Two Types of Intrinsic Oscillations in Neurons of the Lateral and Basolateral Nuclei of the Amygdala

HANS-CHRISTIAN PAPE, 1 DENIS PARE, 2 AND ROBERT B. DRIESANG 1

1 Institut für Physiologie, Medizinische Fakultät, Otto-von-Guericke-Universität, D-39120 Magdeburg, Germany; and 2 Département de Physiologie, Faculté de Médecine, Université Laval, Québec G1K 7P4, Canada

Pape, Hans-Christian, Denis Paré, and Robert B. Driesang. Two types of intrinsic oscillations in neurons of the lateral and basolateral nuclei of the amygdala. J. Neurophysiol. 79: 205–216, 1998. Intracellular recordings in the guinea pig and cat basolateral amygdaloid (BL) complex maintained as slices in vitro revealed that a subpopulation of projection neurons (79%) in the lateral (AL) and basolateral (ABL) nuclei generated two types of slow oscillations of the membrane potential upon steady depolarization from resting potential. The cells were of a stellate or pyramidal-like shape and possessed spiny dendrites and an axon leaving the local synaptic environment, and thus presumably represented projection neurons. Similar oscillatory activity was observed in projection neurons of the cat AL nucleus recorded in vivo. Oscillatory activity with a low threshold of activation (low-threshold oscillation, LTO) appeared as rhythmic deflections (amplitudes, 2–6 mV) of the membrane potential positive to −60 mV. Fast Fourier transformation (FFT) demonstrated a range of frequencies of LTOs between 0.5 and 9 Hz, with >80% occurring at 1–3.5 Hz and an average at 2.3 ± 1.1 Hz. LTOs were more regular after pharmacological blockade of synaptic transmission and were blocked by tetrodotoxin (TTX). Blockade of LTOs and Na+ spikes revealed a second type of oscillatory activity (high-threshold oscillation, HTO) at depolarizations beyond −40 mV, which was capable of triggering high-threshold spikes. HTOs ranged between 1 and 7.5 Hz, with >80% occurring at 2–6 Hz and an average at 5.8 ± 1.1 Hz. HTOs vanished at a steady membrane polarization positive to −20 mV. Current versus voltage relations obtained under voltage-clamp conditions revealed two regions of negative slope conductance at −55 to −40 mV and at around −30 mV, which largely overlapped with the voltage ranges of LTOs and HTOs. TTX abolished the first region of negative slope conductance (−55 to −40 mV) and did not significantly influence the second region of negative slope conductance. Neuronal responses to maintained depolarizing current pulses consisted of an initial high-frequency discharge (up to 100 Hz), the frequency of which depended on the amplitude of the depolarizing current pulse, followed by a progressive decline (‘adaptation’) toward a slow-rhythmic firing pattern. The decay in firing frequency followed a double-exponential function, with time constants averaging 57 ± 28 ms and 3.29 ± 1.85 s, and approached steady-state frequencies at 6.3 ± 2.9 Hz (n = 17). Slow-rhythmic firing remained at this frequency over a wide range of membrane polarization between approximately −50 and −20 mV, although individual electrogenic events changed from Na+ spikes and underlying LTOs to high-threshold spikes and underlying HTOs. Rhythmic regular firing was only interrupted at an intermediate range of membrane polarization by the occurrence of spike doubles. In conclusion, the integrative behavior of a class of neurons in the BL complex appears to be largely shaped by the slow-oscillatory properties of the membrane. While LTOs are likely to synchronize synaptic signals near firing threshold, HTOs are a major determinant for the slow steady-state firing patterns during maintained depolarizing influence. These intrinsic oscillatory mechanisms, in turn, can be assumed to promote population activity at this particular frequency, which ranges well within that of the limbic theta (θ) rhythm and the delta (δ) waves in the electroencephalogram during slow-wave sleep.

