Properties of Carbachol-Induced Oscillatory Activity in Rat Hippocampus

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Williams, John H. and Julie A. Kauer. Properties of carbachol-induced oscillatory activity in rat hippocampus. J. Neurophysiol. 78: 2631–2640, 1997. The recent resurgence of interest in carbachol oscillations as an in vitro model of theta rhythm in the hippocampus prompted us to evaluate the circuit mechanisms involved. In extracellular recordings, a regularly spaced bursting pattern of field potentials was observed in both CA3 and CA1 subfields in the presence of carbachol. Removal of the CA3 region abolished oscillatory activity observed in CA1, suggesting that the oscillatory generator is located in CA3. An N-methyl D-aspartate receptor antagonist, 6,7-dinitroquinoxaline-2,3-dione (DNQX), blocked carbachol oscillations, indicating that AMPA receptor-mediated synaptic currents are necessary for the population oscillation. Moreover, the spread of oscillatory activity into CA1 required intact N-methyl-D-aspartate receptors. These data are more consistent with epileptiform bursting than with theta rhythm described in vivo. In the presence of carbachol, individual CA3 pyramidal cells exhibited a slow, rhythmic intrinsic oscillation that was not blocked by DNQX and that was enhanced by membrane hyperpolarization. We hypothesize that this slower oscillation is the fundamental oscillator that participates in triggering the population oscillation by exciting multiple synchronically connected CA3 neurons. γ-Aminobutyric acid-A (GABA_A) receptors are not necessary for carbachol to elicit synchronous CA3 field events but are essential to the bursting pattern observed. Neither GABA_A nor metabotropic glutamate receptor subtypes appear to be necessary for carbachol oscillations. However, both nicotinic and M1 and M3 muscarinic cholinergic receptors contribute to the generation of this activity. These results establish the local circuit elements and neurotransmitter receptors that contribute to carbachol-induced oscillations and indicate that carbachol-induced oscillations are fundamentally distinct from theta rhythm in vivo.

INTRODUCTION

Synchronization of neural activity in the mammalian brain is postulated to underlie a variety of normal and pathophysiological functions (see Jefferys et al. 1996; Traub and Jefferys 1994). The study of synchronized neuronal discharge has been facilitated greatly by the propensity of the hippocampus to exhibit oscillatory electrical behavior under a variety of conditions (Traub and Miles 1991; Traub et al. 1996a). More than four different oscillatory patterns have been reported in the hippocampus in vivo, expressed during distinct patterns of animal behavior (Bragin et al. 1995; Ylinen et al. 1995a). Furthermore, epileptiform electrical activity, characterized by synchronous cell discharge, has also been described in this brain region (see Mody and Staley 1994; Traub and Jefferys 1994 for review). To understand the mechanisms used to generate oscillatory activity, it is essential to know which cellular elements of the hippocampal circuit participate in the oscillation and how they interact. Although efforts to address these issues have been made in vivo (Buzsáki and Eidelberg 1983; Leung and Yim 1986; Soltesz and Deschenes 1993; Ylinen et al. 1995b), a detailed analysis requires the development of in vitro models that are more accessible to physiological and pharmacological manipulations.

Physiological theta rhythm in vivo requires intact cholinergic input to the hippocampus (Krames et al. 1975; see Bland and Colom 1993 for review). The cholinergic agonist, carbachol, produces a stereotypical pattern of oscillatory behavior in the in vitro hippocampal slice that occurs at a frequency similar to that of theta rhythm (Bland et al. 1988; MacVicar and Tse 1989). Cholinergic oscillations have also been described in neocortical slices in vitro (Lukatch and MacIver 1997). Recently, carbachol oscillations in hippocampal area CA1 have been implicated in the induction of specific patterns of synaptic plasticity. This in vitro result suggests that theta activity could enhance synaptic modification in vivo (Huerta and Lisman 1993, 1995, 1996). Although earlier studies have partially characterized and modeled the synaptic elements contributing to carbachol-induced oscillations in area CA3 of the hippocampus (MacVicar and Tse 1989; Traub et al. 1992), pharmacological features of carbachol oscillations are not entirely understood. It is essential to determine whether carbachol oscillations are a good in vitro model of theta rhythm or whether they represent a form of epileptiform bursting activity. Whereas epileptiform bursting is generated by CA3 neurons firing synchronously to synchronously excite each other and CA1 pyramidal neurons, theta rhythm apparently is generated by rhythmic inhibitory potentials in area CA1 itself with a minimal contribution from the CA3 region.

