therefore produce multiple forms of the protein by alternative RNA splicing. From an evolutionary standpoint, it is interesting that for both K^+ channels and muscarinic acetylcholine receptors, the *Drosophila* protein-coding regions are interrupted by introns (*2, 21*), whereas the corresponding vertebrate proteins are encoded by single exons (*10, 18, 20*).

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Transplanted Suprachiasmatic Nucleus Determines Circadian Period

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The pacemaker role of the suprachiasmatic nucleus in a mammalian circadian system was tested by neural transplantation by using a mutant strain of hamster that shows a short circadian period. Small neural grafts from the suprachiasmatic region restored circadian rhythms to arrhythmic animals whose own nucleus had been ablated. The restored rhythms always exhibited the period of the donor genotype regardless of the direction of the transplant or genotype of the host. The basic period of the overt circadian rhythm therefore is determined by cells of the suprachiasmatic region.

THERE IS CONSIDERABLE EVIDENCE to suggest that the suprachiasmatic nucleus (SCN) of the hypothalamus is the site of circadian pacemaker cells that generate overt circadian rhythms in mammals. The evidence that supports this view is diverse. (i) The SCN is the target of direct and indirect retinal projections required for entrainment of circadian rhythms to environmental cycles (*1, 2*). (ii) The SCN exhibits strong circadian rhythms of glucose utilization in vivo (*3*). (iii) Ablation of the SCN or its surgical isolation within the brain eliminates overt behavioral rhythmicity (*4–6*) and rhythmic electrical activity in the brain (*7*). (iv) Tissue explants containing the SCN continue to express circadian rhythms in electrical activity (*8, 9*) and vasopressin release (*10*) in vitro. (v) Circadian rhythmicity can be restored to SCN-lesioned arrhythmic hosts by implantation of fetal brain tissue containing SCN cells (*11–14*).

Despite this evidence, however, the pacemaker role of the SCN circadian oscillator has not been confirmed. In addition, the role of the nucleus has come into question because methamphetamine given on a long-term basis to arrhythmic, SCN-lesioned rats will restore circadian rhythmicity (*15*). Moreover, in the rat (*16*) and in lower vertebrates (*17*), structures outside the SCN are able to generate circadian rhythms.

Although in the aggregate the evidence is compelling, final proof that the SCN is the site of a central driving oscillator for mammalian circadian systems requires that characteristics of the overt rhythm such as phase and period be unambiguously attributable to the activity of SCN cells. The discovery of the τ mutation in hamsters provided the opportunity to test directly the pacemaker role of the SCN by tissue transplantation. The mutation has the primary behavioral effect of reducing the period of the circadian rhythm from 24 hours to about 22 hours in heterozygotes and to about 20 hours in homozygotes (*18*). If the SCN drives overt behavioral rhythmicity in hamsters, then the period of the rhythm that is restored by SCN transplantation should reflect the genotype of the donor tissue and not that of the lesioned host.

All animals used in these experiments were raised in our colony, and only male animals were used as hosts. These were placed in running wheel cages for activity recording after reaching 8 weeks of age and were kept in constant dim light or constant dark for the duration of the experiment. After the period of the host rhythms had been established (7 to 21 days), animals were anesthetized and placed in a Kopf model 900 stereotaxic instrument for SCN ablation. Lesions were made by current in-

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jection (4 mA for 10 s) through a platinum/iridium (90/10) wire electrode insulated except for 0.3 mm at the tip (electrode placement: anteroposterior, +0.6 mm from bregma; dorsoventral, 8.4 mm from the top of the skull; tooth bar, −2 mm). Functional ablation of the SCN was determined by visual analysis of the subsequent locomotor activity record. As is common with this type of lesion, ultradian rhythmicity persisted in many records after SCN ablation. Lesions were considered to be functionally complete if 24-hour periodicity was not visible in the activity record. The location and extent of the lesion was determined later during histological examination.