INTRODUCTION

Recent anatomic, physiological, and behavioral findings have lead to significant insights into the functions of the amygdala in emotional and social behavior (Aggleton 1992; Davis 1992; LeDoux 1991). In addition, there is growing evidence that dysfunctions of the amygdala contribute to certain clinical conditions, including anxiety disorders, dementia, and schizophrenia (Aggleton 1993). In view of the important role of the amygdala, a more detailed understanding of the underlying cellular mechanisms is required, particularly of the intrinsic membrane properties of the neurons. In fact, neurons in the mammalian brain have been found to possess a large variety of distinctive membrane conductances, which regulate the flow of ionic currents across the plasma membrane and thereby determine the generation of spontaneous electrical activity (Llinás 1988). One consequence of these interactions is the generation of rhythmic membrane potential deflections in individual neurons that may give rise to oscillatory population rhythms through the synchronizing action of the synaptic network (Jefferys et al. 1996; Steriade et al. 1990). Synchronized rhythmic electrical activity, in turn, is considered an important element in the specific integrative properties of defined assemblies of neurons, which may either dictate or reflect the functional state of the brain (Adrian and Matthews 1934; Jefferys et al. 1996; Steriade et al. 1990). One form of rhythmic activity, occurring at a frequency between 2 and 15 Hz, appears to be a widespread phenomenon in the CNS (Alonso and Klink 1993; Alonso and Llinás 1989; Llinás 1988; Silva et al. 1991). These oscillations can act as a functional synchronizing or timing device of intrinsic and synaptic input signals (Lampl and Yarom 1993) to promote population activity at that preferred frequency (Gutfreund et al. 1995). One electroencephalogram (EEG) correlate of this oscillation, termed theta (θ) rhythm, is most prominent in limbic structures during EEG-activated states (Bland and Colom 1993; Buzsáki et al. 1994).

Of particular interest here is the recent finding (Paré et al. 1995) that the majority of projection cells (84%) encountered in the cat lateral amygdala (AL) in vivo generated slow membrane potential oscillations (2–10 Hz) at a range subthreshold to the generation of action potentials. Because
the basolateral amygdaloid (BL) complex is reciprocally connected with the hippocampal formation (Krettek and Price 1977a,b; Russchen 1982a,b) and because this structure displays δ-oscillations during brain-activated states (see Buzsáki et al. 1994), it was hypothesized that the propensity of AL neurons to generate intrinsic oscillations in the same frequency range may favor the emergence of recurring time windows when synaptic interactions would be facilitated in this limbic network (Paré et al. 1995). A recent study demonstrated the presence of δ-related activity in the BL complex (Paré and Gaudreau 1996). However, the ionic mechanisms underlying the slow oscillation of AL neurons are still unknown. Moreover, the patterning influence it exerts on the activity of amygdaloid neurons remains obscure. Therefore the present study was carried out to investigate the relationship of membrane potential oscillations and firing patterns in neurons of the BL complex, whereas the accompanying paper focuses on the ionic mechanisms underlying rhythmic electrical activity.

Part of this work has been published in abstract form (Pape and Paré 1995).

Methods

Preparation and maintenance of amygdaloid slices

Experiments were performed in slices of the BL complex from guinea pigs (200–350 g) and two cats (4–6 mo old). The animals were deeply anesthetized (pentobarbital sodium, 60 mg/kg ip) and decapitated and underwent a wide craniotomy. Both hemispheres were rapidly removed, and slices were prepared in physiological saline (at a temperature of 2–4°C) as 500-μm-thick coronal sections on a vibratome (Model 1000, Ted Pella, Redding, CA). The BL complex was dissected before or after the slicing procedure in cats and guinea pigs, respectively. The slices were maintained in an interface type chamber at 36 ± 1°C during continuous perfusion of a solution containing (in mM) 126 NaCl, 2.5 KCl, 2 MgSO4, 26 NaHCO3, 1.25 NaH2PO4, 2 CaCl2, and 10 dextrose, buffered to a final pH of 7.4 through continuous perfusion of 95% O2–5% CO2. Epi-illumination of the coronal slices readily revealed the boundaries of the AL and basolateral amygdala (ABl) nuclei. Slices were allowed to equilibrate for ∼ 2 h before recording commenced.

Electrophysiological techniques

Glass microelectrodes were prepared on a Flaming-Brown micropipette puller (Model P-87, Sutter Instruments, San Rafael, CA) from thin-walled capillaries (TW-100F, World Precision Instruments, Sarasota, FL). Electrodes were back-filled with 1% biocytin in 2 M potassium acetate, or with 4 M potassium acetate. Electrode resistances ranged between 40 and 70 MΩ when filled with 4 M potassium acetate, and ∼20–30% higher with 2 M potassium acetate. Intracellular recordings under current-clamp or voltage-clamp conditions were controlled through an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). The bridge balance was continuously monitored. During discontinuous current-clamp or voltage-clamp recordings, the headstage output was continuously monitored to ensure adequate settling time. Sampling frequencies ranged between 3 and 5 kHz, and the amplifier gain was between 0.7 and 1 nA/mV. Current–voltage (I–V) relationships were obtained under voltage clamp by applying slow voltage ramps (1–2 mV/100 ms) from a holding potential of around −75 mV to a final potential of around −20 mV. Voltage ramps were repeated every 10–16 s, and individual current traces were averaged to reduce noise. Voltage-clamp experiments were governed by pClamp software (Axon Instruments) operating via a Labmaster DMA interface (Model TL-1-125, Axon Instruments) on an IBM 486 computer. Data were collected on-line with the computer or digitized (NeuroCorder DR-384; Neurodata, New York, NY) and stored on videotape for later analysis. Current-clamp data were analyzed off-line using a CED 1401 interface and Spike2 for Windows V.2.01 software (Cambridge Electronic Design, Cambridge, UK).

