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25. The 3′ untranslated region of the matrin F/G cDNA was removed at the Bam HI site (nucleotide 2055). The remaining cDNA was subcloned into pGEM3Z and linearized with Nco I, and an antisense digoxigenin-labeled cRNA probe was generated with SP6 RNA polymerase. Total RNA was isolated from Sprague-Dawley rat tissues by grinding in liquid nitrogen with a mortar and pestle followed by guanidinium-acid-phenol extraction [P. Chomczynski and N. Sacchi, *Anal. Biochem.* **162**, 156 (1987)]. Polyadenylated RNA was prepared with oligo(dT) magnetic beads (Poly-A Track, Promega). RNA was separated by glyoxal agarose gel electrophoresis and transferred to a Hybond N (Amersham) membrane, which was then incubated with the digoxigenin-labeled probe. After washing the membrane twice with 0.1× standard saline citrate at 65°C, labeled complexes were visualized by chemiluminescence autoradiography (2-hour exposure) (Amersham). The extent of loading of lanes was established by methylene blue staining and by probing for 18*S* RNA or glyceraldehyde-3-phosphate dehydrogenase mRNA.
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A Neuropeptide Gene Defined by the *Drosophila* Memory Mutant *amnesiac*

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Mutations in genes required for associative learning and memory in *Drosophila* exist, but isolation of the genes has been difficult because most are defined by a single, chemically induced allele. Here, a simplified genetic screen was used to identify candidate genes involved in learning and memory. Second site suppressors of the *dunce* (*dnc*) female sterility phenotype were isolated with the use of transposon mutagenesis. One suppressor mutation that was recovered mapped in the *amnesiac* (*amn*) gene. Cloning of the locus revealed that *amn* encodes a previously uncharacterized neuropeptide gene. Thus, with the cloning of *amn*, specific neuropeptides are implicated in the memory process.

Two genes essential for learning and memory in *Drosophila* have been cloned and sequenced. The *dnc* gene encodes a adenosine 3′,5′-monophosphate (cAMP)–specific phosphodiesterase (*1*). A second mutation, *rutabaga* (*rut*), encodes a Ca^{2+}- and calmodulin-sensitive adenylate cyclase (*2*, *3*). Both genes are components of the adenylate cyclase second messenger pathway, and the cloning of these two loci relied on knowledge of the biochemical activities of the gene products. However, full exploitation of the genetic potential to dissect learning and memory mechanisms requires a direct progression from the genetic mutation to the isolation of the gene. Such an approach has been key in other areas of *Drosophila* research, but application of the same techniques to learning mutants is hampered by the labor intensive nature of behavioral testing. For example, mutagenesis by the mobilization of transposable elements or by x-rays greatly facilitates cloning of the disrupted genes. Unfortunately, the lower mutagenesis efficiencies when compared with chemical mutagens, combined with the difficulty of direct behavioral screening, make these traditional approaches problematic.

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Table 1. Fertility of wild-type and mutant flies. Single female flies of the indicated genotypes were placed individually in culture vials, and the percentage of females laying eggs and the average number of progeny produced after 18 days were recorded (*n* refers to the number of female flies assayed). The dnc^{m11} allele was used.

Genotype	*n*	Females laying eggs (%)	Avg. no. of progeny ± SEM
Wild type	50	98	>40
dnc/dnc	84	14	0
dnc P(19A)/dnc P(19A)	70	57	1.7 ± 0.5
dnc P(19A)/dnc	50	74	2.3 ± 0.6
dnc amn/dnc amn	290	66	3.9 ± 1.0
dnc amn/dnc	37	73	1.9 ± 0.7

To circumvent these difficulties a second site suppressor screen was used to isolate *Drosophila* learning and memory mutants. Mutations in *rut* alleles suppress both the female-specific sterility and the behavioral defects of *dnc* (*2*, *4*, *5*). Therefore, it seemed possible that additional learning mutants could be isolated as suppressors of *dnc* female sterility. In such a screen, only the progeny of rare fertile females are candidates for interacting mutants, and the isolation of such flies requires less time and effort than the traditional approach of creating multiple isogenic mutagenized stocks and testing the behavior of each of them individually. The mutagenesis protocol described in (*6*) was used to screen for dominant *P* element–induced X chromosomal mutations that suppressed *dnc* sterility.

