



## Reliability of Spike Timing in Neocortical Neurons

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- screen of the microscope. Between 150 to 300 nerve fibers were analyzed per cross section.
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  14. Addition of progesterone (dissolved in ethanol; final concentration of ethanol, 0.1%) daily to a final concentration of 20 nM to culture medium for 2 weeks did not increase the area occupied by the neurite network extending around DRG explants (control,  $20.6 \pm 1.4$ ; progesterone,  $19.7 \pm 1.2$  mm<sup>2</sup>), the density of neurites (control,  $422 \pm 24$ ; progesterone,  $397 \pm 35$  mm/mm<sup>2</sup>), or the number of Schwann cells (control, 2500  $\pm$  193; progesterone, 2625  $\pm$  104 cells per mm<sup>2</sup>), measured after staining the explants with toluidine blue. Measurements were made with an imaging system (Biocom, RAG version 2). Results are means  $\pm$  SEM of five culture dishes, each containing 10 DRG explants.
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  24. Steroids (pregnenolone, progesterone, and corticosterone) in sciatic nerve and plasma were measured by RIA. Fourteen nerves were pooled for each sample. The RIA procedures have been described and validated (4) [C. Corpéchet *et al.*, *Endocrinology* **133**, 1003 (1993)]. The progesterone antiserum cross-reacts with 5 $\alpha$ -dihydroprogesterone (32%); therefore, our assay includes both progestins, which are potent activators of gene expression via the intracellular progesterone receptor [R. Rupprecht *et al.*, *Neuron* **11**, 523 (1993)].
  25. Rat Schwann cells were prepared from DRG cultures as described (13), with minor modifications. During the first week, DRG explants were cultured in Dulbecco's modified Eagle's medium-Ham's F12 (50:50, v/v) containing 10% fetal bovine serum and nerve growth factor (30 ng/ml), and were treated for 3 days with the antimetabolic agent cytosine arabinoside (10  $\mu$ M) to eliminate all cells other than sensory neurons and Schwann cells. Subsequently, the explants were grown for an additional 3 weeks in defined serum-free medium (8). The outgrowth of axons induces Schwann cell growth [J. L. Salzer and R. P. Bunge, *J. Cell Biol.* **84**, 739 (1980)], resulting in the generation of large numbers of surrounding Schwann cells. The ganglia were then cut out of the cultures with a microscalpel. The remaining pure Schwann cells were stained as described [S. M. Hsu, L. Raine, H. Fanger, *J. Histochem. Cytochem.* **29**, 577 (1981)] with a polyclonal rabbit antiserum to a synthetic peptide common to the four known isoforms of rat 3 $\beta$ -HSD (22).
  26. Schwann cells from DRG explants were harvested by exposure to trypsin and seeded in new culture dishes. After 48 hours, the cells were incubated in triplicate with 100 nM [7-<sup>3</sup>H(N)]pregnenolone (NEN, 925 GBq/mmol) at 37°C for 24 hours. Steroids were extracted from the incubation medium with ethyl acetate:isooctane (1:1, v/v) and separated by thin-layer chromatography (TLC) on silica gel plates developed once in chloroform:ethyl acetate (4:1, v/v). Quantitation of radioactive areas on TLC plates was performed with an automatic TLC linear analyzer. Steroids were further characterized by high-pressure liquid chromatography on a reversed-phase C<sub>18</sub> octadecylsilane column and by recrystallization (4) [Y. Akwa *et al.*, *J. Cell Biol.* **121**, 135 (1993)].
  27. Neuron-Schwann cell cultures of rat DRG were prepared as described (25). After 4 weeks in defined medium, cells were cultured for an additional 2 weeks in myelination-promoting medium [defined medium containing 10% fetal bovine serum and L-ascorbic acid (50  $\mu$ g/ml)], in which Schwann cells differentiate rapidly and myelinate the DRG axons. Ascorbic acid is necessary for the Schwann cells to assemble a basal lamina, a prerequisite for myelination (13).
  28. Myelinated fibers were stained with Sudan black after fixing the cultures with paraformaldehyde (4%) and osmium (0.1%) [N. Kleitman, P. M. Wood, R. P. Bunge, in *Culturing Nerve Cells*, G. Banker and K. Goslin, Eds. (Bradford Books, London, 1991), pp. 337-377]. Myelin formation was quantified as described (13) with an imaging system (Biocom, RAG version 2). Electron microscopic studies confirmed that the myelin formed in vitro was morphologically normal.
  29. We thank Roussel-Uclaf for antisera to corticosterone (Ab 524) and progesterone (Ab 1093); J. Fiet for the antiserum to pregnenolone; B. Delespierre for technical assistance; C. Corpéchet for help with the RIAs; V. Meremans for help with the analysis of axon diameters; and J.-C. Lambert and L. Outin for editorial assistance. Supported in part by the Association Française contre les Myopathies.

