J. Physiol. (1978), **282**, *pp.* 541–560
With 11 text-figures
Printed in Great Britain

ADAPTIVE PROPERTIES OF OLFACTORY RECEPTORS ANALYSED WITH ODOUR PULSES OF VARYING DURATIONS

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(*Received 17 January 1978*)

SUMMARY

1. The adaptive properties of salamander olfactory receptors have been analysed in extracellular unit recordings. Stimulation has been by step pulses of odour of varying durations for 1–10 sec.

2. The most common response was a prolonged impulse discharge that continued throughout the duration of the pulse and terminated abruptly within 1 sec of the end of the pulse. The interval for termination was relatively independent of the pulse duration. Pulses were frequently followed by a period of impulse inactivity lasting 1–3 sec, usually independent of previous pulse duration.

3. The impulse discharges were typically slowly adapting. Initially, during the first 1–2 sec, the frequency rose to 5–10 impulses/sec, at threshold concentration. In some cases, the initial level was maintained throughout the pulse, with little or no adaptation. More commonly, there was a distinct initial phasic peak, followed by decay to a lower level of 4–8 impulses/sec, which was maintained during the pulse. It was concluded that most olfactory receptors are slowly adapting, with variable phasic responsiveness dependent on odour concentration and other factors.

4. Reductions in impulse activity, compared with background, during a pulse were rarely seen. Methods for increasing the level of background activity and the use of very long duration pulses were necessary in order to bring out this type of response. Uniformly reduced activity throughout a pulse was seen clearly in only one case. A pattern consisting of a waning and then recovery of impulse frequency during a pulse was also observed in rare cases.

5. The results have shown that olfactory receptor discharges characteristically have a relatively precise relation to step pulses of odour of varying duration. The properties of the response have implications for the steps involved in the overall processes of activation and inactivation of receptor mechanisms at the olfactory mucosa.

INTRODUCTION

An important problem in olfactory physiology is the adaptive properties of the receptors. On the basis of recordings of summed potentials in the olfactory epithelium, Ottoson (1956) suggested that olfactory receptors are slowly adapting. Although this suggestion has been generally accepted, there has been no single unit

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study specifically concerned with this property. As in other sensory systems, such a study requires appropriate control of the stimulus. In the present experiments we have attempted to fulfill the essential conditions for such a study, in terms of steady levels of odour stimulation delivered over varying periods of time, and with appropriate monitoring of the stimulus pulse (Kauer & Shepherd, 1975; Getchell & Shepherd, 1978). The results have shown that, under these conditions, most olfactory receptors are slowly adapting. The precise control of the stimulus pulse has permitted detailed analysis of the time course and properties of the slowly adapting responses. The implications of these results for transductive mechanisms at the level of the olfactory receptors and for sensory processing of olfactory signals are discussed.

METHODS

The salamander preparation, recording techniques, methods of data analysis, odours and odour delivery methodology are thoroughly described in the preceding paper (Getchell & Shepherd, 1978). The unit responses described in this paper were selected from the population of forty-five olfactory receptors which responded to at least one odour in the previous study. In order to investigate the adaptive properties of the receptors to odour pulses of long duration, i.e. up to 10 sec, the most stable unit recordings were selected for detailed study. Recording stability and response fidelity were ensured by delivery of odour pulses at just over threshold concentrations and long interstimulus intervals, typically 3 min. This allowed investigation of characteristic response properties uncomplicated as much as possible by overstimulation (this problem is discussed by Getchell, 1974; Getchell & Shepherd, 1978).

RESULTS

Excitatory response patterns

The most common pattern of response of an olfactory receptor cell to odour pulses of different durations is illustrated by the recordings in Fig. 1. This unit had an asynchronous, relatively high rate of spontaneous activity, of 2–3 impulses/sec. The threshold for activation by guaiacol was estimated from the intensity-response function to be approximately $1{\cdot}4 \times 10^{-10}$ M. The response (Fig. 1*A*, *b*) to a 2 sec pulse of guaiacol at this concentration consisted of a burst of impulses followed by a period of impulse inactivity. The responses to subsequent pulses, of increasing duration, delivered at 3 min intervals, are shown in *c–f*. The properties that are common to these responses include the following: (1) an early build-up in impulse frequency followed by a discharge that was maintained throughout the duration of the pulse; (2) abrupt termination of the discharge approximately 1 sec after the termination of the pulse; (3) an ensuing period of impulse inactivity which ranged in duration from 3 to 6 sec, and tended to increase with increasing pulse duration. These over-all properties are also shown graphically in the accompanying histograms (Fig. 1*B*).

More detailed analysis has been carried out by the construction of instantaneous frequency plots of the spike discharges, as in Fig. 2. These show that the latency ranged from 400 to 700 msec after the onset of the pulse. There was an initial rapid rise in frequency from about 1·5 impulses/sec to a maximum of 5–10 impulses/sec, that occurred within approximately 2 sec of the onset of the pulses. This corresponds to the phasic period of the response (cf. Getchell & Shepherd, 1978). This initial period was followed by a rather steady tonic discharge of 4–8 impulses/sec that lasted until the termination of the pulse. In the period just after the termination of the

