

Repeating triplets of spikes and oscillations in the mitral cell discharges of freely breathing rats

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Abstract

The olfactory bulb responses to odours display evident temporal organization, both in the form of high-frequency oscillations and precisely replicating triplets of spikes. In this study, the frequency of replicating triplets in a sample of 118 individual responses from 45 cells was compared with that in simulations of non-homogeneous Poisson processes, constructed from the experimental post-stimulus time histograms (PSTHs). In a large majority of the records, replicating triplets (to a precision of 0.5 ms) are found to be more numerous in the physiological records; in some of them, they are ~ 10 times more abundant. An excess of precisely replicating triplets is also found in records where no oscillations are apparent in the autocorrelograms. Triplet replication thus seems a more robust phenomenon than transient oscillation. Not unlike fast oscillations observed in other preparations, replicating triplets produced by a given mitral cell are generally observed only during a restricted period of time of the respiratory cycle (at least in the case of the responses under olfactory stimulation). No relation was found, however, between the nature and strength of the olfactory stimulus and the frequency of replicating patterns. In the absence of olfactory stimulation, some mitral cell discharges also contain more replicating triplets than the non-homogeneous Poisson simulations. Thus, replicating triplets in single-cell discharges seem to play only an indirect role in the coding of olfactory information at the mitral cell output level.

Introduction

Over the last 10 years, much attention has been paid to the possible role that the temporal structure of neuronal discharges may play in the processing of sensory information. Oscillations and synchronization are perhaps the two paradigms on which the greatest attention has been concentrated (for a review, see Singer, 1993). The olfactory bulb seems to be the first structure for which evidence has been presented that neuronal responses may display high-frequency oscillations in the γ range (30–70 Hz, Adrian, 1950; Freeman, 1985). Mitral cells and their glomeruli constitute the first synaptic relay for incoming olfactory information. Detailed studies of mitral cell responses have shown that their discharges usually display characteristic time courses as a function of the phases of the respiratory cycle (Chaput *et al.*, 1992). More recent studies suggest that in fact odours are probably coded by using a complex spatiotemporal code, whereby both the place of individual mitral cells discharges and the temporal order of their firing may carry information about the nature of the stimulant (Laurent & Davidowitz, 1994; Wehr & Laurent, 1996; MacLeod *et al.*, 1998).

The neuronal code used by most neurons in general, and by mitral cells in particular is, however, not precisely known. Several studies of single-unit spike trains in the visual system have revealed a high proportion of temporal patterns (doublets of spikes or higher order patterns) that replicate themselves in the same spike train with submillisecond precision, in proportions incompatible with various null hypotheses, e.g. locally steady Poisson process, shuffling of actual

intervals and renewal models based upon the overall time interval distributions (Strehler & Lestienne, 1986; Lestienne & Strehler, 1987; 1988; Lestienne, 1996). Recently, Lestienne & Tuckwell (1998) have compared the rate of production of precisely replicating patterns in various central nervous system centres in a variety of mammals. They observed that mitral cells of the olfactory bulb, and cells of the lateral geniculate nucleus in the visual system (both centres being located at the level of the first synaptic relay of the incoming sensory information) were particularly inclined to display precisely replicating patterns in their spike trains (Lestienne & Tuckwell, 1998).

Replicating triplets suggest a more complex temporal organization than simple oscillations. Although a functional use of replicating triplets in terms of repeated synchronous discharges at sets of synapses of the downstream neurons (Strehler & Lestienne, 1986; Lestienne & Strehler, 1987) might be found in the future, such a role has not yet been demonstrated. We attempted to assess the propensity of individual mitral cells to produce precisely replicating triplets, by using a new and probably better null hypothesis, in the form of non-homogeneous Poisson process (NHPP)-simulated spike trains (H. C. Tuckwell & R. Lestienne, unpublished results), and studied the possible relationship between these replications and the occurrence of characterized oscillations in their discharges.

Materials and methods

Physiological recordings

Single-unit mitral cells were recorded in the olfactory bulbs of anaesthetized adult Wistar rats, as determined by a careful examination of spike waveforms during recordings and after

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computer acquisition at 16 kHz. Single units were triggered using this waveform criterion and their time of occurrence was recorded. Anaesthesia was provided through injections of equithesin (mixture of pentobarbital sodium and chloral hydrate at 3 mL/kg). Stimulation was provided by exposing the animal to various chemical odours over 3-min periods interspersed by several minutes of pure air delivery using a flow dilution olfactometer. Odours used were camphor, cineole, isoamyl acetate, limonene and methyl amyl ketone, and concentrations were varied in seven steps, ranging from 2×10^{-4} of the saturated vapour pressure up to a maximum concentration of 10^{-1} of this value. Respiration cycles were monitored at the same time as spike recordings, the corresponding signal being recorded by a thermistor placed just at the entrance of the nostril. Mitral cell responses to olfactory stimulations within their range of sensitivity typically consist of a characteristic pattern of discharges, where the firing rate varies with the phase in the respiratory cycle. The times of initiation of the animal's respiratory cycle served as reference points for the construction of 'post-stimulus' time histograms (PSTHs). Details of the preparation and electrophysiological observations may be found in Buonviso & Chaput (1990), and Chaput *et al.* (1992).

Detecting replicating triplets

A triplet is a set of three spikes, consecutive or not, and is characterized by the pair $\{a, b\}$ of time intervals between these spikes (Fig. 1). By definition, the occurrence of a replicating triplet is detected whenever in a spike train two triplets with constituent intervals $\{a, b\}$ and $\{a', b'\}$ are detected within the same time window T (here chosen as 200 ms), and are such that all constituent intervals are smaller or equal to an upper limit w (here 50 ms). Homologous intervals reproduce each other in the sense that $|a - a'| \leq \Delta$ and $|b - b'| \leq \Delta$, where Δ is the specified precision of replication (here 0.5 ms). The maximum span of intervals constituting a replicating triplet was $w = 50$ ms (notation follows that of Abeles & Gerstein, 1988). The use of limited interval spans and replication delays results from previous observations (Lestienne & Strehler, 1987) that this type of replication is essentially a transient phenomenon, and has been shown to improve the signal/background ratio, i.e. the ratio between the number of repeated patterns observed and the number of repeated patterns expected on the basis of the chosen null hypothesis. Both the physiological data and the NHPP-generated events were processed in exactly the same way. The number of replicating triplets per respiratory cycle was computed for all 118 records, together with their standard deviations (SDs).

