Cholinergic plasticity in the hippocampus

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Tests were made for use-dependent plasticity in the cholinergic projections to hippocampus. Transient infusion of the cholinergic agonist carbachol into hippocampal slices induced rhythmic activity that persisted for hours after washout. Comparable effects were obtained with physostigmine, a drug that blocks acetylcholine breakdown and thereby enhances cholinergic transmission. It thus seems that activation of cholinergic synapses induces lasting changes in hippocampal physiology. Two lines of evidence indicated that cholinergic synapses are also the sites at which the plasticity is expressed. First, the induction and expression of the rhythms were not blocked by the *N*-methyl-D-aspartate receptor antagonist D-2-amino-5-phosphonovaleric acid, indicating that a long-term potentiation effect between pyramidal cells was not involved. Second, a muscarinic antagonist (atropine) completely abolished stable rhythmic activity after agonist washout. This result indicates that endogenous cholinergic activity is responsible for the persistence of rhythmic oscillations. These experiments suggest that short periods of intense cholinergic activity induce lasting changes in cholinergic synapses and thus extend such forms of plasticity to beyond the glutamatergic system.

It is now well established that brief periods of intense synaptic activity cause marked and lasting changes in the strength of glutamatergic transmission at sites throughout the forebrain (1–3). A similar consensus does not hold for other transmitters. Several reports indicate that fast, inhibitory transmission mediated by γ-aminobutyric acid type A receptors exhibits use-dependent, lasting changes (4–7), but others have failed to obtain such effects (8). Activity-induced synaptic changes in aminergic and cholinergic contacts in brain seem not to have been reported. Although it is possible that glutamatergic transmission is unique with regard to plasticity, it is also the case that it is much more accessible to conventional stimulation and recording techniques than other systems. In the present study, we used a somewhat different approach involving conventional hippocampal slice preparations to test for activity-dependent changes at endogenous cholinergic synapses.

The hippocampus receives a moderately dense cholinergic projection from the medial septum and basal forebrain (9–11) and has both nicotinic and muscarinic acetylcholine receptors (mAChRs) in its target zones (12, 13). These pathways generate rhythmic activity of various frequencies (14–18), some aspects of which can be reproduced in hippocampal slices by infusing cholinergic agonists (19–23). The *in vitro* rhythms are mediated by mAChRs (19, 20, 22, 23) and originate in the CA3 region from whence they move along intrahippocampal pathways to other subfields (22, 23). Although there is sizeable literature suggesting that the cholinergic projections and the rhythms they generate make essential contributions to plasticity in glutamatergic plasticity (2, 21, 24, 25) and learning (26, 27), there is no evidence that the projections themselves change after intense use.

Given that cholinergic activation triggers rhythms in hippocampus, plasticity could be manifested as a change in oscillations that outlasts the activation itself. For such an effect to be an analogue of the use-dependent changes seen in glutamatergic synapses it would have to depend on cholinergic transmission as opposed to being a secondary effect on some other transmitter system induced by intense cholinergic activity. In other words, it would be necessary to show that cholinergic synapses both induce and express changes in rhythmic oscillations. The following studies addressed each of these issues with results indicating that the cholinergic projections to hippocampus do undergo lasting, physiologically important changes after transient activation.

Methods

Slice Preparation. Transverse slices (350 μm) were cut from the mid- to ventral hippocampus of 3- to 4-week-old male Sprague–Dawley rats by using a vibrating tissue slicer (Leica VT1000). Animals were killed under an accredited animal protocol from the University of California Institutional Animal Care and Use Committee with guidelines from the National Institutes of Health. Artificial cerebrospinal fluid was composed of 124 mM NaCl, 3 mM KCl, 1.25 mM KH_2PO_4, 5 mM $MgSO_4$, 3.4 mM $CaCl_2$, 10 mM D-glucose, and 26 mM $NaHCO_3$ for dissection. Hippocampal slices then were placed on an interface recording chamber and infused at a rate of 60 ml/h with oxygenated recording artificial cerebrospinal fluid of the same composition as described above except that the $CaCl_2$ and $MgSO_4$ concentrations were lowered to 3 and 1 mM, respectively. Humidified 95% O_2/5% CO_2 was additionally blown into the chamber throughout the recovery and recording periods. Slices were allowed to recover for a period of at least 1 h.

Field-Potential Recording. Field potentials were recorded by using extracellular recording electrodes filled with 2 M NaCl (1–5 MΩ). For recording of spontaneous and rhythmic activity, electrodes were placed in stratum pyramidale of field CA3. For monitoring the excitatory postsynaptic potential (EPSP) evoked by Schaffer collateral/commissural stimulation, a recording electrode was placed in CA1 stratum radiatum. Stimulation was delivered through bipolar stimulating electrodes (twisted 64-μm insulated nichrome wires), and stimulation current intensity was set to evoke a response that was $<$50% of the maximum monophasic response (biphasic pulses of 0.1- to 0.3-ms duration, 10–50 μA). Samples of 800 ms were recorded every 30 s; in the cases involving stimulation, stimulation pulses were delivered every 30 s. Stable baseline conditions were monitored for at least 10 min before infusion of drug solutions. Data were recorded by using a differential alternating current amplifier (Model 1700, A-M Systems, Carlsborg, WA) and digitized at 5 kHz. Experiments were conducted at physiological temperatures (32 $\pm$ 2°C).