Neurons were recorded in the AL and ABl, and only those having stable membrane potentials negative to −55 mV, input resistances above 40 MΩ and action potentials overshooting 0 mV were collected for analysis. At the end of each experiment, possible changes in electrode tip potential were monitored to ensure the DC offset of the electrode in the bathing medium, and the value of the measured neuronal membrane potential was corrected accordingly. Changes in electrode tip potential were usually <4 mV. Pharmacologically active substances were applied with the bathing medium or by local superfusion on parts of the slice containing the AL and ABl through pipettes of a larger tip diameter (10–20 μm) by constant low pressure (Picopritzer II, General Valve, Fairfield, NJ). δ-2-Amino-5-phosphonovaleric acid (APV), (−)-bicuculline methiodide (bicuculline), scopolamine hydrochloride (scopolamine), and tetrodotoxin (TTX) were obtained from Sigma, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 3-tropenyl-indole-3-carboxylate methide (ICS 205,930) were obtained from RBI (Research Biochemicals International, Natick, MA), and CDP 35348 was a generous gift from Ciba Geigy.

In vivo recordings

Intracellular recordings in the AL and ABl in vivo were performed in adult cats (2.5–3.5 kg) as described previously (Paré et al. 1995). Brieﬂy, animals were anesthetized with pentobarbital sodium (40 mg/kg ip), paralyzed with gallamine triethiodide (Flaxedil, iv), and artificially ventilated. End-tidal CO2 was kept at 3.7 ± 0.2%, body temperature was maintained at 37–38°C. To ensure sufﬁcient anesthesia, the EEG was monitored from the precruciate region for the entire duration of the experiment, and additional doses of pentobarbital (5–7 mg/kg iv) were administered to maintain a synchronized EEG pattern. In addition, a local anesthetic (Lidocaine, 2%) was applied to pressure points and injected in tissue to be incised. Stability of the recordings was ensured by cisternal drainage, bilateral pneumothorax, and hip suspension. Intracellular recordings were performed with micropipettes filled with 4 M potassium acetate, to which N-(2-aminoethyl) biotinamide hydrochloride (Neurobiotin) (1–2%; Vector Labs, Burlingame, CA) had been added in some of the experiments. Electrode resistances ranged between 25 and 50 MΩ. Intracellular electrical activity was recorded with a high-impedance amplifier with active bridge circuit (Neurodata, New York, NY). Signals were observed on a digital oscilloscope, printed on a chart recorder, digitized and stored on tape. Analyses were performed off-line with homemade and IGOR software (Wavemetrics, Lake Oswego, OR). The stereotaxic coordinates of deeply lying brain structures were obtained from the atlas of Berman and Jones (1982). Micropipettes were inserted obliquely in the amygdaloid complex with a lateromedial trajectory coursing through the posterior sylvian gyrus. Tungsten stimulation electrodes were inserted in the entorhinal/perirhinal cortex and in the basal forebrain to activate amygdaloid projection neurons. The location of the stimulation electrodes was marked with small electrolytic lesions (0.5 mA, 2–5 s) and verified on thionin-stained sections (see Paré et al. 1995 for further details).

Oscillation analysis

The rhythmic nature of the oscillations and their dominant frequency were analyzed by calculating autocorrelograms or fast Four-
rier transformations (FTTs) of the membrane voltage. The oscillation frequency was determined from the interval between the peaks of a voltage auto correlogram of 2 s duration. For FFT analyses, data were digitized at 2 or 4 kHz and filtered with antialiasing filter at 25 or 80 Hz upper corner frequency, respectively. Amplitudes of frequency components corresponding to ± 0.5 sampling rate were reduced to <1% through this procedure.

Staining procedures

At the end of the experiments, the brains were perfused with cold saline solution (0.9%; 500 ml) and fixative (2% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate-buffered saline at pH 7.4; 1 l). The brains were then stored for 24 h in a 30% glucose solution and sectioned at 80 µm on a freezing microtome. Slices containing Biocytin-labeled cells were fixed (2% paraformaldehyde and 2% glutaraldehyde), transferred to 10–20% sucrose in 0.2 M phosphate-buffered saline (pH 7.4), frozen, and sectioned at 80 µm. Sections of the amygdaloid complex were incubated in the avidin-biotin-horseradish peroxidase (HRP) complex solution (ABC kit; Vector Labs) and processed to reveal the intracellular HRP staining (Horikawa and Armstrong 1988).

