the Calvin–Benson (C3) cycle[15]. When the supply of CO_2 from outside the leaves is cut off by closure of the stomata and the C2 cycle does not work either, the C3 cycle will not operate, so light energy may be diverted to produce active oxygen, which could damage the photosynthetic apparatus[12,16]. This may explain why plants suffer severe photoinhibition when photorespiration is suppressed. An important function of photorespiration may be to derive the Calvin–Benson cycle when the supply of CO_2 is limited, a new role for photorespiration.

Under high-intensity light, recycling of the ammonia produced by the glycine decarboxylase complex may be the rate-limiting step (Fig. 1). If there is sufficient GS2 to recycle the ammonia, photorespiration may drive the Calvin–Benson cycle and protect plants from photooxidation (Figs 4–6). However, when there is insufficient GS2 and high-intensity light is supplied continuously, ammonia would accumulate and the amino donor would be depleted[17], which could slow down the Calvin–Benson cycle (that is, the ETR) and lead to photoinhibition (Fig. 5). Rapid photoinhibition is known to occur when the chloroplast NADP system is completely reduced[9,18].

Most algae and all cyanobacteria have a CO_2 pump to concentrate CO_2 around Rubisco; the glycolate formed is metabolized or excreted into the surrounding medium[2,19]. Submerged aquatic plants in unshaded shallow water and land plants have to live under higher-intensity light and lower levels of CO_2 than those experienced by most algae or cyanobacteria, and may have developed photorespiration in order to drive the Calvin–Benson cycle under such conditions. □

Methods

Mature young leaves were used for all experiments. The amount of ammonia was estimated by using aeration and Nessler's reagent[20]. Amino-acid concentrations were estimated according to ref. 21. A *Sma*I–*Sac*I fragment containing a *GUS* gene in pBI121 (ref. 22) was removed and replaced by rice GS2 cDNA[7] (RGS38, in the sense orientation) to give 35S-GS2 cDNA. Ti-binary plasmids were mobilized from *Escherichia coli* to *Agrobacterium tumefaciens* LBA4404 by triparental mating[23]. The *Agrobacterium*-mediated transformation of tobacco was carried out by the leaf disc method[24].

Received 2 September; accepted 29 October 1996.

1. Ogren, W. L. *Annu. Rev. Plant Physiol.* **35,** 415–442 (1984).
2. Husic, D. W., Husic, H. D. & Tolbert, N. E. *CRC Crit. Rev. Plant Sci.* **5,** 45–100 (1987).
3. Powles, S. B. & Osmond, C. B. *Plant Physiol.* **64,** 982–988 (1979).
4. Somerville, C. R. *Annu. Rev. Plant Physiol.* **37,** 467–507 (1986).
5. Häusler, R. E., Blackwell, R. D., Lea, P. J. & Leegood, R. C. *Planta* **194,** 406–417 (1994).
6. Wallsgrove, R. M., Turner, J. C., Hall, N. P., Kendall, A. C. & Bright, S. W. *Plant Physiol.* **83,** 155–158 (1987).
7. Sakamoto, A. *et al. Plant Mol. Biol.* **13,** 611–614 (1989).
8. Matzke, M. A. & Matzke, J. M. *Plant Physiol.* **107,** 679–685 (1995).
9. Wu, J., Neimanis, S. & Heber, U. *Bot. Acta* **104,** 283–291 (1991).
10. Heber, U., Bligny, R., Streb, P. & Douce, R. *Bot. Acta* **109,** 307–315 (1996).
11. Demmig-Adams, B. & Adams, W. W. *Annu. Rev. Plant Physiol.* **43,** 599–626 (1992).
12. Aro, E. M., Vergin, I. & Anderson, B. *Biochim. Biophys. Acta* **1143,** 113–134 (1993).
13. Long, S. P. & Humphries, S. *Annu. Rev. Plant Physiol.* **45,** 633–662 (1994).
14. Brestic, M., Cornic, G., Fryer, M. J. & Baker, N. R. *Plant* **196,** 450–457 (1995).
15. Tolbert, N. E. in *Regulation of Atmospheric CO_2 and O_2 Photosynthetic Carbon Metabolism* (eds Tolbert, N. E. & Preiss, J.) 8–33 (Oxford Univ. Press, Oxford, 1994).
16. Asada, K. in *Causes of Photooxidative Stress and Amelioration of Defence Systems in Plants* (eds Foyer, C. H. & Mullineaux, P. M.) 77–104 (CRC, London, 1994).
17. Wendler, C., Putzer, A. & Wild, A. J. *Plant Physiol.* **139,** 666–671 (1992).
18. Krause, G. H. & Berrend, U. *FEBS Lett.* **200,** 298–302 (1986).
19. Powles, S. B. *Annu. Rev. Plant Physiol.* **35,** 15–44 (1984).
20. Sumner, J. B. in *The Enzymes* (eds Sumner, J. B. & Myrback, K. M.) Vol. 1, 873–892 (Academic, New York, 1951).
21. Sato, K. *et al. J. Agric. Food Chem.* **40,** 808–810 (1991).
22. Jegfferson, R. A., Kavanagh, T. A. & Bevan, M. W. *EMBO J.* **6,** 3901–3907 (1987).
23. Bevan, M. *Nucleic Acids Res.* **12,** 8711–8721 (1984).
24. Horsch, R. B. *et al. Science* **227,** 1229–1231 (1985).
25. Edwards, K., Johnstone, C. & Thompson, C. *Nucleic Acids Res.* **19,** 1349–1350 (1991).
26. Blake, M., Johnson, K. H., Russell-Jones, G. J. & Gotchlich, E. C. *Analyt. Chem.* **136,** 175–179 (1984).
27. Sakamoto, A., Takeba, G. & Tanaka, K. *Plant Cell Physiol.* **31,** 677–682 (1990).
28. Takeba, G. *Plant Cell Physiol.* **24,** 1469–1476 (1983).
29. Genty, B. E., Briantais, J. M. & Baker, N. R. *Biochim. Biophys. Acta* **990,** 87–92 (1989).

