with TRPV3-expressing cells (Fig. 4D).

TRPV3 is activated at warm and hot temperatures and is expressed in skin cells (fig. S2). TRPV3 signaling may mediate a cell-autonomous response in keratinocytes upon exposure to heat. It is also possible that the heat-induced TRPV3 signal is transferred to nearby free nerve endings, thereby contributing to conscious sensations of warm and hot. This hypothesis is supported by indirect evidence that skin cells can act as thermal receptors. For instance, although dissociated DRG neurons can be directly activated by heat and cold, warm receptors have only been demonstrated in experiments where skin-nerve connectivity is intact (*21, 22*). TRPV3 has an activation threshold around 33° to 35°C. The presence of such a warm receptor in skin (with a resting temperature of 34°C) and not DRG neurons (with a resting temperature of 37°C at the cell body) would prevent a warm channel such as TRPV3 from being constitutively active at core 37°C temperatures. The residual heat sensitivity in TRPV1 knockout mice may also involve skin cells: Dissociated DRG neurons from TRPV1-null animals do not respond to moderate noxious stimulus at all, whereas skin-nerve preparations from such animals do respond (*7, 8, 23*). Collectively, these data suggest that a warmth/heat receptor might be present in the skin, in addition to the heat receptors in DRG.

If keratinocytes indeed act as thermal receptors, how then is the information transferred to neurons? Synapses have not been found between keratinocytes and sensory termini; however, ultrastructural studies have shown that keratinocytes contact, and often surround, DRG nerve fibers through membrane-membrane apposition (*19, 20*). Therefore, heat-activated TRPV3 signal from keratinocytes could be transduced to DRG neurons through direct chemical signaling. One potential signaling mechanism might involve adenosine triphosphate (ATP). P2X3, an ATP-gated channel, is present in sensory endings, and analysis of P2X3 knockout mice show a strong deficit in coding of warm temperatures (*24, 25*). Furthermore, release of ATP from damaged keratinocytes has been shown to cause action potentials in nociceptors via the P2X receptors (*26*).

References and Notes

1. D. E. Clapham, L. W. Runnels, C. Strubing, *Nature Rev. Neurosci.* **2**, 387 (2001).
2. C. Montell, L. Birnbaumer, V. Flockerzi, *Cell* **108**, 595 (2002).
3. M. J. Caterina *et al.*, *Nature* **389**, 816 (1997).
4. M. J. Caterina, T. A. Rosen, M. Tominaga, A. J. Brake, D. Julius, *Nature* **398**, 436 (1999).
5. D. D. McKemy, W. M. Neuhausser, D. Julius, *Nature* **416**, 52 (2002).
6. A. M. Peier *et al.*, *Cell* **108**, 705 (2002).
7. M. J. Caterina *et al.*, *Science* **288**, 306 (2000).
8. J. B. Davis *et al.*, *Nature* **405**, 183 (2000).
9. W. Liedtke *et al.*, *Cell* **103**, 525 (2000).
10. R. Strotmann, C. Harteneck, K. Nunnenmacher, G. Schultz, T. D. Plant, *Nature Cell Biol.* **2**, 695 (2000).
11. Materials and Methods are available as supporting material on *Science* Online.
12. J. B. Peng, E. M. Brown, M. A. Hediger, *Genomics* **76**, 99 (2001).
13. M. Funayama, K. Goto, H. Kondo, *Brain Res. Mol. Brain Res.* **43**, 259 (1996).
14. A. Peier *et al.*, data not shown.
15. B. Nilius *et al.*, *J. Physiol.* **527**, 239 (2000).
16. L. Vyklicky *et al.*, *J. Physiol.* **517**, 181 (1999).
17. S. Jordt, D. Julius, *Cell* **108**, 421 (2002).
18. D. G. Wilkinson, in *Essential Developmental Biology, A Practical Approach*, C. Stern, P. Holland, Eds. (Oxford Univ. Press, New York, 1993), pp. 258–263.
19. M. Hilliges, L. Wang, O. Johansson, *J. Invest. Dermatol.* **104**, 134 (1995).
20. N. Cauna, *J. Anat.* **115**, 277 (1973).
21. H. Hensel, A. Iggo, *Pfluegers Arch.* **329**, 1 (1971).
22. H. Hensel, D. R. Kenshalo, *J. Physiol.* **204**, 99 (1969).
23. C. Roza *et al.*, paper presented at the annual meeting of the Society for Neuroscience, San Diego, CA, October 2001.
24. V. Souslova *et al.*, *Nature* **407**, 1015 (2000).
25. D. A. Cockayne *et al.*, *Nature* **407**, 1011 (2000).
26. S. Cook, E. McCleskey, *Pain* **95**, 41 (2002).

