cle, and spermathecae) immediately after copulation. At this point the amount of isotope in other parts did not differ significantly from that in control. Twenty-four hours later a large amount of isotope still remained in the reproductive tracts, although the level was reduced. In *D. melanogaster* females radioactivity decreased in the reproductive tract, and no significant amounts of isotope were detected in any other tissues after 24 hours. *Drosophila* females have been observed to expel material from their reproductive tract after mating (*6*), and we have assumed that this accounts for the reduction in the amount of isotope after 24 hours.

However, *D. mojavensis* females showed significant radioactivity in other body parts, especially unfertilized ovarian oocytes. Since oogenesis requires approximately the same length of time in both these species, and the same female reproductive state existed in both experiments, differential rates of oogenesis cannot be the underlying cause of the presence of isotope in *D. mojavensis* oocytes. The amount of isotope also increases in the somatic tissues of *D. mojavensis* females within 24 hours. The molecular nature of the substances transferred is still unknown, but since male contribution has also been documented with ^{14}C-labeled amino acids (*7*) any major role of tritium exchange in the observation of male contribution in *D. mojavensis* can be ruled out.

After two or three consecutive matings, *D. melanogaster* males are temporarily sterile (*2, 8*). This sterility is caused by a reduction in male accessory gland secretions, not by a reduction in sperm number. After 24 hours, abstinent males regain their fertility (*2*). In contrast, *D. mojavensis* males may mate seven or more times consecutively without any observable reduction in fertility (*1*). It is possible that *D. mojavensis* males transfer less material at each copulation in order to take advantage of the increased mating opportunities in their population.

The phenomenon of males transferring nutrients to females which then appear in female somatic tissues and oocytes has been demonstrated in several species of Lepidoptera (*7*). In these two *Drosophila* species, which differ in their ecology and mating systems, a difference in male contribution to egg production is also apparent. *Drosophila melanogaster*, a cosmopolitan species, can use a variety of substrates for breeding. However, *D. mojavensis* uses necrotic tissue of organ pipe cactus in Sonora, Mexico and southern Arizona, and agria cactus in Baja California. At certain times of the year these resources are limited, and even during times of abundant resources females are selective about the stage and condition of the necrotic tissue on which they will oviposit (*9*). Females must be able to manufacture eggs and also survive until finding an appropriate place to oviposit, and male nutrient contribution may help them do this. The discovery of male nutrient contribution in a genus whose phylogenetic relationships are well defined, whose mating systems vary, and whose ecology is under intensive study (*10*) provides a new opportunity to inquire into the evolution of mating strategies in insects.

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24 October 1983; accepted 23 January 1984

Monoclonal Antibody to Thy-1 Enhances Regeneration of Processes by Rat Retinal Ganglion Cells in Culture

Abstract. *Ganglion cells were dissociated from postnatal rat retinas, identified by specific fluorescent labels, and maintained in culture on a variety of substrates. Regeneration of processes by retinal ganglion cells was enhanced when the cells were plated on glass coated with a monoclonal antibody against the Thy-1 determinant. Plain glass and glass coated with polylysine, collagen, fibronectin, or other monoclonal antibodies supported the growth of neural processes, but were less effective than antibody to Thy-1.*

Detailed studies of differentiated mammalian neurons would be aided by examining identified cells in vitro. This approach requires that viable cells be isolated, unequivocally identified, and cultured. Like other neurons of the central nervous system (CNS), mammalian retinal ganglion cells normally do not regenerate their axons after transection, and indeed many of the cells degenerate (*1*). It is important, therefore, to know whether differentiated retinal ganglion cells can survive and regenerate processes in culture.

Since ganglion cells are the only retinal cells that project to other areas of the CNS, they can be labeled by retrograde transport of markers injected into their projection sites, such as the superior colliculus and lateral geniculate nucleus (*2*). In histological sections Thy-1 antigen is located on cells and processes in the inner retina and can be used to specifically identify the ganglion cells in vitro (*2, 3*). Thy-1 is also expressed on the surface of many neurons not found in the retina as well as on T lymphocytes and embryonic muscle (*4*). Because of sequence homology with immunoglobulins, it has been suggested that molecules displaying the Thy-1 antigen play a role in cellular recognition and morphogenesis in the nervous system (*4*).

In this report we describe aspects of the identification and growth of solitary rat retinal ganglion cells in culture. MacLeish *et al.* (*5*) observed that salamander neurons could be grown and maintained on antibody-coated glass cover slips; we tried this method with several antibodies. Comparing growth on different substrates, we found that, by the second day

in culture, rat retinal ganglion cells plated on cover slips coated with antibodies to Thy-1 (2G12 or MRC OX 7) (*6*) showed enhanced regeneration of processes.