Data are presented as means ± SD and number of observations.

RESULTS

A total of 107 neurons was recorded intracellularly in the AL and ABl nucleus of the guinea pig and cat amygdala maintained as slices in vitro (guinea pig: n = 64 AL, n = 31 ABl; cat: n = 7 AL, n = 5 ABl). The unifying electrophysiological feature of a subpopulation of neurons in ABl (n = 25 of 27 cells that were tested in guinea pig; cat: n = 2/4) and AL (guinea pig: n = 41/43; cat: n = 4/5) was their propensity to generate slow, rhythmic deflections of the membrane potential in response to depolarization beyond approximately −62 mV (Fig. 1), as has been reported earlier for AL neurons in vivo (Paré et al. 1995). Other basic electrophysiological properties of these neurons included an average membrane resting potential and input resistance at −65.9 ± 5.4 mV/74.6 ± 20.4 MΩ (n = 34, AL, guinea pig), −71.2 ± 2.8 mV/50.0 ± 7.9 MΩ (n = 5, AL, cat), −65.0 ± 6.0 mV/73.8 ± 30.5 MΩ (n = 13, ABl, guinea pig), −67.3 ± 5.1 mV/51.3 ± 10.3 MΩ (n = 4, ABl, cat); all of these cells displayed strong inward rectification in the depolarizing direction (Fig. 3) and action potentials overshooting 0 mV (Figs. 4–6). The action potential was typically followed by an afterhyperpolarization (AHP) of 100–200 ms duration (Fig. 4B2). Upon termination of repetitive firing, an additional slow AHP was generated, which lasted for up to 3 s (data not shown). Intracellular staining of oscillating cells in vitro (n = 22) revealed spiny dendrites with heterogeneous spatial arrangement, which gave the cells a stellate or pyramidal-like overall appearance (Fig. 1, D and E). When stained axons could be traced, they were always seen to leave the local synaptic environment. Cells located close to the external capsule tended to appear stellate, whereas pyramidal-shaped forms were encountered more medially (see also Paré et al. 1995). No correlation was found between these different morphological features and the propensity of AL and ABl neurons to oscillate.

Low-threshold oscillation (LTO)

Oscillatory activity with low threshold of activation (referred to as low-threshold oscillation, LTO) could be elicited by the injection of a steady depolarizing current. An example of this behavior is illustrated for a guinea pig ABl neuron in Fig. 1A. At resting level (−69 mV), a stable membrane potential was observed. Depolarization by 9 mV resulted in low-amplitude oscillatory activity. Upon further depolarization, clear rhythmic deflections of the membrane potential were elicited (−57 mV), whose amplitudes (2–6 mV) were sufficient to reach threshold for the generation of action potentials (−55 mV). FFT of the voltage responses revealed a maximal amplitude peak in the power spectrum, thereby indicating the rhythmic nature of the LTO (Fig. 1B). Analyzing the oscillation at all membrane potentials that were tested (−75 to −50 mV) in guinea pig AL (n = 11) and ABl (n = 10) neurons yielded an overall range of frequencies between 0.5 and 9 Hz, of which >80% occurred between 1 and 3.5 Hz. The average frequency of the LTOs was at 2.3 ± 1.1 Hz (n = 11). Moreover, the frequencies observed in an individual neuron at various levels of membrane polarization differed by no more than −2 Hz. Neurons of the cat AL (n = 4) and ABl (n = 2) in vitro displayed LTOs with characteristics similar to those of the guinea pig (data not shown).

A combination of antagonists to ionotropic glutamate receptors (local superfusion: CNQX, 100 µM; AP5, 500 µM), γ-aminobutyric acid (GABA) receptors (local superfusion: bicuculline, 1–3 mM; CGP 35348, 2–5 mM), serotonin 5-HT3 receptors (bathing medium: ICS 205,930; 5 µM) and muscarinic acetylcholine receptors (bathing medium: scopolamine, 5 µM) effectively blocked synaptic transmission (not shown), but did not abolish low-threshold oscillatory activity (n = 26/26). Rather, the oscillations appeared more regular during blockade of synaptic activity (Fig. 1C). Similar results were obtained after blockade of synaptic activity through removal of calcium ions from the bathing medium (n = 3; data not shown). These results and the strong voltage dependence of the LTOs indicated that they represent an electrophysiological property intrinsic to the recorded neurons. By contrast, local application of TTX (10–30 µM) readily and reversibly blocked the LTO (as well as the generation of fast action potentials; n = 21/21; Fig. 1C), which is in line with previous findings on a contribution of sodium (Na+)–dependent membrane conductances to this type of rhythmic activity in neurons from other regions of the brain (Alonso and Llinás 1989; Amitai 1994; Gutfeuld et al. 1995; Klink and Alonso 1993; Leung and Yim 1991) and will be further analyzed in the accompanying paper.