27. We thank E. Gardiner and J. Watson for technical help, and N. Hong, S. Kay, J. Mosbacher, U. Muller, P. Schultz, and C. Song for valuable advice. Supported in part by a grant from Novartis. A.P. is a Basil O'Connor scholar.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1073140/DC1
Materials and Methods
Figs. S1 and S2

22 April 2002; accepted 8 May 2002
Published online 16 May 2002;
10.1126/science.1073140
Include this information when citing this paper.

Correlated Bursts of Activity in the Neonatal Hippocampus in Vivo

Xavier Leinekugel,[1,2,3]*† Rustem Khazipov,[1,4]* Robert Cannon,[1] Hajime Hirase,[2] Yehezkel Ben-Ari,[1] György Buzsáki[2]*

The behavior of immature cortical networks in vivo remains largely unknown. Using multisite extracellular and patch-clamp recordings, we observed recurrent bursts of synchronized neuronal activity lasting 0.5 to 3 seconds that occurred spontaneously in the hippocampus of freely moving and anesthetized rat pups. The influence of slow rhythms (0.33 and 0.1 hertz) and the contribution of both γ-aminobutyric acid A–mediated and glutamate receptor–mediated synaptic signals in the generation of hippocampal bursts was reminiscent of giant depolarizing potentials observed in vitro. This earliest pattern, which diversifies during the second postnatal week, could provide correlated activity for immature neurons and may underlie activity-dependent maturation of the hippocampal network.

Although a variety of oscillatory and intermittent population patterns have been described in the adult central nervous system (*1, 2*), the expression of neuronal activity in the developing brain remains largely unknown. Previous in vitro investigations in hippocampus and neocortex have revealed a number of major developmentally regulated changes in synaptic transmission properties, including a switch from excitatory to inhibitory effects of γ-aminobutyric acid A ($GABA_A$) receptor–mediated signals (*3–7*), and rapid changes in glutamate receptor expression and synaptic connectivity (*7–10*). Therefore, the patterns of activity expressed at these early stages of development may be quite different from those expressed in the adult brain.

In the neonatal hippocampus and neocortex, oscillatory patterns have been described in vitro. These include giant depolarizing potentials (GDPs) (*3, 5, 7, 11–14*) in the hippocampus, cortical early network oscillations [cENOs (*15*)], and synchronized domains (*16–19*) in the neocortex. However, the activities expressed in vivo remain unknown. Correlated patterns of activity, either endogenously generated or initiated by sensory inputs, may be a general requirement for the proper development of central nervous structures (*7, 14, 20–22*). Because the early postnatal period is critical for the activity-dependent maturation of synaptic connections in the cortical structures of the rat (*20–23*), it is important to reveal the physiological patterns expressed in these structures in vivo.

[1]INMED, Institut National de la Santé et de la Recherche Médicale (INSERM) U29, Avenue de Luminy, Boite Postale 13, 13273 Marseille Cedex 09, France. [2]Center for Molecular and Behavioral Neuroscience, Rutgers University, 197 University Avenue, Newark, NJ 07102, USA. [3]INSERM EMI 0224 "Cortex et Epilepsie," Faculté de Médecine Pitié-Salpêtrière, Université Paris 6, 105 Boulevard de l'Hôpital, 75013 Paris, France. [4]Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: xlkg@noos.fr

