

#### CHAPTER 4. SUPPRESSION-RELATED MIXTURE PERCEPTION IN OLFACTORY RECEPTORS

R. DE JONG and J.H. VISSER

It is known from psychophysical and electrophysiological research on olfaction that suppression is a common effect of mixture interaction. In a previous study on olfactory receptors of the Colorado potato beetle, a correlation has been found between the specialization of a receptor cell and the amount of suppression in its response to a mixture. Therefore, it has been suggested that suppression leads to a more quality-dependent and less quantity-dependent response in the receptors. Here, the effects of suppression on the perception of mixtures in olfactory receptors of the Colorado potato beetle are further described. It is demonstrated that the amount of suppression in a receptor cell, which is positively correlated with its degree of specialization, depends on the individual receptor rather than the specific stimulus mixture. Angles between mixture components derived from 2 psychophysical models, the vector model and the U model, are characteristic for receptors, and correlate with the receptors' degree of specialization. Suppression correlates with a decreased sensitivity for a concentration difference, and, in theory, provides receptor responses with a buffer for some variations in stimulus quality.

Most natural olfactory stimuli consist of complex mixtures. Odour perception, therefore, depends on the simultaneous reception of a variety of components. However, most research concerning the ways the information about such stimuli is deciphered by an organism, has been restricted to their pure constituents. The major problem in the interpretation of these studies is that mixtures are often perceived differently than one would expect from addition of receptor responses to the individual components.

In psychophysical experiments the perceived intensities of mixtures are usually lower than would be predicted from summing responses to the single components. The principle of odour interaction at the perceptual level has been described by Berglund et al. (1973). They introduced the vector model to describe intensity summation:

$$\psi_{AB} = \sqrt{\psi_A^2 + \psi_B^2 + 2\psi_A\psi_B\cos\alpha} \quad (1)$$

The perceived intensity of the binary mixture AB is represented

by  $\psi_{AB}$ , and of its pure constituents A and B by  $\psi_A$  and  $\psi_B$  respectively. In this model, the angle  $\alpha$  is thought to depend on the qualitative similarities between components of a mixture. The more similar these components are, the smaller is the angle  $\alpha$ . This psychophysical model has been tested for humans for 2-component mixtures by Cain & Drexler (1974), Cain (1975), Moskowitz & Barbe (1977), Laffort & Dravnieks (1982) and Laing & Willcox (1983). Although the vector model performs very well for binary mixtures, it has shortcomings when applied in an extended form for more complex mixtures. Such an extended vector model has been tested by Berglund (1974), Moskowitz & Barbe (1977) and Laffort & Dravnieks (1979), and may not be able to predict the odour intensities of 3-component and higher-order mixtures when using  $\alpha$ 's which are completely determined in binary mixture experiments (Moskowitz, 1979).

The so-called 'U model' which has been proposed by Patte & Laffort (1979) does not suffer from problems for higher-order mixtures:

$$\psi_{AB} = \psi_A + \psi_B + 2\sqrt{\psi_A\psi_B}\cos\alpha \quad (2)$$

The U model has been tested in an extended form for ternary and quaternary mixtures by Laffort & Dravnieks (1982). Moreover, it fits the results for binary mixtures slightly better than the vector model, and includes cases where the perceived intensity of a mixture is stronger than that expected from simple additivity among the components (Laffort & Dravnieks, 1982). Suppression is, nevertheless, by far the most frequently encountered mixture effect in psychophysical studies.

Central mechanisms seem to be involved in mixture suppression. Gillan (1983) reported suppression of perceived odour intensities in odour-taste mixtures. Odour-odour mixtures, however, produced greater suppression than did the odour-taste mixtures. Furthermore, Cain (1975) demonstrated that 2 substances presented separately to each nostril of humans at the same time, produced suppression, but to a lesser degree than the suppression when the 2 substances were mixed in the vapour phase. This suggests that besides effects in the central nervous system, receptors also

contribute to mixture suppression.

Such interactive effects in the perception of odour mixtures at the level of individual receptor neurones have been described for invertebrates. Gleeson & Ache (1985) recorded from taurine-sensitive chemoreceptors on the antennule of the spiny lobster. They found suppression in the receptor responses to taurine when it was applied in combination with certain amino acids. In some cases these responses were even blocked completely.

