sequences, together with *RARA* should allow a full molecular description of APL.

Note added in proof. Lemmons *et al.* have also reported the cloning of the APL breakpoint region (*31*).

REFERENCES AND NOTES

1. L. K. Hillestad, *Acta Med. Scand.* **159**, 189 (1957).
2. R. A. Larson *et al.*, *Am. J. Med.* **76**, 827 (1984); D. Sheer *et al.*, *Br. J. Cancer* **52**, 55 (1985).
3. R. Dalla-Favera *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7824 (1982).
4. A. De Klein *et al.*, *Nature* **300**, 765 (1982).
5. P. van Tuinen *et al.*, *Oncogene* **1**, 319 (1987); C. W. Miller *et al.*, *Science* **244**, 823 (1989); E. Donti *et al.*, *ibid.*, p. 824; S. C. Weil *et al.*, *ibid.*, p. 825.
6. C. L. Smith and C. R. Cantor, *Methods Enzymol.* **155**, 449 (1987).
7. A. Poustka and H. Lehrach, *Trends Genet.* **2**, 174 (1986).
8. A. M. Frischauf, *Technique* **1**, 3 (1989).
9. A. P. Bird, *Trends Genet.* **3**, 342 (1987).
10. A. Tunnacliffe *et al.*, *EMBO J.* **2**, 1577 (1983).
11. D. Sheer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5007 (1983).
12. G. Moore *et al.*, *Genomics* **4**, 152 (1989).
13. J. Borrow and A. Goddard, unpublished.
14. Y. Bai *et al.*, *Ann. Hum. Genet.* **46**, 337 (1982).
15. GBT8 is a mouse-human somatic cell hybrid constructed from patient APL 511 by P. Gorman and D. Sheer. It contains the APL 15q+ derivative chromosome but no normal chromosome 15 or 17.
16. J. Borrow and A. Goddard, unpublished.
17. P. van Tuinen *et al.*, *Genomics* **1**, 374 (1987).
18. A. Goddard and J. Borrow, unpublished.
19. Colonies (5×10^5) from a PMA-stimulated HL60 cDNA library (a gift from D. Simmons, Oxford) constructed in the plasmid vector CDM8 were screened with LCN4A3/B and five positive clones purified. Sequence data were obtained with the Sequenase Kit from United States Biochemical Corporation and assembled and analyzed with programs from IntelliGenetics Inc.
20. M. Petkovich *et al.*, *Nature* **330**, 444 (1987); V. Giguere *et al.*, *ibid.*, p. 624.
21. M. G. Mattei *et al.*, *Hum. Genet.* **80**, 186 (1988).
22. C. M. Croce, *Cell* **49**, 155 (1987); Y. Ben-Neriah *et al.*, *Science* **233**, 212 (1986); J. Nourse *et al.*, *Cell* **60**, 535 (1990); E. A. McGuire *et al.*, *Mol. Cell. Biol.* **9**, 2124 (1989).
23. S. Green and P. Chambon, *Trends Genet.* **4**, 309 (1988).
24. T. R. Breitman, S. J. Collins, B. R. Keene, *Blood* **57**, 1000 (1981); Z. Y. Wang *et al.*, *Nouv. Rev. Fr. Hematol.* **32**, 25 (1990).
25. A genomic library was prepared from the 15q+ mouse-human hybrid PLTI1S in EMBL3*cos*. LCN4A3/A was used to screen 1.5×10^6 recombinant phage and breakpoint clone fqp12 was isolated.
26. N. Heisterkamp *et al.*, *Nature* **299**, 747 (1982).
27. B. G. Herrmann *et al.*, *Cell* **48**, 813 (1987).
28. A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983).
29. P. G. Sealey *et al.*, *Nucleic Acids Res.* **13**, 1905 (1985).
30. M. K. Yagle *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
31. R. S. Lemmons *et al.*, *Genes Chrom. Cancer* **2**, 79 (1990).

32. We are grateful to A.-M. Frischauf and H. Himmelbauer for advice on the construction of the linking library and use of a genomic cosmid library, and to D. H. Ledbetter for the cell line P12.3B6. We thank A. Lister, A. Rohatiner, and B. Gibbons at St. Bartholomew's Hospital, London, J. Chessels and F. Katz at Great Ormond Street, London, and I. Dube at the University of Toronto for collecting samples from APL patients. We also thank S. Rider and P. Hedge for pulsed-field blocks of DNA and for helpful discussions on APL.

25 June 1990; accepted 14 August 1990

Flip and Flop: A Cell-Specific Functional Switch in Glutamate-Operated Channels of the CNS

BERND SOMMER,* KARI KEINÄNEN,*† TODD A. VERDOORN, WILLIAM WISDEN, NAIL BURNASHEV, ANNE HERB, MARTIN KÖHLER, TAMAKI TAKAGI, BERT SAKMANN, PETER H. SEEBURG‡

In the central nervous system (CNS), the principal mediators of fast synaptic excitatory neurotransmission are L-glutamate–gated ion channels that are responsive to the glutamate agonist α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA). In each member of a family of four abundant AMPA receptors, a small segment preceding the predicted fourth transmembrane region has been shown to exist in two versions with different amino acid sequences. These modules, designated "flip" and "flop," are encoded by adjacent exons of the receptor genes and impart different pharmacological and kinetic properties on currents evoked by L-glutamate or AMPA, but not those evoked by kainate. For each receptor, the alternatively spliced messenger RNAs show distinct expression patterns in rat brain, particularly in the CA1 and CA3 fields of the hippocampus. These results identify a switch in the molecular and functional properties of glutamate receptors operated by alternative splicing.

L-GLUTAMATE (L-GLU), THE MAJOR excitatory neurotransmitter in the mammalian CNS exerts its diverse effects through pharmacologically distinct receptors (*1*). Fast synaptic neurotransmission is thought to be mainly mediated by cationic channels intrinsic to AMPA-gated receptors. Kainate has been suggested to gate a second subtype of glutamate receptor mediating fast neurotransmission. Synaptic transmission mediated by a third receptor subtype, the *N*-methyl-D-aspartate (NMDA) receptor, is characterized by a slow rise time, voltage-dependent block by Mg^{2+}, and Ca^{2+} permeability; these channels induce various forms of activity-dependent synaptic plasticity such as long-term potentiation (LTP) (*2*). However, maintenance of the LTP-associated synaptic enhancement can require, at least in part, a change in postsynaptic AMPA receptors (for example, in the CA1 area of the hippocampus) (*2, 3*).