One X chromosomal line, *P(19A)*, that suppressed *dnc* sterility contained a *P* element inserted in region 19A on the X chromosome, as determined by in situ hybridization to polytene chromosomes (*7*). The memory mutant *amn*, whose primary effect is on memory in the first few hours after associative training (*8*, *9*), was mapped by genetic recombination to a region proximal to *forked* and near *carnation* (*8*). Deficiency chromosome mapping refined the localization of *amn* to the 19A1-2 region of the X chromosome (*10*). The correspondence of the position of *amn* with the new *P* element mutation suggested that this *P* element may have inserted into the *amn* gene. The autosomes of the insertion strain were replaced with autosomes from the standard wild-type Canton-S stock to minimize any effects of other mutations introduced during the screen. The strain was tested for possible allelism to the original *amn* mutation in three ways: by assaying the ability of the original *amn* mutation to suppress *dnc* sterility, by characterizing the behavior of the strain having a *P* element inserted at 19A, and by carrying out complementation tests with the various phenotypes.

Homozygous *dnc* females laid very few eggs, none of which survived to adulthood (Table 1). Females carrying both the *dnc* mutation and *P(19A)* were weakly fertile. The effect was dominant, because a single copy of *P(19A)* also suppressed the sterility. To test the ability of the original *amn* mutation to suppress *dnc* sterility, we constructed a recombinant chromosome with both the *dnc* and *amn* mutations (*11*). The original *amn* allele also relieved *dnc* sterility (Table 1). Like the *P* element insertion, *amn* acted dominantly, as shown by suppression of *dnc* sterility by one copy of the *amn* chromosome (Table 1).

The 19A suppressor also affected behavior. Flies homozygous for *P(19A)* had memory intermediate between wild-type and *amn* flies (Figs. 1 and 2) (*12*). The *P(19A)* allele failed to complement the memory defect of *amn*. Flies heterozygous for *P(19A)* and *amn* had memory as poor as homozygous *amn* flies. Like *amn*, the memory defect of *P(19A)* was recessive (Fig. 2). The poor memory of *P(19A)/amn* flies was not the result of deficient initial learning, because the flies displayed immediate learning indistinguishable from *amn* or wild-type flies (Fig. 2). Memory deficits were also unlikely to result from sensory abnormalities given intact sensitivities to odors and electric shock in *amn* (*8*), and the good immediate learning of the *P* element mutant. Thus, the *P(19A)* flies appeared to carry a hypomorphic (partial function) *amn* mutation.

P elements often create hypomorphic mutations by inserting into regulatory regions and altering the levels of gene expression. Remobilization of such an element and selection for excision events that also remove surrounding DNA can produce severe alleles. To this end, transposase was reintroduced into the germ line of *P(19A)* flies. Isogenic X chromosome lines were created from approximately 200 progeny from individual dysgenic flies (*13*). About 10% of the lines displayed alterations of the 19A region as indicated by Southern (DNA) blot analysis, and an allele that disrupted surrounding DNA, $P(19A)^{EX}$, was selected for behavioral and molecular analysis. The $P(19A)^{EX}$ flies learned as well as the *amn* flies, but their memory was severely curtailed (Figs. 1 and 2). The defect failed to complement *amn* and

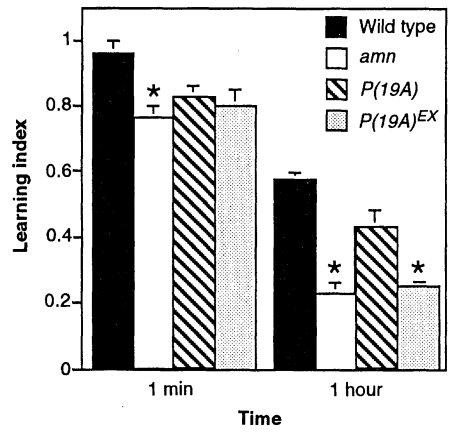


Fig. 1. Learning and 1-hour memory in homozygous normal and mutant flies. Flies of the indicated genotype were trained with the use of the associative, negatively reinforced classical conditioning paradigm of Tully and Quinn (*12*) and tested immediately or after 1 hour. Before training, autosomes from dysgenesis-derived mutant strains were replaced with autosomes from the Canton-S wild-type strain. An asterisk (*) indicates learning or memory values significantly different from the wild type [analysis of variance (ANOVA), $P < 0.01$, one-way analysis of variance with supplementary Newman-Keuls test]. Error bars indicate standard errors of the mean (SEMs) for seven to nine determinations per point.