High-threshold oscillation (HTO)

A second type of oscillatory activity with more positive threshold of activation (referred to as high-threshold oscillation, HTO) could be elicited by steady depolarization beyond the threshold for generation of Na+ spikes and could be studied best after blockade of LTOs and spike activity by TTX (10–30 µM, local application). Figure 2A shows an example of this behavior in a guinea pig AL neuron. HTOs appeared as low-amplitude (5–10 mV), sinusoidal deflections of the membrane potential at around −40 mV. At further increased depolarization, the sinusoidal deflections triggered high-threshold spikes with maximal amplitudes averaging 40 mV, which rhythmically re-
curred at the predominant frequency of the oscillation. Spike amplitudes continuously decreased upon further increases in current injection. At a given level of current injection, oscillatory activity usually persisted with no significant alteration in amplitude or frequency as long as the recording lasted (up to 6 h). HTOs vanished at a steady membrane polarization positive to approximately −20 mV. FFT corroborated the rhythmic nature of these voltage responses (Fig. 2B). The overall range of frequencies (analyzed between −40 and −20 mV) was between 1 and 7.5 Hz in AL (n = 16), and between 2 and 6.5 Hz in ABI (n = 6) neurons of the guinea pig, with >80% of the HTOs ranging between 2 and 6 Hz. The average frequency of the HTOs was at 5.8 ± 1.1 Hz (n = 7). HTOs were routinely studied during blockade of synaptic transmission through application of TTX or receptor antagonists (for pharmacological details see Electrophysiological techniques) and were strongly voltage dependent, thus presumably reflecting intrinsic properties of the cells. Very similar HTOs occurred in neurons of the cat ABI (n = 3/4) and AL (n = 3/4) maintained in vitro.

HTOs were observed in 84% of AL (n = 41) and 63% of ABI (n = 10) neurons that were tested, and LTOs were found in 95% (n = 37) and 83% (n = 25) of AL and ABI cells, respectively. Of these, ∼79% generated both LTOs and HTOs, thereby presumably enabling an interaction be-
between these two types of rhythmic electrical activity in the shaping of spike patterns (see Sculpturing of firing patterns by LTOs and HTOs).

Two regions of negative slope conductance

To corroborate the existence of two different types of oscillations at different ranges of the membrane potential, I-V relationships were obtained from voltage-clamp experiments in AL neurons (n = 3). Application of depolarizing voltage ramps (1–2 mV/100 ms) from slightly hyperpolarized values of the membrane potential evoked a slow inward shift of the membrane current positive to zero-current potential (~59 mV), which was followed by inward current activation at approximately ~45 mV (Fig. 3A; note that unclamped repetitive spikes occurring at around ~40 mV have been eliminated from the figure through low-pass filtering). Further depolarization resulted in the voltage-dependent activation of outward current, and a second region of negative slope conductance at ~30 mV (Fig. 3A). Local application of TTX (10–30 μM) induced a hyperpolarizing shift of the zero current potential by 5–8 mV, abolished the first region of negative slope conductance (~55 to ~40 mV), and did not significantly influence the second region of negative slope conductance (Fig. 3B). The existence of two regions of negative slope conductance in the I-V relationship, which largely overlap with the voltage ranges of LTOs and HTOs observed under current-clamp conditions, and the selective blocking effect of TTX on the low-voltage-activated mechanism, strongly support the notion of two sets of inwardly directed ionic mechanisms underlying the depolarizing phases of these rhythmic activities (see accompanying paper). In any case, these results corroborate the existence of two different types of oscillations with different voltage ranges of activation in amygdaloid neurons.