In this study, we focus on three major issues relating to carbachol-induced oscillations. First, we have asked whether features of carbachol oscillations are more like theta rhythm or like epileptiform bursting by examining the site from which oscillations are generated within the slice, and by testing the effects of glutamate and γ-aminobutyric acid (GABA) receptor antagonists on carbachol oscillations. Second, we have identified an intracellular intrinsic rhythm elicited by carbachol that may be responsible for synchronous firing of CA3 pyramidal neurons that underlies the population oscillation. Finally, we have attempted to identify the cholinergic receptor subtypes necessary to generate and maintain carbachol oscillations.

METHODS

Hippocampal slice preparation

All experiments were carried out in strict accordance with a protocol approved by the Duke University Medical Center Institu-
tional Animal Care and Use Committee. Slices were prepared from male Sprague-Dawley rats aged 21–28 days as previously described (McMahon and Kauer 1997). Briefly, coronal slices (400 μM) were cut from the middle third of the hippocampus using a vibratome, into ice-cold artificial cerebrospinal fluid [ACSF; containing (in mM) 119 NaCl, 26 NaHCO,
3, 2.5 KCl, 1.0 NaH$_2$PO$_4$, 2.5 CaCl$_2$, 1.3 MgCl$_2$, and 11 $\mu$-glucose] saturated with 95% O$_2$–5% CO$_2$. Slices were transferred to an interface chamber maintained at room temperature (22–25°C) for ≈90 min before use.

The cortex and midbrain were dissected away, and the isolated hippocampal slice placed in a submersion recording chamber, where it was pinned across a silicone elastomer (Sylgard) insert and perfused on both sides at a rate of 2–3 ml/min with warmed, oxygenated ACSF (33–34°C). Temperature was monitored using a bead probe close to the slice. The data presented here are based on recordings from 192 slices obtained from 80 animals.

**Extracellular recording**

Field potentials in CA1 and CA3c were recorded with glass microelectrodes filled with 2 M NaCl (1–2 MΩ) using an Axoclamp 2A amplifier. Extracellular recordings of carbachol oscillations were amplified (Brownlee Instruments) and filtered at 25–30 Hz (Frequency Devices) before being displayed on-line on an oscilloscope and chart recorder ( Gould TA240 ). Carbachol-induced field potentials were stored unfiltered on video tape for subsequent off-line analysis (Vetter AD adapter). Data shown for extracellular oscillations are AC coupled except where noted, for clarity. We refer to single events in the extracellular record as “field events”; groups of these are called “bursts.” Field events appear to represent synchronous firing of multiple pyramidal cells, with each field event correlated with three to four intracellular action potentials in CA3 neurons (and thus with a somewhat longer duration than electrically evoked population spikes reflecting a single action potential).

**Single cell recording**

Intracellular recordings were made from CA1 and CA3 pyramidal cells using an Axoclamp 2A in current clamp (bridge mode) with microelectrodes filled with 2 M potassium acetate (80–100 MΩ) or using patch electrodes (borosilicate glass; Sutter, 1.5 mm OD) with resistances of 4–5 MΩ when filled with a potassium gluconate-based solution [containing (in mM) 130 K-gluconate, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 0.6 ethylene glycol-bis(β-aminooethyl ether)-N,N,N’,N’-tetraacetic acid, 2 ATP-Na, 0.3 GTP-Na, and 1 MgCl$_2$] (McBain et al. 1994). Cells were impaled as close as possible to a previously positioned extracellular electrode located in stratum radiatum.

Pulses of current (0.2–0.4 nA for 200–300 ms every 5 s) were delivered through the intracellular electrode to monitor input resistance, action potentials, and series resistance (estimated by using bridge balance). Cells were accepted if they had resting membrane potentials larger than −60 mV, and overshooting action potentials. Series resistances ranged typically from 20 to 45 MΩ and were monitored throughout the experiment. Small changes (≥20%) in series resistance were compensated for using bridge balance; when larger changes occurred the experiment was terminated. Potential values cited in the text are not corrected for junction potential errors.

**Characterization of carbachol-induced oscillations**

Quantification of carbachol-induced oscillations was performed only on those slices that showed regular periods of oscillatory activity, defined as more than five consecutive regularly spaced trains. Interburst interval was measured as the time between the start of one burst and the start of the next. The peak frequency of oscillation within an individual burst was measured during three to five oscillatory cycles.