ACKNOWLEDGEMENTS. We thank N. Murata, K. Asada and K. Satou for discussions. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas from Ministry of Education, Science and Culture, Japan.

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Sensitization of meningeal sensory neurons and the origin of headaches

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THE headaches that accompany certain intracranial pathologies (such as meningitis, subarachnoid haemorrhage and tumour) have been considered to result from mechanical or chemical stimulation of pain-sensitive structures of the intracranial meninges[1,2]. Although the recurrent headache of migraine is of unknown origin and is not accompanied by an identifiable pathology, it shares with intracranial headaches features that suggest an exaggerated intracranial mechanosensitivity (worsening of the pain by coughing, breath-holding or sudden head movement[1,3]). One possible basis for such symptoms would be a sensitization of meningeal afferents to mechanical stimuli. Previous studies of neuronal responses to meningeal stimulation have focused primarily on cells in the central portion of the trigeminal pathway, and have not investigated the possible occurrence of sensitization[4–12]. We have recorded the activity of primary afferent neurons in the rat trigeminal ganglion that innervate the dural venous sinuses. Chemical stimulation of their dural receptive fields with inflammatory mediators both directly excited the neurons and enhanced their mechanical sensitivity, such that they were strongly activated by mechanical stimuli that initially had evoked little or no response. These properties of meningeal afferents (chemosensitivity and sensitization) may contribute to the intracranial mechanical hypersensitivity that is characteristic of some types of clinically occurring headaches, and may also contribute to the throbbing pain of migraine.

Anatomical studies in man and animals have shown that major blood vessels of the intracranial meninges, including the dural venous sinuses, receive a sensory innervation from the trigeminal nerve which originates primarily from cells in the medial (ophthalmic) part of the trigeminal ganglion[13–15]. Direct stimulation of these vessels in neurosurgical patients can evoke painful sensations, which some investigators have described as headache-like, and which are typically referred to the ophthalmic region of the trigeminal dermatome[1]. We used single-unit extracellular recording techniques to study the sensory properties of cells in the rat trigeminal ganglion that supply this intracranial meningeal innervation.

Trigeminal ganglion neurons with A-delta and C-fibre axons were identified as meningeal afferents by their response to electrical stimulation of the dura overlying the ipsilateral transverse sinus (Fig. 1*a*, *b*). The transverse sinus, which is a venous space between the inner and outer layers of the intracranial dura, is supplied by the tentorial nerve[13,14], and was found to be a particularly well innervated region in our previous studies of central *fos* expression evoked by dural stimulation[16]. The majority of neurons (33/45) that were activated by dural shock exhibited mechanosensitive receptive fields on the dura, from which they could be activated by punctate probing with von Frey hairs or by stroking with a blunt rod (Fig 1*c*, *d*). Receptive fields were restricted to the dura overlying and immediately adjacent to the transverse sinus, and did not extend across the midline. Mechanosensitivity was found more frequently in neurons with C-fibres

