Table 1. Detection of antibodies to OV-16 in a longitudinal study of children (ages 1 to 14) from a savanna region in Mali highly endemic for onchocerciasis. ND, not done.

Child	Anti–OV-16 Ab* in year				Skin mf present in year			
	1	2	3	4	1	2	3	4
1	−	+	+	+	−	−	+	+
2	−	+	+	+	−	−	+	+
3	+	+	+	+	−	−	+	+
4	−	+	+	ND	−	+	+	ND
5	−	+	+	ND	−	+	+	ND
6	−	−	+	+	−	−	+	+
7	−	+	+	+	−	+	+	+
8	−	−	−	−	−	−	−	−

*A positive value (+) is defined as >3 SD above the geometric mean of 13 normal individuals run simultaneously.

insensitivity in detecting prepatent and low-level infections. Over most of the large area of the Onchocerciasis Control Program in West Africa, *O. volvulus* transmission has been interrupted by vector control (*1, 2, 19*). However, reinvasion of infective black flies occurs in some border areas and is responsible both for recurrent infections (*1, 20*) and for infections of previously unexposed children (born after the establishment of effective vector control). The use of OV-16, or of similar antigens, should allow the early and specific diagnosis of new infections or reinfections in such vector reinvasion areas, as well as the detection of light infections in areas where control is being attempted by widespread use of ivermectin (*21*). Such a capability will be of paramount importance in monitoring, evaluating, and consolidating onchocerciasis control by both vector control and chemotherapeutic strategies.

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14. Levels of anti–OV-16 were determined by ELISA. Briefly, Immulon 4 plates (Dynatech Laboratories) were coated with 300 ng of OV-16 per milliliter of coating buffer, pH 7.6, and incubated overnight at 4°C. The plates were blocked with 5% bovine serum albumin for 1 hour at 37°C. All sera were run in duplicate at a 1:100 dilution and incubated overnight. For assays of total IgG, Fc-specific, alkaline phosphatase–conjugated goat antibody to human IgG (Sigma) was added. Thirteen uninfected samples were used to determine the normal range (mean ± 3 SD). A high-titer standard reference onchocerciasis serum pool was used to generate calibration curves against which all sera were compared for antibody levels (Flow Cytometric Programme 1.5, Munich, Germany). Levels are expressed as arbitrary units per milliliter. There was no recognition of OV-16 by antibodies of the IgE isotype.
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A Multisubunit Ribozyme That Is a Catalyst of and Template for Complementary Strand RNA Synthesis

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Derivatives of the *sunY* self-splicing intron efficiently catalyzed the synthesis of complementary strand RNA by template-directed assembly of oligonucleotides. These ribozymes were separated into three short RNA fragments that formed active catalytic complexes. One of the multisubunit *sunY* derivatives catalyzed the synthesis of a strand of RNA complementary to one of its own subunits. These results suggest that prebiotically synthesized oligonucleotides might have been able to assemble into a complex capable of self-replication.

RNA MOLECULES CAPABLE OF SELF-replication are postulated to have been important in the early evolution of life (*1–4*). However, an RNA replicase probably requires a folded structure to carry out efficient catalysis, while an optimal template is unstructured. These conflicting requirements may be accommodated by a multisubunit RNA replicase, with the dissociated components serving as templates and the assembled complex as an RNA polymerase. The group I self-splicing introns catalyze phosphodiester exchange reactions similar to those catalyzed by DNA and RNA polymerases (*1*). On the basis of this natural catalytic capacity, these introns might be modifiable to function as RNA polymerases. Indeed, the intron from *Tetrahymena* catalyzes complementary strand RNA (cRNA) synthesis on external templates (*5*). However, this reaction is quite inefficient. Less than 1% of a 40-nucleotide (nt) template is copied to a full-length complementary strand. Self-replication of a ribozyme as large as the *Tetrahymena* intron (413 nt) is clearly impossible with such an inefficient reaction. Smaller ribozymes that are more efficient at cRNA synthesis are required if self-replication is to be demonstrated. We have addressed this question by studying the properties of modified forms of the self-splicing intron *sunY* from bacteriophage T4.

