Theta oscillation coupled spike latencies yield computational vigour in a mammalian sensory system

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Theoretical work carried out almost a decade ago proposed that subthreshold oscillations in membrane potential could be used to convert synaptic current strength into a code reliant on action potential (AP) latencies. Using whole-cell recordings we present experimental evidence for the occurrence of prominent network-driven subthreshold theta oscillations in mitral cells of the mouse olfactory bulb. Activity induced by both injected current and sensory input was accurately reflected in initial AP latency from the beginning of each oscillation cycle. In a network model we found that an AP latency code rather than AP number or instantaneous firing rate provided computational speed and high resolution, and was easily implemented. This coding strategy was also found to be invariant to the total input current as long as the relative input intensities to glomeruli remained constant. However, it was highly sensitive to changes in the ratios of the input currents and improved by lateral inhibitory mechanisms. Since the AP latency-based coding scheme was dependent on the subthreshold oscillation we conclude that the theta rhythm serves a functional role in temporally reformatting the strengths and patterns of synaptic input in this sensory system.

Anatomical (Ramón y Cajal, 2001), molecular (Buck & Axel, 1991; Mombaerts et al. 1996) and functional imaging studies (Kauer, 1988; Joerges et al. 1997; Friedrich & Korsching, 1997; Rubin & Katz, 1999; Uchida et al. 2000; Meister & Bonhoeffer, 2001; Wachowiak & Cohen, 2001) provide convincing evidence that different odours activate spatially distributed combinations of glomeruli across the olfactory bulb (OB). However, due to the comparatively slow dynamics of odour stimuli it has also been suggested that time could be used as a computational variable for odour representation (Laurent, 1999; Friedrich & Laurent, 2001). In this way action potential (AP) timing or rate could code non-temporal stimulus features such as quality or intensity. Recent studies in olfactory bulb slices have shown that mitral cells receiving synaptic input within a common glomerulus laterally excite one another through chemical and/or electrical transmission (Isaacson, 1999; Carlson et al. 2000; Urban & Sakmann, 2002; Schoppa & Westbrook, 2002). In response to extracellular stimulation of the olfactory receptor neuron (ORN) input, this lateral excitation preferentially synchronizes the activity of intra-glomerularly coupled mitral cells at approximately 2 Hz (Schoppa & Westbrook, 2001; Urban & Sakmann, 2002). Thus the early olfactory network provides an intrinsic amplification mechanism that could preferentially boost activity in the theta range.

In mammals the respiration cycle provides a mechanism for repetitive and periodic sampling of odour stimuli and is the source of much of the theta band activity (Macrides & Chorover, 1972; Bressler, 1987; Gray & Skinner, 1988). During nasal inhalation, in response to either odorous room air or controlled odour stimuli, electro-olfactogram recordings from ORNs show changes in potential that are coupled to respiration (Ottoson, 1959; Chaput, 2000). This rhythmic ORN activity propagates to the main OB and is reflected in the patterning of mitral cell discharge (Adrian, 1950; Walsh, 1956; Gault & Coustan, 1965; Macrides & Chorover, 1972; Onoda & Mori, 1980; Chaput & Holley, 1985; Doving, 1987; Ravel & Pager, 1990; Sobel & Tank, 1993; Philpot et al. 1997; Chalansonnet & Chaput, 1998; Lestienne et al. 1999; Charpak et al. 2001; Luo & Katz, 2001; Belluscio et al. 2002). The respiration-coupled theta rhythm displays spatiotemporal dynamics in response to odour stimuli (Spors & Grinvald, 2002) and is present in downstream piriform cortex (Bressler, 1987; Wilson, 1998a,b) indicative of a functional role in the representation of odour stimuli. Here we have therefore examined the possibility that individual mitral cells could use the theta rhythm as a coding device to temporally reformat the strength of synaptic input.
METHODS