The number of replicating patterns in the discharge is a highly non-linear function of the firing rate as exemplified by a Poisson process, in which the number of replicating triplets rises roughly as the sixth

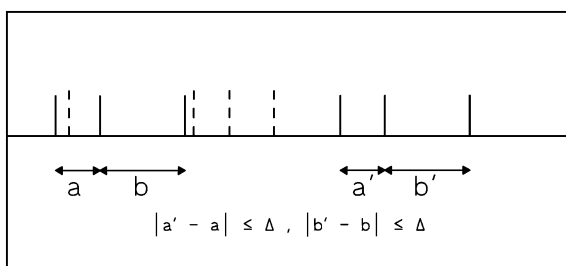


FIG. 1. Definition of replicating triplets. Nine spikes are present in the window of duration $T = 200$ ms here depicted. A triplet with constituent intervals a and b , each of them of duration shorter than $w = 50$ ms, was replicated by a triplet whose interspike intervals a' and b' reproduced, within the tolerance Δ , the intervals of the former triplet. Spikes not belonging to the replicating triplet are shown as dashed lines.

power of the firing rate (Abeles & Gerstein, 1988; Lestienne, 1996). For temporally non-homogeneous spike trains, a steady Poisson process is likely to be a poor model with regard to the frequency of replicating patterns. A better candidate is a NHPP, which is locally in time a Poisson process with a rate that is a function of time $\lambda = \lambda(t)$, chosen so that the process reproduces the normalized physiological PSTH. Determining the function $\lambda(t)$ from the PSTH is an analytically solvable problem (see Tuckwell, 1988).

However, such NHPP sequences do not contain refractory periods as their time interval distributions start at zero. In contrast, mitral cell responses usually display a significant minimum interval, t_{\min} , varying from about 2 to 10 ms. Because small intervals strongly influence the number of replicating patterns (the shorter the intervals, the easier it is for them in relative value to match the replication criteria, uniformly set to 0.5 ms), we have modified the NHPP sequences to introduce a refractory period, the value of which was defined from the TIH histogram of physiological data. This was achieved by remapping the sequences of events in each respiratory cycle by means of a linear transformation, such that a zero interval would be transformed into t_{\min} , and the time of the first and last events in the respiratory cycle would remain unchanged. This transformation was found to introduce very little modification in the PSTHs of the simulated sequences, which remain practically indistinguishable from the original PSTHs, within statistical fluctuations (Fig. 2, left versus right panels).

Left panels of Fig. 2 display the PSTHs of two cells responding to an olfactory stimulation. In both cases, the stimulation was performed with isoamyl acetate at a concentration of 2×10^{-2} of the saturated vapour pressure. Right panels display the PSTHs for the corresponding NHPP simulations, after the introduction of refractory periods, as explained above (broken-line histograms).

One should note that although the modified NHPP sequences had the same 'minimal interspike interval value' as the physiological data, the rising slope of the time interval histograms (TIHs) of these simulations was always steeper than that of physiological data (as can be seen from the shapes of the central troughs in the autocorrelograms of Fig. 6, below) – a circumstance that, because it provides more short intervals in simulations than in real data, should promote rather than diminish the production of replicating triplets in the simulations. The differences in content of replicating patterns observed here, where replicating patterns are consistently more numerous in physiological data than in our simulations, can thus be considered in fact as a minimal effect.

Results

In a vast majority of the physiological records, the frequency of replicating triplets was higher than in the corresponding NHPP simulations (Fig. 3A and B). In a restricted sample of 24 records (out of 118), hereafter called 'sample A', the count of replicating triplets in the physiological data was more than 2.5 SD above the mean for simulations. A comparison of replicating triplet counts per cycle for physiological data and simulations for this sample is displayed in Fig. 3A. Note that variations in replicating triplet rates are approximately parallel for the two sets of spike trains, suggesting that the PSTH shapes are responsible in part (but only in part) for the propensity to produce replicating patterns. This suggests interpreting PSTHs as an *a priori* statistical factor, with a second mechanism producing replicating patterns (in an analogous manner, in particle physics, the cross-section for the production of a resonance is the product of a 'phase space term' by a Breit–Wigner function describing the dynamic production of the resonant state).

