Specificity-related suppression of responses to binary mixtures in olfactory receptors of the Colorado potato beetle

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Responses of antennal olfactory receptors of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) to stimulation with 5 general green odour components, i.e. *cis*-3-hexen-1-ol, *trans*-2-hexenal, *cis*-3-hexenyl acetate, *trans*-2-hexen-1-ol and 1-hexanol, were recorded extracellularly. Response spectra derived from these recordings cannot be classified into distinct reaction types. The spectra overlap in their sensitivity to individual stimuli, but there are differences in their degree of specialization with a gradual conversion from generalist to specialist receptors. Moreover, specialization is found to different stimuli. Receptor reactions to stimulation with binary mixtures of 3 of these compounds indicated that suppression of the response to one chemical by another is very common in olfactory receptor cells. The more a receptor is specialized, the stronger is this suppression. Suppression in narrowly tuned olfactory receptor neurones, therefore, is expected to play a fundamental role in the recognition of natural odour blends.

INTRODUCTION

The invertebrate's coding mechanism for olfactory cues has been investigated by examining responses of neurones at various levels of the nervous system[2,8,24]. Responses of peripheral olfactory neurones in different insect species to various test stimuli have been studied in fair detail. They have been classified into reaction groups on the basis of similarities among the reaction spectra[3,13,15,16,25,28]. Receptors with differences in their degree of specificity and with specializations to different stimuli have been described. Examples of receptors which are extremely narrowly tuned to only one or a few compounds have been given for pheromone receiving neurones in several species[6,14,17,20,23]. Most food odour receptors, on the other hand, are more broadly tuned in their perception of stimuli[4,13,24,28].

Phytophagous insects exhibit specific behavioural responses either to a host odour-specific component or to the mixture of different non-specific host odour components[31]. Flea beetles *Phyllotreta cruciferae*, for example, are attracted by allylisothiocyanate which is a specific component of their cruciferous host plants[9]. However, in the Colorado potato beetle, *Leptinotarsa decemlineata*, the quantitative ratio of several odour components is important for host odour recognition. 'Green' odour, which forms a significant part of all leaf odour blends, is composed of C-6 alcohols, aldehydes and the derivative acetate[30]. Different plant species may show different proportions of the individual components of the green odour complex. Distortion of the composition of the green odour of potato leaf, *Solanum tuberosum*, by addition of small amounts of its components to the odour of potato plants, prevented upwind orientation of Colorado potato beetles towards the plants[32]. Furthermore, the odour of their host plant, potato, is masked for the beetles when it is mixed with other plant odours[27]. Nevertheless, a general problem in

studies on olfactory coding is the lack of knowledge about the precise composition of food odour blends. Although most biologically relevant odours consist of complex mixtures, an analysis of olfactory coding, therefore, has been based on neuronal responses to individual odour components[7,24,31].

The olfactory receptors in the antennae of Colorado potato beetles are sensitively tuned to C-6 compounds[15,29]. In previous work we used 5 green odour components, i.e. cis-3-hexen-1-ol, trans-2-hexenal, cis-3-hexenyl acetate, trans-2-hexen-1-ol and 1-hexanol, to study the responses of neurones in the deutocerebrum of the Colorado potato beetle[5]. In this first relay station, synaptic connections between receptor neurones and interneurones are formed. Characterization of deutocerebral neurons revealed the existence of two groups: one group (A) of neurones showed specific responses to individual leaf odour components, and another group (B) of less specific responding neurones. After stimulation with a potato leaf extract, group B neurones responded, while group A neurones hardly changed from their spontaneous firing activities. Since the potato leaf extract is a mixture of odour components, the lack of response in group A to this mixture implies an important role of suppression as a mixture effect. A coding mechanism for the odour of potato leaf has been proposed on the basis of this suppression[5].

The present study was carried out to characterize the receptor responses to stimulation with the same 5 C-6 components. In order to examine if mixture interactions like those in specialized deutocerebral neurones also exist at the peripheral level, we additionally characterized the olfactory receptors by their responses to some artificial mixtures.