Sculpturing of firing patterns by LTOs and HTOs

In a first attempt to investigate how the intrinsic slow oscillations influence the firing patterns of amygdaloid neurons, cells were progressively depolarized through current injection from resting potential, without prior application of TTX (Fig. 4). LTOs occurring at a slightly depolarized value of the membrane potential were capable of triggering Na⁺ spikes, resulting in a synchronization of spike activity (Fig. 4, B1 and B2). With further increases in depolarizing cur-
rent, the spike frequency increased, and two alterations of the shape of the action potential occurred (Figs. 4, B3–B6, and 6, B and C): 1) the amplitude of the spike progressively decreased, presumably due to Na⁺ inactivation, and 2) the duration of the spike increased, often resulting in a depolarizing afterpotential and the generation of “spike doublets.” This modification presumably reflected an increasing influence of the mechanisms underlying the HTOs. Indeed, with further depolarization, fast spikes were fully inactivated and replaced by HTOs crowned by high-threshold spikes (Fig. 4B7). Depolarization positive to −20 mV abolished the HTOs (Fig. 4B8). Hyperpolarizing the membrane by −5−10 mV reinstated the HTOs, which existed in a voltage range between approximately −20 and −40 mV, and hyperpolarization below this range induced the reappearance of Na⁺ spikes (Fig. 4, B9, B10, and filled circle with 2).

To reveal the contribution of intrinsic oscillating properties to the patterning of repetitive firing during maintained levels of membrane polarization, long (>60 s) depolarizing current pulses of increasing amplitude were injected from resting levels. The typical response of the cells consisted of an initial high-frequency discharge, the frequency of which depended on stimulation strength, followed by a progressive decline toward a slow-rhythmic firing pattern. Slow-rhythmic firing occurred in a narrow range of frequencies, between 2 and 10 Hz, with very little variation at different stimulation intensities. Responses of an AL neuron are exemplified in Fig. 5. Depolarization to a just suprathreshold level evoked an initial discharge of action potentials with frequencies at ~40 Hz, which rapidly declined toward slow-repetitive discharges (Fig. 5Aa). Cessation of spike activity revealed the underlying LTOs at 4.5 Hz (Fig. 5, Ab and Ac). With increases in the depolarizing current step (Fig. 5B), the initial spike frequency increased (up to maximal frequencies at ~100 Hz), followed by adaptation toward repetitive firing at 4.5 Hz. Plots of instantaneous frequency versus time obtained from a larger sample of neurons (n = 17) revealed that the decay in firing frequency followed a double-exponential function, with time constants averaging 57 ± 28 ms and 3.29 ± 1.85 s, respectively, and approached steady-state frequencies at 6.3 ± 2.9 Hz. With even further increases in depolarizing current strength, ongoing activity following the initial accommodating period remained within a narrow range of frequencies at 6 Hz and was associated with a progressive decrease in spike amplitude and an increase in spike duration, due to Na⁺ spikes being fully inactivated and replaced by HTOs within 1–2 min, which in turn resulted in a constant frequency of electrogenic activity at 6 Hz determined by the HTOs (Fig. 5C2). During maintained current injection, a slow depolarizing shift of the prevailing membrane potential was often observed, which could move the membrane potential out of the range of HTOs (Fig. 5C3). Hyperpolarization of the membrane by 5–10 mV reinstated the HTOs (not shown). The slow shift was associated with an increase in apparent input resistance, thereby presumably reflecting the slow inactivation of an outwardly directed membrane conductance, which had been deinnactivated at rest and activated upon depolarization. In support of this conclusion is the observation that the depolarizing shift was not observed, when the membrane was depolarized from depolarized levels (cf. Fig. 6).

To reveal the influence of Na⁺ inactivation on firing pat-
terns in more detail, the neurons were depolarized from different levels of membrane polarization after steady-state values of slow-rhythmic, firing had been reached. A typical experiment is illustrated in Fig. 6. Just-suprathreshold currents evoked slow-repetitive firing of Na\(^+\) spikes triggered by the LTOs. Further depolarization from this level (by doubling the amount of injected current) resulted in a transient increase in firing frequency and a rapid adaptation toward slow-regular firing at 6 Hz. During further stepwise increases in membrane polarization between approximately -50 and -20 mV, the rhythmic pattern of electrogenic activity remained within a narrow range of frequencies between 6 and 10 Hz, although individual electrogenic events changed from fast Na\(^+\) spikes to high-threshold spikes and underlying HTOs. Rhythmic regular firing was only interrupted at an intermediate range of membrane polarization by the occurrence of spike doublets, which possessed an intra-spike frequency between 25 and 80 Hz and gave rise to a hyperpolarizing afterpotential of 200–300 ms duration.

**Existence of LTOs and HTOs in amygdaloid neurons in vivo**

Intracellular recordings from cat AL neurons in vivo demonstrated the existence of LTOs (n = 48/57) and HTOs (n = 11/14), with properties similar to those recorded in the slice preparation in vitro (Fig. 7). Upon injection of a depolarizing constant current, LTOs were elicited at mem-
brane potentials positive to $-62$ mV. The depolarizing phase of the LTOs was capable of triggering the production of action potentials, resulting in a synchronization of discharges with the peak of the oscillation. With increasing current intensities, the production of repetitive trains of action potentials vanished (due to Na$^+$ inactivation), and slow-rhythmic deflections of the membrane potential occurred, indicative of HTOs. Autocorrelograms demonstrated predominant frequencies of the low-threshold and the high-threshold rhythmic activities at 2.5 and 10 Hz, respectively, which are well within the range of frequencies of LTOs and HTOs observed in vitro.

