quency of the dopamine pressure pulses or to the relative dopamine concentration. The generation of rhythmic bursting by the two interneurons is not a function of the continuous presence of dopamine but rather requires the phasic application of dopamine, which presumably mimics the effects of giant dopamine cell activity.

We have shown that it is possible to culture a neural network that is sufficient to produce rhythmic activity similar to that seen in vivo. It can be argued that networks of neurons constructed in vitro cannot be used to prove that a similar circuit functions in vivo. However, no obvious differences in the excitability and connectivity of the interneurons were apparent between our in vivo and in vitro preparations. The experiments of this report provide evidence that three identified interneurons are sufficient to account for a CPG. Furthermore, dopamine is necessary for rhythm generation in this network. Such tests are impossible to perform in the intact nervous system. Our in vitro model system may provide an opportunity to test the cellular basis of rhythm generation in a manner unapproachable in vivo.

REFERENCES AND NOTES

1. A. G. M. Bulloch, Ed., *Cellular Basis of Neuronal Plasticity*, vol. 7 of *Studies in Neuroscience*, W. Winlow, Series Ed. (Manchester Univ. Press, Manchester, UK, 1989).
2. J. Camardo, E. Proshansky, S. Schacher, *J. Neurosci.* **3**, 2614 (1983).
3. S. G. Rayport and S. Schacher, *ibid.* **6**, 759 (1986).
4. D. Kleinfeld, F. F. Raccuria-Behling, H. J. Chiel, *Biophys. J.* **57**, 697 (1990).
5. J. D. Jones, *Comp. Biochem. Physiol.* **4**, 1 (1961).
6. N. I. Syed and W. Winlow, *J. Physiol. (London)* **403**, 78p (1988).
7. N. I. Syed, R. L. Ridgway, A. G. M. Bulloch, K. Lukowiak, in preparation.
8. W. Winlow, P. G. Haydon, P. R. Benjamin, *J. Exp. Biol.* **94**, 137 (1981).
9. N. I. Syed, D. Harrison, W. Winlow, in preparation.
10. N. I. Syed and W. Winlow, in preparation.
11. N. I. Syed, thesis, University of Leeds, Leeds, United Kingdom (1988).
12. T. G. Brown, *Proc. R. Soc. London Ser. B.* **84**, 308 (1911).
13. A. I. Selverston, Ed., *Model Neural Networks and Behavior* (Plenum, New York, 1985).
14. J. W. Jacklet, Ed., *Neuronal and Cellular Oscillators* (Dekker, New York, 1989).
15. R. Lydic, *FASEB J.* **3**, 2457 (1989).
16. Properties tested include resting membrane potential, action potential amplitude, and wave form.
17. R. G. Wong, R. D. Hadley, S. B. Kater, G. Hauser, *J. Neurosci.* **1**, 1008 (1981).
18. G. Audesirk, *Comp. Biochem. Physiol.* **81A**, 359 (1985).
19. H. H. Boer *et al.*, *Cell Tissue Res.* **202**, 231 (1979).
20. R. D. Hadley, D. A. Bodnar, S. B. Kater, *J. Neurosci.* **15**, 3145 (1985).
21. P. G. Haydon, C. S. Cohan, D. P. McCobb, H. R. Miller, S. B. Kater, *J. Neurosci. Res.* **13**, 135 (1985).
22. Dissected ganglia were treated with a mixture of 0.2% collagenase and dispase (Boehringer Mannheim) and 0.2% trypsin (Sigma type III) in a ratio of 2:1, respectively, for 30 to 40 min in defined medium (7), and then with 0.2% trypsin soybean inhibitor (Sigma) for 10 min, also in defined medium. After microdissection of the outer sheath, inner connective tissue sheaths were removed from the ganglionic ring. Identified neurons were removed via gentle suction with fire-polished pipettes from appropriate ganglia maintained in high-osmolarity medium (defined medium containing 30 mM glucose). Neurons were then transferred to poly-L-lysine-coated Falcon 3001 culture plates containing brain-conditioned medium prepared as described (*20, 21*). The pH of the medium was adjusted to 7.9 with 1 N NaOH.
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A Magnesium Current in *Paramecium*

ROBIN R. PRESTON

Recent reappraisals of the role of ionized magnesium in cell function suggest that many cells maintain intracellular free Mg^{2+} at low concentrations (0.1 to 0.7 mM) and that external agents can influence cell function via changes in intracellular Mg^{2+} concentration. Depolarization and hyperpolarization of voltage-clamped *Paramecium* elicited a Mg^{2+}-specific current, I_{Mg}. Both Co^{2+} and Mn^{2+} were able to substitute for Mg^{2+} as charge carriers, but the resultant currents were reduced compared with Mg^{2+} currents. Intracellular free Mg^{2+} concentrations were estimated from the reversal potential of I_{Mg} to be about 0.39 mM. The I_{Mg} was inhibited when external Ca^{2+} was removed or a Ca^{2+} chelator was injected, suggesting that its activation was Ca^{2+}-dependent.

