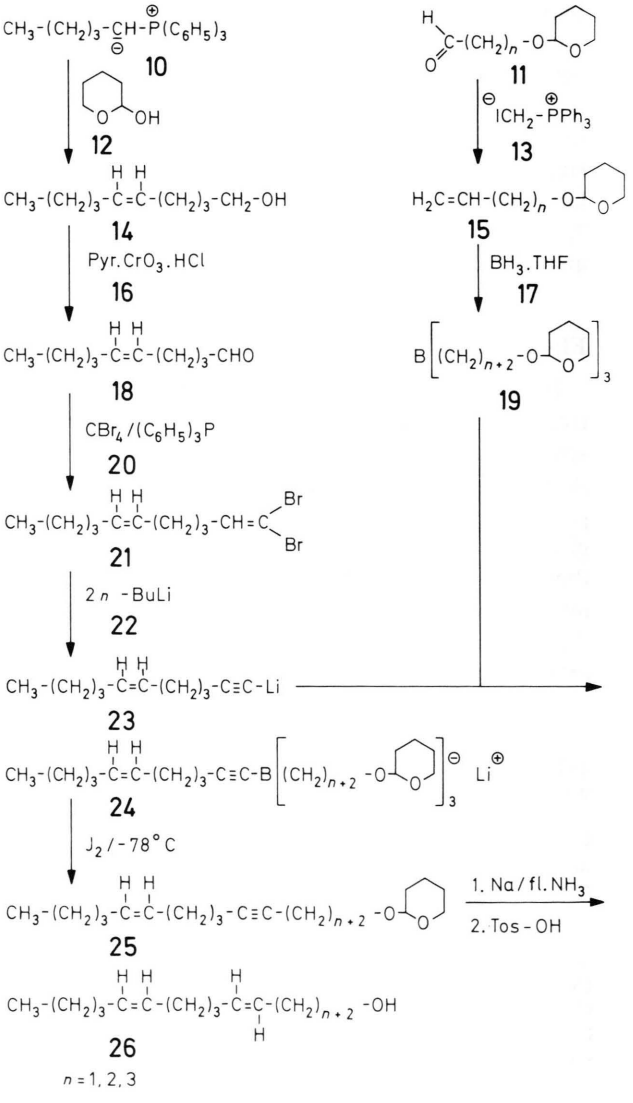
9 *E*-4, *Z*-9-TDAl

not only an ester function (framed with dashed lines in formula 1) but also partially represents an aldehyde function (solid frame). **3** has the same C-chain length of 16 carbon atoms in the alcohol moiety as the pheromone **1**. In **4**, **6** and **7** it is shortened by one C-atom in the part between the (*E*)-double bond and the functional end group, and in **5**, **8** and **9** by two carbon atoms. **6** and **8** are shortened acetates and represent structural variations of **1**, while **7** and **9** are analogs of **2**.

3 has almost the same total chain length (including the ester oxygen atom) as the acetate of the original pheromone **1**. However, the methyl group of the acetate moiety is substituted by a hydrogen atom. **5** corresponds in the total chain length to the natural aldehyde, **2**, whereby, however, the last CH_2-group before the carbonyl function of **2** is substituted by an oxygen atom.

Synthesis

For the synthesis of **3**–**5** the synthetic route which we used previously for the preparation of **1** and **2** [8] was chosen [9].



The ylide **10** was reacted with 2-hydroxytetrahydropyrane **12** to give (*Z*)-5-decenol (**14**), which was oxidized with **16** to the aldehyde **18**. Subsequent reaction with triphenylphosphine-carbontetrabromide **20** yielded the vicinal dibromodiene **21**, which was converted into the alkynide **23** using butyl lithium **22**. The second synthon is the tetrahydropyranyl ether of ω-hydroxyaldehydes **11** ($n = 1, 2, 3$) which were olefinated with methylene phosphorane **13** to give **15**. Subsequent hydroboration with **17** gave the boranes **19**, which reacted with **23** to the ate-complex **24** and subsequently with iodine at −78 °C to the enynes **25**. (All necessary literature references for each synthesis step up to this step are given in l.c. [8]). The triple bond of **25** was partially hydrogenated (*E*)-stereoselectively using sodium in ammonia and then the tetrahydropyranyl group split off with *p*-toluene sulfonic acid in methanol. The alcohol **26** thus generated was converted into the formates **3** (from **26**, $n = 3$), **4** (from **26**, $n = 2$) and **5** (from **26**, $n = 1$) by formylimidazole [10]. The acetylation of **26** ($n = 2$) gave **6**; the oxidation the aldehyde **7**. The acetate **8** and the aldehyde **9** are obtained analogously from **26** ($n = 1$).