Suppression and enhancement in the responses of pheromone receptors of different insect species have been reported by O'Connell (1971) and O'Connell et al. (1986). Etcheto et al. (1982) applied psychophysical models to electroantennogram (EAG) recordings from honey bee workers. These recordings are thought to reflect summated responses of the population of olfactory receptors in an antenna. These authors also demonstrated synergy and suppression in the EAG responses to mixtures. De Jong & Visser (1988) described for the Colorado potato beetle a positive correlation in the degree of specialization of the olfactory receptors with the amount of suppression in their responses to binary mixtures.

Electrophysiological experiments indicate that suppression is the most common mixture effect, and that some psychophysical effects originate from peripheral processes. Mayer et al. (1984) suggested that an insect's behavioural response to odour stimulation, reflects the intensity of olfactory sensation in the central nervous system, which in turn would be a measure for the summed responses of receptors. Psychophysical models, therefore, may be relevant to receptor responses by interpreting the  $\psi$  in the models as the response magnitude.

Little is known about the function of mixture suppression in the perception of olfactory information. Bartoshuk (1975) postulated that the mixture suppression observed in psychophysical taste experiments, plays an important role in encoding a potentially large stimulus concentration range into a much smaller psychological range. Such a mechanism which encodes a broad range of concentrations into a fairly small range of response intensities may be important for lobsters as well (Johnson et al., 1985; Carr & Derby, 1986). De Jong & Visser

(1988) proposed an important qualitative role of mixture suppression in the recognition of potato plant odour by the Colorado potato beetle. According to their hypothesis, the coding of olfactory information involves 2 separate channels. One channel contains neurones which are highly specialized in the perception of particular odour components, and at the same time show strong suppression in their responses to mixtures. The other channel consists of neurones which are more broadly tuned and respond to mixtures with less suppression. Broadly tuned receptors are supposed to detect the presence of compounds, while information about their ratios in a stimulus mixture is obtained by narrowly tuned receptors. The response level to mixtures in narrowly tuned receptors, therefore, is expected to be more quality-dependent and less quantity-dependent than in broadly tuned receptors.

In this report we evaluate data obtained from single cell recordings of Colorado potato beetles' antennal neurones, in order to investigate whether the suppression in a receptor depends primarily upon certain mixture combinations or on receptor characteristics. Furthermore, the effects of a change in stimulus concentration on the responses of these receptors are studied. The role of suppression in narrowly tuned receptors, with respect to olfactory coding, is discussed with use of the psychophysical models (1) and (2).

#### ELECTROPHYSIOLOGICAL DATA

The data used in this report represent the responses of 39 olfactory receptors of the Colorado potato beetle to different stimuli (De Jong & Visser, 1988). The degree of specialization (DS) of an olfactory receptor, was expressed as the specialization in the cell's responses to 5 pure compounds, i.e. *cis*-3-hexen-1-ol, *trans*-2-hexenal, *cis*-3-hexenyl acetate, *trans*-2-hexen-1-ol and 1-hexanol (at a source dilution of  $4 \times 10^{-2}$  in paraffin oil, v/v). These compounds are part of the so-called 'green odour' of potato leaves (Visser, 1983), and are thought to be essential for plant odour recognition by the beetle (Visser & Avé, 1978). A receptor's relative response spectrum was

obtained by setting the cell's 'best' component on a 100%-level. DS was calculated by (De Jong & Visser, 1988):

$$DS = \frac{100n - \sum_{i=1}^n R_i}{n - 1}$$

$R_i$  represents the cell's relative response to compound  $i$ . This calculation expresses DS as a percentage which ranges between 0 for cells without any specialization, and 100 for cells responding to only one of the test compounds. The number ( $n$ ) of different compounds that was tested is 5.

The mixture effect was quantified as the average mixture response of a receptor (De Jong & Visser, 1988). This was done by measuring the cell's responses to three 1:1 binary mixtures, each at 2 dilutions of the total amount of volatiles ( $4 \times 10^{-2}$  and  $8 \times 10^{-2}$ , v/v, at the source). Compounds used in the 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 mixture's 'best' pure component (at a dilution of  $4 \times 10^{-2}$ , v/v, at the source). The average mixture response ( $AMR_6$ ) was calculated for each cell as the average of the 6 values. Variables were compared using the non-parametric Spearman rank correlation test (Siegel, 1956). A negative correlation between  $AMR_6$  and DS has been described previously (De Jong & Visser, 1988).