We have analyzed by molecular cloning the family of AMPA receptors in the CNS (*4*). These receptors bind AMPA, and their channels are gated by AMPA, L-Glu, and kainate, indicating a blurred distinction between AMPA and kainate receptors that varies depending if they are classified by gating or by ligand binding. The properties of these receptors are consistent with electrophysiological data from hippocampal neurons that suggest that AMPA and kainate act predominantly on the same receptor (*5*). We now describe the existence and function of a second molecular version of the four AMPA receptors, generated by alternative splicing.

Molecular cloning and detailed analysis of cDNAs encoding the AMPA-selective glutamate receptors GluR-A, -B, -C, and -D (*4*) revealed that in each receptor a segment of 115 bp exists in one of two sequence versions. This segment encodes 38 amino acid residues within a conserved receptor domain preceding the predicted fourth transmembrane region (Fig. 1) and hence is probably located intracellularly (*4, 6*). The alternative versions were named "flip" and "flop," with the previously described primary structures (*4*) as the flop forms. In each of the receptors, the sequences of the two alternative segments are very similar, and most nucleotide substitutions are silent changes with respect to the protein sequence. Accordingly, the flip and flop versions of each polypeptide differ in only a few (9 to 11) amino acids, and these substitutions are often conservative. A tetrapeptide is consistently different between the two versions of the four receptors. Neither of the alternative tetrapeptides conforms to known consensus sequences.

The exchange of small homologous domains in four glutamate receptors suggested that the two receptor versions for each of the four family members arose from alternative splicing. Such a mechanism was further implied by high resolution mapping and DNA sequence analysis of the murine gene for the GluR-B receptor. In this gene, the flip and flop sequences are on adjacent exons, separated from each other by an intron of approximately 900 bp and from their neigh-

B. Sommer, K. Keinänen, W. Wisden, A. Herb, M. Köhler, T. Takagi, P. H. Seeburg, Laboratory of Molecular Neuroendocrinology, Center for Molecular Biology, University of Heidelberg, 6900 Heidelberg, F.R.G.
T. A. Verdoorn, N. Burnashev, B. Sakmann, Abteilung Zellphysiologie, Max-Planck-Institut für Medizinische Forschung, 6900 Heidelberg, F.R.G.

*The first two authors contributed equally to this report.
†Present address: Biotechnical Laboratory, Technical Research Center, Espoo, Finland.
‡To whom correspondence should be addressed.

boring exons by similarly sized introns (Fig. 2). All exon-intron junctions display the consensus structures of splice sites. Dot matrix analysis of the DNA spanning the entire region (3.5 kb) did not reveal additional coding sequences related to flip and flop. These results and the sequence similarity between GluR-A, -B, -C, and -D, particularly in the domain affected by the module exchange, indicate that the flip and flop forms of all four receptors are generated by alternative splicing. The duplication and functional specialization of this exon sequence apparently preceded in evolution the expansion of the receptor family.

We found that different functional properties were imparted by flip and flop modules. The electrophysiological characteristics of the different receptor forms were analyzed (*7*) in cultured mammalian cells engineered for the transient simultaneous expression (*8*) of GluR-A and GluR-B, as coexpression of two receptors consistently resulted in larger whole-cell currents (*4*). For both flip and flop versions of these receptors, kainate activated a nondesensitizing current, whereas L-Glu caused an initial fast desensitizing current followed by a steady-state plateau (Fig. 3). The steady-state currents showed outwardly rectifying whole-cell current-voltage relations, small unitary conductance (≈1 pS), and gating properties characterized by a relaxation time of 5 to 10 ms (*7*). These properties are very similar to those of native receptors in cultured neurons (*9*). Moreover, the nootropic drug aniracetam potentiated L-Glu– and AMPA-activated currents, but not those elicited by kainate. Aniracetam enhances synaptic currents in rat brain and produces similar agonist-dependent potentiation of Glu-gated channels expressed from brain RNA in *Xenopus* oocytes (*10*).

The flip and flop forms differed in their response to L-Glu and AMPA relative to kainate (Fig. 3A). Whereas saturating concentrations of kainate and L-Glu (*11*) evoked currents with similar amplitudes in cells expressing the flip versions, kainate-evoked currents mediated by flop versions were much larger than those produced by L-Glu, as described (*4*). This difference between the flip and flop channels was observed for both the desensitizing and steady-state components of the current response to L-Glu, although much faster desensitization of flop versions cannot be ruled out. To quantify this difference, we compared the amplitude of the steady-state component of whole-cell currents elicited by saturating concentrations of kainate, AMPA, and L-Glu (*11*) and plotted the amplitude of L-Glu–evoked currents as a function of the kainate response amplitude (Fig. 3B). L-Glu activated channels four to five times more effectively when interacting with the flip versions. AMPA and L-Glu produced similar amplitude differences between the flip and flop variants and similar rapid desensitization. A difference in the responses of the flip and flop versions was also seen upon coexpression of GluR-A and GluR-C, indicating that the flip receptors as a class behave differently from the flop class.