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## Reliability of Spike Timing in Neocortical Neurons

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It is not known whether the variability of neural activity in the cerebral cortex carries information or reflects noisy underlying mechanisms. In an examination of the reliability of spike generation using recordings from neurons in rat neocortical slices, the precision of spike timing was found to depend on stimulus transients. Constant stimuli led to imprecise spike trains, whereas stimuli with fluctuations resembling synaptic activity produced spike trains with timing reproducible to less than 1 millisecond. These data suggest a low intrinsic noise level in spike generation, which could allow cortical neurons to accurately transform synaptic input into spike sequences, supporting a possible role for spike timing in the processing of cortical information by the neocortex.

Neurons transmit information by transforming continuously varying input signals into trains of discrete action potentials. The coding scheme used in this process is an unresolved issue that is critical to computational theories of brain function. Codes that utilize spike timing (1, 2) can make more efficient use of the capacity of neural connections than those that simply rely on the average rate of firing (3). The simplest spike-timing code would be one output pulse for each input pulse, but synaptic currents in the cortex are too small and intracellular recordings in vivo look very noisy (4). Furthermore, cortical activity is characterized by highly irregular interspike intervals in both spontaneous (5) and stimulus-evoked conditions (6). These observations have led some to conclude that only statistical averages of many inputs carry useful information between cortical neurons (7). Another possibility, which we explore here, is that cortical neurons may respond reliably to relatively weak input fluctuations. Irregularity in spike timing may then reflect the presence of information. This is possible only if the intrinsic noise within neurons is small. Although some earlier

studies have suggested that neurons may have low intrinsic noise (8, 9), others have argued to the contrary (10).

The aim of the present report was to determine directly the temporal precision with which cortical neurons are capable of encoding a stimulus into a spike train. A rat cortical slice preparation was chosen so that the state of a single neuron and its input could be well controlled experimentally (11). Somatic whole-cell recordings were made in the current-clamp configuration (12), and spike trains were elicited with current injected through the recording electrode, near the presumed site of generation of action potentials (13). We assessed reliability by repeatedly presenting the same stimulus and evaluating the consistency of the evoked spike sequences.

First, repetitive firing was evoked with flat (dc) current pulses (0 to 250 pA, 0.9 s; Fig. 1A). The variability of spike counts from trial to trial was small [coefficient of variation (CV) =  $0.10 \pm 0.13$ ; mean  $\pm$  SD;  $n = 10$  cells]. However, the small variances in interspike intervals (ISIs) summed to increase the desynchronization of corresponding action potentials over the course of the stimulus. The first spike of each train was tightly locked to the onset of the pulse (SD =  $0.62 \pm 0.25$  ms;  $n = 8$ ), whereas the timing of the last spike in the train was highly variable (SD =  $31 \pm 19$  ms;  $n = 8$ ). Thus, responses to flat pulse stimuli indicate reliability of spike count or average firing

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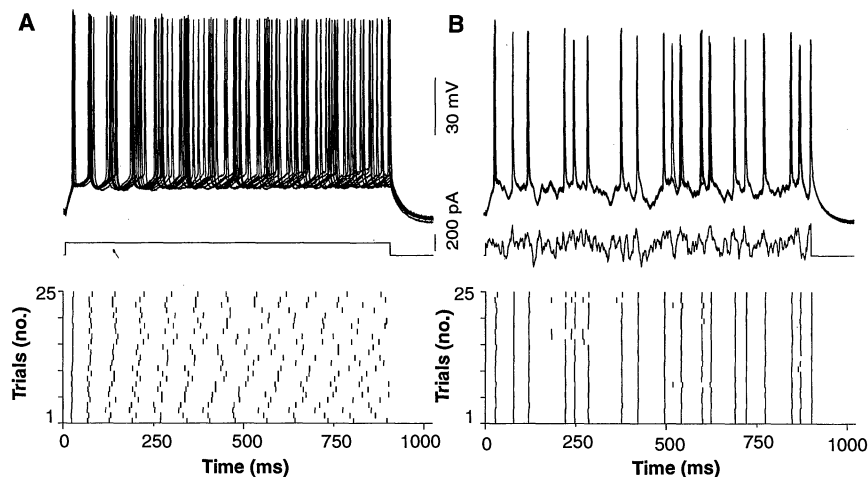
rate but lack of reliability in precise timing, as measured relative to stimulus onset.

