Inhibition of Backpropagating Action Potentials in Mitral Cell Secondary Dendrites

GRAEME LOWE
Monell Chemical Senses Center, Philadelphia, Pennsylvania 19104-3308

Received 14 November 2001; accepted in final form 4 March 2002

Lowe, Graeme. Inhibition of backpropagating action potentials in mitral cell secondary dendrites. J Neurophysiol 88: 64–85, 2002; 10.1152/jn.00941.2001. The mammalian olfactory bulb is a geometrically organized signal-processing array that utilizes lateral inhibitory circuits to transform spatially patterned inputs. A major part of the lateral circuitry consists of extensively radiating secondary dendrites of mitral cells. These dendrites are bidirectional cables: they convey granule cell inhibitory input to the mitral soma, and they conduct backpropagating action potentials that trigger glutamate release at dendrodendritic synapses. This study examined how mitral cell firing is affected by inhibitory inputs at different distances along the secondary dendrite and what happens to backpropagating action potentials when they encounter inhibition. These are key questions for understanding the range and spatial dependence of lateral signaling between mitral cells. Backpropagating action potentials were monitored in vitro by simultaneous somatic and dendritic whole cell recording from individual mitral cells in rat olfactory bulb slices, and inhibition was applied focally to dendrites by laser flash photolysis of caged GABA (2.5-μm spot). Photolysis was calibrated to activate conductances similar in magnitude to GABA A-mediated inhibition from granule cell spines. Under somatic voltage-clamp with CsCl dialysis, uncaging GABA onto the soma, axon initial segment, primary and secondary dendrites evoked bicuculline-sensitive currents (up to ~1.4 nA at ~60 mV; reversal at ~0 mV). The currents exhibited a patchy distribution along the axon and dendrites. In current-clamp recordings, repetitive firing driven by somatic injection was blocked by uncaging GABA on the secondary dendrite ~140 μm from the soma, and the blocking distance decreased with increasing current. In the secondary dendrites, backpropagated action potentials were measured 93–152 μm from the soma, where they were attenuated by a factor of 0.75 ± 0.07 (mean ± SD) and slightly broadened (1.19 ± 0.10), independent of activity (35–107 Hz). Uncaging GABA on the distal dendrite had little effect on somatic spikes but attenuated backpropagating action potentials by a factor of 0.68 ± 0.15 (0.45–0.60 μM flash with 1-mM caged GABA); attenuation was localized to a zone of width 16.3 ± 4.2 μm around the point of GABA release. These results reveal the contrasting actions of inhibition at different locations along the dendrite: proximal inhibition blocks firing by shunting somatic current, whereas distal inhibition can impose spatial patterns of dendrodendritic transmission by locally attenuating backpropagating action potentials. The secondary dendrites are designed with a high safety factor for backpropagation, to facilitate reliable transmission of the outgoing spike-coded data stream, in parallel with the integration of inhibitory inputs.

INTRODUCTION

In the mammalian olfactory system, odors are encoded by the differential activation of a large multigene family of olfactory receptors expressed in different subsets of olfactory receptor cells (Buck and Axel 1991; Malnic et al. 1999). Cells expressing the same receptor project to a small subset of glomeruli within a large glomerular array on the surface of the olfactory bulb, creating a stimulus-specific two-dimensional spatial representation of olfactory receptor activation (Moebaerts et al. 1996; Wang et al. 1998). This pattern of glomerular activity is relayed to layers of projection neurons, the mitral and tufted cells (Price and Powell 1970a). A mitral cell receives glomerular synaptic input via the distal tuft of a primary (=apical) dendrite extending vertically from its soma. The mitral soma also radiates secondary (=basal or lateral) dendrites which extend horizontally ~1,000 μm across the external plexiform layer of the bulb (Mori et al. 1983; Orona et al. 1984). These dendrites are linked laterally by an extensive network of reciprocal dendrodendritic synaptic connections with granule cells (Jackowski et al. 1978; Rall et al. 1966). The lateral connections mediate excitatory-inhibitory interactions, and the current view is that they can shape both the spatial and temporal patterns of mitral cell activity that are thought to encode the intensity and quality of odors (Laurent 1999).

Mitral cell activity is controlled by a complex interplay between intrinsic conductances and synaptic inputs. Excitatory postsynaptic potentials (EPSPs) originating in the distal tuft initiate action potentials either in the soma or in the primary dendrite, depending on the level of somatic inhibition (Chen et al. 1997). The mitral cell membrane exhibits subthreshold bistability with a depolarized plateau potential (Heyward et al. 2001), and action-potential timing can lock to subthreshold membrane potential oscillations, which can be reset by inhibitory postsynaptic potentials (IPSPs) (Chen and Shepherd 1997; Desmaisons et al. 1999). Action potentials activate voltage-sensitive Ca2+ channels (Cinelli and Salzberg 1990, 1992; Mori et al. 1981; Wang et al. 1996), triggering glutamate release from the mitral cell at reciprocal synapses (Isaacson and Strwobridge 1998). The glutamate activates granule cell spines, which release GABA to inhibit the mitral cell (Isaacson and Strwobridge 1998; Jahr and Nicoll 1980; Nowycky et al. 1981; Rall et al. 1966). In addition to this negative feedback inhibition, mitral cells receive lateral inhibition from granule cells activated independently by other mitral cells (Isaacson and Strwobridge 1998; Margrie et al. 2001; Rall et al. 1966). This inhibitory circuitry is augmented by other types of GABAergic interneurons distinguished by parvalbumin immuno-
noreactivity, which make dendrodendritic synapses with the mitral cell soma and primary dendritic shaft (Crespo et al. 2001; Toida et al. 1994, 1996). Glutamate released from mitral cells also activates glutamate autoreceptors on mitral cells, which can modulate burst firing (Friedman and Strowbridge 2000; Salin et al. 2001), and there is evidence for a positive feedback excitatory pathway between mitral cells and interneurons (Didier et al. 2001).

The effect of lateral inhibition on a mitral cell depends on the strength and location of the inhibitory input, and its impact on local signaling processes. Anatomical studies have demonstrated symmetric, presumably inhibitory synapses on the mitral cell membrane (Crespo et al. 2001; Price and Powell 1970a,b; Rall et al. 1966; Sasso-Poggetto and Ottersen 2000; Toida et al. 1994, 1996). On the secondary dendrite, the ability of such synapses to block firing depends on their distance from the soma, the range of current shunting along the dendrite, and the local density of functional postsynaptic receptors. The range of lateral inhibition is of special interest because of its presumed role in shaping spatial activity patterns. The extensiveness of the secondary dendrites suggests that more distal dendritic elements may be electrotonically decoupled from the soma. Distal GABAergic inhibition could then regulate dendritic electrical signaling locally, independent of the soma. Mitral cell dendrites are presynaptic structures, and presynaptic GABA receptors are well known to modulate neurotransmitter release (Dudel and Kuffler 1961; Eccles et al. 1963; MacDermott et al. 1999; Nicoll and Alger 1979). Inhibition can alter the amplitudes and waveforms of action potentials invading the soma, the range of current shunting along the dendrite, and the local density of functional postsynaptic receptors. The range of lateral inhibition is of special interest because of its presumed role in shaping spatial activity patterns. The extensiveness of the secondary dendrites suggests that more distal dendritic elements may be electrotonically decoupled from the soma.

Lateral inhibition could then regulate dendritic electrical signaling locally, independent of the soma. Mitral cell dendrites are presynaptic structures, and presynaptic GABA receptors are well known to modulate neurotransmitter release (Dudel and Kuffler 1961; Eccles et al. 1963; MacDermott et al. 1999; Nicoll and Alger 1979). Inhibition can alter the amplitudes and waveforms of action potentials invading presynaptic terminals (Baxter and Bittner 1991; Segev 1990; Zhang and Jackson 1995), which can strongly impact calcium influx and transmitter release (Sabatini and Regehr 1997). Invasion of mitral cell dendrites by backpropagating action potentials provides the depolarization required to initiate calcium influx for triggering dendritic glutamate release (Bischofberger and Jonas 1997; Isaacsen and Strowbridge 1998; Margrie et al. 2001). Focal modulation of action potentials and calcium influx in the secondary dendrites by lateral inhibition has the potential to modify spatial patterns of dendrodendritic transmission across the bulb.

This paper describes the effect of localized inhibition on the initiation and backpropagation of action potentials in the secondary dendrites of rat mitral cells. Using laser flash photolysis of caged GABA, inhibition was applied locally to the soma and dendrites of mitral cells in olfactory bulb slices during somatic and dendritic whole cell recording. The advantage of this approach is that inhibition can be applied reproducibly with high spatial and temporal resolution (Katz and Dalva 1994; Wang and Augustine 1995). First, the conductance activated by uncaging GABA on the soma and dendrites of mitral cells was characterized. The magnitude of photoinhibition was then adjusted to approximately match physiological levels of inhibition inferred from inhibitory postsynaptic currents (IPSCs) from presynaptic spines of interneurons. This laid the groundwork for applying the method to probe the range of lateral inhibition and study the effect of inhibition on backpropagating action potentials.

**METHODS**

**Slice preparation**

Horizontal olfactory bulb slices (350-μm thick) were prepared from 21- to 28-day-old male CD rats (Charles River). Animals were killed by overdose of halothane anesthesia (saturated vapor), and the olfactory bulbs were removed immediately into ice-cold sucrose artificial cerebrospinal fluid (ACSF) containing (in mM) 240 sucrose, 2.5 KCl, 10 Na-HEPES, 10 n-glucose, 1 CaCl2, 4 MgCl2, and 0.2 ascorbic acid, pH 7.2 with HCl. 317 mOsm, bubbled continuously with oxygen. Slices were cut in ice-cold sucrose ACSF with a vibrating razor blade (60 Hz) and allowed to recover for 1–3 h in an enclosed interface chamber containing high-Mg2+ ACSF (which was composed of, in mM: 124 NaCl, 2.5 KCl, 26 NaHCO3, 1.25 NaH2PO4, 10 D-glucose, 1 CaCl2, and 3 MgCl2, 298 mOsm) bubbled continuously with 95% O2-5% CO2. A 20-gauge needle outlet allowed the gas to escape from the chamber under slight positive pressure. The recovery chamber was prewarmed to 30°C and left to cool slowly to room temperature (22°C). Slices were subsequently transferred to a second enclosed interface chamber containing standard ACSF (in mM): 124 NaCl, 2.5 KCl, 26 NaHCO3, 1.25 NaH2PO4, 25 D-glucose, 2 CaCl2, and 1.3 MgCl2, 312 mOsm, bubbled with 95% O2-5% CO2, at 22°C. Slices remained in storage in the second chamber for ≤4 h before recording.