Reagents. All drugs were obtained from Sigma. Drugs were added to the infusion line by using an injection pump with the exception of D-2-amino-5-phosphonovaleric acid (AP5) and tetrodotoxin, which were bath-applied. Solutions were prepared freshly on the day of the experiment.

Statistics and Measures. Paired, two-tailed *t* tests and two-way ANOVA with repeated-measure tests were used to assess statistical significance. The data are expressed as means $\pm$ SEM. Power spectra were estimated from 400-ms samples by using the fast Fourier transformation function in MATLAB (Mathworks,

Abbreviations: mAChR, muscarinic acetylcholine receptor; EPSP, excitatory postsynaptic potential; AP5, D-2-amino-5-phosphonovaleric acid; CCh, carbachol; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; 5-HT, serotonin.

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Natick, MA). Power was normalized to the last 5 min of agonist infusion.

Spike and burst counts were computed by finding positive peaks in the second derivative of smoothed time-series recordings within 400-ms time windows, again by using MATLAB. Spikes occurring at intervals ≤5 ms constituted a "burst." Spike and burst counts were normalized to the last 5 min of agonist infusion.

Results

Cholinergic Agonists Cause Long-Lasting Increases in Rhythmic Activity. Earlier studies using horizontal slices (22) or slices taken from dorsal hippocampus (20) have reported that carbachol (CCh) application produced oscillations with primary frequencies in the γ and θ ranges, respectively. In contrast, the present report describes cholinergic rhythms that resemble those observed in an earlier study conducted under experimental conditions similar to those used here (23). Infusion of CCh (20 μM) into field CA3 caused the rapid appearance of fast rhythmic activity with a dominant frequency in the β band (27.4 ± 0.2 Hz at 10–20 min of infusion, $n = 28$) and a secondary frequency in the γ range (48.2 ± 0.2 Hz; Fig. 1*A*). Power in the β range increased >30-fold during infusion ($P < 0.0001$, $n = 28$). The waveform of the CA3 rhythm is illustrated in Fig. 1*B*. Each cycle begins with a large spike or burst of spikes followed by a steep positive-going potential, which gradually declines until the cycle begins again. The spiking activity was largely absent from CA3 under baseline conditions. The increase in rhythmic activity was associated with a reliable decrease in the amplitude of the field EPSP recorded in the apical dendrites of field CA1 in response to stimulation of Schaffer collateral/commissural projections.

The depression of the field EPSP produced by CCh reversed quickly after washout. Although recovery was never quite complete, this provided a measure of the time course for CCh washin and washout. In contrast to reversible effects observed with the evoked response, the rhythmic activity continued at a high level even after hours of washout. Fig. 1*C* shows normalized power in the β and γ frequency ranges during washout for a group of 14 slices. Power in the β band after 50–60 min of washout was >20-fold greater than that recorded in the pre-CCh period ($P < 0.0001$, $n = 14$) and gave no evidence of decreasing with time. In three cases, recordings were continued for >3 h of washout, at which time high levels of rhythmic activity still were present. Similar effects were obtained in the γ frequency range. Long-lasting rhythms were eliminated completely after infusion of tetrodotoxin (1 μM) 1 h post-CCh washout ($n = 3$, data not shown).

The induction of persistent rhythms was accompanied by long-lasting changes in firing patterns of CA3 pyramidal neurons. Spiking and bursting spontaneously recorded in CA3 stratum pyramidale, relatively infrequent under baseline conditions, increased after transient CCh application (Fig. 2*A*) following the same time course as ongoing rhythms. In Fig. 2*B* the spike rate (*Upper*) has been normalized to the mean rate during the last 5 min of CCh infusion. As can be seen in these group data, spiking increased ≈3-fold with infusion and remained close to that level throughout 60 min of washout ($P < 0.0001$ pre-CCh vs. 60 min after, $n = 14$). The increased number of short, high-frequency bursts of spikes produced by CCh also persisted without evident change throughout the washout period (Fig. 2*B Lower*). In all, the rhythms induced by cholinergic stimulation, as well as the spiking activity associated with them, remained in place for hours after the stimulation was removed.