In vivo electrophysiology

Whole-cell recordings were carried out on mitral and granular cells of the olfactory bulb of freely breathing mice (C57BL6, P21–P35) anaesthetized with urethane (1.2 g kg$^{-1}$ i.p.) or with a ketamine (50 mg kg$^{-1}$)/xylazine (5 mg kg$^{-1}$) mixture as described previously in accordance with the ethical guidelines of the Max-Planck-Gesellschaft (Margrie et al. 2001, 2002). Patch pipettes (3–6 MΩ) were filled with (mM) potassium gluconate 130, sodium gluconate 10, Hepes 10, Mg$^{2+}$-ATP 4, GTP 0.3, NaCl 4, biocytin 0.3–1 %, pH 7.2. The respiration cycle was recorded with a plethysmograph constructed from a piezoelectric strap fitted around the thorax to measure the distension signal (Kent Scientific Corporation, Litchfield, CT, USA). Mitral cells were identified by their membrane potential, input resistance, firing profile and the appearance of a recurrent IPSP (Margrie et al. 2001). Granule cells were identified by their distance from the surface of the bulb (>300 μm), their hyperpolarized membrane potential (~71±2 mV, n = 41), input resistance (429±21 MΩ, n = 41) and in some cases their recovered morphology. A custom built olfactometer was used to present odor to the nares. For odour presentation positive pressure pulses (50 mbar; usually 5 s in duration) of filtered room air (the carrier stream) were propelled through a glass chamber containing the odour solution into the stimulus air line. A series of Teflon valves allowed for rapid redirection of the pulsed air to alternative odorant chambers. For current step injection-evoked AP experiments (Fig. 3B), current steps were of 100 ms duration (40–450 pA). Naris occlusion was achieved under visual guidance. With a micromanipulator a small piece of silicon gel was firmly placed against and/or marginally inside the cavity. All pharmacology experiments were carried out on cells that exhibited the oscillation regularly for at least 10 min. Data are means ± S.E.M. unless stated otherwise. ANOVA and unpaired t tests were used to determine significance values. Agents used were TTX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX), 2-amino-5-phosphonovalerate (D-APV) and n-amy lactate (1 %), ethyl butyrate (1 %), 2-methylbutyric acid (1 %) (Sigma); lavender and cherry (50 or 100 % of stock) was obtained from a local retailer. All odorants were diluted in 100 % mineral oil (Sigma).

Network model

Simulations were performed in Matlab 6.0 (The MathWorks, Natick, MA, USA). The network consisted of an interconnected mitral cell layer each receiving independent input within an individual glomerulus. Thus each mitral cell reflected the activity of all cells associated with a glomerulus (Schoppa & Westbrook, 2001; Urban & Sakmann, 2002). Mitral cells were modelled as leaky integrate-and-fire neurons with an AP threshold of 15 mV, input resistance of 100 MΩ and τ = 10 ms. Subthreshold oscillation was mimicked by a 4 Hz sinusoidal current injection with a peak amplitude of 125 pA. If threshold was reached at t = t₀, an AP was elicited (V$_{\text{mem}}$(t₀) = 100 mV), the membrane potential (V$_{\text{mem}}$) was then reset to 12 mV (80 % of threshold) and hyperpolarizing current was injected to mimic an after-hyperpolarization (AHP) (linearly decreasing from an initial amplitude of 200 pA within 50 ms). Incorporating heterogeneity in membrane potentials (changes by up to ± 5 mV) did not alter the results. AP-evoked lateral inhibition in all other mitral cells (White et al. 1992) was delayed by 25 ms and followed a bi-exponential time course $i(t) = 200 \text{pA} \cdot (1 - e^{-t/100\text{ms}}) e^{-t/100\text{ms}}$ as extracted from Urban & Sakmann (2002). Stimulation was mimicked by a 1 s step current injection starting at $t = 1000 \text{ms}$ locked to the beginning of the oscillations. With these parameters current injections of 30–200 pA evoked 1–5 APs per oscillation cycle. Using theta locked current injection in addition to the step current did not affect the described computational properties (not shown). To analyse to what extent oscillation-mediated AP latencies can indeed readily represent stimulus features, an explicit mechanism was designed to extract stimulus quality (the pattern of mitral cell activation) from action potential latencies. To obtain this, ‘analysis cells’ were introduced (Fig. 4C) that received input with varying delays from all mitral cells, such that a mitral cell AP resulted in a postsynaptic potential in the analysis cell. To obtain this, ‘analysis cells’ were introduced (Fig. 4C) that received input with varying delays from all mitral cells, such that a mitral cell AP resulted in a postsynaptic potential in the analysis cell. To assess the computational power of this oscillation/AP latency-based scheme it was compared to (i) a spatial scheme relying only on the identity of the activated glomeruli and (ii) a spatial scheme relying on the number of APs per cycle in the mitral cells. Comparison was made in a network with four glomerular units using a template matching procedure: A stimulus pattern S₀ was selected and presented to the mitral cells. The resulting (i) glomerular activation pattern, (ii) number of action potentials and (iii) depolarization in ‘analysis cell’ AC₀ were taken as templates for the respective coding strategies. A stimulus pattern S₁ was assumed to be identical to S₀ if (i) glomerular activation or (ii) action potential numbers matched the respective template or (iii) the resulting depolarization in AC₀ was larger than or equal to the template depolarization. This was done for all S₁ fulfilling the following conditions: (1) two of the four glomeruli receive input and (2) input is 5, 10, ... or 200 pA. This resulted in a number of erroneous matches that could be expressed as a fraction of the entire stimulus space (with six possible combinations of active glomeruli, stimulus space consisted of 40 × 40 × 6 stimuli). This was repeated for 14 different S₀ (150 or 200 pA; 50, 75, 100,... or 200 pA). To study robustness, Gaussian noise ($σ = 50 \text{ pA}$) was added point by point independently of the 1 kHz-sampled signal, resulting in an amplitude similar to the experimentally observed intrinsic noise. Control simulations in a compartmental model of a mitral cell (Shen et al. 1999) were performed in NEURON 4.1.2 (http://www.neuron.yale.edu) and yielded similar results.
Figure 1. Odour-evoked theta oscillations in mitral cells depend on synaptic transmission in the olfactory bulb

