mounting (4). However, I observed 39 occasions when copulations among apparently bonded individuals were not preceded by head-pumping. In such cases, the male grasped the female by the nape and simply attempted to mount; in 36 of the 39 instances, the female visibly struggled, but in no case did she flee. Thus, such behavior has some, but not all, of the characteristics of rape among unpaired individuals. I call this behavior "forced pair copulations" (FPC's) after Barrett (5), who described the phenomenon in captive mallards. It is particularly noteworthy that of the 39 FPC's observed in the present study, 30 occurred within 10 minutes of an observed rape attempt on the female in question. An average of .03 rape per female per hour equates to one rape per female per 33 hours. This provides for an average of 198 10-minute intervals between rapes, so that if FPC's were randomly distributed during the observation periods, only 1 in 198 (.5 percent) would be expected to follow within 10 minutes of a rape. Instead, 30 of 39 did so (77 percent). Confidence that the male performing the FPC was indeed the female's mate was provided in each case by some combination of at least two of the following: (i) individual recognition-three mated pairs were recognizable by feather peculiarities; (ii) defense of the female during a preceeding rape; (iii) failure of the female to avoid the male, both before and after the FPC; and (iv) continued mutual display between the pair after the FPC.

Although a high proportion of FPC's clearly followed rapes, many rapes (.59, or .66) were not followed by FPC's. Important postcopulatory displays among mallards are "bridling" and "nod-swimming," which have been assumed to signal successful copulations (4). Of the 59 observed rapes not followed by FPC's within 10 minutes, I have data on 14; only one of these resulted in the postcopulatory displays indicative of successful copulation. By contrast, of the 30 rapes followed by FPC within 10 minutes, I have data on postcopulatory displays of 12; of these, five showed evidence of sperm transfer [$\chi^2 = 38.45$, $\alpha = .01, P < .001$ (one-tailed test)]. Hence, FPC's, when they occur, are (i) significantly more likely to follow within 10 minutes of rape and (ii) significantly more likely to follow successful rape than unsuccessful rape.

A lower proportion of FPC's result in apparent sperm transfer than do normal copulations; of the 39 FPC's observed in the present study, only 10 (.26) were fol-19 AUGUST 1977

lowed by postcopulatory display, as opposed to 144 of 185 normal copulations among apparently mated pairs (.78) (Fisher's exact probability test, P =.052). Given the strong selective pressures that have doubtless favored the evolution of female insistence upon accurate, species-typical male courtship (6), the low success rate of FPC's is not surprising. Nevertheless, FPC's probably represent efforts by the mates of just-raped females to make the best of a bad situation. Given the usual excess of males over females in surface-feeding ducks (7) mates of rape victims do not have the option of an aggressive response, as reported for mates of seemingly adulterous mountain bluebirds (8), since replacement females are presumably unavailable. Under this circumstance, optimum male strategy is probably to stick with his mate, but also to introduce his sperm as quickly as possible—hence the forcing of a copulation.

An evolutionary perspective on behavior suggests that individuals will behave so as to maximize the difference between the benefits and costs associated with any potential act, with both benefits and costs evaluated in units of inclusive fitness. Rape of one's mate imposes a potential cost, in that it increases the likelihood of another individual's fathering her offspring. The responses available to a rape victim's mate also carry benefits and costs, and the observed pattern suggests that the mate behaves in accord with evolutionary prediction. Thus, aggressive intervention has the benefit of reducing the likelihood of the rape's being successful, but at the cost of pos-

sible injury to the male. Accordingly, such behavior occurs most often when the costs are low (a single rapist rather than several) and less often when the benefits are low (the female is unlikely to have been fertilized as indicated by her repulsion behavior). Similarly, forcing a copulation with a just-raped female conveys the benefit of introducing his sperm as quickly as possible to compete with those of the rapist, but at the possible cost of weakening the pair bond. Accordingly, FPC's occur only when their benefit is likely to outweigh their presumably high cost. Because of the close association of reproductive success with fitness, behaviors associated with reproduction should be especially susceptible to the action of natural selection. Hence, they should be especially amenable to sociobiologic analysis (9). The response of male mallards to rape of their females would appear to be a good example.