In most cases, transplants were performed between 3 and 4 weeks after SCN ablation. When transplants involved hosts and donor tissue with the same genotype, the operation was performed at least 3 weeks after ablation so that there would be ample opportunity to identify rhythmicity that persisted in incompletely lesioned animals. In four cases, in which transplants involved donor and host of different genotype, the implantation was performed 1 week after SCN ablation to make a preliminary assessment of whether the timing of the two operations influenced the success rate of the transplantation procedure.

Fetal tissue was obtained on embryonic day 13.5 (day 1 is the first 24 hours after conception) from donors of known circadian genotype. Pregnant females were anesthetized with sodium pentobarbital, and fetuses were removed and decapitated immediately. The brains were removed quickly and placed in culture medium [60% Eagle's minimal essential medium, with Earle's salts; 40% Hanks balanced salt solution (MEM-BSS)] maintained at 36°C. After all of the brains had been collected, blocks of tissue containing either SCN or control tissue (cortex) were excised and placed in separate dishes (*19*). For implantation, the SCN from two donors were drawn into a 5-μl Wiretrol micropipette (Drummond Scientific, Broomall, Pennsylvania) for stereotaxic placement in the third ventricle of the host near the site of the SCN lesion. This site was chosen to be consistent with tissue placement in other SCN transplant studies. The total volume of material implanted was about 1 μl (*20*).

Circadian rhythmicity was restored unambiguously in about 80% of the arrhythmic hosts that received SCN implants. Only rhythmicity that was visible to naïve observers of the raw activity data is presented in this report. Rhythmicity was not restored in animals that received cortical tissue implants (n = 4), although apparently healthy implants were found later during immunocytochemical analysis. Time series (fast Fourier) analysis was used to confirm the presence of rhythmicity restored by SCN transplantation and to confirm the absence of donor rhythmicity in the activity of control animals. This analysis indicated the presence of residual rhythmicity in some animals after SCN lesions. Because of the difference in period length between host and donor phenotype, this residual rhythmicity did not interfere with the interpretation of the rhythmicity restored by transplantation.

The period of restored rhythms always matched that predicted by the genotype of the donor tissue. Examples of rhythmicity restored by transplantation are shown in Fig. 1. When wild-type tissue was used, the period of the restored rhythm was always about 24 hours; heterozygous donor tissue always produced rhythms with periods close to 22 hours; and homozygous mutant tissue always produced periods close to 20 hours. This result was obtained regardless of the genotype of the host. No influence of the host genotype on the period of rhythms could be detected.

Our data for SCN transplants between different genotypes are summarized in Fig. 2. For all combinations of donor and host, the restored period was significantly different from that of the host ($P < 0.01$), and for each genotype of donor tissue the periods of the restored rhythms always fell within the range shown by intact adults of that genotype.

Perhaps the most parsimonious interpretation of our results, when taken together with the work of others (*1–14*), is that the SCN contains cells that are not only able to generate oscillations with a circadian period, but are also responsible for the generation of overt behavioral circadian rhythms in intact mammals. Al-