Intracellular recordings from cortical neurons *in vivo* reveal large and rapid fluctuations of the membrane potential from many synaptic events (4). The integration of many independent excitatory and inhibitory synaptic currents would be expected to approach a Gaussian distribution at the soma. Accordingly, sequences of filtered Gaussian white noise generated by computer were added to the constant depolarizing pulse (14). As with flat pulse stimuli, spike count showed little variability ( $CV = 0.052 \pm 0.029$ ;  $n = 10$ ). In contrast to the case

with flat pulse responses, when any particular fluctuating current waveform was repeatedly injected the pattern of spikes elicited showed precise and stable timing throughout the length of the trial (Fig. 1B). Occasionally, from trial to trial, spikes could appear, disappear, or abruptly shift tens of milliseconds. In some cases, a single dropped or added spike disrupted the timing of several consecutive subsequent spikes. This behavior made it problematic to compute directly the variability in ISIs or the timing of a particular spike number; therefore, in further analysis we used the peristimulus time histogram (PSTH; Fig. 2A).

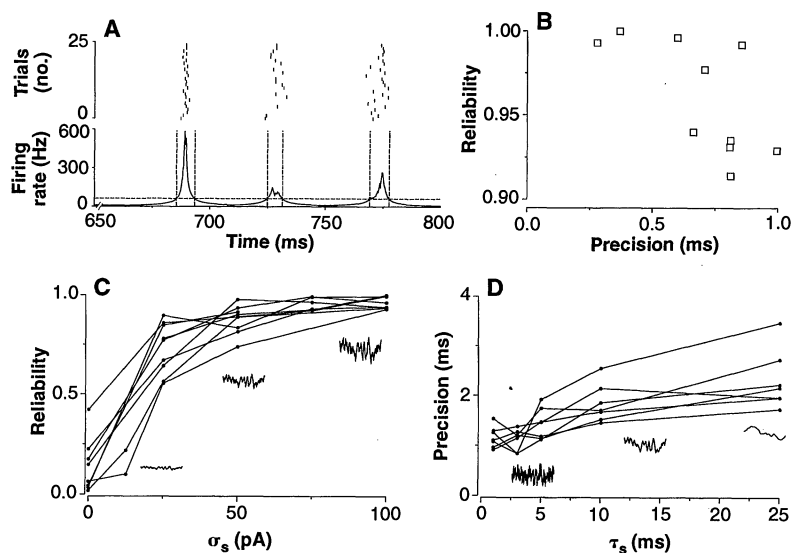
Highly reproducible firing patterns were a robust phenomenon in the presence of stimulus fluctuations. Two measures of spike timing were calculated from the PSTH, which we termed the "reliability" and the "precision" (Fig. 2A). According to these measures, all the cells analyzed were capable of responding to fluctuating input currents with nearly 100% of spikes (high reliability) in clusters with an SD of less than 1 ms (high precision; Fig. 2B). The reliability of spike patterns was strongly correlated with the amplitude of stimulus fluctuations,  $\sigma_s$  (Fig. 2C). The firing rate also increased with the amplitude of fluctuations, particularly for cells showing strong adaptation to dc stimulation (15). The precision of spike timing depended on the time constant of stimulus fluctuations,  $\tau_s$  (Fig. 2D). Precision and reliability dropped as stimuli were filtered at time constants increasing from 1 to 25 ms. The precision of most responses was in the range of 1 to 2 ms, a time scale much smaller than both the maximum firing rate of these cells and the time constant of fluctuations in the stimuli (16). The decrease in precision was paralleled by a reduction of reliability (15).