Changes in the Cholinergic Projections Are Responsible for Long-Lasting Rhythms. A plausible explanation for the long-lasting changes induced by CCh is that the rhythms increase synaptic strength among CA3 collaterals and thereby raise the probability

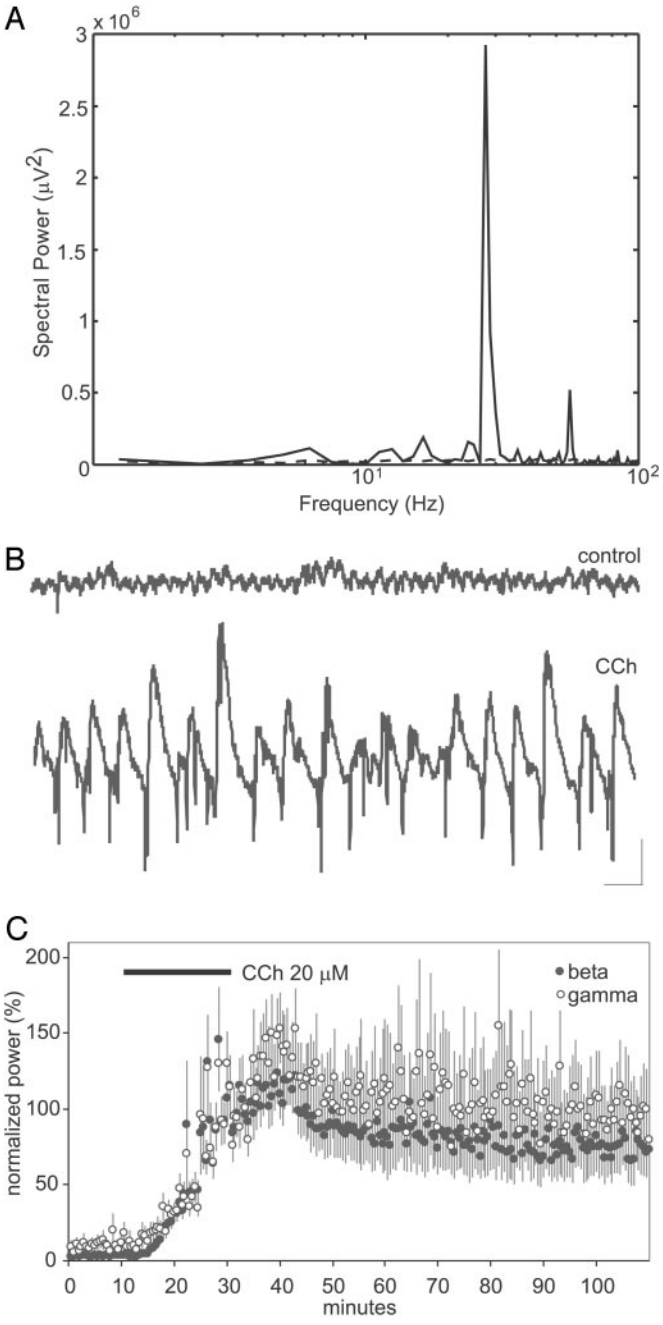

Fig. 1. Induction of fast oscillations in field CA3 of hippocampal slices. (*A*) Example power spectra of control (dotted line) and extracellular oscillations induced by 20 μM CCh (solid line). (*B*) Example traces of extracellular recordings before (*Upper*) and after (*Lower*) infusion of 20 μM CCh. (Calibration: 50 ms, 100 μV.) (*C*) Persistence of oscillations after CCh washout. Each point represents the average normalized power (mean ± SEM) within the β or γ frequency band. CCh (20 μM) was infused for a 20-min period (indicated by black bar) after at least 10 min of baseline recording.

of synchronized cell firing. Indeed, the spontaneous bursting of CA3 neurons associated with the rhythms is reminiscent of stimulation patterns used to induce long-term potentiation (LTP) (2). However, neither induction nor expression of stable rhythmic activity was blocked by *N*-methyl-D-aspartate (NMDA) receptor antagonists. Fig. 3*A* summarizes results for a set of five experiments in which 100 μM AP5 was added before, during, and for 25 min after CCh infusion. The mean β power after 50–60