**Drugs**

All drugs were bath applied at known concentrations and took 45–60 s to reach the chamber from the reservoir. All salts were obtained from Fluka; nucleotides were from Boehringer-Mannheim. Carbachol, glutamate receptor antagonists, bicuculline, and cholinergic agonists and antagonists were obtained from RBI (with the exception of dicyclomine, which was obtained from Sigma). CGP55458 was a gift provided by Ciba-Geigy. All compounds used were water soluble and prepared daily from frozen aliquots.

**Results**

In the majority of naive hippocampal slices, bath application of 50 μM carbachol (CCH) generated extracellularly recorded events grouped into regularly spaced bursts (mean time to onset of field oscillations: 116 ± 13 (SE) s; n = 11; range: 75–230 s). Extracellularly recorded carbachol bursts in the CA1 region occurred at regular intervals (Fig. 1A, top; mean interburst interval: 17.5 ± 0.8 s; range: 11–22 s; mean burst duration: 4.3 ± 0.4 s; range: 1–6 s; n = 9). Each burst typically was preceded by a single field event followed within several hundred milliseconds by a burst of field events at approximately theta frequency (4–12 Hz). Individual field events, grouped into bursts, could be recorded in either s. pyramidale or s. radiatum and are likely to represent population spikes (action potentials fired synchronously in multiple pyramidal cells). We refer to the entire pattern of bursting during many minutes as carbachol oscillations. Oscillations remained stable for prolonged peri-
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Simultaneous intracellular recordings from CA1 pyramidal cells revealed that each field burst was associated with an intracellular cluster of depolarizations that did not reach action potential threshold (Fig. 1A, bottom). Carbachol depolarized CA1 neurons by 2–7 mV, as recorded either with microelectrodes or patch pipettes (n = 20). Each individual intracellular depolarization within the oscillatory burst was paralleled by a negative deflection in the extracellular record (Fig. 1A, right). These bursts closely resemble interictal bursts recorded in hippocampal slices in a variety of in vitro models of epileptiform activity (Swann et al. 1993; Traub and Wong 1982).

The relatively short and well-organized oscillatory bursts that we observed here contrasted with the more extended periods of oscillation previously reported in carbachol (Bland and Colom 1993; Huerta and Lisman 1995, 1996), possibly due to the relatively high levels of extracellular potassium used in previous studies (5 mM). To test this idea, carbachol oscillations were established in normal ACSF and the external potassium concentration was then elevated to 5 mM (Fig. 1B). In three slices, switching from low- to high-potassium-containing ACSF resulted in a prolongation of each oscillatory burst, accompanied by a reduced amplitude of the oscillation. Under these conditions, the oscillatory activity closely resembled that described previously (e.g., Huerta and Lisman 1995).

**Where is the oscillation generated?**

During theta oscillations in vivo, CA3 neurons rarely reach action potential threshold, and thus excitatory synaptic input from CA3 is unlikely to contribute to theta rhythm in CA1 neurons. However, carbachol oscillations we recorded simultaneously in CA3 and in CA1 were essentially identical and each individual field event recorded in CA1 had a correlated field event in CA3 (Fig. 2A). Because the CA1 region receives its primary excitatory input from the CA3 pyramidal cells, these data suggested that carbachol oscillations might originate in area CA3 and be propagated synaptically to CA1, in contrast to previous reports (Bland and Colom 1993; Paulsen and Vida 1996). To address this issue, we performed two types of experiment. First we uncoupled CA1 from CA3 by cutting across the full width of s. radiatum and s. lacunare, severing the axons connecting the two regions. Recording electrodes were placed in s. radiatum on either side of the cut in CA3c and CA1. When carbachol was introduced into the chamber, robust oscillations were induced in CA3, but not in CA1 (Fig. 2B; n = 4). In a second set of experiments, CA1 mini-slices were prepared by dissecting away both CA3 and dentate gyrus; recording electrodes then were placed in both s. pyramidale and s. radiatum of CA1. Carbachol was applied, and again, organized bursts of oscillatory activity were absent (Fig. 2C; n = 6). These observations demonstrate that the CA1 region on its own is incapable of reacting to carbachol with oscillatory activity and that CA3 is the locus from which carbachol oscillations are initiated, an essential difference from in vivo theta rhythm.

**Intracellular oscillations that accompany population oscillations**

Given that CA3 is essential to carbachol oscillations in the hippocampal slice, we examined the phenomenon in CA3 at both the single cell and population level (Fig. 3A). Carbachol produced a gradual depolarization of the pyramidal cell (10–15 mV; n = 6), which after a few seconds depolarized abruptly to threshold. The pyramidal cell entered a repetitive cycle of depolarization-hyperpolarization lasting for the duration of the recording. The initial depolarization of the cell preceded the extracellular recording, but as the cell began to fire in a regular rhythm, bursts of extracellular activity emerged paralleling those in the cell (Fig. 3A, right). The extracellular oscillations gradually grew in amplitude, probably representing the recruitment of more and more synchronously active pyramidal cells, before reaching a stable level.