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FIG. 1 *a*, Experimental setup. *b*, Responses of two trigeminal ganglion cells to single-shock stimulation of the dura overlying the ipsilateral transverse sinus. Each trace shows 3 superimposed sweeps. Response latencies indicate that the axons of these cells conduct in the A-delta and C-fibre range, respectively ($12.5\,\mathrm{m\,s^{-1}}$ and $1.2\,\mathrm{m\,s^{-1}}$, respectively) based on a conduction distance measured at 12.5 mm. Conduction velocities (c.v.) of cells that responded to dural shock ranged from 0.26 to $20.8\,\mathrm{m\,s^{-1}}$. The mean c.v.s were 11.6 ± 4.1 for fast A-delta fibres, 3.5 ± 0.89 for slow A-delta fibres, and 0.76 ± 0.38 for C-fibres. *c*, Map of the response thresholds (mA) of a trigeminal ganglion cell (c.v. $4.8\,\mathrm{m\,s^{-1}}$) at different dural stimulation sites on and around the ipsilateral transverse sinus. The area from which a response to stroking could be evoked is also indicated by the boundary line. *d*, Histogram of discharge of the cell shown in *c* during stroking of the dura over the ipsilateral transverse sinus in the bounded area, and during stroking of the corresponding contralateral dura. *e*–*g*, Responses of the cell shown in *c* and *d* to chemical stimulation (500 mM NaCl in SIF) delivered either by topical application to the dural surface of the transverse sinus (*e*) or by infusion into the superior sagittal sinus (*f*), and absence of response to control infusion delivered into the jugular vein (*g*) (see 7Methods for further explanation).

($\leq 1.5\,\mathrm{m\,s^{-1}}$) (13/15) and slowly conducting A-delta fibres ($1.5 >$ conduction velocities (c.v.) $< 5\,\mathrm{m\,s^{-1}}$) (10/10) than in neurons with rapidly conducting A-delta fibres (5–$21\,\mathrm{m\,s^{-1}}$) (10/20) ($P < 0.01$, chi-square). Among 26 mechanosensitive neurons that were tested with von Frey monofilaments, mechanical thresholds ranged from 0.03 to >4.0 g (monofilaments stiffer than 4.0 g were not usually tested, to avoid puncturing the dura). Among neurons whose thresholds could be determined (≤ 4.0 g; $n = 23$), the mean threshold was 0.86 ± 0.98 g (mean $\pm$ s.d.), with no significant differences between neurons with fast A-delta fibres (1.8 ± 2.0 g; $n = 5$), slow A-delta fibres (0.78 ± 0.97 g; $n = 8$) and C-fibres (1.0 ± 1.1 g; $n = 10$).

A subset of the neurons that responded to dural shock (18/22 mechanosensitive and 1/5 mechanically insensitive neurons) also responded to topical application of algesic or inflammatory agents to the dura (9/11 C-fibres, 7/8 slow A-delta and 3/8 fast A-delta fibres). Responses were found to hypertonic sodium chloride (400 mM, 12/18), potassium chloride (150 mM, 13/21), pH-neutral buffer solutions of low (10/16) or high (4/5) osmolarity, capsaicin (3/9), and a soup of inflammatory agents (histamine, serotonin, bradykinin, and prostaglandin E_2, pH 5) (10/18) (Fig. 2*a*–*d*). Responsiveness to these 'chemical stimuli' (inflammatory, osmotic (low-osmolarity buffer), and ionic (KCl and NaCl)) usually occurred together. Of the 15 neurons that were tested with each of these 4 agents, 7 responded to all, 5 responded to none, and 3 responded to all except the inflammatory soup. Nearly all (18/19) of the neurons that could be activated by chemical stimuli were also mechanosensitive.

Five of the neurons that responded to topically applied chemical agents were also tested for responses to intravascular infusion