MAGNESIUM HAS LONG BEEN RECognized to be a necessary cofactor for numerous enzymatic reactions (*1*), but there is now considerable evidence to suggest that intracellular free Mg^{2+} ($[Mg^{2+}]_i$) may also be a key physiological regulator of cell activity (*2, 3*). In the past, researchers have been reluctant to consider such a role for Mg^{2+} because it was believed that $[Mg^{2+}]_i$ was of the order of 10 to 30 mM (*1*). Recently, however, techniques have been developed that allow $[Mg^{2+}]_i$ to be measured with accuracy (*4*). These methods suggest that, although total intracellular Mg^{2+} concentration is indeed high, the majority of it exists in a bound form. Intracellular free Mg^{2+} concentration is actually 0.1 to 0.7 mM (*1–3*). Since the Michaelis constant (K_m) values for Mg^{2+} activation or inhibition of many enzymes fall within this range (*1*), relatively small changes in $[Mg^{2+}]_i$ could have large effects on cell activity. Although the short half-life of $^{28}Mg^{2+}$ and the lack of Mg^{2+}-sensitive dyes has hampered attempts to characterize possible $[Mg^{2+}]_i$ regulatory mechanisms, it is

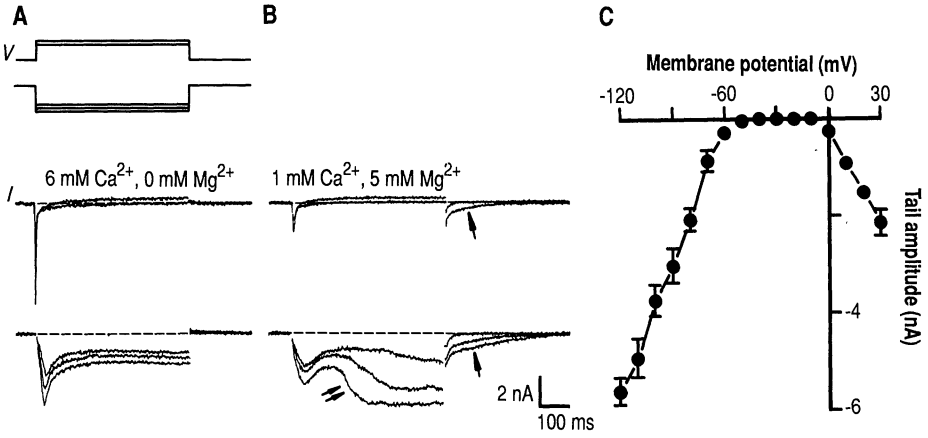

Fig. 1. I_{Mg} in *P. tetraurelia*. (**A**) Voltage steps (V) (500 ms) from −30 mV to 10 and 20 mV, or to −80, −90, and −100 mV, were used to elicit currents (I). (**B**) Repetition of the voltage steps in (A) after adding 5 mM Mg^{2+} to the bath. Arrows, slow inward tail currents of I_{Mg}. Traces have been corrected for linear leakage current, estimated from repeated small (3 to 9 mV, 20 ms) hyperpolarizations. Broken lines represent holding currents (about −0.2 nA in both Mg^{2+} and Ca^{2+} solutions). (**C**) I_{Mg} tail current amplitudes at the moment of return to the holding potential as a function of membrane potential. Currents activated during the voltage steps contained minor active components in addition to I_{Mg}; thus, studies of I_{Mg} under various conditions usually focused on tail currents. These tails represent pure I_{Mg}: note the lack of similar tail currents in the absence of extracellular Mg^{2+} (Table 1A). Values are means ± SEM from 12 cells.