The conserved core of the *sunY* intron

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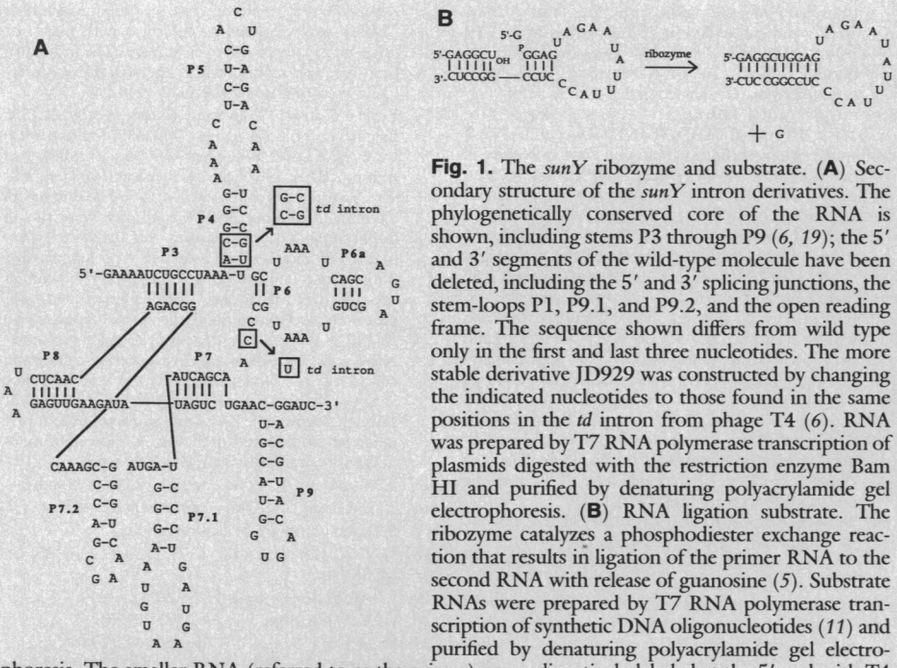
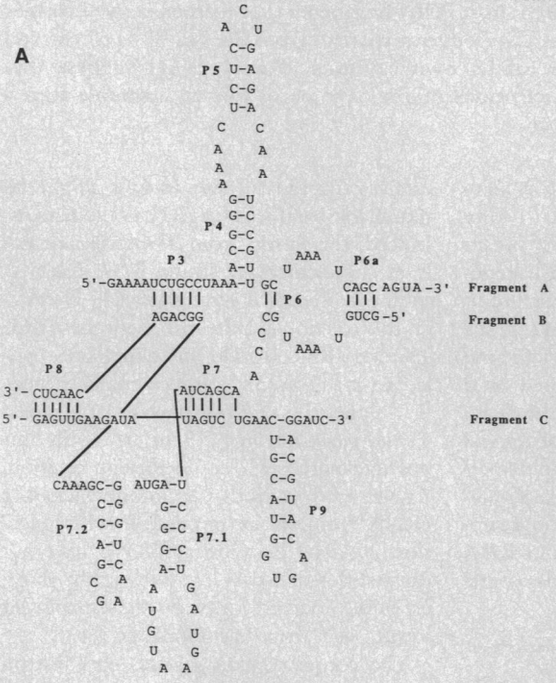


Fig. 1. The *sunY* ribozyme and substrate. (**A**) Secondary structure of the *sunY* intron derivatives. The phylogenetically conserved core of the RNA is shown, including stems P3 through P9 (*6, 19*); the 5′ and 3′ segments of the wild-type molecule have been deleted, including the 5′ and 3′ splicing junctions, the stem-loops P1, P9.1, and P9.2, and the open reading frame. The sequence shown differs from wild type only in the first and last three nucleotides. The more stable derivative JD929 was constructed by changing the indicated nucleotides to those found in the same positions in the *td* intron from phage T4 (*6*). RNA was prepared by T7 RNA polymerase transcription of plasmids digested with the restriction enzyme Bam HI and purified by denaturing polyacrylamide gel electrophoresis. (**B**) RNA ligation substrate. The ribozyme catalyzes a phosphodiester exchange reaction that results in ligation of the primer RNA to the second RNA with release of guanosine (*5*). Substrate RNAs were prepared by T7 RNA polymerase transcription of synthetic DNA oligonucleotides (*11*) and purified by denaturing polyacrylamide gel electrophoresis. The smaller RNA (referred to as the primer) was radioactively labeled at the 5′ end with T4 polynucleotide kinase and γ-[^{32}P]ATP (adenosinc triphosphate).