Spontaneous population-field and multiunit activity was recorded from the hippocampus of freely moving rat pups [postnatal days 4 to 6 (P4 to P6)] (*24*). The most characteristic field pattern at this age was the hippocampal sharp wave (SPW), which reversed across the CA1 pyramidal layer and was associated with multiple unit discharges (n = 3 animals, Fig. 1). Unit firing in the CA1 pyramidal layer occurred mainly in population bursts (58 ± 10% of all units belonged to multiunit bursts) lasting from 0.5 to 3 s and separated by periods of relative silence (Fig. 1E). Hippocampal bursts were often (40 ± 10%) associated with SPWs. The distribution of interburst intervals (Fig. 1F) revealed a sharp peak at 3 s and a wider peak at 10 s, indicating the influence of slow rhythms (0.33 and 0.1 Hz, respectively) in their generation. The level of synchronization as well as the frequency range of the bursts are reminiscent of the oscillatory activities described in in vitro preparations at this age (*3, 5, 7, 11–14*). Bursts occurred mainly during immobility periods, sleep, and feeding. During crawling, the field was largely flat, a state associated with irregular unit activity.

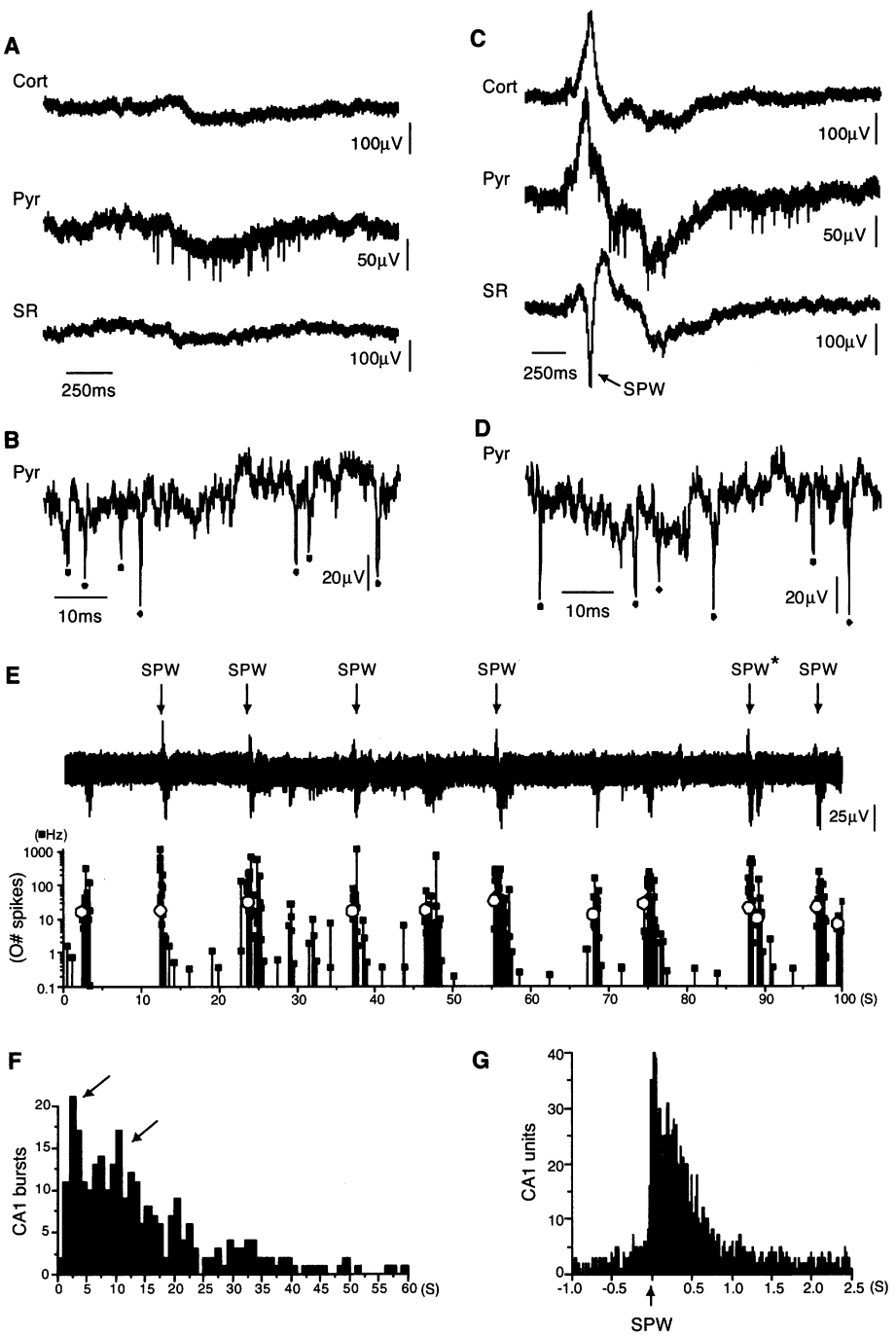

Fig. 1. Hippocampal bursts in freely moving rat pups (P4 to P6). **(A)** Multiple-unit burst discharge in CA1 pyramidal layer (Pyr) and field activity in the neocortex (Cort) and stratum radiatum (SR). Part of the burst is expanded in **(B)**, showing single units (dots). **(C)** Field SPW (arrow) associated burst recorded from the same three sites as in (A). Part of the burst is expanded in **(D)**, showing single units (dots). **(E)** Filtered trace (>200 Hz) of multiunit activity in CA1 pyramidal layer. Arrows indicate simultaneously recorded field SPWs. Below the trace is the corresponding multiple unit activity over time. Each vertical line terminated with a black square represents the firing of one spike (height proportional to the instantaneous frequency of spikes). Vertical lines terminated with a white circle correspond to multiunit bursts (heights proportional to the number of spikes in the burst). Note that population-field