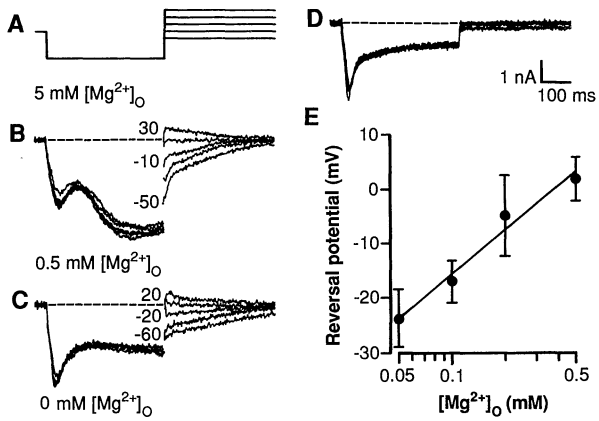

Fig. 2. Reversal potential of I_{Mg}. (**A**) Stimulation protocol that was used to determine the reversal potential of I_{Mg}. Cells were hyperpolarized for 400 ms to activate I_{Mg}, then the voltage was stepped to various test potentials (−70 to 30 mV) to elicit tail currents. Positive increases in voltage elicited depolarization-activated currents in addition to I_{Mg}, causing the amplitude of I_{Mg} to be underestimated. Since I_{Mg} is Ca^{2+} dependent, and since the hyperpolarization-activated Ca^{2+} current is inactivating (*12*), both it and its dependent Mg^{2+} current could be inhibited by a conditioning, inactivating hyperpolarization (4.5 s at −110 mV or −120 mV) before the reversal protocol. Subtraction of currents that remained after a conditioning step from those activated before the conditioning hyperpolarization yielded Ca^{2+} current and I_{Mg}. (**B**) Reversal of tail currents elicited in 5 mM $[Mg^{2+}]_o$. Holding potential was −30 mV, and a 400-ms step to −110 mV was used to activate I_{Mg}. Numbers adjacent to tail currents indicate corresponding test potentials (in millivolts). I_{Mg} reversed at about 10 mV, indicating that $[Mg^{2+}]_i$ was about 2 mM. This value reflects the fact that manipulation of membrane potential in Mg^{2+}-rich solutions increases $[Mg^{2+}]_i$ via I_{Mg}. (**C**) Tail current reversal in 0.5 mM $[Mg^{2+}]_o$. A 400-ms step to −120 mV from −40 mV was used to elicit I_{Mg}. (**D**) In the absence of extracellular Mg^{2+}, repetition of the stimulus protocol used in (C) elicited Ca^{2+} current alone; note the lack of tail currents (*27*). (**E**) Tail current reversal potential as a function of $[Mg^{2+}]_o$. The line was fitted by regression analysis; data are means ± SD from 5 to 11 cells.

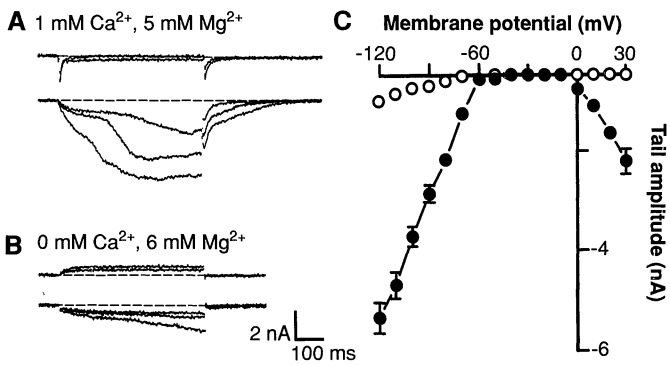

Fig. 3. Ca^{2+}-dependence of I_{Mg}. (**A**) Currents elicited from a single cell in the presence (1 mM Ca^{2+}, 5 mM Mg^{2+}) or (**B**) absence (0 mM Ca^{2+}, 6 mM Mg^{2+}) of extracellular Ca^{2+}. Currents were elicited by steps identical to those used in Fig. 1A. (**C**) Current-voltage relations in the presence (●) or absence (○) of 1 mM $[Ca^{2+}]_o$. Tail currents were elicited by 500-ms steps. Values are means ± SEM from ten cells.

clear that many cells maintain low levels of $[Mg^{2+}]_i$ against a concentration gradient from outside to inside the cell (*5*). Further, $^{28}Mg^{2+}$ influx into several types of cell is hormonally regulated, supporting the notion that changes in $[Mg^{2+}]_i$ are physiologically relevant (*2, 6*). Here I describe a Mg^{2+}-specific ion current that, by changing $[Mg^{2+}]_i$, may regulate cellular processes in *Paramecium* (*7*).