In some intracellular records, carbachol oscillations were accompanied by a slower oscillation (Fig. 3B; n = 4). The action potential discharge in the cell often preceded the population carbachol oscillation (Fig. 3B, left), suggesting that...
this rhythmic activity in individual cells may be involved in initiating the extracellular oscillation. The slow intrinsic oscillation was elicited most easily when a CA3 neuron was hyperpolarized in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX), an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist (Fig. 3C).

The slow oscillation was characterized by a gradual depolarization to threshold, where the cell discharged at high frequency. A prolonged plateau potential followed before the cell hyperpolarized sharply to its new resting level and the cycle repeated itself. The slow oscillation is clearly distinct from the carbachol oscillation, as DNQX blocks the latter (see next section). No reflection of the slow intracellular rhythm was observed extracellularly. We hypothesize that the carbachol-induced, DNQX-insensitive oscillation seen in single pyramidal neurons may excite neighboring CA3 cells and contribute to the synchronous firing observed when excitatory synaptic transmission is intact.

AMPA, but not NMDA, receptors are required for carbachol oscillations

Previous work demonstrated that a broad-spectrum glutamate receptor antagonist, kynurenate, blocks carbachol oscillations (MacVicar and Tse 1989). We therefore tested which glutamate receptor subtypes are required. Carbachol oscillations were abolished by perfusion of the slice with low concentrations of the AMPA receptor antagonist, DNQX (Fig. 4A). The graded reduction in the extracellularly recorded oscillations probably reflects both reduction of excitatory postsynaptic potential (EPSP) amplitudes and a progressive decrease in the synchronization of pyramidal cells. [In the same neuron (Fig. 3C), subsequent hyperpolarization in carbachol and DNQX elicited the slow intracellular oscillation described in the previous section.]

In contrast to the block of carbachol oscillations by DNQX, a N-methyl-D-aspartate (NMDA) receptor antagonist had little impact on carbachol oscillations in either CA3 or CA1 once they were established (n = 4 slices, data not shown). Surprisingly however, if slices were pretreated with 2-amino-5-phosphonovalerate (AP5) before the carbachol application, normal oscillations developed in CA3, but propagation of oscillations into CA1 was markedly attenuated (n = 8; compare Fig. 4B with Fig. 2A). These data suggest that NMDA receptor activation is necessary to establish carbachol oscillations in CA1 but not to maintain them.

The role of metabotropic glutamate receptors also was examined. Preincubation of slices for 10 min in 500 μM (±)α-methyl-4-carboxyphenylglycine (MCPG), a broad spectrum mGlur antagonist, had little impact on oscillations in either CA3 or CA1 (n = 4; data not shown).

GABAergic transmission

Bath application of bicuculline (10 μM), a γ-aminobutyric acid-A (GABA_A) receptor antagonist, resulted in a breakdown in both the regularity and the structure of the extracellularly recorded oscillatory burst so that single field events remained but were no longer clustered into bursts (Fig. 5; n = 8). Washing bicuculline from the bathing medium restored the regular pattern of oscillations, although burst duration was prolonged somewhat compared with those observed prebicuculline. Intracellular recordings from CA3 neurons showed a similar disassembly of the bursting
Which cholinergic receptors are required for carbachol oscillations?

Carbachol is a broad spectrum cholinergic agonist, acting at both nicotinic and muscarinic receptors. We therefore investigated which cholinergic receptors contribute to the generation of carbachol oscillations. Preincubation of slices in atropine (1 μM), a broad spectrum muscarinic receptor antagonist, prevented carbachol oscillations (Fig. 6A; n = 4). In addition, as previously reported, atropine also abolished preestablished oscillations (n = 4, data not shown) (MacVicar and Tse 1989). In contrast to the effects of DNQX, atropine did not reduce the amplitude of the oscillation but caused the burst to disassemble into individual field events, which eventually ceased altogether.