Our observations confirm that AMPA and kainate act on a common receptor (*1, 5*) and reveal a pharmacological picture more complex than anticipated. Thus, the proper-

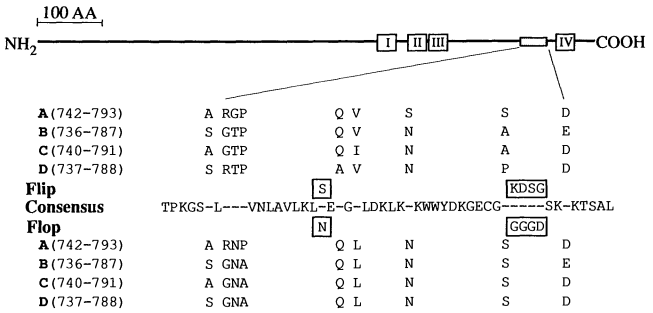

Fig. 1. Comparison of polypeptide sequences in the flip and flop segments of four glutamate receptors. The locations of four putative transmembrane regions (I to IV) and of the alternative sequence segment (stippled box) are indicated on top. AA, amino acids. The lines emanating from the stippled box demarcate the boundary of the alternative sequence in the four receptors. The amino acids (*22*) that are conserved in both forms of the receptors throughout the alternative segment are shown in the middle (Consensus). Above and below the dashes all amino acid substitutions are listed in the flip (above) or flop (below) versions of GluR-A, GluR-B, GluR-C, and GluR-D. Numbers in parentheses refer to amino acid positions within the mature polypeptides (*4*). Boxed residues occur in positions consistently exchanged in all four flip and flop sequences. All amino acids were predicted from DNA sequence analysis of multiple cDNAs isolated from libraries constructed in λZAPII (Stratagene) and λgt10 from mRNA of rat brain (*4*). For each glutamate receptor, approximately half of the cloned cDNAs specified the flip, the other half the flop, sequence. The complete nucleotide sequences encoding the flop-containing polypeptides are deposited at EMBL/GenBank under accession numbers M36418 (GluR-A), M36419 (GluR-B), M36420 (GluR-C), and M36421 (GluR-D) and the corresponding flip versions under M38060 (GluR-A), M38061 (GluR-B), M38062 (GluR-C), and M38063 (GluR-D).

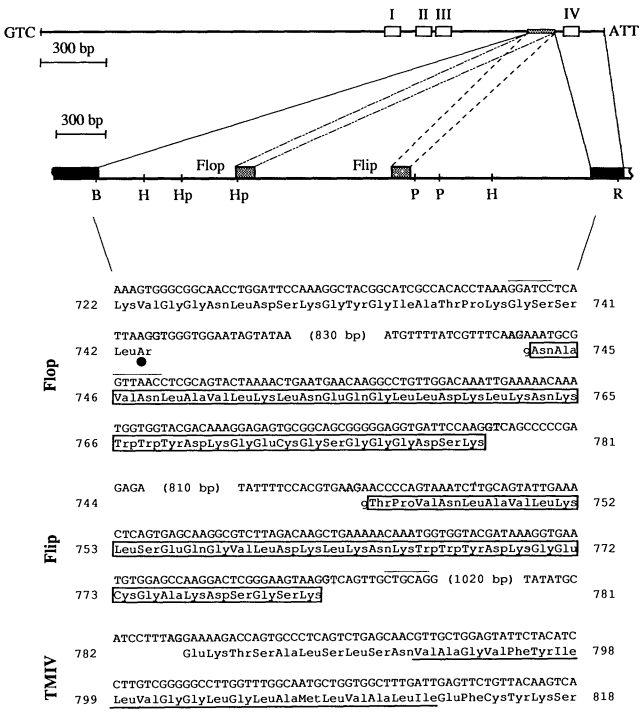

Fig. 2. Flip-flop exon arrangement of the murine GluR-B gene. The upper part depicts linear representations of cDNA (above) and gene (below). In the cDNA, shown from first (GTC, valine) to last (ATT, isoleucine) codon for the mature rat polypeptide of 870 residues (*4*), regions encoding putative transmembrane segments (TM) (I to IV) are boxed, and the alternative sequence element is depicted by a stippled bar. Across the latter region, lines (broken and continuous) connect to exons within the cognate murine gene. In this gene, the flip and flop exons are shown as stippled boxes separated on both sides by introns. The positions of several restriction sites (B, Bam HI; H, Hind III; Hp, Hpa I; P, Pst I; R, Eco RI), predicted from DNA sequence analysis, are marked. The murine gene was isolated from a genomic library constructed in λ-FIX (Stratagene) (*23*). The DNA sequence of the depicted gene segment was determined and the sequence of flip and flop exons, portions of the neighboring exons, and the corresponding intron junctions are shown below the partial gene map. The amino acid sequences corresponding to flip and flop structures are boxed, the predicted fourth transmembrane segment is underlined, and all amino acid sequences are numbered according to positions within the mature rat GluR-B polypeptide (*4*). The single amino acid substitution between the rat and murine GluR-B polypeptide in the sequenced region is indicated by a filled circle (position 743, glycine in rat). Consensus intron-exon junctions are stippled, intron lengths are indicated in parentheses, and three restriction endonuclease sites within the nucleotide sequence are overlined.

ties of channels with flip or flop modules are different when gated by L-Glu or AMPA than when gated by kainate. Further, with L-Glu and AMPA, different current responses are evoked from receptors that differ only in the flip-flop domain. This domain exchange does not substantially affect the ligand binding properties (*4*) of these receptors (not shown). Hence, the flip and flop modules could be important in determining the current responses to L-Glu, the principal excitatory neurotransmitter in the CNS.

Additional experiments revealed (Fig. 3C) that, in channels formed by coexpressing GluR-A flip and GluR-B flop, the fast desensitizing component of L-Glu–evoked currents is large and thus arises from the flip-containing GluR-A, whereas the small sustained current seems to be derived from GluR-B flop (Fig. 3C). In channels composed of GluR-A flop and GluR-B flip, the fast desensitizing component is that of flop and the steady-state current component is mediated by flip. No difference was seen in the kainate-gated currents of these flip-flop channels (Fig. 3D). Our results indicate that native Glu-operated channels may be heteromeric assemblies of both receptor subtype and flip-flop module. In assemblies of two receptor types, one partner can be dominant with respect to the fast desensitizing current component, whereas the other can determine the steady-state component. Such dominance does not correlate with module type.