The reproducibility of spike patterns suggested that spikes were triggered preferentially by particular patterns of depolarizing and hyperpolarizing current in the stimulus. A reverse correlation of spike train and stimulus [spike-triggered average of the stimulus; see (17)] can reveal the stimulus waveform that tends to precede the generation of an action potential and can indicate the length of stimulus history that is relevant. Reverse correlations showed a strong tendency for spikes to be preceded by



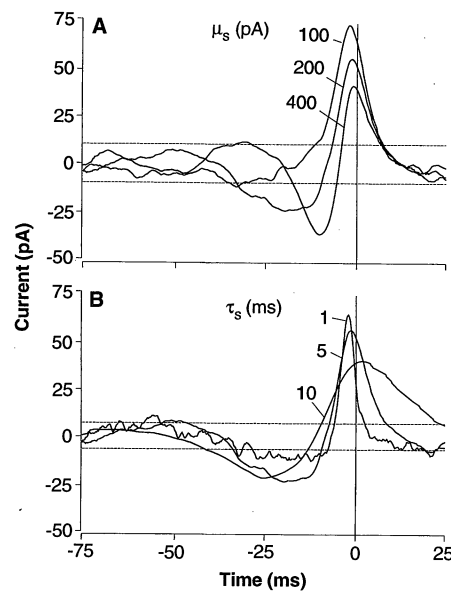
**Fig. 1.** Reliability of firing patterns of cortical neurons evoked by constant and fluctuating current. **(A)** In this example, a superthreshold dc current pulse (150 pA, 900 ms; middle) evoked trains of action potentials (approximately 14 Hz) in a regular-firing layer-5 neuron. Responses are shown superimposed (first 10 trials, top) and as a raster plot of spike times over spike times (25 consecutive trials, bottom). **(B)** The same cell as in **(A)** was again stimulated repeatedly, but this time with a fluctuating stimulus [Gaussian white noise,  $\mu_s = 150$  pA,  $\sigma_s = 100$  pA,  $\tau_s = 3$  ms; see (14)].

**Fig. 2.** Dependence of the reliability and precision of spike timing on stimulus current statistics. **(A)** The PSTH of spikes collected over 20 to 25 successive presentations of a particular stimulus waveform was used to quantify the consistency of spike patterns evoked by fluctuating stimuli of different types. Spikes during the first 100 ms after stimulus onset, during which most spike frequency adaptation occurred, were discarded. We smoothed the PSTH using an adaptive filter (centered on each time step and widened to capture 10 spikes) to yield an estimate of the instantaneous firing rate. A threshold (horizontal dotted line, set at three times the mean firing rate of the cell over a given block of responses) was used to select dramatic elevations in instantaneous firing rate, or "events." Because the minimum ISI was long compared to the duration of these events, at most one spike occurred during any event on any trial. We defined "reliability" as the fraction of total spikes that occurred during such periods of elevated firing rate. We defined temporal "precision" as the SD of spike times within any event, averaged over all events during a response. **(B)** Each square represents the most reliable block of responses recorded in one of ten cells (eight regular firing, two intrinsic bursting). For these responses, a stimulus mean,  $\mu_s$ , between 100 and 300 pA and a fluctuation amplitude,  $\sigma_s$ , between 50 and 100 pA were used, yielding firing rates between 14 and 24 Hz. **(C)** Estimates of reliability for stimuli with various amplitudes of stimulus fluctuations. Each line on the graph connects measurements made for one of nine cells examined at four or five different values of  $\sigma_s$  ( $\mu_s$  for these blocks was 100 to 300 pA, giving firing rates between 8 and 24 Hz). The input resistance of neurons examined was  $222 \pm 85$  megohms;



peak-to-peak voltage transients produced by these currents were less than 25 mV. **(D)** The temporal precision of responses obtained in seven cells for stimuli filtered at different time constants ( $\tau_s = 1$  to 25 ms,  $\sigma_s = 25$  to 50 pA,  $\mu_s = 100$  to 200 pA). The average membrane time constant for these cells was  $29.7 \pm 5.9$  ms.