A, 10 voltage traces superimposed and aligned to the first corresponding respiration cycle (red) of each trace demonstrate the dominance of the subthreshold rhythm. Below, the averaged mitral cell membrane voltage shows that the depolarization follows the inhalation phase of the respiration cycle. B, an auto-correlation of the membrane voltage indicates the theta rhythmic nature of the mitral cell membrane voltage. A cross correlogram of the membrane voltage with the respiration signal shows the coherence of the two signals (bottom). C, membrane potential and AP discharge in a mitral cell in response to overt odour stimulation (1% amyl acetate). Taking the cell away from threshold with DC current injection reveals an underlying odour-induced theta rhythm. APs are clipped, odour presentation was 5 s. D, normalized FFTs of the mitral cell membrane potential for four cells from four different animals. Note the prominent theta activity (2–5 Hz) in all cells. E, summary data of the reduction in oscillation amplitude by superfusion of blockers of synaptic transmission (control 6.3 ± 0.9 mV, n = 11; TTX 2–20 μM, 0.2 ± 0.3 mV, n = 5; NBQX (50–200 μM) plus D-APV (200–400 μM), 0.9 ± 0.4 mV, n = 5) and naris occlusion, 0.6 ± 0.4 mV, n = 3; all P values were < 0.05).
RESULTS