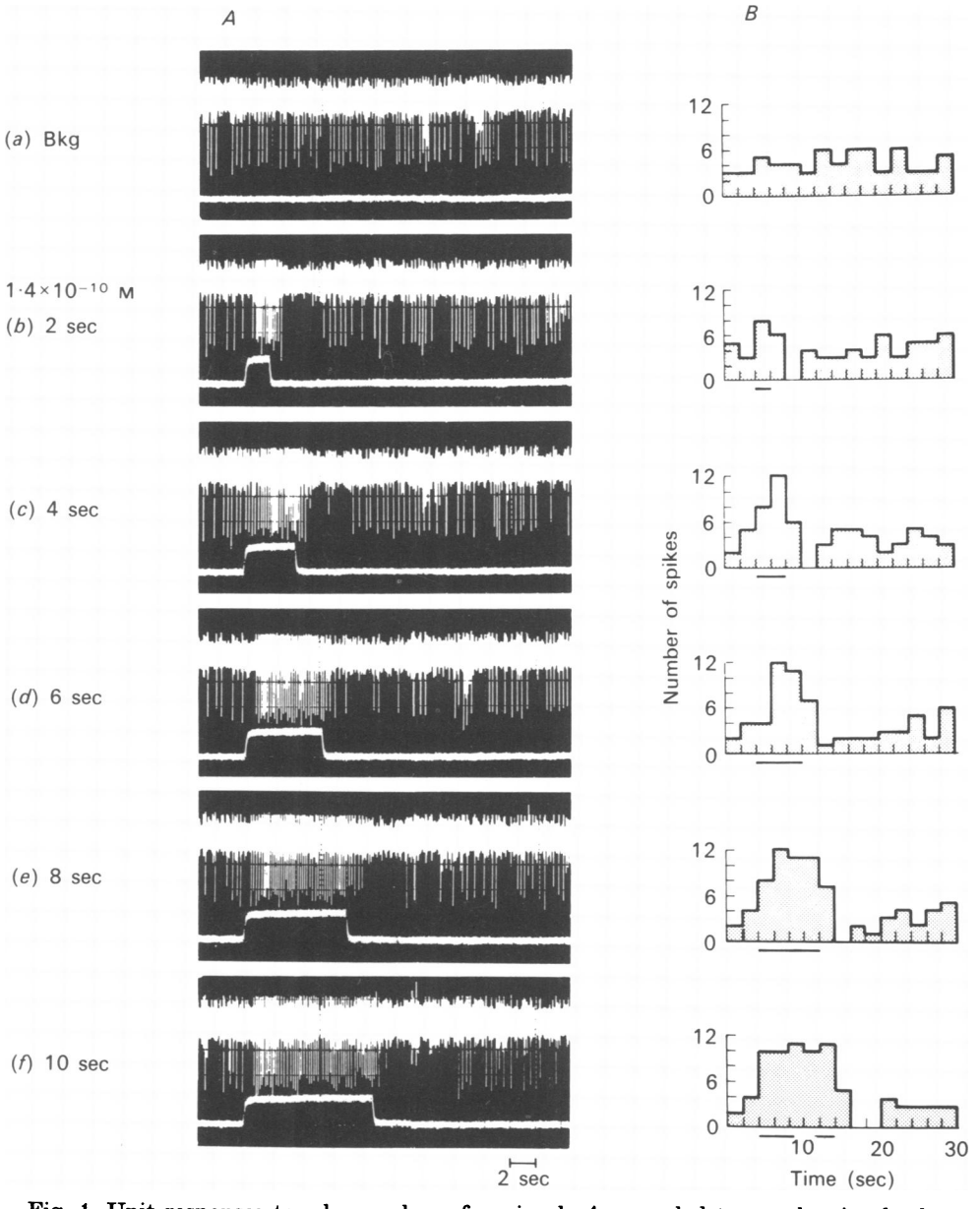

Fig. 1. Unit responses to odour pulses of guaiacol. *A*, recorded traces showing background spontaneous activity (*a*) and responses to odour pulses of increasing duration (*b–f*) at just over threshold concentration of $1{\cdot}4 \times 10^{-10}$ M guaiacol. *B*, peristimulus histograms of the recorded activity, 2 sec time bins.

pulses, it appears that the frequencies began to fall before the abrupt termination of impulse firing which ushered in the period of inactivity.

The ability of olfactory receptors to respond with a maintained discharge throughout pulses lasting as long as 10 sec was an outstanding characteristic of the main category of units in our results. Comparison of the traces in Fig. 1 and graphs in Fig. 2

shows in addition the regularity of the responses to repeated stimuli, which was particularly close in this experiment between the 6 and 8 sec pulse trials. In view of the relatively long exposure of the receptor to the odour during these pulses, as well as the long intervals between stimuli, the reproducibility of these responses seems remarkably close. This also applies to the results in later Figures below. The other responses (in Fig. 1*a*, *b*, and *e*) illustrate the degree of variation that was typical of

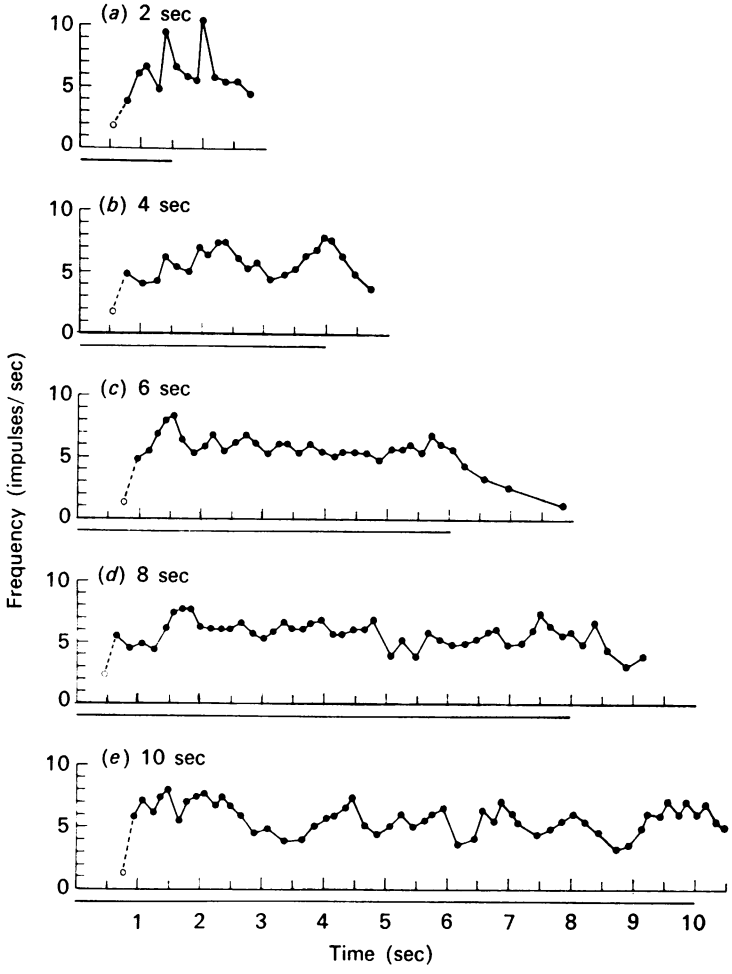

Fig. 2. Instantaneous frequency plots of the responses evoked by guaiacol shown in Fig. 1. Stimulus duration shown by the bar under each graph. Occurrence of the initial spike in the discharge indicated by ○ and plotted as the reciprocal of the latency following the onset of the pulse. See text for full discussion.

most of the units which were studied. A slight tendency to rhythms imposed on the discharge may be discerned in some cases (Fig. 2*d* and *e*): this however was not a general property of the unit population.

Another example of the common discharge pattern is shown in Fig. 3. This is a different receptor responding to pulses of a different odour. The threshold of the unit to a 3 sec pulse of estragole was estimated to be $3{\cdot}9 \times 10^{-8}$ M. A recording of the response

at threshold is shown in the top trace of Fig. 3. As seen in the instantaneous frequency plot (*a*), the response consists of a discharge of impulses with an onset latency of approximately 600 msec. The peak frequency of approximately 8 impulses/sec occurs 1 sec after the pulse onset. With increasing pulse durations (*b*), the initial phasic peak appears to be reached within 1–2 sec. In all trials the impulse frequency then gradually declined into a nearly steady tonic discharge, and this was generally maintained as the pulse duration increased. For the longest duration pulse (9 sec:*d*)

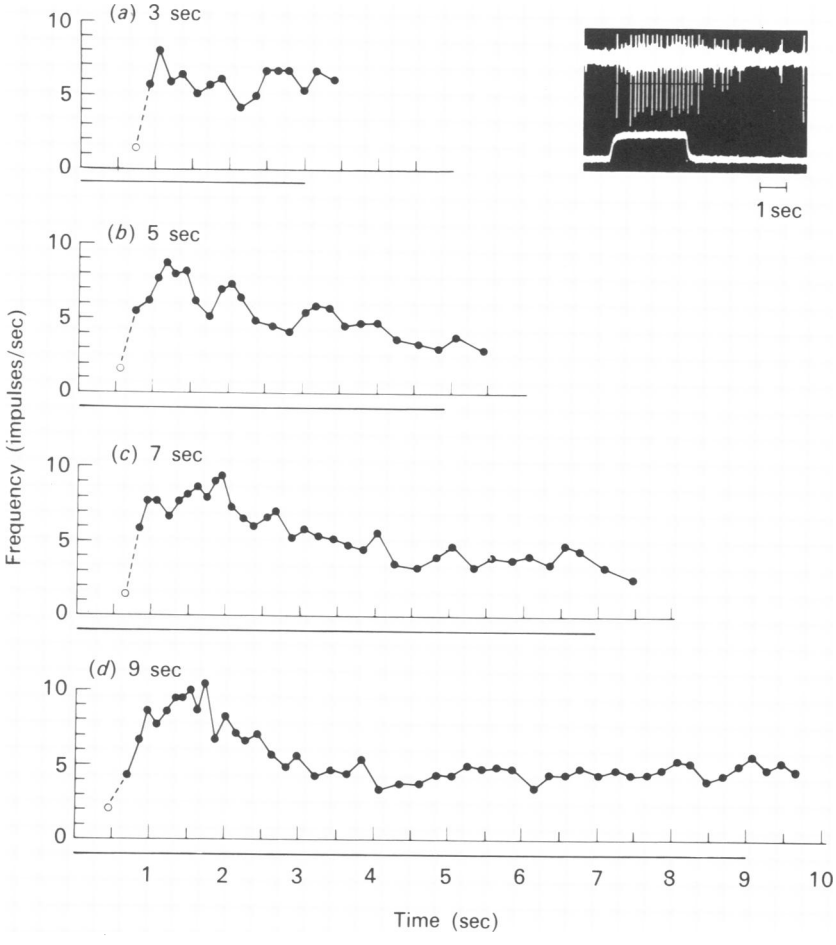

Fig. 3. Instantaneous frequency plots of the responses (e.g. insert, right) evoked by odour pulses of estragole at just over threshold concentration of $3{\cdot}9 \times 10^{-8}$ M and increasing duration. Note separation of the excitatory responses into initial phasic and later tonic components (*b–d*).

the decline from the phasic peak lasted from 2 to 4 sec; the receptor then maintained a nearly constant discharge of 5 impulses/sec for the last 5 sec of the response. In each trial, the response terminated abruptly within 700 msec of the termination of the pulse. This unit had a very low rate of background activity; hence the features of the responses related to the period of impulse inactivity following the impulses and to effects on spontaneous activity could not be evaluated.