SPWs (arrows) co-occur with multiunit bursts. The SPW burst marked by an asterisk corresponds to the event illustrated in (C). **(F)** Histogram of interburst intervals (bin: 1 s). Note peaks at 3 and 10 s (arrows), indicating the influence of slow rhythms (0.33 and 0.1 Hz, respectively) in the occurrence of hippocampal bursts. **(G)** Cross-correlogram between SPWs (reference) and CA1 multiunit discharge (bin: 10 ms).

Because adult SPW bursts are generated in the CA3 recurrent network (*25*), we investigated the SPW events in more detail. Depth distribution of spontaneous and evoked field potentials was recorded by 16-site silicon probes (*26*) placed in the CA1-dentate gyrus axis of urethane-anesthetized rat pups (P3 to P6). Similar to freely behaving pups, the dominant hippocampal pattern was the field SPW followed by a long "tail" of population unit firing (interburst interval range 2 to 86 s, 17.5 ± 19 s in average, n = 12 rats, Fig. 2). These long, multiunit bursts were no longer observed in rats older than P10 (n = 11 rats at P10 to P30), in contrast to other hippocampal patterns (theta, dentate spikes) that progressively emerged during the second postnatal week (*27, 28*). Amplitude-versus-depth profiles of SPWs in P3 to P6 animals revealed a sharp phase-reversal just below the CA1 pyramidal layer. The largest amplitude negative deflection occurred in the middle of stratum radiatum. Stimulation of the ventral hippocampal commissure evoked field responses with a depth profile identical to that of the SPW events (Fig. 2D). These observations suggest that SPWs and associated bursts of CA1 neurons were brought about by population bursts of the CA3 region and conveyed by the glutamatergic Schaffer collaterals to the apical dendrites of CA1 pyramidal cells, similar to the SPW bursts of the adult hippocampus (*2, 26*). A major difference between the developing and adult forms of SPWs was the absence of fast field "ripples" in the pyramidal layer in neonates. These 140- to 200-Hz CA1 pyramidal layer oscillations, which are a hallmark of adult SPWs (*29*), were first observed in P10 animals (n = 11 animals at P10 to P30) (*28*).