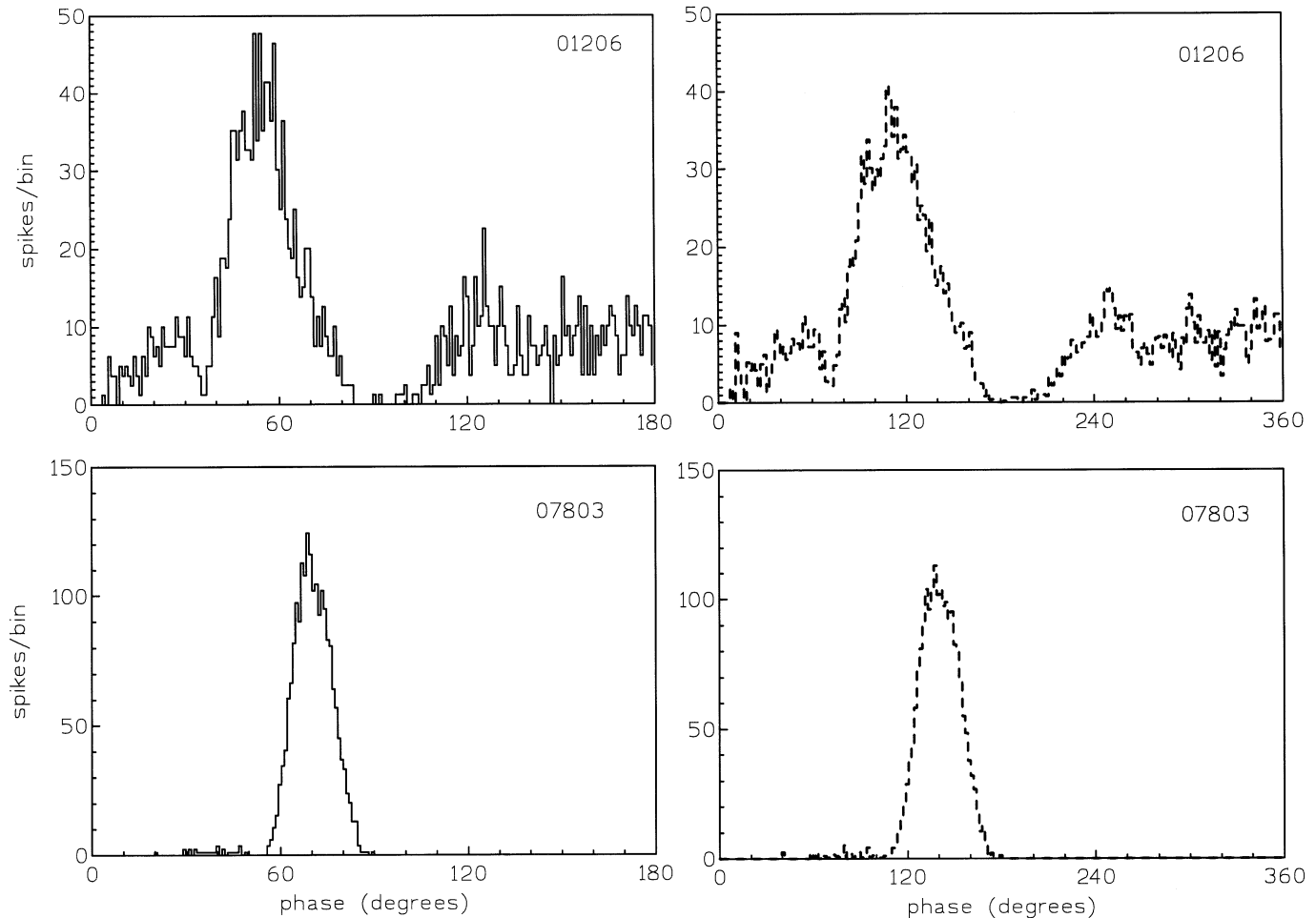


Fig. 2. Examples of the time course of mitral cell responses to olfactory stimulation. Left panels display the physiological responses with respect to the phase of the respiratory cycle (full-line histograms). Right panels display the corresponding time course of spike trains from a simulated NHPP, after introduction of refractory periods as explained in the text. Introduction of the refractory period does not appreciably disturb the fidelity of the reproduction of the physiological PSTHs, the differences being smaller or similar to those due to statistical fluctuations. Upper and lower parts refer to two different examples (records 01206 and 07803, respectively). The bin size is 2° .

Sixty-six other physiological records, containing replicating triplets to varying degrees (although generally smaller than in sample A) constitute sample B. A comparison of replicating triplet rates in physiological data and in simulations for this sample is displayed in Fig. 3B (panels B1 and B2). Here again the rate of replicating triplets is generally higher in the physiological data than in the NHPP simulations (scales in Fig. 2B have been drawn proportionally to the square root of the number of events in order to facilitate the visual comparison of data and simulations in the regions of small ratios of triplets/cycle).

The 28 remaining records out of 118 had no replicating triplets. The corresponding NHPP files also had no replicating triplets, or a number not significantly different from zero. These 28 records were classified as 'sample C'.

The high rates of production of replicating triplets in many mitral cell records suggest that there may be a fine temporal organization in these discharges. One possibility is that replicating patterns reflect some oscillatory process.

In accordance with common practice, the autocorrelograms and their Fourier transforms were examined to detect oscillatory states. In this investigation, we were aware that the standard theory of spectral analysis of stationary random processes (Cox & Lewis, 1966) may

not be directly applicable to non-homogeneous processes. We nevertheless followed the same kind of procedures for this analysis as have been performed in many other studies (Ghose & Freeman, 1992; Bair *et al.*, 1994; Bringuier *et al.*, 1997). In several cases, both autocorrelograms and their Fourier transforms were clearly suggestive of oscillatory states, when, e.g. the autocorrelograms presented at least two secondary peaks beside the peak after the central trough, and/or the power spectra had one or several bumps of various widths apparent above a fluctuating background. This is illustrated in Fig. 6 below, which gives an example of an autocorrelogram considered to be indicative of oscillations (record 15404) and one which is not (record 13714). Records which could be considered to contain oscillations are listed in Table 1, together with the main oscillatory frequency ranges as read from the power spectra. There are often two distinct frequency ranges where peaks are observed in the power spectra. It seems that these can be simply interpreted. Oscillations in the γ range, usually between 30 and 70 Hz, are to be related to the modal or second modal value of the TIH distribution, and oscillations at higher frequency to the presence of burst firing, with spikes succeeding each other with intervals approximately equal to t_{\min} (the 'refractory period') (Bair *et al.*, 1994).

