

is not known, but might be due to position effects resulting from different locations of the transferred DNA in the genome of tobacco.

The level of resistance of plants mediated by fungitoxic substances such as the phytoalexin resveratrol is believed to depend on the ability to produce a high concentration of the compound within a short time after infection^{1,2,9}. For this reason we analysed the levels of resveratrol and the kinetics of resveratrol accumulation in leaves of transgenic tobacco after fungal infection. The results support the notion that a resveratrol-based increased resistance depends on rapid synthesis of high amounts of the phytoalexin in transgenic plants (Fig. 3a-c). In transgenic SR1 plants containing stilbene synthase genes from grapevine up to 400 µg resveratrol per g fresh weight were detected (Fig. 3a-c). This concentration of resveratrol also affected fungal growth *in vitro* (data not shown). A correlation between amounts of resveratrol and disease incidence in transgenic tobacco leaves could be demonstrated (Fig. 3a). Furthermore a comparison of transgenic SR1 tobacco leaves with high or low disease incidence with the accumulation of resveratrol within the leaves indicates that a high concentration of resveratrol 48 h after inoculation is required for enhanced disease resistance (Fig. 3b).

Phytoalexins are vitally important in certain pathogen resistance interactions; in particular, virulence of *Nectria haematococca* on pea depends on its ability to degrade the host phytoalexin²⁰. Furthermore, an avirulent *Cochliobolus heterostrophus* transformed with a gene encoding the phytoalexin-degrading enzyme of *Nectria* became virulent on pea²¹. Thus our work confirms and extends the findings that phytoalexins can determine resistance by moving a phytoalexin from one species to another rather than moving detoxification genes from one pathogen to another. This demonstrates an increased disease resistance of transgenic plants expressing defence-related stilbene synthase genes and raises the possibility of induction of resistance against fungal pathogens by inserting foreign genes coding for phytoalexins. Moreover, we provide the first evidence, to our knowledge, that biosynthetic pathways of plants can be successfully modified or altered by recombinant DNA technology, resulting in pathogen-induced synthesis of a foreign phytoalexin in tobacco plants. Because phytoalexins are fungitoxic substances acting against a variety of different pathogenic fungi, it seems reasonable to assume that there is a general relevance of the described system for other host-pathogen interactions. □

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Ocular regression conceals adaptive progression of the visual system in a blind subterranean mammal

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THE mole rat, *Spalax ehrenbergi*, is an extreme example of natural visual degeneration in mammals: visual pathways are regressed and incomplete¹, and the absence of visual cortical potentials or an overt behavioural response to light have led to the conclusion that *Spalax* is completely blind²⁻⁴. But structural and molecular investigations of the atrophied, subcutaneous eye suggest a functional role for the retina in light perception^{5,6}, and entrainment of circadian locomotor and thermoregulatory rhythms by ambient light demonstrates a capacity for photoperiodic detection⁷⁻⁹. We report here that severe regression of thalamic and tectal structures involved in form and motion perception is coupled to a selective hypertrophy of structures subserving photoperiodic functions. As an alternative to the prevalent view that ocular regression results from negative or nonselective evolutionary processes¹⁰⁻¹², the differential reduction and expansion of visual structures in *Spalax* can be explained as an adaptive response to the underground environment.

We used retrograde and anterograde tracing techniques to study retinal ganglion cells and retinal projections in the blind

mole rat, which has the most rudimentary eye of any mammal^{5,13}. We found less than 900 ganglion cells, which is inferior to the number of sensory fibres of the third eye (pineal complex or frontal organ) in certain vertebrates¹⁴. Ganglion cells are uniformly distributed throughout the retina and form a morphologically homogeneous population (Fig. 1). This lack of retinal heterogeneity could represent a global effect on the morphology of the entire ganglion cell population or may reflect the selective elimination of particular classes of cells.

Intraocular injection of anterograde tracer in the minute eye shows that bilateral retinal projections to all visual nuclei are present in *Spalax*, although compared with non-fossorial rodents, visual pathways are not simply scaled down as a function of eye size. Retinal input to the dorsal lateral geniculate nucleus (dLGN; Figs 1 and 2) is restricted to a thin sheet of label on the superficial dorsal thalamus, within a nucleus no more than 3-5 cells wide. As in other mammals, these cells project to the primary visual cortex¹⁵. The pretectum (PRT), ventral lateral geniculate nucleus (vLGN) and accessory optic system (AOS) also contain a sparse retinal input (Fig. 1). Despite the substantial reduction in absolute size of these structures in *Spalax* as compared with other rodents (87-93%), relative retinal input is proportionately equivalent in both aboveground and underground species (Fig. 3b-c).