It is interesting to compare the frequency plots of the responses in Fig. 3 with those of the previous unit (Fig. 1*A* and Fig. 2). The basic discharge patterns were remarkably similar, despite the different units, different stimuli, and different thresholds. It is important to note that the responses were to stimuli that were just over threshold in concentration. Note also that the spikes maintained a nearly constant amplitude and did not show decrement as the discharge proceeded. These aspects give confidence that the concentrations were within the physiological range, and that the responses did not reflect any appreciable degree of overstimulation. Given this evidence, the differences between the responses in Figs. 1 and 3 take on possible significance; one notes in particular that the responses from the unit in Fig. 3 have a clearer initial phasic component, a more prominent second component of declining frequency, and a final prolonged tonic component more constant in firing frequency. The significance of these components for the adaptive properties of the receptors are discussed below.

Variations on excitatory patterns

A few units gave responses which differed in one or more respects from the general pattern just described. These have been studied in some detail in order to determine the basis for the variations and the possible insights they might give into receptor properties.

The responses shown in Fig. 4 were elicited from a unit by pulses of the carrier air plus CO_2. Each response consisted of three distinct periods. First was a burst of impulses rapidly building up to a high frequency; at these slow sweep speeds the individual spikes cannot be discerned (see Fig. 5 below). This discharge terminated abruptly at the point at which the rising phase of the pulse reached the steady plateau. This was followed by a distinct pause in the impulse discharge. The third period was one of a relatively prolonged constant tonic discharge, which terminated virtually simultaneously with the termination of the pulse. There ensued a period of impulse inactivity, which lasted approximately 3 sec regardless of pulse duration. There was finally a period of several seconds of slow impulse activity which also appeared the same following all the pulses, and was distinctly above the level of background activity before the pulse.

Instantaneous frequencies for the 2 sec and 8 sec responses are plotted in the graph of Fig. 5. Note that the peak frequency of the phasic component of the response reached very high values, of up to 53 impulses/sec. Despite this high frequency, there was only slight decrement in spike amplitudes. The pause that separates the phasic and tonic components is represented by the dip in frequency to approximately 5 impulses/sec. During the ensuing tonic period the frequency declined very gradually from 11 to 7 impulses/sec over a time of 8 sec. Note the very close superimposition of the frequency plots for the 2 and 8 sec pulse trials (open and closed circles, respectively). During the tonic period the spike amplitudes are between the largest value of the first phasic spike and the smallest values of the latter phasic spikes.

Additional details regarding the phasic parts of four of the responses are shown in the frequency plots on the expanded scale in the inset in Fig. 5. Note the relatively brief latencies of onset, within a rather narrow range of 125–160 msec following the onsets of the pulses. For the 8 sec pulse the latency is the longest and the discharge

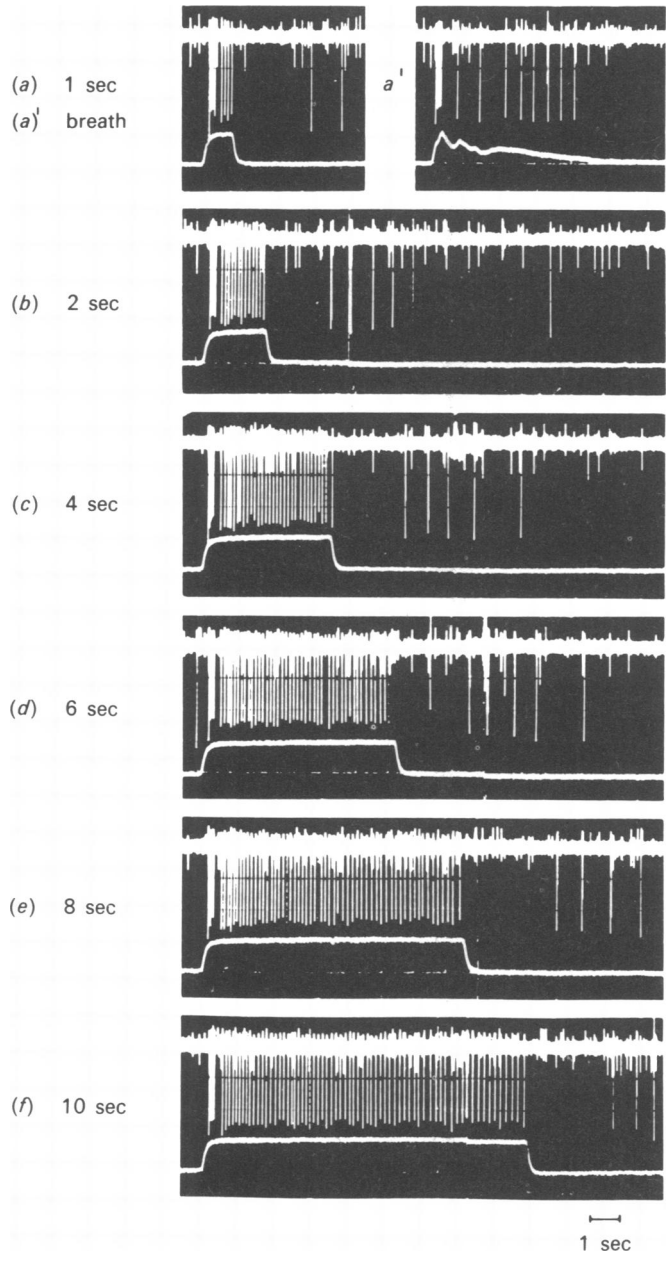

Fig. 4. Unit responses to pulses of the carrier air plus 5% CO_2 (*a–f*) and exhaled breath (*a'*). In response to pulses of increasing duration (*a–f*) the discharge consisted of three components: an initial phasic discharge, a distinct pause, and a tonic discharge.

reaches its peak relatively late; for the other pulses, the latencies are shorter and the peak is reached with the second impulse.

These responses were unusual in the sharp differentiation of the discharge pattern, and with respect to the several properties we have described above. This may be correlated with the fact that this is the only unit among the several hundred

encountered in this study which was sensitive to any significant degree to the control pulse. Since the control pulse contained pure air plus CO_2, it suggests that this particular unit was stimulated either mechanically by the pulse, or by the CO_2 dissolved in it. The only additional evidence we have is that the unit was sensitive to a breath stimulus, as shown in the recording labelled *a'* in Fig. 4, and in the inset in Fig. 5. The significance of the properties of these responses for understanding receptor mechanisms will be discussed below.