Electrophysiological Investigations

Receptor potentials and action potentials were extracellularly recorded from the receptor cells of the sensilla trichodea on the antennae of *A. pernyi* and *A. polyphemus* using the technique described in ref. [11]. The relative effectiveness of a compound for a given cell can be estimated by comparing the loads of the stimulus sources eliciting equal numbers of nerve impulses. A higher load means a smaller activity of the compound. The effectiveness cannot be given more precise than by a factor 2 or 3 due to variability of cell responses and of stimulus delivery. The expected differences in release rates of the stimulus compounds are here neglected because they are, undoubtedly, much smaller than the observed differences in stimulus source loadings for equal responses. Stepwise structural changes with constant functional group produced differences in effectiveness between 10 and 1000. The step from aldehyde to acetate produces a change in effectiveness of at least 10000. Almost identical patterns of specificity were found for the acetate cell of both species and also for the aldehyde cells, respectively.

Table I shows the qualitative results of the responses of both cell types (acetate and aldehyde cell) when stimulated with the molecules **1**–**9**. Examples of nerve impulse recordings from receptor cells of single hairs are given in Fig. 1–3. Each experiment was 10 times repeated.

Fig. 1 and 2 demonstrate that in both species E-6,Z-11-HDFo (**3**) stimulated the acetate cell and was about 10 times less effective than the acetate component **1** of the pheromone. However, with higher concentrations (stimulus source loading 10 μg) the aldehyde cell of *A. pernyi* also responded. Sometimes the (small) spikes of the aldehyde cell were also observed with *A. polyphemus*, with strong stimulus.

Both cells respond clearly to stimulation with the E-5,Z-10-PDFo (**4**), with a carbon chain of one carbon atom less, whereby statistically in different experiments the stimulation of the aldehyde cell predominated. The estimated effectiveness of this compound **4** was about 1000-fold less than of the pheromone acetate **1**. When the molecule was shortened by one further carbon atom to E-4,Z-9-TDFo (**5**) for both species only the aldehyde cell responded. The estimated effectiveness of **5** on the

Table I. Responses[a] of pheromone receptor cells (acetate cells, aldehyde cells) at the antennae of *Antheraea polyphemus* and *A. pernyi* stimulated with molecules **1**–**9**.

Chain length in the alcohol moiety of **1**–**9**	Functional group								
	Acetate			Formate			Aldehyde		
	Molecule no.	Acetate cell	Aldehyde cell	Molecule no.	Acetate cell	Aldehyde cell	Molecule no.	Acetate cell	Aldehyde cell
C-16	**1**	+	−	**3**	+	−	**2**	−	+
C-15	**6**	+	−	**4**	+	+	**7**	−	+
C-14	**8**	+	−	**5**	−	+	**9**	−	+

[a] + = Positive response; − = no response.