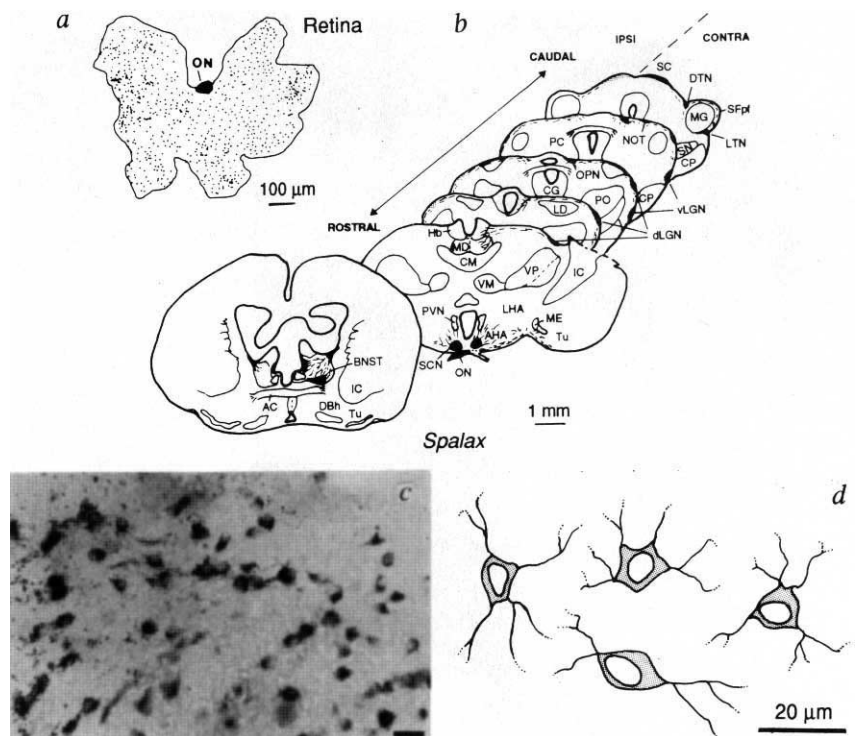
The superior colliculus in *Spalax* receives a sparse, superficial retinal innervation and is severely reduced in both absolute and relative size (Figs 1 and 2). The superficial lamina are collapsed into a single layer 1-2 cells thick. In other rodents, the superior colliculus typically receives the densest retinal innervation (up to 74%), whereas in *Spalax*, the superior colliculus accounts for only 20% of the total retinal projection, a relative decrease of 50%. The absolute reduction in size is even more noticeable: the volume of the retino-recipient layers in *Spalax* is only 3%

FIG. 1 *a*, After horseradish peroxidase (HRP) application to the exposed optic nerve, a total of 823 retrogradely labelled ganglion cells were found homogeneously distributed throughout the retina (surface area, 1.3 mm²; ON, optic nerve head). *c*, High-power photomicrograph of HRP-labelled ganglion cells (scale bar 20 μ m), shown in greater detail in the camera lucida drawings in *d*. The cell soma size distribution is unimodal, with diameters ranging from 6–15 μ m (mean, 10.37, s.d. = 1.79). Although this method may not have fully revealed cell morphology, ganglion cells are all similar in shape, with a large nucleus, scant cytoplasm and limited dendritic arborization. We also observe that all optic nerve fibres are unmyelinated (personal observation). *b*, After intraocular injection of cholera toxin subunit B conjugated with HRP (CT-HRP); more than 20 different structures in the hypothalamus, thalamus, mesencephalon and basal telencephalon receive a projection from the retina (labelled fibres, fine lines; terminal-like label, shaded areas). The encapsulated region of the bed nucleus of the stria terminalis (BNST) contains a diffuse plexus of labelled fibres and terminals, which also extends to several adjacent regions (anterodorsal, anteroventral, thalamic paraventricular and mediodorsal nuclei, lateral habenula). The suprachiasmatic nucleus (SCN) receives a dense bilateral innervation, and retinal fibres are present in the anterior hypothalamic area (AHA), lateral hypothalamic area, subparaventricular zone and retrochiasmatic area. A few labelled fibres are