We next used a series of more selective antagonists of muscarinic receptors (Auerbach and Segal 1996) (Fig. 6, B–E). Preincubation of the slices in pirenzipine, a selective M1 antagonist, prevented the development of oscillations (Fig. 6B; 1 μM, n = 4). Reaplication of CCH after wash of pirenzipine resulted in occasional oscillatory bursts, indicating that the slice was indeed capable of supporting oscillatory behavior (data not shown). Similarly, a selective and irreversible M3 antagonist completely prevented the expression of carbachol oscillations [Fig. 6D; 2-(4,4′-diacetoxyphenylmethyl) piridine (DAMP) mustard, 1 μM, n = 4]. In contrast, preincubation with a M2 antagonist, methoc-
triamine, did not affect carbachol oscillations (Fig. 6C: 1 μM; n = 4). In the presence of a M4 antagonist, dicyclomine, individual field events were generated but failed to assemble into rhythmic bursts (Fig. 6E: 10 μM, n = 4). Taken together, these data suggest that the M1/M3 muscarinic receptor subtypes are essential for initiating carbachol oscillations, and activation of the M4 receptor may be involved in the organization of bursts. In contrast, M2 receptors are not necessary for the generation of carbachol oscillations.

Available muscarinic agonists are not specific for M2, M3, and M4 receptor subtypes. McN-343, a selective M1 agonist, failed to generate oscillatory activity (Fig. 7A: 50 μM, n = 4), although subsequent application of carbachol to the same slice produced robust oscillations (Fig. 7B). We also tested two broad-spectrum muscarinic agonists, oxotremorine-M (50 μM, n = 4) and 5-methylfurmethiodide (50 μM, n = 4), both of which induced rhythmic bursting similar to that induced by carbachol (Fig. 7C and D). Low concentrations of oxotremorine-M, reported to be selective for the M2 receptor subtype, generated occasional individual events but failed to produce regular bursting (5 μM, n = 3).

One feature of the oscillations generated by the muscarinic agonists was that each burst was prolonged compared with those generated by carbachol (burst duration, seconds: carbachol: 4.3 ± 0.8; nicotine: 11.0 ± 3.3; 5-methylfurmethiodide: 12.7 ± 0.8; McN-A-343: 12.7 ± 0.8; n = 4 each; carbachol: 4.3 ± 0.4, n = 9). We hypothesized that this might be due to carbachol’s action at both nicotinic and muscarinic receptors. To investigate this further, we tested the effects of nicotinic acetylcholine receptor (nAChR) agonists and antagonists. Bath application of nicotine produced no obvious oscillatory activity by itself (100 μM, Fig. 8A: n = 4); however, a subsequent application of carbachol in the continued presence of nicotine produced only individual field events that did not organize into bursts (Fig. 8B: n = 4). Nicotine also disrupted preestablished carbachol oscillations in a manner similar to bicuculline’s disassembly of the bursts into field individual events (Fig. 8C: n = 6).

Results using nicotinic antagonists were less clear-cut; methyllycaconitine citrate, a potent and specific antagonist of the α7 nicotinic receptor, had little impact on carbachol oscillations, even at high concentrations (n = 8 slices; 10 nM to 1 μM; data not shown). A second nicotinic receptor antagonist, dihydro-β-erythroidine (DHβE), did disrupt preestablished oscillations when used at relatively high concentrations (250–500 μM, Fig. 8D: n = 4).Taken together, our data indicate that both muscarinic and nicotinic receptors play a role in burst organization.

**DISCUSSION**

We have characterized the cellular and pharmacological properties of carbachol oscillations in the hippocampal slice.
The parallels between extracellular and intracellular data suggest that extracellular recording is a reliable source of information about pyramidal cell activity during carbachol oscillations. Individual field events that make up a burst appear to represent the synchronous firing of groups of pyramidal cells. During carbachol oscillations, synchronous firing becomes organized into bursts within which individual events occur at 4–12 Hz.

**Locus of the oscillatory generator**

In contrast to some previous reports, we find that carbachol fails to elicit oscillatory behavior in CA1 when it is isolated from the CA3 region (Bland and Colom 1993; Paulsen and Vida 1996). We find no obvious difference in the likelihood of carbachol oscillations between low-versus high-potassium-containing ACSF solutions, suggesting that this cannot account for the discrepancy. Perhaps the mini-slices used in a previous study retained a part of area CA3 capable of generating carbachol oscillations (Konopaki et al. 1987). Our data, in agreement with other studies, suggest that CA1 cannot support carbachol oscillations in the 4- to 12-Hz range on its own but requires oscillations generated in CA3 to be propagated synaptically to CA1 (Bianchi and Wong 1994; Borroni and Levy 1996; Traub et al. 1992).