#### APPLICATION OF THE MODELS

We evaluated the electrophysiological data with respect to the vector model (1), and were able to calculate an  $\alpha$  for 82 of the 117 cases (Fig. 1A-C). In 6 cases, the responses to mixtures were stronger than the summed responses of the corresponding components, and since the vector model can not handle cases of synergism, these  $\alpha$ 's could not be determined. In the remaining 29 cases, the responses to mixtures showed stronger suppression than can be explained by the vector model. Using the U model (2), 106 of the 117  $\alpha$ 's could be calculated (Fig. 1D-E). No synergism was

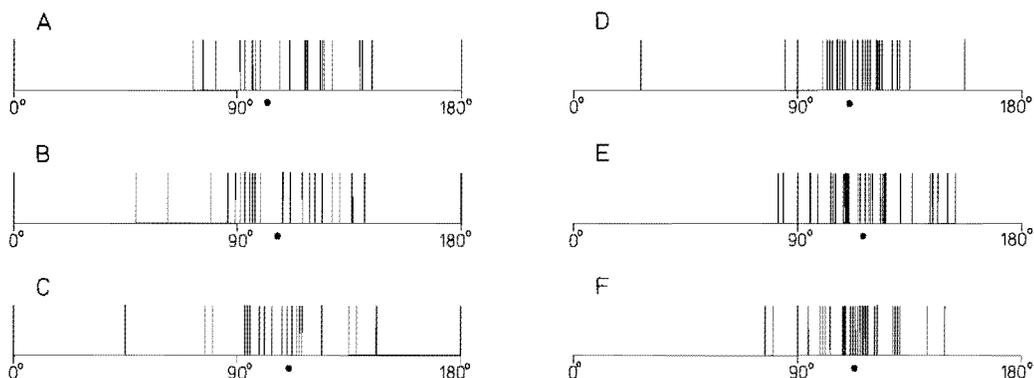


Fig. 1 Distribution of calculated angles for binary mixtures using the vector model (A-C) and the U model (D-F). Angle values are represented by bars, and mean values by filled circles. A and D, mixtures of trans-2-hexenal and cis-3-hexenyl acetate; B and E, mixtures of trans-2-hexenal and 1-hexanol; C and F, mixtures of cis-3-hexenyl acetate and 1-hexanol.

found to be too strong for this model. In 11 cases we calculated  $\cos\alpha$  values which were  $< -1$ . Besides a greater number of  $\alpha$ 's, the results of the U model show less variation in the  $\alpha$  values. The mean values of the  $\alpha$ 's for the 3 mixtures and for both models do not differ very much (Fig. 1).

Some  $\alpha$ 's could not be calculated because some mixtures showed stronger suppression than either model can handle. This may be due to the fact that most receptors showed a background firing rate which was too low to reveal possible inhibitory responses. As a result the magnitude of an inhibitory response could not be expressed as a decrease in frequency. Therefore, instead of expressing the receptor responses by their spike frequencies, the receptor potential might be a better response criterion, but this would require intracellular recordings.

When the  $\alpha$ -values for different pairs of mixture components are compared, it appears that  $\alpha$ -values for the same receptor are correlated with each other. Receptors with a high or a low  $\alpha$ -value for one mixture combination subsequently showed high or low values for other mixture combinations.  $\alpha$ -Values, therefore, are characteristic for these olfactory receptors. Table 1 gives for both models an overview of correlations between different pairs of  $\alpha$ 's for the same receptor population. For the U model we found

Table 1. Correlations between different pairs of  $\alpha$ 's for the same receptor population. Variables were compared using the non-parametric Spearman rank correlation test (Siegel, 1956).

	Vector model	U model
$\alpha_{ab}^1$ vs $\alpha_{ac}$	$r_s^2 = 0.72$ ; N = 21; P < 0.001	$r_s = 0.60$ ; N = 33; P < 0.001
$\alpha_{ab}$ vs $\alpha_{bc}$	$r_s = -0.21$ ; N = 23; P = 0.343	$r_s = 0.47$ ; N = 33; P < 0.010
$\alpha_{ac}$ vs $\alpha_{bc}$	$r_s = 0.29$ ; N = 23; P = 0.180	$r_s = 0.50$ ; N = 34; P < 0.005

<sup>1</sup> a, trans-2-hexenal; b, cis-3-hexenyl acetate; c, 1-hexanol.  $\alpha_{ab}$ ,  $\alpha_{ac}$  and  $\alpha_{bc}$  stand for angles between a and b, a and c, and b and c, respectively.