observed in the basal telencephalon near the amygdaloid region and olfactory tubercle³¹. The vLGN and dLGN are extremely thin, superficial structures and no cytoarchitectural subdivisions could be distinguished. In the pretectum, the olivary pretectal nucleus and the nucleus of the optic tract receive retinal projections. The superior colliculus is highly reduced. A sparse retinal projection to the dorsal (DTN) and lateral terminal nuclei (LTN) of the accessory optic system is also present. The density of label in ipsilateral thalamic and tectal nuclei is less than 2% of the contralateral label, in contrast to the SCN which receives a balanced bilateral innervation. **METHODS.** Six mole rats from the Anza region of central Israel were studied. This population belongs to the recently evolved chromosomal species ($2n = 60$) of the *Spalax ehrenbergi* superspecies (Spalacidae, Rodentia) which actively speciated during the Pleistocene into four sibling species. Animals were anaesthetized (30–40 mg kg⁻¹, ketamine hydrochloride; 2 mg kg⁻¹, xylazine) before receiving an intraocular injection of a 0.2% solution of CT-HRP dissolved in 0.5–1.5 μ l distilled water. An incision was made in the skin slightly above the location of the subcutaneous eye and the HRP conjugate injected with a 50 μ m tipped glass pipette sealed to the needle of a Hamilton syringe. Care was taken to avoid damage to the retina which fills most of the eyecup. After survival periods of 48 to 72 h, animals received a lethal dose of anaesthetic (sodium pentobarbital) and were subsequently perfused through the heart with 300 ml warm saline (0.9%)

of that observed in the hamster and other rodents (Fig. 3*a–c*).

In contrast to the severe hypoplasia, poorly differentiated cytoarchitecture and extremely reduced retinal input of thalamic and mesencephalic structures, the morphology and retinal innervation of the suprachiasmatic nucleus of the hypothalamus (SCN; Figs 1 and 2) in *Spalax* is identical to that in other rodents^{16,17}. The relative size of the retinal projection to the SCN is, however, significantly expanded. The density of retinal input to the SCN in *Spalax* accounts for nearly 20% of the total visual projection, which is equivalent to the amount of label found in the superior colliculus and exceeds that of any other primary visual structure (Fig. 3). In other species, the density of retinal projections to the SCN constitutes a minute fraction (less than 1%) of the total projection. Retrograde tracing studies in rodents have shown that only 30–130 ganglion cells from each retina project to the SCN, compared with the total ganglion cell population of 65,000 (mouse)¹⁸ to 100,000 (hamster)¹⁹. This



followed by 1 l cold 4% paraformaldehyde in 0.1 M phosphate buffer and a post-fixation rinse of 10% sucrose in the same buffer. Coronal sections (30–40 μ m) were cut on a freezing microtome and reacted for HRP³². To obtain retrograde labelling of retinal ganglion cells, the intact eye was removed during anaesthesia in some animals and a crystal of HRP placed on the exposed optic nerve. After 2 min the HRP was washed away and the eye allowed to incubate in oxygenated Ringer's solution. The retina was then removed, reacted as above for HRP and flat mounted. **Abbreviations:** AC, anterior commissure; AHA, anterior hypothalamic area; BNST, bed nucleus of the stria terminalis; CG, central periaqueductal grey; CM, centromedian nucleus; CP, cerebral peduncle; DBH, diagonal band of Broca, horizontal limb; dLGN, dorsal lateral geniculate nucleus; DTN, dorsal terminal nucleus; Hb, habenula; IC, internal capsule; LD, laterodorsal nucleus; LHA, lateral hypothalamic area; LTN, lateral terminal nucleus; MD, mediodorsal nucleus; ME, medial amygdala; MG, medial geniculate nucleus; NOT, nucleus of the optic tract; OPN, olivary pretectal nucleus; ON, optic nerve; PC, posterior commissure; PO, posterior complex of the thalamus; PVN, paraventricular hypothalamic nucleus; SC, superior colliculus; SF-pf, superior fasciculus, posterior fiber branch; SN, substantia nigra; Tu, olfactory tubercle; vLGN, ventral lateral geniculate nucleus; VM, ventromedial nucleus; VP, ventroposterior nucleus.

suggests that in *Spalax*, ganglion cells projecting to the hypothalamus constitute a substantial proportion of the total ganglion cell population.