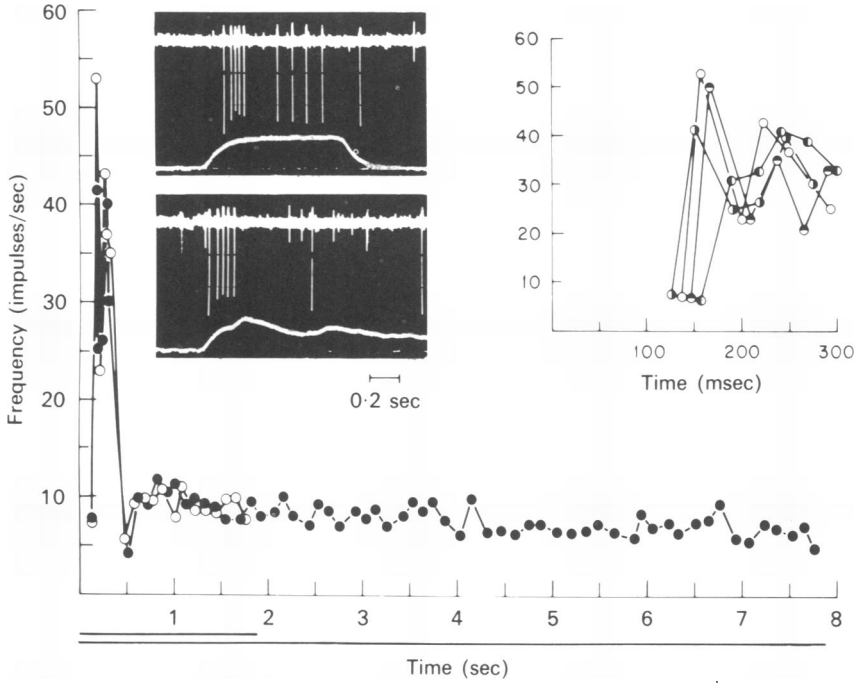

Fig. 5. Unit responses shown in Fig. 4 on an expanded time scale. Traces of the discharge (inset, left) show the very brief latency initial phasic component of the response followed by a pause. The later tonic component required a steady level of stimulus delivery (upper trace) which was observed (lower trace) as shown by the declining CO_2 concentration of the exhaled breath. Instantaneous frequency plots of the responses evoked by the 2 (○) and (●) 8 sec stimulus pulses. Instantaneous frequency plots of the initial phasic component (inset, right) evoked by four stimulus applications.

The responses described thus far, and in the previous paper (Getchell & Shepherd, 1978), showed a close temporal relation between the impulse discharge and the stimulus pulse. A unit which differed significantly in this property is shown in Figs. 6 and 7. The traces in Fig. 6*a* show the intensity-response relation of this unit to a 1 sec pulse of anisole. The threshold was judged to be approximately $1{\cdot}8 \times 10^{-7}$ M (trace *b*); this is within the range of threshold values previously reported (Getchell, 1974; Getchell & Shepherd, 1978). The onset latency at this concentration was rather long, 2·4 sec, and occurred about 1·5 sec after the termination of the pulse. With increasing concentration the latency systematically decreased to 1 sec (Fig. 6, *b–f*). At the highest concentration the initiation of the response (*f*) was nearly coincident with the termination of the pulse.

In addition to its long latency, the response also was of long duration, which increased with increasing concentration. The duration ranged from nearly 3·5 sec at threshold (*b*) to about 19 sec at the highest concentration (*f*). As can be seen in the traces, and as is shown graphically in the accompanying histograms (Fig. 6*B*), the

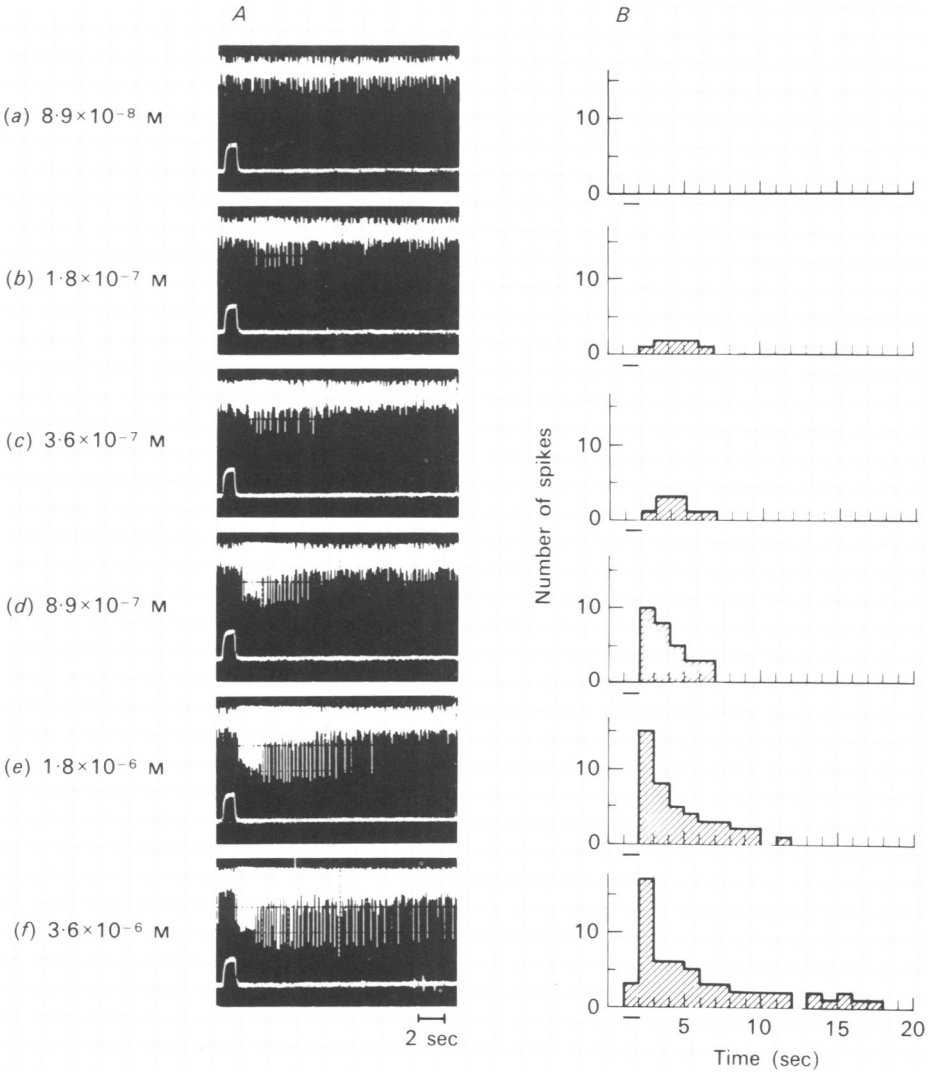

Fig. 6. Unit responses to odour pulses of anisole of increasing concentrations. Recorded traces shown in *A* and peristimulus histograms in *B*. Note that the excitatory responses appeared to be initiated near the termination of the pulse (*a–f*) and were out of phase with the pulse monitor.

responses showed little temporal correlation with the stimulus pulses. It is also evident from inspection of the traces (Fig. 6*A*) and the histograms (Fig. 6*B*) that at higher concentrations the responses initially rose in frequency to between 10 and 15 impulses/sec and then gradually declined. Not only was there an increase in spike

amplitudes during the bursts, but also an increase in spike amplitudes in the responses at higher concentrations. This was the only instance of this phenomenon in our study.

The results in Fig. 6 give the impression that the initiation of the impulse response

