2) Suprachiasmatic lesions do not affect the clock directly but instead, regardless of photoperiod, produce the perceptual illusion of constant high intensity illumination which may have effects on the three rhythms in question identical to those described in hamsters with lesions (*3, 16*). In many animals with such lesions, patterns of locomotor activity in LD 14:10 differ from those in DD (Fig. 1). If the lesions in these animals produced a perceptual illusion of continuous illumination, one would expect similar locomotor patterns regardless of the ambient photoperiod. We have seen animals in which the lesion did not abolish locomotor rhythmicity, but, rather, altered its phase relationship to the photoperiod. In every animal thus affected, the suprachiasmatic nuclei were only partially destroyed. This suggests that less than total damage to the clock may alter either tau (τ), the free-running period length of the clock, or the relationship of the clock to the entraining light cycle.

3) The suprachiasmatic nuclei may not be the primary clock but rather a coupled oscillator in a two- or multi-oscillator system. To negate this hypothesis, it would be necessary to isolate the suprachiasmatic region from all the neural input (except that from the retinas) leaving efferent connections intact, and then to view maintained entrainment of the rhythms in question. This monumental feat has not yet been accomplished in hamsters. However, these facts remain: (i) in hamsters the primary (self-sustained) oscillator in a multi-oscillator system must receive photic input, whereas other driven oscillators need not necessarily be so endowed, and (ii) the only known direct visual projection outside of the classical primary and accessory optic pathways in both the hamster and the rat is to the suprachiasmatic nuclei.

4) The suprachiasmatic nuclei may be a primary oscillator regulating a variety of rhythms. The strongest supporting evidence is (i) the visual pathway from the retina to the suprachiasmatic nuclei, and (ii) the fact that seemingly unrelated rhythms ranging from locomotor activity to adrenal corticosterone content (*1, 2*) are abolished when the suprachiasmatic nuclei are destroyed. We believe it unlikely, although by no means impossible, that a single driven oscillator would be involved in the expression of so many different rhythms. Although further research is needed, we tentatively conclude that the nucleus suprachiasmaticus is a biological clock in the hamster.

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11. The hamsters were raised from stock purchased from Charles River Lakeview, Newfield, N.J. They were placed in the running-wheel cages for continuous recording of locomotor activity. The photoperiod was LD 14:10. Food and water were available. The animals were anesthetized with pentobarbital (10 mg in 0.5 ml) and placed in a Kopf stereotaxic apparatus. Radiofrequency lesions (100 khz, 50 volts, 20 ma; LM4 Lesion Maker, Grass Instruments, Quincy, Mass.) were made bilaterally with an electrode (size 0 insect pin insulated except for its tip with Insl-x (Insl-x Products Corp., Yonkers, N.Y.). The animals were held overnight in small plastic cages and returned to their running-wheel cages the following morning. All animals survived the operation. Activity was recorded for at least 70 days after the operation, during which the photoperiod remained LD 14:10. The animals were then transferred to continuous darkness (DD), and activity was again recorded for 14 to 21 days, after which the animals were removed from activity cages and housed individually in small plastic cages for the duration of DD treatment. When the experiment was completed, animals were anesthetized with pentobarbital, perfused via the left ventricle with saline followed by 10 percent formalin or Bouin's fluid. The gonads were weighed and preserved with the brain in 10 percent formalin.
12. Brains were washed with 70 percent ethanol, trimmed to give a uniform plane of section, dehydrated in ethanol, cleared in toluene and embedded in Paraplast. Serial 10-μm sections were mounted on glass slides and stained [H. Klüver and E. Barrera, *J. Neuropathol. Exp. Neurol.* **12**, 400 (1953)].
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Selective Production of *cis*- and *trans*-Verbenol from (−)- and (+)-α-Pinene by a Bark Beetle

Abstract. *A unique biological system whereby optical isomers are selectively transformed to geometrical isomers is demonstrated in* Ips paraconfusus. *Exposure of adult male and female beetles to vapor of (−)-α-pinene resulted in the production of (+)-*cis*-verbenol, a pheromone of this species, whereas (+)-α-pinene was oxidized to (+)-*trans*-verbenol. It appears, therefore, that the ability of a bark beetle to produce its aggregation pheromone can be governed by the chirality of a precursor in the host tree.*

Bark beetles that attack coniferous trees are exposed to high concentrations of monoterpenes as they encounter resin exuding from the injured tissues of their hosts. Exposure of adult beetles to vapors of individual terpenes results in the formation of oxidation products, including pheromones, which are detected in the hindgut (*1*). The host monoterpene α-pinene has been converted to *cis*- and *trans*-verbenol and myrtenol by every species that we studied.