Markers injected for retrograde transport included the fluorescent dyes granular blue (*7*), Lucifer yellow VS, and Evans blue (*8*). Two days later we dissociated the retinas by trituration after digesting them with papain (*9*). Ganglion cells were identified in culture (*10*) by fluorescence of transported dye (Fig. 1). This figure also demonstrates the health of the cell with its smooth surface and phase-bright appearance. In several experiments a monoclonal antibody against Thy-1 was used as a second probe to identify the cells. Double labeling of unfixed ganglion cells revealed virtually complete overlap of the two markers (Fig. 2, a and b). This finding confirmed the reliability of each method for identifying ganglion cells and is consistent with previous observations (*2*). We used 5- to 12-day-old rats as a source of ganglion cells for plating in vitro; at this age central projections are similar to those in the adult (*11*).

In culture some ganglion cells that had been previously labeled by retrograde transport and that were in direct contact with other cells remained fluorescent and viable for up to 2½ weeks. Viability of cells was determined by a highly refractile appearance under the phase-contrast microscope and the capability of accumulating fluorescein from fluorescein diacetate (*12*). In addition, physiological recordings from ganglion cells with this appearance displayed stable electrical properties. With intracellular pipettes the resting potential was measured at a mean of −60.1 (standard deviation, 7.9 mV) for 27 cells. The input resistance of cells without long processes was 250 to 300 megohms. Intracellular and patch-clamp records revealed tetrodotoxin-sensitive action potentials and evidence for other ionic currents (*13*). Our results are based on ganglion cells that were maintained in culture for no more than several days and whose somas had no direct contact with other cells.

Freshly dissociated retinal ganglion cells usually retained processes up to 100 μm in length (Fig. 1), but these disappeared 4 to 6 hours after plating. By 12 hours in culture some ganglion cells had begun to grow new processes. A variety of substrates have been used to increase process outgrowth by retinal and other neurons (*14, 15*). At least some of the ganglion cells regenerated processes on all substrates that we tested (Table 1). For each substrate the percentage of solitary ganglion cells that regenerated processes was directly proportional to the plating density. At each density, however, the proportion of ganglion cells that regenerated processes was consistently greater for those plated on cover slips coated with 2G12 or MRC OX 7 than for those plated on plain glass or on glass coated with other antibodies, polylysine, collagen, or fibronectin. For cultures of relatively high density about 80 percent of the solitary ganglion cells regenerated processes within 24 hours after plating on 2G12 or MRC OX 7 (Table 1) (*16*). In some cultures plated on 2G12 or MRC OX 7 as many as 95 percent of the ganglion cells regenerated one or more processes. Poly-L-lysine (molecular weight, 20,000 to 30,000; Sigma) was

Table 1. Effect of substrate on the regeneration of processes by rat retinal ganglion cells. Each experiment consisted of a single dissociation of cells cultured in multiple dishes. A neuronal process was counted only if it was longer than the diameter of its cell body. To allow for slight variations among experiments, we analyzed the data summarized in the percentage column with an extension of Fisher's exact test for several 2 × 2 tables (*28*). Each table contained the number of ganglion cells with and without processes on two different substrates for a given retinal dissociation. In determining process length, primary and secondary branches but not fine networks were included. This procedure may have underestimated the effect of 2G12 and MRC OX 7, since ganglion cells on these substrates tended to have far more complex arborizations.

Substrate	Cells with processes (percent)	Mean length of processes per cell	Number of experiments	Number of cells counted
2G12	81*	87	15	371
MRC OX 7	74*	70	4	175
Polylysine	49*†	31	4	157
Collagen	45†	30	5	73
Plain glass	33†	29	15	329
IV2G10	32†	24	5	105
RET-N2	24†	27	3	112
Fibronectin	20†	5	4	62

*Significantly greater than value for plain glass ($P < 0.001$). †Significantly less than value for 2G12 ($P < 0.001$) (but polylysine less than value for 2G12 at $0.001 < P < 0.005$).

