duced by training is due, at least in part, to an increased number of sarcolemmal Ca$^{2+}$ binding sites (*6*). These two independent findings are mutually consistent.

Sarcolemmal fatty acid composition was determined by gas-liquid chromatography (*13*). As shown in Table 1, the concentration of linoleic acid (18:2) and the saturation percentage were significantly higher in exercised rats ($P < .05$). Phospholipid acyl chain length and degree of saturation also have a prominent effect on the physical properties of the lipid bilayer. For example, increasing saturation of natural fatty acids decreases membrane fluidity (*1*).

It is clear that exercise can induce substantial alterations in the lipid composition of the plasma membrane. Because these compositional changes are likely to affect sarcolemmal control of E-C coupling, they may be related to the physiological adaptation that the heart undergoes in response to a stressor.

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11. Two-dimensional thin-layer chromatography was carried out with 20 by 20 cm silica gel G (Merck) plates. The solvent system was that of G. Rouser and S. Fleischer [*Methods Enzymol.* **10**, 385 (1967)]. A 0.5-ml portion of the sample (1.0 mg of lipid) in chloroform was applied to the origin. After saturation of the chamber, plates were developed at 18°C for approximately 90 minutes per side. Phospholipid spots were visualized in $I_2$ vapor and identified by use of the standards and techniques described by V. P. Skipski and M. Barclay [*Methods Enzymol.* **14**, 530 (1969)].
12. After identification of each phospholipid, the spots were scraped and the phospholipids were extracted in 2:1 chloroform and methanol. Inorganic phosphorus release was determined by the method of M. Rockstein and P. W. Herron [*Anal. Chem.* **23**, 1500 (1951)]. It was assumed that each mole of inorganic phosphorus released was equivalent to 1 mole of phospholipid—except for cardiolipin, for which this ratio is 2:1.
13. Gas-liquid chromatography separation of sarcolemmal fatty acids began with the hydrolysis and preparation of fatty acid methyl esters. One-tenth milligram of lipid was incubated with 3 ml of methanol and five drops of concentrated $H_2SO_4$ at 70°C for 2.5 hours. This was followed by a series of petroleum ether extractions and the addition of 1.0 ml of $CH_2N_2$. All samples were run with pentadecanoic acid (15:0) as the internal standard. Details of column temperature, pressure, and packing are described in T. Nagatomo, K. Hattori, M. Ikeda, and K. Shimada [*Biochem. Med.* **23**, 198 (1980)].
14. We thank G. A. Langer and K. D. Philipson for their contributions during these experiments and in preparing this report, T. Tsuruhara and M. Ikeda for technical advice, M. M. Bersohn and J. Vinten-Johansen for helpful editorial suggestions, and C. Heteniak for typing the manuscript. G.F.T. was the recipient of research fellowship 610 from the American Heart Association (Los Angeles affiliate).

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## Selective Herbivory in Tassel-Eared Squirrels: Role of Monoterpenes in Ponderosa Pines Chosen as Feeding Trees

Abstract. *Ponderosa pine twigs collected from trees used by tassel-eared squirrels as sources of cortical tissue for food contained smaller amounts of monoterpenes than twigs from similar trees not used by the squirrels as food sources. Of the 18 monoterpenes isolated from the twig samples, α-pinene was the best single predictor of food source trees. In experiments with captive tassel-eared squirrels, consumption of a preferred food was inversely correlated with the concentration of α-pinene added to the food.*

During winter, tassel-eared squirrels (*Sciurus aberti*) feed predominantly on the cortical tissue of small twigs of certain ponderosa pine (*Pinus ponderosa* Laws) trees (*1*). Such trees are easily distinguished by their defoliated appearance and the accumulation of needle clusters beneath them (Fig. 1). To obtain cortical tissue, the squirrels remove terminal twigs in the upper portions of the tree from larger branches by biting through the stem several centimeters from the terminal needle cluster (Fig. 2). The needle cluster is clipped from the end of the twig and drops to the ground. The rough outer bark is then removed with the teeth while the twig is rotated with the forepaws to expose the phloem and also the cambium and some current year's xylem.