**Electrophysiological recording**

Slices were transferred to a small custom-designed Plexiglas chamber for submerged perfusion at 2 ml/min with standard ACSF at 25°C, bubbled with 95% O2-5% CO2. Temperature was regulated by a custom-built stage heater and an indium tin oxide heated coverslip (Cell Micro Controls) forming the bottom of the recording well. Mitral cell soma and dendrites were visualized with a Nikon E600 FN upright microscope equipped with a Leica HCX APO L 63X/0.90 water-immersion objective, visible and infrared differential interference contrast (IR-DIC) optics, and an infrared video camera (C2400-79H, Hamamatsu Photonics K. K.). For whole cell recordings, voltage-clamp measurements were made with a CsCl pipette solution containing (in mM) 126.3 CsCl, 4.9 KC1, 25.2 K-HEPES, 0.2 K-EGTA, 1.9 Mg-ATP, 0.3 Na-GTP, 1 MgCl2, 3.9 Na2-phosphocreatine, and 6.3 biocytin, pH 7.2, 312 mOsm, \( E_{Cl} = 1.2 \) mV. Current-clamp measurements were made with a K-methylsulfate pipette solution containing (in mM): 123 K-CH3SO3, 4.7 KC1, 24.6 K-HEPES, 0.2 K-EGTA, 1.9 Mg-ATP, 0.3 Na-GTP, 0.9 MgCl2, 3.8 Na2-phosphocreatine, and 6.1 biocytin, pH 7.2, 312 mOsm, \( E_{Cl} = -59.8 \) mV. Pipette resistance was 3–8 MΩ for somatic recordings and 8–15 MΩ for dendritic recordings. In some recordings, pharmacological agents were added to the bath: 1 μM TTX, 50 μM bicuculline methiodide (BMI), 60 μM 2-amino-5-phosphonopentanoic acid (AP-5), or 10 μM 6-cyano-7-nitroquinoline-2,3-dione (CNQX; all from Sigma RBI).

Whole cell voltage-clamp recordings were made with an EPC-8 patch-clamp amplifier (HEKA Electronics), and current-clamp recordings were made with two BVC-700 microelectrode amplifiers (Dagan) in bridge mode with electrode capacitance and series resistance compensation or an EPC-8 patch-clamp amplifier in fast current-clamp mode. Amplifiers were tested pairwise with dual recordings on the mitral cell soma to verify that there was no significant action potential distortion between the instruments. Spike waveforms recorded by two BVC-700 amplifiers were closely matched. With 100-pA current pulse injection, the ratio of spike amplitudes measured between the two was 0.996 ± 0.005 (n = 9 spikes) and the ratio of spike widths was 0.97 ± 0.01 (n = 9 spikes). Amplifiers were controlled through analog output boards driven by patch-clamp software written in LabVIEW (National Instruments). Data were acquired at 50-kHz, 16-bit resolution by two simultaneous-sampling dynamic signal analysis A/D boards (National Instruments), also controlled by software written in LabVIEW.

**Laser flash photolysis**

Once stable whole cell recordings were achieved, the perfusion system was switched to recording mode (4.3 ml total volume of...
bubbled standard ACSF) to allow introduction of caged compounds and pharmacological agents. In this mode, a peristaltic pump removed ACSF from the chamber downstream of the slice and returned it upstream into an enclosed, elevated inlet channel with bubbling port; from there the solution drained by gravity back into the slice recording well. Caged neurotransmitter (O-CNB-caged GABA, at 270 μM or 400 μM; or γ-CNB-caged glutamate, at 320 μM; Molecular Probes) was added to the bath by injection into a loop manifold. Stock solutions of the caged compounds (6 or 9 mM) were made in standard ACSF at pH 4.00 to inhibit spontaneous hydrolysis, stored in the dark at −25°C, and thawed immediately before introduction into the recording chamber. Pharmacological agents were similarly injected. For focal flash photolysis of caged neurotransmitter, the beam from an Innova 90C argon ion laser (Coherent, Santa Clara, CA) was steered by the side-scan provided a more accurate measurement of the re-
the series. A least-squares orthogonal to the structure and that intersected it near the midpoint of
estimated by side-scan photostimulation of axons, taking advantage of
fl
n
of current amplitude versus position was 2.43
image-acquisition functions were integrated
into the LabVIEW patch-clamp/motion-control program. Afterward, frames were cropped and tiled to reconstruct a z-axis projection of the live cell, and an outline of the cell was traced and superimposed onto the photostimulation coordinates recorded by the XYZ positioning system. After each experiment, slices were fixed overnight at 4°C in phosphate-buffered saline with 2% glutaraldehyde, and the biocytin-filled cells were processed with the Vectastain Elite ABC kit and stained with a VIP peroxidase substrate kit (Vector Laboratories). Slices were cleared as whole-mounts in 80% glycerol, and fixed cell morphology was obtained by two methods: a Nikon Microphot microscope equipped with a CCD camera recorded images of sections of dendrites in different focal planes, which were cropped and tiled to yield a z-axis projection of the overall morphology; the XY positioning system and 63× objective were used to reconstruct the dendritic geometry in detail; images were captured by the frame grabber at a series of points along the dendrites separated by <10 μm, and the dendritic diameter at each point was estimated by a LabVIEW program that obtained mean densitometric profiles along parallel axes oriented orthogonal to the dendrite. The overall fixed-cell morphology was used to confirm that the recorded neuron was a mitral cell, to positively identify the dendrites as primary or secondary, and to verify that photolysis data were not complicated by dendritic branches of the recorded cell extending above and below the focal plane. The detailed reconstruction data were used in compartmental models of the dendrites to correct space-clamp errors (see following text).

Data analysis
Physiological responses were analyzed off-line with custom software written in LabVIEW and Origin 6.1 (OriginLab), and with the Mini Analysis Program (Synaptosoft). Corrections to pipette-bath liquid junction potentials were made using the JPCalc Program (Cell MicroControls).
To compare inward currents and spike modulation effects induced by different levels of caged GABA photolysis, data were correlated with a “flash-concentration” stimulus parameter: FC = (laser power at the cell) × (flash duration) × (caged GABA concentration) expressed in units of μl·mM. The laser power at the cell is the nominal value calculated by multiplying unattenuated output power, as measured by the internal power meter, by the attenuation factors associated with the microscope optics. The FC value is a proportional measure of the total quantity of GABA released by photolysis in a fixed focal volume. It is proportional to peak concentration, provided that the rate of diffusion of photoproduct out of the focal volume, and local depletion of caged compound during the flash can be neglected.
Voltage-clamp data obtained by the side-scan method consisted of families of responses obtained from photolysis of caged GABA by 1.24-ms flashes, applied to a series of equally spaced points (0.5–1.5 μm apart) along an axis approximately orthogonal to a dendrite or axon. The current amplitude was measured at t = 2 ms after shutter opening (i.e., 0.76 ms after shutter closing), around the middle of the rising phase of the response. The part of the rising phase after shutter closure and termination of photolysis represents the continued increase in current due to spatial summation of GABA-activated conductance as the photoreleased GABA spread locally by diffusion. Taking the larger current value at this time point reduced the relative contribution of whole cell noise to the error in measurement, at the expense of slightly reduced spatial resolution. Assuming an initial Gaussian distribution of photoreleased GABA of width w = 2σ in the focal plane, and diffusion in two dimensions with coefficient D ~ 7 × 10−5 cm2·s−1, a time delay of δt ~ 0.76 ms would reduce resolution by increasing the effective width to: w′ ~ 4σ2 + 8Dδt = 5.2 μm. In various high-resolution mapping experiments, consecutive side-scans were spaced on average 1.4–3.7 μm apart, a resolution similar to or finer than that set by diffusion. Each scan profile was fit to a Gaussian function by the Levenberg-Marquart algorithm to extract the peak amplitude. In a few cases, two local

Cell morphology
Live cell morphology was recorded on-line using a frame grabber board (PCI-1407, National Instruments) to capture a DIC image for each recorded response. Image-acquisition functions were integrated

J Neurophysiol • VOL. 88 • JULY 2002 • www.jn.org

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maxima were present in the scan profile. Examination of the biocytin
reconstructed neurons showed that the second peak was correlated
with a dendritic branch that was not visible under IR-DIC. In these
instances, the second peak was removed manually from the data, and
the remaining peak was fit.

Space-clamp errors associated with dendritic photolysis currents
recorded under somatic voltage clamp were estimated by constructing
compartamental models of recorded cells based on the dendritic ge-
ometry obtained from biocytin-stained cells. This error estimation was
possible because the positions of dendritic photolysis sites are pre-
cisely known. Models of mitral cells were constructed in the NEU-
RON simulator (Hines and Carnevale 1997). The soma was repre-
sented as a single cylindrical section (length, 20–30 μm; diameter,
20–25 μm), and the primary and secondary dendrites as segments
(compartment length, 1 μm) with piece-wise linear tapering.
Taper intervals were variable, ranging from 15 μm proximally to 200
μm distally, depending on the cell and dendrite. In the primary
dendrites, tapering was significant only proximally (less than ~40 μm
from the soma), whereas in the secondary dendrites, tapering occurred
along the length of the dendrite (Mori et al. 1983). For primary
dendrites, mean diameter was 3.5 ± 0.6 μm at 20 μm from the soma
and 3.0 ± 0.4 μm at 60 μm (n = 6 dendrites); for secondary
dendrites, mean diameter was 2.7 ± 0.3 μm at 20 μm and 2.0 ± 0.3
μm at 60 μm (n = 8 dendrites). The model values of passive
electrotonic parameters were taken as those determined in a recent
modeling study to best-fit data from dual current-clamp recordings of
action potentials in mitral cell primary dendrites (Shen et al. 1999):
intracellular resistivity, $R_i = 70 Ω·cm$; membrane resistance, $R_m =
30,000 Ω·cm^2$; membrane capacitance, $C_m = 1.2 μF·cm^{-2}$. In
the voltage-clamp experiments being simulated here, K+ was replaced
by Cs+ in the intracellular solution, which would slightly reduce $R_i$ by
the ratio of the electrophoretic mobilities of the cations (~5%) (Hille
1984); this had a negligible impact on the computed space-clamp
errors. To simulate the recordings of photolysis currents, a perfect
voltage-clamp electrode was applied to the model soma, and the
conductance activated by focal uncaging of GABA at a given position
along a dendrite was simulated by locating an AlphaSynapse, $g(t) =
g_{\text{max}} \cdot \left(\frac{t}{\tau}\right) \exp[-(t - \tau)/\tau], $ with zero reversal potential, on
the corresponding dendritic compartment (at 1-μm accuracy). The value
of $\tau$ was set equal to the time-to-peak of the response to somatic
photolysis (5–15 ms), and the somatic current was computed at the
measurement time, $t_{\text{meas}} < \tau$. This phenomenological model was able
to closely fit the time course of the photolysis-activated conductance
during its rising phase (when the current amplitude measurement was
made), without explicitly modeling the diffusional spread of GABA at
later times. The value of $g_{\text{max}}$ that generated a measured somatic
current was then obtained by iterative bisection of a conductance
interval bracketing the measured current, and the predicted somatic
voltage-clamp current with perfect space-clamp was calculated by
multiplying the corresponding $g(t_{\text{meas}})$ by the holding potential.

Action potential amplitudes were measured as the difference be-
tween the voltages at two points: the spike peak determined by
quadratic fit of five consecutive data points (50-kHz sampling) and
a prespike inflection point, defined arbitrarily as the point where the
second derivative of the voltage is equal to 1/10 of the local maximum
value of the second derivative of the voltage on the rising phase of
the spike (also located by 5-point quadratic fitting). This procedure al-
ways yielded a reproducible inflection point near the end of the
depolarizing ramp preceding each spike. Spike width was measured as
the time difference between half-peak amplitude points on the rising
and falling phases of the action potential, the amplitudes being mea-
sured from the prespike inflection point. To reduce noise and improve
the reliability of inflection point estimation, data arrays were
smoothed with a second-order Chebyshev filter (ripple, 0.1 dB; cutoff
frequency, 1.5 kHz). Applying this filter did not result in significant
errors in the measurement of spike parameters: filtered action poten-
tials with width 1.18 ± 0.03 ms (n = 9) had their peak voltages
changed by a factor of 0.993 ± 0.002 relative to the unfiltered peaks,
and their widths changed by a factor of 1.01 ± 0.02 relative to peaks
filtered at 3 kHz.