**DISCUSSION**

The results of the present study demonstrate that the majority of neurons of the cat and guinea pig BL complex, recorded under in vitro and in vivo conditions, are capable of generating two different types of slow-rhythmic electrical activity, which 1) represent intrinsic membrane phenomena rather than influences of the synaptic network, 2) share the same range of frequencies between $\sim2$ and 10 Hz, 3) prevail at a wide range of membrane potentials subthreshold and suprathreshold to the generation of action potentials, respectively, and thereby 4) substantially contribute to the patterning of action-potential discharges.

**Morphological classification of oscillating cells**

Literature relating to the intrinsic characteristics of amygdaloid neurons is surprisingly inconsistent. Morphologically, the BL complex, comprised of the AL, the ABl, and the basomedial (ABm) nuclei, contains two major types of neurons (Carlsen 1988; McDonald 1982, 1992; Tömöl and Szafranska-Kosmal 1972): 1) spiny, pyramidal-shaped or stellatelike cells that presumably use glutamate as a neurotransmitter (McDonald 1996; Paré and Smith 1994; Smith and Paré 1994) and that provide the major output to extrinsic targets of the complex and 2) a morphologically heterogeneous class of sparsely spiny cells immunoreactive for GABA and various neuropeptides that are assumed to mediate local synaptic interactions (Carlsen 1988; McDonald 1985; Paré and Smith 1993). Oscillating neurons of the AL and the ABl were identified as a morphologically heterogeneous class of spiny multipolar neurons, both in the cat in vivo (Paré et al. 1995) and in guinea pig and cat in vitro (present study). While most of the oscillating cells were...
cortically projecting neurons (Paré et al. 1995), dendritic arborizations varied between cells from pyramidal-shaped to stellate. A similar heterogeneity of pyramidal and stellate morphologies was reported for regular firing ABl neurons with different degrees of spike frequency adaptation (Rainnie et al. 1993). Thus, rather than reflecting two discrete types of cells, pyramidal-shaped and stellate neurons may represent two extremes of a continuum. Several observations are in line with this conclusion: 1) bursting neurons of the ABl could appear stellate or pyramidal-like, depending on the plane of section with respect to the orientation of the

FIG. 7. LTOs and HTOs in a cat AL neuron in vivo. A: intracellular recording demonstrates the presence of LTOs (4) and HTOs (1 and 2) in a range of membrane potentials subthreshold and suprathreshold to spike generation. B: autocorrelograms calculated from traces in A show the rhythmic nature of the membrane potential deflections with predominant frequencies between 2.5 and 10 Hz. Note the sculpturing of spike firing at frequencies corresponding to that of the slow oscillation (3).
dendritic tree (Paré et al. 1995), 2) nuclear boundaries and adjacent bundles of fibers appeared to constrain the shape and orientation of the dendritic tree (cf. Paré et al. 1995; Washburn and Moises 1992), 3) Golgi staining revealed a morphological continuum of spiny neurons in the BL complex, ranging from stellate- to pyramidal-like (e.g., McDonald 1992), and 4) in the visual cortex, stellate neurons were suggested to emerge continuously from pyramidal neurons due to a progressive shortening of the apical dendrite (Peters and Kara 1985a,b). Following from this is the conclusion that, unless the dendritic tree of a given neuron is fully reconstructed, the most reliable criterion presently available to morphologically classify amygdaloid neurons is the presence or the absence of dendritic spines (cf. Paré et al. 1995).

Electrophysiological features of oscillating neurons

There are many similarities between the oscillating neurons presented here and the “nonaccommodating” type of regular-firing (class 1) cells described in the rat ABl by Rainnie et al. (1993). Both types of neurons produce an initial high-frequent discharge, which slows within 100 ms toward a regular discharge of action potentials at ~10 Hz, irrespective of the intensity of the depolarizing current pulse (Fig. 5) (cf. Fig. 7 of Rainnie et al. 1993). Other similarities include the membrane resting potential (~67.4 vs. ~65.8 mV), the relatively high resting input resistance (62.4 vs. 58.1 MΩ), long-duration action potentials (half-width at ~0.8 ms), and, as noted before, spiny morphology. The regular firing pattern observed in amygdaloid neurons thus appears to reflect the intrinsic oscillatory membrane properties, as is discussed in more detail below.