MATERIALS AND METHODS

Two-day-old female Colorado potato beetles were obtained from the laboratory stock culture and used for the experiments. Recordings from antennal olfactory receptor cells were made as described previously[15]. We modified the method of stimulus delivery by placing the preparation in a continuous airflow (40 cm/s, 30 ml/s) in which odour stimuli were injected by flowing air through a Pasteur pipette (1 ml/s, 2 s). The Pasteur pipette contained a piece of filterpaper (6.0 × 0.5 cm) loaded with a stimulus solution (25 μl).

We used as stimuli 5 C-6 components of the potato leaf odour, namely cis-3-hexan-1-ol, trans-2-hexenal, cis-3-hexenyl acetate, trans-2-hexen-1-ol and 1-hexanol (at a dilution of 4 × 10⁻² in paraffin oil, v/v). The test chemicals were obtained from commercial sources (Roth, Koch Light) and were >97% pure. Paraffin oil alone was used as the control. An interstimulus time of at least 1.5 min was used.

The receptors were recorded extracellularly. In most of the recordings, only one spike amplitude was visible. In a few cases we used recordings with two clearly distinguishable spike amplitudes. The number of spikes in the first reaction second was counted in order to obtain the response level to a stimulus.

The relative response spectrum for each cell was obtained by setting its 'best' component on a 100% level. The degree of specialization of a receptor cell (DS) was calculated by:

$$DS = \frac{500 - \sum\limits_{i=1}^{5} R_i}{4}$$

R_i represents the cell's relative response to component i. This calculation expresses DS as a percentage which, theoretically, ranges between 0 for cells without any specialization, and 100 for cells responding to only 1 of the 5 test stimuli. Once the degree of specialization of a cell was known, its responsiveness to mixtures was determined. This was done by measuring its reaction to three 1:1 binary mixtures, each at two dilutions of the total amount of volatiles (4 × 10⁻² and 8 × 10⁻² M, v/v). The components used in mixtures were trans-2-hexenal, cis-3-hexenyl acetate and 1-hexanol. Each mixture response was expressed as a percentage of the response to the 'best' component that was also present in the mixture, and from these 6 values an average mixture response (AMR₆) was calculated for each cell. Stimulation sequences started with the lowest concentration stimuli. Variables were compared using the non-parametric Spearman rank correlation test[26].

RESULTS

Thirty-nine receptor neurones on the antennae of Colorado potato beetles were recorded. Most of these neurones revealed a low background firing rate of 3 ± 4 spikes/s (mean ± S.D.) which increased to 26

± 16 spikes in the first reaction second after stimulation with the cell's 'best' component. The response spectra of the receptors were classified in 5 groups, according to their 'best' stimulus (Fig. 1A–E). Some spectra (Fig. 1, nos. 29, 30, 36) do not show a 'best' stimulus and, therefore, can be classified in more groups. Within a group the spectra were arranged according to their DS-values. The degree of specialization in one group increases from left to right. The degree of specialization of the receptors for the 5 stimuli ranges from DS-values of 19 to 94 (Fig. 1, nos. 1 and 28 respectively). We did not find a correlation between the sensitivity of cells to their 'best' component (in number of spikes in the first reaction second) and the DS, nor between the background frequency and the DS. The response spectra in Fig. 1 show that there are no distinct receptor types to the 5 stimuli. The recorded population of olfactory receptors responded differentially to the set of stimuli and individual spectra show overlap. Nevertheless, considerable differences in receptor responses are found.

Some receptors showed non-specific responses, while others were specialized to particular stimuli. In 13 cases out of 39 we were able to repeat the stimulation series demonstrating the consistent character of the spectra. Newly calculated DS values, then, did not differ significantly from previous values (Wilcoxon matched-pairs signed-ranks test) ($\overline{\Delta DS}$ = 5.8).