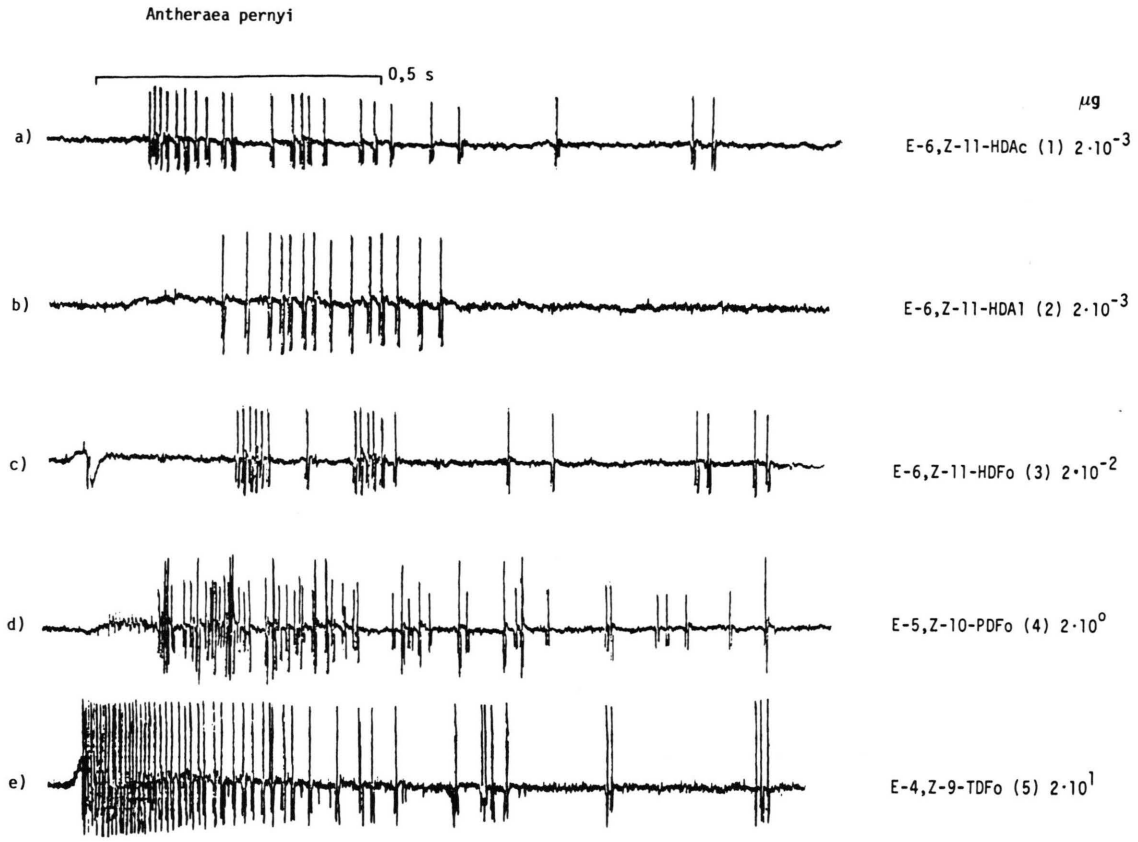


Fig. 1. Action potentials recorded from a sensillum trichodeum of *A. pernyi*. Small action potentials from acetate cell responding to compounds **1** and **3**; large action potentials from aldehyde cell responding to **2** and **5**. Both cells respond to **4**. Stimulus source loading given in µg. Horizontal bar: stimulation period.

aldehyde cell was almost equal to the one of **4** on this cell.

Fig. 3 reflects the responses for the acetates **6** and **8** as well as those for the aldehydes **7** and **9** with *A. polyphemus* (A) and *A. pernyi* (B). It becomes clear, that even with different chain length of the acetates **6** and **8** only the acetate cell (A. large, B. small spikes) is stimulated and on stimulation with the aldehydes **7** and **9** only the aldehyde cell responds (A. small, B. large spikes). Shortening of the carbon chain of the pheromone components **1** and **2** by one carbon atom reduced the estimated effectiveness by a factor of 1000. Further shortening of chain length produced still 10–100 times smaller effectiveness.

Discussion

These results confirm the idea that the molecular and electronic structure of the polar, functional end group of the stimulus molecule is essential whether the molecule is accepted or not by the receptor region. Acetates with chain length C-14 to C-16 are only accepted by the acetate cells of *A. pernyi* and *A. polyphemus*, and the corresponding aldehydes stimulate only the aldehyde cells (Fig. 1, 2 and 3).

We note, however, that with a 10000-fold increase of the stimulus concentration of the pheromone components **1** and **2** the other ("wrong") cell also responded. If this response is due to a direct interaction of the stimulus molecule with the "wrong" cell or to a transfer of the strong excitation of the "right" cell, cannot be decided.

