boring regions of the audio spectrum and enhance (exaggerate) the representation of any imbalance present (*3, 4*). Psychophysical evidence for this type of inhibition can be found in studies of auditory masking, where a pure tone's ability to interfere with the perception of subsequent stimuli (its "effective energy") can be greatly reduced in the presence of another sine wave a few semitones different in frequency [two-tone unmasking (*5*)]. A similar process is seen in single auditory neurons, whose tone-driven firing rates can be markedly reduced by adding a second tone [two-tone inhibition or lateral suppression (*6*)]. That speech formants may be more clearly defined in the perceptual representation than in the physical stimulus (*7*) suggests the biological significance of this type of sensory processing.

Evidence that a neural correlate of unmasking can be observed in peripheral nerve responses from nonhumans (*8*) led us to carry out the physiological and psychophysical studies outlined here. The neural data reported are wave V brainstem responses from humans (*9*). The psychophysical data are measurements of two-tone unmasking obtained from the same subjects.

Three shaped tone bursts served as stimuli: a 17.5-msec masker, a 17.5-msec unmasker, and a 5-msec probe. Probes were presented 2 msec after stimulating with either a masking tone or the two-tone combination [masker plus unmasker (*10*)]. These stimulus complexes were presented repeatedly at a rate of 20 per second. In all conditions, masker and probe frequencies were 2.0 kHz. Unmasker frequencies were spaced at 1/6-octave intervals from 1.414 to 3.175 kHz.

Physiological masking was assessed by averaging 4096 or 8192 samples of probe-evoked electrical activity in the presence of a masker or masker-plus-unmasker (*11*). Psychophysical masking was measured by determining threshold shifts for probe tones under the same conditions.

Figure 1 shows the effect of a masking tone on wave V responses to probes. The masker (at 40 dB re threshold in quiet) reduced probe response amplitudes by approximately the same amount for each probe intensity. Probe response latencies increased in similar fashion.

Figure 2 illustrates two-tone unmasking of wave V responses. The release from physiological masking that occurs when a 2.245-kHz unmasking tone is added can be seen in the increased amplitudes and decreased latencies of responses to the probe. (Masker, unmasker, and probe are 50, 70, and 25 dB re threshold in quiet.)

Figure 3 shows how an unmasker's effectiveness depends on its frequency. Psychophysical and physiological unmasking are plotted together to show their similarity. Both functions show a 5- to 10-dB release from masking when the unmasker is placed 1/6 to 1/3 octave above the test frequency.

A substantial inhibitory effect can thus be observed in auditory population responses from the human central pathways. The inhibitory phenomenon consists of an interaction between frequencies a few semitones apart. For the range of conditions examined to date, its magnitude and form agree reasonably well with psychophysical measurements from the same subjects.

These observations do not necessarily mean that neurons in the wave V population are themselves subject to inhibition, only that inhibitory phenomena occur at some stage of the process leading to activity in the population. In fact, the extremely brief time-course of the phenomenon is consistent with [but does not necessarily imply (*12*)] inhibitory processing carried out by a preneural mechanism (*4*).

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10. This is forward masking: The masking stimulus precedes the probe. Psychophysical two-tone unmasking is not observed when a probe and masker-plus-unmasker complex are presented simultaneously [T. Houtgast, in *Facts and Models in Hearing*, E. Zwicker and E. Terhardt, Eds. (Springer-Verlag, New York, 1974)]. Similarly, we have been unable to demonstrate "physiological unmasking" in the simultaneous condition.
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14. We thank Bruce Masterton and David Harris for their comments on earlier versions of this report. Portions were presented at the 97th meetings of the Acoustical Society of America, Boston, 11 to 15 June 1979.