Since the verbenols are dissymmetric molecules and the biological activity of an insect pheromone may depend on its chirality (*2*), we were interested in determining whether bark beetles might preferentially oxidize (+)- or (−)-α-pinene and whether the optical rotation of the products would be dependent on the rotation of the precursor. The species chosen for this study was *Ips paraconfusus* Lanier, since *cis*-verbenol is a component of the pheromone system of this beetle (*3*).

Adult male and female beetles were exposed to the vapors of (+)-α-pinene

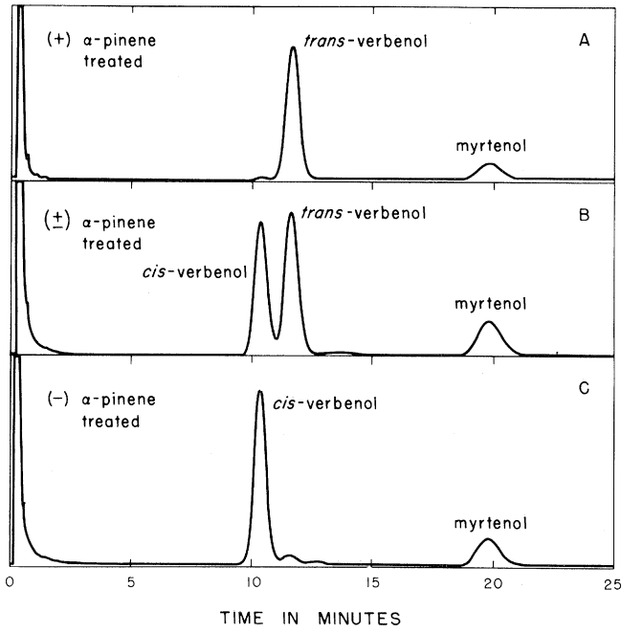

Fig. 1. Gas chromatograms of hindgut volatiles from *Ips paraconfusus* males after exposure of the beetles to vapors of optically purified and racemic α-pinene.

(K & K Laboratories) ($[\alpha]_D^{23} = +20.2$) and (−)-α-pinene (Chemical Samples Co.) ($[\alpha]_D^{23} = -45.6$) for a period of 24 hours (*4*). The beetles' hindguts were then removed and stored at −70°C until approximately 5000 were collected from each sex treated with each optical form of α-pinene. The hindgut samples were extracted with ethyl ether, and the extracts were subjected to gas chromatography [15 percent free fatty acid polyesters (FFAP) on Varaport 30; 1.85 m by 2 mm internal diameter, glass; 120°C]. The results indicated that the treatment with (+)-α-pinene resulted in the production of predominantly *trans*-verbenol by both sexes. However, predominantly *cis*-verbenol was produced by both sexes after treatment with (−)-α-pinene. Approximately the same amount of myrtenol was formed in each case. The experiment was repeated several times with only five beetles for each sample, and the same results were consistently obtained. The identities of the verbenols and myrtenol were confirmed by mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance spectroscopy.

Each hindgut extract was subjected to short-path distillation, and the major volatile products were collected by preparative gas chromatography (15 percent FFAP on Varaport 30; 1.85 m by 4.5 mm internal diameter, stainless steel; 140°C) (*5*). The collected fractions were rechromatographed in order to obtain each compound in a high degree of purity, and the optical rotation of each product was measured. The *cis*-verbenol obtained from the (−)-α-pinene treatment of both sexes was dextrorotatory, $[\alpha]_D^{23} = +5.9$ (0.1, methanol) for the female sample and +5.6 (0.07, methanol) for the male sample. The *trans*-verbenol resulting from (+)-α-pinene exposure was also dextrorotatory, $[\alpha]_D^{23} = +127$ (0.41, methanol) for the female material and +125 (0.34, methanol) for the male material. The sign of rotation of the myrtenol was the same as that of the α-pinene used in each case. From (−)-α-pinene, the specific rotation of the myrtenol was $[\alpha]_D^{23} = -45$ (0.06, methanol) for the female sample and −50 (0.03, methanol) for the male sample. The myrtenol from (+)-α-pinene had a specific rotation $[\alpha]_D^{23} = +11$ (1.4, methanol) for the female sample and +6.7 (0.04, methanol) for the male sample (*6*).