The responses of the cells in Fig. 1 to the binary mixtures are represented in Fig. 2, and, in addition, are related to the response to the 'best' mixture component. The classification of these responses in groups corresponds with the one in Fig. 1. In 63 of the 117 cases. one component alone elicited a stronger response than the mixture in which the same concentration of that component was present. On the other hand, only in 6 cases the sum of the responses to individual components was smaller than the responses to the corresponding mixtures. Suppression, therefore, is found to be the most common mixture effect. The strength of this suppression varied in the different receptors and was sometimes found to be very pro-
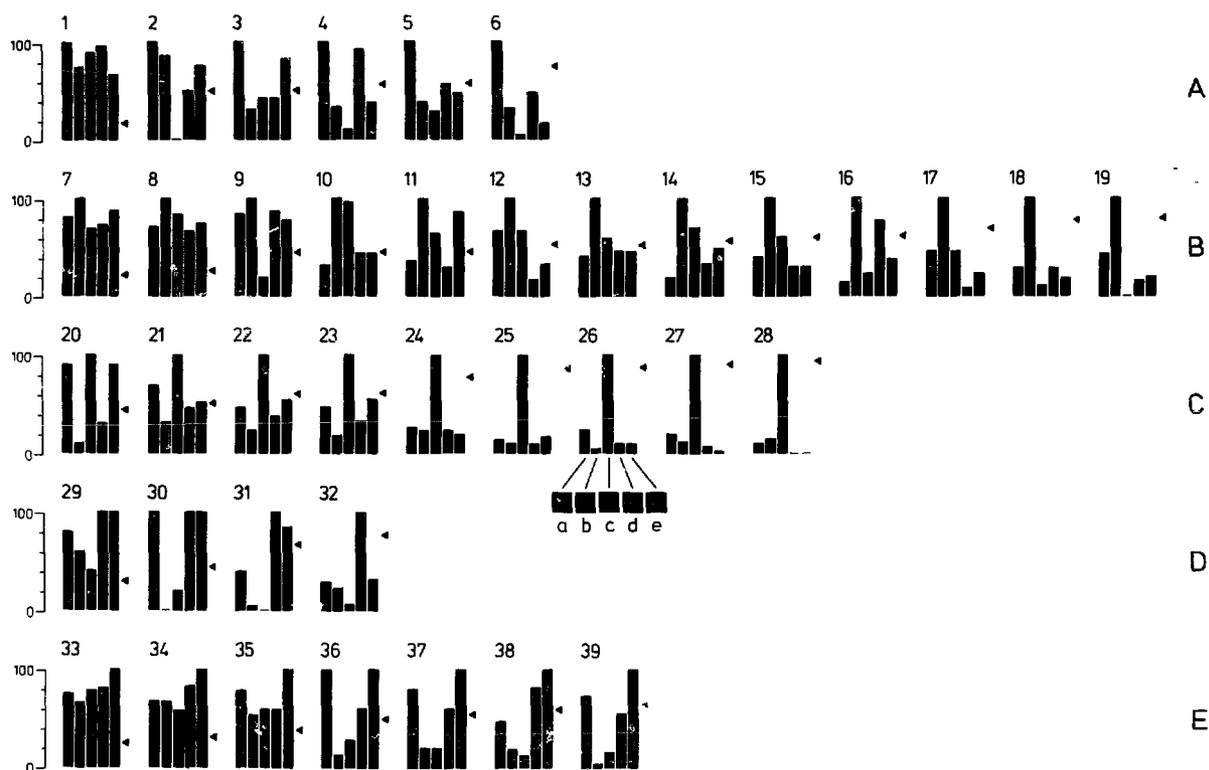