GluR-A, -B, and -C mRNAs are prominently expressed and widely distributed in the CNS, whereas GluR-D mRNA has a more restricted expression (*4*). This distribution was determined with oligonucleotide probes (pan probes) complementary to sequences not affected by the flip and flop module exchange. By using oligonucleotides specific for the alternatively spliced versions of each receptor mRNA (*12*), we investigated where and to what extent the exon switch occurs in the adult rat brain (Fig. 4). The neuronal populations decorated by the cognate flip- and flop-specific probes together make up the structures visualized by the respective pan probes (*4*). Differences in signal intensities relative to those of pan probes reflect the differential stringencies used for these probes (*12*). This finding is best illustrated for GluR-D mRNA: the flip version is only observed in the cerebellum (Fig. 4g), whereas in forebrain (Fig. 4h) the flop version of this mRNA has the same expression pattern as seen with the pan D probe (*4*) but at a reduced signal strength.

The cell-specific nature of the alternative splice is particularly evident in the hippocampus (Fig. 4, i to k). The CA3 neurons synthesize only the flip version of three glutamate receptors (GluR-A, -B, and -C), while both mRNA versions of these and of GluR-D are seen in CA1 neurons. However, we observed a higher expression of flop relative to flip species in the dentate gyrus. A switch in expression between the alternatively spliced mRNAs occurs precisely at the boundary between the CA1 and CA3 areas (Fig. 5). For GluR-A and -B mRNAs, the smaller and more densely packed CA1 pyramidal cells express lower amounts of the flip versions than the larger, less densely packed CA3 pyramidal cells. In contrast, CA1 pyramidal cells express all flop versions. There are virtually no silver grains over CA3 pyramidal cells hybridized with any flop-specific probe. However, many putative interneurons in the oriens, pyramidal, and radiatum layers in both the CA1 and CA3 sectors strongly express flop sequences.

Other CNS regions showing differential expression of flip and flop modules (Fig. 4) illustrate the widespread use of the alternative splice in the rat brain. In the neocortex, flip versions of the GluR-A, -B, and -C mRNAs are expressed in a laminated pattern, with high expression in layers II, III, and VI, while expression of the flop forms is more uniform. The anterior olfactory nucleus expresses predominantly the flip mRNAs, whereas the granule layer of the olfactory bulb contains more flop mRNA than flip mRNA. In the tenia tecta, we saw only GluR-A and -B flip mRNAs but no flop sequences. The septal nuclei express GluR-B flip mRNA at much higher levels than the respective flop form.

Alternative splicing generates diversity in

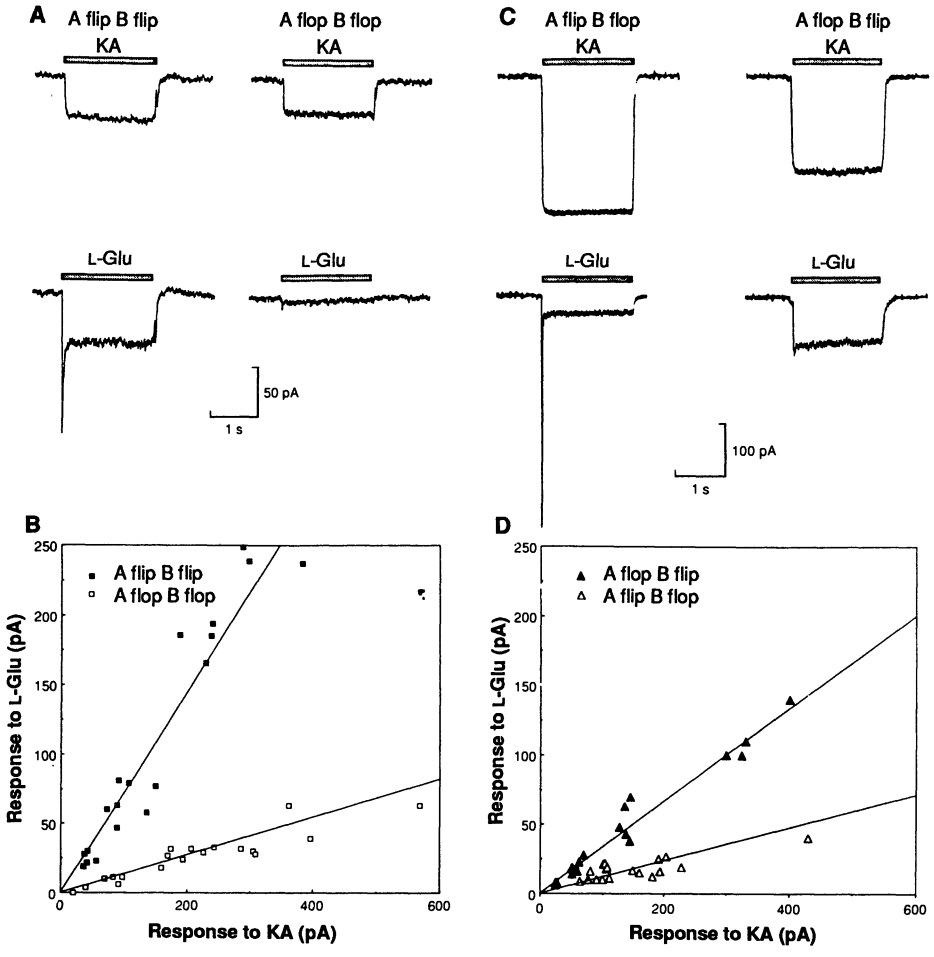

Fig. 3. Differential effectiveness of L-Glu (300 μM) and kainate (KA) (300 μM) on flip and flop versions of recombinant glutamate receptors. (**A**) Inward currents at −60 mV evoked by rapid application of 300 μM L-Glu and 300 μM kainate in cells expressing the flip forms (left) and the flop forms (right) of GluR-A and -B. (**B**) Scatter plot of the amplitude of steady-state currents elicited by 300 μM L-Glu as a function of the amplitude of currents evoked by 300 μM kainate. Responses elicited by AMPA (30 μM) closely matched those evoked by L-Glu (300 μM) in both receptor forms. Each point represents a separate experiment in which kainate and L-Glu were applied to the same cell or cluster of cells. (■) Cells expressing GluR-A and -B flip, (□) GluR-A and -B flop. Linear regression (solid lines) through the origin indicates that the relative potency of L-Glu with respect to kainate is about four- to fivefold higher in the flip version. (**C**) Currents evoked by rapid application of 300 μM L-Glu and 300 μM kainate in cells expressing GluR-A flip and -B flop (left) and GluR-A flop and -B flip (right). (**D**) Scatter plot as in (B) from cells expressing (▲) A flop and B flip and (△) A flip and B flop.