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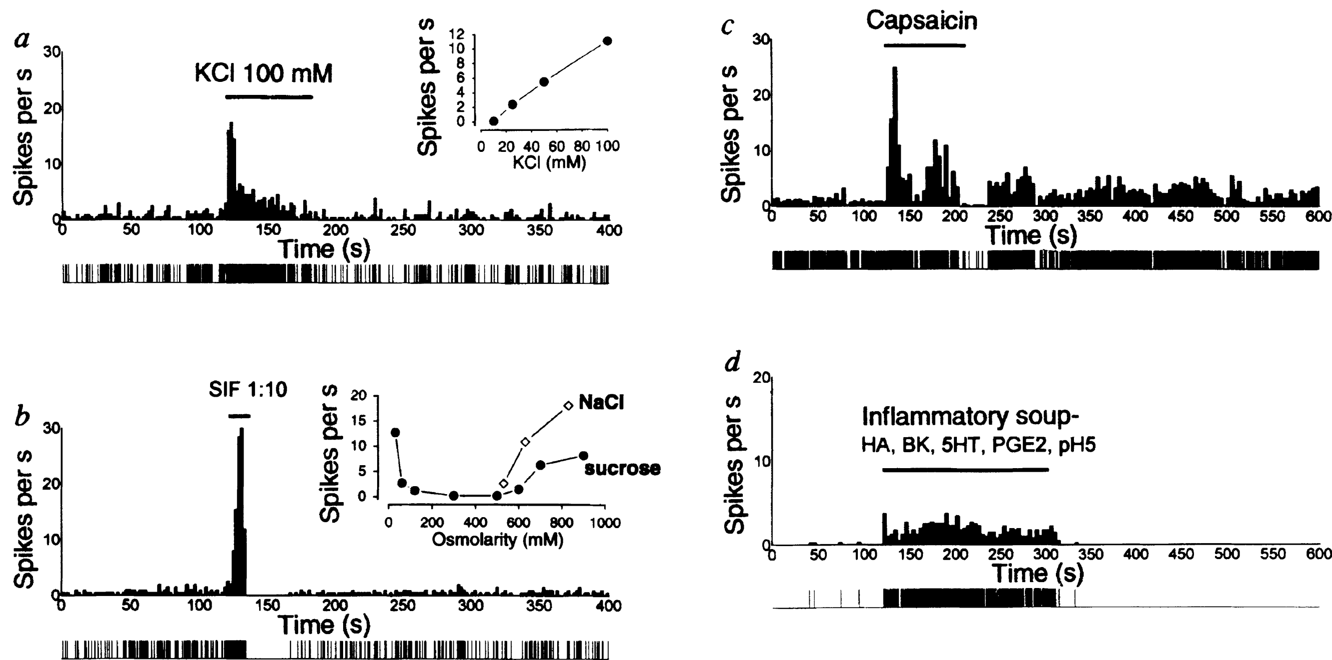

FIG. 2 Responses to topical application to the dura of algesic and inflammatory agents. *a*, Response of a neuron (same cell shown in the right-hand trace of Fig. 1*b*, c.v. 1.2 ms^{-1}) to 100 mM KCl in SIF, pH 7.2. Inset shows a plot of neuronal discharge against KCl concentration for this neuron. In neurons tested at lower concentrations, responses were found to 25 mM (3/6) and 50 mM KCl (6/6). *b*, Response of the cell shown in *a* to SIF diluted 10-fold with distilled water, pH 7.2. Inset shows plot of neuronal discharge against osmolarity for this neuron. Filled symbols: osmolarity of isotonic SIF was increased by adding increasing concentrations of sucrose (up to 600 mM), and was decreased by diluting the isotonic SIF with distilled water. Open symbols: osmolarity was increased by increasing NaCl concentrations (up to 400 mM) in SIF. The neurons responded to both high and low osmolarity. Increased NaCl concentrations produced a greater response than sucrose solutions of equal osmolarity in 5/5 neurons, indicating that the response to NaCl is not solely an effect of hyperosmolarity. Responses were found in 5/5 neurons to 250 mM NaCl, which was the lowest NaCl concentration tested. In neurons tested at multiple osmolarities (raised or lowered with sucrose or water, respectively), responses were found to 120 mOsm (5/8), 60 mOsm (6/8), 30 mOsm (8/8), 600 mOsm (1/4), 700 mOsm (3/5), and 900 mOsm (4/5). *c*, Response of a cell (c.v. 3.5 ms^{-1}) to 1% capsaicin. No response was produced by capsaicin vehicle, which was tested immediately before the capsaicin. *d*, Response of a cell (c.v. 4.2 ms^{-1}) to the soup of bradykinin, serotonin, and prostaglandin E_2, all 10 μM, and histamine, 100 μM, SIF, pH 5.0.

into the lumen of the sinus, using hypertonic sodium chloride as a stimulus. Four of these 5 neurons were found to respond to both routes of application (Fig. 1*e*–*g*). The response for both routes of chemical application provides evidence that the neurons have receptive endings within or adjacent to the walls of the sinus, consistent with anatomical data[13].

Sensitization was investigated by determining mechanical sensitivity before and after topical application to the receptive field of low pH buffer ($n = 2$) or the inflammatory soup ($n = 15$), both of which are effective for sensitizing cutaneous nociceptive afferents to mechanical stimuli[17,18]. Application of these agents for 5 min enhanced the sensitivity to subsequent testing with mechanical stimulation in 10 of 15 mechanosensitive neurons tested, and also resulted in the appearance of mechanosensitivity in 1 of 2 neurons that initially had been unresponsive to our mechanical stimulation. This chemically induced sensitization to mechanical stimuli was significant ($P < 0.01$, Wilcoxon's signed rank test). Such sensitization to mechanical stimuli was observed both in neurons that discharged in response to the sensitizing agent ($n = 6$) as well as in neurons that did not ($n = 5$).