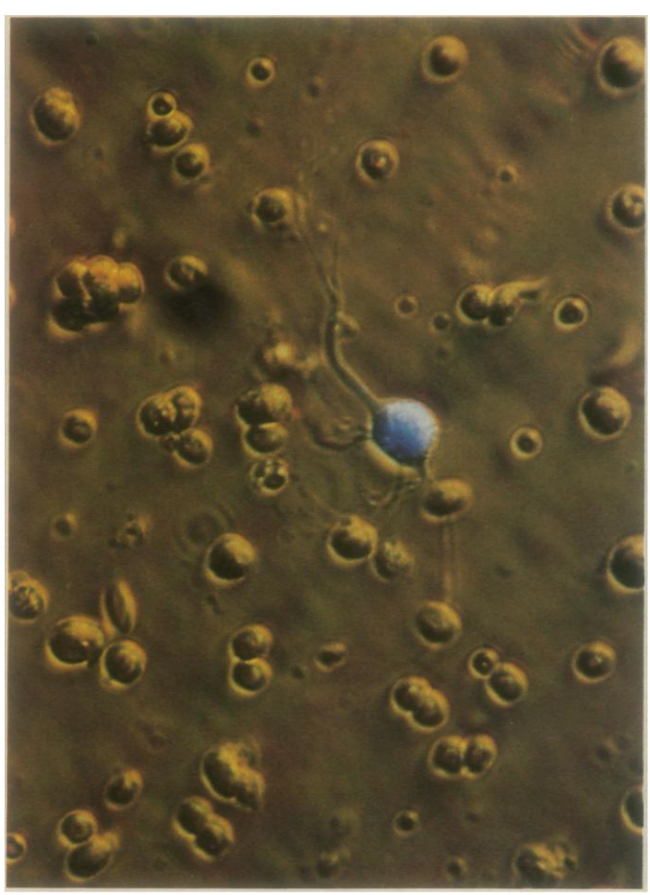

Fig. 1. A freshly dissociated rat retinal ganglion cell, labeled before dissociation by retrograde transport of the fluorescent dye granular blue, lies among other retinal cells. The presence of the blue marker allows rapid and reliable identification of the ganglion cell. Photographed with epifluorescence and phase microscopy.

also significantly better than plain glass, with 49 percent of the ganglion cells regenerating processes—an effect appreciably less than that of 2G12 or MRC OX 7. The average length of processes per cell was substantially greater on 2G12 or MRC OX 7 than on the other substrates, including polylysine (Table 1 and Fig. 2, c and d).

The effect of the monoclonal antibodies to Thy-1 was not an artifact of the presence of immunoglobulin on the cover slips, since coating them with IV2G10, a monoclonal antibody that interacts with intermediate filaments and not the cell surface, did not enhance process regeneration (Table 1) (*17*). In addition, not every antibody that binds to the ganglion cell surface promoted the outgrowth of processes. The monoclonal antibody RET-N2 reacts with all neurons in the retina including ganglion cells (*18, 19*). By day 2 in culture there was, if anything, slightly less process outgrowth on RET-N2 than on plain glass [the difference was not significant (Table 1)].

In other experiments we tested the specificity of the effect of 2G12 for ganglion cells. Amacrine cells are presynaptic to ganglion cells in the retina and were presumptively identified in our cultures autoradiographically by their uptake of tritiated glycine and γ-aminobutyric acid (GABA) (*20*). The growth of these amacrine processes on plain glass was similar to that on glass coated with 2G12 or IV2G10. In each case about 45 percent of the solitary amacrine cells had processes by the second day in culture. On the other hand, plating on polylysine affected putative amacrine cells that accumulated [^{3}H]GABA; 77 percent of these solitary cells had processes. For cells accumulating GABA, the difference between polylysine and plain glass was significant at $P < 0.002$ (Fisher's exact test; $N = 60$ cells for each substrate). This experiment suggests that the effect of 2G12, but not polylysine, is specific for ganglion cells (*21*). The lack of specificity of polylysine is corroborated in other systems in which this large, highly charged molecule enhances outgrowth of processes by many types of neurons (*14*).

To be effective the antibody to Thy-1 had to be bound to the glass cover slip, since supplementing the medium with various concentrations of 2G12 about 4 hours after plating on plain glass did not increase the percentage of ganglion cells with processes. Under these conditions, little if any 2G12 became attached to the glass (as determined with ^{125}I-labeled antibody to mouse immunoglobulin G).

A possible explanation for the effect on regeneration is that antibodies to Thy-1 selectively increase the adhesion of retinal ganglion cell bodies or processes to the substrate. In other systems, adhesion has been implicated in process outgrowth (*14*). We performed a crude test to ascertain whether a cell body was attached to the substrate by agitating the dish and observing the cell. Attachment to the substrate was confirmed for about 100 cells by attempting to penetrate them with a recording micropipette. Insofar as the number of ganglion cells attached to the substrate after these manipulations is a measure of adhesion of the soma (*22*), we found no appreciable differences among the various substrates. However, the adhesion of processes and growth cones rather than the cell body may be important in process regeneration (*14, 23*). The question of adhesion to various substrates should be further studied, perhaps with calibrated pulses of air and interference-reflection optics to observe the points of attachment of processes and growth cones (*14*).

It is also possible that the effect of 2G12 and MRC OX 7 on process regeneration reflects the Thy-1 antigen's function. Recently, other monoclonal antibodies have been shown to inhibit process outgrowth by retinal ganglion cells in the chick (*24*) and sympathetic ganglion cells in the rat (*25*). Specific cell-surface molecules are probably important in the growth and maintenance of neuronal processes (*26*). The methods used here may provide a way of screening for antibodies that interact with these molecules—and hence of identifying them.

