to proline and hydroxyproline, glycine is normally excreted in mouse urine; we did not observe abnormally high amounts of glycine in the urine of PRO/Re mice. The mice do excrete a substance(s) that causes the pine shavings to exhibit a bright yellow color (*10*). This might be indicative of some type of abnormal renal transport mechanism (*11*), although the plasma appeared clear on visual examination. Proline did not stain pine shavings. When the shavings were treated with aqueous proline, proline mixed with urine from the parental lines, or proline mixed with urine from PRO/Re mice, no unusual stain was observed when compared to control shavings treated with only water or urine.

In summary, this report describes the discovery of an unusual biochemical characteristic of proline metabolism, hyperprolinemia, occurring in a new inbred strain of mice now designated the PRO/Re strain. An elevation of the blood concentration several times that in the normal animal is indicative of an "over-flow" type disorder of amino acid metabolism, perhaps similar to one of the types of hyperprolinemias known to occur in human beings. We believe that the PRO/Re strain may serve as an animal model for similar types of biochemical disorders in man and may also be useful in studies on (i) the comparative biochemistry and physiology of mammalian proline metabolism, (ii) the genetic transmission of biochemical traits, and (iii) the structural and functional organization of the genome in *Mus musculus* (*12*).

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6. The PRO/Re strain was developed from crosses between 129/ReJ (A^w/A^w, c^{ch}/c^{ch}, p/p) and C57BL/6J (a/a) inbred strains. After the genotype of particular mice was determined to be a/a, c^{ch}/c^{ch}, p/p, they reproduced by brother-sister inbreeding for over 25 generations without interruption to produce a highly inbred strain now designated the PRO/Re strain. Gene symbols referred to here are as follows: a, nonagouti; A^w, white-bellied agouti; c^{ch}, chinchilla; p, pink-eyed dilution.
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9. The amino acids in urine were first resolved by paper chromatography using the water-saturated phenol solvent that effectively separates proline from hydroxyproline. The developed chromatograms were dipped in the isatin reagent, dried, and then dipped in freshly prepared Ehrlich aldehyde reagent. A red-purple spot of increasing intensity appears if hydroxyproline is present.
10. We thank Ethel Anthony who helped maintain the PRO/Re colony of mice and first noticed the staining of the pine shavings, and Margaret Singleton and Marilyn Dolliver for their technical assistance.
11. In type I hyperprolinemia in man, there is a renal defect which occurs simultaneously with a deficiency of liver proline oxidase. In type II hyperprolinemia, there is no evidence of a renal defect, and the enzyme studies suggest a deficiency of Δ^1-pyrroline-5-carboxylate dehydrogenase. The existence of renal abnormalities or of enzyme lesions in the liver of the PRO/Re mice is yet to be determined. The mode of inheritance of the hyperprolinemia is also undetermined at this time. In type I hyperprolinemia, the renal disease appears to be transmitted from generation to generation as a single unit factor. However, the hyperprolinemia is not present in either the parents or the children of affected patients, a suggestion of a more complex genetic mechanism for the expression of the metabolic abnormality.
12. During the preparation of this manuscript, we observed that both the hyperprolinemia and prolinuria can be detected in PRO/Re mice at 4 weeks of age.
13. We thank NIH for financial support (grants AM 14769-01 and CA 01074), and the Southwaite Foundation for partial support. The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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Unit Responses to Moving Visual Stimuli in the Motor Cortex of the Cat

Abstract. *Neurons in the pericruciate cortex of the cat were tested with moving visual stimuli for responses to specific properties of the visual receptive field. Specific response patterns were shown by cells of origin of the pyramidal tract as well as by other cells.*

Processing of visual information at the cortical level has been investigated, and complex receptive field properties (that is, specificity to movement, orientation, and shape) have been demonstrated for unit responses in areas 17 to 19 of the cat visual cortex (*1, 2*). Similar studies of unit responses to stationary and moving visual stimuli in the anterior middle suprasylvian association area (AMSA) of the cat have revealed movement and orientation specificity for a number of "association" neurons (*3*).

