dient, was enriched with glia. Small glial cells (microcytes and oligodendrocytes) appeared in fractions 17 and 18 (Fig. 1A) and 16 to 18 (Figs. 1B and 2A), whereas larger glial cells (astrocytes) appeared in fractions 18 to 20 (Fig. 1A) and 18 to 19 (Fig. 1B). The latter fractions also contained capillary tubes and endothelial nuclei which were seen particularly in highest incidence with astrocyte cells (Fig. 2B). The third peak [fractions 25 (Fig. 1A) and 24 (Fig. 1B)], containing purified neuronal perikarya (Fig. 2, C–F) was banded at or near the interface between 58 percent sucrose and potassium citrate, and was cleanly separated from the tail of the second peak. Fraction 22 (Fig. 1B), which precedes fraction 24 (Figs. 1B and 2, E and F) is the result of a moving peak whose purified neuronal contents (Fig. 2, E and F) disappear at $50{,}002 \times 10^6\ \omega^2 t$, including acceleration and deceleration, into the interface of 58 percent sucrose and potassium citrate forming fraction 24. The purity of the recovered neuronal fraction was also assessed by measuring carbonic anhydrase activity (*12*), which is a marker of glial cell concentrations in brain tissue (*11*). The distribution and highest occurrence of enzyme activity (Fig. 1C) are associated with the glial-enriched band (tail of second peak); activity was not detected in the neuronal fraction (third peak).

Our experiments illustrate the potential resolving power and flexibility of zonal ultracentrifugation for fractionation of brain into whole cells. It may become practical to obtain increasingly purified cellular populations of brain in quantities necessary for classical biochemical measurements of metabolism.

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References and Notes

1. Neurons and glia vary in their shape, in mass, and in relation to each other in an intact brain. Thus, it is difficult to relate neurochemical data of a gross sample to its anatomical function. It is therefore relevant to attempt to separate the neurons from the glia and to characterize the chemical properties of each cell type.
2. H. Hyden, *Nature* **184**, 433 (1959).
3. O. H. Lowry, *J. Histochem. Cytochem.* **1**, 420 (1953).
4. B. I. Roots and P. V. Johnston, *Ultrastructure Res.* **19**, 350 (1964).
5. S. P. R. Rose, *Nature* **206**, 621 (1965); *Biochem. J.* **102**, 33 (1967); M. Satake and S. J. Abe, *Biochemistry* **59**, 72 (1966).
6. Pharmacia Ltd., New York, New York; dextran derivative with molecular weight of 400,000.
7. N. G. Anderson, *Nat. Cancer Inst. Monogr.* **21**, 9 (1966).
8. ———, H. P. Barringer, E. F. Babelay, C. E. Nunley, M. G. Bartkus, W. D. Fisher, C. T. Rankin, Jr., *ibid.*, p. 137; N. G. Anderson, D. A. Waters, C. E. Nunley, G. B. Cline, *Fed. Proc.* **25**, 421 (1966).
9. Centrifugal force (number of times gravity) used for particle sedimentation has been expressed in a variety of ways including *g* minutes (number of times gravity × time), speed and time with centrifugal force at either *R* av (mean, radial distance from the axis of rotation to the middle of the gradient cell) or *R* min (minimum, radial distance from the axis of rotation to the miniscus of the gradient cell) and *R* max (maximum, radial distance from the axis of rotation to the bottom of the gradient cell) specified or simply time, speed, and the manufacturers rotor number. We use the quantity $\omega^2 t$ [where rotor speed per second (rev/sec) $=$ π radian/sec $=$ ω], derived from earlier work in which the rotor speeds were accurately determined at intervals during acceleration and deceleration, and the square of the angular velocity (ω^2) was plotted against time to obtain a value proportional to $\omega^2 t$ by simple integration (*7*, *8*). Accordingly, in these studies at 35,000 rev/min for 45 minutes $\omega^2 t$ is approximately $40{,}000 \times 10^6$, not including acceleration and deceleration, where ω equals 1200 π radian/sec (600 rev/sec) and *t* equals 2700 seconds. The Spinco Model L centrifuge used was equipped with an electronic integrator which indicated the integral of $\omega^2 dt$ continuously in digital form (*8*). The advantage of such integration is that the total centrifugal force (number of times gravity × time) applied in an experiment can be controlled for reproducibility (that is, between experiments to reach a certain predetermined value), and the value is indicated at once.
10. R. E. Dill and J. H. Martin, *Texas Rep. Biol. Med.* **24**, 720 (1966).
11. E. Giacombi, in *Morphological and Biochemical Correlates of Neural Activity*, M. M. Cohen and R. S. Schneider, Eds. (Harper and Row, New York, 1964), p. 15. Giacombi has estimated carbonic anhydrase in neurons and glia of Dieter's nucleus, isolated by microdissection, and has calculated that the enzyme is 100 to 120 times more active in the glia than in the neurons. Carbonic anhydrase thus represents a glial marker by which we could determine glial contamination of the fractions.
12. Carbonic anhydrase activity was determined by the electrometric method of R. E. Davis [*J. Amer. Chem. Soc.* **80**, 5209 (1958)].
13. Supported by PHS grant MH13258, University of Wisconsin, and by the Biophysical Separation Laboratory at Oak Ridge National Laboratory, Oak Ridge, Tennessee. We thank Dr. Norman G. Anderson for facilities during July and August 1967. We also thank C. T. Rankin, Jr., L. H. Elrod, and K. Wilcox for technical assistance and operation of the zonal-ultracentrifugation equipment; Dr. D. Brown for electrometric analysis of carbonic anhydrase activity; and Dr. R. E. Canning for cooperation in procurement of chemicals and supplies.