DAVID P. BARASH Departments of Psychology and Zoology, University of Washington, Seattle 98195

References

- K. Z. Lorenz, J. Ornithol. 89, 194 (1941); T. Lebret, Ardea 49, 97 (1961).
 F. McKinney, in Function and Evolution in Behavior (Oxford Univ. Press, London, 1975), p. 321
- 3. D. P. Barash, Sociobiology and Behavior (Elsevier, New York, 1977). K. Raitasue, Pap. Game Res. (Helsinki) 24, 1
- 4. K. (1964). 5.
- Barrett, thesis, University of Minnesota (1973).
- *Surv.* **27**, 385 (1961). 8. D. P. Barash, *Am. Nat.* **110**, 1097 (1976). 9. _____, *Am. J. Psychol.* **89**, 195 (1976).

17 March 1977

Identification of the Female Japanese Beetle Sex Pheromone: Inhibition of Male Response by an Enantiomer

Abstract. (Z)-5-(1-Decenyl)dihydro-2(3H)-furanone, isolated from virgin female Japanese beetles (Popillia japonica) attracted males of the species in field bioassays. However, the synthesized racemic mixture of this compound did not attract male Japanese beetles. The Z and E isomers and the saturated analog of both enantiomers of this compound were synthesized stereospecifically. Pure synthetic (R,Z)-5-(1-decenyl)dihydro-2(3H)-furanone was competitive with live females and with the pheromone isolated from live females in attracting males. Male response was strongly inhibited by small amounts of the S,Z isomer. Although the E isomer and the saturated analog of the pheromone are present in the material obtained from females, the role of these compounds in mediating the insect's behavior is unclear.

The Japanese beetle, Popillia japonica Newman (Coleoptera: Scarabaeidae), was introduced to North America about 1916 and has since become well established in the eastern part of the United States (1). The adults are devastating

pests of a variety of trees, ornamentals, and cultivated crops, and the larvae attack the roots of grasses. We report the isolation, identification, and synthesis of a sex pheromone produced by female Japanese beetles. We also report the inTable 1. Male Japanese beetles captured in Ellisco traps baited with mixtures of synthesized (R,Z)- and (R,E)-5-(1-decenyl)dihydro-2(3H)-furanone (Wooster, Ohio; July 1976).

Ratio* $R,Z:R,E$	Mean number of males captured† (five replicates)
100:0	845 a
90:10	981 a
85:15	825 a
80:20	926 a
Four females	442 b
Empty trap	26 c

*The quantity of the R,Z isomer was held constant at 5 μg . †Means followed by unlike letters differ significantly at the 5 percent level of probability (Duncan's multiple range test).

hibition of a behavioral response to a pheromone by its enantiomer.

The synthesized pheromone, (R,Z)-5-(1-decenyl)dihydro-2(3H)-furanone (Fig. 1), was very attractive to male Japanese beetles in field tests, but a racemic mixture of the synthesized Z isomer was inactive. Several pheromones that contain asymmetric carbons have previously been identified, and in most cases in which careful studies have been conducted only one enantiomer elicits a behavioral response (2). On the other hand, Borden et al. (2) reported that the enantiomers of 6-methyl-5-hepten-2-ol were synergistic and both were necessary to attract Gnathotrichus sulcatus LeConte, and Wood et al. (2) reported that Dendroctonus brevicomis LeConte responded to mixtures containing specific enantiomers of exo-brevicomin and frontalin.

In replicated field tests traps baited with 5 μ g of the pure synthesized pheromone (the *R*,*Z* isomer) captured about twice as many males as traps baited with four virgin females (3). However, admixture of as little as 1 percent of the synthesized *S*,*Z* isomer significantly reduced the response of males to the *R*,*Z* isomer. Although females contain smaller amounts of the *E* isomer and the saturated analog of the pheromone (see below), the role of these compounds could not be determined in our tests.