For backpropagated dendritic action potentials, a backpropagation
attenuation factor (BPAF) was defined for each spike, as the ratio of
dendritically to somatically recorded amplitudes. The BPAF decayed
after breakthrough into whole cell mode, and the initial value, BPAF,
was estimated by extrapolation back to 1 min prior to breakthrough (the
approximate time taken to achieve a dual recording by breakthrough at
the soma, after initial breakthrough at the dendrite). Extrapolation was by
linear or exponential fit to the monotonic change occurring during the first
1–10 min of recording. SDs were obtained from the 68% prediction
interval for the least-squares fit. For each backpropagated spike, a back-
propagation broadening factor, BBPF, was defined as the ratio of den-
dritically recorded to somatically recorded spike widths. The BBPF
increased after breakthrough into whole cell mode and the initial value,
BBPF, was also estimated by a similar back-extrapolation procedure.
Backpropagation conduction velocity was computed from the time dif-
fERENCE between the somatically and dendrically recorded action poten-
tial peaks and also decayed over time, so a similar extrapolation was
applied to extract the initial conduction velocity. For each somato-
dendritic pair of spike trains, the broadening and attenuation factors
were calculated as the average BPAF and BBPF for the first three or four
spikes (prior to the flash, if GABA was applied by photolysis).

When analyzing the effect of caged GABA photolysis on dendritic
spike trains, flash timing usually fell between action potentials, so to
correct for variations in spike timing relative to shutter opening, a
corrected photolysis attenuation factor (PAF) for dendritic spike
trains was estimated by back-extrapolating, to the flash time, the
amplitude differences between the last prespike flash and a series of
4–10 postflash spikes. Referencing the postflash amplitudes relative
to the last prespike flash was justified by the observed lack of activity-
dependent attenuation in the dendritic spike train. Measurements were
not taken from spikes that coincided with or overlapped a period of
several milliseconds during or following the flash because during this
period, both local GABA concentration and membrane voltage were
changing rapidly. Back-extrapolation of amplitudes was performed by
using the Levenberg-Marquart algorithm to fit an exponential decay to
the postflash amplitude difference, and a standard error was estimated
from the prediction band for 68% confidence level at the flash time.
In cases where an exponential fit did not converge because the scatter in
the postflash amplitudes masked the curvature in the plot of amplitude
recovery versus time, extrapolation was performed by a linear fit, with
standard errors in the extrapolated values taken from prediction bands
at 68% confidence level. The effect of dendritic photoinhibition on
somatic spike amplitude was too small to allow reliable curve fitting
and extrapolation, and a somatic photolysis attenuation factor (PAF) was
calculated directly by dividing the amplitude of the first postflash
spike by the mean amplitude of all the preflash spikes. Again, this
was justified by the observed lack of activity-dependent attenuation in
the somatic spike trains. The standard error in PAF was obtained from
the amplitude variance of the preflash spikes.

RESULTS

Caged GABA photolysis activates a GABA$_A$ receptor-mediated conductance in mitral cells

The membrane current evoked by photolytic release of GABA onto the mitral cell soma was recorded under whole cell voltage clamp while dialyzing with CsCl to block K+ conductances and shift the chloride reversal potential to near 0 mV. The bath included TTX and Cd$^{2+}$ to block regenerative Na$^+$ and Ca$^{2+}$ currents. Under these conditions, laser flash photolysis of caged GABA evoked large transient currents that were inward at negative holding potentials (Fig. 1A). The amplitudes
and decay rates of these currents varied considerably between cells: at −60 mV, with a 0.725-μM flash (1.24 ms, FC = 0.29 μJ · mM), the peak inward current was 646 ± 267 pA (range, 275–1082 pA; n = 8 cells, 1 flash/cell), and decay time constants were 64 ± 30 ms (range, 36–119 ms, monoexponential fit to decay; n = 8 cells). Responses to laser flashes were not observed when caged GABA was omitted from the bath solution, and introducing 400-μM caged GABA into the bath did not activate a significant inward current in the absence of laser irradiation. The photolysis responses were quite stable during repeated stimulation of the same cell: the peak current recorded from one cell subjected to repeated somatic photolysis (n = 19 trials, 2 s apart) was 529 ± 11 pA, with the 2% variation being attributable to baseline noise in the whole cell current. The mean percentage variation in amplitudes for repeated stimulation of the soma and proximal dendrite was 3.9 ± 1.1% (average from n = 4 cells, 5 flash trials/cell). The current-voltage relation was nearly linear, with reversal potential near zero (Erev = 1.9 ± 2.7 mV, n = 10 cells), consistent with activation of a chloride current (Fig. 1B). Responses were strongly blocked by 50 μM BMI (attenuation factor for peak current 0.057 ± 0.023, n = 4 cells at −60 mV; FC = 0.29 μJ · mM), indicating they were mediated by GABA_A receptors (Fig. 1C). The recovery kinetics were significantly slower at positive holding potentials (Fig. 1D): the decay time at +40 mV was 2.19 ± 0.55 times longer than the decay time at −40 mV (P < 0.001, paired t-test, n = 7 cells; monoexponential fit to decay). This slowing is consistent with the known voltage-dependent prolongation of decay kinetics of GABA_A receptor-mediated IPSCs (Otis and Mody 1992). The photolysis responses were most likely due to a direct action of the uncaged GABA on the membrane of the recorded mitral cells, without contributions from polysynaptic pathways involving glutamatergic excitation. Indeed, there were no significant differences, with or without 50 μM AP-5, 50 μM CNQX in the bath, between: decay times at −60 mV, reversal potentials, BMI attenuation factors, or the ratio of recovery kinetics at ±40 mV (P > 0.1, n = 5).

Spatial distribution of currents activated by caged GABA photolysis

Flash photolysis was applied at different points on the mitral cell membrane to determine the spatial distribution of the receptors underlying the GABA-activated inward current recorded somatically under whole cell voltage clamp with CsCl dialysis. Figure 2 shows typical data obtained from a mitral cell (photolysis sites 1–3 μm apart), in which the primary dendrite...
was mapped out to 170 μm from the soma, and the secondary dendrite out to 140 μm (the maximum distances at which a current was detectable by somatic recording). For both dendrites, the response was largest proximally and declined progressively with increasing distance from the soma. Exponential fits to these declines yielded decay lengths of 75 ± 28 μm for primary dendrites (range, 24–95 μm; n = 6 cells) and 78 ± 48 μm for secondary dendrites (range, 42–166 μm; n = 7 cells). The responses evoked by dendritic stimulation were compared relatively low (70 Ω · cm), and the rising phases of the photolysis-activated currents were relatively slow (times to

FIG. 2. Mapping of GABA receptors on mitral cell dendrites. A, top: the x-y coordinates of photostimulation points along the dendrites of a mitral cell (○), 41 points on the secondary dendrite (left) and 58 on the primary dendrite (right), overlayed onto a tracing of the Nomarski image of the cell (position of the somatic recording electrode is indicated). Scale bar: 50 μm. Bottom: plots of the magnitudes of the inward currents evoked by local photolysis of 270-μM O-CNB-caged GABA (3.5-ms flashes, 0.4 μl), measured at the end of shutter opening (○); also shown are the same currents after compensation for space-clamp errors (●). Superposed over the same abscissas are plots of the percentage space-clamp error (% SCE: ○) and the space-clamp error-compensated currents divided by the dendritic diameter that provide a measure of the local conductance density (●). The cell was dialyzed for 90 min with a CsCl-based pipette solution, and the nominal holding potential was ~60 mV. The abscissas in these plots represent integrated distance along the dendrites, Σ√(dx² + dy² + dz²), with 0 distance taken at a point where the base of the dendrite joins the soma. B: raw data showing photostimulation responses evoked at each of the points plotted in A. Numbers on the left of every 10th trace are also labeled on the corresponding photolysis sites and amplitudes in A. B1, 1 and 2: responses along the secondary (1) and primary (2) dendrites superimposed by rescaling to show similarity in time courses. Integrated distances for traces in B1 are (in μm): 0, 23.4, 46.5, 64.4, 90.8, 101.1, 109.5, 118.2, and 130.8; for traces in B2 are (in μm): 0, 29.1, 54.6, 76.7, 105.6, and 161.2. C: bright-field image of the whole-mount of the fixed slice containing the cell in A and B visualized by biocytin staining. The approximate locations of the distalmost photostimulation points, 41 on the secondary dendrite and 58 on the primary dendrite, are marked. Scale bar: 100 μm.

One factor that might contribute to these declines is a reduced ability of the somatic electrode to clamp the dendritic membrane voltage at more distal locations (Bhalla and Bower 1993; Spruston et al. 1993). The contribution of space-clamp errors to the spatial profile was estimated by numerical simulation of reconstructed cells and was found to be relatively small (○ and ● in plots of Figs. 2A, 3A, and 4, A and B). Estimated space-clamp errors increased approximately linearly with increasing distance from the soma (∇, Fig. 2A). For primary dendrites, the relative error was 2.7 ± 1.7% at 20 μm from the soma and 6.8 ± 4.2% at 60 μm (n = 6 dendrites); for secondary dendrites, it was 2.5 ± 0.9% at 20 μm and 7.5 ± 2.9% at 60 μm (n = 8). The computed space-clamp errors were modest because the photolysis locations were <200 μm from the soma, where both dendrites are fairly wide (diameter of primary dendrite more than ~3 μm, of proximal secondary dendrite more than ~1.5 μm), the assumed intracellular resistivity was relatively low (70 Ω · cm), and the rising phases of the photolysis-activated currents were relatively slow (times to
The similar time courses of the responses along the dendrites (Fig. 2B, I and 2) is consistent with a weak effect of cable filtering. After compensation for space-clamp errors, the mean exponential decay lengths were increased by 12% (to 84.3 ± 31.4 μm) for primary dendrites (n = 6) and 28% (to 100 ± 64 μm) for secondary dendrites (n = 8).

The spatial profiles of the space-clamp compensated responses were divided by the dendritic diameters to obtain a proportional measure of the GABA-activated conductance per unit membrane area (Figs. 2A, 3A, and 4, A, B: plotted as A). This revealed a significant difference between the conductance density profiles of the primary and secondary dendrites. In primary dendrites, dividing by the diameter made the profile more shallow proximally, where the dendrite tapers off from the soma (less than ~30 μm) but had much less effect on the profile at more distal locations where the diameter is more uniform. Decay lengths for exponential fits to primary dendritic profiles at >30 μm from the soma were increased by only 16 ± 14% (means increased from 80 ± 48 to 91 ± 49 μm, n = 6) after diameter division. Linear regression indicated that the conductance density of the primary dendrite decreased significantly with increasing distance from the soma (slope, −11.3 ± 9.3 pS · μm⁻²; P < 0.01, n = 6 dendrites, probed lengths 50–300 μm). By contrast, in secondary dendrites, which taper continuously, dividing by the diameter strongly reduced the decay rate of the profile of conductance versus distance. For half of the dendrites tested, exponential decay lengths were increased by 43 ± 10% (means increased from 94 ± 64 to 131 ± 81 μm, n = 4), and linear regression of the conductance density profile revealed a significant residual decay (slope, −10.3 ± 7.9 pS · μm⁻²; P < 0.05, n = 4 dendrites, probed lengths 50–150 μm); in the remaining four dendrites, the density profiles were too shallow to allow exponential fitting, and linear regression did not indicate a significant decrement of conductance density with distance (−1.6 ± 5.6 pS · μm⁻², P > 0.05, n = 4 dendrites; probed lengths, 60–90 μm).

