Subcellular fractionation of rat brain showed the greatest enrichment of opiate receptor binding in the microsomal fraction, which is rich in membrane fragments (Table 2). About half of the total opiate receptor activity could be recovered in the crude mitochondrial-synaptosomal fraction. The crude nuclear fraction contained the least opiate receptor binding of all fractions examined. No opiate receptor binding could be demonstrated in the soluble supernatant fraction (Table 2).

In what type of nervous structures is the opiate receptor localized? Effects of opiates have been demonstrated on acetylcholine (*7*), serotonin, and norepinephrine (*8*). Thus, as with most psychoactive drugs, effects can be demonstrated on several neurotransmitter systems so that it is impossible to ascertain which of these represents the primary action of the drug. Our regional study of opiate receptor binding in rat brain revealed the most binding in the corpus striatum, whose binding exceeded that of the cerebral cortex more than fourfold. No opiate receptor binding was detectable in the cerebellum. Of the known neurotransmitters only dopamine and acetylcholine are highly concentrated in the corpus striatum. Dopamine concentrations are low in most other regions of the brain, and its regional variations do not parallel those found for the opiate receptor (*9*). By contrast, regional differences in acetylcholine concentrations more closely parallel the variations we have observed in opiate receptor binding. Both for opiate receptor binding and acetylcholine concentrations, there is a marked difference between corpus striatum and cerebral cortex, and negligible concentrations of acetylcholine and opiate receptor binding occur in the cerebellum (*10*). If the opiate receptor is associated with a known neurotransmitter, acetylcholine is the most likely candidate.

Goldstein *et al.* (*11*) reported binding of radioactive levorphanol to mouse brain homogenates, about 2 percent of which showed stereospecific differences between levorphanol and dextrorphan. It is unlikely that the binding of these two compounds was pharmacologically relevant for the following reasons. Maximum differences between levorphanol and dextrorphan were obtained at $3.9 \times 10^{-3} M$, concentrations at which we found that the specific opiate receptor sites are fully saturated for both drugs so that no differences can be detected. Moreover, Goldstein *et al.* (*11*) reported that the degree of stereospecificity increased with increasing concentrations of levorphanol and dextrorphan, whereas we found that stereospecific differences decrease with increasing concentrations. Our results differ from those of Goldstein *et al.* (*11*) in several other ways. They reported the greatest amount and activity per unit weight of binding in the crude nuclear fraction, whereas we observed the highest specific activity of receptor binding in the microsomal fraction and the greatest recovery in the crude mitochondrial-synaptosomal fraction (Table 2). Moreover, they failed to find any regional variations of binding in the brain.

As A. Burgen (*12*) has pointed out, "Contemporary ideas of drug action and drug specificity are all based on the assumption that the initial process in drug action is the formation of a reversible complex between the drug and a cell component generally known as the drug receptor." Only drugs that present a high degree of molecular complementarity toward the site at which they act are believed to be able to form this drug-receptor complex (*12*). We have shown that a component of nervous tissue can selectively form complexes with opiate drugs at very low concentrations. Since this binding is highly stereospecific and corresponds well with the previously reported pharmacological potencies of opiates, we believe that our results represent a direct demonstration of "opiate receptor" binding.

Identification of the opiate receptor provides new insight into the mechanism of action of opiates. Our binding assay affords a rapid means of determining the relative potencies of potential narcotic agonists and antagonists, with attendant theoretical and practical implications.

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Genetic Mechanisms Determining the Central Visual Pathways of Mice

Abstract. *Albino mammals have abnormal visual pathways. "Flecked" mice have a variegated pigment distribution, with roughly half the cells normally pigmented and half albino. They do not show the albino abnormality in the visual pathways. The gene at the albino locus, which determines the course of the visual pathways must, thus, have an extracellular action.*

The central visual pathways of albino mammals are abnormal (*1*, *2*). Parts of the retina that normally give rise to uncrossed retinofugal axons send axons across the midline in albinos. These segments of abnormal retina, which have well-defined borders (*2*), must contain ganglion cells that are wrongly specified concerning the central course of their axons. If the primary abnormality involved a deviation of fibers at the level of the chiasm, where, normally, the retinofugal fibers are not arranged in strict retinotopic order, then the borders of the abnormally connected retina would not be well defined.

Since an abnormally small ipsilateral pathway has been found in albinos of

several unrelated mammalian species, the albino locus must be playing a part in controlling the central growth of retinofugal axons. It is known that the albino locus determines the formation of tyrosinase (*o*-diphenol oxidase, E.C. 1.10.3.1), and thus controls the intracellular formation of melanin (*3*). The manner in which the albino gene acts upon the retinal ganglion cells to affect the chiasmatic growth of their axons is not known. The action may involve tyrosinase; however, since retinal ganglion cells do not contain melanin, there may be a quite different action. Cattanach's translocation in the mouse (*4, 5*), in which the normal allele at the albino locus has been transferred to the X chromosome and so has become subject to random inactivation in the female (*6*), offers an interesting opportunity for studying the development of the abnormality. Female mice that are homozygous for the allele for albinism and heterozygous for this translocation display the characteristic albino-variegated or flecked coat, and in the pigment layer of the retina one sees small patches of pigmented cells interspersed with patches of albino cells (*7*).