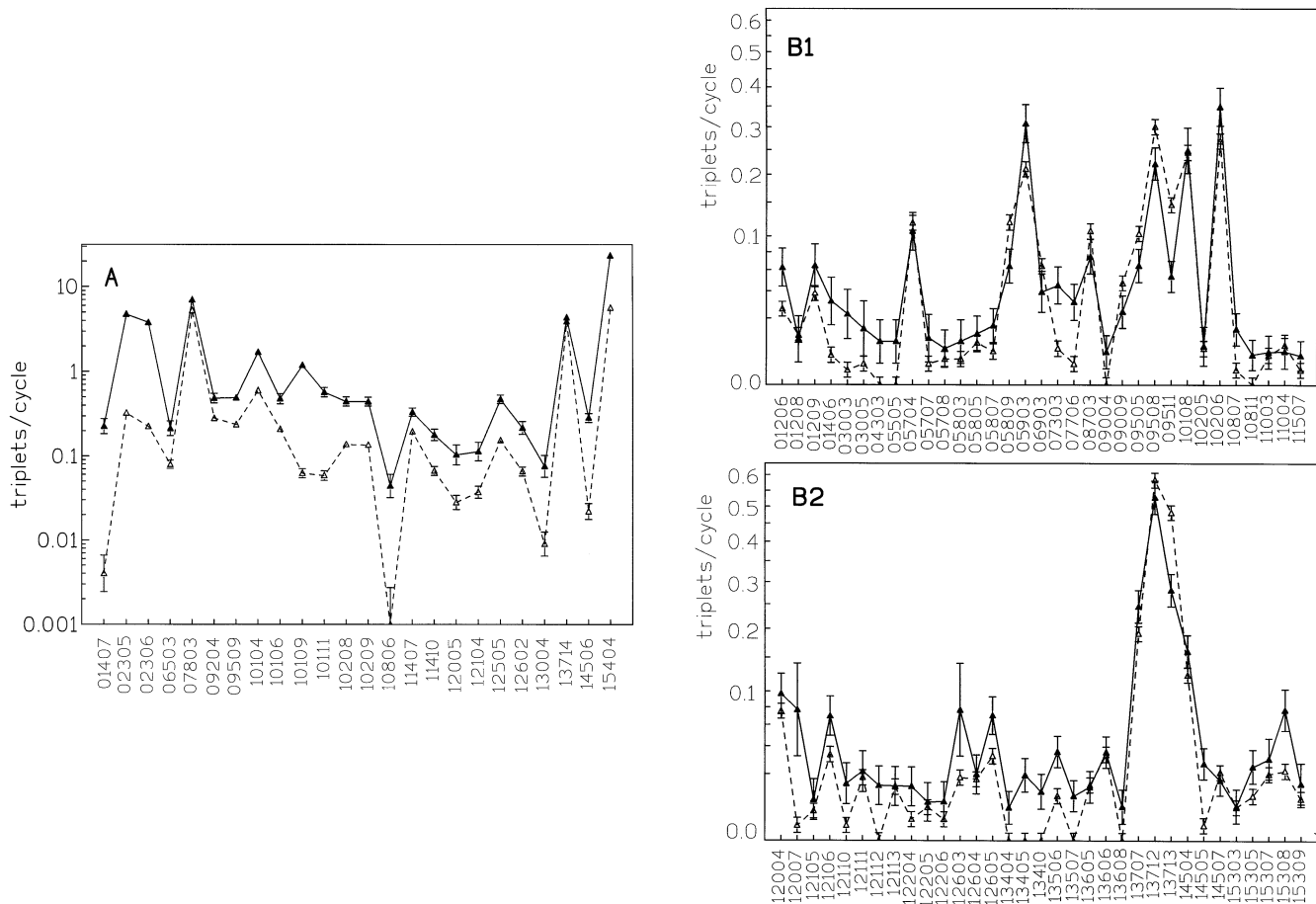


FIG. 3. Replicating triplets can be produced at unexpected rates. Comparison of the rate of production of replicating triplets per spike in physiological data (full triangles and full lines) with those in NHPP-generated spike trains, having the same PSTHs and similar refractory periods (open triangles and broken lines). (A) The 24 records for which the production rate of replicating triplets in physiological data exceeded by more than 2.5 SD (bars) that observed in simulations are grouped. Note the logarithmic scale used, which emphasizes the logarithmic parallelism in the trends of production rates in the two sets of spike trains, and the fact that in physiological data replicating patterns can be as much as 10 times more numerous than in the simulations. Typical values for the number of respiratory cycles were ~200 for physiological recordings and 1000 for simulations. (B1 and B2) The 66 other physiological records for which replicating triplets have been found have been grouped. The rate of production of replicating patterns is clearly more often higher in the data, as compared with simulations, than the reverse, even for those records where the production of replicated triplets was very small (an ordinate scale proportional to the square root of the number of replicating triplets per cycle has been adopted to allow for a better visualization of those small rate records).

The structure of replicating triplets

Once replicating triplets are detected, one would like to know more about their structure. Two-dimensional plots of particular interest are ‘*a* versus *b*’ plots (joint interval scattered plots) and ‘*a* versus δt ’ plots (first interval of first triplet versus time interval between the first spike of the first copy and the first spike of the second copy). The first plot gives a sense of the internal structure of replicating triplets, and the second one about the distribution of the interval between copies. Two such representative plots for the record labelled 15404 are displayed in Fig. 4.

In panel A, the frequency of occurrences of replicating triplets as a function of their constituent intervals *a* and *b* is given. A complex landscape of peaks appears, dominated by a huge peak in the region of small *a*- and *b*-values, with a sharp maximum at ~ (7, 7) ms. Other peaks occur at low intervals as a chessboard-type pattern. This is to be expected from oscillatory discharges, where time intervals of any order tend to be a multiple of a fundamental. Note, however, that there is still a noticeable proportion of replicating patterns which appear in regions where *a* and *b* are not multiples of each other; such

triplets do not seem to be explicable in terms of simple oscillatory mechanisms. The replicating triplets with constituents (*a*, *b*) where neither of the conditions $a = kb$ or $b = ka$ ($k = 1-3$) apply to within 0.5 ms are called incommensurable triplets. We observed that our physiological data do contain a lesser proportion of ‘incommensurable’ triplets versus all triplets than the simulations.

Figure 4B shows the relation between the length *a* of the first interval of a triplet and the time interval δt separating the two copies. A striking concentration of events is observed along the first diagonal, implying that triplets and their copies are intermingled; the first interval of the second copy starts with the same spike as the second interval of the first copy, as in the sequence of spikes that follows, where the | represent spikes, and their spacing (like *a*, *b* and *a* - *b*) represent interspike intervals:

$$| \quad a \quad | \quad b \quad | \quad a - b \quad | \quad b \quad |,$$

which contains a replicating triplet of the form (*a*, *b*). Many ‘*a* versus δt ’ plots actually display this sort of alignment. It should be noted, however, that the NHPP-simulated events also display this sort of