Another point to consider relates to the various degrees of spike frequency adaptation in response to steady depolarizing stimuli described for regular firing cells in the BL complex. In ~30% of the cells, an initial barrage of action potentials was followed by a complete cessation of discharge, whereas the remainder of the cells displayed a progressive slowing of the discharge or a sustained repetitive discharge throughout a depolarizing current injection (Rainnie et al. 1993; Washburn and Moises 1992). A systematic correlation of these firing patterns with distinctive (sub-)classes of cells is complicated, however, because 1) all of these neurons displayed an initial decay in firing frequency, 2) a steady-state level of spike activity may have been reached in the cells displaying a progressive slowing of the discharge, if depolarizing stimuli of a longer duration had been used, and 3) the membrane current abt to regulate repetitive spike activity (a Ca2+-dependent K+ current, termed slow I_AHP) is under the control of a number of transmitters and metabolic systems in neurons from various regions of the brain in different species (for reviews see Sah 1996; Storm 1993), including the rat ABl (Womble and Moises 1993). From this it is tempting to speculate that cessation of spike activity during an ongoing depolarizing stimulus (“adaptation”) may reflect the functional inactivation of the oscillatory mechanisms, resulting from the counterefect of a transmitter or metabolically induced increase in the slow I_AHP. In support of this conclusion is the observation (Pape and Driesang, unpublished observations) that the few neurons encountered in the present study, displaying strong spike frequency adaptation with cessation of discharge during injection of strong depolarizing current and a pronounced slow AHP, were found to not express HTOs.

Possible functional significance of oscillatory behavior

The results of the present study demonstrate that neurons of the BL complex generate slow-rhythmic spike trains within a narrow range of frequencies between 2 and 10 Hz. Slow-rhythmic firing occurs upon membrane depolarization to levels near spike threshold, as well as in response to stronger depolarizing stimuli following an initial period of adaptation from high-frequency activity. This repetitive firing behavior must be regarded as an expression of the strong, intrinsic oscillatory properties of the cells. A number of recent studies in neurons from various regions of the mammalian brain demonstrated the presence of sinusoidal oscillations at 2–15 Hz in the subthreshold range of potentials (Alonso and Klink 1993; Alonso and Llinás 1989; Gutfreund et al. 1995; Leung and Yim 1991; Llinás and Yarom 1986; Silva et al. 1991; Takakusaki and Kitai 1997; Williams et al. 1996). LTOs in amygdaloid neurons are basically similar to these subthreshold oscillations in terms of the range of membrane potentials and frequencies at which they occur. The subthreshold oscillatory properties have been shown to act as a functional synchronization and timing device of intrinsic signals and synaptic inputs (Alonso and Klink 1993; Lampl and Yarom 1993), and to bias the response of the neuron to synaptic signals occurring at the same frequency (Gutfreund et al. 1995). HTOs, by comparison, appear to predominantly determine spike frequency adaptation toward slow-rhythmic firing patterns during maintained depolarizing influence (see accompanying paper). In view of the strong tendency of amygdaloid neurons to generate voltage signals at a preferred frequency over a wide range of membrane polarization, it seems feasible to speculate that they are capable of synchronizing synaptic input signals and to reinforce the neuronal output at this particular frequency, which in turn will lead to the promotion of population activity at that frequency. It is important to keep in mind that this frequency tuning occurs progressively during maintained depolarization and is usually preceded by an accommodating high-frequency discharge, the frequency of which is dependent on the stimulus size.

The range of frequencies of rhythmic electrical signals between 2 and 10 Hz brings up the issue of their possible role in the limbic θ-rhythm or the δ-waves in the EEG during slow-wave sleep. Although most prominent in the hippocampal EEG, the θ-rhythm is characteristic also of other limbic structures, including the mammillary bodies, the medial septum/vertical limb of the diagonal band of Broca, and the cingulate and entorhinal cortices (as reviewed by Bland and Colom 1993; Buzsáki et al. 1994). The limbic structures, due to their large proportion of common inputs and reciprocal interconnections, were proposed to function as multiple synchronizing systems (Bland and Colom 1993; Steriade et al. 1990), where large subsets of neuronal circuitry are entrained into a common oscillatory mode, for instance through recruitment by cellular pacemakers (Alonso and Klink 1993; Alonso and Llinás 1989) and syn-
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Address for reprint requests: H.-C. Pape, Institut für Physiologie, Medizinische Fakultät, Otto-von-Guericke-Universität, Leipziger Str. 44, D-39120 Magdeburg, Germany.

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