Although overlooked in previous studies of subterranean mammals^{1,20,21}, an exceptionally dense retinal projection is observed in the bed nucleus of the stria terminalis (BNST). Contralaterally, labelled retinal fibres profusely invade several regions within the encapsulated part of the BNST, although some fibres continue from this region to the anterodorsal and anteroventral nuclei, the mediodorsal nucleus, the lateral habenula, and the thalamic paraventricular nucleus (Figs 1 and 2). Compared to other rodents in which these regions merely contain a few scant retinal fibres²², in the mole rat these pathways are hypertrophied and show both a relative and absolute expansion (Fig. 3).

Despite an acute peripheral reduction these results confirm the conservation of a basic pattern of primary visual

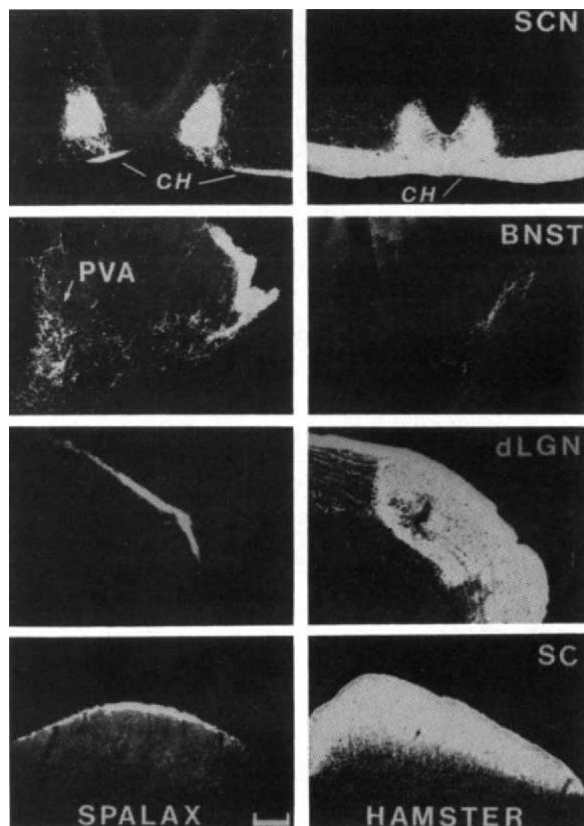


FIG. 2 Dark-field illustrations of retinal projections to contralateral visual structures in the blind mole rat (left side) and in the hamster (right side) following intraocular injection of CT-HRP. For direct comparison, all photographs are scaled to the same size (scale bar, 300 μm). The size of the suprachiasmatic nucleus (SCN) is similar in the mole rat and in the hamster. In both species, anterogradely labelled HRP terminals fill the entire nucleus, although the density is greatest in the ventral region. Note the difference, however, in the size of the optic chiasm (CH). The retinal projection to the lateral and medial regions of the bed nucleus of the stria terminalis (BNST) is hypertrophied in *Spalax* as compared to the presence of a few scant fibres within this region in the hamster. In *Spalax*, labelled fibres also invade the paraventricular thalamic nucleus (PVA) along the wall of the third ventricle. The extent of the dorsal lateral geniculate nucleus (dLGN) is drastically reduced in *Spalax* and the width of the entire nucleus is no greater than the width of the optic tract in the hamster (80–100 μm wide). The superior colliculus (SC) in *Spalax* is also severely degenerate and the superficial lamina (stratum zonale, stratum griseum and stratum opticum) are fused into a single layer 50 μm thick. The total volumes (in mm^3) occupied by retinal fibres within of the different visual structures in *Spalax* are: SCN, 0.0280; BNST, 0.0411; dLGN, 0.0388; SC, 0.0514; and in the hamster: SCN, 0.0253; BNST, 0.0012; dLGN, 0.5884; SC, 1.9840.

organization, and demonstrate that evolutionary regression is uniquely focused on non-hypothalamic structures involved in the analysis of spatial parameters and visuomotor integration. This selective reduction in *Spalax* is coherent with the inability of the subcutaneous eye and degenerate lens to focus an image on the retina, and with the lack of extraocular eye muscles^{5,13}. By contrast, the diffuse, low-level illumination of the subcutaneous eye is entirely compatible with the function of ambient light detection by the SCN. In mammals, photic input to the SCN synchronizes hormonal, physiological and behaviour rhythms to the photoperiodic light cycle²³. SCN neurons respond to large-field, low-level, diffuse light (0.1–500 lux)²³ and a single, short daily exposure (<3 s) is sufficient to synchronize and maintain the complete 24-h cycle of circadian locomotor activity²⁴. Photic entrainment of locomotor and thermoregulatory activity constitutes the exclusive visual capacity in