The recorded currents exhibited variations in amplitude as a function of distance along the more proximal parts of the dendrites (Fig. 2A), which contributed to the scatter in decay lengths obtained from exponential fits to the overall decay. A possible source of such variation is random error in IR-DIC-guided positioning of the laser focus onto the dendrite. To reduce the contribution of random positioning errors, data were also acquired by the side-scan method, and a least-squares test to four repeated scans was 10% error in Gaussian fits. Applying this test to secondary dendrites (Fig. 4B), 10 local peaks were detected (5 with P < 0.05, 3 with P < 0.01, 2 with P < 0.005; mean valley/peak amplitude ratio, 0.57 ± 0.13), from a total of seven cells (6 side-scan, 1 IR-DIC; maximum distances 53–147 μm from soma; summated length over all cells 579 μm); on primary dendrites (Fig. 4A), 9 local peaks were detected (5 with P < 0.05, 4 with P < 0.005; mean valley/peak amplitude ratio, 0.62 ± 0.07), from a total of four cells (3 side-scan, 1 IR-DIC; maximum distances 54–300 μm from soma; summated dendritic length over all cells 830 μm).

Sometimes it was possible to visualize and trace the axons of the mitral cells under IR-DIC illumination, and a few of these were also probed for GABA responses. Figure 4, B and C, illustrates side-scan data obtained from axons, showing local peaks and valleys in the profiles. A total of four local peaks were detected on axons (1 with P < 0.05, 3 with P < 0.01; mean valley/peak amplitude ratio, 0.54 ± 0.03), from a total of three cells (all by side-scan; maximum distances 19–34 μm from soma; summated length from all cells 84 μm). Compared to dendrites, the amplitudes of currents evoked by axonal photostimulation declined on average more strongly with increasing distance from the soma. The mean decay length from exponential fits to axon profiles from n = 3 cells was 29 ± 4 μm (range, 25–31 μm). This mean includes the profile shown in Fig. 4B but not that in Fig. 4C, which was not well fit by an exponential decay due to the large spatial fluctuations. Space-clamp corrections are not shown for the axon data because of the difficulty in accurately quantifying the small diameter of the axon initial segment, and the uncertainty in the Rm value. Trial calculations showed that predicted space-clamp errors were quite sensitive to diameter variations around ~1–2 μm and to the unknown distribution of a large leak conductance (Rm = 1000 Ω · cm²) along the initial segment (Shen et al. 1999).

**Currents activated by caged GABA photolysis are comparable in magnitude to IPSCs from presynaptic spines**

To address the question of whether caged GABA photolysis induces an unphysiologically large-conductance change, currents evoked by photolysis at the soma were compared with currents induced by GABA release from presynaptic spines of dendrodendritic synaptic contacts at the same location. Making the comparison at the soma rules out any cable filtering of dendritic postsynaptic currents caused by loss of voltage clamp. Spine activation was spatially restricted by local photolysis of caged glutamate at the soma, and the bath ACSF contained 0 added Mg²⁺ to facilitate activation of N-methyl-
abolished by 50 μM TTX, 200 μM Cd²⁺, 50 μM AP-5, and 50 μM CNQX. Serial photolysis of 400-μM O-CNB caged GABA (1.24-ms flash duration, 0.73 μJ) was performed by moving the center of the focal volume along linear tracks approximately orthogonal to the secondary dendrite. A: IR-DIC tracing, projected onto x-y plane, of a recorded mitral cell showing primary dendrite (PD) and secondary dendrite (SD). The recording pipette is shown at the soma, and the series of ○ overlayed along the secondary dendrite indicates the x-y coordinates of peaks obtained from Gaussian fits to side-scan photolysis response profiles (scale bar: 20 μm). The plot above these points shows the corresponding peak amplitudes as a function of position, before (○) and after (●) compensation for space-clamp errors. The un-normalized conductance density (compensated current divided by diameter) is plotted on the same abscissa (●), * putative local peaks in the amplitude profile. The left-most peak satisfied the spatial average nonmonotonicity criterion (P < 0.05). Total integrated distance along the dendrite covered by 36 side-scans was 73.7 μm, and mean interscan distance was 2.0 μm. The plot abscissa represents integrated distance ∑√(dx² + dy² + dz²), which is larger than the x-y projection distance. B: raw side-scan data from the cell in A. Magnitude of inward current at 2 ms after shutter opening is shown as line plots of y-axis scans, stacked along the x-axis. The secondary dendrite is approximately parallel to the x-axis. C: raw data and Gaussian fits to 4 side-scans repeated across the 1 location on a secondary dendrite. The scatter in the peak amplitudes of these fitted curves is 4.7%. The relative error for side scans of dendrites from n = 3 cells was 10 ± 7%.

The error bars on the plots reflect this error estimate.

d-aspartate (NMDA) receptors. Figure 5A shows a series of currents recorded from a mitral cell dialyzed with CsCl with 1 μM TTX in the bath. Baseline control recordings (top) exhibited a low rate of spontaneous postsynaptic events. These events could be blocked by 50 μM BMI, indicating that they were reversed IPSCs mediated by GABA_A receptors (Kirilillova and Lin 1998; Wells and Kauer 1993). Glutamate photostimulation evoked a prolonged barrage of asynchronous events, continuing >1 s. This barrage became more intense as more glutamate was released by higher laser energies, and it was abolished by 50 μM BMI (bottom). This indicated that the evoked events were reversed IPSCs mediated by GABA_A receptors and that photostimulation triggers local GABA exocytosis from presynaptic spines. After addition of BMI, a residual slow inward current remained that was blockable by 60 μM AP-5 (data not shown), indicating involvement of NMDA autoreceptors (Petralia et al. 1994). The caged glutamate responses resembled other responses caused by asynchronous release of GABA onto the mitral cell: i.e., responses evoked by puff pipette application of NMDA or KCl depolarization of the inhibitory interneurons (Friedman and Strowbridge 2000), stimulation of the mitral cell by voltage pulse (Isaacson and Strowbridge 1998; Schoppa et al. 1998; Wells and Kauer 1993) or uncaging calcium in the mitral cell to release glutamate (Chen et al. 2000; Isaacson 2001). Figure 4B shows the results of an analysis of the glutamate-evoked IPSCs recorded from the cell in Fig. 4A. Mean event amplitude was 103.5 ± 81.6 pA, with many events in the 200- to 300-pA range; mean decay time was 13.6 ± 10.6 ms. Analysis of data from a second cell yielded larger amplitudes, and similar decay kinetics (Fig. 5B, right).

After recording glutamate responses, the bath solution was switched to ACSF containing TTX and caged GABA, and GABA was uncaged at the same somatic site. Figure 4C compares the postsynaptic events recorded from the cell of Fig. 4A with GABA-activated currents recorded from the same cell over a range of flash energies. Uncaging of GABA resulted in currents that were several hundred picoampere in amplitude; the dose-response relationship could be fit to a Hill equation: $I = I_{max} \cdot FC^n/(K_{50}^n + FC^n)$, $K_{1/2} = 0.37 ± 0.02 μJ \cdot mM$, $I_{max} = 562 ± 26 pA, n = 1.7 ± 0.1$. Hill fits to dose-response relationships for the somatic GABA-activated current obtained from three additional cells gave consistent values for parameters $K_{1/2} (0.36 ± 0.04 μJ \cdot mM)$, range, 0.31–0.40 μJ \cdot mM,
n = 4) and n (1.85 ± 0.14; range, 1.69–2.03, n = 4), which characterize the GABA receptor, whereas I_{max} varied widely (522–1,436 pA). The wide variation in maximal somatic current probably reflects in part the technical difficulty in specifying the three-dimensional geometry of overlap between the photolysis beam and the irregularly shaped somatic membrane.

The peak GABA concentration associated with FC = K_{1/2} ≈ 0.37 μM is difficult to know precisely because the EC_{50} of GABA_{A} receptors under nonequilibrium conditions (as might occur during flash photolysis) depends on entry of receptors into desensitized states. For the currents measured several milliseconds after photolysis, the GABA concentration corresponding to FC = K_{1/2} might lie between EC_{50} ~ 10–40 μM at equilibrium (Feigenspan et al. 2000), and EC_{50} ~ 185 μM for 1-ms transient pulses (Galarreta and Hestrin 1997). If the receptors are assumed to be near equilibrium, a rough estimate of the peak GABA concentration after uncaging can be deduced from the observed attenuation of the peak current by the competitive inhibitor bicuculline. Assuming a Hill coefficient of 1 for the GABA_{A} receptor (Feigenspan et al. 2000; Ueno et al. 1997), at a bicuculline concentration of 50 μM, an attenuation factor of ~0.06 corresponds to IC_{50} ~ 3.2 μM, which lies between the IC_{50} values at 10 μM GABA (1.6 μM) and 30 μM GABA (5.8 μM) for the GABA_{A} receptor with subunit composition α1β2γ2 (Ueno et al. 1997). The latter data are applicable here because mitral cells strongly express mRNA for α1, β1, β2, β3, and γ2 subunits of the GABA_{A} receptor (Laurie et al. 1992), and receptors with different subunits have similar affinities for GABA and bicuculline (Ebert et al. 1997; Krishek et al. 1996). Interpolation between the IC_{50} values gives ~20 μM GABA as a peak concentration. This number is consistent with the cited range of EC_{50} values at equilibrium because the attenuation by BMI was measured at FC = 0.29 μM, only slightly below the K_{1/2} of the photolysis response. Thus an equilibrium approximation may be useful for describing the rising phases of photolysis responses, which are relatively slow (5–15 ms) compared with unitary IPSCs from granule cells (rise times, <1 ms).

The tests with caged glutamate showed that the magnitudes of the inhibitory conductances resulting from the uncaging of GABA can be comparable to the range of inhibitory conductances of IPSCs received from presynaptic spines. Photolysis with FC values of ~0.45–0.90 μM generated inward currents of ~300–600 pA, which is in the range of the amplitudes of individual IPSC events evoked by direct activation.
of NMDA receptors on spines. A significant difference was the slower decay of GABA photostimulation currents compared with spine-evoked IPSCs (Fig. 4C). Spine IPSCs are expected to decay more rapidly because the GABA released into the synaptic cleft is much more localized than the GABA released by photolysis. The peak concentration of GABA in the synaptic cleft following exocytosis may be more than ~500 μM (Jones and Westbrook 1995; Maconochie et al. 1994). This is considerably higher than would be attainable by flash photolysis of 400-μM caged GABA, which may generate peak tran-

**FIG. 5.** Comparison of GABA photostimulation-evoked currents and inhibitory postsynaptic currents (IPSCs) evoked by spine activation. A: GABAergic IPSCs evoked by spine activation, recorded from the mitral cell soma under whole cell voltage-clamp at −60 mV, with CsCl dialysis. The bath included 1 μM TTX to block regenerative Na⁺ currents. Top: a control recording with no photostimulation. Lower traces: responses to photolysis of 320 μM γ-CNB-caged glutamate at a site on the soma (5 ms flashes, 0.37–2.25 μJ). Bottom: the response to caged glutamate photolysis (5 ms, 2.25 μJ) with 50 μM BMI in the bath. B, left: event histograms of IPSC amplitude (bottom, 40-pA bins) and IPSC decay time (top, 5-ms bins) for the first 1-s period of glutamate responses (5-ms flash, 2.25 μJ) recorded from the cell in A: (cell 1, mean amplitude, 103.5 ± 81.6 pA; mean decay time, 13.6 ± 10.6 ms; n = 57 events). Right: similar event data recorded from another cell (cell 2, n = 114 events) with a more sustained response (2.4 s; 3 repeats; stimulus, 7 ms, 2.0 μJ). The number of events at larger amplitudes was greater (mean, 171.1 ± 122.4 pA), and the mean decay time was similar (9.8 ± 6.9 ms). C: comparison of IPSCs evoked by glutamate photostimulation (top, same as 2.25 μJ trace in A), and inward currents evoked by GABA photostimulation (270 μM, 5 ms, 0.22–2.2 μJ) at the same location on the soma of the mitral cell in A. The bath included 1 μM TTX. D: plot of the magnitude of the inward current at 4 ms after shutter opening, as a function of flash energy, for the family of GABA photostimulation-evoked currents in C. The data were fit to a Hill equation: inward current, I = Iₘₐₓ · FCⁿ/[kⁿ + FCⁿ], where FC = (flash energy) × (caged GABA concentration). Fit parameters were: k = 0.37 ± 0.02 μM · mM, Iₘₐₓ = 562 ± 26 pA, n = 1.7 ± 0.1.
sients of only $\sim 10-100 \, \mu M$. However, the uncaging of GABA can activate conductances of comparable magnitude by spatial summation over a larger membrane area.