Several previous studies have described responses of central neurons in the spinal trigeminal nucleus and thalamus to electrical, mechanical, and chemical (bradykinin, capsaicin) stimulation of the dura, although sensitization was not examined[4–12]. These studies showed that central dura-responsive neurons typically had nociceptive facial receptive fields with a distribution reminiscent of the pattern of pain referral evoked by dural stimulation, consistent with a role for these neurons in the mediation of referred pain of intracranial origin. The only prior electrophysiological study of meningeal primary afferents examined responses to mechanical and thermal, but not chemical, stimuli[11]. We report here the first direct observations, to our knowledge, of chemosensitivity and chemically induced sensitization to mechanical stimuli in afferents supplying the intracranial meninges, properties that have been found previously for other types of somatic and visceral afferents[17–20] and that have long been hypothesized in theories of headache pathogenesis[1,15], owing to their parallels to the symptomatology of some types of clinically occurring headaches. The headache that accompanies meningitis, as well as migraine headache, is characterized by features that suggest an exaggerated intracranial mechanosensitivity. Normally innocuous activities that produce an increase in intracranial pressure or altered intracranial haemodynamics, such as sudden head movement, coughing, or breath-holding, evoke dramatically increased head pain during such headaches[1,3].

Mechanical sensitization might also contribute to the throbbing pain of migraine. Although this pain has long been attributed to the pulsations of abnormally dilated blood vessels[1,2,21,22], more recent studies of vessel diameter during migraine attacks have failed to find dilatation greater than that known to occur in the absence of headache[15,22–24]. It thus appears that cerebral vasodilatation could only contribute to migraine headache if it were accompanied by sensitization of intracranial afferents (or possibly of central neurons[25,26]) to respond to normally innocuous mechanical stimuli. Our results show that chemically induced sensitization to mechanical stimuli can occur in meningeal afferents. It is generally assumed that the location of the intracranial afferent endings in question must be peri-arterial, because of the throbbing quality of the headache[1,2,21]. But we note that this need not be the case, because the arterial pressure pulse is transmitted mechanically throughout the subarachnoid space and meninges[27].

How might sensitization occur during migraine? Alternatives to

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the vasodilatation theory have implicated intracranial processes such as cortical spreading depression[28] or meningeal neurogenic inflammation[15,29]. Cortical spreading depression consists of a slowly propagating depression of neural activity which is accompanied by chemical disturbances, including elevated levels of extracellular potassium[28]. Meningeal neurogenic inflammation apparently results from release of neuropeptides (for example, substance P, calcitonin-gene-related peptide) from the peripheral endings of dural afferents, which in turn induces degranulation of dural mast cells[15] (whose released contents may include histamine, serotonin and cytokines). Thus, dural sinus afferents may be affected by chemical alterations in the cerebral cortex and the dura itself, because the sinuses collect the venous drainage from much of the cortex. In addition, recent evidence suggests that substances may move between the dura and underlying cortex by diffusion through the subarachnoid space[30].

Our results show that exposure to chemical agents associated with tissue injury or inflammation produces discharge in some meningeal afferents. Such exposure also causes some of these afferents to become more sensitive to mechanical stimuli for up to an hour or more. We suggest that such chemically induced transient mechanical sensitization could lead these neurons to respond to relatively small, normally innocuous mechanical alterations in the intracranial space such as might be produced by haemodynamic changes or head movement, and that excitation of such afferents could contribute to or worsen certain headaches. □

