containers that were half-filled with pond water and then placed inside a Styrofoam cooler.
10. A newt was placed in a release device located in the center of the arena floor in total darkness. When the lid of the arena was closed, a light centered above the release device was turned on. The newt was released after a delay of 30 seconds. Its movements were observed through the frosted Plexiglas floor of the arena by means of a mirror located underneath. Directional responses were measured to 5° accuracy at a distance of 10 cm from the center of the arena. A detailed description of arena design is provided elsewhere (*2*).
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14. The testing sequence was one animal in mag N = north (vertical component down), the next in mag N = east (vertical component up), then one in mag N = west (down), mag N = south (up), mag N = east (down), mag N = north (up), mag N = south (down), and mag N = west (up).
15. Newts exposed to a mean temperature of 18°C and fluctuations of less than ±5°C in the temperature of training tank water before the water temperature was increased for testing exhibited *y*-axis orientation [Fig. 2, A and C; (*2, 4*)]. However, newts exposed to diel fluctuations in water temperature of greater than 20°C (from 3° to 5°C at night to 25° to 27°C during the day) for one or more days before testing responded to the increase in water temperature by orienting in the direction of their home pond [Fig. 2E; (*4*)]. The adaptive significance of the newt's response to differing water conditions is discussed elsewhere (*2, 4*).
16. In two earlier test series, an abrupt drop in training tank water level just before testing elicited a unimodal magnetic response lasting about 30 minutes (*20*). Newts exposed to this treatment exhibited either *y*-axis or homeward orientation, with the type of orientation varying between tests; in these early tests, the factors responsible for the switch in behavior had not been identified. Newts orienting along the *y*-axis reversed their orientation when the vertical component of the magnetic field was inverted. Newts orienting relative to the home pond direction did not respond to an inversion of the vertical component.
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Molecular Cloning of the Chicken Progesterone Receptor

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To define the functional domains of the progesterone receptor required for gene regulation, complementary DNA (cDNA) clones encoding the chicken progesterone receptor have been isolated from a chicken oviduct λgt11 cDNA expression library. Positive clones expressed antigenic determinants that cross-reacted with six monospecific antibodies derived from two independent sources. A 36–amino acid peptide sequence obtained by microsequencing of purified progesterone receptor was encoded by nucleotide sequences in the longest cDNA clone. Analysis of the amino acid sequence of the progesterone receptor deduced from the cDNA clones revealed a cysteine-rich region that was homologous to a region found in the estrogen and glucocorticoid receptors and to the avian erythroblastosis virus *gag-erb*-A fusion protein. Northern blot analysis with chicken progesterone receptor cDNA's indicated the existence of at least three messenger RNA species. These messages were found only in oviduct and could be induced by estrogens.

STEROID HORMONE REGULATION OF gene expression in eukaryotic cells is mediated by specific intracellular receptors (*1*) that have a high affinity for their respective steroid hormone ($K_d = 10^{-10}M$) and are present in low concentrations in target cells (*2*). In the chick oviduct, the interaction of progesterone with its receptor results in increased transcription of a defined set of genes coding for egg white proteins (*3*). In addition to its role as gene regulator, the progesterone receptor (PR) itself appears to be regulated by estrogen (*4*). The PR in chick oviduct consists of two hormone-binding moieties (*5*). Protein A, which has a molecular mass of 79 kD, binds to DNA with high affinity. Protein B, which has a molecular mass of 108 kD, binds less well to DNA but interacts with certain nonhistone chromosomal proteins (*2*). The progesterone-responsive ovalbumin gene has sequences in its 5′ flanking region that may be involved in the regulation of its expression by the PR-progesterone complex (*6*). However, direct analysis of the relation between PR proteins A and B and of their interactions with target gene elements has been hampered by their low concentration and the difficulty of purifying substantial quantities of receptor. Therefore, to define the functional domains of the PR required for such interactions with gene elements, we have cloned a complementary DNA (cDNA) encoding the chick progesterone receptor.