Consistent with previous studies in the mammalian olfactory bulb (Adrian, 1942; Ottoson, 1959; Gault & Leaton, 1963; Macrides & Chorover, 1972; Onoda & Mori, 1980; Bressler & Freeman, 1980; Chaput & Holley, 1985; Gray & Skinner, 1988; Charpak et al. 2001; Luo & Katz, 2001) whole-cell recordings from mitral cells show a dominating rhythm synchronized to the respiration cycle \((n = 23, \text{Fig. 1A and B})\). This locking of membrane potential to the respiration cycle was observed for periods ranging from tens of seconds up to for as long as the recording persisted \((>1 \text{ h})\) and could be evoked by room air or by overt odour stimuli \((n = 23; \text{Fig. 1A–C})\) consistent with recent voltage-sensitive dye measurements in rodents (Spors & Grinvald, 2002). In single cells, the subthreshold oscillation had an amplitude (peak to trough) of \(6.9 \pm 1.3 \text{ mV}\), an average frequency of \(3.7 \pm 0.2 \text{ Hz}\) \((n = 23; \text{Fig. 1D})\) and was abolished following superfusion of the sodium channel blocker TTX or NBQX and D-APV over the surface of the olfactory bulb \((\text{control } 6.3 \pm 0.9 \text{ mV}, \ n = 11; \ \text{TTX } 2–20 \text{ } \mu\text{M}, \ 0.2 \pm 0.3 \text{ mV} \ n = 5; \ \text{NBQX} \ (50–200 \text{ } \mu\text{M}) \ \text{plus D-APV} \ (200–400 \text{ } \mu\text{M}), \ 0.9 \pm 0.4 \text{ mV} \ (n = 5); \ \text{all } P \text{ values were } <0.05, \text{Fig. 1E})\). Closure of the ipsilateral naris also diminished the subthreshold theta rhythm in mitral cells \((\text{control } 6.3 \pm 0.9 \text{ mV}, \ n = 11; \ 0.6 \pm 0.4 \text{ mV}, \ n = 3; \ P < 0.05; \text{Fig. 1E})\). Fast Fourier transform (FFT) analysis of membrane potential in mitral cells revealed that the respiration-locked activity occurs in the theta bandwidth \((\text{Fig. 1D})\). The LFP measured in the upper third of the external plexiform layer exhibited identical pharmacological properties, power spectrum and correlation structure \((n = 7; \not\text{shown})\). This theta rhythm is therefore dependent on nasal airflow, requires AP generation/propagation and glutamatergic transmission within the olfactory bulb. Furthermore the general kinetics of sensory input-evoked synaptic current in mitral cells is defined by the inspiration–exhalation cycle \((\text{Fig. 1C})\).

The influence of subthreshold membrane potential oscillations on AP timing was then investigated in detail by direct current input via the patch pipette \((\text{Fig. 2A})\). Progressively more positive constant current resulted in a decreased latency to the first AP and in an increase in AP number \((\text{Fig. 2B})\). Since current intensity and AP number covaried, AP number can be used as an indicator of input strength. Figure 2C shows that odour-evoked and direct current injection-evoked APs exhibit the same relationship.

![Figure 2](image-url)
between initial AP latency and AP number per oscillation cycle. In the case of synaptically evoked APs, the majority of APs occurred towards the end of the inhalation phase of the respiration (Fig. 3A1). In 95% of the odour-evoked suprathreshold oscillation cycles five or less APs were evoked (Fig. 3A2).

When coding of information by individual neurons is considered, there exist a number of features of individual spike trains that may convey information about a stimulus. Within each oscillation cycle these may include inter-spike interval (ISI), AP timing or latencies, AP number and higher-order features such as changes in sustained firing rate (Laurent, 1999; Laurent et al. 2001).

Figure 3B shows that the average ISI for mitral cells receiving this oscillatory drive remains constant throughout the range from two to five APs both for odour- and current injection-evoked suprathreshold activity ($F_{(3,221)} = 1.8, P > 0.1$; $39 \pm 3.9 (2 \text{ APs})$ vs. $42.7 \pm 5.8 (5 \text{ APs})$; $n = 4$ for odour-evoked APs; $46.7 \pm 4.9 (2 \text{ APs})$ vs. $51.1 \pm 5.1 (5 \text{ APs})$ for current injection-evoked APs; $n = 5$). As this held true for non-oscillating cells ($56.4 \pm 4.2 (2 \text{ APs})$ vs. $61.6 \pm 2.7 (5 \text{ APs})$; $n = 7$), the observed constant firing rate is likely to be due to intrinsic (Desmaisons et al. 1999) or possible feed-forward inhibitory mechanisms, rather than the consequence of the oscillatory drive. Since the instantaneous firing rate remains constant for less than six APs per cycle (which accounts for over 95% of all observed cycles) firing rate cannot represent the strength of network-driven input during the vast majority of respiration cycles (Fig. 3A2 and B). Thus, we investigated whether the distribution of spikes across each cycle efficiently conveys information about input strength.