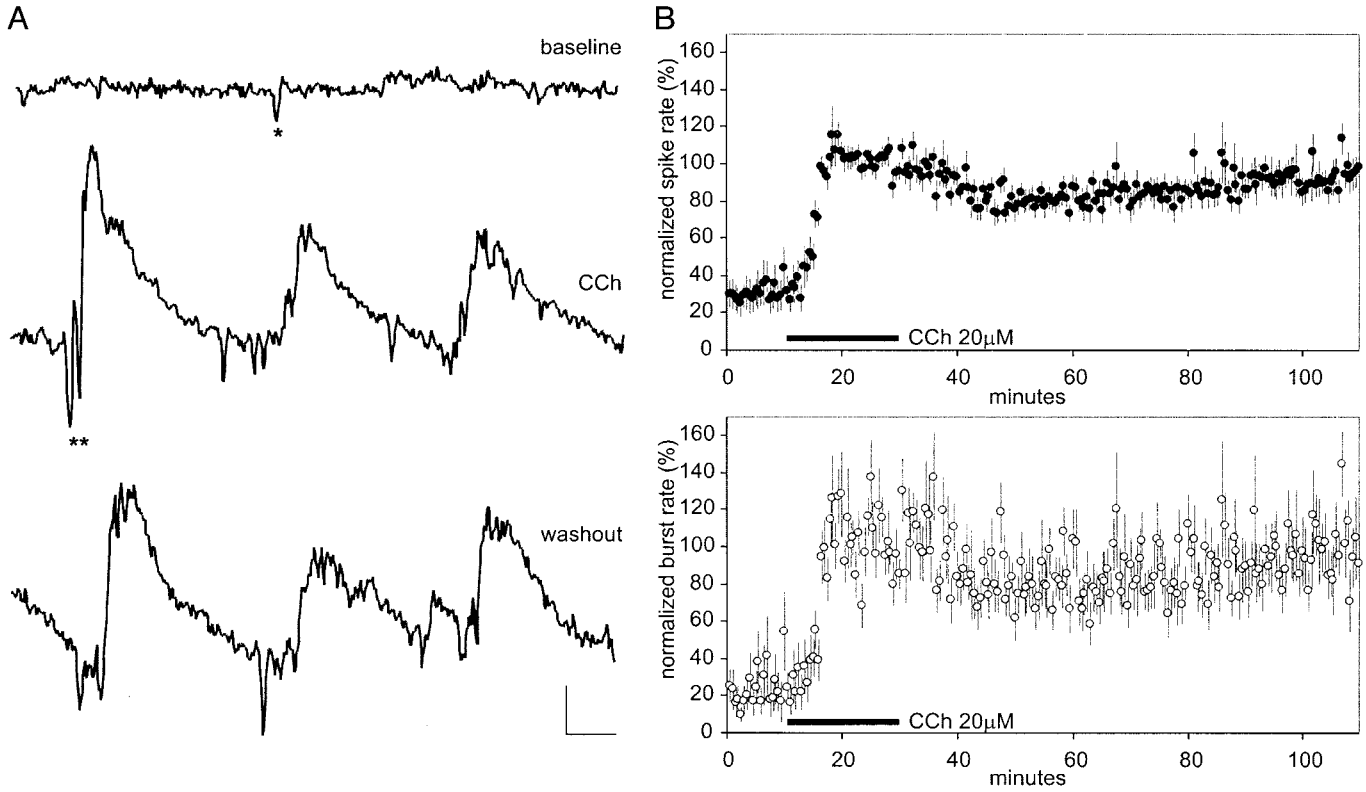

Fig. 2. Lasting increases in CA3 pyramidal cell activity levels after transient CCh application. (*A*) Representative traces showing examples of spikes and bursts, indicated by * and **, respectively. (Calibration: 10 ms, 100 μV.) (*B*) Spike (*Upper*) and burst (*Lower*) rates of CA3 pyramidal neurons plotted across time to demonstrate the sustained increases that result from transient (20-min) CCh application. Each point (mean ± SEM) represents the normalized spike or burst count within a 400-ms time window.

min of CCh washout was 76.0 ± 1.6% of the peak effect for the CCh-alone group and 109.0 ± 4.7% for the CCh-plus-AP5 group ($P < 0.0001$). If anything, then, NMDA receptors reduced the power of the β activity.

An alternative explanation is that modifications to the cholinergic terminals themselves are responsible for long-lasting rhythms. This idea has the great advantage that it could explain why the rhythms found after washout of CCh so closely resemble those triggered in its presence. On the other hand, unlike the case for CA3 associational synapses, there is no evidence for activity-dependent changes in cholinergic synapses. The acetylcholinesterase inhibitor, physostigmine, was used to test whether enhancing cholinergic transmission induces long-lasting rhythmic activity. As shown in Fig. 3*B*, physostigmine caused an increase in rhythmic activity that, other than being smaller in scale, was not detectably different from that produced by CCh. The peak effect was again in the β range, with a secondary increase in the γ band, and the resultant waves had the same spike-on-trough pattern seen with the agonist. Most importantly, the increased rhythmic activity persisted without evident change for the entire duration of the washout period; power in the β and γ ranges was approximately three times baseline power at the end of the recording session ($P < 0.0001$, $n = 5$).

The above experiment establishes that cholinergic synapses underlie a use-dependent, long-lasting change in a naturally occurring aspect of hippocampal physiology. The question then arises as to whether the same synapses also express the change or trigger a reaction in a noncholinergic system that drives rhythms. If the former hypothesis were true, then blocking cholinergic transmission after washout of CCh (or physostigmine) would be expected to have a profound effect on the induced rhythms. Fig. 3*C* summarizes results confirming this prediction. The muscarinic antagonist atropine was infused starting 45 min after CCh washout, allowing long-term rhythms to stabilize and, as is evident, completely abolished the oscillations. Normalized power of the β and γ activity was reduced to near baseline levels by the last 10 min of atropine infusion ($P < 0.0001$ for β and $P < 0.0001$ for γ, $n = 5$). EPSPs were recorded in CA1 stratum radiatum to monitor the health of the slice and appeared to remain strong and healthy during atropine washin and washout, the same period during which oscillations were lost (Fig. 3*C Inset*).