Fig. 1. Relative response spectra of 39 olfactory receptor cells in Colorado potato beetle antennae for 5 leaf odour components. The receptor's 'best' component is 100%. a: cis-3-hexen-1-ol. b: trans-2-hexenal. c: cis-3-hexenyl acetate. d: trans-2-hexen-1-ol. e: 1-hexanol. Chemicals were pipetted on filterpaper at a dilution of 4 × 10⁻² v/v. Triangle indicates DS value.
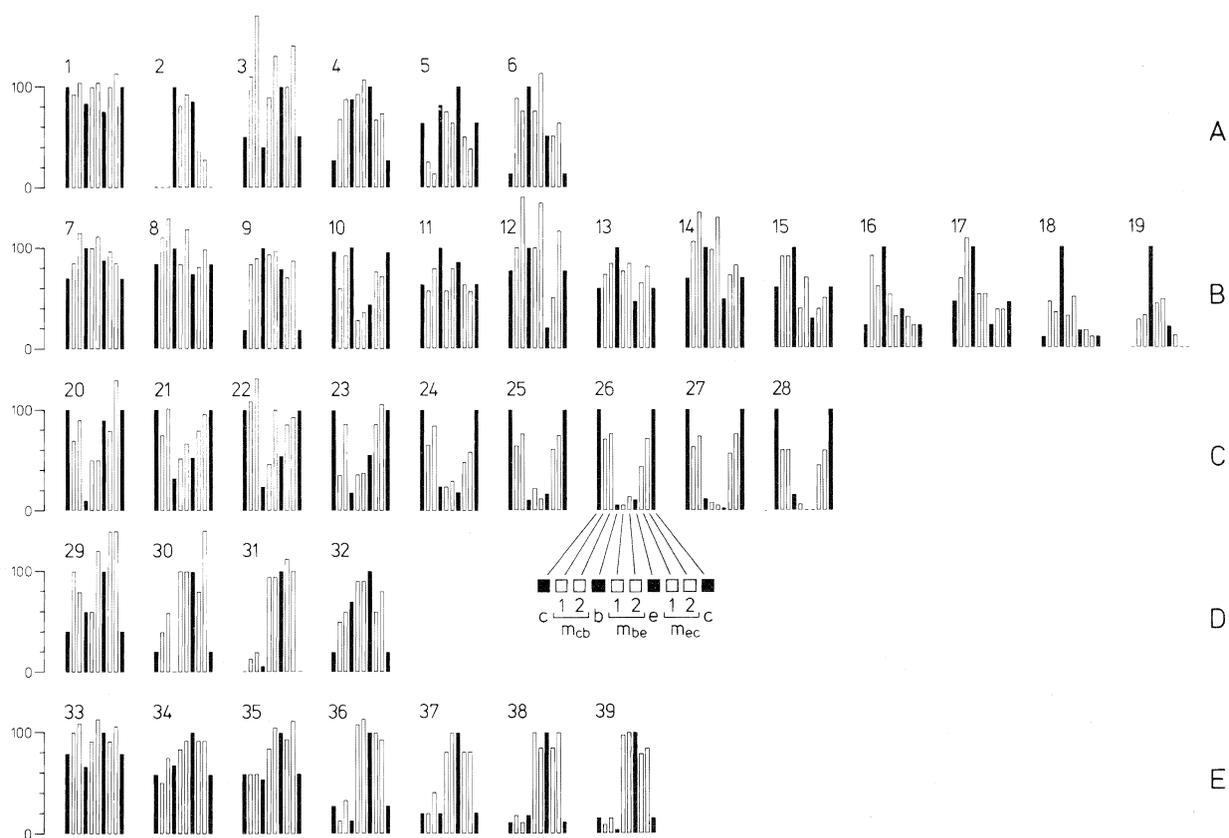
Fig. 2. Relative response spectra of the receptors shown in Fig. 1 for 3 binary mixtures at two concentrations, and their individual components. The 'best' component is 100%. c: *cis*-3-hexenyl acetate. b: *trans*-2-hexanal. e: 1-hexanol, m_{cb} = 1:1 mixture of c and b, m_{be} = 1:1 mixture of b and e, m_{ec} = 1:1 mixture of e and c. 1 = mixture at a dilution of 4×10^{-2} v/v; 2 = mixture at a dilution of 8×10^{-2} v/v. Pure compounds were at a dilution of 4×10^{-2} v/v.
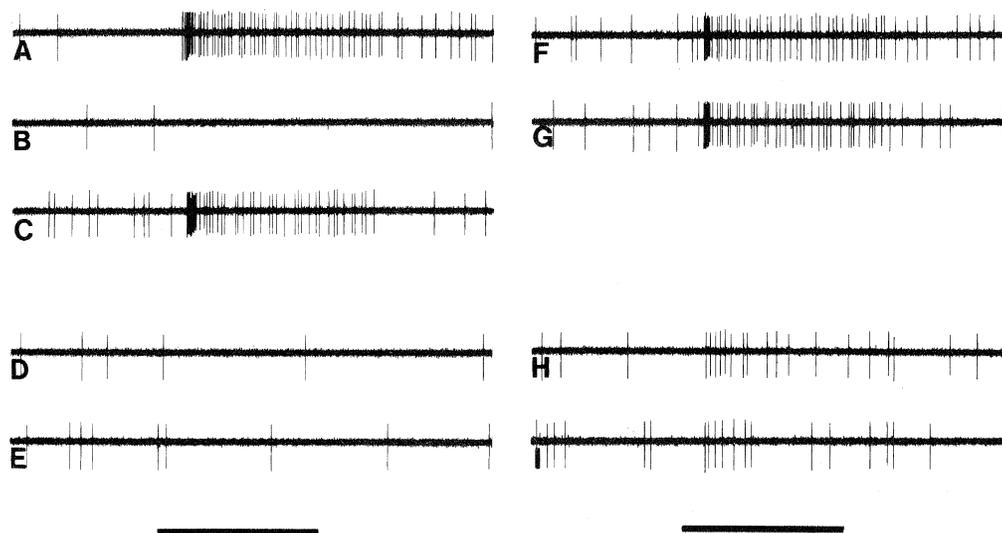