If AP latencies were to play any role in sensory representation in the olfactory system, then computation

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**Figure 3. Synaptically evoked AP firing rates and latencies in individual mitral cells**

A1, a frequency histogram (fitted with a Gaussian) of the normalized latencies of odour-evoked APs. A2, a cumulative histogram showing the percentage of odour-evoked oscillation cycles evoking 1 to more than 8 APs per cycle (data are from 398 cycles, $n = 4$ cells; fitted with a single exponential). B, the mean ISI of each oscillation-evoked burst was calculated and converted to a mean firing rate (closed circles; $n = 4$). Triangles indicate the firing rate for oscillating cells that were driven across threshold by DC current injection ($n = 5$ cells). The firing rate for current step-evoked APs (from non-oscillating cells) is depicted by open circles ($n = 7$; current step was 0.1–0.3 nA, 100 ms duration). Lines of best fit for data from 2 to 5 APs, are plotted. C1, (top) superimposed voltage traces of synaptically evoked suprathreshold responses in a cell (top traces) showing the latency to the initial spike for theta cycles containing 2 (green trace) and 5 APs (black trace; APs are clipped). Membrane voltage recordings are aligned to the simultaneously recorded respiration cycle (bottom traces, superimposed). C2, a plot of the synaptically evoked latencies to the initial AP as a function of the number of APs elicited within each oscillation cycle (mean ± S.D.; $n = 186$ cycles; $P$ values **< 0.001 and * < 0.05). D, a plot of synaptically evoked spike times for all evoked APs plotted against the number of APs elicited per cycle.
Figure 4. A network model with firing properties based on experimental data

A, a network of interconnected integrate-and-fire models of mitral cells and postsynaptic analysis cells. A stimulus pattern consists of 1 s current injection of varying intensity into the mitral cells in addition to an ongoing theta subthreshold oscillation. Resulting APs (clipped) evoke lateral inhibition in other mitral cells (not shown). B, comparison of the model and the experimental data. B1, in the model, 150 pA direct current was injected to yield on average 3 APs per cycle. Note the AHPs in comparison to the experimental data. B2, initial (filled circles) and mean (open circles) AP latencies were determined, normalized to the cycle length and plotted as a function of the AP number. Results from the model are displayed in black and compared to the experimental data (grey, see Fig. 2C). B3, firing rates were calculated in the same manner as in the experiment (grey) and displayed as a function of the AP number per cycle. C, a layer of analysis cells (AC) each receiving excitatory (red) or inhibitory (green) synaptic input from all mitral cells. D, a network of 10 mitral cells and 20 analysis cells was presented 20 stimulus patterns for 1 s each as in A. Connectivity was set to ensure that analysis cell 1 responded to stimulus pattern 1 with depolarization. The depolarization of analysis cell 1 is depicted both as a voltage trace and in a false colour plot. Membrane potential for cells 2–20 is depicted in false colour only and normalized to the maximal response in the individual analysis cell. For displaying purposes voltage traces were binned in 100 ms bins.
in downstream neuronal elements would rely on the differential latencies of incoming APs. To assess this possibility experimentally it is therefore necessary to use an external reference that is insensitive to sensory input and olfactory bulb activity. We found that when the mitral cell membrane potential was aligned to the beginning of the respiration cycle (Fig. 3C1) the latency to the initial spike decreased as the total number of spikes per cycle increased (1 AP (104.2 ± 3 ms) vs. 5 APs (46.9 ± 4 ms), \( P < 0.0001 \); Fig. 3C2). This covariance between AP number and latency to the initial spike was highly significant (latency to initial AP for the range of 1–8APs; \( F_{(7,179)} = 30.3, P < 0.0001 \) which was not the case for the average AP latency per cycle (103 ± 2 ms (2 APs) vs. 108 ± 5 ms, (6 APs); \( F_{(7,940)} = 1.7, P > 0.1 \). Due to the activity-dependent distribution of spikes (Fig. 3D) the initial AP latency may provide a reliable and rapid means of representing input strength.