Because early network patterns in the developing hippocampus in vitro are characterized by recurrent GDPs mediated by the synergistic excitation of GABA-mediated (GABAergic) and glutamatergic synapses (*3, 7, 11–14*), we combined extracellular and patch-clamp recordings from pups at P3 to P6 to investigate synaptic currents during hippocampal SPW bursts in anesthetized animals. SPWs were associated with large complex synaptic events in CA1 pyramidal cells (n = 11 neurons). In the voltage clamp mode with a low-chloride intracellular solution (E_{Cl} = –70 mV), we identified $GABA_A$ receptor–mediated postsynaptic currents (PSCs) at glutamate reversal potential (0 mV) and glutamatergic PSCs at $GABA_A$ reversal potential (–70 mV). The large synaptic currents included both glutamatergic and GABAergic components (n = 7) (Fig. 3). The prominent glutamatergic component of SPWs probably reflects an excitatory drive mediated by Schaffer collaterals. Although SPWs are not expressed in the in vitro preparation, and the glutamatergic component of in vivo bursts is more pronounced than during in vitro GDPs, the duration, the relatively rhythmic recurrence of the burst events, and the associated $GABA_A$ receptor–mediated synaptic currents all suggest that these events are the in vivo counterparts of GDPs described in vitro (*3, 5, 7, 11–14*).

Our results suggest that hippocampal SPW bursts that occur in awake and sleeping rat pups as well as in urethane-anesthetized animals are the main hippocampal field pattern during the first postnatal days. First, patterns other than SPWs (theta and dentate spikes) were also observed in this study, but only in rats older than 7 days of age. Second, isolated hippocampal transplants display only sharp wave burst events (*30*), supporting the view that this is an endogenous hippocampal network pattern. Third, the SPW burst sequence observed in the hippocampus in vivo is compatible with the in vitro observation that synchronous discharges of CA3 pyramidal cells occur spontaneously and propagate to the CA1 area in hippocampal slices and isolated hippocampi excised from animals younger than 2 weeks old (*5, 7, 13*). Although the immature CA1 subnetwork is able to generate synchronized GABA-mediated bursts by itself (*5, 13*), GDPs in CA3 most often precede CA1 bursts in vitro (*13*).

We have shown here that spontaneous SPW bursts in the immature CA1 region are driven by synaptically activated GABA and glutamate receptors. Trophic actions of GABA and glutamate in the immature hippocampus have been suggested by previous experiments (*7, 21*). Because CA3 neurons provide the main afferent pathway to the CA1 region, the high correlation between CA1 multiunit activity and SPWs in neonates suggests that SPW bursts provide synchronized pre- and postsynaptic firing, a condition that may favor Hebbian modification of developing synapses. Because neurons rarely discharged between bursts at this age, these synchronous events represent the major source of correlated neuronal activity for the neonatal hippocampus.

Few studies have examined the temporal and spatial organization of neuronal activity in developing brain structures in vivo. Recently, involvement of the thalamus and visual cortex has been shown in the generation of synchronized activity in the developing lateral geniculate nucleus (LGN) (*31*), challenging the classical view that sensory visual structures were the exclusive generator of the propagating waves of activity observed in the immature LGN (*32*). The present work in the intact animal shows that the hippocampus also generates endogenous synchronous activities during early postnatal life. Because these population bursts represented the majority of hippocampal activity at the earliest develop-