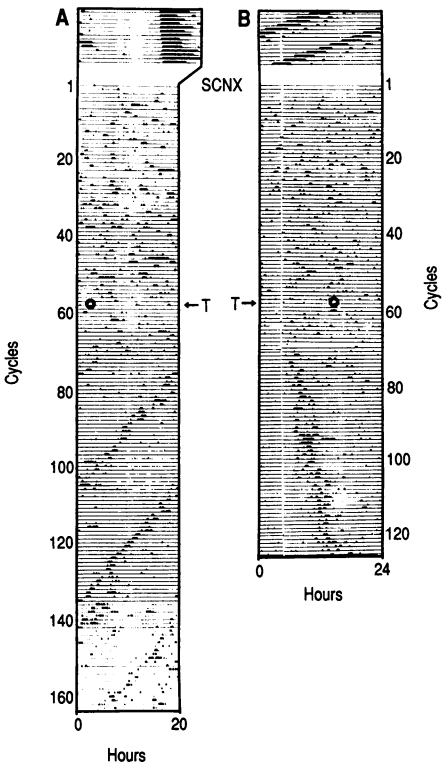

Fig. 1. (**A**) Expression of homozygous mutant rhythmicity in a wild-type host. The endogenous rhythm of the intact host is shown at the top of the activity record (period, 24.05 hours). SCNX is SCN ablation; at this point, the plotting interval was changed from 24 hours to 20 hours to help visualize rhythmicity that was restored later by neural transplant. Implantation of fetal SCN tissue was performed on the day indicated by a T at the time indicated by the circle. The period of the restored rhythm in this example was 19.5 hours. (**B**) Expression of wild-type rhythmicity in a heterozygous mutant host. The period of the host rhythm was 21.7 hours. Transplantation resulted in the restoration of a 24.2-hour rhythm seen in the lower third of the record.

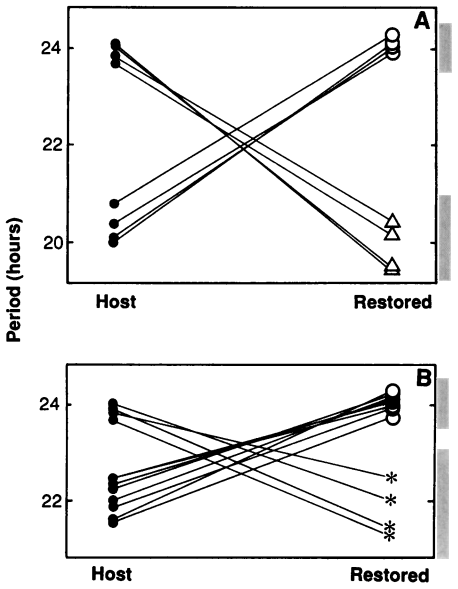

Fig. 2. Reciprocal transplantation of SCN tissue between wild-type and mutant animals. Periodicity was determined from eye-fit lines drawn through activity onsets on at least 20 consecutive days of data and was confirmed later with time series analysis. To reduce measurement error, data were plotted at intervals close to their free-running period before the final period determination was made. This procedure reduced the inherent variability of the measurement to less than 1% for a single determination of period. For each host, the endogenous rhythm (left) was eliminated by SCN ablation and restored by SCN implants (right). The range of period of the intact adult population for each genotype is indicated by vertical shaded bars (right axis). (**A**) Reciprocal transplants between wild-type and homozygous mutants. (**B**) Reciprocal transplants between wild-type and heterozygous mutants. Symbols represent the following: (●) host; (○) SCN tissue from wild-type donor; (*) SCN tissue from heterozygous donor; and (△) SCN tissue from homozygous mutant donor.

though behavioral patterns can be modified by transplantation of large brain regions early in the development of some vertebrates (*21*), our work shows that a discrete behavioral pattern can be transplanted with a neural gift of limited size and well-defined function.