Smith and Hadley (4) first reported that large numbers of searching male Japanese beetles were attracted to emerging females. Later, Ladd (5) demonstrated that single virgin female beetles emitted a volatile sex pheromone and that traps baited with virgin females captured large numbers of males. We obtained a benzene solution highly attractive to males in field bioassays by rinsing the glass vessels used to hold virgin females (6). The bioassays were conducted by pouring 50 to 100 female-day equiva-

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lents (FD) of the benzene washings into a glass petri dish placed on the ground in an open field or golf-course fairway (7). The number of males responding in 5 minutes was counted and compared with the number responding to three females in a small cage placed in the same area during the same period. There were at least three replicates of each test, and all steps in the isolation procedure were monitored with this assay.

The benzene washings were filtered to remove solids and concentrated by vacuum distillation (150 mm, 36°C) through a 10-cm Vigreaux column: The concentrated benzene washing (50 FD) was as attractive to males as were three virgin females. It was fractionated by gel permeation liquid chromatography (8) with hexane as the mobile phase, and the hexane was removed from the active fraction by distillation at atmospheric pressure through a 10-cm Vigreaux column. Neither distillate was active. The concentrated liquid chromatographic fraction was then purified by sequential gas chromatography on OV-101, Carbowax 20M, SP 2300, SP 2340, and finally a second Carbowax 20M column (9). One compound was obtained from the final Carbowax 20M column that contained all the activity of the original flask washings; and, as shown by rechromatography on all of the columns, its purity was greater than 99 percent.

The active compound was identified by mass, infrared, and nuclear magnetic resonance (NMR) spectroscopy and by chemical transformations. The methane ionization mass spectrum of the phero-

Table 2. Male Japanese beetles captured in Ellisco traps baited with mixtures of synthesized (R,Z)- and (R,E)-5-(1-decenyl)dihydro-2(3H)-furanone and the R-saturated analog (Wooster, Ohio; July 1976).

Ratio* R,Z:R,E: R-saturated	Mean number of males captured† (seven replicates)
100 : 0 : 0	69 bc
98:0:2	90 bc
96:0:4	108 c
94:0:6	57 b
92:0:8	90 bc
90: 0: 10	68 bc
80 : 0 : 20	52 b
85:15:2	58 b
85:15:4	63 b
85:15:6	53 b
85:15: 8	58 b
85:15:10	62 b
0: 0:100	6 a
Empty trap	3 a

*The quantity of the R,Z isomer was held constant at 500 ng. †Means followed by unlike letters differ significantly at the 5 percent level of probability (Duncan's multiple range test).

Table 3. Male Japanese beetles captured in traps baited with mixtures of synthesized (R,Z)- and (S,Z)-5-(1-decenyl)dihydro-2(3H)-furanone (Wooster, Ohio; July 1976).

Ratio* R,Z:S,Z	Mean number of males captured† (six replicates)
100 : 0	168 a
99.5 : 0.5	106 ab
99: 1	91 bc
98: 2	96 bc
95: 5	52 bcd
90:10	30 cd
80:20	9 d
50:50	6 d
Empty trap	4 d

*The quantity of the R,Z enantiomer was held constant at 5 μ g. †Means followed by unlike letters differ significantly at the 5 percent level of probability (Duncan's multiple range test).

mone had the following diagnostic peaks: (M + 1), 225; (M - 1), 223; (M + 29), 253; (M + 41), 265; [(M +(1) - 18], 207; [(M + 1) - 36], 189;[(M + 1) - 60], 165; and a typical straight chain, unsaturated hydrocarbon series of peak clusters from m/e 67 to 169. The infrared spectrum (10) obtained with about 25 μ g of pure pheromone showed strong absorptions at 1790 cm⁻¹ (C=O) and 1172 cm⁻¹ (C-O). The remainder of the infrared spectrum consisted of hydrocarbon and olefinic absorption bands. A band at 980 cm⁻¹ suggested that a trans olefinic bond might be present although it was much weaker than would be expected. The evidence suggested a γ -lactone of a 14-carbon hydroxy acid with one double bond, and this was supported by the NMR spectrum (CCl₄, internal tetramethylsilane standard, δ , ppm): 0.87, triplet, 3H [CH₃]; 1.27, broad singlet, about 14H $[-(CH_2)_7-]$; 2.06 to 2.42, broad multiplet, 4 to 5H [ring protons]; and multiplets at 5.06, 1H, and 5.46, 1H [olefinic].