A panel of six monoclonal antibodies, each recognizing the chick PR, was used to screen a λgt11 cDNA library for expression of PR epitopes. Five of these antibodies, raised against the chick receptor, have been characterized (*7*). Each of the antibodies appears to react with a different antigenic determinant of the receptor. The additional monoclonal antibody was raised against human PR from T47D cells and cross-reacted with chick PR. We analyzed the antibodies by immunoblotting to determine their specificity for PR under conditions used to screen the expression products of the cDNA clones (Fig. 1A). Crude cytosolic extracts from chick oviduct were subjected to electrophoresis in 10% sodium dodecyl sulfate (SDS)–polyacrylamide gels and transferred to nitrocellulose filters. Filter strips containing resolved cytosolic proteins were then incubated with each of the monoclonal antibodies. All six antibodies reacted specifically with PR protein B, and all but two reacted equivalently with both protein A and protein B. Antibody PR22 had the highest apparent affinity for PR under these conditions and was chosen for primary screening of the cDNA library (*8*).

To screen large numbers of recombinants, we constructed a λgt11 cDNA expression library (*9*) with size-selected polyadenylated RNA (larger than 2 kb) from chick oviduct. The library contained approximately 6×10^6 members, 95% of which were recombinants. Approximately 3×10^6 phages

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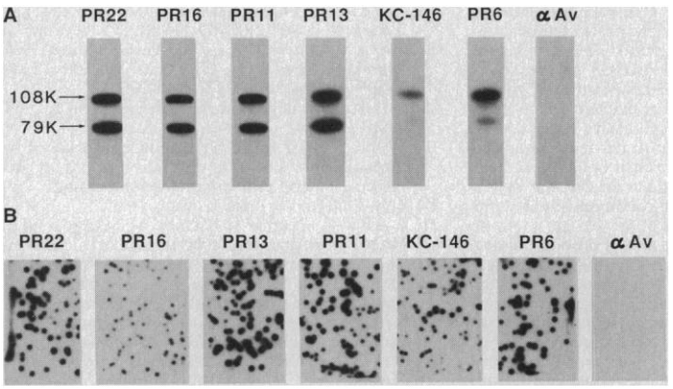

Fig. 1. (A) Immunoblot characterization of mouse monoclonal antibodies to chick PR. Cytosolic extracts from oviducts of estrogen-stimulated chicks were resolved on 10% SDS-polyacrylamide gels and transferred to nitrocellulose filters. Filter strips were cut and incubated with monoclonal antibodies PR22, PR16, PR11, PR13, KC-146, PR6, and mouse immunoglobulin (IgG) avidin (αAv). This IgG to avidin was used as an irrelevant antibody control because there is no detectable avidin in oviducts of chicks treated with estrogen. (B) Expression of PR epitopes by λgt11 clones. *Escherichia coli* Y1090 cells were infected with λgt11 clones, transferred onto agar plates, and incubated at 42°C for 4 hours. The plate was overlaid with a dry nitrocellulose filter that had been presoaked in 10 m*M* isopropyl β D-thiogalactopyranoside and incubated to 37°C for 4 hours. The filters were cut into strips, and each strip was incubated with the indicated antibody to PR, then with rabbit antibody (IgG) to mouse IgG, and finally with ^{125}I-labeled protein A.

were screened with PR22. We selected putative PR clones by screening primary positive plaques with PR22 alone and also with a mixture of the remaining antibodies in the absence of PR22. Three independent cross-hybridizing clones were obtained. They were designated CPR2 (1.3 kb), CPR4 (800 bp), and CPR5 (1.1 kb). The expression products of CPR2 reacted with each of the individual monoclonal antibodies (Fig. 1B). No immunoreactivity was obtained with an irrelevant monoclonal antibody to avidin, indicating that the positive signals obtained were specific for antibodies to PR.

To confirm the identity of the immunopositive clones, a peptide obtained from purified homogenous PR protein B was sequenced for comparison with the deduced amino acid sequence of these three clones. The B protein was purified by the steroid affinity chromatography procedure of Renoir *et al.* (*10*), followed by phosphocellulose chromatography, preparative SDS–polyacrylamide gel electrophoresis (PAGE) and electroelution of the 108-kD band (*8*). Analysis of the purification at various steps is shown in Fig. 2A. We used the synthetic progestin [^{3}H]R5020 to elute the receptor from the affinity chromatography column, thereby allowing photocoupling of the radiolabeled ligand to the receptor (*11*). The presence of PR in the 108-kD protein band after the phosphocellulose step was confirmed by fluorography (Fig. 2A).