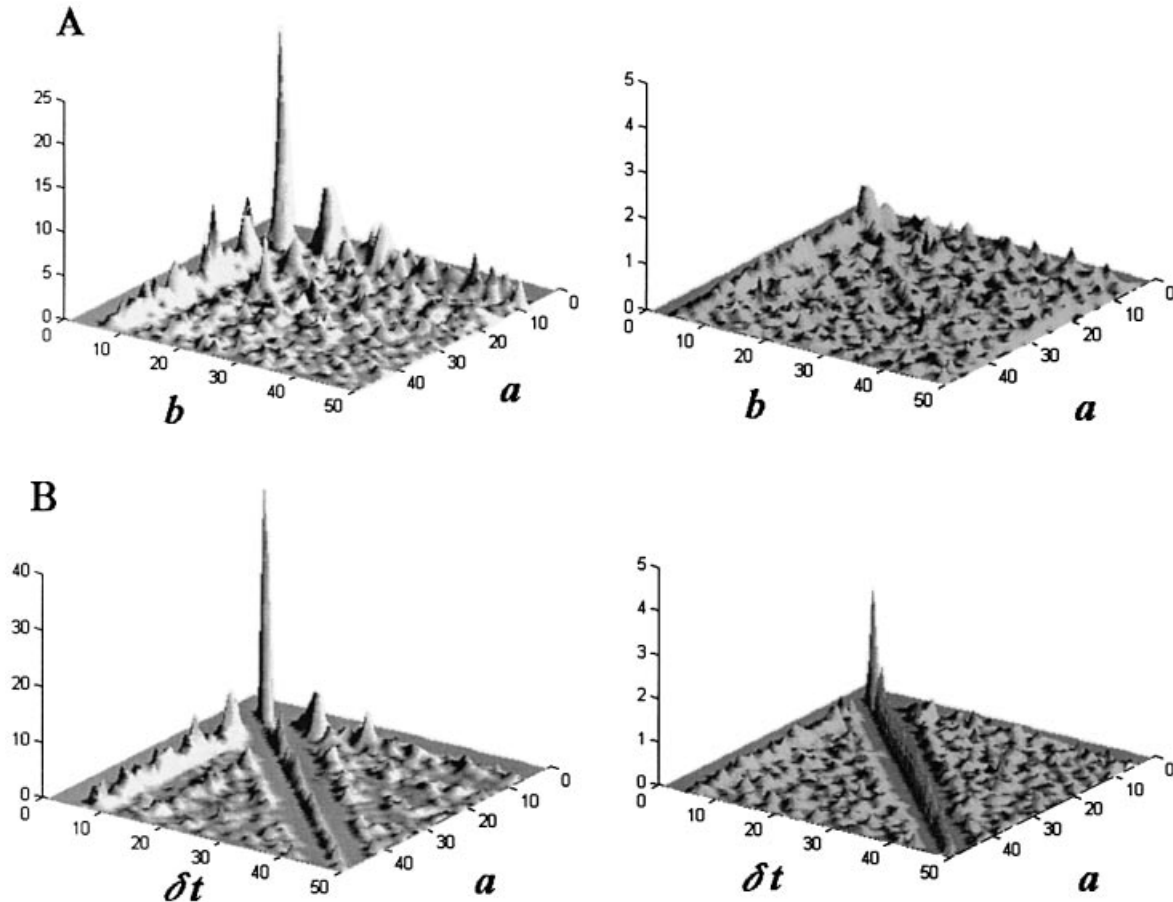


FIG. 4. Internal structure of replicating triplets. Left panels: study of the internal structure of replicating triplets of one record (15404, last record in sample A). (A) Plot of first interval versus second interval, a versus b . Note the sharp peaks positioned on the squares of a grid, and the high concentration of events at small intervals, with a global maximum about the point (7, 7) ms. (B) Plot of first interval versus time interval between the two copies of the replicating triplet, a versus δt . Note again the sharp peak, at the point (7, 7) ms, denoting replicating triplets composed of small and equal intervals, of the *lalala* kind. The striking alignment along the diagonal denotes closely packed, intermingled triplets (see text). The diagonal is flanked by a valley on each side reflecting the effect of refractory periods. In the right panels, similar plots are drawn for the corresponding NHPP spike train. Note the change in scale (all scales are in terms of events per spike per ms^2), exaggerating the relative size of the structures seen in the (a , b) and (a , δt) plots of the simulations.

TABLE 1. List of records where oscillations are suspected from inspection of autocorrelograms and power spectra

Record number	Oscillation frequencies (ranges in Hz)	
02305	25–75	
02306	25–75	
07803	35–125	190–235
10104	25–90	150–240
10106	0–110	160–210
10108	20–70	170–200
10109	35–125	175–210
10111	0–125	170–210
12505	20–90	140–175
15404	20–90	135–175

Taking into account the non-stationarity of evoked discharges, frequency ranges reported are only approximate.

alignment, which is made even more readily visible because the refractory period creates valleys on both sides of the diagonal.

Figure 5 displays further examples of (a , b) plots, shown as 2D contours. The chessboard-type patterns are again observed in those records for which oscillations were detected (records 02305, 07803,

10109 and 15404), with varied spacing between peaks. The other records display much more variable and less stereotyped patterns.

Finally, triplets can also be distinguished by whether or not their time span contains spikes not belonging to the triplet ('foreign spikes'). Triplets that do not contain foreign spikes are called 'clean'. We found that the physiological data contain a significantly greater proportion of 'clean' triplets than the simulated spike trains (on average over sample A, 17% of the detected triplets in our data were clean, but only 8% in the corresponding NHPP spike trains). It is probable that the production of clean triplets is related to the intrinsic properties of neurons. The overall small proportion of clean triplets indicates that an extrinsic origin is more likely for most of the replicating triplets in our spike trains, perhaps due in some way to the properties of the networks in which the recorded cells were involved. The present tests are, however, very crude. Presumably, a more definite answer as to the intrinsic or synaptic origin of replicating triplets would require intracellular recordings for comparing the triplets occurring spontaneously or during odour presentation with those that could be elicited through current injection in the recorded cells.

Autocorrelograms of limited range

A large production rate of replicating triplets seems to be correlated with a transient dynamic state of the neurons in response to olfactory