Microozonolysis (11) of the pure pheromone in carbon disulfide at -78° C, reductive cleavage of the ozonide with triphenylphosphine, and gas chromatography of the product on OV-101 yielded one major peak that was identical in retention time and mass spectrum to nonanal. Thus, the pheromone was tentatively identified as (Z)- or (E)-5-(1decenyl)dihydro-2(3H)-furanone.

Racemic (Z)- and (E)-5-(1-decenyl)dihydro-2(3H)-furanone were synthesized by the addition of the lithium salt of 1-decyne to methyl 4-oxobutyrate. The γ -lactone was formed in the course of this reaction, and the resulting acetylenic lactone was reduced to the olefinic and saturated lactones (12). The Z, E, and saturated lactones were purified by high-

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resolution, high-pressure liquid chromatography on silica with hexane-diethvl ether as the mobile phase and by gas chromatography on OV-101 and Carbowax 20M. The resulting synthetic lactones were more than 99.5 percent pure when analyzed on all five gas chromatographic columns. The synthesized racemic (Z)-5-(1-decenyl)dihydro-2(3H)-furanone had a retention time identical to that of the natural pheromone on all five gas chromatographic columns, had the same mass, NMR, and infrared spectra, and gave the same ozonolysis product. In addition, the synthesized racemic saturated lactone was identical in every respect to another compound obtained from females and eluted just prior to the pheromone on the SP 2340 column. Similarly, the synthesized racemic E lactone was identical in gas chromatographic retention times on all five columns, in mass spectra, and in the ozonolysis product to a third compound from the female Japanese beetle that eluted just after the pheromone on the final Carbowax 20M column. The saturated analog and the Eisomer amounted to about 3 and 15 percent, respectively, of the Z isomer (the pheromone) in the material obtained from females.

Samples containing 5, 50, 500, and 5000 ng of the pure synthesized racemic Z isomer dissolved in 0.25 μ l of hexane were subjected to bioassay; all failed to attract male beetles to the petri dish. In some instances, males appeared to orient and fly upwind toward the synthesized lactone, but they always stopped 30 cm or more from the petri dish. Admixture of 10, 13, 15, 17, and 20 percent of the E isomer to the Z isomer also failed to produce a response equivalent to that elicited by 10 to 20 ng of the pheromone isolated from females. However, one to three males occasionally moved closer and crawled into the petri dishes baited with 85:15 or 83:17 mixtures of the Z and E isomers. In the same test, four females or 100 FD of the pheromone isolated from females attracted 20 to 40 males that moved into the petri dish. In a similar test, admixture of 3 to 7 percent of the saturated lactone to the mixtures of Z and E isomers failed to elicit a response from males.

During these tests, we noted that the synthetic racemic olefinic lactones inhibited the response of males to virgin females. Although 5 ng of the Z isomer failed to inhibit response, the addition of 50 to 5000 ng of the Z isomer to a petri dish containing a cage of three virgin females reduced the response of males to females by 80 to 100 percent. The E iso-19 AUGUST 1977





Fig. 1. Synthesis scheme for Japanese beetle pheromone.

mer was an effective inhibitor only at the 5000-ng level.

Since the purity of the natural pheromone and the synthesized lactone had been rigorously examined by gas chromatographic, spectroscopic, and chemical methods and since the two compounds were identical chemically, the most likely explanation for the lack of activity of the synthesized material seemed to be inhibition of attraction by one of the enantiomers in the synthetic racemic mixture. Insufficient material was available to determine the optical rotation of the natural pheromone so we synthesized both enantiomers stereospecifically. The synthesis route for the R enantiomer is given in Fig. 1 (13). The S enantiomer was synthesized by the same method starting with S(+)-glutamic acid. The Z and E isomers of each synthesized enantiomer were obtained at a purity of more than 99 percent by liquid chromatography on silica and gas chromatography on Carbowax 20M. The optical rotations of the R,Z and S,Zenantiomers were $[\alpha]_{\rm D}^{26^{\circ}{\rm C}} = -69.6^{\circ}$ and $[\alpha]_{D}^{26^{\circ}C} = +70.5^{\circ}$, respectively (14). The Z enantiomers were identical chromatographically, spectroscopically, and chemically to the natural pheromone.