**Relationship between carbachol oscillations and other examples of oscillatory and bursting behavior**

The CA3 region of a hippocampal slice can generate oscillatory electrical activity in response to a variety of stimuli (Traub et al. 1996a). These disparate stimuli converge on a common target, the richly reciprocally innervated CA3 pyramidal cells. The physiological phenomenon of theta rhythm observed in vivo shares a common oscillatory frequency with carbachol oscillations. This suggests that the circuit or intrinsic properties of neurons in CA3 are “tuned” to this frequency band in vivo (Traub et al. 1992). A useful in vitro model of theta rhythm should exhibit cellular interactions similar to those in vivo, for example, between CA3 and CA1 pyramidal cells and between pyramidal cells and GABAAergic interneurons. As previously indicated from simulations (Traub et al. 1992), theta rhythm in vivo differs significantly from carbachol oscillations we observe in slices. For example, in the majority of studies in vivo, the pyramidal cells are silent during the peak of theta while the interneurons discharge phasically, resulting in a powerful inhibitory drive to the principle neurons (Soltész and Deschénes 1993; Ylinen et al. 1995b). In addition, the firing of CA3 neurons does not appear to be critical for theta rhythm in area CA1, as CA3 neurons in vivo generate little or no theta rhythm, and generally fail to reach threshold during theta oscillations (Bland and Wishaw 1976; Bland et al. 1975; Fox and Ranck 1981). Moreover, rhythmic GABAergic inhibitory postsynaptic potential (IPSPs) underlie the theta rhythm, possibly due to rhythmically active septal neurons (Stewart and Fox 1990), and the phase and amplitude of theta oscillations shift with the chloride reversal potential in both CA1 and CA3 pyramidal cells, as expected if IPSPs dominate the rhythm (Fox et al. 1983; Leung and Yim 1986; Ylinen et al. 1995b). Recently theta rhythm has been mimicked in a septohippocampal slice preparation using rhythmic electrical stimulation of the medial septal nucleus (Toth et al. 1997). Rhythmic septal stimulation results in theta-frequency oscillations in hippocampal pyramidal cells driven by rhythmic IPSPs; importantly, the oscillations are not blocked by muscarinic and glutamate receptor antagonists. In contrast, our data show that during carbachol oscillations, the output of CA3 pyramidal cells essentially controls the theta-frequency rhythm, whereas inhibition organizes the firing into discrete bursts. Thus carbachol oscillations appear fundamentally distinct from theta observed in vivo.

Carbachol oscillations more closely resemble the interictal bursting reported in a variety of epilepsy models (McBain et al. 1993; Nagao et al. 1996; Swann et al. 1993; Traub and Wong 1982; Traub et al. 1996a); in fact, a muscarinic receptor agonist, pilocarpine, is used in vivo to produce chronic pathologies that resemble clinical epilepsy (Turkski et al. 1983). Carbachol oscillations in CA3 neurons appear more controlled than other examples of interictal bursting, perhaps due to partial suppression of glutamate release by muscarinic receptors present on CA3 nerve terminals (Scanziani et al. 1995). Under our recording conditions, evoked field EPSPs in both CA1 and CA3 are reduced by about half (unpublished observations), suggesting that although glutamatergic transmission is still present in the slice (and required for carbachol oscillations), it is attenuated.

**Intracellular oscillations underlie the population oscillation**

Although AMPA receptors are necessary for population carbachol oscillations, DNQX failed to block a second, slower oscillation in individual CA3 pyramidal cells. This DNQX-sensitive rhythm is distinct from the originally reported rhythmic slow activity in CA3 neurons, which was blocked by kynurenate (MacVicar and Tse 1989). Rhythmic bursts similar to the DNQX-insensitive rhythm we observe have been reported previously in CA3 pyramidal cells when all fast synaptic transmission has been blocked and in the presence of 4-aminopyridine and carbachol (Bianchi and Wong 1994). Our data indicate that 4-aminopyridine is not essential to initiate this rhythm, which instead appears to be produced by carbachol alone. Taken together, these results indicate that the slow rhythm observed in synthetically uncoupled CA3 pyramidal cells represents the fundamental oscillator that entrains pyramidal cells into the characteristic bursts that comprise carbachol oscillations (Traub et al. 1992). Carbachol must block or activate conductances intrinsic to CA3 neurons that promote rhythmic activity, and via excitatory connections between CA3 neurons, the intracellular oscillations can become entrained into synchronous excitatory activity. DNQX simply disconnects the pyramidal cells from one another but does not interfere with the underlying intracellular rhythm in individual cells.