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Mice: Fighting by Neonatally Androgenized Females

Abstract. *Administration of testosterone propionate to female mice on the day of birth resulted in increased fighting after administration of testosterone during adulthood. This fighting, comparable to fighting among normal male mice, suggests that early androgenic stimulation organizes neural structures mediating aggression in the mouse.*

When two male mice that have been socially isolated for some time are placed together, aggression is invariable. Relatively little is known about the development of the neuroendocrine determinants of this behavior. One report (*1*) indicates that, if the male mouse is castrated before puberty, fighting is rare. Treatment of the castrate with exogenous testosterone results in arousal of the propensity to fight. Unlike males, adult female mice do not fight when testosterone is administered (*2*). This difference in response by male and female mice to testosterone may reflect differences in the nature or sensitivity of brain systems underlying aggression. Much research on the neural mechanisms underlying sexual behavior (*3*) indicates that males do differ from females with respect to some brain characteristics, and that these differences result from differential androgenic stimulation during a limited critical period of development. I sought to determine whether or not differences between adult males and females in fighting also are determined by the presence or absence of early stimulation by androgens.

Some Swiss-Webster litters of mice were injected subcutaneously with 0.5 mg of testosterone propionate within 24 hours of birth. The result was genital virilization characterized by small vaginal opening and hypertrophied clitoris in all females. Control litters received injections of peanut oil within 24 hours of birth, without genital effect. The young mice were kept with the mother until 30 days of age, when all were weaned and gonadectomized; they remained in the litter of birth until 60 days of age, when the males that had received testosterone at birth were eliminated from the study, the remainder being individually caged.

The remainder constituted three groups: (i) males treated with oil at

Table 1. Percentages of pairs of mice fighting at least once, and mean latencies to fighting when fighting occurred.

Group	Pairs		Latency (min)
	No.	Fighting (%)	
OM	16	100.0	5.1
TPF	16	93.8	5.0
OF	12	8.3	

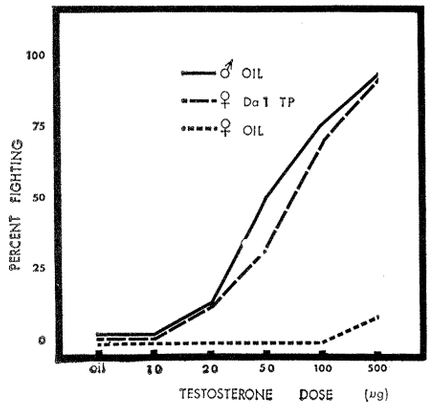

Fig. 1. Effects of neonatal stimulation by testosterone on adult fighting in mice. Represented are the percentages of pairs in each group fighting at various dosages of testosterone during adulthood.

birth (OM), (ii) females treated with oil at birth (OF), and (iii) females treated with testosterone propionate at birth (TPF). During the 1st week of isolation all mice were injected subcutaneously daily with 0.05 ml of oil. On the 6th and 7th days of the 1st week, all mice were tested for fighting; none fought. Successively during each of the subsequent 5 weeks, all subjects received daily injections of 10, 20, 50, 100, and 500 μg of testosterone; on the 6th and 7th days of each week they were tested for fighting. Thus during successive weeks they were tested under progressively higher doses of testosterone.