<sup>2</sup>  $r_s$ , correlation coefficient; N, number of data pairs; P, probability (2-tailed).

significant correlations for all 3 combinations, while for the vector model this was the case for only one combination. The latter result may be due to a smaller number of data pairs.

The correlations between pairs of  $\alpha$ 's indicate that the correlation between  $AMR_6$  and DS does not depend on the presence of one specific compound in the mixture, i.e. the most stimulating compound. In other words, the amount of suppression in a receptor cell, which is positively correlated with its degree of specialization, depends on receptor cell characteristics, rather than on the presence of one specific stimulus.

The correlation between  $AMR_6$  and DS, therefore, is not due to the presence of the cell's 'best' stimulus in a mixture. Of the 39 cells tested, 31 showed the highest sensitivity to 1 of the 3 components used in the mixtures, and thus 4 of the 6 mixtures contained this compound. When the  $AMR_4$  is calculated as the mean of these 4 mixtures containing the cell's 'best' compound, the correlation between  $AMR_4$  and DS for these 31 cells is better ( $r_s = -0.70$ ; N = 31; P < 0.001, 2-tailed; Fig. 2A) than that for  $AMR_6$  and DS for the whole cell population ( $r_s = -0.52$ ; N = 39; P < 0.001, 2-tailed). The  $AMR_2$ s of the same 31 cells, resulting

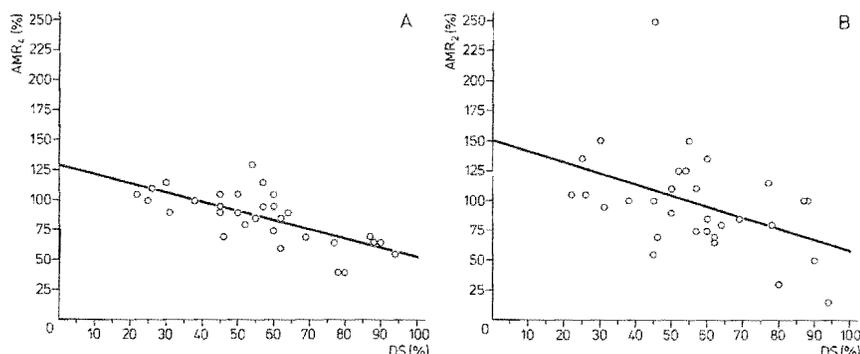


Fig. 2 Scatterdiagram for the average mixture response (AMR) and the degree of specialization (DS) in olfactory receptors of the Colorado potato beetle. A, mixtures containing the cell's 'best' compound ( $AMR_4$ ) ( $r_s = -0.70$ ;  $N = 31$ ;  $P < 0.001$ , 2-tailed); B, same cells, mixtures not containing their 'best' compound ( $AMR_2$ ) ( $r_s = -0.48$ ;  $N = 31$ ;  $P < 0.01$ , 2-tailed).

from the 2 mixtures not containing the receptor's 'best' component, still show a negative correlation with DS ( $r_s = -0.48$ ;  $N = 31$ ;  $P < 0.01$ , 2-tailed; Fig. 2B), even though the accuracy of measurements is less than in the foregoing procedure. This decrease in accuracy is caused by the weaker responses to mixtures lacking the 'best' compound, particularly in the more specialized cells, and to the smaller number of mixtures used for calculating the cell's AMR. With both models (1) and (2),  $\alpha$ 's are correlated with the receptor's DS. Using the vector model we found:  $r_s = 0.24$ ;  $N = 82$ ;  $P < 0.05$  (2-tailed), and with the U model:  $r_s = 0.31$ ;  $N = 106$ ;  $P < 0.001$  (2-tailed). These analyses demonstrate that suppression in response to binary mixtures is correlated with the extent of specialization of the receptor cells, and that suppression does not depend on the presence of one specific compound, e.g. the 'best' stimulus.