Reversal of Long-Lasting Increases in Rhythmic Activity. An unexpected feature of the effects of atropine on the tonic rhythms was the absence of a washout effect. This could reflect the state of the slices late into the experiment; that is, after several hours the slices may not be capable of regenerating autonomous activity. However, loss of rhythmic activity in the atropine cases was not accompanied by reductions in the size of EPSPs during antagonist infusion and washout. An alternate but related idea is that the rhythms are self-regenerative and therefore disappear if interrupted for more than a few moments. Serotonergic activation is reported to desynchronize hippocampal electroencephalogram (28) and was used to test whether rhythms recovered after a transient suppression unrelated to cholinergic receptors. As shown in Fig. 3*D*, serotonin (5-HT, 10 μM) caused an immediate reversal of rhythmic activity, but in marked contrast to the atropine result the rhythms returned essentially unchanged after washout of the agonist. Reversal and subsequent recovery of long-lasting rhythms after 5-HT application were accompanied by corresponding changes in CA3 pyramidal cell activity levels (Fig. 4 *B–D*). In contrast, the complete elimination of rhythms due to atropine was associated with a permanent

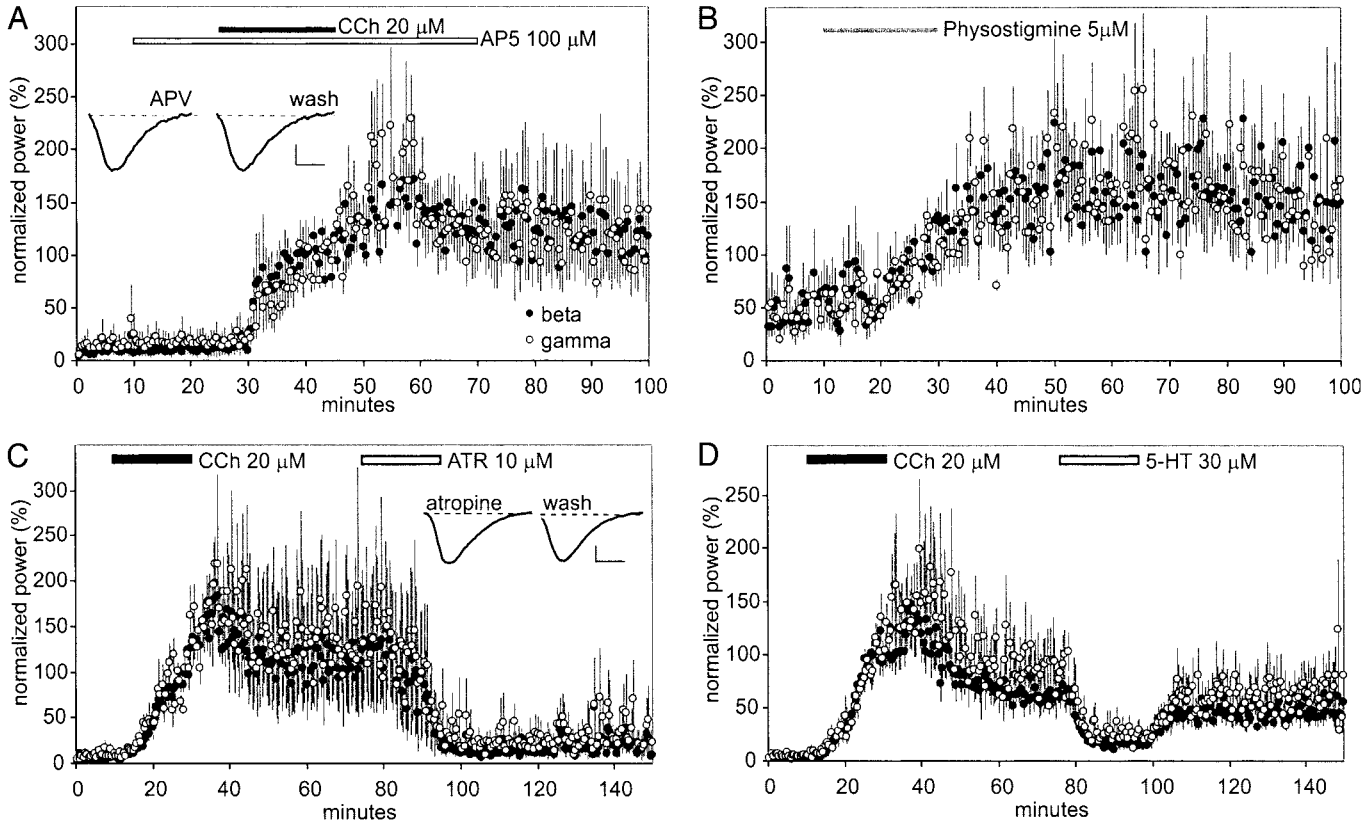

Fig. 3. Effects of pharmacological manipulations on long-lasting rhythms. (*A*) NMDA-independence of long-term increases in oscillatory activity. Shown are five cases in which slices were infused with CCh (20 μM) for 20 min within a 50-min bath application of AP5 (100 μM). Representative EPSPs from CA1 stratum radiatum are shown (*Inset*) at the end of the AP5 infusion and 30-min post-AP5 washout. (Calibration: 5 ms, 400 μV.) (*B*) Long-term increases in rhythmic activity caused by elevation of endogenous acetylcholine levels. Physostigmine was applied for 20 min at a moderate concentration (5 μM). (*C*) Necessity of mAChRs for maintenance of β and γ rhythms. Atropine (ATR) was infused at a concentration of 10 μM for a period of 20 min beginning 45 min after CCh washout. Representative EPSPs from CA1 stratum radiatum are shown (*Inset*) during atropine washin and washout. (Calibration: 5 ms, 500 μV.) (*D*) Temporary depression of rhythmic activity brought on by 5-HT infusion. 5-HT (30 μM) was infused for 20 min after 45 min of CCh washout.

reversal of CCh-induced increases in pyramidal cell activity (Fig. 4 *A–C*). Although additional experiments with other transmitter agonists are needed, these experiments suggest that the loss of rhythmic activity with atropine is due to an effect unique to cholinergic transmission; i.e., intense activation of cholinergic receptors induces stable rhythms, whereas blockade of the receptors eliminates them.

Discussion