The slower time course of the conductance change produced by uncaging GABA might actually be a better simulation of physiological conditions. Coordinated firing of granule cells in vivo during oscillations of populations of mitral and granule cells (Freeman and Baird 1987; Li and Hopfield 1989) would be expected to result in strong spine depolarization by back-propagating action potentials in the granule cell apical dendrites. This would provoke stronger, more synchronous GABA release driven by voltage-sensitive calcium channels, facilitating a temporal summation of IPSCs and magnifying and prolonging the inhibitory input to mitral cell dendrites. Focal depolarization of spines by KCl has been shown to activate a large ($>1 \, \mu A$), slow ($>100 \, ms$) Cd$^{2+}$-sensitive dendrodendritic IPSC in mitral cells (Halabisky et al. 2000), similar to IPSCs evoked by GABA photostimulation at higher laser power. IPSCs much larger than 1 nA can also be evoked by glomerular shock (Schoppa et al. 1998). Under such conditions, it is expected that IPSCs received from granule cells and other interneurons would be more closely mimicked by the currents evoked here by uncaging of GABA.

### Inhibition of somatic action potentials by photolysis of caged GABA

The mitral cell soma initiates spike trains in response to depolarizing current conducted from the primary dendrite during synaptic excitation of the apical dendritic tuft. The EPSC from the primary dendrite is counteracted by GABAergic inputs on the soma and secondary dendrites, activated during self- and lateral inhibition. In the voltage-clamp recordings described in the preceding text, functional GABA receptors were found distributed over the secondary dendrites $\leq 150 \, \mu m$ from the soma. At what range do these receptors influence action potential firing at the soma? In general, it is expected that distal inhibition would be less effective at shunting the somatic EPSC, but the actual range depends on the density of the GABA receptors and the magnitude of depolarizing current. This was demonstrated by recording from mitral cells under somatic current clamp, dialyzing with low internal chloride solution, and initiating spike trains by injecting square current pulses into the soma. During the spike trains, inhibitory input was applied locally along the dendrite by caged GABA photolysis, using IR-DIC imaging to position the laser focus on the dendrite (Fig. 6B).

Figure 6A illustrates the different spatial patterns of inhibition obtained when varying the injected current at a fixed level of photolysis (FC = 0.85 \, \mu A \cdot mM). For 50-pA current pulses, barely suprathreshold for repetitive firing (40 pA was sub-threshold), the spike train was terminated by focal inhibition at points extending out as far as 137 \, \mu m from the soma (left); at 100 pA (middle), inhibition applied further than 78 \, \mu m from the soma failed to terminate somatic firing; and at 150 pA (right), only inhibition on the most proximal site (8 \, \mu m) was effective in terminating the spike train. A larger depolarizing current requires a larger conductance shunt to prevent spiking. Thus the decrease in the maximal spatial range of spike termination with higher somatic current (Fig. 4C) shows that distal inhibition is indeed less effective at shunting somatic current.

For the purposes of comparing data from different cells, the range of spike termination was defined arbitrarily as the maximal range for termination of repetitive firing without recovery of spiking during the 100-ms time period after GABA release. Trains of action potentials exhibited spike frequency adaptation, so the release of GABA was timed at 100 ms after the beginning of the current pulse, when frequency adaptation was largely complete (mean adaptation time constant, 49 \, ± 26 ms, exponential fit, $n = 6$ cells). The spike frequency adaptation was present when CNQX and AP-5 were included in the bath to block self inhibition by dendrodendritic feedback.

Different cells exhibited different firing thresholds depending on their input impedance and resting potential; so to compare data from several cells, the injected current was corrected by subtracting out a threshold current estimated from subthreshold pulses applied to each cell. Data from several cells confirmed that at approximately equal levels of photolysis, the maximal range of spike termination was negatively correlated with threshold-corrected current ($r = -0.57$, range slope $=-0.33 \pm 0.11 \, \mu m/pA^{-1}$, $n = 4$ cells; FC = 0.75–0.91 $\mu J \cdot mM$). Conversely, at approximately equal magnitudes of current, the maximal range of spike termination was positively correlated with the level of photolysis ($r = 0.70$, range slope $=1.8 \pm 0.8 \, \mu m$; $\mu J^{-1} \cdot mM^{-1}$, $n = 5$ cells; $I_{\text{corrected}} = 90–110 \, pA$). The maximum observed range ($<140 \, \mu m$) was limited by the technical difficulty of visualizing the finely tapered secondary dendrites at more distal locations. The considerable cell-to-cell variability in these range measurements may be caused by several factors, such as the spatial heterogeneity in GABA receptor density along the secondary dendrite (Figs. 3 and 4) and the high sensitivity to shunting when the somatic membrane potential lies close to firing threshold. Spatial heterogeneity in dendritic conductance would also contribute to the patchiness of the spatial profiles of inhibition shown in Fig. 6.

### Action potential backpropagation in the secondary dendrites

Beyond a certain range, which depends on the relative levels of excitation and inhibition, distal GABAergic inhibitory inputs to the secondary dendrite are ineffective at blocking somatic action potentials. However, GABAergic granule-mitral synapses are paired with reciprocal glutamatergic mitral-granule synapses through which the distal dendrite can send output to granule cells. Activation of these outputs requires sufficient distal depolarization to activate presynaptic Ca$^{2+}$ channels. Such depolarization could be provided by laterally backpropagating action potentials. In the mitral cell primary dendrite, apically backpropagating action potentials are well characterized because of the ease of recording from the relatively thick dendritic trunk (Bischofberger and Jonas 1997; Chen et al. 1997; Shen et al. 1999). The finer, tapered secondary dendrites have only recently been shown to support active backpropagation of action potentials (Charpak et al. 2001; Margrie et al. 2001).

Dual whole cell current-clamp recordings from soma and dendrite confirmed that trains of action potentials initiated at the soma do backpropagate laterally into the secondary dendrite (Fig. 7). In these recordings, dendrodendritic feedback inhibition was blocked by glutamate receptor antagonists. The backpropagated spikes in the secondary dendrite were found to be attenuated relative to the somatic spikes. The backpropaga-
Action potential inhibition in mitral cell dendrites. A: families of responses recorded from the soma of a mitral cell under whole cell current clamp (−52 mV resting potential), while injecting 200-ms current pulses of different magnitudes (left to right column: 50, 100, and 150 pA) and applying GABA photostimulation (270-μM caged GABA, 7.2 ms, 3.2 μL) at different points along the secondary dendrite. On the left, stimulus points are numbered, and the corresponding integrated distances from the soma \( \sqrt{(dx)^2 + (dy)^2 + (dz)^2} \) are indicated in μm. Bottom traces are controls without photostimulation. ↑, timing of photolysis. B: the x-y coordinates of photostimulation points along the secondary dendrite of the cell in A overlayed onto a tracing of the Nomarski image of the cell (position of the recording electrode is indicated). The dendrite extending to the right is the primary dendrite, which could be followed up to the glomerular layer with IR-DIC imaging. Scale bar: 50 μm. C: collapse of the range of somatic inhibition with increasing strength of excitation. The histogram plots the maximum observed distance at which GABA photostimulation was able to terminate a 200-ms spike train evoked by injected current pulses of various amplitudes.

One other cell, the BPAF decay was also due to a slow growth in the somatic spike (0.67 ± 0.10 mV/min). The initial attenuation factor (BPAF), estimated by back-extrapolation (see methods) was 0.75 ± 0.07 (range 0.70–0.86; \( P < 0.005, \) t-test for BPAF = 1; \( n = 6 \) cells) at distances of 93–152 μm (mean 112.3 μm) from soma. The backpropagated spikes were slightly broadened, and the backpropagation broadening factor...
FIG. 7. Action potential backpropagation in the mitral cell secondary dendrite. A: whole cell current-clamp recording of a train of action potentials recorded simultaneously from the soma (left), and the secondary dendrite (right) at a point 152 μm from the soma. Action potentials were evoked by a 200-ms, 200-pA current pulse injected into the soma. Mean firing frequency was 39.3 Hz (initial, 50 Hz; final, 38.6 Hz). The pipettes contained K-methylsulfate, and the bath solution contained 60 μM 60 μM 2-amino-5-phosphonopentanoic acid (AP-5) and 10 μM 6-cyano-7-nitroquinolxalene-2,3-dione (CNQX) to block dendrodendritic feedback inhibition. Resting potential was −48 mV. In this cell, intrinsic membrane potential oscillations were visible. B: the x-y projection of the recorded cell, reconstructed by biocytin staining. The positions of the somatic and dendritic recording sites are shown by pipette symbols. Scale bar: 100 μm. C: expanded plots of the first (left) and last (right) action potentials in A, with somatic and dendritic recordings superimposed. D: plot of initial backpropagation attenuation factor (BPAF) vs. distance from the soma (data are from dual whole cell recordings from the soma and secondary dendrite of n = 6 mitral cells).

(BPBF) increased following breakthrough (rate 0.06 ± 0.04 min⁻¹, range 0.006–0.1 min⁻¹, linear regression, n = 5 cells). The estimated initial broadening factor (BPBF), was 1.19 ± 0.10 (range, 1.06–1.29; P < 0.05; n = 6 cells). For each cell, the gradual broadening of dendritic spikes after whole cell breakthrough was directly correlated with attenuation: linear regression of BPBF against 1-BPAF gave a mean correlation coefficient of r = 0.962 ± 0.039 (BPAF range, 0.4–0.86, n = 5 cells). Like the amplitudes, the gradual increase in relative broadening was due primarily to an increase in the width of dendritically recorded spikes with the somatic spike widths being more stable. The peak of a dendritically recorded spike always occurred after the peak of the corresponding somatically recorded spike, showing that action potentials were initiated at the soma. Backpropagation conduction velocities calculated from the peak time difference exhibited a gradual decrease during whole cell recording, and the extrapolated initial velocity (BPCV₁) was 0.44 ± 0.14 m/s (n = 6 cells).