After behavioral observation we attempted to determine the extent of SCN lesions, the amount of extra-SCN donor tissue within the implant, and whether neural connections had been established between the host brain and implant. For each animal examined (*22*) (n = 16) in which rhythmicity had been restored, the SCN lesion appeared complete [no evidence of the host SCN when antisera directed against vasoactive polypeptide (VIP), vasopressin, or neuropeptide Y (NPY) were used], and a plug of donor tissue was found within the third ventricle. These plugs were always found in close apposition to the ependymal wall. VIP-positive perikarya and fibers were always identified within these implants, and in most cases cells and fibers formed a discrete "ball" (Fig. 3, A and B) reminiscent of the organization of VIP within the SCN. We could never clearly trace VIP fibers extending from the graft and crossing the host-graft border, although this was strongly suggested in some sections (Fig. 3C). Vasopressin-positive perikarya that resemble the vasopressin immunoreactive perikarya within the SCN were also consistently found in the implant (Fig. 3, E to G). These perikarya were often difficult to identify because of their small size (long axis around 10 μm) and weak immunostaining. In these cells, much of the soma was occupied by the nucleus (Fig. 3E). Vasopressin immunoreactive perikarya were often associated with a fine plexus of varicose fibers showing weak vasopressin immunostaining. These SCN-like vasopressin perikarya and fibers contrast with vasopressin cells of the magnocellular system, which show large, strongly immunoreactive perikarya (long axis around 25 μm) and fibers. Such cells were also identified within some of the implants (Fig. 3D), suggesting that we had occasionally transplanted part of the magnocellular system. NPY-positive fibers were always found crossing the host-graft border (Fig. 3, H and I), but cell bodies were rarely found within the implant. As NPY perikarya have not been identified within the SCN and were not identified within the graft, we assume that the majority of NPY fibers within the graft came from the host. In four unsuccessful SCN implants, we identified weakly stained VIP cells and fibers within the graft, but found no evidence of vasopressin immunoreactive