The present results indicate that transient periods of cholinergic stimulation induce long-term rhythms, associated with lasting increases in cell spike and burst rates. In that they involve naturally occurring physiology, are triggered by relatively brief episodes of intense activity, and remain long after the triggers are removed (3 h at least), the tonic rhythms resemble LTP. A plausible explanation for lasting increases in rhythmic activity would be that an induction of LTP at glutamatergic synapses, resulting from burst patterns induced by CCh, maintained the rhythmic activity via strengthened collateral connections. However, NMDA receptor antagonists at concentrations that completely suppress LTP did not interfere with the formation of stable rhythms. Subsequent experiments showed that facilitation of endogenous cholinergic activity resulted in the formation of stable rhythms, whereas antagonism of endogenous cholinergic transmission eliminated already developed rhythms. In all, the long-term rhythms appear to be both induced and expressed by endogenous synapses, probably within field CA3. This observation leads to the further conclusion that the cholinergic projections to hippocampus are plastic and in particular undergo use-dependent changes that bear some resemblance to those found in LTP.

Because the long-term rhythms are not notably different from the acute oscillations in the presence of CCh or physostigmine, it is likely that they are due to the same end points produced by the drugs, namely stimulation of above-normal numbers of cholinergic receptors. This could be achieved by enhancing either (*i*) spontaneous release of acetylcholine from the within-slice projections arising from the medial septum/diagonal-bands complex or (*ii*) the response of postsynaptic receptors to released transmitter. Although there is little evidence favoring either of these ideas, CCh is reported to affect the distribution of postsynaptic mAChRs in cortex (29). Equilibrium-binding and immunocytochemical studies should quickly narrow the range of possible postsynaptic mechanisms underlying the expression of long-term rhythms, and intracellular recordings should be useful in determining whether presynaptic release kinetics play a role.

One of the more surprising features of the rhythms was their erasure by transient exposure to a muscarinic antagonist. That is, not only were the rhythms blocked by the antagonist, as expected if the oscillations reflect ongoing cholinergic activity, but they also failed to return after washout of the antagonist. This was not due to suppression of the rhythms *per se*, because noncholinergic agents blocked the oscillations only while present in the tissue. These results suggest that periods of subnormal cholinergic activity reset the lasting changes induced by periods of intense activity. This observation should be of help in iden-