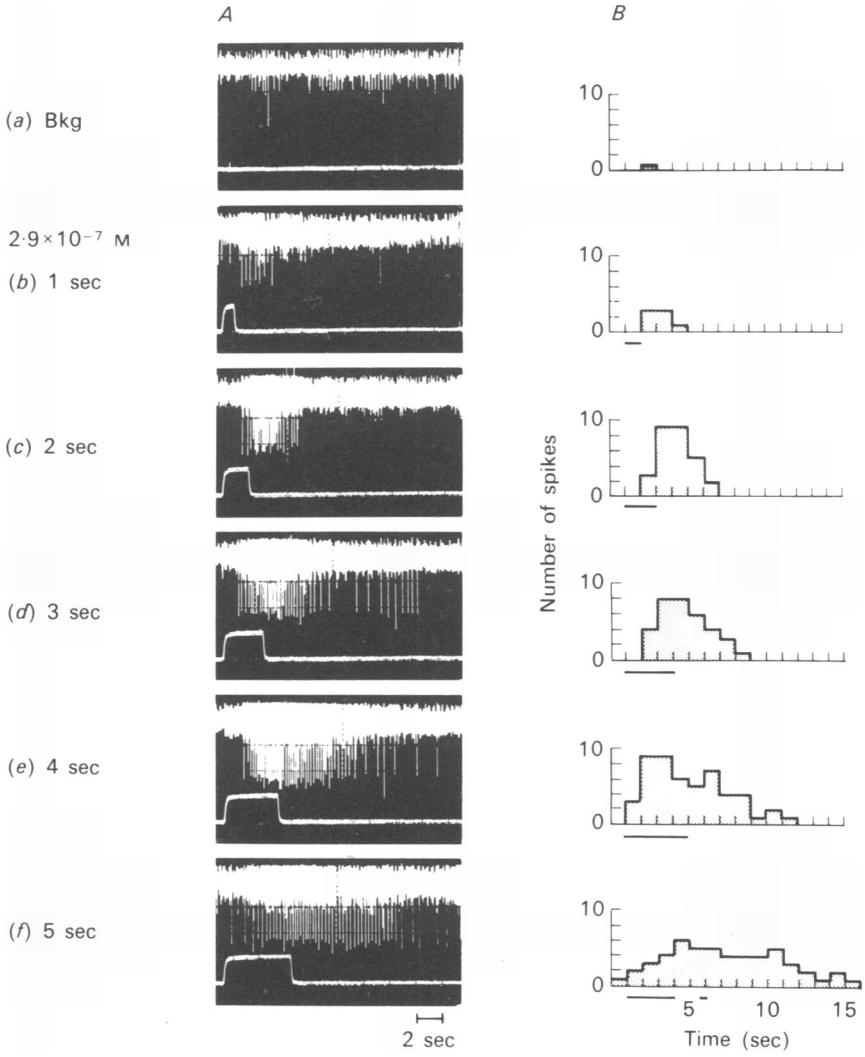

Fig. 7. Responses of the unit shown in Fig. 6 to odour pulses of anisole at increasing durations. The just over threshold concentration was $2{\cdot}9 \times 10^{-7}$ M. With increasing pulse duration (*A*, *b–e*) the discharge was initiated approximately 2 sec after the end of the pulse. The reduced response shown in (*A*, *f*) may be related to fatigue. Peristimulus histograms (*B*) of the recorded activity, 1 sec time bins.

is time-locked to the termination of the stimulus pulse, and the question therefore arises whether this could represent a category of 'off' response. In order to test this possibility, the responses were investigated using pulses of increasing duration at just over threshold concentrations of anisole at $2{\cdot}9 \times 10^{-7}$ M. Inspection of the traces in

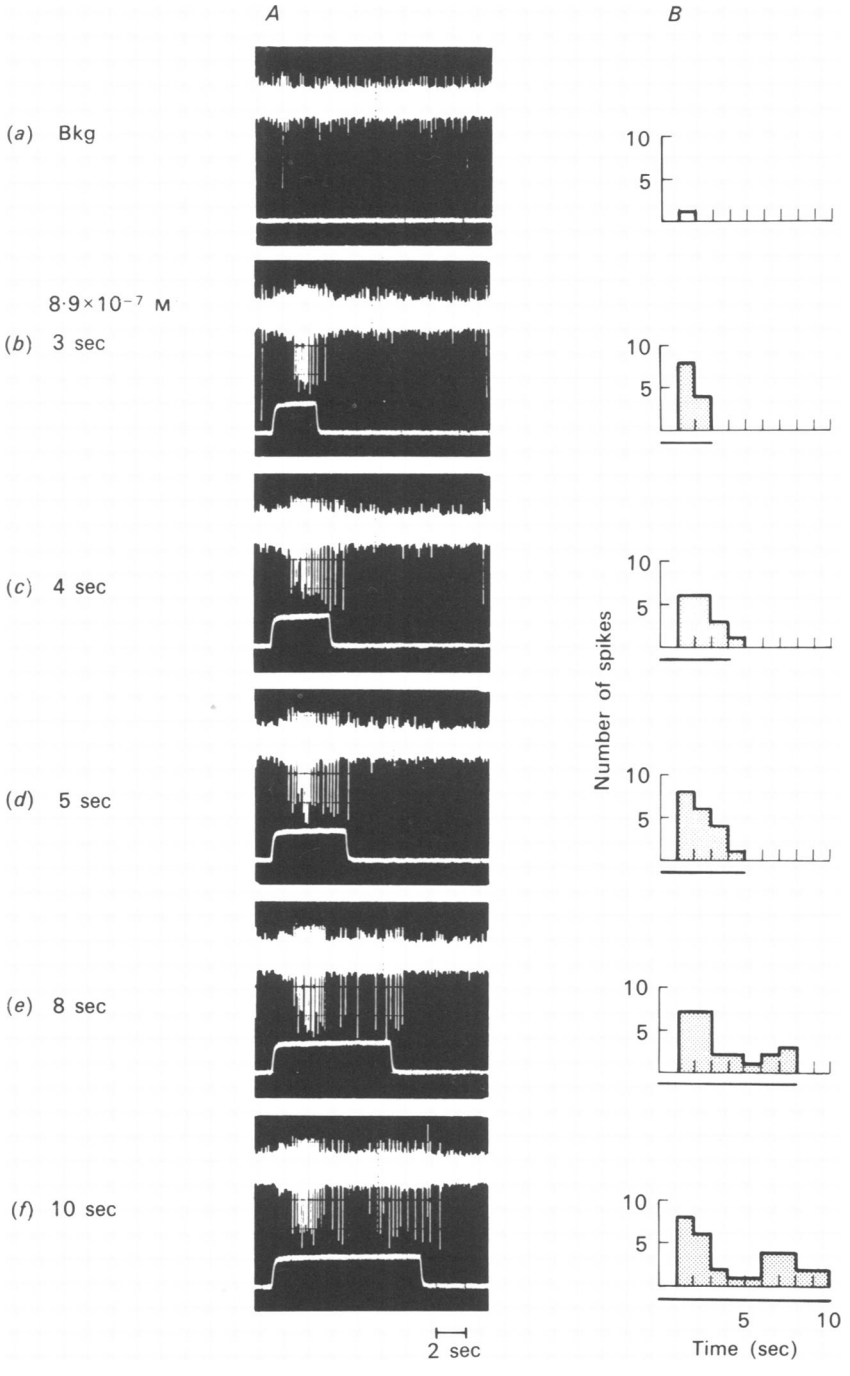

Fig. 8. Unit responses to odour pulses of anisole of increasing duration. The just over threshold concentration was $8{\cdot}9 \times 10^{-7}$ M. *A*, the traces show spontaneous activity (*a*), and responses to relatively short duration pulses (*b* and *c*) consisting of an excitatory discharge of impulses. As the pulse duration was increased (*d–f*) the responses consisted of three sequential components: excitation, reduced impulse activity, and excitation, which were time locked to the odour pulse. Peristimulus histograms shown in *B*, 1 sec time bins.

Fig. 7*A* clearly shows that the onset of the response remained relatively constant, between 1 and 2 sec, as the pulse duration was systematically increased from 1 to 5 sec (*b* to *e*). Thus, the ability to vary pulse duration enables one to demonstrate that this discharge is not time-locked to the termination of the pulse, and therefore is not a