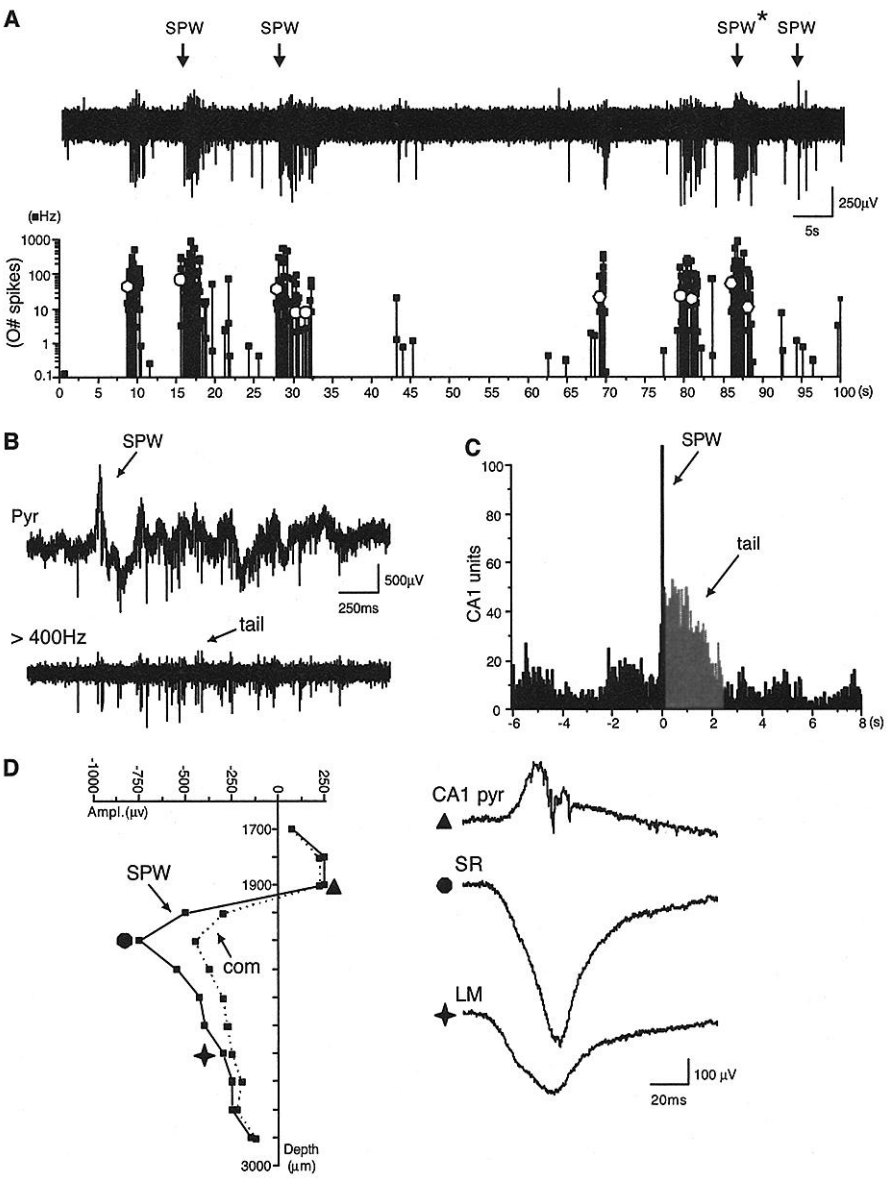

Fig. 2. SPW bursts under urethane anesthesia. (**A**) Filtered trace (>300 Hz) of multiunit activity in CA1 pyramidal layer. Arrows indicate simultaneously recorded field SPWs. Below the trace is the multiple unit activity over time, as in Fig. 1E. (**B**) Wide-band (1 Hz to 5 kHz) and filtered (>400 Hz) traces of the SPW marked by an asterisk in (A). Note prolonged firing ("tail") after the field SPW. (**C**) Cross-correlogram between SPWs (reference) and CA1 multiunit discharge (bin: 10 ms). (**D**) Amplitude-versus-depth distribution (left plot) of spontaneous SPWs and evoked field responses to commissural electrical stimulation (com), recorded with 16-site silicon probe. Example of SPW event in CA1 pyramidal layer (CA1 pyr, triangle), stratum radiatum (SR, circle), and stratum lacunosum moleculare (LM, star) is shown on the right.