Oscillations with properties quite similar to those of carbachol oscillations also can be generated in the CA3 region after application of metabotropic glutamate receptor agonists (Taylor et al. 1995). These oscillations also are blocked by AMPA-receptor antagonists, are insensitive to NMDA-receptor antagonists, and have a burst pattern that is modulated by GABA_A-receptor agonists. However, unlike car-
bacterial oscillations, the metabotropic glutamate oscillation was blocked by MCPG. M1/M3 receptors and mGluR1/ mGluR5 receptors share a common phosphotyrosinolinositol intracellular signaling pathway likely to be responsible for CA3 cell excitation (Tse and MacVicar 1989; see Felder 1995; McKinney 1993 for reviews). Together these data suggest that activation of phosphotyrosinolinositol breakdown is a potent fundamental mechanism sufficient to produce rhythmic oscillations in CA3 pyramidal cells.

**NMDA receptors are necessary for spread of oscillations from CA3 to CA1**

Preestablished carbachol oscillations in CA3 and CA1 were unaffected by application of AP5, even at relatively high concentrations (see also MacVicar and Tse 1989). In contrast, preincubation with AP5 markedly attenuated oscillations in only the CA1 region. These data suggest that the generation of oscillations in CA3 does not require NMDA receptors but that propagation of the oscillations into CA1 does require NMDA receptors. Intertitial events (similar in structure to the single field events seen during carbachol oscillations) generated in CA3 by elevated extracellular potassium concentrations also are unaffected by AP5; in contrast, the seizures they trigger in CA1 are disrupted (Traynelis and Dingledine 1988). We speculate that synaptic connections between CA3 and CA1 trigger rhythmic excitation of the CA1 region at the start of carbachol oscillations. Muscarinic receptor potentiation of the NMDA receptor (Markram and Segal 1990) may enable the receptor to mediate transient synaptic strengthening, allowing oscillations to become established in CA1. These data suggest that NMDA receptors play a pivotal role in controlling the spread of rhythmic activity from CA3 to the CA1 region and indicate another similarity to epileptiform bursting rather than to theta rhythm.

**GABA receptors are important for burst organization**

MacVicar and Tse (1989) originally reported that blockade of GABAergic transmission had no impact on carbachol oscillations recorded intracellularly. In recent years, however, considerable evidence has accumulated pointing to the importance of GABAergic transmission in the generation and shaping of synchronized neuronal discharge in a variety of brain structures (Cobb et al. 1995; Michelson and Wong 1994; Traub et al. 1996b; Whittington et al. 1995). We found that blockade of GABA<sub>α</sub>-receptors disassembled each burst into nonphasic field events. One possible reason for the difference between our results and previous data are the high levels of extracellular potassium (which erodes inhibition) used in previous studies (Korn et al. 1987; McCarren and Alger 1985); our relatively low potassium levels may allow inhibitory events to contribute to the generation of oscillations. Another possibility is that different parts of CA3 behave differently from one another. We routinely recorded from area CA3c, and perhaps more of the local circuit remains intact in this part of CA3, enabling GABAergic interneurons to be driven synchronically during the oscillatory event and hence contribute inhibitory drive to the principle neurons. Consistent with this hypothesis, CA3c appears to be the origin of a variety of bursting behaviors (Korn et al. 1987). We conclude that previous work underestimated the role of GABA<sub>α</sub>-mediated inhibition in patterning synchronous bursting activity in the CA3 region.

How might fast inhibitory synaptic transmission govern the assembly of each individual synaptic event into a full-fledged burst? Our results show that bicuculline does not interfere with individual field events that presumably represent the synchronous firing of multiple pyramidal cells, indicating that even when GABA<sub>α</sub> IPSPs are blocked, pyramidal cells can fire together. The clustering of individual field events into bursts, which is absent in bicuculline, may result if one group of interneurons fires synchronously just before a burst, providing sufficient rebound excitation to depolarize pyramidal cells into a range where high-threshold Ca<sup>2+</sup> currents are activated, initiating a burst. Synchronization of pyramidal cells after rebound from a pronounced inhibitory event has been demonstrated directly in area CA1 (Cobb et al. 1995). A simulation of carbachol oscillations previously indicated that blockade of GABA<sub>α</sub> receptors should prolong burst duration and make bursts less frequent (Traub and Dingledine 1990). The most dramatic result we observed in bicuculline was the disassembly of the bursts into individual events but occasional remaining bursts were prolonged. Possibly GABA<sub>α</sub> receptors on GABAergic interneurons themselves make the circuit more complex than the model. Recordings from interneurons during carbachol oscillations could shed light on this question.