Depolarization of *P. tetraurelia* (*8–10*) in the absence of extracellular Mg^{2+} ($[Mg^{2+}]_o$) elicited a rapid inward Ca^{2+} transient ($I_{Ca,d}$) that inactivated within milliseconds (Fig. 1A, upper trace). Adding Mg^{2+} to the bath solution reduced the amplitude of this current, as reported (*11*), but the return to the holding potential was now followed by a slowly decaying, inward tail current (Fig. 1B, upper trace, arrow). Typically, this current's decay could be described by the sum of two exponents ($\tau \approx 14$ ms and 110 ms). Hyperpolarization of *P. tetraurelia* elicited a second inactivating Ca^{2+} current, $I_{Ca,h}$ (*10, 12*) (Fig. 1A, lower trace). Hyperpolarization in Mg^{2+} solutions again activated $I_{Ca,h}$, but this current was now accompanied by a second inward current that activated more slowly and was sustained (Fig. 1B, lower trace, double arrows). Termination of the voltage step at 500 ms again yielded a tail current that decayed biexponentially ($\tau \approx 8$ ms and 90 to 200 ms). These slow inward tail currents were only observed in the presence of extracellular Mg^{2+} (Table 1A) and represented the deactivation of a Mg^{2+}-specific ion current, I_{Mg}. A plot of the tail amplitudes of I_{Mg} as a function of membrane potential yielded a bell-shaped curve (Fig. 1C), reflecting the fact that the current was elicited upon either depolarization or hyperpolarization.

I investigated the selectivity of I_{Mg} by replacing extracellular Mg^{2+} with other divalent cations. Both Mn^{2+} and Co^{2+} could substitute as charge carriers, although the resultant currents were reduced compared with that carried by Mg^{2+} (Table 1B). The tail currents elicited after depolarization and hyperpolarization showed similar selectivity rankings: $Mg^{2+} \geq Mn^{2+} = Co^{2+} \geq Sr^{2+} = Ba^{2+} >> Ca^{2+}$. The Mg^{2+} conductance pathway was impermeable to either K^+ or Na^+ (*13*).

Next I determined the reversal potential of I_{Mg}, since this value would yield information about $[Mg^{2+}]_i$ in *Paramecium*. The reversal potential was calculated from tail currents of I_{Mg}. Cells were hyperpolarized to elicit I_{Mg}, and then I_{Mg} tails were elicited by increasing the voltage stepwise through a range of test potentials (Fig. 2A). Although reversal of I_{Mg} was clearest when $[Mg^{2+}]_o$ was high, and thus the tails of I_{Mg} were large (Fig. 2B), activation of I_{Mg} under these conditions significantly increased $[Mg^{2+}]_i$. This increase was temporary, but it interfered with an accurate measurement of $[Mg^{2+}]_i$ at or near resting conditions. Thus, I used Mg^{2+} concentrations of 0.5 mM or less in determining the reversal potential (Fig. 2, C and D). The reversal potential shifted by 27.1 mV per tenfold change in $[Mg^{2+}]_o$ (Fig. 2E), close to the 29 mV per decade change predicted by the Nernst equation for a pure Mg^{2+} current and supporting the notion that I_{Mg} is indeed carried by Mg^{2+}. From these reversal potentials, $[Mg^{2+}]_i$ was estimated to be 0.39 mM (± 0.14 mM, mean ± SD from 34 cells).

Removing extracellular Ca^{2+} revealed a Ca^{2+} dependence of I_{Mg}. Depolarization of *Paramecium* in nominally Ca^{2+}-free solutions failed to evoke I_{Mg}, whereas hyperpolarization yielded a current that was less than 10% of control values (Fig. 3). Iontophoretic injection of a Ca^{2+} chelator, EGTA, reduced the amplitude of I_{Mg} tail currents by 50% to 60% (Table 1C). This suggests that I_{Mg} was dependent on changes in intracellular, rather than extracellular, Ca^{2+} concentration for its activation.

Similarities between the kinetics of I_{Mg} and the Ca^{2+}-dependent Na^+ current of *Paramecium* (*10*), together with reports of Mg^{2+} currents through Ca^{2+} channels in other systems (*14*), raised the possibility that I_{Mg} was mediated by a conductance pathway in *Paramecium* that has been described previously. This was probably not the case, however. Mutants lacking either the Ca^{2+}-dependent Na^+ current (*fast-2*) (*10, 15*), or the two Ca^{2+}-dependent K^+ currents (*pantophobiac*) (*15*), expressed Mg^{2+} currents that were indistinguishable from those of the wild type (Table 1D). Deciliation of *P. tetraurelia* removed the depolarization-acti-

Laboratory of Molecular Biology, University of Wisconsin–Madison, 1525 Linden Drive, Madison, WI 53706.