The optical purity of the α-pinene used in these studies, although adequate for the original objectives, was relatively low. In view of the results, we were interested in repeating the basic experiment with α-pinene samples of high optical purity. Such samples were obtained by differential complexing of the α-pinene enantiomorphs with silver perchlorate (*7*). After the samples were repeatedly purified by this method, the optical rotation of the (+)-α-pinene was $[\alpha]_D^{23} = +51.22$ and of the (−)-α-pinene was $[\alpha]_D^{23} = -51.30$. These values represent an optical purity of > 97 percent in each case, based on the highest reported value of +52.4 for (+)-α-pinene (*7*).

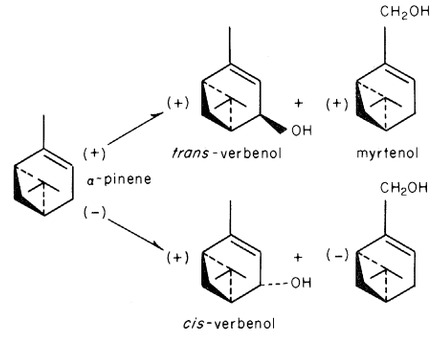

Fig. 2. Oxidation of α-pinene enantiomers by *Ips paraconfusus*.

Ips paraconfusus adults were exposed to the optically purified α-pinene enantiomorphs and to a racemic mixture in the same manner as before. Hindguts were taken from 20 beetles of each sex from each treatment, and the extracts were analyzed by gas chromatography. The chromatograms (Fig. 1) showed that more than 97 percent *trans*-verbenol was produced from the (+)-α-pinene and at least 95 percent *cis*-verbenol was obtained from the (−)-α-pinene, whereas treatment with (±)-α-pinene resulted in approximately equal quantities of the two verbenols. As noted before, the amount of myrtenol produced was unaffected by the chirality of the α-pinene used.

The results suggest that both sexes of *Ips paraconfusus* produce only the *cis* isomer of verbenol from optically pure (−)-α-pinene and only the *trans* isomer from optically pure (+)-α-pinene. The sign of rotation of *trans*-verbenol is the same as that of its precursor, whereas the sign of rotation of *cis*-verbenol is reversed from its precursor (Fig. 2). Significantly, this reversal is restricted to the formation of the one product known to be involved in the pheromone system of this beetle. It appears likely that the *cis*- and *trans*-verbenols produced by the beetles are optically pure, regardless of the optical purity of the α-pinene, whereas the optical purity of the myrtenol is directly related to the optical purity of the α-pinene.

To our knowledge, this is the first report of an organism that is capable of selectively converting enantiomorphs of a precursor into products which are geometrical isomers. The biological implication of this finding is significant. Bark beetles depend on a system of aggregation pheromones for their mass attack and colonization of host trees. This specific precursor requirement means that variations in optical rotation of the α-pinene in the trees can strongly influence the pheromone production by this species.

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5. The analytical gas chromatograph was a Varian 1200, with a flame ionization detector. The preparative model was an Aerograph 700, with a thermal conductivity detector. Mass spectra were obtained with a Hitachi–Perkin-Elmer RMU-6E coupled to a Varian Aerograph 204 gas chromatograph. Optical rotation measurements were made with a Perkin-Elmer 141 polarimeter.
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The Categories of Hue in Infancy

Abstract. *Infant looking time was monitored during habituation to the repeated presentation of a wavelength stimulus selected from one basic adult hue category and after a change in stimulation. Recovery from habituation was greater to a wavelength selected from an adjacent hue category than to a wavelength from the same category even though these two stimuli were equally distant (in nanometers) from the habituation wavelength. Differential responding evidenced infants' categorical perception of hue; that is, infants see the physically continuous spectrum as divided into the hue categories of blue, green, yellow, and red. These results help to resolve the long-standing controversy surrounding the primacy of perception over language in the organization of hue.*

We have found that 4-month-old infants respond to differences in wavelength as though they perceived categories of hue—blue, green, yellow, and red. That is, infants responded differently to two wavelengths selected from adjacent adult hue categories (for example, blue at 480 nm and green at 510 nm) but did not respond differently to two wavelengths separated by the same physical distance but selected from a single adult hue category (for example, blues at 450 nm and 480 nm).

Modern school children still paraphrase Newton's original observations on the categories of hue in the spectrum (*1*).

> The Original or primary colours are, *Red, Yellow, Green, Blew*, and a *Violet-purple*, together with *Orange, Indico*, and an indefinite variety of Intermediate gradations.