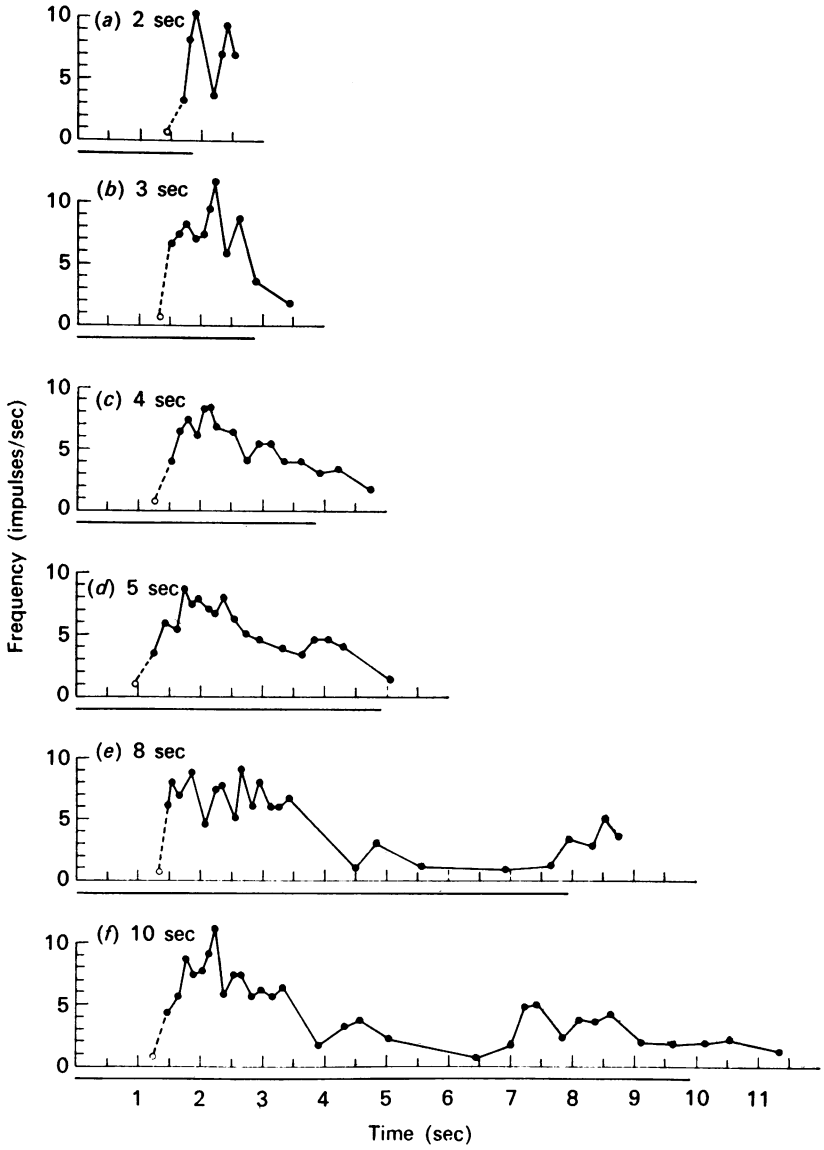

Fig. 9. Instantaneous frequency plots of the responses shown in Fig. 8. Anisole 9×10^{-7} M. See text.

class of 'off' response. There appears to be a nearly linear relation between the duration of the burst discharge and the pulse duration. The accompanying histograms (Fig. 7*B*) show that the maximum frequency of about 8 impulses/sec was attained shortly after the initiation of the responses at intermediate pulse durations (*b*, *c*, *d*),

then followed by a decrease in frequency until the termination of the response. The depressed level of response to the 5 sec pulse (*f*) suggests some degree of receptor fatigue. This aspect will be commented on further in the Discussion.

In the results discussed thus far there was a maintained impulse discharge during the excitatory responses. The responses shown in Fig. 8 have a more complicated pattern. They were evoked by anisole at $8{\cdot}9 \times 10^{-7}$ M, estimated to be just over threshold concentration. The responses therefore were unlikely to be complicated by high odour concentration. Inspection of the traces (Fig. 8*A*) and the accompanying histograms (Fig. 8*B*) shows that the 3, 4 and 5 sec pulses evoked fairly typical burst discharges, as described above. However, with the 5 sec pulse there was not a prolongation of the response beyond that of the response to the 4 sec pulse. The reason for this becomes apparent in the responses to 8 and 10 sec pulses. It can be seen that in these trials the discharge was sharply reduced between 3 and 6 sec, and then showed some recovery during the remainder of the plateau pulse. The final termination of impulse firing was clearly related to the termination of the pulse, as was usual for excitatory responses.

The instantaneous frequencies for this experiment are plotted in Fig. 9. Each response was initiated by a rapid increase in frequency which reached a maximum of 8–12 impulses/sec approximately 1·5–2·5 sec after the onset of the pulse. This early part of the discharge is similar to the corresponding stage described in Figs. 1–3. The plots show the ensuing fall in frequency to about 1 impulse/sec with the longer pulses, and the late recovery to approximately 5 impulses/sec. This clearly departs from the patterns of firing shown in the plots of Figs. 2 and 3. It may be noted that there was no evidence that the access of the stimulus molecules to the receptors was restricted in this experiment, or that the unit was damaged, or that this particular response pattern was peculiar to anisole (cf. Figs. 6 and 7; also Fig. 5 in Getchell & Shepherd, 1978).

Reductions in activity associated with odour pulses

In units with relatively high background rates of activity it was possible to assess reductions in activity as well as excitation associated with the stimulus pulse. We have already noted that a period of impulse inactivity was common following a pulse. In contrast, units which showed reductions in impulse activity during a pulse were rarely encountered. This has been discussed in relation to effects of changing concentration in a companion paper (Getchell & Shepherd, 1978), and we now consider this property in relation to changing pulse duration.

Only one unit showed well-delineated, stimulus locked reductions in background activity during a pulse. The method for demonstrating this type of response is illustrated in Figs. 10 and 11. In Fig. 10, (*A*, *a*) the background spontaneous activity was approximately 1·3 spikes/sec. Two second pulses of increasing concentrations of *n*-butanol were delivered, as shown in *b*–*f*. No effect was seen on the large amplitude spike activity in trials *b*, *c* and *d*. In *e*, there appeared to be a possible slight reduction in frequency associated with the pulse, and this tendency seemed more pronounced at the highest concentration (*f*). Inspection of these traces and the accompanying histograms (Fig. 10*B*) suggests that the threshold for this effect was approximately $7{\cdot}5 \times 10^{-6}$ M-*n*-butanol. It is important to note that other units were excited by these

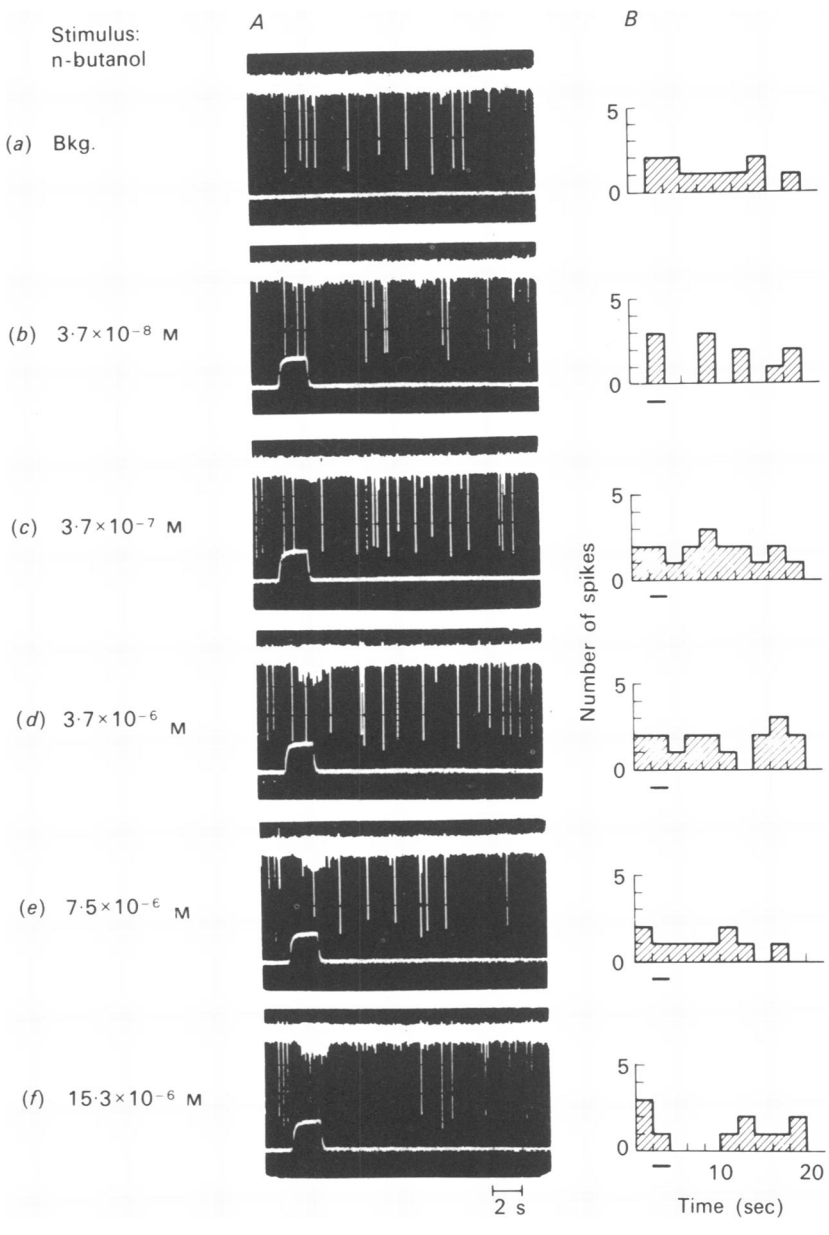

Fig. 10. Unit responses to odour pulses of *n*-butanol of increasing concentration. Traces in *A* and the peristimulus histograms in *B* show that for the responses evoked by the higher concentrations (*e* and *f*) the impulse activity appeared to be reduced.