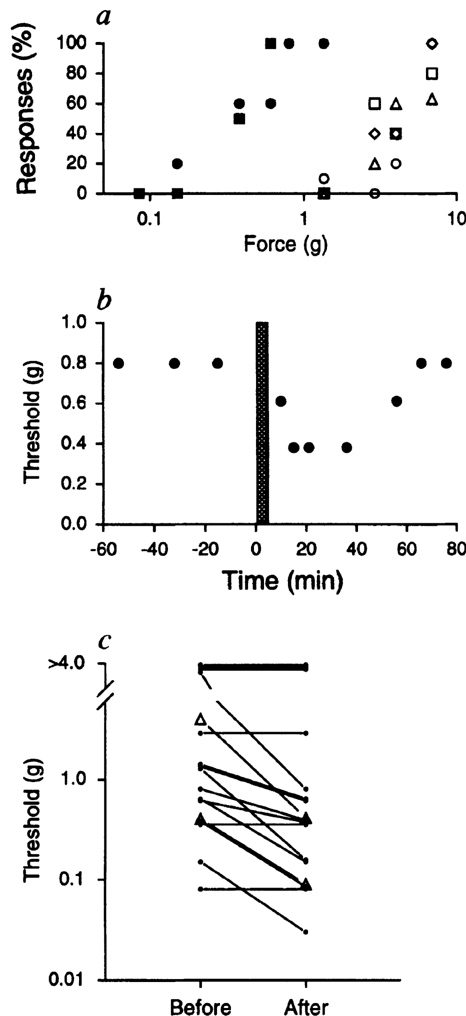

FIG. 3 Mechanical sensitization, as demonstrated by a lowered von Frey threshold, following topical application to the dura of acidic or inflammatory agents. *a*, Decrease in mechanical threshold of a neuron (c.v. $13.9\,ms^{-1}$) following application of pH 5.0 SIF for 2 min. The open and filled symbols represent responses to von Frey monofilaments before and after the chemical stimulation, respectively. A full series of von Frey responses was tested at 65, 45, 20, and 5 min before the chemical application (open circles, squares, diamonds, and triangles, respectively), and at 5 and 15 minutes following the chemical application (filled circles and squares, respectively). The von Frey threshold (>50% incidence of responses) decreased from about 4.0 g to 0.38 g following exposure to the low pH solution. *b*, Time course of changes in mechanical threshold for another neuron (same cell shown in Fig. 2*c*) which received a 5-min exposure (starting at time 0) to the soup of bradykinin, serotonin, prostaglandin E_2, and histamine in SIF, pH 5.0. *c*, Plot of mechanical thresholds before and after application of chemical agents. Circles and triangles represent neurons tested with inflammatory soup ($n = 15$) and pH 5.0 buffer ($n = 2$), respectively.

Methods

Urethane-anaesthetized male rats (400–600 g) were placed in a sterotaxic headholder. The right transverse sinus and the caudal part of the superior sagittal sinus were exposed by craniotomy. The exposed dura was bathed in a synthetic interstitial fluid (SIF) consisting of 135 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5 mM $CaCl_2$, 10 mM HEPES, and 10 mM glucose, pH 7.2. In 5 animals the entire superior sagittal sinus was exposed, and PE-10 tubing was inserted into the rostral end and passed caudally to the caudal end (sinus confluens). Tungsten microelectrodes were advanced into the trigeminal ganglion by a dorsal approach through the cerebral cortex. Single-shock electrical search stimuli (0.5 ms pulses, 5 mA, 0.7 Hz) were delivered through a bipolar stimulating electrode placed on the dura overlying the ipsilateral transverse sinus while the microelectrode was advanced through the ganglion. Chemical agents were delivered by topical application to the exposed dura or by infusion into the superior sagittal sinus. Sinus infusions were followed by control infusions into the jugular vein. Because the direction of venous blood flow is from the dural sinuses through the jugular vein to the heart (Fig. 1*a*), the absence of a response to jugular infusion provides evidence that the response to sinus infusion was from a site of action between the sinus and the jugular vein. The jugular infusate would be expected to access the intracranial circulation only after traversing the heart, at which point it is apparently too diluted to produce a response (Fig. 1*g*). A second sinus infusion was delivered following the jugular infusion, in order to verify that the neuron was still responsive. All agents tested in this study were delivered in SIF, except capsaicin, which was prepared by first dissolving in ethanol, then heating and re-dissolving in 10% Tween-80. Neurons that were tested to more than one agent ($n = 20$) were tested in the following order (following mechanical testing), at 10-min intervals: KCl, NaCl, low osmolarity SIF, high osmolarity SIF, and capsaicin; the inflammatory soup was either tested immediately before the capsaicin or in neurons that were tested for sensitization (Fig. 3), it was the first agent tested. Neuronal responses were quantified by calculating the mean firing rate during the initial 10 s of the response, minus the mean firing rate during the 60 s preceeding the stimulus.

To investigate sensitization (Fig. 3), a graded series of von Frey monofilaments (Stoelting, Wood Dale, Illinois) was applied in order of increasing stiffness to measure mechanical threshold at 10–30-min intervals for 1–2 h before and 1–2 h after the conditioning stimulus. Each data point (Fig. 3*a*) was obtained by applying a single monofilament 5–10 times to the neuron's mechanical receptive field on the ipsilateral transverse sinus. The percentage of times that the neuron responded was plotted against the force of the monofilament. Sensitization was not tested unless the baseline measurements were stable. The plotted post-stimulus measurements (Fig. 3*c*) were taken 5–30 min after the stimulus, which appeared to be the period of maximum sensitization.

Received 1 August; accepted 23 September 1996.