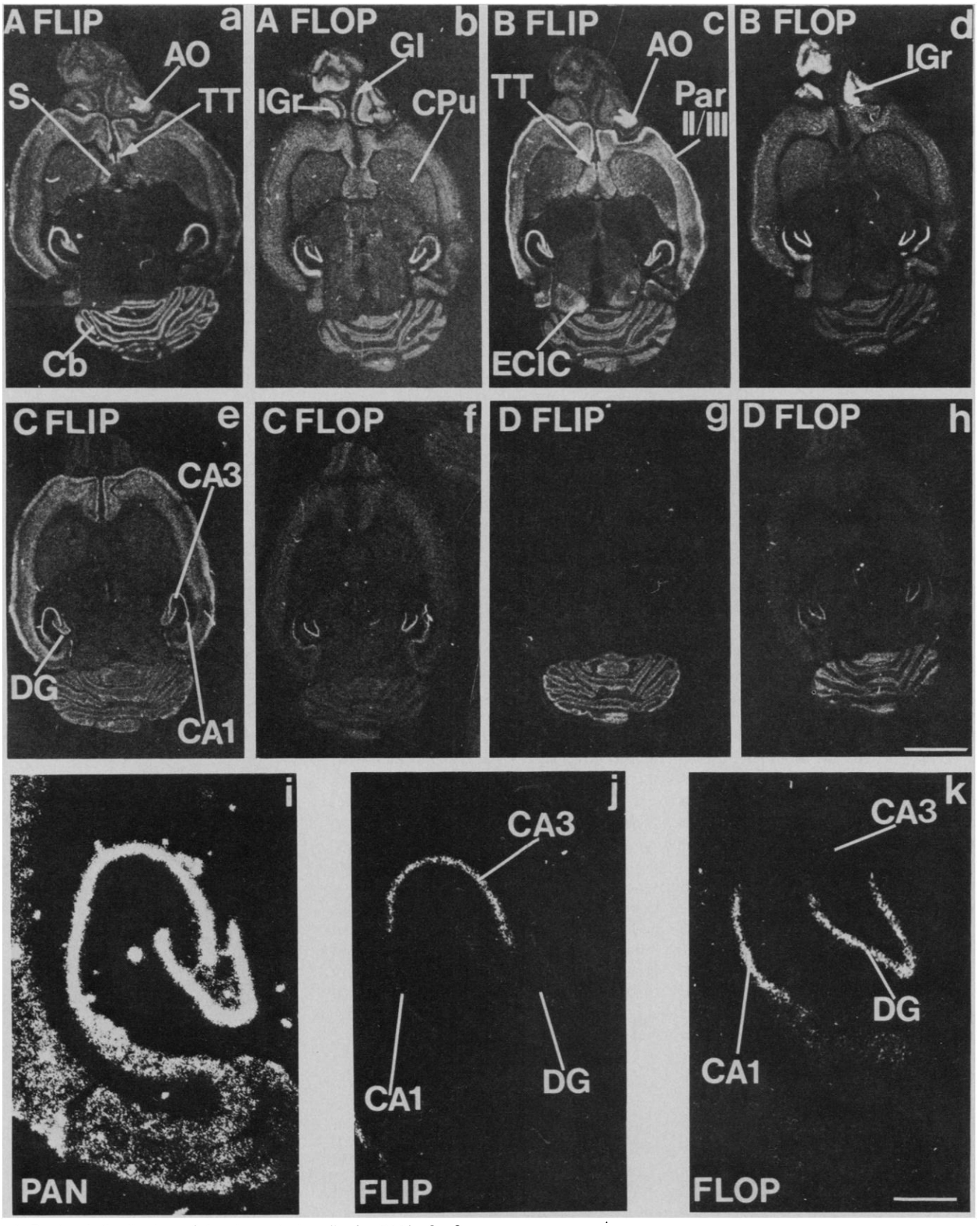

Fig. 4. Regional distribution of the alternatively spliced mRNAs for four glutamate receptors in rat brain. (**a**) GluR-A flip mRNA distribution; (**b**) GluR-A flop; (**c**) GluR-B flip; (**d**) GluR-B flop; (**e**) GluR-C flip; (**f**) GluR-C flop; (**g**) GluR-D flip; (**h**) GluR-D flop; (**i**) GluR-B, both mRNA versions in hippocampus detected with pan B probe (*4*); (**j**) GluR-B flip, hippocampus; (**k**) GluR-B flop, hippocampus; (j and k) higher magnification images of GluR-B flip and GluR-B flop autoradiographs depicted in (c) and (d). The autoradiographs in (c) and (d) were exposed longer to photographic paper in (j) and (k) to better illustrate the expression gradients. AO, anterior olfactory nucleus; Cb, cerebellum; CPu, caudate putamen; DG, dentate gyrus; ECIC, external cortex of the inferior colliculus; GI, glomerular layer of the olfactory bulb; IGr, internal granular layer of the olfactory bulb; Par II/III, parietal cortex (layer II and III); S, septal nuclei; TT, tenia tecta. Scale bars: (a to h), 5 mm; (i to k), 0.8 mm. Oligonucleotides and procedures are as in (*12*).