To assess the potential computational role of AP latency and the subthreshold oscillatory drive (Hopfield, 1995), we constructed a network consisting of leaky integrate-and-fire models of mitral cells receiving independent input that were connected via lateral inhibition (Fig. 4A). In this model, stimulation was mimicked by a 1 s step on top of a sinusoidal subthreshold current injection (Fig. 4A). Stimulus space consists of all combinations of current injection amplitudes into mitral cells that result in up to six APs. Since experimental work suggests that only a subset of glomeruli are activated by an individual stimulus (Kauer, 1988; Joerges et al. 1997; Friedrich & Korsching, 1997; Rubin & Katz, 1999; Uchida et al. 2000; Meister & Bonhoeffer, 2001; Wachowiak & Cohen, 2001), the fraction of simultaneously activated glomeruli in the model was chosen to be 50% (Hopfield, 1999; see discussion).

The intrinsic cellular properties of model mitral cells including AHPs and firing rates were comparable to the experimental data (Fig. 4B1 and B3). Importantly, the initial AP latency decreased logarithmically as a function of AP number while the mean latency remained constant as in the experimental data (Fig. 4B2). We then investigated the possibility that AP latencies could be used by downstream circuits to extract stimulus-related information about the net strength of synaptic input to mitral cells. Therefore, we constructed a second layer of ‘analysis cells’ that receive convergent input from mitral cells with varying delays (Fig. 4C) and should each respond optimally to a unique stimulus \( S \) (see Methods).

Successive presentation of 20 stimulus patterns to a network of 10 mitral cells resulted in analysis cell \( n \) mainly

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Figure 5. AP latency coding provides computational advantage and enables scale invariant pattern detection dependent on subthreshold oscillations

A, percentage of erroneous matches for three coding strategies analysed in a model network with 4 mitral cells (black). Percentages were determined for 14 different original stimulus configurations (see Methods). The influence of simulated noise (50 pA Gaussian noise as described in the Methods) is shown in grey. B, distribution of erroneous matches in stimulus space. Input into combinations of glomeruli other than glomeruli 1 and 2 (the correct combination for the correct stimulus) cannot result in an erroneous match (left). Erroneous matches for the correct combinations of activated glomeruli are shown in detail on the right. The correctly identified stimulus is indicated as a red circle and was (125 pA (glom 1), 150 pA (glom 2), 0 (glom 3), 0 (glom 4)) current injection into the 4 glomeruli. C, reducing the amplitude of the subthreshold oscillation increased the number of erroneous matches.
Figure 6. For legend see facing page
depolarizing following presentation of stimulus pattern \( n \) (diagonal in Fig. 4D). This indicates that 20 stimulus patterns can be distinguished unequivocally. To further assess, quantify and compare the computational vigour of the proposed AP latency coding strategy, we used a smaller network of only four mitral cells so the entire stimulus space could be examined. We chose to compare three coding strategies, namely (1) spatial pattern of activated glomeruli (2) pattern of AP numbers and (3) pattern of AP latency. Template matching showed that the pure spatial strategy wrongly matched 9.08±0.67% of stimulus space whereas the second and third coding strategies matched 1.28±0.14 and 0.73±0.18% respectively (Fig. 5A). Addition of 50 pA noise resulted in 9.47±0.77, 1.33±0.14 and 0.95±0.2% erroneous matches, thus retaining computational supremacy of the AP latency coding scheme (Fig. 5A).

Erroneous matches represent a (usually undesired) generalization from the stimulus template to a larger region of stimulus space. To further assess the nature of the generalization we plotted the entire stimulus space for one analysis cell. From Fig. 5B it is apparent that only sensory input into glomeruli 1 and 2 resulted in erroneous matches in any coding strategy. We next examined in greater detail the subset of stimulus space (1/6 of the total) corresponding to the activation of the two correct glomeruli (Fig. 5B). We find that strategies 1 and 2 simply generalize approximately radially from the template. The AP latency strategy, however, detects not only the template stimulus but also linearly scaled versions of the template. Thus the ratio rather than the total synaptic inputs onto mitral cells is reliably encoded by the subthreshold oscillation-driven AP latencies (Hopfield, 1995). This is consistent with an approximately logarithmic relation between initial AP latency and input strength as seen both in simulation and in the experimental data (Figs 2C and 4B). Decreasing the amplitude of the subthreshold oscillation resulted in an approximately 10-fold increase in the number of erroneous matches (Fig. 5C), indicating that indeed the theta oscillation is necessary to retain high fidelity stimulus pattern encoding.