Fig. 3. Responses of one olfactory receptor cell in the Colorado potato beetles' antenna on stimulation with 3 compounds, and their 1:1 mixtures at two concentrations. Bar indicates stimulation period (2 s). A: stimulation with *trans*-2-hexenal (4×10^{-2} v/v). B: stimulation with *cis*-3-hexenyl acetate (4×10^{-2} v/v). C: stimulation with 1-hexanol (4×10^{-2} v/v). D and E: stimulation with a 1:1 mixture of *trans*-2-hexenal and *cis*-3-hexenyl acetate, respectively at 4×10^{-2} and 8×10^{-2} v/v. F and G: stimulation with a 1:1 mixture of *trans*-2-hexenal and 1-hexanol, respectively at 4×10^{-2} and 8×10^{-2} v/v. H and I: stimulation with a 1:1 mixture of 1-hexanol and *cis*-3-hexenyl acetate, respectively at 4×10^{-2} and 8×10^{-2} v/v.
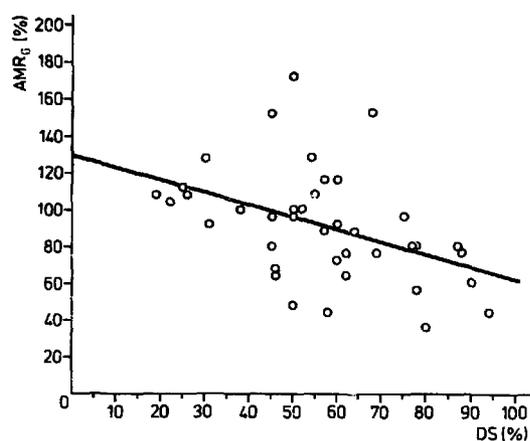
Fig. 4. Scatter diagram for degree of specialization (DS) and average mixture responses (AMR_6) in olfactory receptor cells of the Colorado potato beetle. $r_s = -0.52$; $n = 39$; $P < 0.001$, non-parametric Spearman rank correlation test, 2-tailed[26].
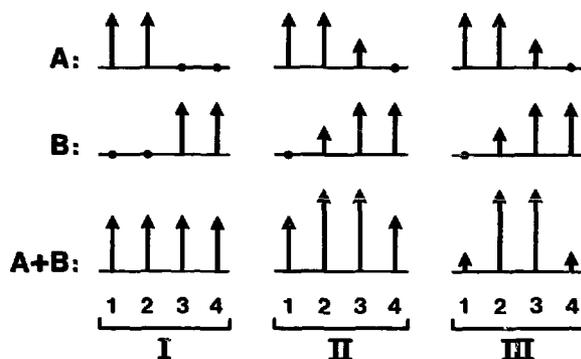

Fig. 5. A schematic diagram representing a population of 4 receptor cells. Receptor cells are thought to respond to compounds A and B applied singly, and the 1:1 mixture of A and B. I = labelled line concept in a cell population with solely specialist receptors. II = across-fibre pattern concept in specialist and generalist receptors. III = as II, involving suppression. The arrow length stands for response intensity.

nounced. Fig. 3 shows a recording in which clear responses to trans-2-hexenal and 1-hexanol were strongly reduced in the presence of cis-3-hexenyl acetate. The response spectrum of this neurone is represented in Fig. 2 no. 2.

The AMR_6 values, which are used as indices for receptors' responses to mixtures, range between 35 and 170 and correlate significantly with receptor DS ($r_s = -0.52$; $n = 39$; $P < 0.001$, 2-tailed). Highly specialized cells show a considerably stronger suppression than the more broadly tuned cells. A scattergram for the AMR_6 and the DS of the receptors is presented in Fig. 4.