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Interspecific Chimeras in Mammals: Successful Production of Live Chimeras Between *Mus musculus* and *Mus caroli*

Abstract. *Live chimeras between two species of mouse,* Mus musculus *and* Mus caroli, *were produced by blastocyst injection. These chimeras were entirely similar to* M. musculus ↔ M. musculus *chimeras in their somatic tissue organization. This is the first report of completely normal development of interspecific chimeras in mammals.*

Mammalian chimeras have proved very useful for investigating early embryonic development (*1, 2*), but precise clonal analysis of cell lineages has been limited by the lack of a cell marker that is ubiquitous and that distinguishes the two parental cell types in situ (*3*). One solution to this problem is to make chimeras from embryos of two different species so that there are sufficient genetic differences for unequivocal identification of the two cell types in any tissue (*4*). This approach has been applied to mammals with various degrees of success. Although aggregation chimeras between rat and mouse (*5*) and between bank vole

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Table 1. Glucose phosphate isomerase analysis of different tissues from 18.5-day-old *M. caroli* ↔ *M. musculus* chimeric fetuses. Tissue samples were diluted with water, frozen, thawed, and subjected to electrophoresis in 12 percent Electrostarch gels by the method of Peterson (*18*). Staining was performed on nitrocellulose filters that were cleared for quantitative densitometry measurements. The numbers represent the percentages of *M. caroli* enzyme as calculated from densitometer tracings. The numbers in parentheses are percentages for hybrid bands. N.D., not detectable.

Chimera No.	Placenta	Yolk sac	Liver	Lung	Heart	Gut	Skeletal muscle	Remainder of carcass
24	6	1	38	11	23	16	4 (5)	21 (9)
25	10	50	87	78	81	83	83 (5)	73 (N.D.)
26	9	51	81	60	48	52	57 (13)	45 (12)
27	N.D.	N.D.	31	10	17	6	47 (4)	38 (9)
28	1	N.D.	17	25	34	27	36 (5)	11 (6)
29	11	5	39	52	36	26	56 (19)	65 (10)

and mouse (*6*) can be implanted successfully in the mouse uterus (*6, 7*), development after implantation is limited, and no live births have been reported. Greater success has been achieved in producing rat ↔ mouse chimeras by blastocyst injection (*8*): immunofluorescence analysis with species-specific antiserums revealed the presence of mixed rat and mouse embryonic tissues until at least the middle of gestation (*9*). However, the few offspring born were runted and showed little evidence of rat tissue (*9*), which suggests that there was selection against the rat cells as development proceeded. We report here the successful production of live chimeras between two more closely related species, *Mus musculus* and *Mus caroli*. These chimeras showed no evidence of selection against one cell type or the other, and they should prove useful for the clonal analysis of development.

Mus musculus, the laboratory mouse, and *M. caroli*, a wild species from Southeast Asia (*10*), show a variety of genetic differences (*11*) and do not normally interbreed (*12*). The morphological changes associated with embryogenesis are very similar in the two species (*13*), but the preimplantation development of *M. caroli* is completed 16 to 20 hours earlier than that of *M. musculus* and the gestation period is 1 to 2 days shorter (*12*). Chimeras were made by injecting *M. caroli* inner cell masses into *M. musculus* blastocysts. *Mus musculus* blastocysts were obtained on the afternoon of the fourth day after the natural mating of HA(ICR) albino, non-agouti mice homozygous for the *b* allele of the glucose phosphate isomerase (GPI) gene (*Gpi-1^b*/*Gpi-1^b*). *Mus caroli* females were superovulated (*14*) and mated with males. Blastocysts of cell numbers similar to those of the *M. musculus* blastocysts were flushed from the uteri 76 hours after the injection of human chorionic gonadotropin (hCG) [The randomly bred *M. caroli* stock used has agouti coat pigmentation and a single GPI-1 isozyme indistinguishable from the GPI-1A isozyme of *M. musculus* (*12*).] Inner cell masses were then dissected immunosurgically (*15*) from the *M. caroli* blastocysts and injected microsurgically into *M. musculus* blastocysts. The injected embryos were transferred into the uterine horns of *Gpi-1^b*/*Gpi-1^b* HA(ICR) females on the third day of pseudopregnancy. Nine conceptuses were dissected after 18.5 days (just before birth) so that all fetal tissues and extraembryonic structures could be analyzed.