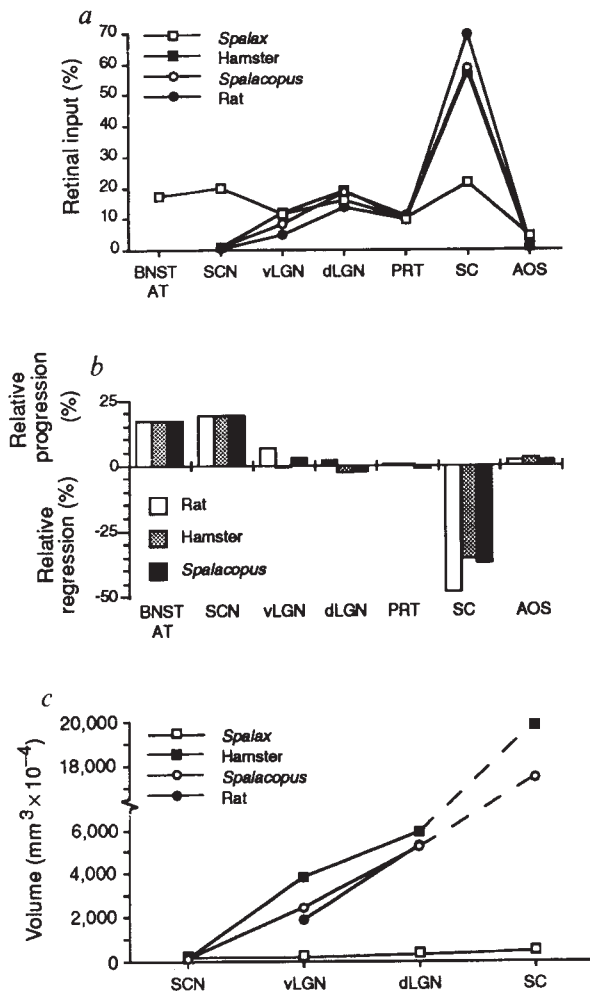


FIG. 3 *a*, Relative degree of retinal input to primary visual structures compared to the total quantity of retinal projections in *Spalax*, hamster, rat and *Spalacopus cyanus* (South American Octodontidae, 'cururo'). All these rodents are of similar body size (120–140 g). The density of retinal projections in each primary visual structure was quantified using a previously described method¹⁷. *b*, Relative degree of change in the proportions of retinal input to different primary visual structures in *Spalax* compared to measures obtained in the other rodents. A relative progressive development in *Spalax* is seen in structures involved in photoperiodic functions (SCN, BNST). The main regressive feature is the drastic reduction of retinal input to the superior colliculus. The relative size of other visual structures in *Spalax* are unmodified compared to that of the other species. *c*, Comparison of the absolute size (volume, $\text{mm}^3 \times 10^{-4}$) of visual structures in *Spalax* and other rodents. The size of the SCN is equivalent in all species. The vLGN and dLGN are reduced by 87–93% in *Spalax*. The retino-recipient layers of the superior colliculus are reduced by 97%. Values for the rat are adapted from Toga and Collins³³ and Sugita and Otani³⁴.

Spalax^{2,7–9,25} and we have previously shown this response is abolished if the eyes are removed^{7,8}. Because a considerable amount of light penetrates mammalian skin, activity at the ground's surface while excavating dirt through mounds could provide adequate light stimulation to the retina. For solitary species like *Spalax*, in which individuals are spatially isolated by exclusive territorial domains, photoperiodic synchronization of neuroendocrine physiology and behaviour may be a necessary prerequisite for successful reproduction²⁵.

The BNST is also involved in neuroendocrine regulation and reproductive functions²⁶ and plays a crucial role in seasonal temperature regulation. Vasopressinergic innervation of the lateral septum by the BNST controls both the maintenance of normal body temperatures and hypothermia^{27–29}. In *Spalax*, the

thermoregulatory capacity to adapt to cold temperatures increases with the decrease in day length preceding winter conditions² and retinal innervation of the BNST provides a putative pathway for mediation of this response. The presence of melatonin receptors in the SCN, BNST and several rostral thalamic regions³⁰, which in *Spalax* also contain retinal fibres (anteroventral, thalamic paraventricular and lateral habenular nucleus), provides further evidence for the involvement of these structures in photoperiodic functions.