Table 1. Ion selectivity of I_{Mg} and effects of mutations. Cells were held at −30 mV and stepped for 500 ms to 20 mV or −110 mV to elicit I_{Mg}. Data are means ± SEM. (**A**) Demonstration of I_{Mg}. The concentrations used were as follows: Ca^{2+}, 6 mM Ca^{2+}; Ca^{2+} + Mg^{2+}, 1 mM Ca^{2+} and 5 mM Mg^{2+}. (**B**) Ion selectivity of I_{Mg}. Tail currents were measured 25 ms after returning to the holding potential of −40 mV and were elicited in 1 mM Ca^{2+} plus 1 mM concentrations of the various cations. A concentration of 1 mM was used to minimize possible inhibitory effects of these cations on the two Ca^{2+} currents. (**C**) Effect of EGTA on I_{Mg}. Tail currents were elicited in 1 mM Ca^{2+}, 5 mM Mg^{2+} before and after an iontophoretic injection of EGTA (−7 nA, 60 s). Such an injection produced an intracellular EGTA concentration of about 5 mM, assuming a cell volume of 200 pl. Injection techniques are described (*9, 10*). (**D**) I_{Mg} in mutant paramecia. The mutant *fast-2* lacks a Ca^{2+}-dependent Na^+ current (*10, 15*), whereas *pantophobiac* lacks Ca^{2+}-dependent K^+ currents (*15*). (**E**) Effects of deciliation on I_{Mg}. Deciliation by agitation in 5% (v/v) ethanol removed the depolarization-activated I_{Ca} (*16*) without simultaneously eliminating I_{Mg} activation upon hyperpolarization. However, this I_{Mg} was reduced by deciliation; this could reflect a reduced Ca^{2+} signal or localization of a portion of the channels that mediate I_{Mg} to the ciliary membrane. (**F**) Effect of 1 mM amiloride on I_{Mg}.

Condition	Mg^{2+} (mM)	Tail current amplitudes (nA)		(*n*)
		20 mV	−110 mV	
A				
Ca^{2+}	0	0.00 ± 0.02	0.04 ± 0.02	(11)
Ca^{2+} + Mg^{2+}	5	−1.33 ± 0.09	−4.44 ± 0.23	(11)
B				
Mg^{2+}	1	−1.53 ± 0.10	−1.49 ± 0.09	(24)
Co^{2+}	0	−0.89 ± 0.05	−0.59 ± 0.07	(8)
Mn^{2+}	0	−0.74 ± 0.09	−0.88 ± 0.08	(8)
Ba^{2+}	0	−0.14 ± 0.06	−0.49 ± 0.05	(6)
Sr^{2+}	0	−0.18 ± 0.01	−0.16 ± 0.04	(7)
Ca^{2+}	0	−0.01 ± 0.08	−0.05 ± 0.05	(5)
C				
Control	5	−1.67 ± 0.10	−5.05 ± 0.45	(5)
EGTA injected	5	−0.71 ± 0.11	−2.84 ± 0.14	(5)
D				
fast-2	5	−1.07 ± 0.16	−4.78 ± 0.89	(5)
pantophobiac	5	−1.06 ± 0.55	−4.91 ± 0.56	(3)
E				
Control	5	−1.55 ± 0.11	−4.25 ± 0.29	(5)
Deciliated	5	−0.05 ± 0.5	−1.74 ± 0.34	(5)
F				
Control	5	−1.26 ± 0.09	−4.93 ± 0.43	(6)
Amiloride	5	−1.09 ± 0.17	−4.16 ± 0.56	(6)

vated I_{Ca} (*16*) and its dependent I_{Mg} ($I_{Mg,d}$), but failed to concomitantly eliminate I_{Mg} upon hyperpolarization ($I_{Mg,h}$) (Table 1E). Also, 1 mM amiloride reduced the hyperpolarization-activated I_{Ca} by >95% (*12*) but inhibited I_{Mg} by <15% (Table 1F). These observations, together with the kinetic dissimilarities between the Mg^{2+} and Ca^{2+} currents, make it unlikely that I_{Mg} was mediated by the same permeability as either of the two Ca^{2+} transients. $I_{Mg,h}$ was relatively unaffected by the near complete suppression of $I_{Ca,h}$ by amiloride (Table 1F). This observation may indicate that I_{Mg} is sensitive to even small changes in intracellular Ca^{2+} concentration, a notion that is supported by the inability of EGTA to inhibit I_{Mg} by >60% (Table 1C). $I_{Mg,d}$ was lost when $I_{Ca,d}$ was suppressed completely, however (Table 1E) (*17*), reaffirming the contention that I_{Mg} is Ca^{2+} dependent. Finally, Mg^{2+} permeates a mechanosensitive channel in *Paramecium* (*18*); since this permeability is also Ca^{2+}- and Sr^{2+}-selective, it is unlikely that it accounts for I_{Mg}. Thus, I_{Mg} probably represents a new conductance pathway in *Paramecium*.