Trains of backpropagating action potentials in the secondary dendrite did not exhibit the dramatic activity-dependent attenuation that has been reported for spikes in the apical dendrites of some pyramidal neurons. To test for activity-dependent attenuation or facilitation, spike trains of various frequencies were evoked by somatic injection of square current pulses of constant duration and various amplitudes. A spike train generates a sequence of BPAF values. To detect activity-dependent changes in attenuation, linear regression was applied to BPAF sequences as a function of time. For 30 spike trains, the mean value of the regression coefficient, \( b_{xy} \) (BPAF), was \( -0.042 ± 0.123 \) s⁻¹, which was not significantly different from zero (P > 0.05; initial firing frequency, 35–107 Hz; n = 3 cells), and the \( b_{xy} \) (BPAF) values were not correlated with the initial firing frequency (r = 0.047; regression coefficient \( (1 ± 4) × 10^{-4} \)). Thus there was no evidence of activity-dependent attenuation or facilitation. A similar analysis was applied to test for activity-dependent changes in the broadening factor. A small but significant decrease in BPBF was detected during spike trains in some cells, \( b_{xy} \) (BPBF) = \( -0.25 ± 0.29 \) s⁻¹ (P < 0.05; 21 trials, initial firing frequency, 34–71 Hz; n = 3 cells), while other cells showed no trend, \( b_{xy} \) (BPBF) = \( -0.08 ± 0.26 \) s⁻¹ (P > 0.05; 10 trials, initial firing frequency, 26–88 Hz; n = 2 cells). In the former group, the broadening trend in spike trains was positively correlated with initial firing frequency (r = 0.35, regression coefficient, 0.004 ± 0.002, P > 0.1).

Inhibition of backpropagating action potentials in the secondary dendrites

Action potentials backpropagating into the secondary dendrites may encounter GABAergic inhibition from granule cells or other interneurons. Such inhibition can take the form of dendrodendritic feedback elicited by previous backpropagating
action potentials, or it may arise extrinsically, as lateral inhibition from interneurons excited by another mitral cell. To study the effect of local inhibition on the backpropagating spikes, dual whole cell current-clamp recordings were made from the soma and dendrite of an individual mitral cell. Spike trains were initiated by somatic current injection, and inhibition was applied directly to the dendrite by flash photolysis of caged GABA during the spike train; glutamate receptors were blocked to prevent complications from activation of polysynaptic pathways, such as synaptic feedback. The injected current and the level of caged GABA photolysis were adjusted so that photoinhibition at the dendritic recording site did not terminate firing at the soma. Figure 8 shows the effect of applying GABA at the site of dendritic recording, which in this cell was 113 μm from the soma. The peak amplitude of the dendritic action potentials was markedly attenuated immediately following GABA release and recovered progressively over the remaining period of the spike train. The hyperpolarizing afterpotentials of the dendritic spikes were also attenuated and recovered with a similar time course, suggesting that both the sodium and potassium currents of the action potentials were being shunted by the inhibitory conductance. In contrast, the somatic action potential was only very slightly attenuated by the inhibition applied to the dendrite.

When more GABA was released by increasing the flash energy, the attenuation of the dendritic spike became more pronounced, while the effect on the somatic spike remained very small (Fig. 8E). The change in PAF at the dendrite with increasing photolysis was quantified by linear regression, and it varied substantially between cells: ($-158 \text{ to } -33 \times 10^{-4} \mu J^{-1} \cdot mM^{-1}$) ($n = 3$ cells, 93–113 μm from soma). Some of the variation in the sensitivity of dendritic spikes to localized GABA application may be due to the large spatial fluctuations in the local density of GABA-activated conductance seen in the side-scan experiments. Fluctuations over a distance of 20 μm along a dendrite could cause the photoactivated conductance to vary by several-fold (Figs. 3 and 4).

The photoactivated conductances that significantly attenuated the dendritic spikes are likely to be comparable to inhibitory conductances received from granule cell spines. For example, in Fig. 5, spine IPSCs in the range of ~300–400 pA correspond to photolysis levels FC ~0.45–0.60 μJ · mM. For this range of FC

![Image](http://jn.physiology.org/)

**FIG. 8.** Attenuation of backpropagating action potentials in the secondary dendrites. *A*: whole cell current-clamp recording of a train of action potentials recorded simultaneously from the soma (*left*), and the secondary dendrite (*right*) at a point 113 μm from the soma. Action potentials were evoked by a 200-ms, 150-pA current pulse injected into the soma. Mean firing frequency was 43.5 Hz (initial, 50.5 Hz, final, 40.6 Hz). Pipettes contained K-methylsulfate, and the bath solution contained 50 μM AP-5 and 50 μM CNQX to block dendrodendritic feedback inhibition. The arrow below the right (dendritic) trace indicates the time of flash photolysis of 400-μM caged GABA (2.22-ms flash, 1.3 μJ; FC = 0.52 μJ mM). *B*: the x-y projection of the recorded cell reconstructed by biocytin staining. The positions of the somatic and dendritid recording sites are shown by pipette symbols. Scale bar: 100 μm. Arrow, site of caged GABA photolysis. *C*: expanded view of traces in *A*, showing superimposed somatic and dendritic action potentials before and after uncaging of GABA on the dendrite. Arrow indicates time of shutter opening. *D*: expanded view of action potentials in *C* with pre- and post-photolysis spikes superimposed at their inflection points. ⋯⋯(with reduced amplitude), the post-photolysis spikes (soma and dendrite). *E*: decrease in photolysis attenuation factor (PAF) with increasing level of photolysis (FC) for the cell in *A–D*. The PAF is plotted for spike attenuation at the soma (●) and dendrite (●). The rate of decrease in PAF estimated by linear regression was ($-2.9 \pm 1.0 \times 10^{-4} \mu J^{-1} \cdot mM^{-1}$) at the soma and ($-52 \pm 2 \times 10^{-4} \mu J^{-1} \cdot mM^{-1}$) at the dendrite.

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values, the mean attenuation factor was $PAF = 0.68 \pm 0.15$ ($n = 7$ trials; 3 cells). Because the amplitude of dendritic spikes decayed progressively following breakthrough into whole cell mode, there was a concern that the attenuation of dendritic spikes by GABA might be a property of decayed spikes. However, this is unlikely because it was possible for GABA to cause significant reductions in spike amplitude early in the recording sessions. For example, the data in Fig. 8, A, C, and D, were acquired ~2.5 min after establishment of dual whole cell recording, when $BPAF = 0.71$ (the extrapolated initial $BPAF$ was estimated as 0.86). The mean value of $PAF$ for $BPAF > 0.60$ was $0.75 \pm 0.11$ ($n = 5$ trials, 2 cells). The decay of dendritic spikes corresponded to a decreasing $BPAF$, but overall there was no significant correlation between $PAF$ and $BPAF$ ($r = 0.16$, $P > 0.05$; $PAF = 0.47–0.91$; $BPAF = 0.45–0.71$; $n = 63$ trials, 5 cells).

Spatial profile of the inhibition of backpropagating action potentials

How does the inhibition of backpropagating action potentials vary with the position of inhibitory input on the secondary dendrite? To determine the spatial profile of inhibition, flash photolysis was applied to a series of points along the dendrite during dual whole cell recording (Fig. 9). As the inhibitory input was moved away from the dendritic recording site in either direction, the attenuation of the dendritically recorded spikes became less pronounced (Fig. 9C). Gaussian fits to these attenuation profiles yielded a $2\sigma$ width of $16.3 \pm 4.2 \mu m$ ($n = 4$ cells; 150–300 pA at soma, FC range = 0.51–1.52 $\mu J \cdot mM$; dendritic electrode 93–113 $\mu m$ from soma; $PAF$ at electrode = 0.51–0.64); thus the attenuation was twofold less effective

![Fig. 9. Spatial profile of attenuation of backpropagating action potentials. A: whole cell current-clamp recordings of trains of action potentials recorded simultaneously from the soma (left), and the secondary dendrite (right) at a point 93 $\mu m$ from the soma. Action potential trains were evoked by 200-ms, 200-pA current pulses injected into the soma (initial firing frequency was 96.4 $\pm 2.9$ Hz). On the left, stimulus points are numbered, and the corresponding integrated distances from the soma are indicated in micrometers. Arrow, the time of flash photolysis of 270-$\mu M$ caged GABA ($3.2$-ms flash, $1.87 \mu J$; FC $= 0.51 \mu J \cdot mM$). B: outline of the $x$-$y$ projection of the soma and secondary dendrite of the recorded cell traced from the IR-DIC image. The recording sites are shown by pipette symbols, and numbered open circles indicate the photolysis sites corresponding to traces in A. Scale bar: 25 $\mu m$. C: plot of photolysis attenuation factor (PAF) for the dendritic action potentials as a function of distance from the soma. The position of the dendritic recording electrode is indicated by a vertical line and symbol. The curve is a Gaussian fit to the data ($2\sigma$ width = 14.2 $\mu m$).](http://jn.physiology.org/)

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when the photolysis site was moved 19.2 ± 4.9 μm away from the recording site. The spatially localized nature of the attenuation is consistent with a current shunting mechanism. The attenuation always became weaker when the photolysis site was moved in a proximal direction from the dendritic recording electrode toward the soma. This means that, for the preceding range of FC values, the backpropagating action potentials were not blocked and recovered their amplitude after passing through the site of localized inhibition. Complete failure of backpropagation was not observed, even with higher levels of photolysis applied after dendritic spikes had decayed over time during whole cell recording.

The spatial organization of inhibition in mitral cells is summarized in Fig. 10. On the primary dendrite, soma and proximal secondary dendrite, focal inhibition blocks spiking at the soma; on the distal secondary dendrite, it has little effect on somatic action potentials and locally attenuates dendritic action potentials. The relative magnitude of excitation and inhibition determines the size of the proximal domain of somatic spike block (Fig. 6) as well as the degree of attenuation experienced by backpropagating spikes. Stronger inhibition is needed to attenuate spikes at the dendritic recording site, but this requires injecting a larger current into the soma to prevent termination of firing. The phenomenon of spike attenuation does not occur at the soma because any local conductance shunt large enough to attenuate somatic spikes will so strongly shunt the depolarizing current that repetitive firing will be blocked. In the distal dendrite, a similarly large conductance shunt can attenuate dendritic spike amplitude, but only has a small effect on the depolarizing current at the soma, and repetitive firing is main-
tained. The patchiness of the transition from the proximal blocking domain to the distal attenuation domain (Figs. 6 and 10) may be a consequence of spatial variations in the local GABA-activated conductance along the secondary dendrite (Figs. 3 and 4).

DISCUSSION

Lateral inhibition in the olfactory bulb relies on bidirectional signaling in the secondary dendrites of mitral cells. These dendrites transmit excitatory output from the soma in the form of centrifugally backpropagating action potentials, and they receive GABAergic inhibitory input, which is conducted centripetally to the soma. In this study, dendritic output was measured by dual whole cell recordings, and local dendritic input was simulated by laser flash photolysis of caged GABA. These experiments provided information on the properties of backpropagating action potentials in the secondary dendrites, the spatial distribution of functional GABA receptors, and the spatial range of lateral inhibition. They also revealed how these output and input signals interact as they propagate through the dendrites.

Distribution of GABA receptors on mitral cells

The objective of this study was to use local photolysis to activate inhibitory conductances along the mitral cell dendrite to estimate the spatial range of lateral inhibition and determine the effect of local inhibition on lateral signaling by backpropagating action potentials. The voltage-clamp experiments validated the method by showing that flash photolysis can activate an inhibitory GABA\(_A\)-mediated conductance rapidly and reproducibly at any point on the soma and proximal dendrites with a spatial resolution as fine as \(\sim 2.5 \, \mu m\). The voltage-clamp recordings provided high-resolution "maps" of the density of GABA receptors per unit membrane area and revealed local heterogeneities in the conductance. This information was useful for interpreting the results of the current-clamp experiments in which the conductance change caused by local photolysis was not directly observable.