Substituting narrow monochromatic radiation for prism-dispersed sunlight, and psychophysical techniques for introspection (*2*), modern research has confirmed the relationship between wavelength and color naming given in Newton's original experiments. Wavelengths are usually described by one or two primary hue names (Fig. 1). Color-naming functions are roughly characterized by plateaus—wavelength ranges which form categories where a single hue term predominates—and by boundaries between the plateaus. Although the physical dimension is continuous, the psychological structure is discontinuous. Moreover, it has been argued that discrimination is typically poor nearer the center of plateaus (these wavelengths look the same and, hence, tend to be called by the same name), but discrimination is good at boundaries between hues (as chromatic distinctions become clearer, color names change) (*3*). The categorical perception of hue may have a biological basis. De Valois (*4*), for example, has demonstrated that discrimination of wavelength in the macaque (which has demonstrated color vision identical with normal human adult trichromats) is a function of chromatic analysis by thalamic neural tissue. Furthermore, Bornstein (*5*) has matched the results of cross-cultural studies on the designation of hue category centers to the sensitivity maxima of these same neural cells. Finally, the observation that at least two infrahuman species (*6*) see hues categorically makes the assumption of a biological basis for qualitative chromatic distinctions more plausible than the alternative assumptions about learning and language.

These logical, psychological, neurological, and ethological considerations led us to hypothesize that very young human infants would see the physical spectrum in a categorical fashion much like that of adults. To test this hypothesis, we made use of the fact that babies look less and less at a visual stimulus that is repeatedly presented (*7*). At the end of the so-called habituation phase, a test stimulus perceived by the child as dissimilar from the first stimulus produces increased looking (dishabituation); if, however, the child sees the test stimulus as similar to the first stimulus, looking time remains low (continued habituation).

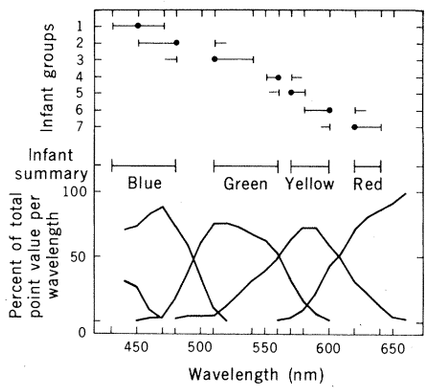

Fig. 1. (Upper panel) Results for seven infant experimental groups. Dots stand for habituation stimuli, vertical bars for category and boundary stimuli; a horizontal connection indicates a lack of difference in mean looking times between wavelengths, and a gap indicates, by comparison, a statistically significant difference (see Table 1) (*18*). The summary gives ranges of wavelengths responded to as similar by infants as well as ranges of probable transition between hues. (Lower panel) Adult color-naming functions replotted after Boynton and Gordon (*14*).

In our experimental situation, infants were shown a given wavelength of light (habituation stimulus, HS) (*8*) for fifteen 15-second trials followed by a series of nine 15-second test trials. Intertrial intervals averaged 7.5 seconds. The test series consisted of three blocks each containing three randomized stimuli: HS and two physically different stimuli, one selected from the same category as HS (category stimulus, CS) and one selected from a category adjacent to HS (boundary stimulus, BS). In this design infants serve as their own controls since they are continuously probed in the test series for their responses to HS (*9*).

Our main hypothesis was that the basic hue categories (blue, green, yellow, and red) and the boundaries between categories (blue-green, green-yellow, and yellow-red) common to adults would exist in infants. Consequently, we chose wavelength stimuli to straddle the color-naming boundaries (HS and BS) or to fall wholly within a single adult color-naming category (HS and CS). Moreover, BS and CS in each test sequence were separated by equal physical distances (in nanometers) from HS. Thus, for example, HS for experimental group 2 was a 480-nm light which adults perceive as mostly blue, CS was a 450-nm light perceived by adults also as blue, and BS was a 510-nm light perceived by adults as mostly green. Consequently, if babies look longer at one or another of the test stimuli of 450, 480, and 510 nm, then we would have inferential evidence about the infants' perception of the similarities and qualitative categorization of these wavelengths. Each boundary was explored by two groups of infants—for one group HS and CS were drawn from the category on the short-wavelength side of the boundary (for example, experimental group 2) and for a second group HS and CS were drawn from the category on the long-wavelength side of the boundary (for example, experimental group 3). Thus, HS in group 3 was 510 nm, BS was 480 nm, and CS was 540 nm.

Eight groups of ten healthy, full-term 4-month-old Caucasian infants were seen.