**Fig. 3.** Reverse correlations (spike-triggered stimulus averages) were computed over 25 consecutive trials for which stimuli were generated with equivalent parameters ( $\mu_s$ ,  $\sigma_s$ ,  $\tau_s$ ) but different random seed [see (14)]. **(A)** Reverse correlations from blocks of trials with mean amplitudes shown ( $\sigma_s = 50$  pA,  $\tau_s = 3$  ms). Firing frequency ranged from 10 to 25 Hz (corresponding to 225 to 554 spikes averaged). **(B)** Reverse correlations obtained with the different time constants of stimulus filtering shown ( $\mu_s = 200$  pA and  $\sigma_s = 50$  pA; 282 to 322 spikes averaged). For (A) and (B) the trigger point ( $t = 0$ , vertical line) was at the inflection in the rising phase of the spike. The current values shown are relative to the mean current ( $\mu_s$ ). For a neuron generating spikes randomly, the average stimulus preceding a spike is not expected to differ from the average stimulus in general, which approaches a flat line with increasing samples. Departure from this expectation reveals a preference for particular stimulus waveforms. Confidence limits (dashed horizontal lines) were calculated as described in (9), and only the widest limits are shown. The data are from a single neuron for which reverse correlations were collected at a variety of stimulus parameters. Similar



a depolarizing transient. At greater mean input currents ( $\mu_s$ ), the average depolarizing transient was reduced while a preceding hyperpolarizing transient was introduced (Fig. 3A). Varying the time constant of stimulus filtering revealed a preference for maximum stimulus slope 5 to 10 ms preceding the spike (Fig. 3B). Therefore, the time course of the reverse correlations was broadened by filtering of the stimulus, but the basic shape was preserved. Transients of this amplitude correspond to the arrival of about 10 excitatory postsynaptic currents of 5 to 10 pA within 10 ms.

These data demonstrate that repetitive firing in neocortical neurons is sufficiently reliable that currents resembling synaptic input may be repeatedly encoded into spike patterns with millisecond precision. Therefore, it is likely that the intrinsic variability of the spike-generating currents and their susceptibility to nonsynaptic background noise make only minimal contributions to interspike interval variability under in vivo conditions.

Spike-triggered stimulus averages suggest that consistent temporal coding follows in part from a greater sensitivity of spike generation to transients than to steady-state depolarization. This analysis also indicates that properly timed hyperpolarizing events may increase the firing probability, possibly through a reduction of sodium channel inactivation. Stimuli without transients may be encoded reliably with respect to the mean rate but not with respect to the exact timing of spikes. The behavior observed is roughly compatible with a deterministic leaky-integrator or Hodgkin-Huxley model with a fixed level of additive background noise (18). However, other mechanisms such as spike frequency adaptation may

contribute to the observed reliability.

We have deliberately isolated one step in the sequence of electrical and chemical events involved in the propagation of a neural signal. Although we find that reliable spike trains may be elicited by injected currents resembling integrated synaptic inputs, we have not addressed unreliability at other steps in the signaling process, particularly in synaptic transmission (19). Such variability would be expected to erode the fidelity of temporal coding but may be mitigated by particular activity patterns (20). Evidence for rapid modulation of firing rate (21) and repetition of particular spike interval patterns (1) suggests that it is possible for the neocortex to overcome these sources of noise.

Neurons in the peripheral auditory system can encode information on the basis of the timing of individual spikes (22). Although our finding that neocortical neurons also have the ability to generate precisely timed firing patterns does not prove that this timing has a physiological significance, it is consistent with theories of cortical information processing in which spike timing is important.