Trees on which the squirrels feed (feeding trees) are usually cone-bearing

Table 1. Major monoterpenes analyzed from cortical tissues of ponderosa pine twigs.

| Monoterpene | Feeding trees | | Nonfeeding trees | |
|---|---|---|---|---|
| | Percent | Range | Percent | Range |
| α-Pinene | 27.2 | 9.0-53.9 | 26.0 | 6.0-59.1 |
| β-Pinene | 16.4 | 5.5-36.4 | 14.6 | 4.7-31.8 |
| 3-Carene | 28.8 | 11.7-58.5 | 31.9 | 16.5-59.0 |
| Myrcene | 5.6 | 1.6- 8.5 | 5.9 | 2.1-11.5 |
| Limonene | 11.1 | 3.4-27.3 | 10.6 | 1.3-24.8 |
| β-Phellandrene | 4.7 | 0.7-11.7 | 4.5 | 0.9-27.0 |
| Terpinolene | 3.3 | 1.6- 7.9 | 3.7 | 1.0- 9.9 |
| Total | 97.1 | | 97.2 | |

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Fig. 1. Ponderosa pine tree (center) that has been heavily used as a feeding tree by tassel-eared squirrels. The trees on either side of the feeding tree do not show signs of defoliation.

trees between 28.0 and 76.0 cm in diameter (*2*). Within this size range, much variability exists as to which trees are chosen by squirrels for twig feeding. In the Enchanted Mesa area (Boulder County, Colorado), trees of virtually the same height, diameter, and age (determined by increment boring), and growing in the same locality, showed marked differences in defoliation; for example, of two trees that were less than 1 m apart at the base, with upper branches interdigitating, one was heavily defoliated and had been a feeding tree for 10 years (from 1969 to 1979), whereas the other showed almost no sign of twig clipping. Some trees had only a few needle clusters beneath them or small debarked twigs still attached in their crowns, suggesting that these trees might have been sampled by squirrels for palatability and subsequently rejected.

Captive tassel-eared squirrels could distinguish (in the laboratory) between twigs collected from known feeding trees and those from trees not used as food sources (*3*). Preferences resulting from the influence of factors such as social facilitation, location and orientation of trees, and other nongustatorial cues were thus eliminated (*4*). These findings support our contention that the squirrels' initial selection of feeding trees was based on factors associated with pine twig palatability.

Monoterpenes ($C_{10}H_{16}$), a major class of secondary compounds present in ponderosa pine and other conifers, have deterrent or even toxic effects on a number of species (*5*). We therefore examined the monoterpene content of ponderosa pine twigs from trees on which the squirrels feed and those on which they do not feed.

Ponderosa pine twigs were collected on Enchanted Mesa from 16 feeding trees and 16 nonfeeding trees. All twigs were cut from laterals and tips of upper branches to simulate the locations of browsing by tassel-eared squirrels. Collections were made from 1977 to 1979 during the months of December, April, August, and October. A total of 74 paired samples, each containing at least ten twigs (from different branches) was analyzed. The outer bark of each twig was removed with a scalpel and the exposed phloem, cambium, and current year's xylem were scraped off for processing. Monoterpenes were separated by a small-scale procedure combining steam distillation and extraction (*6*).

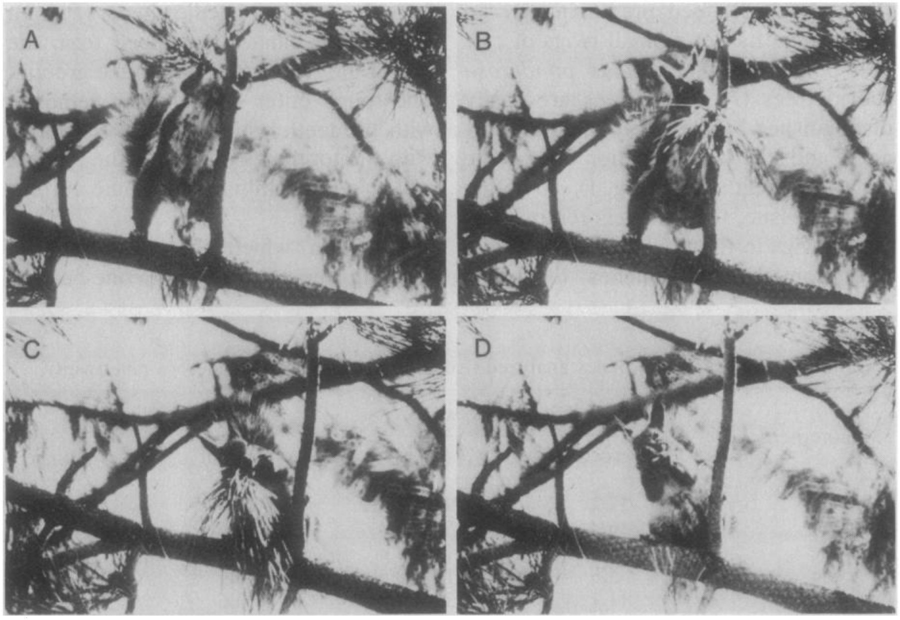

Fig. 2. A tassel-eared squirrel feeding on a ponderosa pine twig. (A and B) The squirrel gnaws through the base of the twig. (C) The terminal needle cluster is removed. (D) The squirrel eats the cortical tissue from the twig after removing the bark.