Six fetuses were judged chimeric on the basis of GPI analysis and eye pigmentation, and each chimera showed both *M. musculus* and *M. caroli* GPI enzymes in all tissues analyzed (Table 1), although the proportions of the two varied somewhat from tissue to tissue. Such variation has also been observed in *M. musculus* ↔ *M. musculus* chimeras (*2*). Perhaps the best evidence for normal interaction of *M. musculus* and *M. caroli* cells in the chimeras was the presence of hybrid GPI enzyme in the skeletal muscle (Table 1), indicating that myoblasts from the two species had fused to form functional myotubes (*16*).

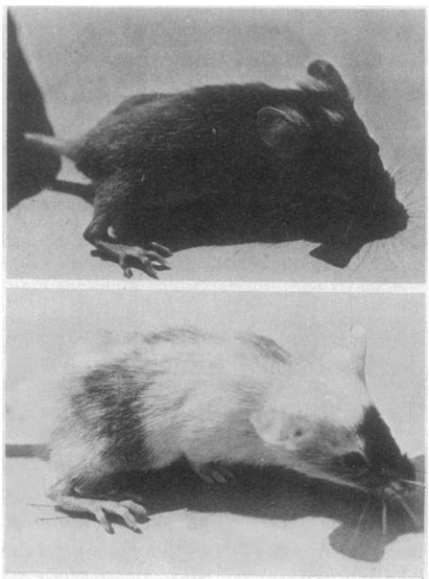

Fig. 1. Two 3-week-old *M. musculus* ↔ *M. caroli* chimeras. Their coats show patches of albino (*M. musculus*), agouti (*M. caroli*), and black, non-agouti pigmentation. The black patches are presumably due to a combination of pigmented *M. caroli* melanocytes in the skin with non-agouti *M. musculus* dermis (*19*). The upper mouse is *M. caroli* on the basis of coat color phenotype but not of GPI analysis (its blood contained only 42 percent of *M. caroli* enzyme).

Five other recipients were allowed to go to term, and 38 of the 48 offspring were judged chimeric on the basis of hair and eye pigmentation and GPI analysis. The pattern and extent of *M. caroli* pigmentation in the coat and the eyes varied from mouse to mouse (Fig. 1), as seen in *M. musculus* ↔ *M. musculus* chimeras (*17*). The percentage of *M. caroli* GPI enzyme in the blood of the chimeras also varied widely, from 2 to 92 percent, with an average of 38 percent. The sex ratio of the 36 interspecific chimeras weaned showed an excess of phenotypic males (27 males to 9 females), which agrees with similar findings that most XX ↔ XY *M. musculus* ↔ *M. musculus* chimeras are phenotypically male (*2*).

Mus musculus ↔ *M. caroli* chimeras thus appear to be very similar to *M. musculus* ↔ *M. musculus* chimeras in their somatic tissue organization, and there is no evidence for selection against *M. caroli* cells during development. If species-specific antiserums can be raised, or other genetic differences between the two species exploited so as to provide an in situ cell marker system, these interspecific chimeras should be very useful for many studies in mammalian development. The chimeras are also of potential interest for studies of maternal-fetal interactions and the genetic control of mating behavior.

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Unequal Alternating Monocular Deprivation Causes Asymmetric Visual Fields in Cats

Abstract. *Kittens were reared so that each eye received normal patterned vision on alternate days. If the eyes received equal periods of stimulation, the visual fields were normal. If one eye received much more experience than the other, the field of the less experienced eye was restricted to the temporal hemifield. This change, which differs from that observed when one or both eyes are deprived continuously of patterned input, suggests that an imbalance in the duration of stimulation can influence the outcome of the normal competitive interaction between pathways from the two eyes and can cause a selective suppression of a portion of the input from the less experienced eye. This suppression may involve the ipsilateral retino-geniculo-cortical pathways or it may involve the entire cortical pathway from the less experienced eye, leaving the colliculus to control responses to visual targets.*