For testing, two mice from the same group were paired in a neutral cage. If they fought within 10 minutes, the latency from the start of the test to the beginning of the fight was recorded to the nearest minute, and the test was terminated by restoration of each mouse to its home cage. A "fight" was scored if one mouse (or both) persistently attempted to bite the other. The same individuals were paired for each test.

More than 90 percent of the pairs of virilized females showed fighting during at least one test. One hundred percent of the pairs of males fought. Only one pair of group OF ever fought. Once fighting occurred, it persisted in most subsequent observations under conditions of increased dosage of testosterone.

For each pair in the OM and TPF groups, a mean latency to fighting was calculated for those tests on which fighting occurred. Analysis of variance of the data on latency (Table 1) failed to indicate any significant difference between males and virilized females.

When the dose of testosterone during adulthood was increased, the proportions of pairs of males and of virilized females showing fighting were increased (Fig. 1). The close correspondence of the curves for the OM and TPF groups indicates that these groups were little different in their response to testosterone; at no dose level was the difference in proportion of pairs fighting statistically significant. In constrast, females treated with only oil at birth failed to fight even when given large doses of exogenous testosterone in adulthood. Chi-square comparisons of group OF with group TPF are significant beyond the 0.05 level at all dose levels greater than 20 μg per day.

These data clearly indicate that administration of testosterone to the neonatal female mouse facilitates the display of testosterone-aroused fighting in adulthood; they suggest that the usually observed difference between normal male and female mice, with respect to fighting, is due to the fact that males are stimulated by testicular androgens early in life. One may presume that the stimulation by endogenous testosterone in the male (and exogenous testosterone in the female) can "organize" or cause the differentiation of a neural substrate for fighting.

The ability of early androgenic stimulation to affect adult patterns of behavior has been clearly documented in several species for a variety of sexually dimorphic behaviors (*3*). In these instances, androgenic stimulation affected behavioral differentiation only when this stimulation occurred during a particular and limited period during maturation. It is not yet clear to what extent the "organizing" capacity of androgens with respect to adult fighting in mice is similarly time-limited. Data now being collected in our laboratory (*4*) suggest that the period, during which administration of testosterone promotes fighting in the female mouse, may extend well beyond the 10th day after birth.

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References and Notes

1. E. A. Beeman, *Physiol. Zool.* **20**, 373 (1947).
2. D. A. Edwards, in preparation.
3. C. H. Phoenix, R. W. Goy, A. A. Gerall, W. C. Young, *Endocrinology* **65**, 365 (1959); R. W. Goy, *J. Anim. Sci.* **25**, 21 (1966); R. E. Whalen and D. A. Edwards, *Anat. Rec.* **157**, 173 (1967).
4. J. Tollman and J. A. King, *Brit. J. Anim. Behav.* **6**, 147 (1956).
5. Supported by PHS predoctoral fellowship 2F1-MH 30315-03. I thank R. E. Whalen for advice and encouragement. The hormones were supplied by P. Perlman, Schering Corp., Bloomfield, N.J.
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Red and Far-Red Light Effects on a Short-Term Behavioral Response of a Dinoflagellate

Abstract. *Cessation of movement (stop response) is used as a criterion for light reception by the dinoflagellate* Gyrodinium dorsum *Kofoid. Brief irradiation (2 seconds at 470 nanometers) elicits a stop response in cells any time during the 6-minute interval after removal from growth lights. This stop response is inactivated by exposure for 4 minutes to 470-nanometer light prior to stimulation. Red light (620 nanometers) reactivates this stop response, and far-red light (700 nanometers) reverses this reactivation. This red–far-red photo reversibility is taken as evidence for phytochrome involvement.*

Phytochrome mediates several long-term growth responses (flowering, seed germination, stem elongation) in higher plants (*1*). Studies of leaf movement in *Mimosa* (*2*) and in *Albizzia* (*3*), and of algal chloroplast movement (*4*), suggest the participation of this pigment in short-term light responses, not mediated by way of effects on RNA metabolism. Phytochrome, however, has not been implicated in the control of movement in motile lower plants, perhaps because the effects of prior irradiation on the light-induced behavioral response have been ignored. We now report the possible involvement of phytochrome in a short-term response of the dinoflagellate *Gyrodinium dorsum* Kofoid.

Our culture procedure and experimental apparatus remain as reported (*5*) with the following modifications. Experiments were only conducted 8 hours after the beginning of a light period, on 5-day-old cultures grown on a 16-hour-light, 8-hour-dark cycle. Stimulation sources were a Bausch and Lomb No. 33-86-02 grating monochromator with a 20-nm bandpass between 400 and 740 nm, and a Toyoda microscope lamp in combination with