#### CONCENTRATION EFFECTS ON RESPONSES

Concentration shifts lead to changes in receptor responses. A change in mixture concentration is proportional to the changes in concentrations of its components. Assuming that this would change  $\psi_A$  and  $\psi_B$  by the same factor  $n$  to  $n\psi_A$  and  $n\psi_B$  respectively, the

response  $\psi'_{AB}$  to the new mixture concentration can be expressed for both models (1) and (2) as:

$$\psi'_{AB} = n\psi_{AB} \quad (3)$$

Since, according to (3),  $\psi'_{AB}/\psi_{AB} = n$ , concentration shifts should lead to relatively similar changes in the responses of different receptors. However, the relative change in response to an increase in stimulus concentration (from  $4 \times 10^{-2}$  to  $8 \times 10^{-2}$ , v/v, at the source), averaged for the 3 mixtures, is significantly correlated with  $AMR_6$ :  $r_s = 0.40$ ;  $N = 39$ ;  $P < 0.005$ , 2-tailed, (Fig. 3). Thus, at strong suppression, the relative increase of receptor response caused by an increase in mixture concentration is small.

Differences between receptors in their dose-response relations can explain this effect. Dose-response relations are characterized by sigmoid-shaped curves when plotted on semi-logarithmic scales. The response intensity levels off at high stimulus concentrations. Visser (1979a) found, using the EAG recording technique, sigmoid-shaped dose-response curves for the Colorado potato beetle's antenna to the 'green odour' components. EAG recordings reflect the responses of the complete receptor cell population and, therefore, do not reveal differences between the receptors within this population. When, for instance, specialized neurones are more sensitive than generalized neurones, test concentrations sometimes lie at the right asymptotic region of the dose-response curves for specialized neurones, and in the steep region for the generalized neurones. This would cause relatively smaller effects by concentration shifts in specialized receptors. Although we did not find a relation between the absolute response levels of receptors to their 'best' compound and their DS (or between absolute response levels to their 'best' compound and  $AMR_6$ ), this possibility cannot be excluded completely.

Another explanation could be differences between the steepness of dose-response curves for cells with high and low  $AMR_6$  values. The steeper the curve, the smaller the concentration range in which the cell's response intensity is affected. A relationship

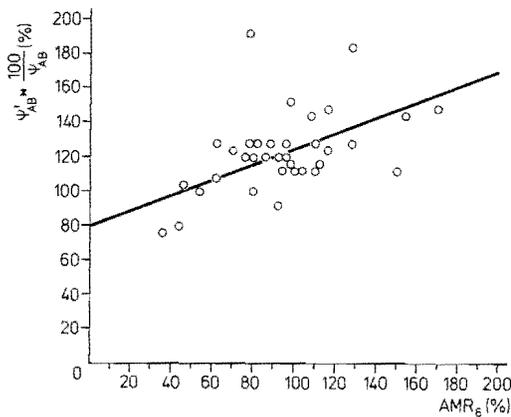


Fig. 3 Scatterdiagram for the relative change in receptor response to an increase in mixture concentration ( $\psi'_{AB}$  at  $8 \times 10^{-2}$ , v/v) related to responses to low mixture concentrations ( $\psi_{AB}$  at  $4 \times 10^{-2}$ , v/v) and the average mixture response ( $AMR_G$ ).  $r_s = -0.40$ ;  $N = 39$ ;  $P < 0.005$ , 2-tailed.

between the receptor's  $AMR_G$  and the steepness of its dose-response curves, thus explains the observed correlation between  $AMR_G$  and the relative change in response to a concentration increase (Fig. 3). Specialized receptors may have steeper curves than more generalized ones. This could cause synergy at low concentrations and suppression at high concentrations, and thus would reduce the concentration dependency of the responses. When the test concentrations fall in the steep region of their dose-response curves, the situation would be reversed of course, since steeper curves then show stronger effects. However, since the test concentrations used in our experiments, are relatively high compared with the EAG dose-response curves (Visser, 1979a), the latter possibility does not seem very likely.

Concentration dependency of mixture interaction in receptor cells, has been described by Johnston et al. (1985) for some types of chemoreceptors in lobsters. These receptors showed synergy when mixtures were applied at low test concentrations, and suppression at high test concentrations.