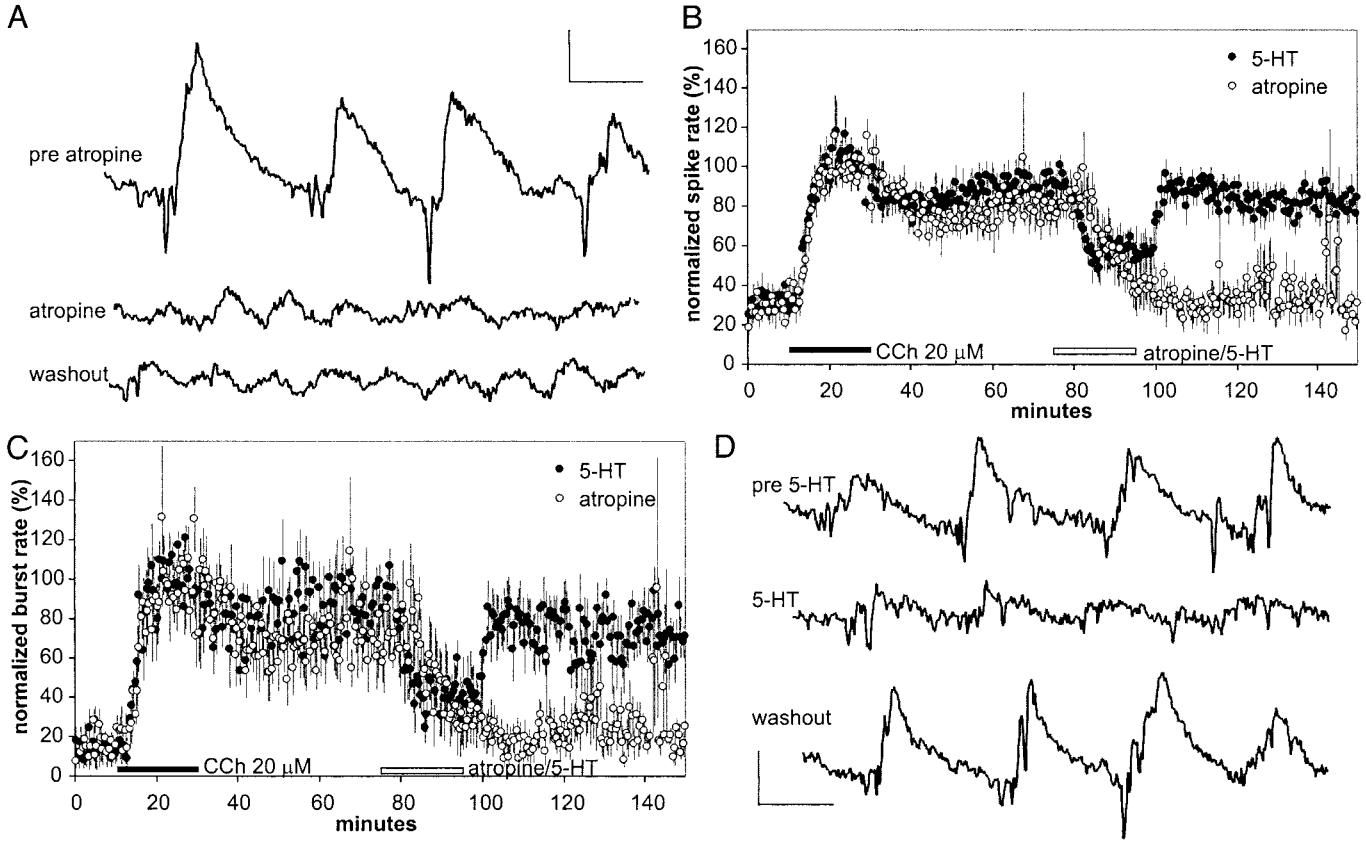

Fig. 4. Effects of atropine and 5-HT on spike and burst frequency. (*A*) Examples of spontaneous activity recorded in CA3 stratum pyramidale after induction of long-term rhythms immediately before, during, and 30 min after atropine infusion. (Calibration: 20 ms, 100 μV.) (*B*) Reversible effects of 5-HT on spike frequency contrasted with irreversible effects of atropine. (*C*) Recovery and lack of recovery of lasting increases in burst frequency after 5-HT application and atropine application, respectively. (*D*) Representative samples of spontaneous activity recorded in the CA3 pyramidal cell layer immediately before, during, and 30 min after 5-HT infusion. (Calibration: 20 ms, 100 μV.)

tifying the changes that express the long-lasting rhythms. For example, activation and/or deactivation of signaling cascades may be involved, given that mAChRs are G protein-coupled. Beyond this, reversibility has important implications for the possible functional significance of cholinergic plasticity. Possibly, the strength of the connections between the ascending cholinergic projections and their forebrain targets undergoes continuous adjustment, with the most recent period of (high/low) activity serving to set up activity for the events that follow.

The functional significance of tonic rhythmic activity presumably is related to whatever roles the rhythms play in their acute occurrences. The most widely discussed hypothesis regarding this is that fast rhythms synchronize interconnected brain areas and thereby allow for transfer of information between them (30–32). For example, two-way interactions between hippocampus and entorhinal cortex are proposed to be necessary for the formation and retrieval of memory (33–35); in accord with this idea, frequency-dependent transfer of activity from superficial layers of entorhinal cortex to hippocampus has been described in studies using real-time optical imaging and voltage-sensitive dyes (36). The effects described in this article could preserve temporal relationships between the two areas across the extended periods of time needed for complex behaviors without requiring that animals maintain behavioral modes (e.g., searching) associated with intense cholinergic activity. According to this hypothesis, animals would initiate synchronizing rhythms with one set of behaviors and then use these rhythms during a second, quite different set of behavioral activities.

In all, the present studies provide evidence that long-lasting, use-dependent plasticity is not restricted to glutamatergic synapses and raise new questions about how such phenomena are used during behavior. Additionally, these data link cholinergic fast rhythms to lasting changes in neuronal activity, consistent with a primary role for fast rhythms, and the cholinergic system in general, in mnemonic processing.