It has been proposed that lateral inhibition provides the olfactory bulb with a mechanism for amplifying differences in mitral cell activity patterns (Shepherd & Greer, 1998). Consistent with the notion that the theta rhythm-locked AP firing in mitral cells evokes lateral inhibition across the bulb we observed EPSPs in granular cells (amplitude = 12.5 ± 2.2 mV, \( n = 18 \), Fig. 6A1 and A2). In the limited number of cases when granular cells reached threshold, AP firing was also locked to the respiration cycle. Consistent with very low overall firing rates (Margrie et al. 2002) in both mitral and in particular granule cells (< 0.01 Hz, \( n = 18 \)), we observed respiration-coupled inhibitory postsynaptic potentials in 3/23 mitral cells (Fig. 6B). In line with previous in vitro studies on recurrent inhibition in mitral cells (Isaacson & Strowbridge, 1998) the respiration-locked inhibition was mediated by fast putatively unitary events (Fig. 6B2). The majority of these events occurred between 20 and 40% of the respiration cycle length (0.32 ± 0.09, mean ± S.D., \( n = 162 \); Fig. 6B2) and thus on the early phase of the distribution of mitral cell firing.

Removing lateral inhibition in the model (amplitude = 0) resulted overall in a 2-fold increase in the number of erroneous matches (Fig. 6C). This was not significantly altered if a tonic hyperpolarizing current was added to account for the overall loss of inhibition. As noted above, the AP latency scheme predominantly generalizes from a stimulus to linearly scaled versions of the stimulus, detecting the ratio between the input currents rather than the total amount of current input to the glomeruli (Fig. 5B). In Fig. 6D1 the set of linearly scaled versions of the stimulus is indicated by the black line through the origin and the correct stimulus. To assess how lateral inhibition contributes to shaping this profile, we determined the distance of an erroneous match to this ‘constant-ratio’ set of stimuli (Fig. 6D1, dotted line). From Fig. 6D2 it

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**Figure 6.** Theta oscillation synchronous lateral inhibition increases AP latency-dependent coding capacity

A1, the morphology of the granule cell recorded from in A2. GLOM, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; GCL, granular cell layer. A2, an example of a granule cell receiving large EPSPs (presumably from mitral cell dendritic input) and exhibiting AP discharge locked to the respiration cycle (\( n = 18 \)). B1, an example of a mitral cell exhibiting respiration-coupled lateral inhibition during overt odour stimulation (\( n = 3 \)). B2, the latency distribution of respiration-locked IPSPs in mitral cells. Black line indicates a Gaussian fit (mean 0.29, half-width 0.12 of the respiration cycle), for comparison the fit for the distribution of action potential latencies is shown in grey (mean 0.40, half-width 0.21). The mean decay time constant of the IPSPs (fitted with a single exponential) was 6.3 ± 0.2 ms, \( n = 162 \) (\( n = 3 \) cells). C, histogram illustrating the effect of the amount of lateral inhibition on the number of erroneous matches in the model. D1, the distribution of erroneous matches is plotted as in Fig. 5B with (blue) and without (green) lateral inhibition incorporated into the model. The red circle indicates the correct stimulus. D2, a plot of the number of matches as a function of the distance from the stimulus axis (measured as indicated by the dotted line in D1).
becomes apparent that lateral inhibition does not only result in an overall reduction of erroneous matches but predominantly restricts the erroneous matches to a narrow region in stimulus space. This demonstrates that for the AP latency coding scheme, lateral inhibition is not crucial but does indeed enhance pattern separation. In particular, it contributes to restricting the ‘erroneous’ matches to a well-defined region in stimulus space.