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- As the purpose was to isolate the process of spike generation from synaptic transmission and dendritic integration, care was taken to eliminate sources of variability extrinsic to spike-generating currents themselves. To reduce spontaneous synaptic activity, D-2-amino-5-phosphonovaleric acid (D-APV; 20  $\mu$ M) and 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10  $\mu$ M) were used to block glutamate receptors, and bicuculline methiodide (BMI; 5  $\mu$ M) was used to block  $\gamma$ -aminobutyric acid (GABA<sub>A</sub>) receptors. To mitigate the effects of possible long-term drift in recording conditions, reliability was measured over blocks of consecutive trials recorded during a period of less than 2 min, and blocks showing obvious instability (fluctuations in membrane potential or input resistance) were not included in the analysis. Data are reported for cells in which recordings were sufficiently stable and long-lasting to permit examination of repeated responses to stimuli of a range of parameters. Nevertheless, even under these conditions, relatively small sources of uncontrolled noise were still present. For instance, the background voltage noise measured over 200-ms periods at resting potential ( $-68.4 \pm 5.1$  mV,  $n = 10$ ) was in the range of 0.05 to 0.5 mV (root mean square).
- Coronal slices of occipital cortex (400  $\mu$ m) were prepared from Sprague-Dawley rats 14 to 24 days old deeply anesthetized with ether and decapitated. After 1 to 6 hours incubation in an interface chamber, a slice was transferred to a submerged chamber (22° to 24°C) for recording and continuously perfused with oxygenated (95% O<sub>2</sub>, 5% CO<sub>2</sub>) Ringer solution containing (in millimolar) 126 NaCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 10 D-glucose, 2.5 KCl, 2 MgCl<sub>2</sub>, 2 CaCl<sub>2</sub>, and 26 NaHCO<sub>3</sub>. Tight-seal whole-cell recordings were obtained from pyramidal-shaped neurons of layer 5 under visual control [G. J. Stuart, H.-U. Dodt, B. Sakmann, *Pfluegers Arch. Gesamte Physiol. Menschen Tiere* **423**, 511 (1993)]. Patch pipettes (3 to 8 megohms, thin-walled borosilicate glass, wax-coated to reduce capacitance) contained (in millimolar) 100 potassium gluconate, 25 KCl, 5 NaCl, 10 Hepes, 0.2 EGTA, 4 adenosine triphosphate, and 0.3 guanosine triphosphate; pH 7.2 with KOH. We recorded whole-cell potentials using a patch-clamp amplifier (Axopatch 200a) in the "fast" current-clamp mode, filtered at 1 kHz and digitized at 4 to 16 kHz. Trials were 1024 ms in duration and were col-

- lected at intervals of 3 to 4 s. We performed bridge balance digitally off line using hyperpolarizing pulses of 50 to 100 pA preceding each trial. We detected spike times using thresholding of the first or second time derivative of the voltage. For all data collection and analysis we used Sun workstations with custom software written in C++ based on NEURON [M. Hines, in *Neural Systems: Analysis and Modeling*, F. H. Eeckman, Ed. (Kluwer, Boston, 1993), pp. 127–136].
13. G. J. Stuart and B. Sakmann, *Nature* **367**, 69 (1994).
  14. The arrival of many uncorrelated excitatory and inhibitory synaptic events will deliver a total current to a neuron that may be treated approximately as shot noise [S. Rice, in *Selected Papers on Noise and Stochastic Processes*, N. Wax, Ed. (Dover, New York, 1954), pp. 133–294]. The event rates and their amplitude waveforms determine the mean, variance, and frequency spectrum of the net current. Accordingly, the stimuli used were realizations of Gaussian white noise with chosen mean ( $\mu_s$ ) and SD ( $\sigma_s$ ) of fluctuations. Convolution with the function  $f(t) = t \exp(-t/\tau_s)$  gave low-pass filtering with a time constant  $\tau_s$ , as could be expected from synaptic time courses and dendritic filtering. Unless otherwise noted,  $\tau_s$  was 3 ms. The range of  $\sigma_s$  investigated, 0 to 100 pA, produced voltage transients up to about 25 mV peak to peak.
  15. Z. F. Mainen and T. J. Sejnowski, data not shown.
  16. There was no systematic relation between  $\mu_s$  and reliability over the range of values investigated (50 to 300 pA producing a firing rate of 4 to 32 Hz), although in some cells reliability did increase or decrease with  $\mu_s$ .
  17. The reverse correlation reported is similar to the first-

- order response kernel of the neuron [P. Z. Marmarelis and V. Z. Marmarelis, *Analysis of Physiological Systems: The White Noise Approach* (Plenum, New York, 1978)].
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  23. We are grateful to T. Zador, C. F. Stevens, C. Koch, and W. Bair for insightful comments and discussion. Z.F.M. is a Howard Hughes Medical Institute predoctoral fellow.

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## Chemical Characterization of a Family of Brain Lipids That Induce Sleep

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A molecule isolated from the cerebrospinal fluid of sleep-deprived cats has been chemically characterized and identified as *cis*-9,10-octadecenoamide. Other fatty acid primary amides in addition to *cis*-9,10-octadecenoamide were identified as natural constituents of the cerebrospinal fluid of cat, rat, and human, indicating that these compounds compose a distinct family of brain lipids. Synthetic *cis*-9,10-octadecenoamide induced physiological sleep when injected into rats. Together, these results suggest that fatty acid primary amides may represent a previously unrecognized class of biological signaling molecules.