The work of Buser and Imbert (*4*) and others (*5, 6*) revealed that unit responses from the cat motor cortex can be elicited by visual, auditory, and somatosensory stimuli and hence are polysensory. The study reported here indicates that some neurons in the pericruciate "association" cortex (PCA) of the cat are also responsive to oriented and moving stimuli. Both cells of origin of the pyramidal motor system (PT units) and nonpyramidal tract units (NPT units) in the PCA display such specific visual properties.

Fourteen cats were anesthetized with chloralose (70 mg/kg), and standard surgical techniques were used to expose the PCA. The area investigated was limited to the PCA, and the region of the frontal eye fields was not invaded (*7*). The nictitating membranes were removed, and the eyes were dilated with topical methyl atropine. A concentric bipolar electrode was placed in the ipsilateral medullary pyramidal tract for identification of PT cells by antidromic stimulation (*6, 8*). After surgery the animal was placed in a special atraumatic head holder that did not interfere with the presentation of visual or auditory stimuli (*9*). Standard procedures were used in recording individual unit responses from the tungsten microelectrodes.

Units were tested for polysensory properties by presenting an auditory free-field click, a visual flash from a photostimulator, and a tactile single pulse shock to the ipsilateral forepaw. Moving white stimuli were projected on a darkened tangent screen (1 by 1 m), centered 1 m from the eyes, by

a 35-mm projector that was 1 m behind the screen. Stimuli were of constant illumination in an otherwise darkened room. The stimuli were primarily bars measuring from 4° by 0.4° to 24° by 3.4° (in degrees of visual angle), although 4° to 6° circles and various edges were used as well. The stimuli were moved across a 34° (horizontal) by 34° (vertical) visual field with the visual axis centered 12° above the lower edge of the visual field. The stimuli were swept across the visual field and returned to the point of origin, beginning at 0°, 45°, 90°, and 135° from horizontal, at velocities ranging from 10° to 140° sec^{-1}.

After cell identification and polysensory testing the animal was presented with moving visual stimuli by the method of Hubel and Wiesel (*1*); the longest bar (24° by 0.4°) was shown first to determine the optimal size, direction, and orientation of the stimulus in the field and to determine the size of the receptive field itself. Unit responses to moving stimuli were photographed, and the receptive field was sketched on a sheet of transparent paper attached to the screen.

Of the 24 units tested, 20 were polymodal, responding with a 30- to 35-msec latency to auditory, visual, and tactile stimuli. Thirteen of the tested units were identified as PT units, nine were identified as NPT units, and two were unclassified. The most effective visual stimuli for eliciting a unit response were bars of various lengths and widths, although circles and edges could often drive units if these stimuli were presented in the same orientation and direction as the bars.

Of the tested units, ten could be visually driven only by the flash or the onset or offset of steady illumination, while 14 units responded to the flash or to steady illumination, or to both, and gave responses to moving stimuli. Of these 14 units, 6 (4 PT, 2 unclassified) responded to stimuli across a wide portion of the visual field and to most orientations and directions of the moving stimuli. In most of these units, the strongest responses were to stimuli at the center and outer edges of the field. These units typically responded equally well to bar, circle, and edge stimuli. Eight units (three PT, five NPT) responded to moving visual stimuli in a more restricted portion of the field. For these units only one orientation of the stimulus usually proved effective. In addition, response to the stimulus usually was greater for one direction of movement than for another. Of these units, five (two PT, three NPT) gave the strongest response to a horizontal bar moving vertically and crossing the visual field in a region 0° to 12° below center (see Fig. 1 for an example of this type of cell).