P(19A), the original insertion strain, but was fully recessive (Fig. 2). These results suggest that the excision chromosome carried a severe *amn* allele.

Genomic DNA from *P(19A)* flies was cloned, and DNA from the region surrounding the *P* element insertion was analyzed to determine the molecular basis for the behavioral phenotypes and to identify the *amn* transcription unit (Fig. 3A). In the *P(19A)* strain there were two closely spaced *P* elements in the 19A region (*14*). When transposase was reintroduced into the germ line of *P(19A)* flies, all of the resulting lesions were complex local rearrangements (*13*). None of the lines precisely removed one or both of the elements, and none contained large flanking deletions. Molecular cloning and sequencing of the 19A region from the $P(19A)^{EX}$ strain indicated that two genetic rearrangement events occurred. First, the DNA between the two elements was inverted, and second, a deletion removed approximately 800 base pairs (bp) of genomic DNA and most of the right-most *P* element (Fig. 3A).

Drosophila complementary DNA (cDNA) libraries were screened with genomic DNA spanning 10 kb on either side of the *P* elements to identify the transcription unit or units that were disrupted by the *P* element lesions. After extensive screening of cDNA libraries representing multiple tissues and developmental stages, two overlapping 3.6-kb cDNAs were recovered from size-selected adult head cDNA libraries (*15*). Sequence analysis revealed a single region of good *Dro-*

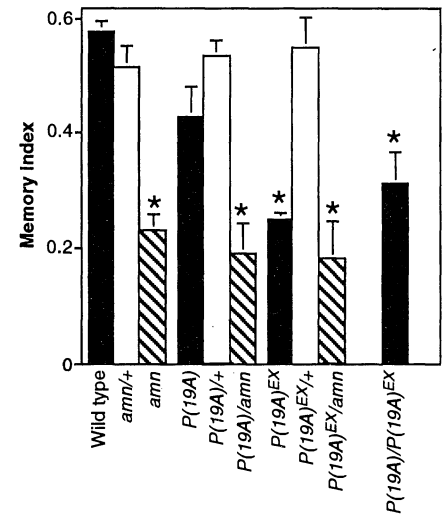


Fig. 2. One-hour memory in normal and mutant flies. Unless specifically indicated in the genotype, flies were homozygous. Flies were trained as described (*12*) and tested 1 hour after training. An asterisk (*) indicates memory values significantly different from the wild type (ANOVA, $P < 0.01$, one-way analysis of variance with supplementary Newman-Keuls test). Error bars indicate SEMs for seven to nine determinations per point.

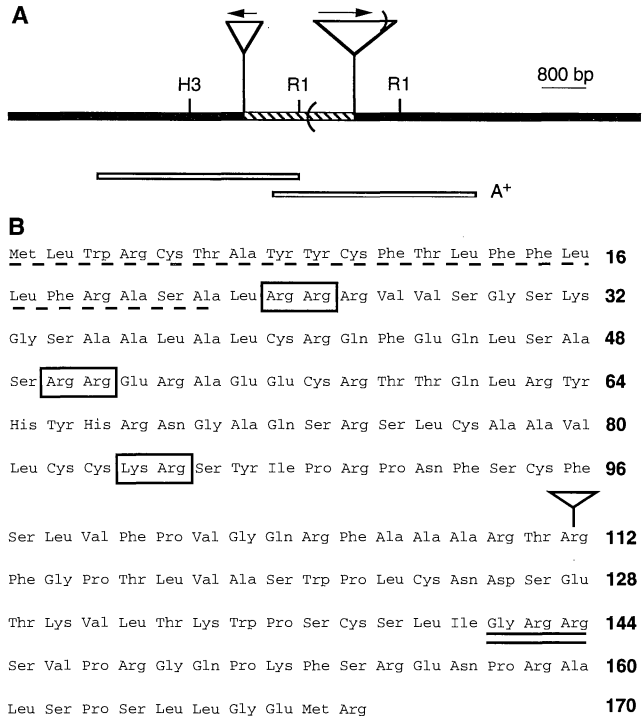
sophila codon usage and an open reading frame that began with a CUG rather than an AUG initiation codon. Non-AUG start codons are unusual, but at least two other nervous system–specific examples are known in *Drosophila* (*16*), and several instances have been identified in mammalian systems as well (*17*). These results suggest that CUG is used to initiate synthesis of the *amn* gene product, but they do not rule out the possibility of alternatively spliced transcripts not recovered in cDNA screens. The cDNAs for such variants may be difficult to identify because they were not detected even with extensive screening (*15*).