If, in the production of the abnormal chiasmatic pathway, each ganglion cell acts independently and specification of a cell as ipsilateral or contralateral depends upon the enzymes produced within that cell, then a flecked mouse will have an abnormal pathway roughly half as large as that of an albino mouse. This is a reasonable expectation because the other effects of the albino gene, that is, on pigmentation, are intracellular. If, however, the mechanism responsible for the abnormality is intercellular, involving materials that diffuse between cells, or mechanical interactions between cells, then the flecked mice could show an abnormality equal to that of the albino mice, no abnormality at all, or an abnormality somewhere between the two in size.

The retinogeniculate pathway has been studied in albino and fully pigmented mice by staining degenerating axons by the Nauta method after section of one optic nerve. The ipsilateral pathway to the lateral geniculate nucleus is reduced in albino mice, as in albinos of other species. The size of the uncrossed pathway has also been determined for flecked, albino, and fully pigmented mice by counting the number of degenerating axons that could be seen in electron micrographs of the optic tract after the ipsilateral eye had been removed. Initial studies of the optic nerve close to the chiasm showed that a number of normal axon profiles are still present in the nerve 7 days after removal of an eye. By 11 days there are very few normal profiles left. Because the degeneration products begin to be cleared quite rapidly and because one can expect the axons in the optic nerve and tract to degenerate at approximately the same rate in all animals of the same age, it was decided to study the degeneration in the tract after 11 days.

Table 1. Number of degenerating axons in optic tracts of pigmented, albino, and flecked mice. Pigmented mice are of the inbred strain JUN/Ct. Albino mice are F_1 hybrids of the cross of the albino strains JU/FaCt (coisogenic with JUN/Ct) and A/H. Flecked mice are the chromosomally unbalanced duplication form of Cattanach's translocation, *T(7;X)Ct* (*4*), maintained by crossing flecked females with F_1 hybrid JU/FaCt × A/H males. Therefore, albino and flecked mice have roughly the same genetic background, half of which is shared by the pigmented mice.

Animal	Type	Degenerating axons (No.)
49	Pigmented ♀	506*
50	Pigmented ♀	554
60	Pigmented ♀	644
52	Albino ♂	400
53	Albino ♂	305
54	Albino ♂	385
55	Flecked ♀	716
65	Flecked ♀	644
66	Flecked ♀	714
67	Flecked ♀	732

* Mean of two independent counts: 492 and 521.

All of the experimental mice were born between 18 and 29 December 1971, and one eye was removed on 8 or 9 June 1972. The mice were briefly anesthetized with ether, one eye was cut out by aseptic procedures, and the animals were given Intramycetin postoperatively. Eleven days later the mice were again anesthetized with ether and were perfused through the heart with a solution of 1 percent paraformaldehyde and 1.25 percent glutaraldehyde in 0.1*M* cacodylate buffer. This was immediately followed by a second, similar solution containing 5 percent glutaraldehyde.

The brains were left in this second solution in a refrigerator (4° to 10°C) for 2 to 3 hours. Thin slices were then taken through the optic tract about 1 mm behind the chiasm, and these were rinsed in buffer, transferred to Dalton's fixative (*8*), dehydrated slowly in ethanol, and embedded in Araldite.

Thin sections containing the whole optic tract were cut perpendicular to the axons and mounted on large-hole grids. Electron micrographs were taken of all parts of the tract. Prints were made at a magnification of 3400 diameters, and these were fitted together so that all of the degenerating axons in one section could be counted without counting any axon twice.

An axon was regarded as degenerating if its axoplasm was electron-opaque, if the myelin was clearly deformed into loops or folds, or if the myelin contained no axoplasm. Only myelinated axons were counted, and the counts were made without knowing the source of the section.

In all of the mice, the degenerating ipsilateral axons are heavily concentrated in the antero-inferior parts of the optic tract. This distribution makes it difficult to use a sampling technique to count the axons. It also demonstrates that the criteria we have used exclude almost all normal axons, since practically no degenerating axons were seen in the posterio-superior parts of the tracts.

The results of the counts are shown in Table 1. In the albino animals the ipsilateral component is, as expected, markedly smaller than in the normal, pigmented mice. In the flecked mice the ipsilateral component is, surprisingly, somewhat larger than it is in the pigmented animals.

The main conclusion to be drawn from these results is that the abnormality is not seen in the flecked mice. All of the retinal segments that are abnormal in the albinos must be normal in the flecked mice, even though only about half or slightly more (*9*) of the cells in these segments form pigment. Our results do not show the mechanism that produces the abnormality, but they do limit the possibilities. If the absence of pigment itself is responsible, then the axonal misrouting could be produced by mechanical deformations of the eye cup or optic stalk that may occur when pigment formation is absent during development. It is possible that pigment formation is irrelevant. The lack of tyrosinase may affect the concentration of some other substance which can diffuse between cells and influence the course taken by some of the ganglion cell axons, or the albino gene may determine a process that is independent of tyrosinase production, and this process may act upon the ganglion cells.