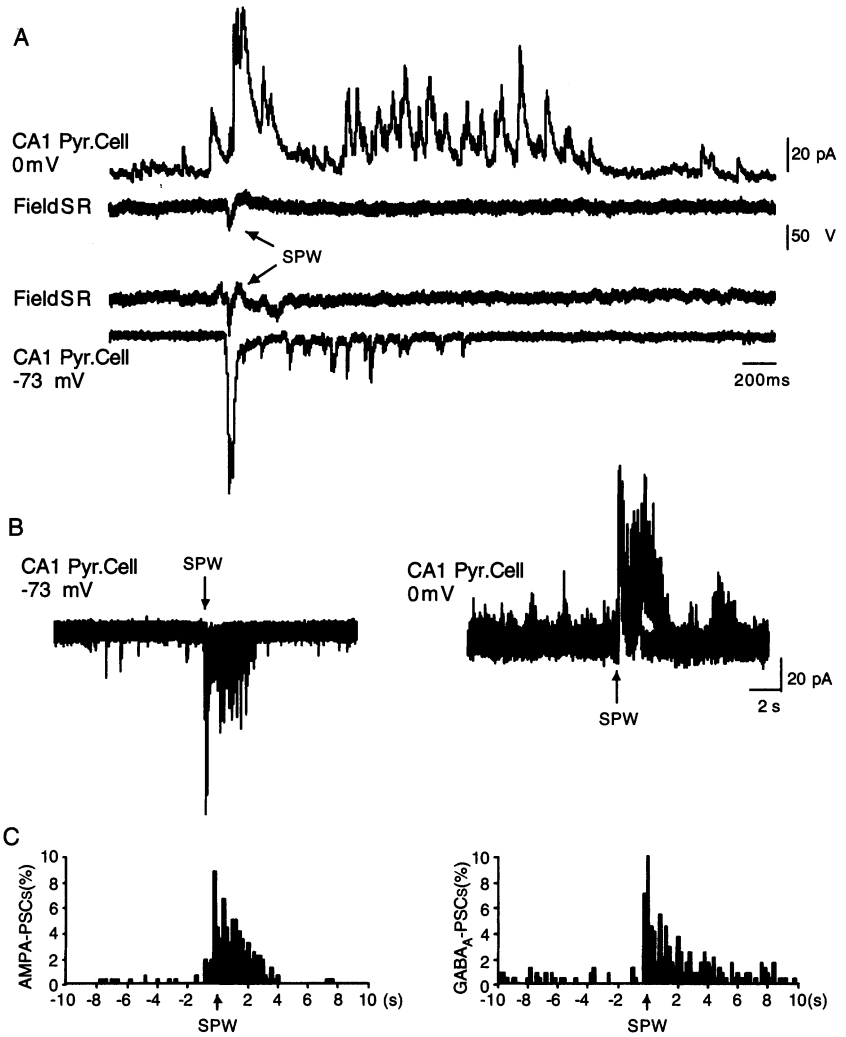

Fig. 3. Intracellular correlates of SPW bursts. (**A**) Intracellular (CA1 pyramidal cell, whole-cell with a low-chloride–containing pipette solution) and extracellular (stratum radiatum, SR) recordings during SPW events in a P5 pup. Upper pair: intracellular voltage clamp at glutamate reversal potential (0 mV), showing presumed $GABA_A$ receptor–mediated postsynaptic currents (upward deflections). Lower pair: intracellular voltage clamp at $GABA_A$ reversal potential (−73 mV), showing presumed glutamate receptor–mediated postsynaptic currents (downward deflections). (**B**) Superimposed traces of intracellular events (left, voltage clamp −73 mV; right, voltage clamp 0 mV) synchronized by the peak of SPWs. (**C**) Cross-correlograms between SPWs and presumed AMPA receptor–mediated PSCs (left) and between SPWs and presumed $GABA_A$ receptor–mediated PSCs (right).

mental stages, we hypothesize that these patterns contribute to the maturation and maintenance of cortical circuits in the newborn rat.