**DISCUSSION**

Studies on various sensory modalities report both beta and gamma frequency activity in response to sensory stimuli (Singer & Gray, 1995; Laurent et al. 2001). Here we report experimental and theoretical evidence that a respiration-coupled subthreshold theta oscillation in mitral cells can provide a temporal platform for reformatting net synaptic input intensities into AP latencies. This subthreshold oscillation in mitral cells was dependent on activation of AMPA and NMDA receptors indicating that excitatory transmission in the olfactory bulb is essential for the generation of the respiration coupling. Since excitatory synaptic input to mitral cells is confined to the glomerulus it is likely that ORN input drives the oscillatory activity (Ottoson, 1959; Chaput, 2000). Intra-glomerular excitation between the tufts of mitral cells may then serve as a mechanism for amplification and synchronization (Schoppa & Westbrook, 2001; Urban & Sakmann, 2002).

In this study we used the respiration cycle as a reference point from which to measure AP latency. Since the respiration cycle length can be reliably recorded and varies only marginally across the recording period it is a useful external reference to determine the relative AP latency across each theta cycle and different cells in different preparations. We found that the latency to the first AP co-varied with AP number but not ISI to reflect input intensity. The logarithmic relationship between AP latency and input intensity (Figs 2C and 4B) might allow efficient encoding of relative input intensities (Hopfield, 1995) as described by our network model.

To reduce the number of free parameters in the model neither short-term dynamics nor variability in synaptic kinetics or efficacy were utilized. Such features can in addition increase computational power of a latency-based scheme. For computational reasons we chose a network with only four glomerular units, two of which were activated by a given stimulus. However, increasing the total number of glomerular units did not significantly change our results while any increase in the number of activated glomeruli actually substantially enhanced the computational supremacy of the latency-based coding scheme (not shown). Analysis cells were introduced in our model predominantly as a direct test of this coding strategy. However, the simplicity of the detection mechanism used in our model (delays and postsynaptic summation) and that simple learning rules can be applied to build the described connectivity make it a biophysically realistic implementation. In addition, the parameters used in the model are consistent with the functional architecture of piriform cortex (Wilson & Shepherd, 1998; Zou et al. 2001). In particular the pyramidal cells of the piriform cortex receive input (both monosynaptic extrinsic and polysynaptic intrinsic) with varying delays up to several hundred milliseconds following afferent activation (Ketchum & Haberly, 1993a). In a previous study (White et al. 1998) a similar delay scheme was used to facilitate odour detection in an artificial nose system demonstrating a direct application of this computational parameter. In our model the analysis cells (ACs) are tuned to particular glomerular activity patterns. As in experimental data from second order olfactory neurons in mammals and insects, our ACs are sparsely activated in a highly stimulus-specific manner (Wilson, 2001; Perez-Orive et al. 2002).

Compared to unit recordings under the same anaesthesia conditions whole-cell recordings from mitral cells and granule cells reveal very low rates of spontaneous activity (Margrie et al. 2002). Consistent with this we observed respiration-coupled IPSPs in a limited set of mitral cells. The distribution of these IPSPs was found to coincide with the early phase of the distribution of APs in mitral cells. This is in agreement with recent in vitro experiments revealing that early action potentials in mitral cells are most efficient at evoking lateral IPSPs (Urban & Sakmann, 2002). This lateral inhibition will contribute to AP timing making it very likely that increasing odour concentration would result in a complex interaction between excitatory ORN input and lateral inhibition. For example, in cells where an increase in lateral inhibition dominates, the initial AP latency will increase rather than decrease with increasing concentration (Kauer, 1974; Yokoi et al. 1995; Margrie et al. 2001). In support of a role of lateral inhibition in tuning mitral cell responses (Yokoi et al. 1995) our data show that lateral inhibition can indeed refine an AP latency-based coding scheme in the early olfactory system. Although this study has not determined directly what features of an odour stimulus might be represented by the initial AP latency we can predict that via lateral inhibition, increasing odour concentration will have a heterogeneous effect on the initial AP latency in individual mitral cells. Further studies ideally presenting a large number of odours, varying odour quality and recording from multiple mitral cells or downstream elements are likely to yield more insight into the aspects of olfactory stimuli that are encoded in AP latencies.

There exist a number of advantages for using the initial AP latencies to represent the strength of synaptic input. By developing a scheme to detect stimulus patterns using AP latencies we have shown that such a strategy is not simply faster but also more efficient than, for example, codes...
Theta rhythm-dependent AP latencies