Functional GABA receptors could be activated by local photolysis of caged GABA at all tested locations on the somatic, proximal dendritic, and axonal membranes. Photolysis-activated responses were bicuculline-sensitive, indicating involvement of GABA\(_A\) receptors. These results are broadly consistent with previous anatomical data. On the mitral cell membrane, GABA\(_A\) receptor subunits are associated with granule-mitral synapses within the dendrodendritic reciprocal synapses between mitral cells and granule cell spines (Sassoe-Pognetto and Ottersen 2000). In electron microscopic studies, reciprocal synapses were found to be widely distributed over the soma, primary and secondary dendrites as well as the axonal hillock and initial segment (Mori 1987; Price and Powell 1970a; Rall et al. 1966; Toida et al. 1994, 1996). The photolysis-activated responses are expected to be correlated with the distribution of dendrodendritic synapses, but they could also include an additional component from extrasynaptic receptors. The estimated diameter of the region of local photolysis (\(\sim 2.5 \, \mu m\)) is larger than the mean dimensions of granule cell spines (\(1.36 \times 0.61 \, \mu m\)) (Woolf et al. 1991), so that extrasynaptic membrane would always be exposed to photoreleased GABA.

The GABA responses detected by photolysis on the mitral soma and proximal primary dendrite are likely to include contributions from GABAergic synapses from parvalbumin immunoreactive interneurons (e.g., short axon cells) (Toida et al. 1994, 1996). These interneurons were once thought to disinhibit granule cell dendrites but are now known to synapse exclusively onto projection neurons (mitral and tufted cells) (Crespo et al. 2001). They have been compared with basket cells, which are important for somatic inhibition of cortical pyramidal neurons, and they might act in concert with the axonal inhibitory input to control mitral cell discharge (Douglas and Martin 1990). Inhibitory synaptic contacts on the axon initial segment are well placed to exert control over neuronal excitability. In cortical pyramidal neurons, the initial segment is the primary site of spike initiation (Stuart and Sakmann 1994; Stuart et al. 1997), and it receives axo-axonic GABAergic input from chandelier cells, a major class of parvalbumin-positive interneuron believed to be critical in controlling epileptic discharge (DeFelipe 1999). In the mitral cell, inhibitory synapses on the initial segment and soma would act to shift the site of spike initiation up to the primary dendrite or completely block somatic spike output (Chen et al. 1997).

The estimated density profiles of the GABA-activated conductance, obtained by dividing the dendritic diameter into the space-clamp error-compensated currents, showed a significant difference between the distributions of inhibitory inputs on the primary and secondary dendrites. In five of six primary dendrites (83%), the density clearly decreased with increasing distance from the soma (\(P < 0.001\)), whereas only four of eight secondary dendrites (50%) exhibited a decreasing profile (\(P < 0.001\)); the slopes of the other secondary dendritic profiles were not significantly different from zero (\(P > 0.05\)). Long-range gradients in the density of GABA-activated conductance have been reported previously in the dendrites of hippocampal neurons, using the laser photolysis method (Pettit and Augustine 2000).

The GABA-activated conductance density displayed significant short-range spatial fluctuations along the mitral cell dendrites and axons when measured by the side-scan method at an axial resolution of 3.2 \(\mu m\). This spatial heterogeneity is corroborated by available anatomical evidence that suggests that the density of dendrodendritic synapses per unit length of secondary dendrite is nonuniform. Granule cell spines are concentrated into tightly packed ribbons separated by several micrometers, with the number of spines per micrometer of dendrite varying from 0.7 to 6.0 (Woolf et al. 1991). The local conductance fluctuations observed in the side-scan data lie within this range of density variation. Another study reported that a higher density of synapses exists in a central region of the secondary dendrite compared with proximal and distal regions (Mori 1987), although detailed data were not presented. Sharp local peaks have also been observed in the density profile of functional glutamate receptors along the apical dendrites of layer V neocortical pyramidal neurons (Frick et al. 2001). The functional significance of such receptor clustering remains to be determined.

Range of lateral inhibition

Local inhibition applied by uncaging GABA onto the soma and a proximal domain of the secondary dendrite blocked...
action potentials initiated by injecting depolarizing current into the soma. This provided a direct measurement of the spatial range of lateral inhibitory input to a mitral cell. The maximal range for block of action potential generation during 100-ms current steps varied from 0 to ~140 μm, corresponding to depolarizing currents ranging from ~150 pA down to near spike threshold, at inhibition levels roughly matched to magnitudes of IPSCs from inhibitory interneurons. The maximal range near threshold may be underestimated because photolysis at greater distances was limited by difficulty of visualizing the fine distal dendrite. In a proximal domain out to ~120 μm, the dendrite overlaps 96% of the mitral cell somata connected to the same glomerulus but also a major fraction of those connected to adjacent glomeruli (Buonviso et al. 1991). Thus many inputs from neighboring glomeruli are positioned to be able to block mitral cell firing driven by a wide range of depolarizing currents.

Previously, the range of lateral inhibition between mitral cells was inferred indirectly from in vivo extracellular single-unit recordings of mitral cell odorant responses. The issue was addressed by spatially mapping single-unit responses during foccal electrical stimulation of the feline olfactory bulb (Free- man 1974). This yielded an estimated mean distance of ~570 μm for mitral-granule or granule-mitral transmission. Later studies were done with odorant stimulation. Sequentially recorded hamster mitral cells 400–600 μm apart exhibited opposite response polarities (excitation versus inhibition of spiking) (Meredith 1986), and it was suggested that mitral cells at this separation could inhibit each other. Recordings from the rat found a significantly lower probability of mitral cell responses of the same polarity at 100- to 200-μm separation (Wilson and Leon 1987). Simultaneous recordings from pairs of rat mitral cells separated by 150–200 μm were more likely to have opposite responses, while pairs <40 μm apart were more likely to have responses of the same polarity (Buonviso and Chaput 1990). In the mouse, when single-unit recordings from mitral cells were correlated with odorant-evoked activity patterns in the glomerular layer detected by intrinsic optical imaging, an extensive inhibitory zone was apparent at lateral distances more than ~200 μm from the apical tuft of the recorded cell (Luo and Katz 2001).

The ranges of lateral inhibition obtained in the single-unit studies are not the same as the range of inhibitory input measured here by photolysis. The former involve disynaptic coupling between pairs of mitral cells with signals passing through the secondary dendrites of both the output and the input cells, whereas the photolysis experiments only involve input to one dendrite. Adding the excitatory output pathway should more than double the range because the output involves active backpropagation of action potentials, whereas the inhibitory input relies only on passive conduction. However, the apparent attenuation of the backpropagated action potentials with distance would place limits on the effective output range. It should be noted that the spatial statistics of paired single-unit responses of opposite polarity overestimates the range of disynaptic coupling due to bias from an unknown fraction of uncoupled pairs. If synapses are distributed at random, more widely separated mitral cells will have a reduced coupling probability because their radially symmetric dendritic fields have less overlap (Kishi et al. 1982). The ability of one mitral cell to inhibit another depends not only on their intersomatic separation but also on other unspecified geometric parameters, such as overlap of secondary dendritic fields and the deployment and strength of granule cell dendrodendritic linkages.

The results in Fig. 6 illustrate how the range of spike block by photolysis depends on the relative strengths of somatic excitation and dendritic inhibition. For inhibitory input of fixed strength, the range is decreased by increasing the depolarizing current injected into the soma. Conversely, for a fixed depolarizing current injected into the soma, the range of inhibition increased for stronger inhibitory inputs. Such properties simply reflect the reduced ability of distal inhibition to shunt a somatic current due to leakage along the dendrite. Restated in terms of the geometry of lateral inhibition, it means that the effective range of lateral inhibitory input on mitral cell dendrites should vary during an odorant response as the balance shifts between somatic excitation and dendritic inhibition.

**Backpropagating action potentials in the secondary dendrites**

Direct measurements of voltage signaling in the finely tapered secondary dendrites have only recently been achieved. Such data are critical for understanding how dendritic electrical signaling controls calcium dynamics and glutamate release during dendrodendritic transmission. Both active and passive backpropagation were considered in computer models by Rall and Shepherd (1968), and the available data on extracellular field potentials were consistent with either possibility. Subsequent models of lateral inhibition assumed passive conduction of voltage transients in these dendrites (Meredith 1992; Shep- herd and Brayton 1979). However, a detailed compartmental model of the mitral cell (Bhalla and Bower 1993) incorporated active conductances for backpropagation of spikes in the secondary dendrites.

The dual whole cell recordings show that somatically initiated action potentials do indeed backpropagate into the secondary dendrites at distances of ~90–150 μm from the soma. The amplitudes of the dendritic spikes were attenuated by a factor of ~0.7–0.8 but exhibited little broadening. There was no activity-dependent attenuation or broadening of backpropagated spike trains. Similar properties were observed recently in another study of backpropagation in the secondary dendrites (Margrie et al. 2001). These data contrast with the broadening and strongly activity-dependent attenuation of backpropagated action potentials seen in the apical dendrites of CA1 hippocampal (Spruston et al. 1995) and layer 5 neocortical pyramidal neurons (Stuart et al. 1997). In those cells, the dendritic spike amplitude drops off sharply during a high-frequency spike train.

At least two factors appear to contribute to attenuation and broadening of spikes in the CA1 dendrite: prolonged inactiva- tion of the dendritic Na⁺ conductance and a high density of A-type K⁺ conductance in the dendrite. The mechanism of spike attenuation in the mitral cell secondary dendrite is unknown. The lack of broadening in the recordings of the backpropagated spike (at initial times) indicates that the attenuation is not caused by capacitative loading by the dendritic recording electrode. The attenuation cannot be explained by dendritic tapering because spike amplitude is predicted to be reduced by flaring, not tapering (Goldstein and Rall 1974). The progressive decay and broadening of the dendritically recorded spikes during whole cell recording, compared with the relative stabil-
ity of the somatically recorded spikes indicates that the spike-generating conductances in the dendrite have different properties from those in the soma. The decay might be caused by “washout” of soluble factors regulating the dendritic conductances. In the CA1 apical dendrite, there is evidence that backpropagation is regulated by Ca$^{2+}$-dependent protein phosphorylation (Colbert and Johnston 1998; Tsubokawa et al. 2000). Progressive spike broadening in the dendrites could reflect either a loss in dendritic K$^+$ conductance or a transition from active to passive propagation of the somatic spike associated with a loss of dendritic Na$^+$ conductance.

In this study, dendritic recordings were established only as far as $\sim150\ \mu m$ from the soma. However, Margrie et al. (2001) achieved dendritic recordings $\leq240\ \mu m$ from the soma and noticed a decaying trend in the amplitude versus distance (BPAF, $\sim0.4$ at $240\ \mu m$). If this trend is extrapolated along the length of the secondary dendrite, the backpropagating spikes would have trouble invading the far distal dendrite $\sim1000\ \mu m$ from the soma. Given the vulnerability of dendritic spikes to washout during whole cell recording, it is possible that some of this spatial decrement is caused by accelerated washout and decay of the dendritic conductances at more distal locations where the cytoplasmic volume is smaller. The taper of the dendrites $300$–$500\ \mu m$ from the soma (Charpak et al. 2001; Margrie et al. 2001) suggests that backpropagating spikes can travel at least this far. A spatial gradient in the amplitude of the Ca$^{2+}$ transient was seen without dendritic recording, which argues that the spatial gradient of spike attenuation is not just an artifact of dendritic washout.