(~200 nt) is about half the size of that of the *Tetrahymena* intron (*6*). This small size suggested that it might be easier to develop an RNA replicase by modifying the *sunY* intron, because fewer catalytic cycles would be required to generate a full-length complementary strand with the ribozyme itself as the template. We have further reduced the size of the *sunY* catalytic domain, and deletion derivatives as small as 164 nt retain some self-splicing catalytic activity (7). These small derivatives appear to be unstable, as activity is only detected in the presence of high concentrations of monovalent cation and magnesium. We therefore took advantage of the demonstration that changing certain bases in the *sunY* intron to the bases found in the corresponding locations in the larger *td* intron (another self-splicing intron from bacteriophage T4) results in increased self-splicing activity (*8*). These changes were incorporated into a *sunY* intron derivative that lacks the P1 stem (its natural 5′ cleavage site) (Fig. 1), and the derivative was assayed for activity in a template-directed oligonucleotide ligation reaction with a separate P1-like substrate (Fig. 1B). The *td* nucleotide changes resulted in a three-to fourfold enhancement of the rate of ligation. We used this *sunY-td* chimera (JD929, 180 nt) in the experiments described below.

We considered the possibility that these ribozymes could be divided into several short RNA chains, which might be better templates, while retaining the ability to assemble into an active ribozyme. Because the sizes and sequences of the L6 and L8 loops in the secondary structure of the *sunY* intron are not conserved phylogenetically, and because interruption at L6 in the *td* intron does not inactivate splicing (*9*), we decided to interrupt the RNA chain at those sites (Figs. 1 and 2A). Although the RNA can be interrupted in other nonconserved loops (*10*), the choice of L6 and L8 yields RNA fragments with minimal secondary structure (to promote template function) and substantial complementarity with the other fragments (to facilitate assembly of the complex). The resulting subunits are referred to as fragment A (5′ end through L6), fragment B (3′ side of P6a to L8), and fragment C (3′ side of P8 through P9). Fragments A, B, and C are 59, 75, and 43 nucleotides in length, respectively. These fragments were synthesized by in vitro transcription of synthetic DNA templates (*11*).

The single-chain ribozyme and the corresponding three-subunit ribozyme were assayed for template-directed oligonucleotide ligation activity (Fig. 2B). The multisubunit enzyme was approximately twofold less active at 42°C than the corresponding single-chain enzyme and had a lower temperature optimum (37° versus 45°C). One possible



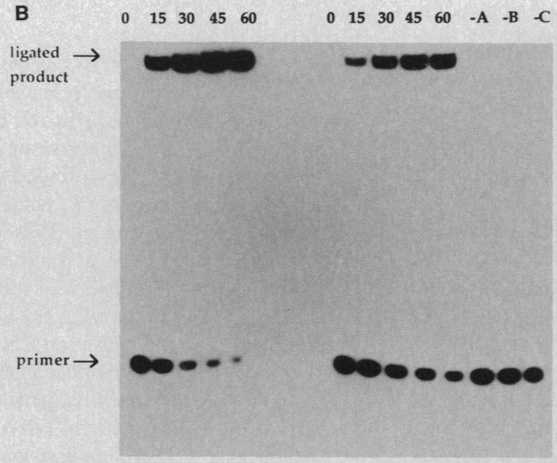


Fig. 2. Structure and activity of multisubunit ribozymes. (**A**) Interruption of the *sunY* intron derivative (JD929) in loops L6 and L8 resulted in the three fragments shown. These RNA molecules were prepared by T7 RNA polymerase transcription of DNA oligonucleotides (*11*) and purified by denaturing polyacrylamide gel electrophoresis. (**B**) Autoradiogram of a 20% acrylamide–7 M urea gel that shows time courses of ligation of the RNA substrate (Fig. 1B). On the left, a time course is shown with the intact *sunY* ribozyme. On the right, the comparable three-subunit enzyme was used (Fig. 2A). Reaction conditions and analysis were as described (*20*). The lanes on the far right show 60-min reactions in which the indicated fragment of the enzyme was omitted.

explanation for these differences is that the multisubunit enzyme is only partially assembled into active complexes.