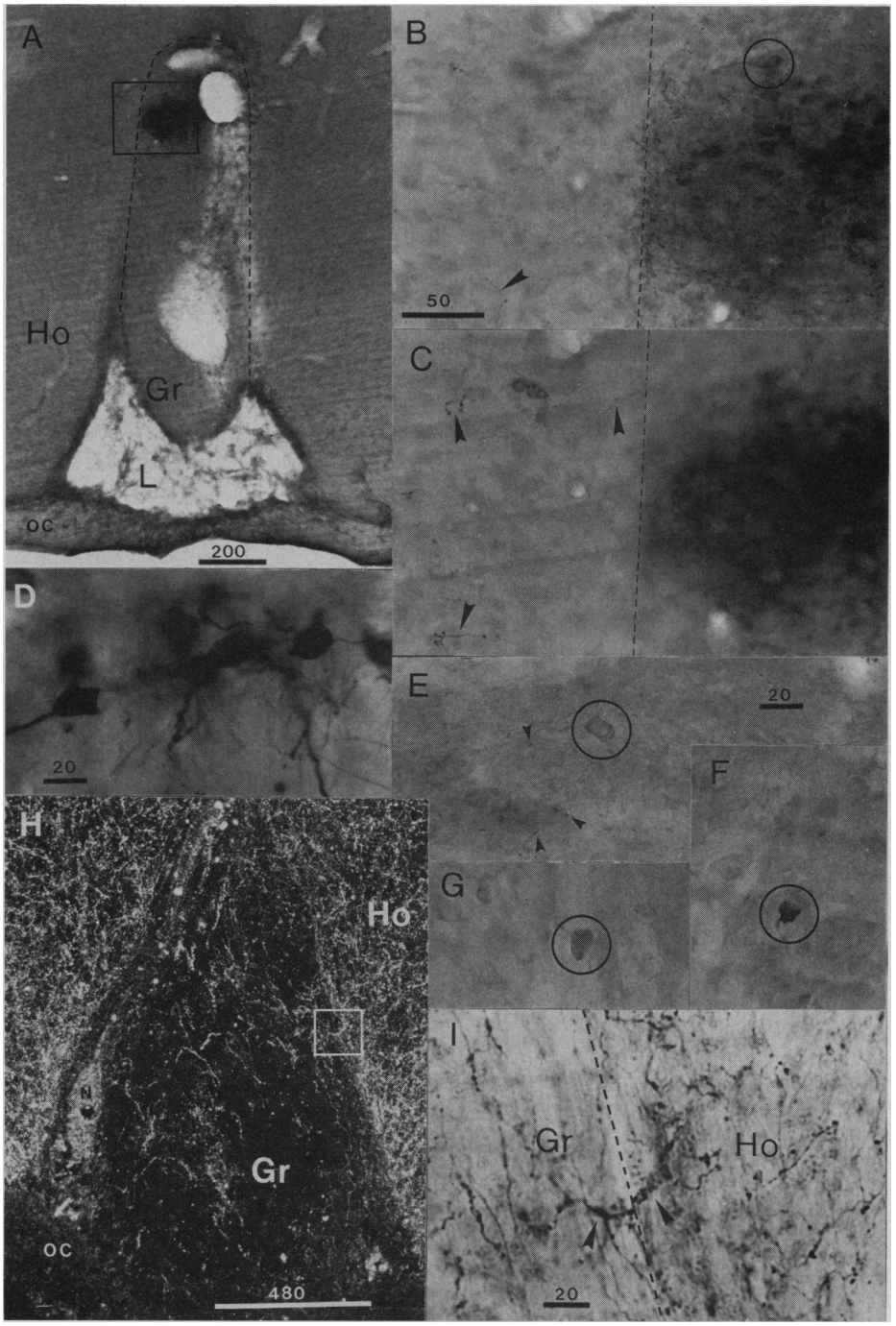

Fig. 3. (**A**) Photomicrograph of a graft placed within the third ventricle that restored a 22-hour rhythm. The approximate borders between the host and graft tissue have been indicated with a dotted line. This section has been immunostained with an antibody to VIP. The area within the square has been enlarged in (B) and (C). (**B**) A "ball" of VIP perikarya and fibers (right side), reminiscent of the organization of VIP within the SCN. (**C**) The same area as (B) but at a different plane of focus. At this plane of focus immunoreactive fibers [indicated with arrowheads in (B) and (C)] are identified that appear to have arisen from the graft and cross the host-graft border (dotted line). (**D**) Magnocellular-like vasopressin cells were occasionally found within the graft. (**E, F,** and **G**) Vasopressin immunoreactive perikarya (circles) and fibers (arrowheads) within the graft that resemble the vasopressin cells of the SCN. Perikarya are faintly stained and small and were often associated with a fine plexus of varicose fibers. These cells contrast with the vasopressin cells of the magnocellular system, which have large densely stained perikarya associated with heavily stained fibers (D). (**H**) Dark-field photomicrograph of a successful graft placed within the third ventricle. This section has been immunostained with an antibody to NPY. The area within the white square has been enlarged in (**I**) to show immunoreactive NPY fibers (arrowheads) crossing the host-graft boundary (dotted line). As no perikarya were identified within the graft we assume that these NPY fibers came from the host tissues. Abbreviations: Gr, graft; Ho, host tissue; N, necrotic tissue; OC, optic chiasma; and L, lesion site and base of third ventricle. Scale bars: (A) 200 μm; (B and C) 50 μm; (D) 20 μm; (E, F, and G) 20 μm; (H) 480 μm; and (I) 20 μm.

perikarya or fibers within the graft or evidence that NPY fibers were entering the graft from the host. In contrast to implants that contained the SCN, cortical implants never restored rhythmicity to the host. Cortical implants always contained a few NPY perikarya and many fibers. In cortical implants, NPY fibers were always seen to cross the host-graft border, and most crossing fibers seemed to originate from the host.

Although most of our implants contained some portion of extra-SCN tissues (Fig. 3, A and D), the immunocytochemical analysis showed that grafts that restored rhythmicity always contained cells with SCN characteristics (VIP and vasopressin). Therefore, the period of the overt rhythm is determined by cells within, or very close to the SCN. This observation is in agreement with reports showing that the SCN is required for successful restoration of rhythmicity (*11–14, 23*).

In most of our locomotor data, rhythmicity was visually apparent within 6 to 7 days after transplantation. Although surprisingly short, this latency does not preclude the possibility that neural reconnections drive the behavior since dense neural outgrowth has been reported from other transplanted tissue with a similar time course (*24*). Immunocytochemical analysis indicates that neural connections have been made between graft and host brain; however, it was not possible to determine the source of fibers crossing the graft boundary.