To rule out the presence of a major contaminating protein of the same molecular weight in the electroeluted 108-kD band, a map of ^{125}I-labeled tryptic peptides of this material was compared with that obtained when receptor from the DEAE Sephacel fractions was immunoprecipitated with the monoclonal antibody PR6. Both preparations yielded identical peptide maps (Fig. 2B). The absence of additional spots in the gel-purified band compared to the immunoprecipitated material confirmed the purity of the gel-purified PR B protein. The electroeluted PR was cleaved by brief treatment with cyanogen bromide to yield a 42-kD peptide that was purified by SDS-PAGE and electroeluted, yielding 120 pmol of this fragment. The peptide was analyzed by vapor-phase microsequencing and a sequence of 36 amino acids was obtained. This was compared with the deduced amino acid sequence from clone CPR2. The complete 36–amino acid sequence was contained in CPR2, providing a perfect match and confirming that CPR2 contained a PR cDNA insert (Fig. 3A).

Comparison of the PR sequence deduced from the CPR clones with the amino acid sequence of human glucocorticoid receptor (GR) (*12*), human estrogen receptor (ER) (*13*), and the avian erythroblastosis virus *gag-erb*-A fusion protein (v-*erb* A) (*14*) revealed a striking homology in a cysteine-rich region contained in all of these proteins (Fig. 3B). A similar homology was observed for chicken estrogen receptor (*15*). Nine of 11 cysteines in PR are conserved among all of the sequences. This observation further

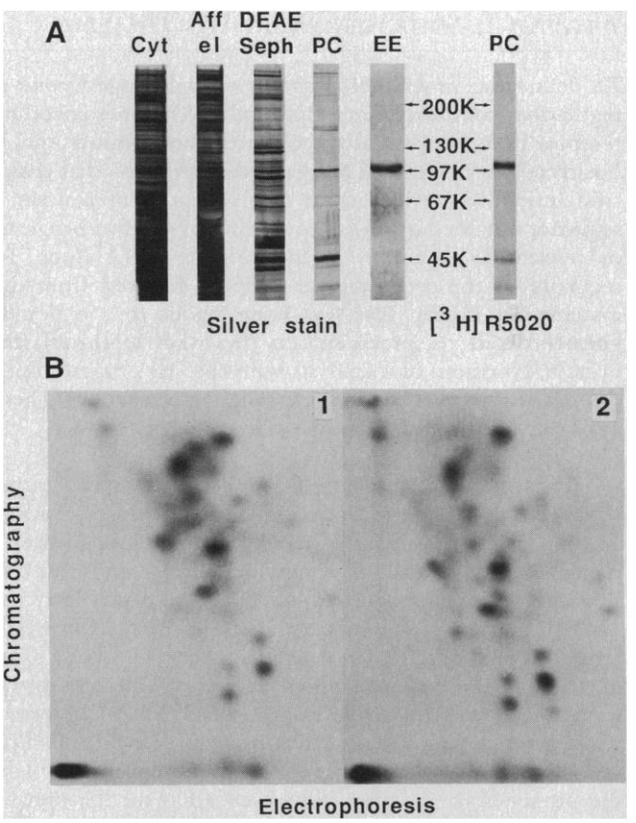

Fig. 2. Purification, photoaffinity labeling, and tryptic peptide map analysis of the B progesterone receptor. (A) The chick oviduct PR B protein was purified from oviduct cytosol (Cyt) to homogeneity by a modification of the steroid affinity chromatographic method (Aff el) and DEAE Sephacel (DEAE Seph) reported by Renoir *et al.* (*9*). Two additional steps were included: phosphocellulose ion-exchange chromatography (PC), and preparative SDS-PAGE and electroelution (EE). Aliquots of samples at the different stages of purification were subjected to SDS-PAGE and visualized by silver staining lanes. To confirm the presence of PR in the 108-kD band, the PC eluate was photoaffinity-labeled with the progesterone analog [^{3}H]R5020, separated by SDS-PAGE, and the radiolabeled proteins visualized by fluorography (PC, far right). Some nonspecific labeling of the 48-kD band was seen, as well as a very strong radioactive band at 108 kD. The weak nonspecific labeling is due to the high concentration (500 n*M*) of [^{3}H]R5020 utilized to elute the receptor from the affinity resin. (B) Photographs of the autoradiographs of the cellulose thin-layer sheets after two-dimensional separation (first dimension: electrophoresis in the horizontal direction, left to right, HOAc : formic acid : H_2O; second dimension: chromatography in the vertical dimension, bottom to top, butanol : pyridine : HOAc : H_2O) of ^{125}I-labeled tryptic peptides obtained according to the technique of Elder *et al.* (*19*). (1) B PR (2 μg) from a preparative 7.5% SDS-polyacrylamide gel. (2) B receptor (2 μg) immunoprecipitated from DEAE Sephacel fractions with PR6 antibody separated from the immunoglobulins in a 7.5% SDS-polyacrylamide gel.