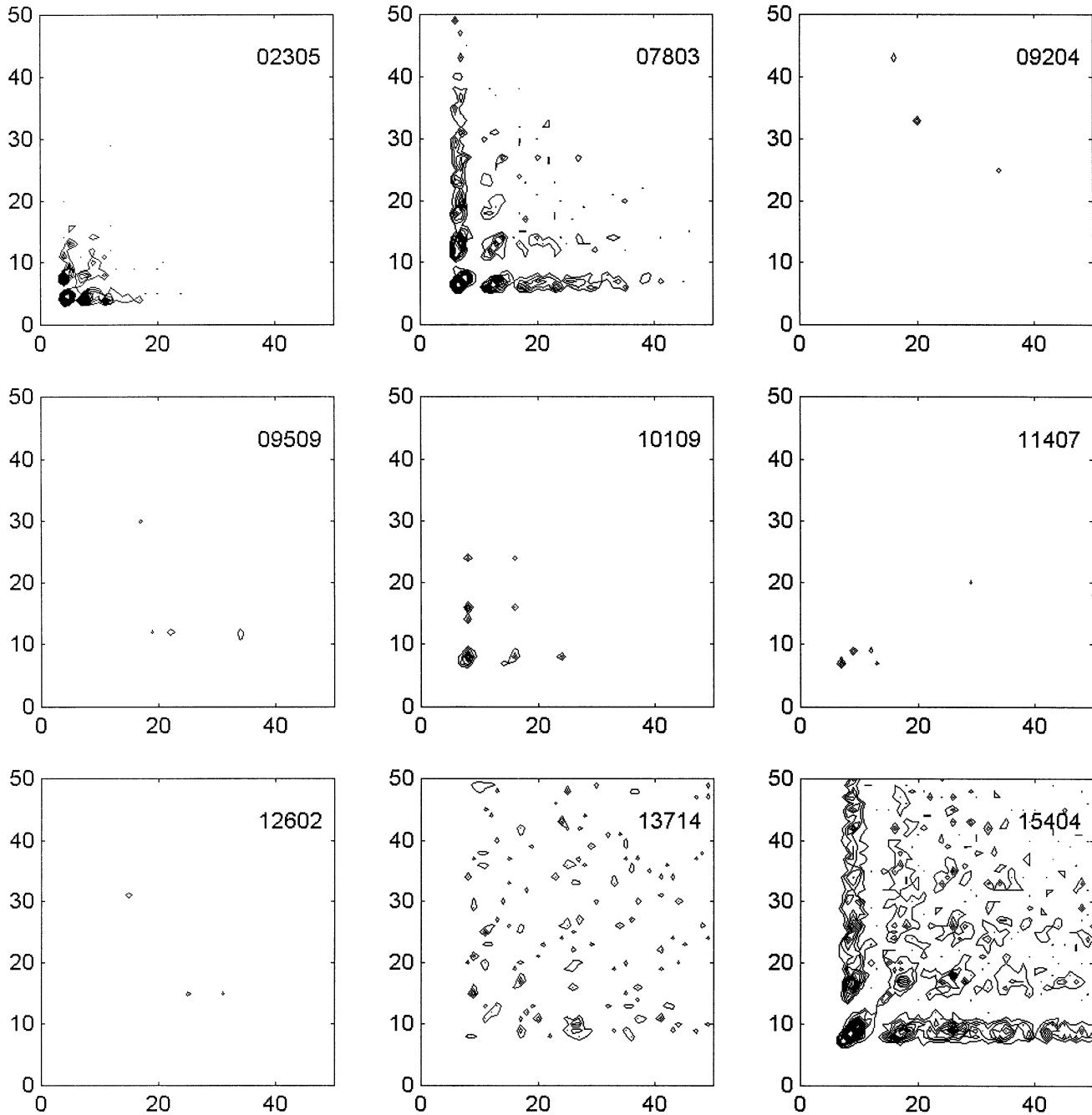


FIG. 5. Internal structure of replicating triplets (continued). Nine examples of the first versus second interval, *a* versus *b*, plots are displayed as 2D contour maps. Time units are in ms. Contours are equally spaced for increasing densities of multiples of two events per ms^2 . Note that records 02305, 07803, 10109 and 15404 were classified as oscillatory (Table 1) and display regular patterns of varied intervals. No such regularities are observed in the five remaining examples.

stimuli. Statistical tools of analysis of spike trains, e.g. autocorrelograms (in the sense of spiking probabilities), although well suited for analysis of steady point processes, are evidently not very well suited for analysis of dynamically changing situations because the range of temporal correlations of the spike discharges here analysed is quite limited.

As a first step towards a possible improvement in the tools of data analysis, we consider autocorrelograms of limited range. In these plots, instead of accumulating intervals between any two spikes, whatever the number of intervening spikes (as in the usual autocorrelogram), one accumulates intervals only up to a limited

order. As an example, Fig. 6 displays the autocorrelograms of order 3 for two spike trains, and compares them with the corresponding autocorrelograms. Note that hardly any temporal structure is visible in the full autocorrelogram of record 13714, top diagram in Fig. 6, but is more visible in the limited autocorrelogram of order 3 (bottom diagram). Of course, in many situations temporal correlations are not only limited in range, but vary with time. Thus, the best approach would probably be to use a limited range autocorrelogram processed in a sliding window of time. Full autocorrelograms processed in a sliding window of time have already been used to detect transient oscillations (MacLeod & Laurent, 1996).