GABA<sub>α</sub>-mediated inhibition is apparently unnecessary to generate carbachol oscillations. In agreement with results of MacVicar and Tse (1989), blockade of GABA<sub>α</sub> receptors had no consistent effect on either the initiation of oscillations or when applied to preestablished oscillations.

**Multiple cholinergic receptor subtypes participate in the generation of carbachol oscillations**

Multiple muscarinic receptor subtypes have been cloned (see McKinney 1993 for review) and exhibit distinct but overlapping patterns of expression within the hippocampus (Levey et al. 1995). In addition to its affinity for the nicotinic receptor (McMahon et al. 1994), carbachol is an agonist at all muscarinic receptor subtypes (Bujo et al. 1988; McKinney et al. 1991). Our data indicate that both M1 and M3 receptors, found on CA3 pyramidal cells (Levey et al. 1995), may be necessary for carbachol oscillations. The M1 receptor may be the primary receptor involved, because at the concentrations of 4-DAMP used here to block M3 receptors, some M1 receptor blockade would be expected (Auerbach and Segal 1996).

In contrast to the localization of M1 and M3 receptors on pyramidal cells, M2 and M4 receptors are localized predominantly to nonpyramidal, presumably GABAergic cells (Levey et al. 1995). Because GABA<sub>α</sub> antagonists disrupted carbachol-induced bursts, we were interested particularly in whether M2 and M4 receptors modulated oscillations. Methoctramine, a putative M2 antagonist, had little impact on the structure of the oscillatory burst even at high concentrations. In contrast, dicyclomine, a M4 receptor antagonist, disrupted the bursting pattern, leaving asynchronous individual events reminiscent of those seen when GABA<sub>α</sub> receptors are blocked. We therefore speculate that M4 receptors present on GABAergic interneurons contribute to the organization of bursting during carbachol oscillations.
All broad-spectrum muscarinic agonists tested here caused oscillations similar to carbachol oscillations, but the bursts were prolonged and not as well defined as in carbachol. This suggests that an additional nicotinic component, contributed by carbachol but not by muscarinic agonists, may be necessary for the full expression of oscillations. Bath application of nicotine rapidly desensitizes nACh receptors (Lester and Dani 1995), essentially removing these receptors from the circuit. Although having little effect itself, nicotine caused the disassembly of bursts, whether applied before carbachol or to preestablished oscillations. Moreover, although a selective α7 nAChR antagonist had no effect on preestablished oscillations, DHβE, a less selective nAChR antagonist (Martin-Barrows and Kellar 1987), attenuated preestablished oscillations. These data strongly suggest that nAChRs play a crucial role in carbachol oscillations. We interpret these results to mean that when nicotinic receptors are either desensitized or blocked, bursts are prevented though individual field events may continue.

Nicotine acts on a variety of pre- and postsynaptic targets in the hippocampus (Gray et al. 1996; Sawada et al. 1994). One possible site of action may be GABAergic interneurons, recently demonstrated to be responsive to nicotine (Frazier et al. 1996; McQuiston and Madison 1996). The disassembly of bursts by nicotine is similar to that of bicuculline, supporting a role of nAChRs on interneurons.

Taken together, our evidence suggests that M1, M3, M4, and the nAChR receptors work together to generate carbachol oscillations. Whereas M1 and M3 receptors are likely to promote membrane potential oscillations in CA3 pyramidal cells, probably by activating a phosphoinositide pathway, M4 and nACh receptors may excite interneurons to shape the oscillation into bursts.

In conclusion, the properties of carbachol oscillations identify them as more closely related to epileptiform bursting than to theta rhythm in vivo. We also have identified a basic oscillatory pattern in CA3 pyramidal cells that is induced by carbachol. This rhythm in individual neurons may initiate the population rhythm by exciting neighboring pyramidal cells via AMPA receptors. GABA-releasing interneurons in the local circuit are also likely targets of carbachol and are responsible for the organization of the bursting pattern characteristic of carbachol oscillations.

We thank S. Douglas for invaluable technical assistance and Drs. Lori McMahon, Richard Mooney, and Felix Schweizer for insightful comments on the manuscript.

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Received 13 May 1997; accepted in final form 11 July 1997.

REFERENCES

Auerbach, J. M. and Segal, M. Muscarinic receptors mediating depression and long-term potentiation in rat hippocampus. J. Physiol. (Lond.) 492: 2: 479–493, 1996.
Markram, H. and Segal, M. Long-lasting facilitation of excitatory post-