A perfect polyadenylation signal occurred exactly 10 bp upstream of the polyadenylate [poly(A)] tail. The open reading frame had an organization characteristic of neuropeptide precursor genes (Fig. 3B). An initial stretch of hydrophobic residues could act as a signal peptide. A potential signal cleavage site that conforms to the consensus (*18*) occurred after Ala^{22}. There were several pairs of basic residues that could be cleavage sites. If Arg-Arg and Lys-Arg pairs serve as cleavage sites, then three potential peptides of 24, 32, and 56 amino acids would be predicted. The last putative Arg-Arg cleavage site was preceded by a glycine that forms the consensus signal for peptide COOH-terminal amidation. Many peptides are amidated, and this modification is often required for biological activity (*19*).

Sequence comparison of the cDNAs and genomic DNA from the *P* element mutant *P(19A)* showed that the smaller (600-bp) *P* element inserted directly into the third putative neuropeptide in the open reading frame (Fig. 3A). The second, larger *P* element inserted into the downstream 3′ untranslated region. Genetic analysis indicated that *P(19A)* is a hypomorphic allele that has residual gene activity (Figs. 1 and 2). Several models can explain the hypomorphic nature of *P(19A)*; these include the possibility that alternative mRNA splicing occurs around the *P* element or elements, or that a fusion protein is produced that terminates in the first *P* element.

The original *amn* mutation was created by ethylmethane sulfonate mutagenesis of a control strain isogenic for the X chromosome and is a genetic null in memory assays (*9*). To characterize this mutation molecularly, we amplified the open reading frame from the isogenic control wild-type strain (Canton-S–derived) and from *amn* genomic DNA with the polymerase chain reaction (PCR) (*20*). Four clones were sequenced from each genotype; these included two independent clones from two separate PCR reactions for each. The genomic DNA sequence from the *amn* mutant contains a single base deletion in the middle of the predicted signal peptide. This change alters the reading frame and creates a stop codon at amino acid 28 of the *amn* predicted protein. No potential peptides can be synthesized from the *amn* chromosome. These results are consistent with the *amn* null phenotype and support the behavioral genetic evidence that identifies the putative peptide-encoding transcription unit as the *amn* gene.

Two of the potential peptides had homology to the genes that encode mammalian adenylate cyclase activating peptide (PACAP) (*21*) and growth hormone releasing hormone (GHRH). The first predicted *amn* peptide was homologous to mature GHRH and to a region of the PACAP precursor protein that shares homology with GHRH (Fig. 4). The 38–amino acid mature PACAP neuropeptide was isolated from hypothalamic tissue on the basis of its ability to stimulate adenylate cyclase from anterior pituitary. This peptide is homologous to the second predicted *amn* peptide. The biochemical activities of the *amn* pep-