Whatever the mechanism by which regrowth is promoted, these results show that identified mammalian retinal ganglion cells can survive and regenerate processes in culture. In addition, certain monoclonal antibodies, such as antibodies to Thy-1, can selectively enhance the growth of specified neurons of the mam-

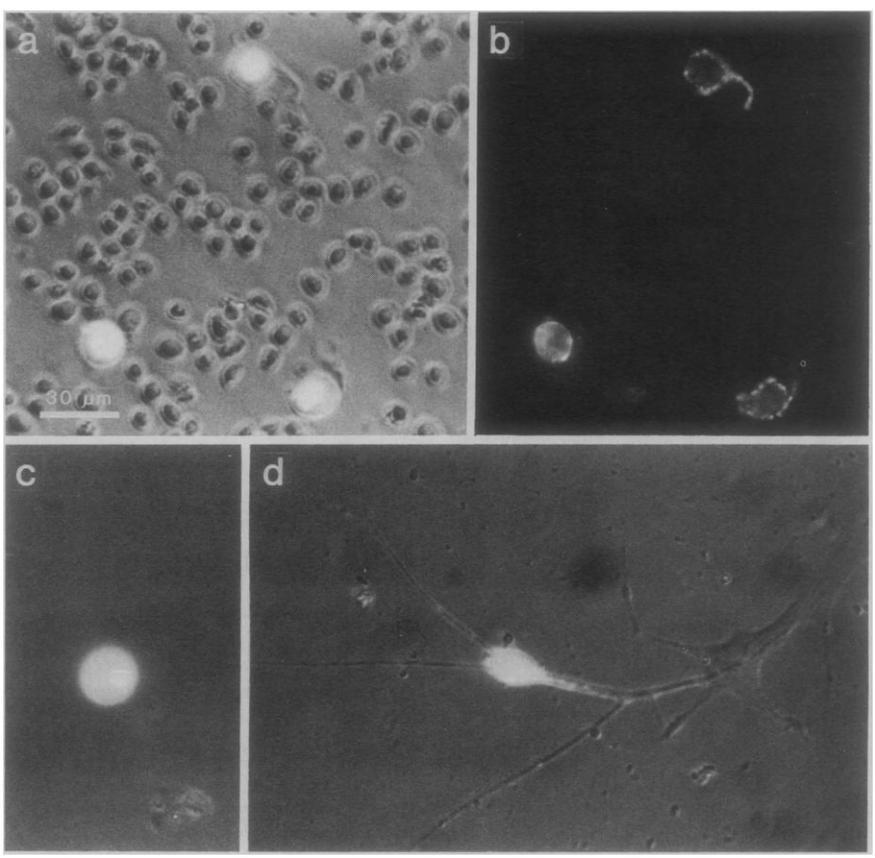

Fig. 2. (a) Three freshly dissociated rat retinal ganglion cells labeled with granular blue after retrograde transport. (b) Cells in the same field labeled by 2G12 and visualized with rhodamine-conjugated goat antibody to mouse IgG. The same three cells were marked by each technique. (c and d) Rat retinal ganglion cells labeled with granular blue and maintained in culture for 3 days. (c) Typical ganglion cell attached to a plain glass surface. It has not developed any neuronal processes. (d) Ganglion cell plated on 2G12. It has developed several processes that contain the fluorescent dye. Many such processes ended with growth cones or with apparent contacts onto other cell types, as shown in this field. Processes grew more than 800 μm in some culture dishes, often forming complicated networks. The photographs in (a), (c), and (d) were made with a combination of epifluorescence and phase optics; (b) with fluorescence only.

malian CNS. Since many human neurons display a Thy-1 molecule on their surface (*5*), these results may have implications for the regeneration of human CNS tissue (*27*).

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29 September 1983; accepted 6 February 1984

Metabolic Mapping of the Brain During Rewarding Self-Stimulation

Abstract. *Local rates of cerebral glucose utilization were measured in rats by the quantitative 2-deoxy-D-[^{14}C]glucose autoradiographic method during electrical stimulation of the ventral tegmental area. Rats trained in intracranial self-stimulation showed a pattern of changes in forebrain metabolic activity distinctly different from the pattern seen in rats stimulated by the experimenter. These findings provide information about the distribution of local cerebral activity specific to reinforced instrumental behavior.*

Rats will perform operant responses to deliver brief trains of electrical pulses directly to their brains. This phenomenon, known as intracranial self-stimulation (ICSS), is frequently used to study the neural mechanisms subserving goal-oriented behavior because the work animals will do to receive electrical stimulation resembles the work they will do to receive more conventional reinforcers such as food and water (*1*). The essence of such behavior is the contingent association between the response and its consequences. By definition, positive reinforcers facilitate behavior only when the rewarding event is made contingent on