Each of the RNAs that composed the multisubunit enzyme is in the size range of RNAs that were used as templates for previously described multiple-oligonucleotide ligations carried out by the *Tetrahymena* ribozyme (*5*). Thus, the ability of the multisubunit ribozyme to catalyze similar oligomer ligations on external template molecules was explored.

As an initial test, a 36-nt RNA was used as a template for complementary strand RNA synthesis. Four short RNA oligomers (one 9-nt and three 10-nt fragments) that could anneal to this template were synthesized with solid-phase RNA phosphoramidite chemistry (*12*) (Fig. 3A). In order to generate a full-length cRNA, the oligomers must be ligated together in three separate phosphodiester exchange reactions in which the 5′ guanosine residue at the end of each 10-nt fragment is released (*5*). We measured over time the ligation of these oligomers catalyzed by three different ribozymes: (i) the *Tetrahymena* intron; (ii) the *sunY-td* single-chain ribozyme; and (iii) the *sunY-td* multisubunit ribozyme (Fig. 3, B, C, and D). The yield of full-length cRNA was markedly greater for the *sunY* catalyzed reactions than for reactions catalyzed by the *Tetrahymena* ribozyme. The efficiency of ligation reactions catalyzed by the *Tetrahymena* enzyme fell off precipitously as the substrate chain length increased (*5, 13, 14*). This difference in enzyme efficiencies was observed under a number of different reaction conditions and may be a function of structural differences between the *sunY* and *Tetrahymena* introns (*15, 16*). The identities of the full-length and intermediate products were confirmed by RNA sequencing with base-specific ribonucleases.

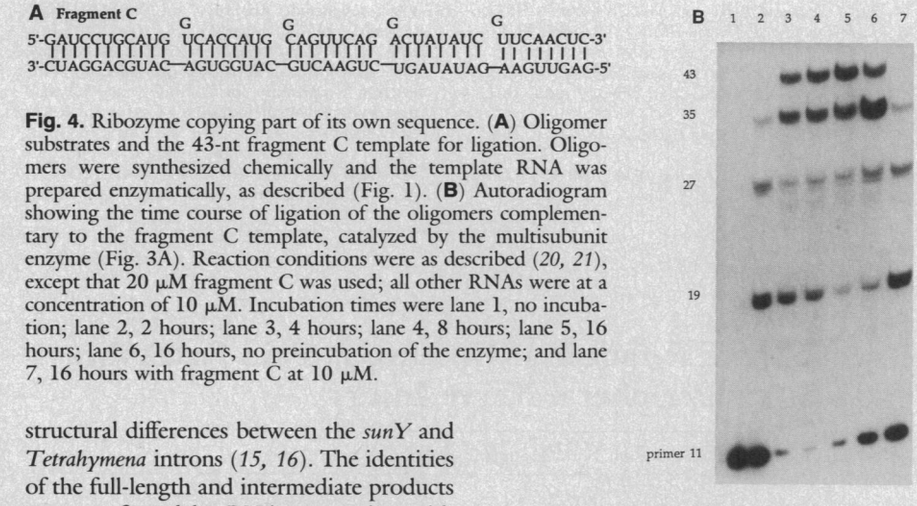


Fig. 4. Ribozyme copying part of its own sequence. (**A**) Oligomer substrates and the 43-nt fragment C template for ligation. Oligomers were synthesized chemically and the template RNA was prepared enzymatically, as described (Fig. 1). (**B**) Autoradiogram showing the time course of ligation of the oligomers complementary to the fragment C template, catalyzed by the multisubunit enzyme (Fig. 3A). Reaction conditions were as described (*20, 21*), except that 20 μM fragment C was used; all other RNAs were at a concentration of 10 μM. Incubation times were lane 1, no incubation; lane 2, 2 hours; lane 3, 4 hours; lane 4, 8 hours; lane 5, 16 hours; lane 6, 16 hours, no preincubation of the enzyme; and lane 7, 16 hours with fragment C at 10 μM.

In order to test whether the multisubunit enzyme could catalyze the synthesis of an RNA strand complementary to one of its own subunits, five oligomer substrates were synthesized that were complementary to fragment C (Fig. 4A). A time course was generated for the ligation of these oligomers catalyzed by the multisubunit enzyme (Fig. 4B). Some ligation of the substrate oligomers occurred in a reaction that contained no additional template beyond that in the enzyme complex itself (Fig. 4B, lane 7). This implies that some fraction of the complexes were sufficiently unfolded to allow annealing of the oligomer substrates. However, the complex was stable enough that its function was not completely inhibited by the presence of these anti-sense substrate oligomers.