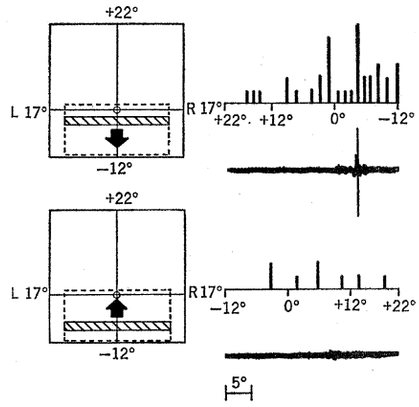

Fig. 1. Responses of a pyramidal tract cell with restricted receptive field to a horizontal bar of light moving downward (upper graph) or upward (lower graph). Above the unit response is a histogram showing incidence of firing as a function of location of the light bar. The dotted area on the representations of the visual field shows the receptive field of this unit, which was driven only by a light bar moving downward. Other directions of movement were as ineffective as the upward motion. Dimensions are in degrees of visual angle (*L*, left; *R*, right).

Dimensions of the receptive field varied from unit to unit; in some units receptive fields encompassed the entire lower visual field while in others they were limited to areas a few degrees high by a few degrees wide. Two units (one PT, one NPT) responded best to a vertical bar crossing the visual field horizontally. Receptive field sizes for these units appeared to be 34° high by 5° to 10° wide in either the right or left visual field. One unit (NPT) responded best to an oblique bar moving at a 45° orientation; the visual field for this unit was a 17° square in the upper right visual field. In all of these units, the best responses were to bars of various dimensions although weaker responses to the circles and edges could be obtained at times. In general the optimal stimulus velocity for each unit was in the range of 25° to 75° sec^{-1}. Although other velocities would drive the unit, the responses fell off drastically as the velocity was increased or decreased from the optimal value. The optimal velocities reported here are less than those reported for receptive field neurons in the AMSA (*3*) and greater than those reported for primary visual cortex (*1*, *2*).

In 9 of the 14 units responsive to movement we tested monocular as well as binocular responses to the optimal stimuli. In all instances we found that each unit could be driven by either eye alone, although in some cases the activity (in terms of incidence of firing) elicited by one eye was greater than the activity from the other eye. We have also observed that units responsive to movement (as well as nonresponsive units) often are located sequentially along a single microelectrode track and that they frequently have similar receptive field characteristics. This observation may suggest a columnar and perhaps functional organization of cells in the PCA similar to that described for the primary visual cortex (*1*, *2*).

Our results indicate that some neurons in the cortical area PCA of the cat exhibit specific receptive field responses to moving visual stimuli. The receptive field properties we described seem in many ways similar to those previously described for neurons in the AMSA (*3*). It seems clear that coding of moving visual stimuli occurs in the PCA, with both PT and NPT cells involved in about equal numbers. The PT units were found to have both wide and restricted receptive fields. All NPT units were found to have restricted fields. The fact that PT cells are involved may suggest they can be described as serving both "sensory" and "motor" functions and may necessitate a reevaluation of the classical notions of the functions of the pyramidal motor system.

The results suggest either that neurons in the PCA, like those in the primary visual cortex and the AMSA, are involved in the processing of visual information, or that the presence of neurons responding to specific aspects of the visual receptive field does not necessarily imply that the cells are performing a simple or specific sensory function. In accord with the latter argument it is conceivable that units in the PCA are responding only to certain properties of the stimulus, such as movement, that convey adaptive information about the environment, and that the response is independent of the details of the stimulus.

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Human Virus Vaccines: Why Monkey Cells?

Petricciani *et al.* (*1*) state that, in regard to the human diploid cell strain WI-38, "reservations" and "theoretical objections" exist "about the use of parenterally administered vaccines made from such cells." Regrettably they do not state what their "reservations" or "theoretical objections" are; and, consequently, such gratuitous, unreferenced pronouncements are pejorative on two counts. First, it could be inferred from the language used, that this opinion is shared by many or all national control authorities and their scientific advisers on human virus vaccines. Human vaccines, prepared in WI-38, currently in use and administered by the parenteral route to more than 1 million people have been licensed in the United Kingdom, France, Yugoslavia, and in the U.S.S.R. (*2*). For this reason and because of other published position statements, such a conclusion is, in my view, unwarranted (*3*). Second, the use of such vague statements as "reservations" and "theoretical objections" without revealing what these are, effectively prevents rebuttal. Whenever these terms are clarified, interested parties should be given an opportunity to reply.