The pursuit of endogenous sleep-inducing substances has been the focus of an extensive, complicated body of research (1). Several compounds, including delta-sleep-inducing-peptide (2) and prostaglandin PGD<sub>2</sub> (3), have been suggested to play a role in sleep induction, and yet, the molecular mechanisms of this physiological process remain largely unknown. We analyzed the cerebrospinal fluid of cats in search of com-

pounds that accumulated during sleep deprivation. A molecule with the chemical formula C<sub>18</sub>H<sub>35</sub>NO was isolated from the cerebrospinal fluid of sleep-deprived cats (4). The compound's structural features, two degrees of unsaturation, a long alkyl chain, and a nitrogen substituent capable of primary fragmentation as ammonia, were most compatible with either a nonconjugated diene in which a primary amine was allylic (4) or a monounsaturated alkane chain terminating in a primary amide (5).

Initial electrospray mass analysis of the natural compound revealed mass peaks of  $m/z$  282 ([M + H]<sup>+</sup>), 304 ([M + Na]<sup>+</sup>), 320 ([M + K]<sup>+</sup>), and 564 ([2M + H]<sup>+</sup>), indicating that the molecular mass of the compound was 281 daltons (4). High-reso-

lution fast atom bombardment-mass spectrometry (FAB-MS) analysis indicated that the exact mass measurement of the [M + Na]<sup>+</sup> ion was  $m/z$  304.2614 ± 0.0006 daltons. This measurement allowed for the determination of elemental composition and a best fit for the molecular formula C<sub>18</sub>H<sub>35</sub>NO, which has a calculated [M + Na]<sup>+</sup>  $m/z$  of 304.2616 daltons. Tandem mass spectrometry analysis (MS-MS or MS<sup>2</sup>) revealed a distinctive fragmentation pattern in the low molecular mass range consistent with other long chain alkanes (Fig. 1A). Sequential neutral loss of 17 and 35 mass units from the parent ion indicated the loss of ammonia followed by the loss of water. Additional MS<sup>3</sup> experiments were performed on the daughter ions of  $m/z$  265 and 247 (4).

Such MS<sup>2</sup> and MS<sup>3</sup> analyses were also performed on various synthetic candidate structures (6), and although several products gave spectra quite similar to those of the natural compound, only the fragmentation patterns generated from monounsaturated fatty acid amides, such as *cis*-9,10-octadecenoamide (Fig. 1B), matched exactly those of the endogenous lipid. Of interest was the neutral loss of 17 mass units from the parent ion of *cis*-9,10-octadecenoamide, indicating that the molecule first fragments at the carbon-nitrogen bond of its terminal amide group to release ammonia. Mass analysis also identified a compound from the cerebrospinal fluid of human and rat with the molecular formula C<sub>22</sub>H<sub>43</sub>NO with MS<sup>2</sup> and MS<sup>3</sup> fragmentation patterns indistinguishable from those of synthetic *cis*-13,14-docosenoamide (Fig. 1, C and D) (7).

*Cis*-9,10-octadecenoamide and the C<sub>18</sub> natural lipid exhibited identical elution properties on thin-layer chromatography (TLC) (8) and gas chromatography-mass spectrometry (GC-MS) (9). However, these techniques proved insensitive to the position and configuration of the olefin of closely related synthetic fatty acid amides, and the *cis*-8,9- (Fig. 2, 3), *cis*-9,10- (1), *cis*-11,12- (4), and *trans*-9,10- (2) octadecenoamides were not distinguishable from the natural compound by TLC and GC (10). Through infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and chemical degradation procedures, the exact structure of the endogenous lipid, including the position and configuration of its olefin, was unambiguously determined. The position of the double bond along the alkyl chain of the natural compound was determined by ozonolysis (11). GC-MS analysis of the ozonolysis reaction mixture derived from the natural lipid revealed nonyl aldehyde as the only CH<sub>3</sub>-terminal aldehyde present. Nonyl aldehyde corresponds to an olefin located at the 9,10 position of the C<sub>18</sub> fatty acid primary amide.

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