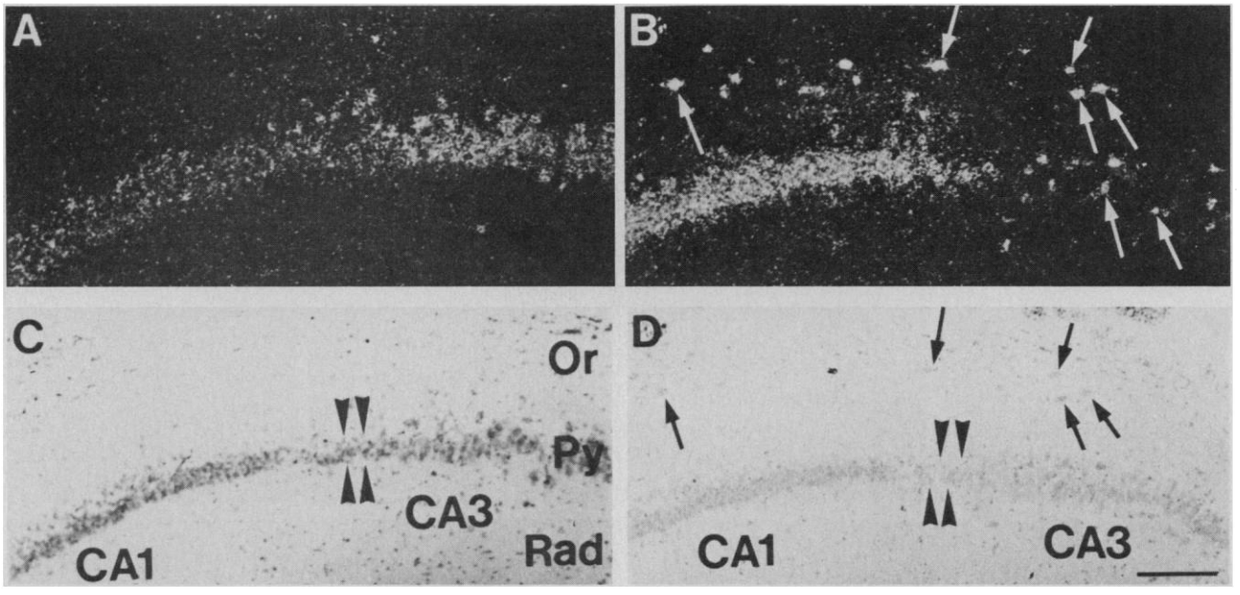

Fig. 5. Emulsion autoradiographs showing differential distribution of the GluR-A flip (**A** and **C**) and flop (**B** and **D**) mRNAs at the CA1-CA3 boundary of rat hippocampus. Shown are dark-field (upper) and bright-field (lower) photomicrographs of GluR-A flip– and GluR-A flop–expressing cells; Or, oriens layer; Py, pyramidal layer; Rad, radiatum layer. Black arrowheads in (C) and (D), the CA1-CA3 border as observed by Nissl stain; arrows in (B) and (D), flop-expressing nonpyramidal (interneuronal) cells. Scale bar, 200 μm. For details see (*12*).

a variety of genes expressed in the brain, including those for the mammalian calcitonin gene–related peptide precursor (*13*), microtubule-associated proteins (*14*), amyloid precursor protein (*15*), and voltage-gated K^+ channels in *Drosophila* (*16*). However, in the few examples of alternative splicing in ligand-gated ion channels (*17*), no function could be assigned to the alternative products. In members of the ligand-gated superfamily of genes, channel diversity seems to be usually achieved by assembling into a functional receptor different subunits encoded by separate genes (*18*).

The functional characteristics of the two alternative exons in the four AMPA receptors and the cell-specific expression of both forms of the receptors suggest an important role for the splicing-directed channel modification. The exon switch may underlie adaptive changes in neurons such as synaptic plasticity. Our data predict that an insertion of the flip module into synaptic AMPA receptors would cause enhanced responses to L-Glu and, hence, produce a synapse operating at an increased gain. Indeed, enhanced current responses at postsynaptic AMPA receptors occur after the induction of LTP in CA1 neurons of the hippocampus (*2, 3*). Postsynaptic receptor modification and full LTP in these cells require a time delay after stimulation (*19*), which may reflect a signal transduction cascade from cell membrane to nucleus. This cascade, possibly initiated at NMDA receptors by Ca^{2+} currents and Ca^{2+}-dependent protein phosphorylation (*3*), could activate splicing factors involved in selecting the appropriate exon.

The conspicuous absence in CA3 pyramidal neurons of receptors containing the flop cassette may reflect the different synaptic inputs to the CA1 (Schaffer collaterals) and CA3 (mossy fibers) areas and suggests disparities in the properties of excitatory channels in the pyramidal cell populations that might be revealed by patch clamp analysis in the hippocampal slice (*20*).

The cell specificity of the observed splicing events implies that it is developmentally regulated. Furthermore, this mechanism for generating functionally modified excitatory synaptic channels could malfunction. For example, a perturbed balance between high and low gain synapses might cause neuronal injury by inappropriately high and sustained excitation (*21*). This injury could contribute to the etiology of many neuropathological conditions, from focal epilepsy to neurodegenerative diseases.