We cannot explain why the numbers are higher in the flecked than in the fully pigmented mice. It remains to be determined whether the large ipsilateral

component characterizes all flecked animals, or whether there are other genetic factors that play a part in determining the size of this component.

The situation described here resembles the ocular albinism that occurs in man (*10*). This is a sex-linked abnormality. Affected males show the visual abnormalities that characterize full albinos, while the carrier females show a patchy distribution of retinal pigment and no visual defects. One could argue that if there were abnormal retinal patches in the females, these could be too small to be identifiable clinically, but it seems more reasonable to conclude that the females are like the flecked mice and have a completely normal visual system.

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Swallowing in Fetal Sheep

Abstract. *Swallowing was measured in fetal sheep by using electromagnetic flowmeter heads chronically implanted in the fetal esophagus. The fetus swallows 20 to 200 milliliters of amniotic fluid in two to seven discrete episodes per day. The episodes are 1 to 9 minutes in duration and occur at seemingly random intervals. Swallowing is influenced by the condition of the fetus and may be the first manifestation of eating and drinking behavior.*

It is well established that many mammalian species, including rats, guinea pigs, sheep, and humans, swallow in utero (*1, 2*). The only quantitative data on fetal swallowing, however, are estimates of volume swallowed per 24 hours by humans (*2*). Fetal swallowing activity may contribute to the control of amniotic fluid volume and may be the forerunner of adult eating and drinking behavior. Before the possible functional significance of fetal swallowing can be understood, the behavior itself must be studied in more detail. In this study swallowing was measured in fetal sheep by means of an electromagnetic flow transducer chronically implanted in the fetal esophagus. The method provides continual data over periods of weeks on the frequency of intrauterine swallowing and the volume of amniotic fluid swallowed at any given time.

Nine dated pregnant ewes in the last third of gestation were operated under sterile conditions. The general surgical procedure is as described in Dawes *et al.* (*3*). A midline abdominal incision was made to expose the uterus. The fetal head was palpated through the uterus and withdrawn through the abdominal incision. The uterus and fetal membranes were incised and the fetal head was delivered. Loss of amniotic fluid was minimized by clamping the uterus and membranes to the fetal neck skin. A cannulated electromagnetic flowmeter head (*4*) with an internal diameter of 3, 4, or 5 mm was inserted through a longitudinal incision into the fetal esophagus. The incision was sutured over the flowmeter cannula. A catheter was inserted into the carotid artery for sampling fetal blood and measuring blood pressure. A catheter was inserted into the fetal trachea for measuring tracheal pressure. Two catheters for injection of chemicals into and sampling from the amniotic fluid were sutured to the fetal head and snout. After implantation of all catheters the fetal head was replaced in the uterus and uterine and abdominal incisions were closed. The ewe was kept in a metabolism cage after surgery. The carotid arterial and tracheal catheters were connected to pressure transducers; the externalized lead from the esophageal flow transducer was connected to the flowmeter. The flowmeter provided a continuous measurement of esophageal flow with very low baseline drift (*5*). Volumes swallowed were obtained by integrating the flowmeter output over successive 1-minute periods. Records of flow rate, volume swallowed, and carotid and tracheal pressures were made continuously on a 24-hour basis.

Records of integrated esophageal flow, or volume swallowed, for one fetus are presented in Fig. 1. These records are characteristic of the swallowing activity of the sheep fetus during the last third of gestation. Over a 24-hour period the fetus swallows in discrete episodes or bouts. A bout consists of a period of swallowing during

Table 1. Average volumes swallowed per day by fetuses with electromagnetic flowmeter heads chronically implanted in the esophagus. Averages do not include volumes swallowed on the day of operation or the first postoperative day.

Age of fetus (days)	Internal diameter of flowmeter cannula (mm)	Volume swallowed per day (mean ± S.D.) (ml)	Number of bouts per day (mean ± S.D.)	Weight of fetus at delivery (kg)
109 to 123	3	126 ± 30 (9)*	2 ± 1	2.49
110 to 125	4	150 ± 68 (9)	3 ± 1	3.00
115 to 129	4	359 ± 147 (9)	5 ± 2	2.88
118 to 141	4	243 ± 86 (20)	4 ± 1	3.92
119 to 123	4	491 ± 0 (1)	9 ± 0	3.92
120 to 125	4	79 ± 51 (3)	1 ± 1	3.18
121 to 135	4	149 ± 60 (9)	2 ± 1	3.10
122 to 128	5	477 ± 203 (3)	6 ± 2	3.10
136 to 143	4	403 ± 207 (4)	5 ± 2	3.15

* The number of days from which the average was calculated is given in parentheses.