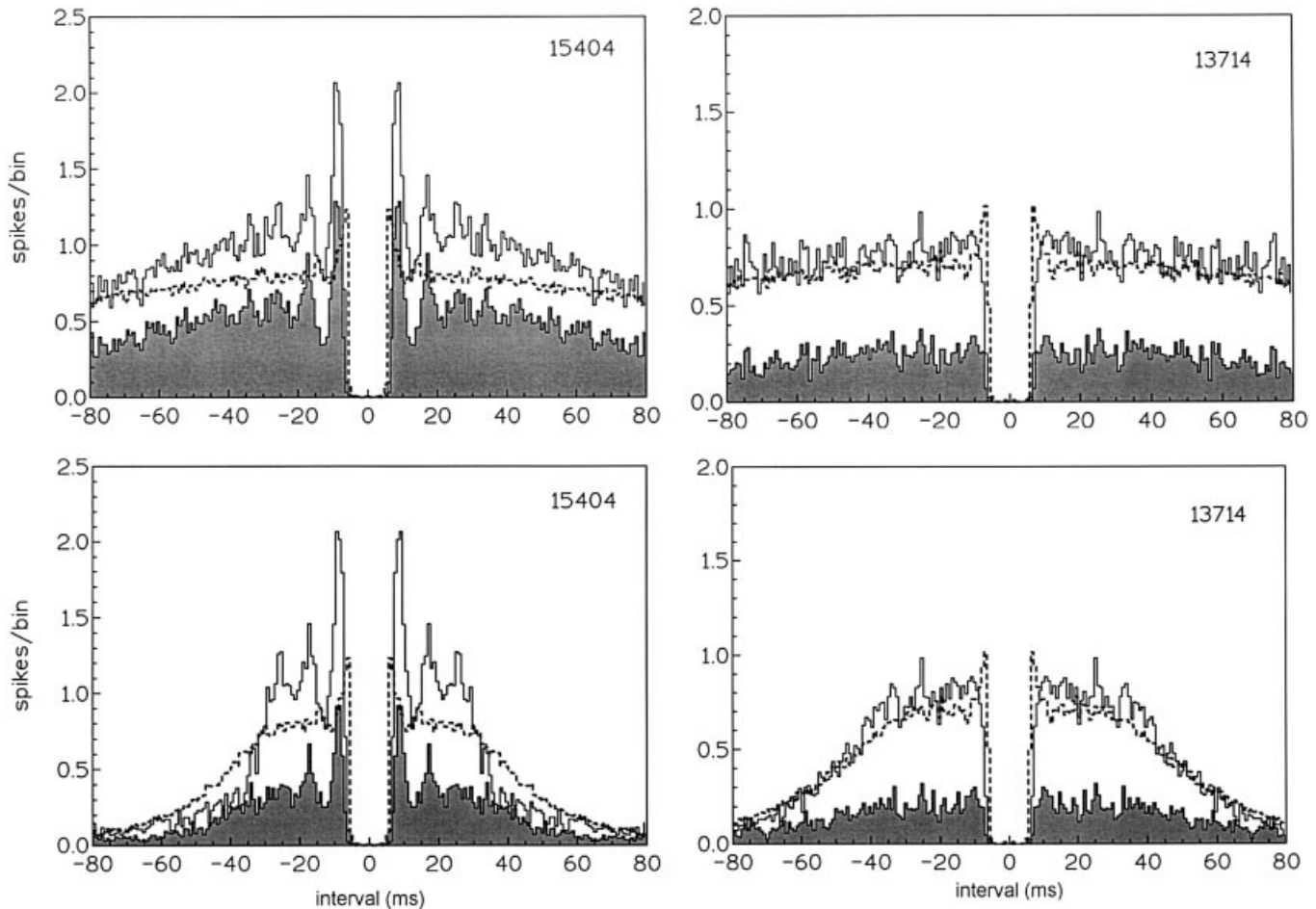


FIG. 6. Limited range autocorrelograms as a tool to detect transient correlations. In the two examples (records 13714 and 15404) shown here, full autocorrelograms are displayed in the top part, and autocorrelograms limited to intervals of order 3 (two intervening spikes at maximum) are displayed below them (full-line histograms). Each plot is contrasted with its counterpart drawn from the corresponding NHPP simulation (broken-line histograms). Although some structure in the autocorrelogram of record 15404 – which was classified as ‘oscillatory’ – is visible, deviation from the NHPP simulations is more evident in the autocorrelogram of range 3, below. The record 13714 was classified as ‘non-oscillatory’ in view of its full autocorrelogram, yet it contains numerous replicating patterns and its limited range autocorrelogram more clearly deviates in shape from its NHPP counterpart. Shaded autocorrelograms were built from only spikes belonging to replicating triplets and essentially display the same structures as correlograms built with all spikes.

Replicating pattern rates and the nature and strength of the stimulation

The question of the correlation between the frequency of replicating triplets and the nature and strength of the olfactory stimulation is a very natural one. We therefore investigated whether any systematic trend was present in our data, whether in terms of the classification in samples A, B and C of high, moderate and vanishing rates of replicating triplets, or in terms of increasing or decreasing odour concentrations.

From the 24 records constituting the A category, 16 were made under stimulation with isoamyl acetate, but this is about the same proportion as in the overall sample (56%). Concentrations of odours in sample A varied from 2×10^{-3} to 10^{-1} of the saturated vapour pressure, and the distribution of concentrations in this sample does not differ significantly from the distribution in the overall sample. Furthermore, no consistent or systematic variation with concentrations was observed when comparing for each odour separately the rates of replicating triplets for all series made with the same cell.

Finally, the rates of occurrence of replicating triplets in cell discharges outside the periods of olfactory stimulation were found to be much lower than during evoked olfactory responses, but the

spontaneous firing rates of mitral cells were also much lower. In terms of replicating triplets rates, discharges outside the periods of stimulation were classified into the same three categories as olfactory responses, but class A (large differences between replicating triplets rates in data and simulations) contained only 12 records, class B (moderate to not significant differences) 44 records and class C (no replicating triplets found in data) 62 records. Thus, no relationship was detected between the presence of replicating patterns and the nature or strength of the stimulation, although replicating triplets were relatively more numerous in odour-evoked responses than in spontaneous mitral cell discharges.

Such a negative result in single-cell discharges should not be taken as a demonstration that replicating triplets do not play a role in the coding of odours, as we know that odours are probably coded through the spatiotemporal organization of the discharges of many mitral cells, taken altogether (Chaput *et al.*, 1992; Laurent & Davidowitz, 1994; MacLeod & Laurent, 1996; Laurent, 1997; MacLeod *et al.*, 1998).

Discussion

The spike sequences of mitral cells in response to olfactory stimulation have been shown to exhibit a complex temporal