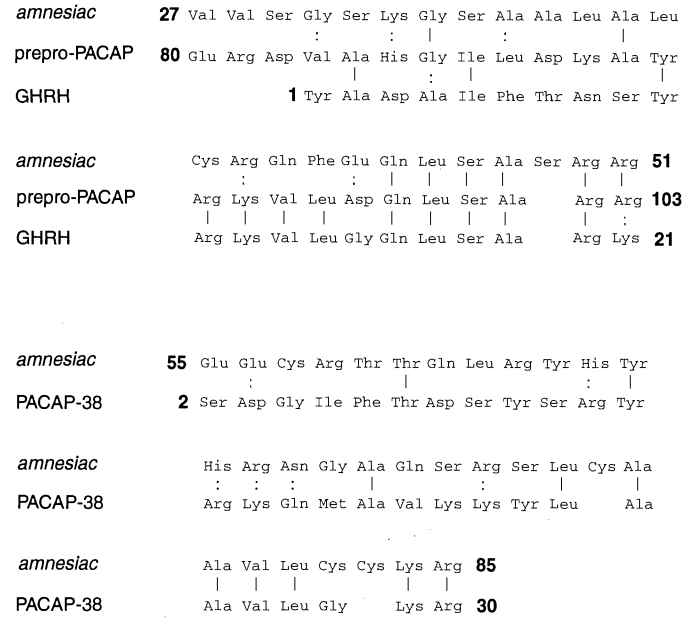


Fig. 3 (**left**). Molecular cloning, cDNA isolation, and sequence analysis of the *amn* gene. (**A**) Two *P* elements inserted 1.8 kb apart in the 19A region of the X chromosome (*14*). Genomic DNA is indicated by the black and striped boxes. Apparently, two gene rearranging events occurred in this strain. (i) The DNA between the two elements was inverted. The striped box shows inverted material. (ii) A deletion removed approximately 800 bp of genomic DNA and most of the right-most *P* element. Parentheses include the extent of the deletion. *P* element insertions are shown as triangles, and the arrows indicate the direction of *P* element transcription. Open boxes denote the approximate position of the two 3.6-kb cDNAs isolated, one of which has a poly(A) tail. (**B**) Protein sequence of the amn^+ open reading frame. The predicted signal sequence is dashed underlined, putative dibasic cleavage sites are boxed, and a COOH-terminal amidation signal is double underlined. The triangle indicates the *P* element insertion. **Fig. 4** (**right**). Homology between the predicted *amn* peptides and the PACAP precursor protein (prepro-PACAP), the mature PACAP (PACAP-38), and GHRH. Vertical lines indicate identical residues; colons identify similar amino acids.

tides are unknown, but both the genetic and molecular evidence suggest that the peptides act through adenylate cyclase to increase the concentration of cAMP. The second site suppressor screen that produced the *P* element *amn* allele selected for mutations that suppressed *dnc* female sterility, which is caused by elevated cAMP concentrations in phosphodiesterase-deficient female flies (*1*, *4*). Suppressor mutations should affect genes that, like the *rut* adenylate cyclase, normally act to increase cAMP concentrations (*2*, *3*).

Identification of the *Drosophila* memory mutant *amnesiac* as a neuropeptide-encoding gene implicates the putative peptide or peptides in neuronal plasticity. Many mammalian peptides have intriguing patterns of expression in the central nervous system, well-characterized receptors, and documented effects on standard second messenger systems. Nevertheless, most of these peptides have no defined physiological or behavioral roles. Mutations, with functional information derived from their phenotypes, address these issues directly. Our present finding that a specific behavioral defect results from a mutation in a probable neuropeptide gene is a first step toward dissecting the functional role of neuropeptides in the brain. The inference that a specific peptide may be involved in extending memory beyond initial response times of second messenger systems activated by classical neurotransmitters is potentially of general importance. Whether mammalian homologs of the *amn* gene products play a similar role in their hosts is an open, inviting question (*22*).