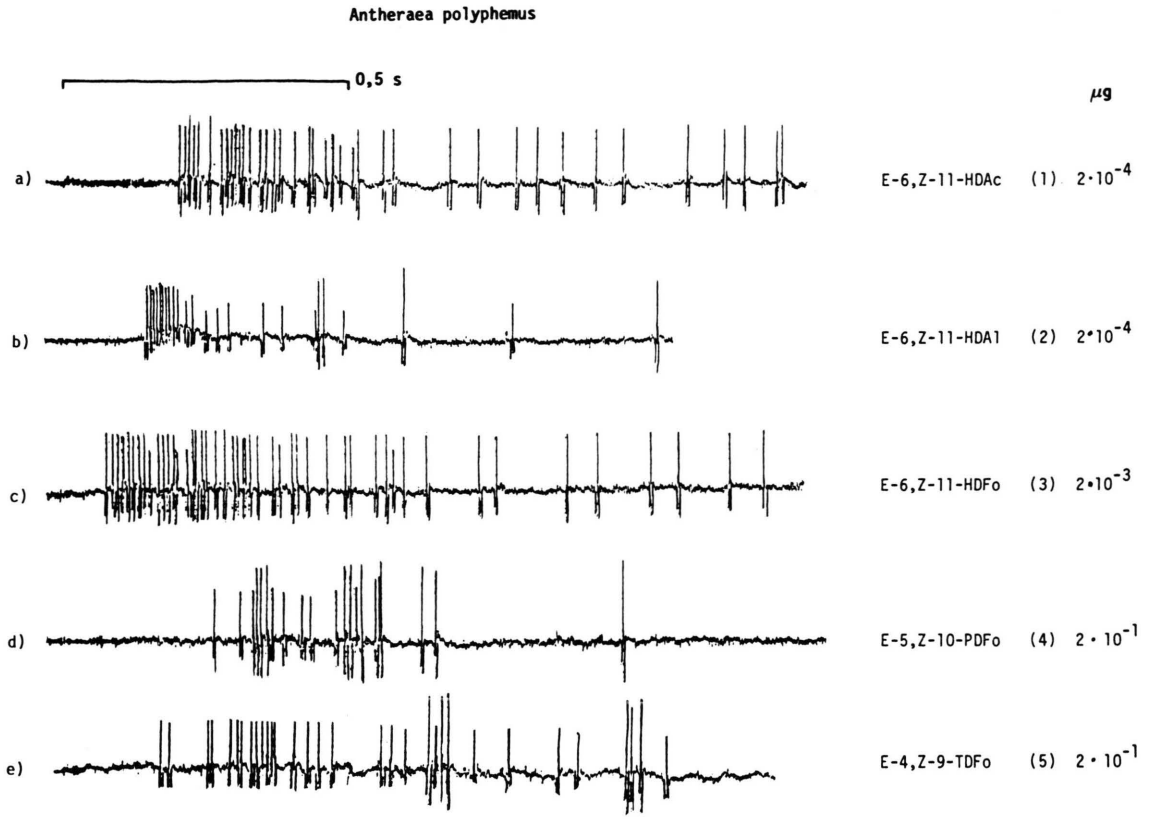


Fig. 2. Action potentials recorded from a sensillum trichodeum of *A. polyphemus*. Large (small) action potentials from acetate (aldehyde) cell responding to compounds **1** and **3** (**2** and **5**). Both cells respond to **4**. Few large action potentials observed with stimulation by **2** and **5** may be attributed to spontaneous activity of the acetate cell. For further explanation see Fig. 1.

The formate group shows a hybrid character, since it possesses an ester function. With a chain length of 16 carbon atoms it is relatively well "recognized" by the acetate cell and only in much higher concentrations by the aldehyde cell. When shortened to a C-15 chain, both the acetate and the aldehyde cell detect the molecule about equally well. With C-14 only the aldehyde cell responds and, astonishingly, this reduction in chain length does not lead to further decreased effectiveness on the aldehyde cell (Fig. 1 and 2). This clearly shows that for the stimulation of the cell the total chain length is of great importance. It is much too short for the acetate cell, but it is equal to the one of the aldehyde pheromone **2**. E-4,Z-9-TDFo (**5**) differs from the pheromone aldehyde **2** because of the replacement of the CH_2-group adjacent to the carbonyl group by an oxygen atom. Obviously, the aldehyde cell accepts the aldehyde part of the formate group if the total chain length is appropriate.

The importance of the chain length for the stimulation is reflected also by the fact that the C-14 aldehyde **9** is significantly less active than the formate **5**. When the formyl group in **5** is substituted by an acetate group, the molecule **8** is no longer recognized by the aldehyde cell, but by the acetate cell.

From these results one can assume that the adaptation of the stimulus molecule to the receptor region to an essential degree occurs *via* the terminal functional group which *e.g.* can enter into stronger π-interactions with amino groups of proteins than the remainder of the molecule.

The aliphatic-olefinic part of the signal, however, is of similar importance for the stimulating of the cell, because the total chain length plays an important role. Van der Waals interactions between the