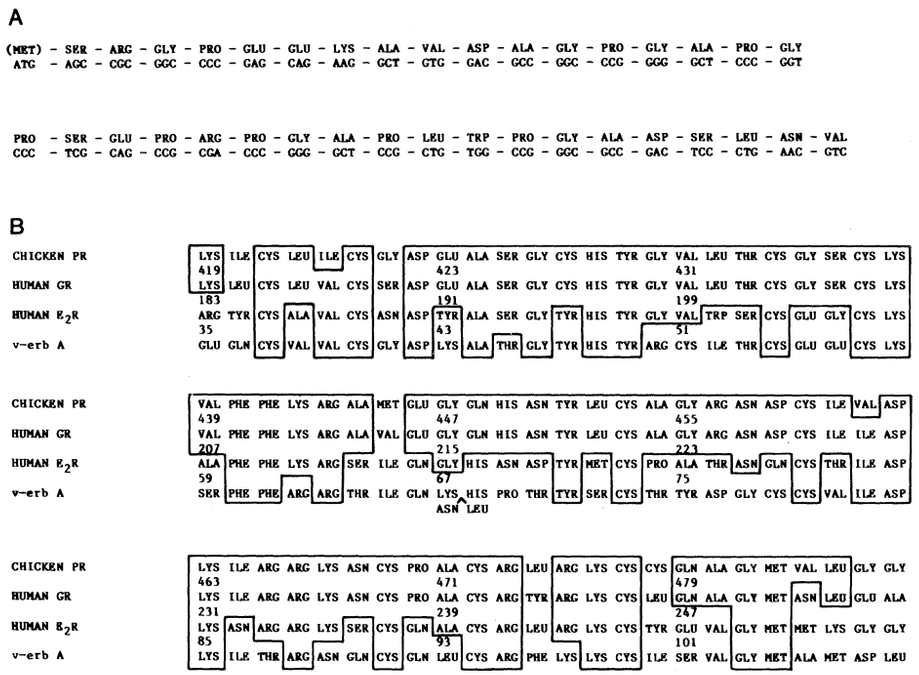

Fig. 3. (A) Partial peptide and nucleotide sequence of the PR. Amino acid sequence obtained directly from the peptide isolated from the purified PR is on the top line; the corresponding cDNA sequence of the CPR2 clone for this region is on the lower line. The amino acid and nucleotide sequences agree completely. This peptide is located approximately 650 amino acids from the carboxyl terminal of the protein. (B) Amino acid sequence homologies between cysteine-rich regions of chick PR, human GR, human ER, and putative v-*erb* A oncogene product.

confirmed that we had cloned sequences encoding an authentic steroid receptor because this cysteine-rich v-*erb* A consensus sequence is the only major region showing homology among steroid receptors. As expected, the highest homology occurs between PR and GR where the overall homology between the two receptors amounts to 86% of the amino acids in the cysteine-rich region. Outside this conserved region the homology with GR drops to approximately 20%. The sequence of CPR2 bears no relation to the "B antigen," which was cloned previously. This was expected because authentic PR has little or no structural homology with B antigen, as indicated by the fact that none of the antibodies to PR cross-react with the B antigen (*16*).

The homology among the steroid receptors and v-*erb* A is consistent with the view that the steroid receptors arise from a family of genes that may be derived from a common primordial gene. The conservation of the cysteine-rich region among the steroid receptors suggests that this region may comprise a major, essential functional domain. In this regard, it has been suggested previously that the cysteine-rich consensus region may represent a DNA-binding domain of GR and v-*erb* A (*17*). Site-directed mutagenesis and expression of PR cDNA's should help to clarify the function of this region and will localize the remaining functional domains of the receptor.