In summary, *P. tetraurelia* possesses a Ca^{2+}-dependent Mg^{2+} current that may be elicited upon either depolarization or hyperpolarization. $I_{Mg,d}$ and $I_{Mg,h}$ could conceivably represent two distinct permeabilities, but it is more likely that a single species of channel or transport protein mediates both currents. The similarities in the kinetics, Ca^{2+} sensitivities, and ion selectivities of $I_{Mg,d}$ and $I_{Mg,h}$ support this contention. The mechanism of Mg^{2+} permeation is uncertain. There are few reports of Mg^{2+}-specific ion channels in other organisms: Späh and Fleckenstein (*19*) proposed such an activity in guinea pig myocardium, but their observations have since been attributed to Mg^{2+} effects on Na^+-current inactivation (*3, 20*). In contrast, there are many reports of Mg^{2+}-specific active transport systems in a variety of organisms (*5, 21*). Regardless of the mechanism of Mg^{2+} entry, I_{Mg} activates sufficiently fast that it could be evoked during normal membrane excitation (*22*). Excitation in *Paramecium* results in altered swimming behavior, effected through a coupling of membrane potential to ciliary beat (*23*). Cilia contain several Mg^{2+}-stimulated enzymes (*24*), including dynein, an Mg^{2+}–adenosine triphosphatase (ATPase) that powers the cilium. Thus, activation of I_{Mg} might influence cell behavior directly. However, Mg^{2+} serves as a physiological regulator of K^+-, Na^+-, and Ca^{2+}-channel permeability in many cells (*3, 25*), raising the possibility that I_{Mg}, through changes in $[Mg^{2+}]_i$, may regulate excitation in *Paramecium*. In fact, action potential recordings suggest that Mg^{2+} entering the cells via I_{Mg} affects the recovery phase of the response (*26*).

REFERENCES AND NOTES

1. T. Günther, *Magnesium Bull.* **1**, 91 (1981); B. M. Altura, J. Durlach, M. S. Seelig, *Magnesium in Cellular Processes and Medicine* (Karger, Basel, 1987).
2. R. D. Grubbs and M. E. Maguire, *Magnesium* **6**, 113 (1987).
3. R. E. White and H. C. Hartzell, *Biochem. Pharmacol.* **38**, 859 (1989).
4. F. J. Alvarez-Leefmans, F. Giraldez, S. M. Gamiño, *Can. J. Physiol. Pharmacol.* **65**, 915 (1987).
5. P. W. Flatman, *J. Membr. Biol.* **80**, 1 (1984).
6. M. E. Maguire and J. J. Erdos, *J. Biol. Chem.* **255**, 1030 (1980); J. J. Erdos and M. E. Maguire, *J. Physiol. (London)* **337**, 351 (1983); M. E. Maguire, *Magnesium* **6**, 180 (1987).
7. R. R. Preston, *J. Cell Biol.* **109**, 255a (1989).
8. Voltage-clamp and cell-culture techniques have been described (*9*). Potassium currents were suppressed by the use of 10 mM tetraethylammonium (TEA) chloride in the bath solution, and by the use of 4 M CsCl in the microelectrodes used for current injection and membrane potential recording. TEA inhibits K^+ channels externally, whereas Cs^+ diffusing from the electrodes inhibits the K^+ channels internally (*9*). Sodium currents are not observed in the absence of external Na^+ (*10*). All solutions contained 10 mM TEA-Cl, 1 mM Hepes, and 0.01 mM EDTA, pH 7.2. Chloride salts of Ca^{2+}, Mg^{2+}, and other divalent cations were added to this solution at stated concentrations. Tail current amplitudes were determined by fitting currents at >10 ms after returning to the holding potential to single or double exponents and then extrapolating back to the instant of returning to the holding potential.
9. R. D. Hinrichsen and Y. Saimi, *J. Physiol. (London)* **351**, 397 (1984); R. R. Preston, Y. Saimi, C. Kung, *J. Membr. Biol.* **115**, 41 (1990).
10. Y. Saimi, *J. Membr. Biol.* **92**, 227 (1986).
11. F. Wehner and E. Hildebrand, *J. Exp. Biol.* **119**, 321 (1985).
12. R. R. Preston and Y. Saimi, *Biophys. J.* **57**, 519a (1990); R. R. Preston, M. A. Wallen-Friedman, Y. Saimi, C. Kung, *J. Membr. Biol.* **115**, 51 (1990); R. R. Preston, Y. Saimi, C. Kung, in preparation.
13. K^+ and Na^+ (10 mM) had no effect on the amplitude of I_{Mg}, nor did these cations induce currents that could be attributed to their permeating the I_{Mg} conductance pathway, as reported (*9, 10*).
14. W. Almers and P. T. Palade, *J. Physiol. (London)* **312**, 159 (1981); K. Nakatani and K. W. Yau, *ibid.* **395**, 695 (1988); K. Narita, F. Kawasaki, H. Kita, *Brain Res.* **510**, 289 (1990).
15. The loss of Ca^{2+}-dependent currents in both *fast-2* and *pantophobiac* results from calmodulin gene mutations (cam^{13} and cam^1, respectively); J. A. Kink *et al.*, *Cell* **62**, 165 (1990).
16. H. Machemer and A. Ogura, *J. Physiol. (London)* **296**, 49 (1979).
17. I_{Mg} may also be suppressed fully by complete inactivation of the corresponding Ca^{2+} currents, as described in the legend to Fig. 2A.
18. Y. Satow, A. D. Murphy, C. Kung, *J. Exp. Biol.* **103**, 253 (1983).
19. F. Späh and A. Fleckenstein, *J. Mol. Cell. Cardiol.* **11**, 1109 (1979).