Nevertheless, in contrast to the assumption made earlier that  $\psi'_A/\psi'_B = \psi_A/\psi_B$ , the concentration shift may have changed  $\psi_A$  and  $\psi_B$  in a dissimilar way. Since suppression may provide receptors with an additional buffer against small changes in the ratio of  $\psi_A$  and  $\psi_B$ , as is discussed later, the response levels of

specialized receptors then would be less influenced by concentration changes. This effect could have contributed to the observed correlation in Figure 3.

#### QUALITY BUFFERED RESPONSES

According to De Jong & Visser (1988), the response level in narrowly tuned receptors depends on odour quality. The chemical composition of a food odour, however, is not always exactly the same, e.g. due to differences in age of the food sources. Although the odour compositions of young and old potato plants differ in their ratios of components (Visser, 1979b), both odours are attractive to Colorado potato beetles (Visser, 1976). This indicates that some tolerance exists in the mechanism of odour quality coding. Potato leaf odour, however, loses its attractiveness to Colorado potato beetles when small quantities of its component odours are added artificially (Visser & Avé 1978; De Jong & Visser, unpubl.), which demonstrates that this tolerance is limited. Nevertheless, a certain buffer capacity in the perception of quality might enable an organism to distinguish between biologically important signals and background noise in olfaction.

Fig. 4 illustrates for both the vector model (Fig. 4A) and the U model (Fig. 4B), the change in response level  $\psi_M$  of an olfactory receptor to a stimulus M when stimulus C is added. Stimulus M alone elicits a response level  $\psi_{M_0}$ . The response levels elicited by stimulus C ( $\psi_C$ ) and combinations of stimuli M and C ( $\psi_M$ ), are expressed relatively to  $\psi_{M_0}$ . The relation between  $\psi_M$  and  $\psi_C$  is given for several angles between M and C. Simple addition of the response levels  $\psi_{M_0}$  and  $\psi_C$  occurs at  $\alpha = 0^\circ$  with the vector model and at  $\alpha = 90^\circ$  with the U model. The response intensity  $\psi_{M_0}$  can be suppressed by stimulus C at high  $\alpha$ -values. At  $\alpha = 180^\circ$ , for example, there can be a severe suppression of  $\psi_{M_0}$ . Cases where the response to a mixture is stronger than predicted from adding responses to the single components are only included in the U model and depend on the  $\alpha$ -value ( $0^\circ \leq \alpha < 90^\circ$ ). Both models show that within a certain range  $\psi_M$  can be relatively unaffected on addition of stimulus C. With the vector model at  $\alpha$

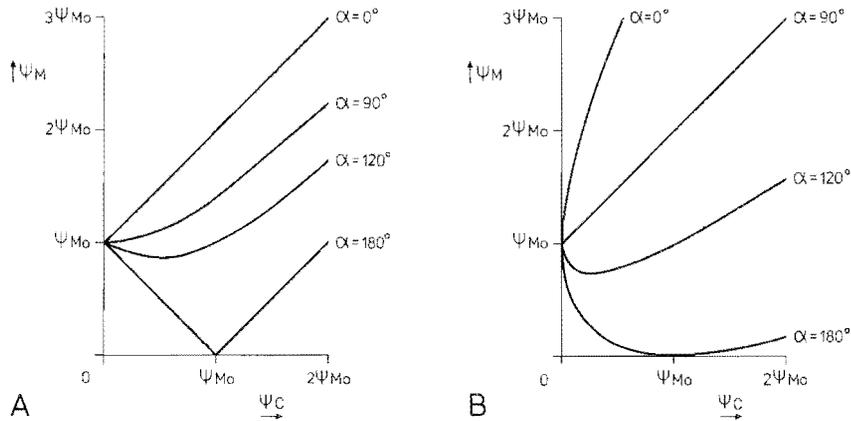


Fig. 4 Response level  $\psi_M$  of a receptor after addition of a stimulus C (with response level  $\psi_C$ ) to a stimulus M with a response level  $\psi_{M0}$ . The relation between  $\psi_M$  and  $\psi_C$  is given for several angles between C and M in the vector model (A) and the U model (B). See text for further explanation.

=  $90^\circ$ , for instance, addition of stimulus C with a relatively small response level  $\psi_C$  does not affect  $\psi_{M0}$  significantly. The capacity of this buffer is in both models determined by the particular angle between M and C, and by the ratio of response intensities  $\psi_C$  and  $\psi_{M0}$ . Suppression, therefore, in theory provides receptor responses with a buffer for some variations in stimulus quality.

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