REFERENCES AND NOTES

1. D. T. Monaghan, R. J. Bridges, C. W. Cotman, *Annu. Rev. Pharmacol. Toxicol.* **29**, 365 (1989); G. L. Collingridge and R. A. J. Lester, *Pharmacol. Rev.* **40**, 143 (1989).
2. R. A. Nicoll, J. A. Kauer, R. C. Malenka, *Neuron* **1**, 97 (1988); M. B. Kennedy, *Cell* **59**, 777 (1989).
3. G. L. Collingridge and W. Singer, *Trends Pharmacol. Sci.* **11**, 290 (1990).
4. K. Keinänen *et al.*, *Science* **249**, 556 (1990).
5. J. F. Blake, R. G. Yates, M. W. Brown, G. L. Collingridge, *Br. J. Pharmacol.* **97**, 71 (1989); N. I. Kiskin, O. A. Urishtal, A. Y. Tsyndrenko, *Eur. J. Neurosci.* **2**, 464 (1990).
6. M. Hollman, A. O'Shea-Greenfield, S. W. Rogers, S. Heinemann, *Nature* **342**, 643 (1989); P. Gregor, I. Mano, I. Maoz, M. McKeown, V. Teichberg, *ibid.*, p. 689; K. Wada *et al.*, *ibid.*, p. 684.
7. The electrophysiological properties of recombinantly expressed glutamate receptors were studied by patch clamp techniques [O. P. Hamill, A. Marty, E. Neher, B. Sakmann, F. J. Sigworth, *Pfluegers Arch.* **391**, 85 (1981)] in the whole-cell configuration essentially as described [T. A. Verdoorn, A. Draguhn, S. Ymer, P. H. Seeburg, B. Sakmann, *Neuron* **4**, 919 (1990)]. Agonists were rapidly applied by means of a theta tubing pipette attached to a stepper motor and placed within 50 μm of the cell. Control solution was pumped through one side of the theta tubing (0.2 ml per minute) to continuously perfuse the cell. Solution containing agonist was perfused through the other side and agonist was applied by stepping the application pipette so that the agonist side of the theta tubing was perfusing the cell. Currents were measured in single cells or in electrically coupled clusters of transfected cells. By lifting single cells off the cover slip while in the whole-cell recording mode, the solution surrounding the cell could be completely exchanged in 20 to 40 ms as judged by the rise time (baseline to peak) of the current upon a change to perfusion solution containing 135 mM KCl. Channel conductance and gating kinetics were estimated by fluctuation analysis of whole-cell currents.
8. The full-length cDNAs were subcloned from recombinant Bluescript phagemids (Stratagene, La Jolla, CA) into the eukaryotic expression vector pCDM8 [B. Seed, *Nature* **329**, 840 (1987)] in which a cytomegalovirus promoter controls the transcription of the cloned cDNAs. Transient expression with these constructs was performed in human embryonic kidney cells 293 (ATCC CRL 1573) as described [D. B. Pritchett, H. Lüddens, P. H. Seeburg, *Science* **245**, 1389 (1989)].
9. P. Ascher and L. Nowak, *J. Physiol. (London)* **399**, 227 (1988); S. G. Cull-Candy, J. R. Howe, D. C. Ogden, *ibid.* **400**, 189 (1988); M. Mayer and L. Vyklicky, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1411 (1989); C.-M. Tang, M. Dichter, M. Morad, *Science* **243**, 1474 (1989); L. O. Trussel and G. Fischbach, *Neuron* **3**, 209 (1989); T. Priestly, G. N. Woodruff, J. A. Kemp, *Br. J. Pharmacol.* **97**, 1315 (1989).
10. I. Ito, S. Tanabe, A. Kohda, H. Sugiyama, *J. Physiol. (London)* **424**, 533 (1990).
11. Saturating steady-state currents were produced by 300 μM kainate, 30 μM AMPA, and 300 μM L-Glu, as determined from concentration-response curves constructed in transfected cells.