1. Wolff, H. G. *Headache and Other Head Pain* (Oxford Univ. Press, New York, 1963).
2. Martin, J. B. in *Harrison's Principles of Internal Medicine* 12th edn (eds Wilson, J. D. *et al.*) 108–115 (McGraw-Hill, New York, 1991).
3. Blau, J. N. & Dexter, S. L. *Cephalalgia* **1,** 143–147 (1981).
4. Strassman, A. S., Mason, P., Moskowitz, M. & Maciewicz, R. J. *Brain Res.* **379,** 242–250 (1986).
5. Davis, K. D. & Dostrovsky, J. O. *J. Neurophysiol.* **59,** 648–666 (1988).
6. Davis, K. D. & Dostrovsky, J. O. *Brain Res.* **446,** 401–406 (1988).
7. Davis, K. D. & Dostrovsky, J. O. *Brain Res.* **454,** 89–100 (1988).
8. Zagami, A. S. & Lambert, G. A. *Exp. Brain Res.* **81,** 552–566 (1990).
9. Zagami, A. S. & Lambert, G. A. *Neurosci. Lett.* **121,** 187–190 (1991).
10. Lambert, G. A., Zagami, A. S., Bogduk, N. & Lance, J. W. *Cephalalgia* **11,** 75–85 (1991).
11. Dostrovsky, J. O., Davis, K. D. & Kawakita, K. *Can. J. Physiol. Pharmacol.* **69,** 652–658 (1991).
12. Kaube, H., Hoskin, K. L. & Hoskin, K. L. & Goadsby, P. J. *Cephalalgia* **12,** 133–136 (1992).
13. Penfield, W. & McNaughton, M. *Arch. Neurol. Psychiat.* **44,** 43–75 (1940).
14. Andres, K. H., von During, M., Muszynski, K. & Schmidt, R. F. *Anat. Embryol.* **175,** 289–301 (1987).
15. Moskowitz, M. A. & Macfarlane, R. *Cerebrovasc. Brain Metab. Rev.* **5,** 159–177 (1993).
16. Strassman, A. M., Mineta, Y. & Vos, B. P. *J. Neurosci.* **14,** 3725–3735 (1994).
17. Steen, K. H., Reeh, P. W., Anton, F. & Handwerker, H. O. *J. Neurosci.* **12,** 86–95 (1992).
18. Davis, K. D., Meyer, R. A. & Campbell, J. N. *J. Neurophysiol.* **69,** 1071–1081 (1993).
19. Baker, D. G., Coleridge, H. M., Coleridge, J. C. G. & Nerdrum, T. *J. Physiol.* **306,** 519–536 (1980).

© 1996 Nature Publishing Group

20. Schaible, H.-G. & Schmidt, R. F. J. *Physiol. (Lond.)* **403,** 91–104 (1988).
21. Graham, J. R. & Wolff, H. G. *Arch. Neurol. Psychiatr.* **39,** 737–763 (1938).
22. Friberg, L., Olesen, J., Iversen, H. K. & Sperling, B. *Lancet* **338,** 13–17 (1991).
23. Iversen, H. K., Nielsen, T. H., Olesen, J. & Tfelt-Hansen, P. *Lancet* **336,** 837–839 (1991).
24. Thomsen, L. L., Iversen, H. K. & Olesen, J. *Cephalalgia* **15,** 109–116 (1995).
25. Woolf, C. J. *Nature* **308,** 686–688 (1983).
26. Burstein, R., Malick, A. & Strassman, A. *Soc. Neurosci. Abstr.* **22,** 864 (1996).
27. Daley, M. L., Pasupathy, H., Griffith, M., Robertson, J. T. & Leffler, C. W. *IEEE Trans. Biomed. Eng.* **42,** 420–424 (1995).
28. Lauritzen, M. *Brain* **117,** 199–210 (1994).
29. Goadsby, P. J. & Edvinsson, L. *Ann. Neurol.* **33,** 48–56 (1993).
30. Orr, E. L. & Stokely, M. E. *Soc. Neurosci. Abstr.* **21,** 1858 (1995).

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From stimulus encoding to feature extraction in weakly electric fish

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ANIMALS acquire information about sensory stimuli around them and encode it using an analogue or a pulse-based code. Behaviourally relevant features need to be extracted from this representation for further processing. In the electrosensory system of weakly electric fish, single P-type electroreceptor afferents accurately encode the time course of random modulations in electric-field amplitude[1]. We applied a stimulus estimation method[2] and a signal-detection method to both P-receptor afferents and their targets, the pyramidal cells in the electrosensory lateral-line lobe. We found that although pyramidal cells do not accurately convey detailed information about the time course of the stimulus, they reliably encode up- and downstrokes of random modulations in electric-field amplitude. The presence of such temporal features is best signalled by short bursts of spikes, probably caused by dendritic processing, rather than by isolated spikes. Furthermore, pyramidal cells outperform P-receptor afferents in signalling the presence of temporal features in the stimulus waveform. We conclude that the sensory neurons are specialized to acquire information accurately with little processing, whereas the following stage extracts behaviourally relevant features, thus performing a nonlinear pattern-recognition task.

The electric fish *Eigenmannia* generates a quasi-sinusoidal electric field with carrier frequencies between 200 and 600 Hz, by regularly discharging an electric organ located in its tail. The fish senses local distortions of the electric field by means of two classes of tuberous electroreceptors distributed on the body surface[3]: T-type and P-type electroreceptors encode changes in phase and electric field amplitude, respectively, which are used for electrolocation[4] and communication[5].