Table 2. Discriminant function analysis of the most abundant monoterpenes found in ponderosa pine twigs collected from feeding and nonfeeding trees.

| Monoterpene | Standardized discriminant function coefficients | |
|---|---|---|
| | December data | Combined data |
| α-Pinene | 0.4604 | 0.2491 |
| β-Pinene | 0.2112 | 0.1113 |
| 3-Carene | −0.0437 | 0.0642 |
| Limonene | 0.0918 | 0.0007 |

Eighteen monoterpenes were isolated from the cortical oleoresin of the ponderosa pine twigs. Of the 16 that were identified, α-pinene, β-pinene, 3-carene, myrcene, limonene, β-phellandrene, and terpinolene constituted about 97 percent of the total (Table 1). In results that were consistent with those on ponderosa pine xylem oleoresin (*7*), there were no significant seasonal differences in percent composition of cortical oleoresin monoterpenes in the trees tested.

The total monoterpene content was greater in nonfeeding trees than in feeding trees. The mean monoterpene content was 281.50 ± 151.65 (standard deviation) for feeding trees and 557.69 ± 310.43 for nonfeeding trees. These values represent the amount of monoterpene relative to 10 percent of a known internal standard (*6*). The difference in total monoterpene content was significant [$t(15) = 3.22$; $P < .01$, one-tailed test]. A discriminant function analysis (*8*) was performed on these data in order to ascertain which monoterpene contributed most heavily in differentiating feeding trees from nonfeeding trees (Table 2). A one-variate model with α-pinene as the predictor for feeding and nonfeeding trees best fits the data; feeding trees have a lower average proportion of this monoterpene than nonfeeding trees.

If monoterpene levels function as repellents to squirrels, preferences for ponderosa pine twigs with lower concentrations of these compounds would be expected. In choice tests with captive squirrels, in which twigs from feeding and nonfeeding trees of known monoterpene content were used, the squirrels preferred the twigs that were lower in monoterpene concentration (*4*).

The extent to which $\alpha$-pinene influences the choice of food by tassel-eared squirrels was investigated in another series of tests in which subjects were allowed to choose between plain mash (made from ground sunflower seeds and laboratory food) and mash laced with varying amounts of $\alpha$-pinene (*9*). Repeated-measures analysis of variance indicated that the $\alpha$-pinene content affected food consumption [$F(2,\ 14) = 3.72$; $P = .025$, one-tailed test]; as the amount of $\alpha$-pinene increased from 0.1 to 0.3 to 0.5 ml per 100 g of mash, the proportion of food consumed decreased from 0.45 to 0.41 to 0.24, respectively. The drop in consumption at 0.5 ml per 100 g was significant (Scheffé test; $P < .05$, one-tailed test) and showed that higher concentrations of $\alpha$-pinene caused tassel-eared squirrels to reduce their intake of a preferred foodstuff.

Thus the amount of $\alpha$-pinene is important in choosing which ponderosa pine trees are to be used as food sources by tassel-eared squirrels. Other factors that may affect twig preferences are nutritional quality of the cortical tissue and possible synergisms between nutrients and combinations of secondary compounds (mono- and sesquiterpenes). Contextual factors such as the visual appearance of the trees, presence of conspecifics, and pheromonal cues may also act as determinants in feeding tree selection if the chemistry of the tree is acceptable.