We suggest that the photoperiodic system, sustaining appropriate reproductive and thermoregulatory responses, has been selectively expanded, whereas the acute metabolic burden of maintaining a large eye and non-functional 'image forming' visual system provides the underlying evolutionary impetus for their morphological regression. Thus, ocular regression, linked to selective progression of photoperiodic pathways, represents one aspect of the unique range of adaptations²⁵ that subterranean mammals have evolved to cope with environmental constraints imposed by the underground niche. □

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Pharmacology of GABA receptor Cl^- channels in rat retinal bipolar cells

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γ -AMINO BUTYRIC acid (GABA), a major inhibitory neurotransmitter in the mammalian nervous system, is known to operate bicuculline-sensitive Cl^- channels through GABA_A receptors and bicuculline-insensitive cation channels through GABA_B receptors^{1,2}. Recent observations indicate that the retina may contain GABA receptors with unusual pharmacological properties³⁻⁵. Here we report that GABA gates bicuculline-insensitive Cl^- channels in rod bipolar cells of the rat retina, which were not modulated by flunitrazepam, pentobarbital and alphaxalone and were only slightly blocked by picrotoxinin. Moreover, the GABA_B receptor agonist baclofen, and the antagonist 2-hydroxysaclofen had no effect. The underlying single-channel conductance was 7 pS and the open time 150 ms. These values are clearly different from those obtained for GABA_A receptor channels recorded in other neurons of the same preparation, and in other parts of the brain^{1,6-8}. The bicuculline- and baclofen-insensitive GABA receptors were activated selectively by the GABA analogue *cis*-4-aminocrotic acid (CACA)⁹. Hence they may be similar to those receptors termed GABA_C receptors¹⁰.

Patch-clamp experiments were performed on organotypic slice cultures¹¹ prepared from 6-day-old rat retinae¹². Neurons were identified by injecting Lucifer Yellow using the patch pipette¹². Small bipolar cells and larger multipolar cells, presumably amacrine cells, were routinely obtained. All cells displayed inward currents in response to externally applied GABA

TABLE 1 Pharmacology of retinal GABA receptors

Drugs		Bicuculline-insensitive	Bicuculline-sensitive
Flunitrazepam	(1 μ M)	-2 \pm 10 (6)	120 \pm 73 (9)
Pentobarbital	(50 μ M)	5 \pm 13 (5)	247 \pm 135 (4)
Picrotoxinin	(10 μ M)	-2 \pm 15 (11)	-52 \pm 10 (9)
	(100 μ M)	-19 \pm 24 (8)	-96 \pm 4 (3)
Alphaxalone	(1 μ M)	-8 \pm 13 (5)	62 \pm 66 (10)
Strychnine	(5 μ M)	+1 \pm 18 (4)	-52 \pm 7 (4)

Numbers are the percentage changes of GABA-induced whole-cell currents observed upon including various drugs in the GABA (20 μ M) solution. Bicuculline-insensitive currents were recorded from bipolar cells in the presence of 100 μ M bicuculline. The open-channel blocker picrotoxinin was applied twice (interval, 2 min) and the second application was evaluated. Numbers are given as means \pm s.d. (*n* cells).

(20 μ M) when voltage-clamped at negative membrane potentials. In most bipolar cells, high concentrations of bicuculline (100 μ M) were unable to completely abolish the GABA response (Fig. 1a). The bicuculline-resistant component, when present, was from 17% to 89% (mean 52% \pm 23%; 28 cells) of the original GABA-induced current. In contrast, GABA receptors in multipolar cells were exquisitely sensitive to bicuculline (Fig. 1b), the half-maximal inhibitory concentration being only 0.4 μ M (not shown). Thus retinal bipolar cells contain GABA receptors which are different from the known GABA_A subtypes. As rodent bipolar cells are mainly targeted by rod photoreceptors¹³, the cells studied here were probably rod bipolar cells.

GABA-induced whole-cell currents recorded with symmetrical Cl^- in the presence of bicuculline revealed reversal potentials around 0 mV (mean -4 mV \pm 3 mV; 4 cells). Upon partial substitution of the intracellular Cl^- by equimolar amounts of gluconate, the reversal potential was shifted to more negative potentials, as expected for Cl^- -selective channels (Fig. 1c).

To characterize the pharmacology of bicuculline-insensitive GABA receptors further, we tested the effects of flunitrazepam,

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