20. T. Kiyosue, M. Arita, S. Imanishi, M. Aomine, *Jpn. Heart J.* **23** (suppl.), 51 (1982).
21. *Escherichia coli* possesses at least two Mg^{2+} uptake systems. One of these also transports Co^{2+} and Mn^{2+}, an ion selectivity reminiscent of I_{Mg} [D. L. Nelson and E. P. Kennedy, *J. Biol. Chem.* **246**, 3042 (1971); S. Silver and D. Clark, *ibid.*, p. 569].
22. I_{Mg} tail currents could be elicited with depolarizations of 10 ms or less. Action potential duration in *Paramecium* may exceed 40 ms, depending on stimulus intensity.
23. C. Kung and Y. Saimi, *Annu. Rev. Physiol.* **44**, 519 (1982); Y. Saimi and C. Kung, *Annu. Rev. Genet.* **21**, 47 (1987); H.-D. Görtz, *Paramecium* (Springer-Verlag, New York, 1988); R. D. Hinrichsen and J. E. Schultz, *Trends Neurosci.* **11**, 27 (1988).
24. L. A. P. Miglietta and D. L. Nelson, *J. Biol. Chem.* **263**, 16096 (1988); S. M. Travis and D. L. Nelson, *Biochim. Biophys. Acta* **966**, 84 (1988); P. A. Mason and D. L. Nelson, *ibid.* **1010**, 116 (1989).
25. J. Golowasch, A. Kirkwood, C. Miller, *J. Exp. Biol.* **124**, 5 (1986); G. L. Collingridge and T. V. P. Bliss, *Trends Neurosci.* **10**, 288 (1987); P. R. Stanfield, *ibid.* **11**, 475 (1988); R. E. White and H. C. Hartzell, *Science* **239**, 778 (1988); I. Duchatelle-Gourdon, H. C. Hartzell, A. A. Lagrutta, *J. Physiol. (London)* **415**, 251 (1989); M. Horie and H. Irisawa, *ibid.* **408**, 313 (1989); M. Pusch, F. Conti, W. Stühmer, *Biophys. J.* **55**, 1267 (1989); M. Tarr, J. W. Trank, K. K. Goertz, *Am. J. Physiol.* **257**, H1663 (1989).
26. Action potentials triggered in the presence of physiological external Mg^{2+} concentrations (0.5 mM) lack the strong, K^+-based afterhyperpolarization characteristic of similar responses in Mg^{2+}-free solutions.
27. Although an outward tail that may correspond to loss of internal Mg^{2+} via I_{Mg} was seen occasionally in response to initial membrane potential change, subsequent steps failed to elicit similar currents. It is not certain whether this reflected inhibition of I_{Mg} or depletion of $[Mg^{2+}]_i$. Activation of I_{Mg} in 0.05 mM $[Mg^{2+}]_o$ did not significantly deplete $[Mg^{2+}]_i$, which was 0.35 ± 0.18 mM before and 0.34 ± 0.19 mM after prolonged activation of I_{Mg} with the conditioning protocol (means ± SD from five cells).
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The Primate Hippocampal Formation: Evidence for a Time-Limited Role in Memory Storage