References and Notes

1. G. Buzsáki, J. J. Chrobak, *Curr. Opin. Neurobiol.* **5**, 504 (1995).
2. G. Buzsáki, L. W. Leung, C. H. Vanderwolf, *Brain Res.* **287**, 139 (1983).
3. Y. Ben-Ari, E. Cherubini, R. Corradetti, J. L. Gaïarsa, *J. Physiol.* **416**, 303 (1989).
4. X. Leinekugel, V. Tseeb, Y. Ben-Ari, P. Bregestovski, *J. Physiol.* **487**, 319 (1995).
5. O. Garaschuk, E. Hanse, A. Konnerth, *J. Physiol.* **507**, 219 (1998).
6. R. Yuste, L. C. Katz, *Neuron* **6**, 333 (1991).
7. Y. Ben-Ari, R. Khazipov, X. Leinekugel, O. Caillard, J. L. Gaïarsa, *Trends Neurosci.* **20**, 523 (1997).
8. G. M. Durand, Y. Kovalchuk, A. Konnerth, *Nature* **381**, 71 (1996).
9. J. T. Isaac, M. C. Crair, R. A. Nicoll, R. C. Malenka, *Neuron* **18**, 269 (1997).
10. A. Agmon, D. K. O'Dowd, *J. Neurophysiol.* **68**, 345 (1992).
11. X. Leinekugel, I. Medina, I. Khalilov, Y. Ben-Ari, R. Khazipov, *Neuron* **18**, 243 (1997).
12. R. Khazipov, X. Leinekugel, I. Khalilov, J. L. Gaïarsa, Y. Ben-Ari, *J. Physiol.* **498**, 763 (1997).
13. S. Menendez de la Prida, S. Bolea, J. V. Sanchez-Andres, *Eur. J. Neurosci.* **10**, 899 (1998).
14. Y. Ben-Ari, *Trends Neurosci.* **6**, 353 (2001).
15. O. Garaschuk, J. Linn, J. Eilers, A. Konnerth, *Nature Neurosci.* **3**, 452 (2000).
16. R. Yuste, A. Peinado, L. C. Katz, *Science* **257**, 665 (1992).
17. K. Kandler, L. C. Katz, *Curr. Opin. Neurobiol.* **5**, 98 (1995).
18. T. H. Schwartz *et al.*, *Neuron* **20**, 541 (1998).
19. A. Aguilo *et al.*, *J. Neurosci.* **19**, 10856 (1999).
20. H. O. Reiter, D. M. Waitzman, M. P. Stryker, *Exp. Brain Res.* **65**, 182 (1986).
21. C. S. Goodman, C. J. Shatz, *Cell/Neuron* **72/10** (suppl.), 77 (1993).
22. L. C. Katz, C. J. Schatz, *Science* **274**, 1133 (1996).
23. A. Kirkwood, H. Lee, M. F. Bear, *Nature* **375**, 328 (1995).
24. Materials and methods are available as supporting material on *Science* Online.
25. J. Csicsvari, H. Hirase, A. Mamiya, G. Buzsáki, *Neuron* **2**, 585 (2000).
26. A. Ylinen *et al.*, *J. Neurosci.* **15**, 30 (1995).
27. M. O. Leblanc, B. H. Bland, *Exp. Neurol.* **66**, 220 (1979).
28. X. Leinekugel, R. Khazipov, G. Buzsáki, data not shown.
29. G. Buzsáki, Z. Horvath, R. Urioste, J. Hetke, K. Wise, *Science* **256**, 1025 (1992).
30. G. Buzsáki, F. Bayardo, R. Miles, R. K. Wong, F. H. Gage, *Exp. Neurol.* **1**, 10 (1989).
31. M. Weliky, L. C. Katz, *Science* **285**, 599 (1999).
32. R. Mooney, A. A. Penn, R. Gallego, C. J. Shatz, *Neuron* **17**, 863 (1996).

33. We thank R. Miles and J. C. Poncer for fruitful comments and discussion on the manuscript and K. D. Harris for decisive help with matlab software. Supported by the Human Frontier Science Program (H.H. and X.L.), Fondation Française pour la Recherche sur l'Epilepsie (X.L.), and NIH grants FO6 TW02290, NS 34994, NOT 43994, NS 43157 (G.B.), and RR09754.

Supporting Online Material

www.sciencemag.org/cgi/content/full/296/5575/2049/DC1

Materials and Methods

21 February 2002; accepted 2 May 2002