The multisubunit ribozyme derived from the *sunY* intron is the smallest and one of the most efficient RNA catalysts yet identified for the synthesis of cRNA. Because the individual RNA molecules that formed the active ribozyme complex are quite short, each one could potentially serve as a template for the synthesis of complementary strands, which in turn would be templates for the synthesis of additional subunits.

Our experiments suggest that the spontaneous assembly of the first RNA replicases from prebiotically produced RNA molecules might not have been as difficult as imagined. Activated nucleotides have been observed to condense nonenzymatically into chains 20 to 40 nt in length (*17, 18*). It now seems that RNA polymers of this modest length could assemble to form a replicase; longer strands of 200 or more nucleotides may not be necessary.

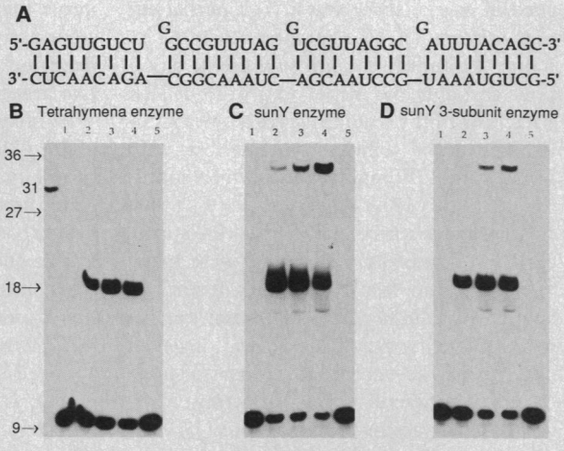


Fig. 3. Template-directed assembly of multiple oligonucleotides. (**A**) Oligomer substrates and the 36-nt template for ligation. The four short oligomers were synthesized chemically with phosphoramidites purchased from Milligen-Bioseearch. Synthetic RNA was purified by anion-exchange high-performance liquid chromatography with a Dionex NA-100 column and a gradient that contained acetonitrile (10%) and ammonium acetate, pH 5.6 (0.01 to 2 M). The template RNA was prepared by T7 RNA polymerase transcription as described (*11*). (**B** to **D**) Autoradiograms showing time courses of ligation of the oligomers to produce full-length cRNA. (B) The *Tetrahymena* intron (*5*) as the catalyst. (C) The single-chain *sunY* derivative as the catalyst. (D) The multisubunit enzyme (Fig. 3A) as the catalyst. Reactions were as described (*20*), except that ethanol (10%) was included (*21*), the $MgCl_2$ concentration was 20 mM, and all RNAs were at a concentration of 10 μM. RNA enzymes were preincubated for 20 min in buffer at 37°C prior to addition of the RNA substrates. Reactions were incubated at 37°C for varying times, quenched as described (*20*), and analyzed on 20% acrylamide–70% formamide gels. The far left lane in (B) contains a 31-nt marker. Incubation times were lane 1, no incubation; lane 2, 2 hours; lane 3, 4 hours; lane 4, 8 hours; lane 5, 8 hours, no enzyme RNA added. The amount of the 27-nt product varied in different experiments but was always lower in abundance than the other products.

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21. In other experiments, ethanol and polyethylene glycol enhanced the activity of these ribozymes.
22. We thank R. Green, M. Hanna, and S. Whoriskey for helpful comments on the manuscript. Supported by a grant from Hoechst AG.

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Adrenergic Excitation of Cutaneous Pain Receptors Induced by Peripheral Nerve Injury

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The mechanisms by which peripheral nerve injuries sometimes lead to causalgia, aberrant burning pain peripheral to the site of nerve damage, are uncertain, although the sympathetic nervous system is known to be involved. Whether such syndromes could be the result of the development of responsiveness by some cutaneous pain receptors (C-fiber nociceptors) to sympathetic efferent activity as a consequence of the nerve injury was tested in an animal model. After nerve damage but not in its absence, sympathetic stimulation and norepinephrine were excitatory for a subset of skin C-fiber nociceptors and enhanced the responsiveness of these nociceptors to tissue-damaging stimulation. These effects were demonstrable within days after nerve lesions, occurred at the cutaneous receptive terminal region, were manifest in sensory fibers that had not degenerated after the injury, and were mediated by α_2-adrenergic–like receptors.