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11. Flies of the genotype *y dnc^{m11} cv v f* were crossed to *amn* flies, which are wild type for these markers. Seven independently derived recombinant males of the genotype *y dnc^{m11} cv v* were crossed individually to balancer *FM7a* females to create individual stocks. These stocks were used to test suppression of *dnc* female sterility. We confirmed the presence of the *dnc^{m11}* allele in the recombinant chromosome by following flanking genetic markers and by subnormal female fertility (Table 1). We inferred the presence of the *amn* mutation by following visible markers and by independent analysis of multiple recombinant lines, which all demonstrated the same degree of suppression of *dnc* female sterility. Similar crosses were carried out with *P(19A)* and the Canton-S background chromosome for behavioral analysis. Retention of the *P* element insertion was monitored by Southern blot analysis.
12. For behavioral testing all fly stocks were kept at 25°C, 45% relative humidity, and on a 16:8 hour light-dark cycle and were maintained on standard cornmeal medium. One hour before testing, the flies were transferred to clean plastic bottles. Olfactory discrimination learning and subsequent memory of the flies was measured in a classical conditioning test [T. Tully and W. G. Quinn, *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **157**, 263 (1985)]. The odors, shocks, and training schedules used were exactly as described there; the apparatus had minor variations. We trained the flies by exposing them separately to two odors: one odor was presented with a negative electric shock reinforcement, and the other odor was an unreinforced control. The flies were then tested with a choice between converging air currents containing the two odorants. The learning index was the fraction of the population that avoided the negatively reinforced odor minus the fraction avoiding the control odor, averaged for two training-testing runs in which flies were conditioned to avoid appropriate odors of a pair [W. G. Quinn, W. A. Harris, S. Benzer, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 708 (1974)]. For the learning test, the flies were given the odorant choice immediately, whereas memory testing was performed 1 hour after training.
13. Females heterozygous for the 19A *P* element insertion, strain *P(19A)*, and the X chromosome balancer *FM7a* [*P(19A)/FM7a*] were crossed to males carrying *Sb Δ2-3(99B)/TM6* third chromosomes. Males of the genotype *P(19A)/Y; Sb Δ2-3(99B)/+* were mated individually to homozygous *FM7a* females. A single *P(19A), Sb$^+$* male or a *P(19A)/FM7a, Sb$^+$* female progeny was selected from each fertile male carrying the *P* element insertion chromosome and the *Sb Δ2-3(99B)* chromosome. Only progeny without the Δ2-3 element were selected to prevent further dysgenic events. These flies were crossed to the appropriate *FM7a* balancer flies and bred into lines isogenic for the X chromosome. Lines were screened by Southern blot analysis, first with the use of *P* element probes, and later with probes from surrounding genomic DNA when they became available. Autosomes from lines selected for further analysis were replaced with autosomes from the inbred Canton-S strain used for behavioral studies to minimize any possible effects of genetic background.
14. Genomic DNA was cloned in the vector EMBL3 after partial digestion with the restriction enzyme Sau 3A and fractionation on sucrose gradients with standard methods [J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989)]. The structure of the original insertion strain and of *P(19A)^{EX}* was determined by Southern blot analysis and by DNA sequencing. Genomic and cDNA subclones were prepared in the vector pBluescript. The two cDNAs recovered were sequenced completely on both strands by creating overlapping deletions with exonuclease III and S1 nucleases [S. Heinkoff, *Methods Enzymol.* **154**, 156 (1987)] and dideoxynucleotide chain termination [F. Sanger, S. Nicklen, A. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)] with Sequenase (U.S. Biochemical). Sequences were compared with sequence data bases with BLAST [S. F. Altschul, W. Gish, W. Miller, W. W. Myers, D. J. Lipman, *J. Mol. Biol.* **215**, 403 (1990)]. The cDNA sequence has been deposited under GenBank accession number U22825.
15. A number of plaques representing the entire complexity (usually ~1 × 10^6) of six adult *Drosophila* head cDNA libraries, an embryonic library, a larval library, a library of whole adult flies, and an ovary-specific library were screened with probes that spanned 10 kb on either side of the *P* element insertions, with the exception of a small, highly repetitive, transcribed region just 5′ to the cDNAs. Given the low frequency of cDNA clones recovered, the embryonic cDNA library and five of the six adult *Drosophila* head cDNA libraries were rescreened extensively to maximize recovery of rare cDNAs. Two cDNAs were ultimately derived from the screenings, and both came from highly size-selected (>2.0 kb) adult head libraries. Of the two cDNAs recovered, the more 5′ cDNA came from the library donated by T. Schwarz [D. M. Papazian, T. L. Schwarz, B. L. Tempel, Y. N. Jan, L. Y. Jan, *Science* **237**, 749 (1987)], and the more 3′ cDNA from the library provided by B. Hamilton [B. A. Hamilton, M. J. Palazzalo, E. M. Meyerowitz, *Nucleic Acids Res.* **19**, 1951 (1991)].
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20. Extensive analysis of the *rosy* gene [C. S. Lee *et al.*, *Genetics* **116**, 55 (1987)] indicates that almost all loss-of-function mutations recovered after chemical mutagenesis occur in the open reading frame; the rest occur at mRNA splice junctions. Sense-altering mutations occurring within the open reading frame are quite rare in a defined *Drosophila* strain, or even between strains. PCR primers were designed to amplify a 630-bp segment of genomic DNA including the entire open reading frame. These primers were used to amplify genomic DNA from *amn* and the background (Canton-S) strain it was derived from. Two separate PCR reactions from two separate DNA preparations were performed on each strain. The PCR products were gel-purified and subcloned into pBluescript. Two independently derived subclones from each PCR reaction were sequenced in their entirety, for a total of four clones sequenced for each genotype. The single base pair deletion was observed in all of the *amn* clones and none of the clones derived from the background strain. The open reading frame was eventually sequenced from four strains, three derived from Canton-S and one derived from Oregon-R. The only change observed