12. The following oligonucleotides served as probes: 5′-CAAAGCGCTGGTCTTGTCCTTACTTCCGGAGTCCTTGCT-3′ (oligo A-flip, complementary to sequences encoding residues 771 to 783 of the mature GluR-A flip polypeptide); 5′-CAAAGCGCTGGTCTTGTCCTTGGAGTCACCTCCCCC-3′ (oligo A-flop, complementary to residues 772 to 783 of GluR-A flop); 5′-GAGGGCACTGGTCTTTTCCTTGGAATCACCTCCCCC-3′ (oligo B-flop, complementary to residues 776 to 787 of GluR-B flop); 5′-TAGAGCACTGGTCTTGTCCTTACTCCCGGAGTCCTT-3′ (oligo C-flip, complementary to residues 780 to 791 of GluR-C flip); 5′-TAGAGCACTGGTCTTGTCCTTGGAGTCACCGCCCCC-3′ (oligo C-flop, complementary to residues 780 to 791 of GluR-C flop); 5′-CAAGGCACTCGTCTTGTCCTTGCTTCCCGAGTCCTT-3′ (oligo D-flip, complementary to residues 777 to 788 of GluR-D flip); 5′-CAAGGCACTCGTCTTGTCCTTGGAGTCACCTCCCCC-3′ (oligo D-flop, complementary to residues 777 to 788 of GluR-D flop). These oligonucleotides incorporated the maximum number of nucleotide differences between the flip and flop versions on one hand and between the four receptor sequences on the other. Due to the high degree of sequence conservation in and around the region affected by the alternative splice, these oligonucleotides, optimized in length versus sequence substitutions, differed in as few as three nucleotides (oligos D-flop and A-flop). Hence, high stringency conditions were employed, resulting in a lower signal than that achieved with the pan probes. At this stringency, the distinction between flop and flip sequences was clear. Some cross-hybridization of a given flop (or flip) probe to other flop (or flip) mRNAs cannot be excluded, but was often negligible as indicated, for example, by the absence of a signal for GluR-D flip mRNA in all brain regions but cerebellum. The probe D-flip (36 nt) differs from A- and B-flip in five, and from C-flip in six, positions. In situ hybridization was as described [W. Wisden, B. J. Morris, S. P. Hunt, in *Molecular Neurobiology—A Practical Approach*, J. Chad and H. Wheal, Eds. (IRL Press, Oxford, in press), vol. 2]. Oligonucleotides were 3′ end-labeled with terminal deoxynucleotidyl transferase (Bethesda Research Laboratories) and α[^{35}S] dATP (1200 Ci/mmol, New England Nuclear) at a 30:1 molar ratio of labeled nucleotide to oligonucleotide. Sections were hybridized overnight at 42°C with probe (1 pg/μl) in 50% formamide, 4× standard saline citrate (SSC), and 10% dextran sulfate and then washed in 0.1× SSC at 60°C for 20 min before dehydration and apposition to Kodak XAR-5 film for 1 week. After film exposure, sections were dipped in Kodak emulsion and exposed for 4 weeks at 4°C, developed in Kodak D19 developer, and counterstained with thionin before viewing through a Zeiss Axioplan microscope. Structures were identified according to I. G. Paxinos and C. Watson [*The Rat Brain in Stereotaxic Coordinates* (Academic Press, Sydney, ed. 2, 1986)] and S. A. Bayer [in *The Rat Nervous System*, G. Paxinos, Ed. (Academic Press, Sydney, 1985) vol. 1, pp. 335–352].
13. S. G. Amara *et al.*, *Nature* **298**, 240 (1982).
14. C. C. Garner and A. Matus, *J. Cell Biol.* **106**, 779 (1988); M. Goedert, M. G. Spillantini, R. Jakes, D. Rutherford, R. A. Crowther, *Neuron* **3**, 519 (1989).
15. P. Ponte *et al.*, *Nature* **331**, 525 (1988); T. E. Golde, S. Estus, M. Uslak, L. H. Younkin, S. G. Younkin, *Neuron* **4**, 253 (1990).
16. D. M. Papazian, T. L. Schwarz, B. L. Tempel, Y. N. Jan, L. Y. Jan, *Science* **237**, 749 (1987); A. Kamb, L. E. Iverson, M. A. Tanouye, *Cell* **50**, 405 (1987).
17. D. Goldman *et al.*, *Cell* **48**, 965 (1987); D. Beeson, A. Morris, A. Vincent, J. Newsom-Davis, *EMBO J.* **9**, 2101 (1990).
18. M. Noda *et al.*, *Nature* **302**, 528 (1983); M. Mishina *et al.*, *ibid.* **321**, 406 (1986); P. R. Schofield *et al.*, *ibid.* **328**, 221 (1987); E. S. Levitan *et al.*, *ibid.* **335**, 76 (1988); E. S. Deneris, J. Boulter, L. W. Swanson, J. Patrick, S. Heinemann, *J. Biol. Chem.* **264**, 6268 (1989); T. A. Verdoorn, A. Draguhn, S. Ymer, P. H. Seeburg, B. Sakmann, *Neuron* **4**, 919 (1990).
19. S. N. Davies, R. A. J. Lester, K. G. Reymann, G. L. Collingridge, *Nature* **338**, 500 (1989).
20. F. Edwards, A. Konnerth, B. Sakmann, T. Takahashi, *Pfluegers Arch.* **414**, 600 (1989).
21. J. W. Olney and L. G. Sharpe, *Science* **166**, 386 (1969); J. W. Olney, O. L. Hu, V. Rhee, *Exp. Brain Res.* **14**, 61 (1971).
22. Single-letter abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
23. Male BALB/c mouse liver DNA was partially digested with Mbo I, fractionated on a 10 to 40% sucrose gradient, and DNA fragments of 16 to 20 kbp were pooled. Cohesive Mbo I termini were partially filled in with dAMP and dGMP with Klenow enzyme (Boehringer Mannheim). This DNA was ligated to λ vector that had been digested with Xho I and partially filled in with dTMP and dCMP. Packaged ligation products were used to infect *Escherichia coli* strain P2 392, and a library of 4.4×10^6 recombinant phage was obtained. This library was screened with 5′ end-labeled oligonucleotides B-flip and B-flop (*12*), as well as with an oligonucleotide (5′-CACTCTCGATGCCATATACGTTGTAACCTTCCTTATAAGTTGC-3′) complementary to sequences encoding residues 546–859 (ATYKEGYNVYGIES) of the mature GluR-B polypeptide (*4*). Four independent, overlapping clones were isolated, mapped, and sequenced across the specified region.
24. P. Werner constructed the rat brain cDNA libraries. We thank S. Grünewald for expert help with cell culture, G. Muncke for the preparation of rat brain sections and assistance with emulsion autoradiography, and J. Rami for excellent secretarial help. K.K. and W.W. were supported by EMBO long-term fellowships, T.A.V. by a von Humboldt fellowship, A.H. by, in part, a Jeantet award to B.S., and T.T. by a fellowship of the Kanagawa Dental College. Funded in part by a grant of the German Ministry of Research and Technology (BMFT BCT 364) to P.H.S.

6 August 1990; accepted 5 September 1990

Coding Channels in the Taste System of the Rat

THOMAS R. SCOTT AND BARBARA K. GIZA

Basic taste qualities are thought to be perceived independently, yet discrete neural coding channels have not been demonstrated in the central nervous system. The response profiles of taste cells in the nucleus tractus solitarius (NTS) of the rat were categorized into four groups, and the effects of amiloride, a passive sodium channel blocker, on each were determined. NTS neurons that responded specifically to sodium chloride (NaCl) or to NaCl and sugars were suppressed by amiloride; those broadly sensitive to salts, acids, and bitter stimuli were unaffected. Moreover, the response profile evoked by NaCl lost its distinctiveness after treatment with amiloride, becoming similar to those evoked by acids and quinine. Receptors that respond to sodium must relay their information through independent coding channels to identifiable subgroups of NTS neurons, the activity of which is responsible for the perception of saltiness.

GUSTATORY RECEPTORS THROUGHOUT the oral cavity form synapses on peripheral neurons whose axons transmit taste-evoked activity to second-order cells in the NTS of the medulla. It is not known if the taste system is composed of information channels, each with a specific coding responsibility, or if all neurons contribute to every afferent signal. Most psychophysicists contend that the basic taste qualities are perceived independently (*1*). Despite a partitioning of peripheral and central nervous system taste cells based on several criteria, however (*2, 3*), electrophysiologists have not demonstrated a corresponding independence among subgroups of neurons (*4*).

We have adopted a strategy that appears to provide a resolution. We have identified presumed subgroups of cells by their distinct response profiles. Then we have altered the significance of a particular chemical to the rat in order to determine if the resulting accommodation in the function of the taste system is distributed among all cells or is restricted to the subgroup with sensitivities that are most relevant to address the new condition.

Three applications of this approach indicate that the taste system is indeed organized into a discrete number of independent coding channels. First, we created a conditioned taste aversion to sodium saccharin and monitored its neural effects on second-order taste cells in the NTS (*5*). Only cells with "sweet" response profiles were affected by this experience. The change in activity was appropriate to address the new situation: the neural response to formerly appetitive sodium saccharin took on a profile more like that of quinine, providing a neural basis for the behavioral rejection that rats show to sodium saccharin after the conditioning procedure.

Next, we altered the rat's physiological need by Na^+ deprivation, generating an intense Na^+ appetite (*6*). The response to

Department of Psychology, University of Delaware, Newark, DE 19716.