same odour pulses, as shown by the low amplitude spike activity time-locked to the pulses in all traces (especially *d*–*f*). This may be taken as evidence that the stimulus was not having an overall adverse effect on the functional activity of the epithelium. As previously noted (Getchell & Shepherd, 1978), analysis of this type of response is hampered by two factors, a low rate of background activity, and a brief duration of

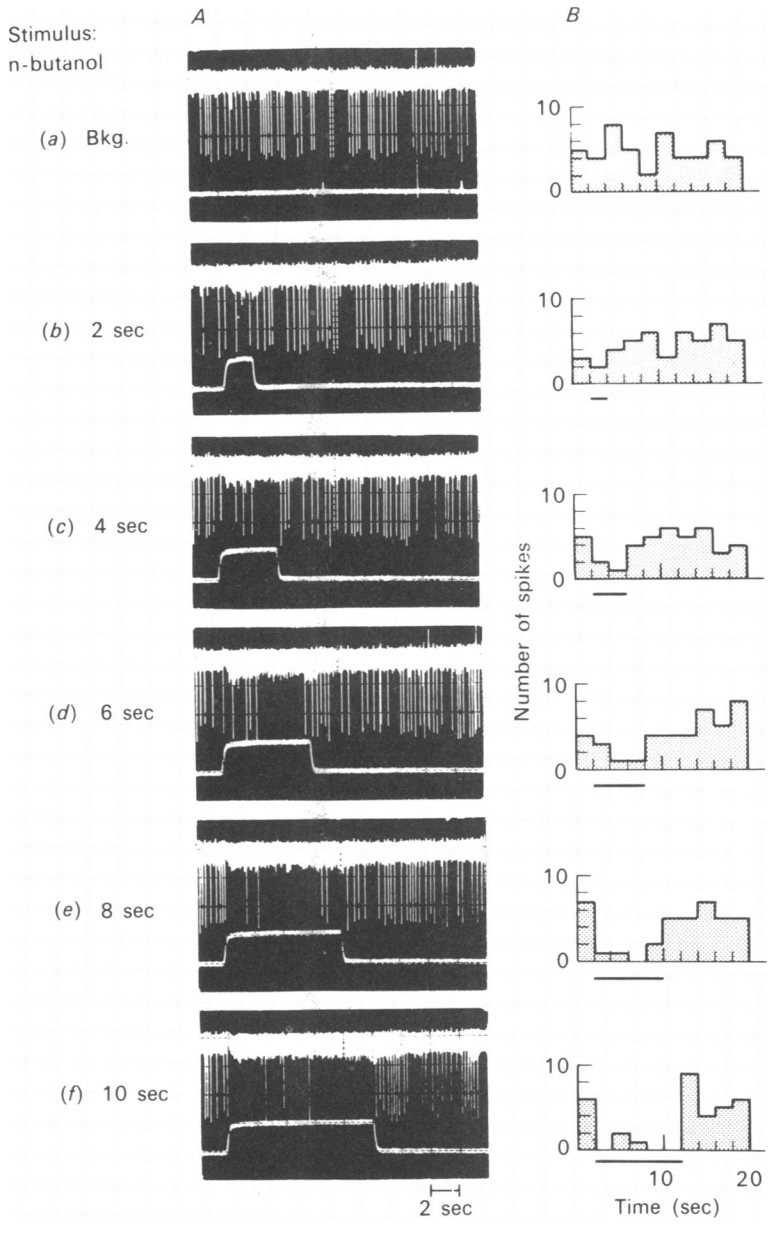

Fig. 11. Responses of the unit shown in Fig. 10 to odour pulses of increasing duration. The concentration of *n*-butanol was $7{\cdot}5 \times 10^{-6}$ M. The traces (*A*) and the peristimulus histograms (*B*) show: an increased level of background spontaneous activity (*a*) by application of an odour mixture (compare with *a* in Fig. 10), time-locked reduction in impulse activity for the longer duration pulses (*c–f*), an occasional breakthrough of impulses of all durations (*b–f*), and a rapid resumption of the spontaneous activity following the pulse.

an odour pulse. In the case of the unit in Fig. 10, it was found that the level of activity could be increased by steady delivery of a low concentration odour mixture. The flow rate of the mixture was kept low (4·5 ml./min) so as not to cause appreciable shift in the DC recording of the electro-olfactogram. With this manipulation, the background activity of this receptor was increased to about 2·5 spikes/sec (Fig. 11*A*, *a*). With regard to the second factor, our stimulus system permitted use of pulse duration that could be varied over a wide range, so that longer pulses could be used to enhance a reduction of activity, if present, against a given background level. A concentration of $7{\cdot}5 \times 10^{-6}$ M-*n*-butanol was selected to investigate the effects of such long term pulses. It may be recalled that at this concentration the effect of a 2 sec pulse was a slight decrease in impulse activity (Fig. 10*A*, *e*); with the higher level of background activity, the same 2 sec pulse had little effect, as is shown in Fig. 11*A*, *b*. However, as the stimulus duration was increased by 2 sec increments from 2 to 10 sec, a reduction of spike activity was clearly revealed (Fig. 11*A*, *B*). This was time locked to the stimulus, and the higher rate of background activity was quickly resumed after the pulse terminated. The effect was most clearly observed during the 10 sec pulse (Fig. 11*A*, *f*), after which the activity resumed within 300 msec. It should be noted that the activity was not completely eliminated during the pulses (*c*–*f*). The fact that these few spikes occurred during the pulses at the characteristic amplitude and voltage conformation for this unit suggests that the reduction of activity was not related to a decrement of spikes, as have been observed by other investigators (see Discussion).

In the unit illustrated in Figs. 10 and 11 reduced activity occurred primarily at higher stimulus strengths. In contrast, one unit showed reduced activity with pulses of estragole at relatively low stimulus concentrations ($7{\cdot}8 \times 10^{-10}$ M). Although background activity was not manipulated in this case, a positive correlation was obtained between the durations of the longer pulses and the durations of the reduced activity. When the trials were repeated, increasing the concentration by 2 log units, the ongoing activity appeared to be shut off more abruptly, and activity was eliminated during the pulses, except for occasional spikes, as in the previous trials. In three other units there was evidence of possible reductions in activity during a pulse, but an adequate analysis, including changes in background activity, under concentration and pulse duration, could not be completed.

DISCUSSION

Characteristic response patterns

The present results indicate that the most common response of olfactory receptors to long duration odour pulses is a prolonged discharge of impulses. A crucial property of this excitatory discharge is that it is time-locked to the pulse. The discharge usually terminated abruptly within 1 sec of the termination of the pulse. In some cases the discharge terminated within several hundred msec, and in one instance it was essentially coterminus with the end of the pulse, within the time resolution of our methods. Of considerable interest was the finding that, for a given unit and given odour, the interval for resumption of background activity was relatively independent of pulse duration, i.e. it was similar following a 2 sec up to a 10 sec pulse.