CAUSALGIA, A DEBILITATING SYNdrome that develops after some peripheral nerve injuries, is characterized by severe burning pain initially localized to the skin innervated by the injured nerve (*1*). Frequently, causalgic pain is associated with sympathetic nervous system effects such as regional alterations in cutaneous blood flow and perspiration. This association, and the relief that can be provided by interruption of the sympathetic nervous supply to the affected body region, have led to characterization of causalgia and its pain as a reflex sympathetic dystrophy (*2*). Injury-induced interactions between sympathetic efferent postganglionic axons and cutaneous sensory fibers have been proposed as the basis of causalgic pain (*1*); however, sympathetic stimulation (SS) and sympathetic chemical mediators neither excite nor enhance the activity of pain receptors (nociceptors, that is, noxious stimulus receptors) of normal skin (*3*). Because SS does increase the responsiveness of some sensory units associated with nonpainful cutaneous mechanoreception (*3*), some proposals suggest central neural processes rather than peripheral interactions between efferent sympathetic neurons and specific sensory receptors for pain as the basis for causalgia (*2, 4*). We examined the influence nerve lesions have on the way nociceptors respond to sympathetic action, to clarify the relation of presumed pain receptors to causalgia.

The great auricular nerve was exposed with sterile surgical procedures in anesthetized New Zealand white rabbits and damaged in one of three ways: (i) a partial cut with scissors; (ii) two ligatures separated by 5 mm tied tightly enough to decrease blood flow through the nerve (*5*); (iii) a 30-s stretching of the connective tissue surrounding the nerve sufficient to interfere with blood flow. The animals were then monitored for 4 to 148 days; no signs of trophic changes in the ear or discomfort were apparent (*6*). Terminally, recordings were made with platinum hook electrodes from fine filaments of the nerve 15 to 20 mm central to the nerve injury; a filament was dissected until discharges were identified from a single cutaneous afferent fiber conducting <1.5 m/s (C-fiber) across the injury site (*7*). Sensory units identified as C-fiber polymodal nociceptors of the skin (CPMs), which have been linked to cutaneous pain, were selected for analysis (*8*). C-fiber sensory units of the great auricular nerve do not regenerate and show recognizable afferent properties for at least 30 days after nerve crush or transsection (*9*). Most of our observations were made on nerves injured less than 30 days previously, and therefore they represented recordings from fibers spared from degeneration by the injury. We stimulated the units with a heating and cooling sequence delivered by a counterbalanced 50-mm^2 thermode (*10*). The CPMs of hairy skin typically sensitize on repeated exposure to moderately noxious heat, that is, on a second test they generate more impulses to a given heat stimulus and the threshold temperature for heat decreases (*8, 10*). Activation and sensitization of the CPM fibers by heat are indices of the responsiveness of their peripheral receptive terminals to skin stimulation (*10*).

In control animals, CPM units sensitized as expected (*8, 10*): the mean number of impulses produced to a second heat cycle doubled compared to values from the initial trial (Table 1). SS rostral to the superior cervical ganglion in control animals (20 stimuli per second for 30 s, 5 min before beginning of the thermal cycle, causing visible vasoconstriction in the ear and a 0.5°C drop in temperature at the receptive field) produced no difference in threshold or in the mean response of CPM units to the initial thermal stimulation (*3*). SS before a second thermal test cycle suppressed the increased response expected during the active heating stage (Table 1).

In control animals, SS by itself did not excite CPM units (*3*). In contrast, after all three kinds of nerve injury, some CPM units were directly excited by SS before the first heat exposure (Table 2). This response was a low-frequency discharge during electrical stimulation of the ascending cervical sympathetic trunk and for a short period afterward (*11*). Direct excitation by SS occurred in about 20% of the units from damaged nerves, as early as 7 days after injury. Of 65 units tested 24 days or less after nerve injury, 10 were directly excited. Close arterial injections of norepinephrine (NE) also never excited CPM units of control animals (*11*). On the other hand, such NE injections activated 27 of 65 units from animals within 30 days or less after nerve injuries (Table 2 and Fig. 1). These evoked responses to SS and NE had long latencies (*11*), suggesting

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