DISCUSSION

Previous work on deutocerebral neurones indicated that mixture interaction is essential in the coding of olfactory information in the Colorado potato beetle[5]. Here we studied response spectra of receptor neurones to pure compounds, and also examined the perception of more complex odours at the receptor level. Therefore, we stimulated additionally with simple 1:1 binary mixtures. These mixtures do not resemble natural food odour blends, but should be considered as a first step in the study of mixture effects.

Qualitative responses as presented in spectra are thought to give more reliable information about neu-

ronal reactions than quantitative responses[13] and, therefore, are used for unraveling the coding of olfactory input. Grouping of responses according to spectra have been applied often to characterize an olfactory receptor population. However, since classification of receptors in this way is not only dependent on the treatment of electrophysiological data but also on the set of stimuli used in the experiments, this approach has its limitations[25]. Within groups rather varied spectra can be present while each spectrum shows significant features. The response spectra of olfactory receptors of the Colorado potato beetle (Fig. 1) show varying degrees of receptor specialization and no clear separation in response types for the 5 stimuli. Our data reveal that suppression is an important feature of the response characteristics of receptor cells. The responses of receptors to the binary mixtures suggest a function of mixture effects in the perception of olfactory information. Such mixture interaction has been discussed previously for the processing of information in the antennal lobe of the Colorado potato beetle[5].

Olfactory coding is generally thought to be realized either by labelled lines, or by across-fibre patterns. The labelled line coding theory was proposed for insect pheromone receptors which possess narrow, non-overlapping chemosensitivities[4]. Each behaviourally relevant compound is thought to have its own receptor type for the detection of its presence.

In this theory the blend composition is determined by the activities in particular types of sensory neurones. A simplified illustration of this situation is presented in Fig. 5(I). Observations on food odour receptors, showing that most receptors have broad and overlapping response spectra[4,31] led to a second theory, the across-fibre pattern hypothesis. This concept holds that each perceptible odour is represented by a unique pattern of activity across the array of sensory neurones (Fig. 5(II)).

Highly specialized chemoreceptors are supposed to serve as inputs for specific detection systems[1,8,12]. In the Colorado potato beetle, the negative correlation between DS and AMR_6, however, shows that the level of excitation in narrowly tuned olfactory receptors in response to mixtures does not solely depend on the presence of their 'best' stimulus (Figs. 3 and 4). As suppression is correlated with the degree of specialization, other chemicals to which these cells do not show a pronounced reaction when applied singly, may contribute to the mixture response. Therefore, the activities of olfactory receptors depend on the total composition of a mixture, and the response pattern differs especially in narrowly tuned neurones from what is expected on the basis of their responses to single compounds (Fig. 5(III)). Similar observations have been made of olfactory receptor responses of other insect species and lobsters. 'Ionine' and 'alcohol' receptor types of the cockroach *Periplaneta americana* showed a smaller response to odour of lemon oil than was expected on the basis of summation of responses to odour of lemon oil fractions[24]. Responses of taurine-sensitive receptors on the antennules of the spiny lobster *Panulirus argus* were suppressed when taurine was presented in mixture with certain amino acids[10]. Enhancement and suppression have been demonstrated in responses of narrowly tuned chemoreceptors in the American lobster, *Homarus americanus*[11]. Although pheromone receptors are generally believed to function as labelled lines, synergism and suppression have also been reported in responses of pheromone receptors in the red-banded leaf roller, *Argyrotaenia velutinana*[18], and the cabbage looper, *Trichoplusia ni*[21]. Such mixture interactions could explain why sometimes certain behaviourally important compounds, when applied in a pure form, fail to elicit clear activity on any of the identified receptor neurones[19,22].

The response levels in the group of highly specific neurones in the deutocerebrum of the Colorado potato beetle are thought to depend on the ratio of certain odour components, rather than on the presence of their 'best' component[5]. The other group containing less specific deutocerebral neurones did not show this sensitivity to different ratios. The reactions of receptors presented in this study suggest that peripheral responses are at least partially responsible for this effect. In general, specialized receptors and specialized deutocerebral neurones show suppression as a mixture effect, unlike the other receptors and deutocerebral neurones. The presumed separation of olfactory information by these groups is more obvious at the deutocerebral level than at the peripheral level.

ACKNOWLEDGEMENTS

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