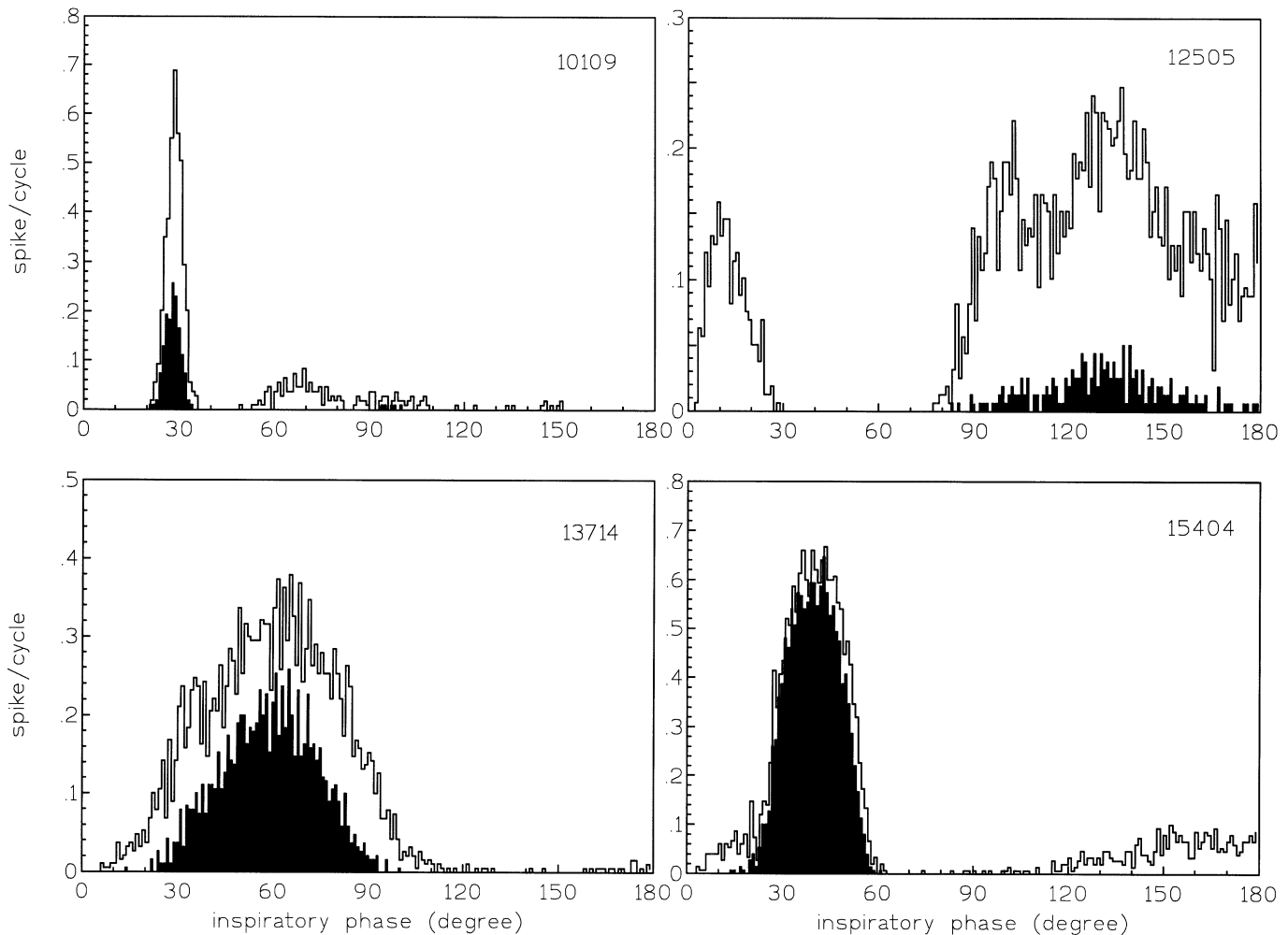


FIG. 7. Time distribution of spikes-in-triplets along respiratory cycles. Respiratory phase distribution of spikes (the 'PSTH') for four examples of cell-odour combinations (unshaded histograms), and restricted to only those spikes that belong to precisely replicating triplets (dark histograms). The proportion of such spikes varies greatly from one example to the other; in the last example (record 15404), nearly all spikes of the peak discharges were part of replicating triplets. Note that, besides the strong correlation between the firing rates and the production of replicating triplets, the latter tend to concentrate in certain phase regions. Units: spike/cycle/degree.

organization, which is probably patterned by network properties and to a lesser extent by intrinsic neuronal properties. Several studies have emphasized the oscillatory behaviour of such cells (Freeman, 1985; Chaput *et al.*, 1992). A train of oscillations at frequencies higher than 20 Hz would generate many replicating patterns made of regular intervals smaller than 50 ms. Conversely, repeated patterns composed of equal or nearly equal intervals, if abundant enough, are likely to generate an oscillatory signal in both the autocorrelogram and the power spectrum, and this is indeed what we observed in several cells. The temporal structures that we have found in mitral cell discharges seem, however, to be more complex and transitory than mere oscillations. Short sequences of repeated intervals may be viewed as short trains of waves, and non-symmetric repeating triplets may also be considered in a sense as a type of generalization of short oscillations, playing on two or more registers at the same time. From this point of view, our finding that replicating triplets in mitral cell discharges are more probably due to extrinsic than intrinsic mechanisms may concur with the conclusion that, in visual cortical neurons, 'as far as frequencies between 10 and 80 Hz are concerned, the temporal pattern in the output of a cell is likely to be much more strongly determined by its synaptic drive than by its membrane properties' (Nowak *et al.*, 1997).

It has been recently shown that an excess of precisely repeating patterns over predictions based on a NHPP model may be produced on stochastic grounds, whenever the distribution of spike count over successively repeated stimuli departs from a Poisson process, in particular when the variance/mean of the spike count distribution is higher than 1 (Oram *et al.*, 1998). In this study, the spike count distribution also departs from Poisson, but in the sense that the variance/mean factor is usually very significantly smaller than 1 (Lestienne *et al.*, 1998). It should be noted that strictly oscillatory discharges, producing regularly spaced spikes in time, necessarily produce narrow spike count distributions. Thus, from this point of view also, precisely repeated patterns and oscillations seem to belong to the same class of phenomena in mitral cell discharges.

Oscillations have been shown to play a crucial role in the fine discrimination of odours in the locust and honey bee: while the population of stimulated antennal lobe cells is clearly correlated with the odour (Faber *et al.*, 1999), the precise time sequences of their excitation is correlated with the fine odour discrimination (Laurent & Davidowitz, 1994; MacLeod & Laurent, 1996; Laurent, 1997; MacLeod *et al.*, 1998). Quite remarkably, the detected periods of oscillations usually last only for a small fraction of the time of odour presentation, typically for ~200 ms. We found that the production of

replicating triplets also is very restricted to a limited period of time of mitral cell discharges. This is shown in Fig. 7, which displays for a set of cells the phase (in the respiratory cycle) of spikes discharges (full line) and the phase of those spikes that belong to a replicating triplet (black histogram). The proportion of spikes belonging to replicating triplets changes from record to record, up to nearly fully occupying the main peak response in record 15404. Nevertheless, triplets production is confined at times in the early peak of discharges in the cycle, and in other records, in the late part of the cycle.

The strikingly large differences in the rates of production of replicating patterns in mitral cell discharges and their NHPP simulations, despite the similarity of their time course, is considered as an indication that they might play a significant role in the processing of information in the brain. The necessity to think of neuronal processing of information in terms of temporal coding in these data is further emphasized by the generality of definite correlations between spikes with intervals of orders higher than 1, as disclosed by the use of autocorrelograms of limited range, even in the cases where discharges can not be classified as 'oscillatory' from the inspection of usual autocorrelograms.

Temporal coding of information in spike trains can refer to the coding of external qualities of the stimulations or to the internal management of the processing. The fact that no correlation was observed between the production of replicating triplets and the nature or strength of olfactory stimulations does not preclude the possibility that spikes belonging to precisely replicating patterns might play a special role in the processing of olfactory information, but points to a more subtle role than simple symbols for the coding of external stimulation. Investigating such a role in information processing would require multisite recordings in the olfactory bulb.

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Abbreviations

NHPP, non-homogeneous Poisson process; PSTH, post-stimulus time histogram; TIH, time interval histogram.

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