With regard to adaptation, the instantaneous frequency plots permit the conclusion that, under conditions of stimulation at just over threshold concentration, most olfactory receptors are slowly adapting. Because of our concern to avoid overstimulation of the receptors, we have not carried out trials which extended over the complete concentration range with the longer pulses. However, on the basis of the low concentrations of stimuli used here and taken together with the results of the previous study (Getchell & Shepherd, 1978), several components of adaptation can be identified. Previously we have shown that with odour pulses of a relatively short duration, i.e. 1–3 sec, and increasing concentration, the response consists of an impulse discharge which rapidly increases to a peak and then declines. This corresponds to the phasic component of the responses to longer duration pulses reported here (cf. Figs. 1–3). A slow decline in impulse frequency from this peak is an identifiable component of adaptation in some receptors (cf. Fig. 3). The discharge during the subsequent steady level also appeared to reflect a distinct component of adaptation. This period lasted for the duration of the pulse and was characterized by a surprisingly constant frequency of discharge in many receptors. In fact, it could be concluded that some receptors showed essentially no adaptation over substantial periods of the pulse (cf. Fig. 3). The implications of this finding for receptor mechanisms are discussed below.

Variations in the typical patterns just described may be significant. For example, the responses of one unit to carrier air plus CO_2 showed an unusually sharp division into phasic and tonic components (Fig. 4 and 5). The discharge patterns and frequency plots are remarkably similar to those of another type of slowly adapting receptor, the frog muscle spindle (Ottoson & Shepherd, 1971). This similarity includes the presence of a distinct pause separating the phasic and tonic components in both cases.

Responses giving evidence of reductions in activity, time-locked to the pulse, were also of interest, though they were not common. In the unit shown in Fig. 8, a characteristic excitatory response declined in frequency from an initial phasic peak and then recovered during a long pulse. This could be regarded as a variation on the pattern of adaptation, or as evidence of partial suppression (see below). Complete elimination of impulse activity throughout a long duration pulse was rarely observed. The dependence of this type of response on various factors under experimental control has been emphasized in Results (cf. Figs. 10, 11). Several mechanisms might account for this type of response: altered excitability due to electrode impingement (see discussion by Moulton & Beidler, 1967; Getchell & Getchell, 1974); potassium accumulation in the extracellular space (Getchell, 1977); activation of inhibitory ionic mechanisms (Gesteland, Lettvin & Pitts, 1965; Takagi, Wyse, Kitamura & Ito, 1968) or nonspecific action of odour molecules on the receptor membrane. We have no direct evidence regarding these factors, other than that they do not appear to be dominant in shaping the patterns of responses to the odour pulses used in the present study.

Mechanisms of excitatory responses

In view of the patterns of excitatory discharges described above, the question arises of the extent to which they may reflect steps involved in the sensory transductive process. Although there is little direct experimental evidence regarding these steps

(for discussion, see Getchell & Getchell, 1977), the following are likely to occur: (1) absorption of odour molecules into the mucus; (2) diffusion through the mucus layer; (3) interaction of odours with molecular receptors on the receptive membrane; (4) electrical responses resulting from the molecular interaction, i.e. the receptor potential and impulse initiation; (5) inactivation of the ligand-receptor molecule complex and subsequent removal of the odour molecules. This by no means exhausts the possible steps, but simply outlines the main sequence.

With regard to the present results, it seems reasonable to assume that steps (1), (2) (and possibly (4)) account for the major part of the initial latency of the impulse response, and may also be implicated in the latency of response termination following the end of the pulse. The present results are too preliminary to characterize further the steps in more quantitative terms, apart from noting that the latency of onset and termination generally occurred within 1 sec. Also, there were instances in which a shorter latency of onset was associated with a shorter latency of termination (e.g. Fig. 4). It is of interest to recall that with pulses of increasing odour concentration, there were shorter latencies of onset (cf. Getchell & Shepherd, 1978), consistent with a steeper concentration gradient driving diffusion in the mucus layer during step (2). The latency of response termination tended to remain relatively constant for most units, and to become shorter or longer in others. This may reflect not only the initial steps, but also other factors.

The impulses presumably arise by spread of the receptor potential from the sensory membrane activated by step (4) (Getchell, 1973; 1977*a*, *b*; Getchell & Getchell, 1977). What then controls the time course of the receptor potential of an individual receptor cell? The two most relevant steps in the sequence above appear to be mainly step (3), which represents the molecular activation of the receptor, and step (5) which represents mechanisms for inactivation and removal of stimuli. It may be postulated that the slowly adapting impulse discharge that is characteristic of the responses during the later stages of prolonged odour pulses reflects a relative balance between the two mechanisms of activation and inactivation. It appears that these two mechanisms can be maintained in a nearly steady state during a constant flow of odour over the mucosal surface. This presumably induces the receptor potential (cf. Ottoson, 1971), which in turn generates an impulse discharge at a nearly constant frequency. Minor rhythmic variations in this tonic level (Fig. 2), and larger variations (prolongation of the discharge, as in Fig. 7, or decline followed by recovery, as in Figs. 8 and 9) may be taken to reflect at least in part changes in the balance between these two competing mechanisms.

Post-stimulus activity

Several aspects of the impulse activity following the termination of the pulses may be noted. No evidence was found for a classical type of off-response, i.e. an impulse discharge directly initiated at the termination of the pulse. We have pointed out the value of variable pulse durations to test for this property (cf. Figs. 6 and 7). The common pattern following an excitatory discharge was a period of impulse inactivity followed by a period of slightly increased impulse frequency somewhat above the prestimulus level. This 'rebound' activity is clearly distinguishable from an off-response because of the long period of intervening impulse inactivity and the absence

of the characteristic pattern of impulses normally observed during a time-locked excitatory discharge.

The period of inactivity was of interest with regard to its possible relation to the steps discussed above. The termination of the pulse causes an abrupt removal of the activating molecules from the headspace above the epithelial surface. This raises the question about the resultant effects on the mechanisms for inactivation related to step (5) above. Two possibilities seem of interest. One is that the inactivation process is restricted in its site of action to the odour molecules themselves; in such a case, it would be expected that the sensory cell would rapidly revert to its prestimulus level of activity following the termination of the pulse. The other possibility is that the inactivation process acts on the sensory membrane, at the sites of the molecular receptors. In such a case, it would be expected that when this process is suddenly unopposed by the stimulus molecules it could cause a direct depression of the receptor cell membrane, with consequent reduction of impulse activity.

The duration of impulse inactivity would be determined by the time required by the inactivation process to decay back to its background level. It is of interest that this period of inactivity tended to be dependent on the duration of the preceding pulse.

Significance of receptor adaptation

The present results confirm Ottoson's (1956) evidence obtained from summated potential recordings that the olfactory receptors are slowly adapting, and extend it to the single receptor cell level. Ottoson (1971) has pointed out that if the receptors are slowly adapting, it implies that the rapid adaptation in our perception of odours under conditions of prolonged stimulation must have a more central location, possibly in the olfactory bulb or more centrally in the olfactory cortex. To the extent that one can apply the results obtained from amphibian olfactory receptors to the human, our results agree with this interpretation. A belief that olfactory receptors are rapidly adapting nonetheless persists; one may note in this regard that physiologists routinely employ long interstimulus intervals to avoid progressive diminution ('adaptation') of responses. We have discussed the possibility that the diminution of responses in some cases may be related to the use of high stimulus strengths, decrement of spikes in a discharge, or inadequate clearing of stimulus molecules after stimulation. The active clearing of the headspace above the epithelial surface between stimuli, used in the present experiments, may be a closer approximation to the physiological mechanisms of inhalation and exhalation through the nasal cavity. This technique, therefore, may be useful for further studies investigating relationships between peripheral and central adaptation mechanisms. It should also be clear that study of adaptation requires the use of a stimulus monitor for determining the temporal relationship of the impulse discharge and its aftermath to the period of stimulation.

The authors thank Dr Marilyn L. Getchell and Dr John Kauer for valuable discussions and critical review of the manuscript. The research was supported by NIH grant NS-11667 and NSF grant BNS-76-81404 to T. V. Getchell and NIH grant NS-07609 to G. M. Shepherd.

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