One is forced to conclude that the "reservations" or "theoretical objections" held by Petricciani *et al.* (*1*) have failed to impress a substantial proportion of the scientific community including several major national control authorities. Furthermore, any "reservations" or "theoretical objections" that will be proposed by Petricciani *et al.* are equally applicable to any other cell population including, and especially, the monkey cells developed by Wallace [reference 5 in (*1*)].

To state that tests for oncogenicity of monkey cells are better because such cells can be inoculated into monkeys and that this "allows a latitude of testing that does not exist for human diploid cells" is inaccurate on several counts. First, viable normal human cells, including human diploid cell strains, have been inoculated into man with no evidence of tumorigenicity (*4*, *5*). Since hypothetical vaccines to be grown in the monkey cell substrates developed by Wallace are apparently intended for human parenteral use, do Petricciani *et al.* intend to inoculate these monkey cells into man to demonstrate presence or absence of tumorigenicity? Anything short of that begs the question. Second, despite this type of test, or any other test, the potential tumorigenicity of cells derived from any animal species cannot be ascertained with absolute certainty. Finally, if the Division of Biologics Standards (DBS) now recognizes the necessity for tumorigenicity testing of cell substrates used for human virus vaccine preparation, why are such tests not required for primary monkey kidney, dog, rabbit, duck, and chicken cell substrates? If the system for tumorigenicity testing that they now advocate, and which has been known for decades, has merit, why are studies on this question only now "in progress"?

As regards WI-38, vaccines produced in these cells have not been found to be tumorigenic in over 1 million individuals parenterally inoculated, nor in nearly 1 million recruits that have received adenovirus vaccine in enteric coated capsules, nor in the several million individuals that have received oral polio vaccine.

Petricciani *et al.* say that "vaccines produced from monkey kidney cell cultures have been overwhelmingly successful" . . . "with no evidence of untoward reactions." There are, however, other factors that should be weighed when the use of monkey kidney cells as a substrate for preparation of human virus vaccines continues:

1) Twenty-three people have died as a result of handling monkeys or their cultured cells (*6*). The most recent incident involving human fatalities (Marburg agent) caused the DBS to halt for several months the licensing of new vaccine lots produced in monkey kidney cells. This incident in which seven individuals died in Germany in 1967 resulted from contact with organs and cell cultures derived from the green monkey and from the tissue culture vessels themselves (*6*).

2) A substantial number (25 to 80 percent) of monkey kidneys processed for vaccine manufacture must be discarded because of extensive contamination with one or more of 20 known viruses.

3) The annual slaughter of monkeys for primary cultures has reached such proportions that several species are endangered (*6*).

4) At least several hundred thousand people in this country have been inoculated with live SV40 found in polio vaccines produced in monkey kidney cells. This virus produces tumors in hamsters and converts normal human cells to cancer cells in vitro.

Petricciani *et al.* justify the expenditure of about a million dollars in contract funds by the DBS to develop monkey diploid cell strains on the basis that "alternatives to WI-38 should be explored." Surely these expenditures and the energies and resources of many scientists should be justified by more than mere unstated "reservations" and "theoretical objections" to a cell substrate now widely used throughout the world for human vaccine preparation. It is noteworthy that when we advocated the use of human diploid cells as an "alternative" to primary monkey kidney (*4*) it was for 10 years unacceptable to the DBS (a U.S. license for the use of attenuated poliomyelitis vaccine produced in WI-38 has just been issued by DBS to Pfizer.)

The work described by Petricciani *et al.* was done under contract to DBS by Lederle Laboratories [reference 5 in (*1*)] and represents one of several examples where DBS engages in activities in which serious conflicts of interest are bound to result. In my view, and in the view of others, no control authority should be in the business of developing products or product components that they themselves will ultimately control. Nor should any control authority be allowed to sit in judgment of products or product components when the choice is between substances developed by that control authority and