Binocular competition almost certainly occurs during the normal development of the visual pathways of higher mammals. Competition is easy to demonstrate, however, only when the stimulation to the two eyes is unbalanced. In experiments in which this imbalance is created by depriving one eye of all patterned input [monocular deprivation (MD)] (*1*), the effects of competition are difficult to separate from those of deprivation. We felt that competition could be more readily studied if the imbalance to the two eyes could be created without continuous deprivation of either eye (*2, 3*). Kittens were therefore reared by presenting each eye with patterned visual input but on alternate days and for different periods of time [unequal alternating monocular deprivation (AMD)]. This manipulation resulted in a striking behavioral asymmetry: the visual field of the less experienced eye was restricted. This asymmetry suggests that a difference in the duration of patterned visual stimulation is sufficient to place one eye at a competitive advantage. A difference in the quality of visual stimulation is not required. The pattern of the visual field deficit seen in cats with unequal AMD is different from those seen previously in MD or binocularly deprived (BD) cats (*4*) and suggests that either (i) the ipsilateral visual pathway is more susceptible to the effects of competition or (ii) competition can suppress the entire geniculo-cortical pathway from one eye.

Kittens were reared in the dark from 3 days to 4 weeks of age, when they were brought out into the light for daily periods of exposure with one eye occluded. For the four experimental animals, the right eye was exposed for 8 hours and, on alternate days, the left eye for 1 hour (AMD 8/1). Six control animals were exposed for equal periods with each eye: two of the animals for 8 hours (AMD 8/8) and four animals for 1 hour (AMD 1/1). Four normally reared cats served as additional control subjects.

Beginning at 2 months of age, the animals were tested for their ability to orient to targets in the visual field (*4*). Animals were taught to fixate on a target (a piece of food on a wire) presented straight ahead at a distance of 40 cm. A novel stimulus (a piece of food on another wire) was introduced at a distance of 20 cm along one of the guidelines, which were placed every 15° to the left or right of the fixation line (0°). A positive response was recorded when, upon being released, a cat turned and immediately approached the novel stimulus. A negative response was recorded if the cat approached the fixation object or if it scanned the field before approaching the novel stimulus (*5*). The novel stimulus was presented at each of 15 positions from 105° left to 105° right except at 0°. For the trials at 0°, only the fixation object was presented. On these trials, failure to directly approach the fixation object was scored as a negative response, and the number of these responses was used as an indication of the background level of nonspecific responses for the other trials. The order in which the trials were presented was determined by a table of random permutations. Each animal was tested monocularly with each eye 12 times at each position.

The visual field of each eye of a normal cat extends 120°, from 90° temporal to 30° nasal (Fig. 1). In control cats given equal periods of stimulation to the two eyes (AMD 8/8 and AMD 1/1), the visual fields for each eye were normal and of equal size (Fig. 1). In contrast, in all cats given unequal periods of stimulation to the two eyes (AMD 8/1), the visual fields for the two eyes were of unequal size (Fig. 1). The visual field of the 8-hour eye was normal. However, the visual field of the 1-hour eye of each of these animals was restricted to the temporal hemifield and extended from 90° temporal to the midline (*6*). None of these cats ever responded to a target in the nasal field of the 1-hour eye. The loss of responses in the nasal field was striking when compared with the responses of the 8-hour eye or to those of any of the cats receiving equal exposure to the two eyes. In particular, the AMD 8/1 cats made fewer responses with the left eye to targets at 15° and 30° nasal than did the AMD 1/1 cats, which had received the same length of exposure with the corresponding eye [t (6) = 11.7, $P < .001$, two-tailed]. Further, the AMD 8/1 cats showed a slight, but significant, reduction in responsiveness to targets presented at the temporal margin of the field of the 1-hour eye. Specifically, the AMD 8/1 cats made fewer responses with the left eye to targets presented at 90° temporal than did the AMD 1/1 cats [t (6) = 3.3, $P < .02$, two-tailed]. Thus, unequal periods of stimulation to the two eyes result in asymmetric visual fields. Since both eyes of the AMD 1/1 cats show normal fields, even though neither eye received any

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