At the level of the electrosensory lateral line-lobe (ELL), the first central nucleus of the electrosensory pathway, amplitude information is processed nearly independently of phase information, and is re-encoded in the output spike trains of E- and I-type pyramidal cells[4,6]. The temporal response of P-receptor afferents and pyramidal cells has been characterized by their average firing rate for step changes and sinusoidal amplitude modulations of an externally applied electric field[4,7]. Under these conditions, the response of P-receptor afferents is slowly adapting (tonic), whereas the response of pyramidal cells is transient (phasic). Furthermore, P-receptor afferents and E-type pyramidal cells raise their mean firing rate when the electric field amplitude is increased, whereas I-type pyramidal cells are inhibited. These mean response characteristics leave open several alternatives for the encoding and processing of time-varying modulations in electric-field amplitude in single-spike trains of ELL pyramidal cells. Pyramidal cells might, for instance, transmit detailed information about the time course of temporal changes in the stimulus waveform[8], or of specific stimulus frequencies[9], combined with half-wave rectification.

To address the question of how modulations in electric-field amplitude are temporally encoded and processed between the first two stages of the amplitude pathway, we studied the responses of P-receptor afferents and pyramidal cells to random distortions of a mimic of the fish's own electric field. Such distortions were simulated by superposing zero-mean, random amplitude modulations, $s(t)$, to an externally applied electric field in fish whose electric-organ discharges were strongly attenuated by intramuscular injection of a curare-like drug (Fig. 1*a*). The carrier frequency of this field was equal to the fish's own frequency before the drug was applied, and the cut-off frequency of the random amplitude modulations[1] (Fig. 1*c*) was varied over a behaviourally relevant range (2–40 Hz) (refs 4, 10).

We assessed the ability of single-spike trains to convey detailed information about the time course of $s(t)$ by smoothing the time series of action potential events with a filter chosen to obtain the best estimate of $s(t)$ (refs 1, 2, 11). This method and its variants are able to detect detailed encoding of a random stimulus or some filtered and/or half-wave rectified version of it (such as temporal derivative encoding, band-pass filtering and on/off responses). The accuracy of the information transmitted about $s(t)$ was characterized in the time domain by the coding fraction, defined as $\gamma = 1 - \varepsilon/\sigma$, where ε is the root-mean-square error between the true and estimated stimulus, and σ is the standard deviation of $s(t)$ (ref. 1). The coding fraction takes the maximum value of 1 when the stimulus is perfectly estimated ($\varepsilon = 0$) and the minimum value of 0 if estimation from the spike train is at chance level ($\varepsilon = \sigma$) (refs 12, 13). We computed the coding fraction for P-receptor afferents and pyramidal cells; single P-receptor afferents were able to encode up to 75% of the stimulus time course, but pyramidal cells encoded less than 30%. Such a poor performance suggests that individual pyramidal cells do not convey detailed information on the time course of the stimulus to higher-order levels of the electrosensory system.

Pyramidal cells might transmit information about temporal features in the stimulus by performing a nonlinear classification task. This possibility can be tested by using neural network models. An artificial neural network can learn from the stimulus and response data to find the 'optimal' sensory input pattern eliciting a spike[14]. We used a more direct method derived from signal-detection theory, which assumes that the nonlinear classification task is implemented by means of a linear summation of incoming information plus a threshold computation[11,15]. To determine the optimal temporal feature **f** predicting the occurrence or non-occurrence of a spike in a pyramidal cell, we binned the data and studied the distributions of amplitude modulation waveforms preceding a bin containing a spike or no spike. We first determined the mean stimulus preceding a bin containing a spike ($\mathbf{m}_1$) and no spike ($\mathbf{m}_0$) (Fig. 1*b*) and then computed the covariance matrices $\mathbf{\Sigma}_1$ and $\mathbf{\Sigma}_0$ (Fig. 1*c*, *d*) characterizing second-order variations and correlations of these distributions around their means. The optimal **f** was determined as the direction in this temporal stimulus space of amplitude modulation waveforms for which the ratio of the mean squared distance between stimuli preceding a spike and no spike (computed from $\mathbf{m}_1$ and $\mathbf{m}_0$) and of their variances (computed from $\mathbf{\Sigma}_1$ and $\mathbf{\Sigma}_0$) was maximized (see equation (1) in 7Methods). This method is illustrated in a two-dimensional example (Fig. 2*a*, inset). For the I-type pyramidal cell depicted in Figs 1 and 2, the optimal sensory stimulus that predicted the occurrence of a spike, **f**, was a downstroke in the

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