was the single base pair deletion found in *amn*, suggesting that random sequence polymorphisms are rare or absent in this reading frame.
21. A. Miyata *et al.*, *Biochem. Biophys. Res. Commun.* **164**, 567 (1989); K. Ogi, C. Kimura, H. Onda, A. Arimura, M. Fujino, *ibid.* **173**, 1271 (1990); C. Kimura *et al.*, *ibid.* **166**, 81 (1990).
22. *Drosophila* appears to possess several neuropeptides homologous to mammalian peptides [R. Nichols, S. A. Schneuwly, J. E. Dixon, *J. Biol. Chem.* **263**, 12167 (1988); T. Lundquist and D. R. Nässel, *J. Comp. Neuro.* **294**, 161 (1990); D. R. Nässel, T. Lundquist, A. Höög, L. Grimelius, *Brain Res.* **507**, 225 (1990)], and an *amn* homolog may occur in mammals (M. B. Feany, unpublished observations). This homolog may not be identical to PACAP given the restricted homologies of the two proteins. *Drosophila* contains a highly related PACAP homolog that is distinct from *amn* [Y. Zhong and L. A. Peña, *Neuron*, **14**, 527 (1995)].

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Control of Proton Sensitivity of the NMDA Receptor by RNA Splicing and Polyamines

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The function of the *N*-methyl-D-aspartate (NMDA)–preferring glutamate receptor can be regulated by extracellular pH, a process that may be important during ischemia in the brain or during seizures. Protons inhibit NMDA receptor function by 50 percent at pH 7.3 through interactions with the NR1 subunit, and both polyamines and NR1 exon 5 potentiate receptor function through relief of the tonic proton inhibition present at physiological pH. A single amino acid (lysine 211) was identified that mediates the effects of exon 5 in the rat brain. Electroneutral substitutions at this position restored pH sensitivity and, consequently, polyamine relief of tonic inhibition. This effect, together with the structural similarities between polyamines and the surface loop encoded by exon 5, suggest that exon 5 may act as a tethered pH-sensitive constitutive modulator of NMDA receptor function.

NMDA receptors serve many functions in the developing and adult central nervous system (*1*). However, activation of these receptors also can contribute to the pathophysiology of epilepsy (*2*) and stroke (*3*). One way the brain protects itself from the potentially harmful actions of NMDA receptors is to tightly regulate their function. Indeed, NMDA receptors are controlled by many endogenous substances as well as second messenger systems (*1*). Of the effects of endogenous ions on NMDA receptor function, inhibition by extracellular protons (*4*, *5*) is particularly interesting for three reasons. First, ion-selective electrodes have provided information that describes changes in extracellular pH during normal brain function (*6*). For example, the acidic and alkaline transients associated with synaptic transmission are sufficient to alter synaptic NMDA receptor activation (*6*). Second, the acidification of the interstitial spaces that occurs during both seizures and ischemia (*7*) will inhibit NMDA receptors. Because NMDA receptor activation is critical to both seizure development and stroke-induced neuronal damage, receptor inhibition by falling pH should serve as negative feedback (*5*, *8*). Third, the sensitivity of NMDA receptors to physiological concentrations of protons suggests that NMDA receptors are tonically inhibited at physiological pH (*5*).