STUART M. ZOLA-MORGAN AND LARRY R. SQUIRE

Clinical and experimental studies have shown that the hippocampal formation and related structures in the medial temporal lobe are important for learning and memory. Retrograde amnesia was studied prospectively in monkeys to understand the contribution of the hippocampal formation to memory function. Monkeys learned to discriminate 100 pairs of objects beginning 16, 12, 8, 4, and 2 weeks before the hippocampal formation was removed (20 different pairs at each time period). Two weeks after surgery, memory was assessed by presenting each of the 100 object pairs again for a single-choice trial. Normal monkeys exhibited forgetting; that is, they remembered recently learned objects better than objects learned many weeks earlier. Monkeys with hippocampal damage were severely impaired at remembering recently learned objects. In addition, they remembered objects learned long ago as well as normal monkeys did and significantly better than they remembered objects learned recently. These results show that the hippocampal formation is required for memory storage for only a limited period of time after learning. As time passes, its role in memory diminishes, and a more permanent memory gradually develops independently of the hippocampal formation, probably in neocortex.

CURRENT UNDERSTANDING OF THE organization and neural foundations of memory has depended importantly on cognitive studies of memory-impaired patients (*1*) and on studies of a primate model of human amnesia (*2*). In humans, neuropathological findings (*3, 4*), together with high-resolution magnetic resonance imaging (*5*), have demonstrated that selective, bilateral damage to the hippocampal formation is sufficient to cause significant memory impairment. Similar findings have been obtained in monkeys (*6–8*) and other mammals (*9*). On the basis of neuropsychological studies of patients with confirmed hippocampal damage, it appears that the hippocampal formation is necessary for establishing a usable record in long-term memory of previously encountered facts and events (*1, 10*).

One useful source of information about the function of the hippocampal formation is the phenomenon of retrograde amnesia, that is, loss of memories acquired before the onset of amnesia. Retrograde amnesia is often temporally graded; patients lose access to the recent past more readily than to the remote past (*11*). Further, as measured by objective tests, memory for the very remote past can be intact in patients with hippocampal damage (*3, 12*), regardless of the difficulty of the test items (*13*). This finding suggests that the hippocampal formation is not a repository of permanent memory. In addition, the phenomenon of temporally graded retrograde amnesia suggests that the role of the hippocampal formation in memory is time-limited. However, more data are needed to confirm and illuminate these ideas. Indeed, the correct interpretation of temporally graded retrograde amnesia depends on the precise shape of the performance curves, which cannot be determined with certainty with the tests available for assessing remote memory retrospectively in humans (*14*).

We have assessed retrograde amnesia prospectively in cynomolgus monkeys (*Macaca fascicularis*) with bilateral lesions of the hippocampal formation (the H^+ lesion) (*15*). Figure 1 shows a cross section from the brain of a monkey in the operated group. Monkeys were trained on five different sets of 20 two-choice object discrimination problems (100 discrimination pairs). Training on each 20-pair set began approximately 16, 12, 8, 4, and 2 weeks before surgery. For training, each object pair was presented for 14 consecutive trials with a 15-s intertrial interval (*16*). Monkeys were trained on two new object pairs each day so that 10 days were required to train monkeys on each of the five sets of 20 object pairs (*17*). The ability to learn simple object discrimination problems like the ones used here is known to depend on the integrity of the hippocampal formation (*7*).

Preoperative performance on the 100 object discrimination problems averaged 54.5% correct (chance, 50%) on the first trial of training and 87.7% correct on trial 14 (average of 18 monkeys and 100 discrimination pairs). The learning curves were numerically very similar for the five training episodes, although some improvement did occur with continuing exposure to discrimination problems (*18*). Tests given at the end of each training episode, which assessed the level of preoperative learning (*17*), showed that virtually the same final level of performance was attained on each of the five sets of discrimination problems. Performance on these tests averaged 78.9, 81.9, 79.4, 79.7, and 78.6% for the first to the last training episode, respectively. A two-way analysis of variance (training episode × group) revealed no significant differences ($F < 2.0$, $P > 0.10$).

Two weeks after surgery, we assessed memory for the preoperatively learned object pairs by presenting a single trial of each of the 100 pairs in a mixed order. This retention test consisted of 50 trials present-

Veterans Affairs Medical Center and Department of Psychiatry, University of California, San Diego, CA 92093.