The efficiency of spike backpropagation in the secondary dendrites seems to improve during a spike train (Margrie et al. 2001), and this was correlated with enhancement of Ca$^{2+}$ transients in the more distal dendrite. The authors proposed that later action potentials in a spike train penetrate further and activate more distal dendritic output, leading to dynamic regulation of the output range of lateral inhibition. Activity-dependent improvement of backpropagation was not seen here perhaps because the smaller injected currents produced a lower firing rate ($<100\ Hz$) and less tonic depolarization.

The active backpropagation of spikes in the secondary dendrites is most important in shaping long-range or global activity patterns in the bulb. It extends the range of dendritic output and enables precise timing information to be relayed over long distances. The function of the backpropagating spikes is to activate voltage-sensitive calcium channels, triggering dendritic glutamate release. The spatial gradients of spike amplitude, and their activity-dependent regulation, would therefore contribute to the spatial patterning of Ca$^{2+}$ dynamics (Margrie et al. 2001) and dendrodendritic transmission. For example, if Ca$^{2+}$ channels were uniformly distributed along a dendrite, then a spatial gradient of spike amplitude would be translated directly into a spatial gradient of Ca$^{2+}$ influx and glutamate release. The absence of any slowing or broadening of the backpropagated spikes means that the transmitter release will be spatially patterned without distorting its timing.

**Attenuation of backpropagating action potentials by inhibition**

What happens when action potential traffic in the mitral cell secondary dendrites encounters GABAergic inhibition from the dendrodendritic synapses? The answer depends on the location and strength of the inhibitory input. When proximal inhibitory input is sufficiently strong, it simply terminates spiking at the soma by shunting the depolarizing current driving repetitive firing. More distal inhibition fails to block spike initiation at the soma, but if it is strong enough, it can locally attenuate the spikes as they backpropagate into the dendrite.

Attenuation of backpropagating action potentials was demonstrated by focal uncaging of GABA onto the distal dendrite. Although this did not directly show that dendrodendritic inhibition can attenuate spikes, the inhibitory conductances activated by photolysis were approximately matched to the magnitudes of IPSCs from granule cell spines. Evidence that dendrodendritic inhibition can actually attenuate backpropagating action potentials in the distal secondary dendrite comes from fluorometric Ca$^{2+}$ measurements. Margrie et al. (2001) found that during backpropagation of spike trains, Ca$^{2+}$ transients at locations $>300\ \mu m$ from the soma were substantially boosted following bath application of the GABA$_A$ antagonist bicuculline, which blocks dendrodendritic feedback inhibition. This suggests that the feedback inhibition does have a shunting effect on backpropagating action potentials. Thus it appears that at least three different mechanisms determine the spatial profile of spike attenuation along the secondary dendrite: intrinsic attenuation by backpropagation (BPAF); extrinsic GABAergic attenuation by lateral inhibitory inputs (measured here as PAF); and activity-dependent feedback attenuation mediated by dendrodendritic GABAergic inhibition.

The voltage-sensitive calcium channels responsible for glutamate release from mitral cell dendrites have yet to be identified. Spike-evoked dendritic Ca$^{2+}$ transients are known to be sensitive to cadmium but not nifedipine or Ni$^{2+}$, indicating the involvement of high-threshold Ca$^{2+}$ channels. Dendrodendritic feedback inhibition is reduced by $\omega$-conotoxin GVIA, a blocker of N-type Ca$^{2+}$ channels, and $\omega$-conotoxin MVIIIC, a blocker of P/Q-type Ca$^{2+}$ channels (Isaacson and Strowbridge 1998). However, these toxins can act on the disynaptic feedback pathway by blocking the release of GABA from granule cell spines (Isaacson 2001). If glutamate release is dependent on N- and P/Q-type Ca$^{2+}$ channels, then it is likely that the dendritic spike attenuation observed here would reduce calcium influx through these channels and suppress dendrodendritic transmission. These types of Ca$^{2+}$ channels are typically activated at intermediate depolarizations. Isaacson and Strowbridge (1998) measured calcium influx in the secondary dendrite, and the associated dendrodendritic feedback IPSC, induced by 5-ms rectangular voltage pulses. They found that both increased steeply from $-40$ to $0\ mV$, which is within the observed range of amplitude variations of the attenuated dendritic spikes. Therefore spatial patterns of spike amplitude attenuation in the mitral cell secondary dendritic arbor can be translated into spatial patterns of Ca$^{2+}$ influx and transmitter release.

Attenuation of backpropagating action potentials has been observed in the dendrites of other neurons. In CA1 hippocampal pyramidal neurons, it has been shown that local inhibition can attenuate backpropagating spikes in the apical dendrite.
neocortical pyramidal neurons, dendritic Na⁺ regenerative currents in the dendrite. In the apical dendrites of neocortical pyramidal neurons, dendritic Na⁺ and Ca²⁺ spikes can be delayed, attenuated, or blocked by IPSPs (Kim et al. 1995). The role of spike attenuation in the mitral cell secondary dendrite should be contrasted with its role in the apical dendrites of pyramidal neurons. In the latter, signal flow is regarded as primarily orthograde with backpropagating spikes acting as retrograde signals that filter EPSP and IPSP inputs, and modulate their integration. By contrast, the mitral cell secondary dendrites support bidirectional signal flow in a proximal domain and somatofugal signaling in an extensive distal domain. Backpropagating spikes are expected to be the primary output signal for triggering fast transmitter release in these dendrites, and their modulation provides a mechanism for imposing spatial patterns of lateral inhibitory signaling across the bulb.

Implications for olfactory coding

In the olfactory bulb, stimulus-specific information may be encoded in both spatial and temporal patterns of spike activity in the output neurons, the mitral and tufted cells. Spiking in a mitral cell is driven by glutamatergic excitation from sensory afferents projecting to the apical tuft of its primary dendrite, and it is patterned by feedback inhibition and lateral inhibition directed onto its expansive radial field of secondary dendrites. The effect of the inhibition on mitral cell output is related to the location and strength of the inhibitory input. Inhibition applied to the soma or proximal secondary dendrites is highly effective at blocking action potentials and thus exerts the strongest influence on temporal patterning. The radius of the proximal spike-blocking domain of the dendrites depends on a balance between excitation and inhibition. Excitatory input is conducted from the glomerulus to the soma, via the primary dendrite, and inhibitory input is received from surrounding interneurons. Within a module or unit of adjacent mitral cells connected to the same glomerulus, proximal inhibitory zones will be closely overlapped, and increasing glomerular excitation is expected to be balanced by increasing inhibition if there is a shared population of interneurons. This tends to make the spatial range of mutual inhibition invariant for the mitral cells within a module, thus preserving the common temporal code established for that glomerular unit (Buonviso et al. 1992, 1996; Carlson et al. 2000; Schoppa and Westbrook 2001).

Neighboring mitral cells connected to different glomeruli can also have overlapping proximal inhibitory zones but can be driven by glomerular excitation of different strengths. Consider two such neighboring cells, subject to strong and weak glomerular excitation. The firing pattern of the strongly excited mitral cell would be relatively unaffected by weaker inhibition received from interneurons connected to the weakly excited mitral cell. Its radius of proximal spike block would be contracted and exclude the weakly excited mitral cell. On the other hand, the weakly excited mitral cell would have an expanded range of proximal inhibition that encompasses the strongly excited mitral cell. Therefore its firing pattern can be directly altered by inhibition from interneurons activated in the vicinity of the soma of the strongly excited mitral cell. This means that lateral inhibition between neighboring modules tends to be polarized, and it should be described by a vector field over the glomerular array. The vector field may be dynamic, because lateral inhibition may switch polarity when the local ratio of excitation to inhibition varies over time during a phasic odor stimulus or following feedback inhibition. Such polarity switching might enhance the sensitivity of the system to subtle shifts in complex blends of odors.

Lateral inhibition between neighboring cells has been proposed to sharpen the spatial contrast of odorant-specific glomerular activity patterns (Buonviso and Chaput 1990; Meredith 1992; Rall and Shepherd 1968; Shepherd 1991). A strongly activated mitral cell suppresses the activity of neighboring mitral cells receiving weaker input in a radial scheme reminiscent of the center-surround organization of ganglion cells in the retina (Kuffler 1953; Luo and Katz 2001). The polarizing effect of differential excitation on the range of lateral inhibition would tend to augment this radial suppression of surrounding cells. The relevance of spatial contrast enhancement depends on how mitral cell output is packaged and delivered to neurons upstream in olfactory cortex. Spatial contrast would be preserved, for example, by uniform encoding and decoding of data as absolute firing rates across all glomeruli with lateral inhibition acting to subtructively reduce relative firing rates (Holt and Koch 1997). The picture becomes more complicated if meaningful information is contained in time-correlated firing of ensembles of mitral cells. Precise temporal correlations in the spike trains may be folded into the raw spatial pattern relayed through the bulbofugal projections and read out by coincidence detection mechanisms in the dendrites of cortical neurons. In the insect antennal lobe, it has been proposed that such temporal correlations are responsible for fine odor discrimination (Stopfer et al. 1997), and computer models show that fast GABAergic inhibition from local interneurons is capable of transiently synchronizing the projection neurons (Bazhenov et al. 2001). In mitral cells, transient inhibition could have a dual function: it could modulate a rate code, and it could alter temporal correlations between interconnected cells by resetting the timing of intrinsic membrane oscillations (Desmaisons et al. 1999).

Long-range lateral signaling in the distal domain of the secondary dendrites relies exclusively on backpropagated action potentials. In this domain, inhibitory input is unlikely to exert a strong influence on the temporal coding of spike output from the soma, but it is likely to affect the spatial patterning of lateral inhibitory output, by locally attenuating action potentials and reducing glutamate release at dendrodendritic synapses. In this study, local inhibition by GABA photorelease attenuated but did not cause a complete conduction block of dendritic action potentials. A high safety factor for propagation allows the secondary dendrite to receive and integrate inhibitory inputs without disrupting transmission of long distance output signals. It remains to be seen whether inhibition from granule cells or other interneurons in the more distal secondary dendrite can be strong enough to completely block backpropagation (Bhalla and Bower 1993). Complete block would disrupt global signaling and multiply the number of states available to the olfactory bulb neural network.

NOTE ADDED IN PROOF

While this manuscript was in press, Xiong and Chen (2002) reported that inhibition of the secondary dendrite can indeed block the

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backpropagation of action potentials. They applied inhibition by puffing 1 mM GABA onto the dendrite and by delivering electric shocks to the underlying granule cell layer, methods which are likely to activate stronger, more widespread inhibition than was achieved by the focal uncaging method used here. Thus varying levels of inhibitory input to the dendrite might exert a full range of effects on backpropagating spikes, from partial attenuation to complete blockade. They were also able to record unattenuated spikes up to ~600 μm from the soma and suggested that amplitude attenuations observed in some dendritic spikes may be due to varied recording conditions.

The author is very grateful to B. M. Salzberg, L. M. Masukawa, M. Meister, and A. Gelperin for valuable comments on the manuscript. D. J. Perkel and K. Khodakhah provided early advice and discussion about technical issues, and their help was greatly appreciated. Critical comments from anonymous reviewers of this and an earlier version of the manuscript were also most helpful and are gratefully acknowledged.

This work was supported by a gift from the Ambrose Monell Foundation, the Morley R. Kare Fellowship, and National Institute on Deafness and Other Communication Disorder Grants RO1 DC-00505-12 and DC-04208-01.

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