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1. Bliss, T. V. & Lomo, T. (1973) *J. Physiol. (London)* **232,** 331–356.
2. Larson, J., Wong, D. & Lynch, G. (1986) *Brain Res.* **368,** 347–350.
3. Staubli, U. & Lynch, G. (1987) *Brain Res.* **435,** 227–234.
4. Korn, H., Oda, Y. & Faber, D. S. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 440–443.
5. Otis, T. S., De Koninck, Y. & Mody, I. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7698–7702.
6. Brussaard, A. B., Kits, K. S., Baker, R. E., Willems, W. P., Leyting-Vermeulen, J. W., Voorn, P., Smit, A. B., Bicknell, R. J. & Herbison, A. E. (1997) *Neuron* **19,** 1103–1114.
7. Nusser, Z., Hajos, N., Somogyi, P. & Mody, I. (1998) *Nature* **395,** 172–177.
8. Arai, A., Silberg, J. & Lynch, G. (1995) *Brain Res.* **704,** 298–306.
9. Lewis, P. R. & Shute, C. C. (1967) *Brain* **90,** 521–540.
10. Mosko, S., Lynch, G. & Cotman, C. W. (1973) *J. Comp. Neurol.* **152,** 163–174.
11. Woolf, N. J., Eckenstein, F. & Butcher, L. L. (1984) *Brain Res. Bull.* **13,** 751–784.
12. Levey, A. I., Edmunds, S. M., Koliatsos, V., Wiley, R. G. & Heilman, C. J. (1995) *J. Neurosci.* **15,** 4077–4092.
13. Albuquerque, E. X., Pereira, E. F., Alkondon, M., Schrattenholz, A. & Maelicke, A. (1997) *J. Recept. Signal Transduct. Res.* **17,** 243–266.

14. Petsche, H., Stumpf, C. & Gogolak, G. (1962) *Electroencephalogr. Clin. Neurophysiol.* **14,** 202–211.
15. Gogolak, G., Stumpf, C., Petsche, H. & Sterc, J. (1968) *Brain Res.* **7,** 201–207.
16. Macadar, O., Roig, J. A., Monti, J. M. & Budelli, R. (1970) *Physiol. Behav.* **5,** 1443–1449.
17. Apostol, G. & Creutzfeldt, O. D. (1974) *Brain Res.* **67,** 65–75.
18. Vertes, R. P. & Kocsis, B. (1997) *Neuroscience* **81,** 893–926.
19. Konopacki, J., MacIver, M. B., Bland, B. H. & Roth, S. H. (1987) *Brain Res.* **405,** 196–198.
20. MacVicar, B. & Tse, F. W. Y. (1989) *J. Physiol. (London)* **417,** 197–212.
21. Huerta, P. T. & Lisman, J. E. (1993) *Nature* **364,** 723–725.
22. Fisahn, A., Pike, F. G., Buhl, E. H. & Paulsen, O. (1998) *Nature* **394,** 186–189.
23. Shimono, K., Brucher, F., Granger, R., Lynch, G. & Taketani, M. (2000) *J. Neurosci.* **20,** 8462–8473.
24. Blitzer, R. D., Gil, O. & Landau, E. M. (1990) *Neurosci. Lett.* **119,** 207–210.
25. Auerbach, J. M. & Segal, M. (1994) *J. Neurophysiol.* **72,** 2034–2040.
26. Landfield, P. W. (1977) *Physiol. Behav.* **18,** 439–445.
27. Winson, J. (1978) *Science* **201,** 160–163.
28. Assaf, S. Y. & Miller, J. J. (1978) *Neuroscience* **3,** 539–550.
29. Van der Zee, E. A., Strosberg, A. D., Bohus, B. & Luiten, P. G. (1993) *Brain Res. Mol. Brain Res.* **18,** 152–162.
30. Freeman, W. (1975) *Mass Action in the Nervous System* (Academic, New York), pp. 429–435.
31. Gray, C. M., Konig, P., Engel, A. K. & Singer, W. (1989) *Nature* **338,** 334–337.
32. Bressler, S. L., Coppola, R. & Nakamura, R. (1993) *Nature* **366,** 153–156.
33. Squire, L. R. & Zola-Morgan, S. (1991) *Science* **253,** 1380–1386.
34. Witter, M. P., Wouterlood, F. G., Naber, P. A. & Van Haeften, T. (2000) *Ann. N.Y. Acad. Sci.* **911,** 1–24.
35. Fell, J., Klaver, P., Lehnertz, K., Grunwald, T., Schaller, C., Elger, C. E. & Fernandez, G. (2001) *Nat. Neurosci.* **4,** 1259–1264.
36. Iijima, T., Witter, M. P., Ichikawa, M., Tominaga, T., Kajiwara, R. & Matsumoto, G. (1996) *Science* **272,** 1176–1179.