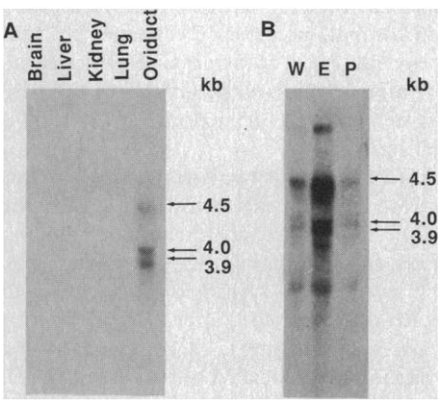

Fig. 4. (A) Northern hybridization analysis of PR mRNA distribution in chick tissues obtained from estrogen-treated chicks. Total cellular RNA (20 μg) from brain (lane 1), liver (lane 2), kidney (lane 3), lung (lane 4), and oviduct (lane 5) were electrophoresed in a 1.5% agarose gel in the presence of 2.2*M* formaldehyde. Ethidium bromide staining showed that the RNA in each lane was equivalent in mass and was largely intact. The RNA was transferred to nitrocellulose and hybridized to 1×10^7 cpm of ^{32}P-labeled antisense RNA probe obtained from the CPR2 cDNA. Molecular sizes were determined with RNA size standards (BRL). The variability in this gel system is ±300 bases. (B) Estrogen inducibility of PR mRNA's. Total cellular RNA (20 μg) from oviducts of estrogen withdrawn (W) chicks (lane 1) and oviducts of animals stimulated with the synthetic estrogen diethylstibestrol (E) for 8 hours or with progesterone (P) for 18 hours were subjected to Northern analysis as described for (A).

To determine the size of the messenger RNA (mRNA) encoding the PR, Northern blot hybridization analysis was carried out with polyadenylated RNA from oviducts of chicks that had been treated with estrogen. The CPR2 insert was ligated to the plasmid vector PGEM2 (*18*) and a ^{32}P-labeled antisense RNA probe was prepared. This probe detected at least three mRNA bands of approximately 4.5 kb, 4.0 kb, and 3.9 kb (Fig. 4A). The mRNA's were distributed in a tissue-specific manner, with detectable levels appearing only in oviduct. No detectable PR mRNA was found in brain, lung, liver, or kidney under these conditions. Furthermore, the oviduct mRNA's were inducible by estrogen (Fig. 4B). After estrogenic stimulation, we frequently saw a large band (greater than 6.0 kb) and a small band of approximately 3.0 kb. The origin of these species is unknown. Little or no change was seen after progesterone administration. These data are consistent with the tissue specificity and estrogen inducibility of the PR that is seen with hormone-binding assays (*4*).

Availability of a cDNA clone for the PR will facilitate elucidation of the structure-function relation of the avian PR and of the origin of the A and B progesterone-binding proteins. In addition, structural analyses of the steroid-binding region of the PR should permit the development of new agonists and antagonists of progesterone that could be used to treat reproductive disorders or malignancies, as well as to develop new contraceptive or fertility enhancement drugs.

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Distinct Pathways of Viral Spread in the Host Determined by Reovirus S1 Gene Segment

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The genetic and molecular mechanisms that determine the capacity of a virus to utilize distinct pathways of spread in an infected host were examined by using reoviruses. Both reovirus type 1 and reovirus type 3 spread to the spinal cord following inoculation into the hindlimb or forelimb footpad of newborn mice. For type 3 this spread is through nerves and occurs via the microtubule-associated system of fast axonal transport. By contrast, type 1 spreads to the spinal cord through the bloodstream. With the use of reassortant viruses containing various combinations of double-stranded RNA segments (genes) derived from type 1 and type 3, the viral S1 double-stranded RNA segment was shown to be responsible for determining the capacity of reoviruses to spread to the central nervous system through these distinct pathways.