The fact that the genotype of the host does not appear to affect significantly the expression of the transplanted rhythm is somewhat surprising, especially in view of evidence for the existence of oscillators outside the SCN in the mammalian brain (*15, 16*). We interpret the absence of a host contribution to the circadian period to mean that either the SCN is essentially autonomous in determining the primary characteristics of rhythmicity in hamsters or that the host brain fails to make the connections with the tissue graft that are required for the brain to influence this period. In either case, our results strengthen the view that the SCN occupies a position at the top of the circadian hierarchy in mammals.

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19. Tissue for implantation was obtained in the following manner. Pregnant females were anesthetized and prepared for surgery on day 13.5 of gestation. Fetuses were located and decapitated in utero, and the heads were removed to a sterile petri dish containing BSS. The entire litter was collected at one time. Fetal brains were then removed under dissecting microscope and placed in a second dish. After all of the brains had been collected, each was oriented with the ventral surface visible under the microscope. All dissections were performed with the tissue immersed in MEM-BSS. This procedure allowed the ends of the developing optic nerves to float in the media so that the optic chiasm could be located easily. A coronal incision was made where the two nerves fused, and a second, parallel cut was made 1 to 1.5 mm caudal to this. Two parasagittal cuts were then made about 1.5 mm on either side of midline, with the scissors held at a 45° angle so that these cuts passed into the third ventricle. This resulted in the excision of two small blocks of tissue connected by the optic chiasm. Neural tissue for implantation was then teased away from the chiasm and pia mater.
20. Tissue blocks for implantation were placed in a group at the tip of a Wiretrol micropipette. The pipette was graduated in increments of 1.0 μl so that the total volume to be injected could be estimated. The tissue occupied about 1 μl of the 1.5 to 2 μl volume that was injected into the host brain.
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22. Animals were perfused intracardially with 350 ml of 4% paraformaldehyde in 0.01*M* phosphate buffer containing 15% picric acid; the brain was removed and placed in the same fixative for an additional 24 to 48 hours. Frontal sections (80 μm) were cut on a vibratome and processed for immunocytochemistry as described [R. G. Foster, G. Plowman, A. Goldsmith, B. Follett, *J. Endocrinol.* **115**, 211 (1987)] with antibodies directed against NPY (1:1000; Peninsula Laboratories, Belmont, CA), vasopressin (1:500; Incstar, Stillwater, MN), and VIP (1:1000; Peninsula Laboratories). Controls were performed by incubating sections in absorbed primary antisera [40 nm of peptide (Peninsula Laboratories) added to 1 ml of diluted primary antisera for 24 hours at 4°C] or omitting the primary antisera. In both controls, immunostaining was abolished in the graft and host brain, except for faint staining within necrotic tissue and astrocytes around the lesion site, suggesting artifactual staining within these tissues. Necrotic tissue and astrocytes around the lesion site also showed immunostaining with VIP, NPY, and vasopressin antibodies (Fig. 3).
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Regularization Algorithms for Learning That Are Equivalent to Multilayer Networks

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Learning an input-output mapping from a set of examples, of the type that many neural networks have been constructed to perform, can be regarded as synthesizing an approximation of a multidimensional function (that is, solving the problem of hypersurface reconstruction). From this point of view, this form of learning is closely related to classical approximation techniques, such as generalized splines and regularization theory. A theory is reported that shows the equivalence between regularization and a class of three-layer networks called regularization networks or hyper basis functions. These networks are not only equivalent to generalized splines but are also closely related to the classical radial basis functions used for interpolation tasks and to several pattern recognition and neural network algorithms. They also have an interesting interpretation in terms of prototypes that are synthesized and optimally combined during the learning stage.

MOST NEURAL NETWORKS ATtempt to synthesize modules that transduce inputs into desired outputs from a set of correct input-output pairs, called examples. Some of the best known applications are a network that maps English spelling into its phonetic pronunciation (*1*) and a network that learns the mapping corresponding to a chaotic dynamical system, thereby predicting the future from

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Transplanted suprachiasmatic nucleus determines circadian period

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