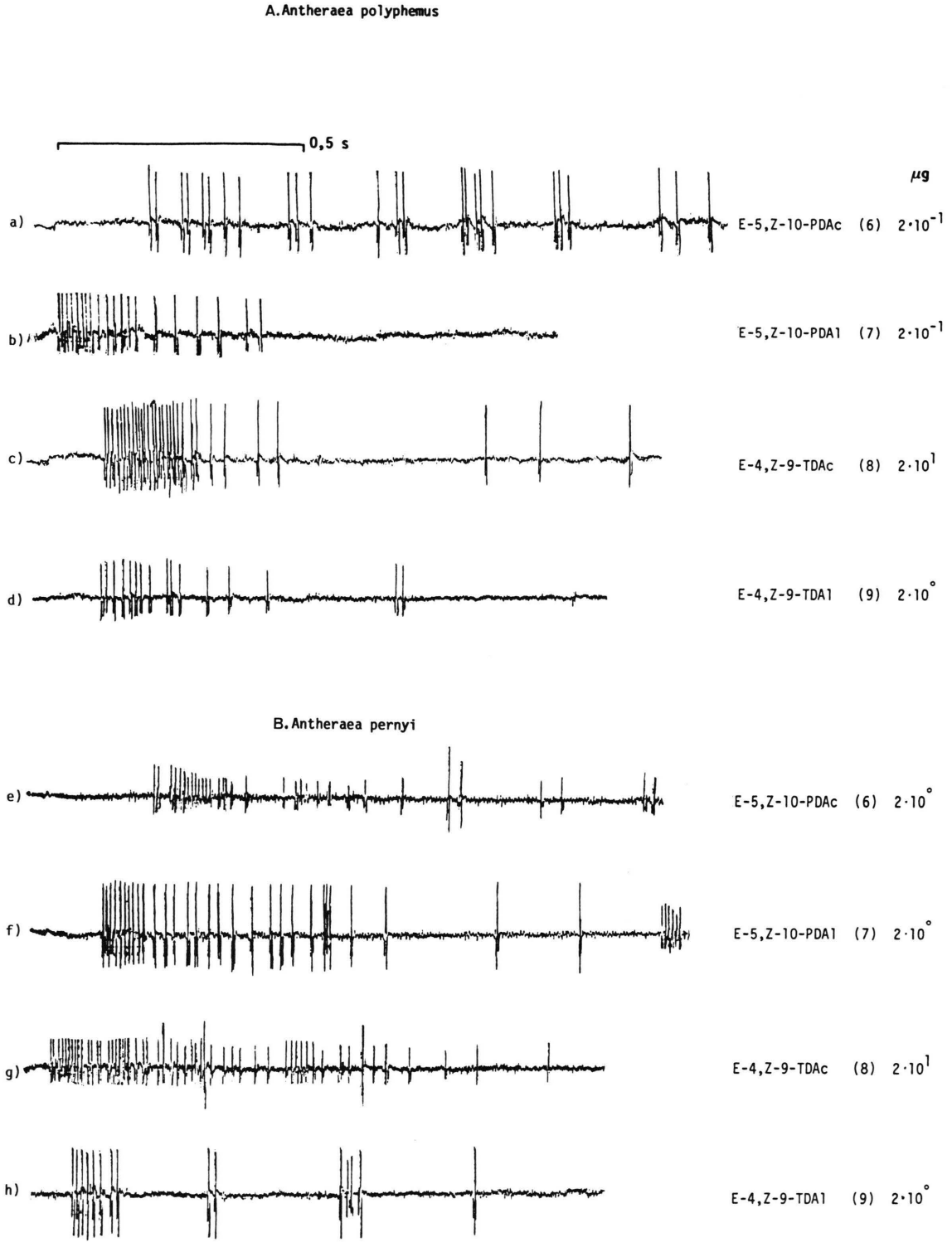


Fig. 3. Action potential responses of acetate and aldehyde receptor cells of A. *A. polyphemus* and B. *A. pernyi* with the substances **6**–**9**. Acetate cells respond to the acetates (**6** and **8**), aldehyde cells to the aldehydes (**7** and **9**). For further explanation see Fig. 1.

hydrogen atoms of the signal molecule and those of the receptor region may determine the effectiveness of the stimulus molecule.

The replacement of a CH_2-group by an oxygen atom in the α-position relative to the aldehyde function, transforming **2** into **5**, results in a large decrease of effectiveness not only because of the influence of π-electron interactions (diminished carbonyl activity in the formate compared with the aldehyde group) but also because of the lack of van der Waals interactions of one CH_2-group as well as because of changing of conformational structures. This shows clearly that the region adjacent to the carbonyl function of the aldehyde strongly influences the interaction with the receptor structure.

When the acetate group is replaced by the formate group (transition **1** to **3**) the consequences for the interactions with the receptor region are not very large. The electronic structure of the end group is not changed as much as in the transition from **2** to **5**. The van der Waals interactions in the aliphatic-olefinic region in **3** are the same as in **5**. The same is also valid for the three-dimensional structures of the various possible conformations which, as mentioned above, are of great importance for the flexible insertion of the signal molecule into the receptor region [2, 3]. Because of this, loss of activity from **1** to **3** is not as large as that from **2** to **5**.

We will later discuss the consequences of systematic changes in the olefinic part of the molecule for its biological activity.

Acknowledgement

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