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3. Feeding trees were determined in the field by extensiveness of defoliation and number of freshly clipped needle clusters; the nearest neighbor of the same age and size as the feeding tree that did not show evidence of squirrel-caused defoliation was designated as a nonfeeding tree. In choice experiments and monoterpene analysis, twigs from a specific feeding tree were compared with twigs from its counterpart nonfeeding tree.
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6. A 2.0-g sample of cortical tissue was frozen under liquid nitrogen, ground to a powder, transferred with 50 ml of distilled water, and steam-distilled with *n*-pentane for 1 hour. The pentane extract was dried over anhydrous $Na_2SO_4$, and the pentane was removed (micro Vigreaux column). When the volume of the extract was less than 0.1 ml, an internal standard of 10.0 $\mu$l of *n*-tetradecane in *n*-heptane (200 $\mu$l of $C_{14}H_{30}$ in 10 ml of $C_7H_{16}$) wa added, and the volume was then increased to 0.1 ml with *n*-heptane. The concentrated extract was analyzed by gas chromatography (F and M model 810), with dual-flame ionization detection and a 104-m glass capillary column coated with Carbowax 20M). A 5.0-$\mu$l sample of the concentrated extract with added internal standard was injected into a glass-lined injection port at a split ratio of approximately 100 : 1. The oven conditions were 5 minutes at 65°C, then increased 1° per minute to 165°C, and isothermal thereafter. Helium make-up gas was supplied at 30 ml/min at the exit end of the glass capillary column. The data were recorded on a Varian CDS-111 chromatography data system, which gave the area percent composition for the various components and the area of each component relative to 10 percent of the area of the internal standard. Presentation of data in this manner permitted the evaluation of amounts of each component in the samples relative to the internal standard as well as the determination of the (relative) total amounts of all monoterpenes in each sample. Kovats' indices were determined on the glass capillary Carbowax 20M column at 70°C for the monoterpenes, and mass spectra were obtained from the total essential oil with a Carbowax 20M glass capillary column in a quadrupole gas chromatography–mass spectrometer (Finnigan model 1015C) with a model 6000 data system [H. Maarse and R. E. Kepner, *J. Agric. Food Chem.* **18**, 1095 (1970)].
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9. The mash consisted of 50 percent ground sunflower seeds and 50 percent mouse food (Charles River); 5 ml of safflower oil was added to each 100 g of mash. The mash containing monoterpene was made up in concentrations of 0.1, 0.3, and 0.5 ml of $\alpha$-pinene per 100 g of mash. On each testing day, after 23 hours of food deprivation, each squirrel was given two food dishes; one contained 20 g of plain mash, the other 20 g of mash laced with $\alpha$-pinene. Once a squirrel began to feed from either dish, it was given 20 minutes to eat; then both dishes were removed and the amount of each type of food eaten was recorded. Each squirrel received five trials at each of the three concentration levels. The monoterpene was obtained from Glidden, Organic Chemicals Division, SCM Corporation, Jacksonville, Fla.
10. We thank J. R. McKnight, J. C. Pederson, R. M. Potter, and K. Wuensch for their help with the study and A. J. Cooper, S. D. Berry, A. J. Pantle, and J. C. Jahnke for reading the manuscript. Supported by NSF grants BNS 76-05069 and DEB 76-80423.

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## Spatial Knowledge and Geometric Representation in a Child Blind from Birth

Abstract. *A series of experiments demonstrated that a congenitally blind 2½-year-old child—as well as sighted but blindfolded children and adults—can determine the appropriate path between two objects after traveling to each of those objects from a third object. This task requires that the child detect the distances and the angular relationship of the familiar paths and that she derive therefrom the angle of the new path. Our research indicates that the locomotion of the young blind child is guided by knowledge of the Euclidean properties of a spatial layout and by principles for making inferences based on those properties.*

We have had the opportunity to study the spatially guided locomotion of one 2½-year-old blind child in several experimental settings. After the child had been taken along several paths connecting four objects in a small room, she was able to move directly between the objects along paths she had never taken. Sighted adults and 3-year-old children, all blindfolded, performed with similar accuracy. These observations demonstrate that the locomotion of children, with or without visual experience, is guided by metric knowledge of space. This knowledge makes possible the derivation of further spatial information.

These observations were undertaken to address a classical issue in psychology, the development of human knowledge of space. Descartes (*1*) suggested that the geometric principles underlying spatial knowledge are innate and accessible to any perceptual mode. He offered the example of a blind man exploring objects with a stick. For the man to discover the shapes and arrangement of those objects, he must refer each tactual

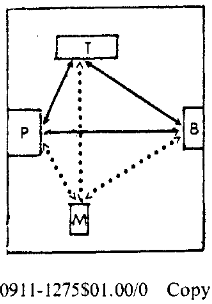

Fig. 1. Room layout for spatial inference experiment. The room measured 2.44 m by 3.05 m. Dashed lines, trained routes; solid lines, test routes. Landmarks: *M*, mother; *P*, pillows; *T*, table; *B*, basket.

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