FOR A VIRUS TO PRODUCE SYSTEMIC illness, it must first spread from its site of initial entry and primary replication in the host to distant target tissues (*1*). This aspect of viral pathogenesis is particularly well exemplified by neurotropic viruses which, after entering the host through a number of divergent portals (for example, respiratory, gastrointestinal, or venereal), subsequently spread to reach the central nervous system (CNS) (*2*). Two principal pathways of spread to the CNS have been identified for neurotropic viruses. Most neurotropic viruses (for example, arboviruses, enteroviruses, measles virus, mumps virus, and lymphocytic choriomeningitis virus) spread through the bloodstream to reach the CNS (hematogenous spread), but several viruses, including rabies virus, herpes simplex virus, herpesvirus simiae, and poliovirus can reach the CNS by traveling through nerves (*2, 3*). Almost nothing is known about the viral genes and proteins responsible for determining the capacity of viruses to spread through specific pathways within the infected host. Similarly, the precise cellular mechanisms utilized by viruses as they spread have not been identified.

The mammalian reoviruses are neurotropic viruses that have been useful in identifying genetic and molecular mechanisms of viral pathogenesis (*4*). For example, the reovirus hemagglutinin has been identified as the viral cell attachment protein that determines the capacity of reoviruses to bind to red blood cells, lymphocytes, and specific populations of cells within the CNS (*5–7*). The viral hemagglutinin has also been shown to mediate binding of reoviruses to isolated microtubule preparations in vitro (*8*). Our prior success in studying viral pathogenesis by using reoviruses encouraged us to investigate viral-host interactions involved in viral spread.

We report that two serotypes of reovirus spread by different pathways to reach the CNS after peripheral (forelimb or hindlimb footpad) inoculation into newborn mice. Reovirus type 3 spreads through nerves to reach the spinal cord after footpad inoculation, and this spread is mediated by the microtubule-associated system of fast axonal transport. In contrast, reovirus type 1 spreads to the spinal cord through hematogenous pathways. By using reassortant reoviruses containing specific combinations of double-stranded RNA (dsRNA) segments (genes) derived from type 1 and type 3, we have been able to show that the reovirus S1 dsRNA segment, which encodes the viral hemagglutinin, is responsible for determining the capacity of these viruses to spread by different routes to the CNS.

Reovirus type 3 is a neurotropic virus that infects neurons and produces a lethal necrotizing encephalitis after intracerebral inoculation into newborn mice (*6, 7, 9*). Following intracerebral inoculation, type 3 is able to spread to the retina, where it selectively infects and injures retinal ganglion cells (*10*). The axons of retinal ganglion cells, which form the optic nerve, synapse directly on intracerebral neurons. These facts suggested that type 3 is capable of being transported via nerves within the CNS and that a similar type of transport could be used by type 3 to spread to the CNS after its entry at a peripheral site. Type 1, in distinction to type 3, does not infect neurons or retinal ganglion cells after intracerebral inoculation, but instead infects ependymal cells and produces hydrocephalus (*6, 7, 10, 11*). The failure of type 1 to infect neurons suggested that this reovirus would utilize nonneural pathways to spread to the CNS.

To investigate our hypothesis that types 1 and 3 use different pathways of spread to the CNS, we took advantage of the fact that the motor and sensory neurons innervating the hindlimb and forelimb footpads are located in different regions of the spinal cord. If type 3 spreads via nerves, it should appear preferentially in the region of the spinal cord containing the neurons innervating the skin and musculature at the site of viral inoculation. If type 1 spreads through the bloodstream, it should appear in all regions of the spinal cord in equivalent amounts and with similar kinetics. Therefore, we injected type 3 and type 1 into the forelimb and hindlimb footpads of neonatal mice and studied the pattern of spread of these two viruses to the spinal cord. We found that after either hindlimb or forelimb inoculation, type 3 appeared first, and in up to 1000-fold higher titer, in the region of the spinal cord innervating the injected limb (Fig. 1, A and B). In contrast, type 1, after either hindlimb or forelimb inoculation, appeared at essentially the same time and in equivalent titer in all regions of the spinal cord (Fig. 2, A and B). These results support our hypothesis that type 3 spreads through nerves, and type 1 via the bloodstream, to reach the CNS.

To confirm these results, we studied the spread of type 1 and 3 from the hindlimb to the spinal cord in neonatal mice after section of the sciatic nerve. Since the sciatic nerve is the principal neural pathway from the hindlimb to the spinal cord, its section should completely prevent spread of virus from the hindlimb through nerves but should not affect its capacity to spread through the bloodstream. As predicted, sciatic nerve section completely inhibited spread of type 3 to the spinal cord. Nerve section contralateral to the injected limb and sham operation had no significant effect on the spread of type 3 (Fig. 1C). Section of the sciatic nerve had

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