We studied the proton sensitivity of recombinant rat NMDA receptors expressed in *Xenopus laevis* oocytes (*9*) and in HEK 293 cells (*10*). Similar to native NMDA receptors (*5*), rat homomeric NR1 (*11*) function is strongly inhibited by physiological concentrations of protons (Table 1). This observation suggests that the NR1 subunit contains molecular entities that regulate receptor function in a pH-sensitive manner.

At least three exons (Fig. 1A) can be

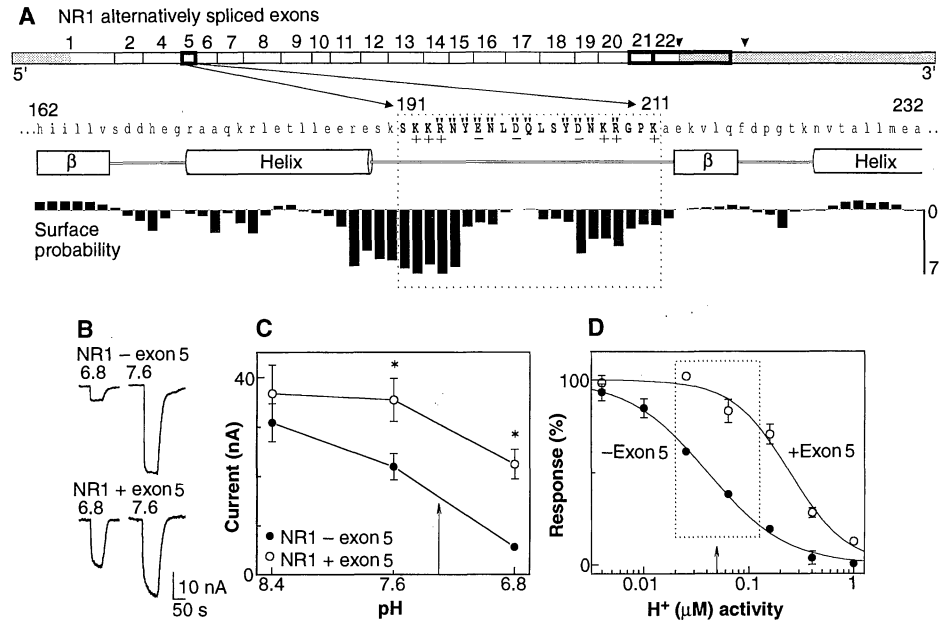

Fig. 1. Control of NMDA receptor proton inhibition by NR1 exon 5. (**A**) NR1 is constructed from 22 exons, four of which can be alternatively spliced (bold) to form nine isoforms (*11*, *21*); stop codons are shown as arrowheads. The third exon, which contains a stop codon, is not shown. Many of the amino acids encoded by exon 5 (uppercase letters) possess side chains that contain π molecular orbital systems (••). Helix and β sheet regions shown were predicted (*12*) according to Rost and Sander; accuracy was <72% if this region of NR1 behaved as a soluble protein. Other algorithms predict helices at Ala^{174} to Ser^{189}, Val^{225} to Arg^{238} (Chou and Fasman), His^{171} to Lys^{193}, and Val^{225} to Leu^{242} (Garnier *et al.*), with an estimated accuracy of about 50 to 55%. β sheets were predicted at Lys^{214} to Phe^{218} (Chou and Fasman) and Glu^{213} to Phe^{218} (Garnier *et al.*). Nearly identical results were obtained for NR1 – exon 5. The surface probability index was calculated from the amino acid surface probabilities (*12*). Downward denotes the increasing likelihood that residues are accessible to water. (**B**) Current responses at −60 mV in Ba^{2+} to glutamate + glycine (pH 6.8 and pH 7.6) are shown from oocytes injected with NR1 – exon 5 and NR1 + exon 5. Scale bars are 10 nA and 50 s. (**C**) Mean agonist-induced responses were determined for NR1 ± exon 5. Identical quantities of splice variant cRNA (from four preparations) were injected into oocytes pairwise (n = 19 cells per point; the asterisk denotes $P < 0.05$). For all figures, vertical arrows mark physiological pH (7.3) and error bars are SEM (whenever larger than the symbol). (**D**) The composite H^+ inhibition curves for NR1 ± exon 5 are shown as a percent of the fitted maximum (see Table 1 for IC_{50} values). The physiological range is superimposed as a box. Data are from 77 oocytes.