Samples of 5, 50, and 500 ng and 5, 50, and 500 μ g of the *R*,*Z*-, *R*,*E*-, and *R*-saturated and the *S*,*Z*-, *S*,*E*-, and *S*-saturated isomers were bioassayed in the field near Wilmington, North Carolina, in June 1976 and near Wooster, Ohio, in July 1976. (*R*,*Z*)-5-(1-Decenyl)dihydro-2(3*H*)furanone was very attractive to male Japanese beetles. A 50-ng sample of the R,Z isomer was about equal to four virgin females in the bioassay. Traps (three) baited with 5 μ g of the R,Z isomer attracted twice as many males in 1 day as traps baited with four virgin females (Table 1). In replicated tests, traps baited with 50 and 500 ng of the R,Z isomer caught more males than traps baited with equivalent amounts of the natural pheromone.

When traps were baited with the other five isomers, only the R,E isomer attracted male Japanese beetles, and the captures, while significantly greater than those captured by an empty trap, were only about 10 percent of those in traps baited with the R,Z isomer. However, admixture of 10, 15 and 20 percent of the R, E with the R, Z isomer did not significantly increase trap captures over those of the pure R, Z isomer (Table 1). Similarly, admixture of 2, 4, 6, 8, 10, and 20 percent of the R-saturated analog to the pure R, Z isomer or to a mixture of the R,Z and R,E isomers did not significantly increase trap captures (Table 2).

Since the racemic mixture of the synthesized pheromone was inactive, we prepared mixtures of the R,Z and S,Zenantiomers in which the S,Z enantiomer amounted to 0.5, 1, 2, 5, 10, 20, and 50 percent of the total mixture. The amount of R, Z enantiomer was held constant at 5 μ g in each mixture. In replicated field tests, as little as 1 percent of the S, Z enantiomer significantly reduced the number of males captured by traps baited with the pure R,Z enantiomer, and the number of males captured generally decreased with increasing quantities of the S, Z enantiomer (Table 3). Considerably less than 50 percent of the S,Zenantiomer was required to reduce trap captures to the level of an empty trap. This correlates well with our previous findings that the racemic mixture of the Zisomer did not attract males.

It is unusual in Coleoptera to find a pheromone consisting of only one compound, and inhibition of response to a pheromone by its enantiomer is unique among pheromones reported thus far. Investigation of the material obtained from females revealed no synergists though the E and saturated isomers were present in smaller amounts, and the synthesized R,E isomer showed slight activity. Possibly, these isomers have some subtle undetected role in the chemical communication of this species or they may have a role in species isolation in the beetle's native habitat. The enantiomeric composition of the natural product remains to be established. Synthesized (R,Z)-5-(1-

decenyl)dihydro-2(3H)-furanone is a potent attractant for male Japanese beetles and appears to have considerable potential for survey and control of this serious pest.

J. H. TUMLINSON Insect Attractants, Behavior and Basic Biology Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, Florida 32604

M. G. Klein

Japanese Beetle Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Ohio Agricultural Research and Development Center, Wooster 44691 R. E. DOOLITTLE

Insect Attractants, Behavior and Basic Biology Research Laboratory

T. L. LADD

Japanese Beetle Research Laboratory A. T. PROVEAUX

Insect Attractants, Behavior and Basic Biology Research Laboratory

References and Notes

- 1. W. E. Fleming, "Biology of the Japanese Beetle," U.S. Dep. Agric. Tech. Bull. No. 1449
- H. Borden, L. Chong, J. A. McLean, K. N. Slessor, K. Mori, *Science* 192, 894 (1976); D. L.
 Wood *et al.*, *ibid.*, p. 896; S. Iwaki, S. Marumo,
 T. Saito, M. Yamada, K. Katagiri, *J. Am.* em. Soc. 96, 7842 (1974)
- 3. Ellisco traps were placed on the ground in areas where male beetles were active and baited by placing a stainless steel planchet (2.5 cm in di-ameter) containing the test sample in 0.25 ml of hexane in the bait well. All tests were run as randomized complete blocks with five or more replicates and 11 m between traps. Data were analyzed statistically, and mean captures were sep 172ed statistically, and mean captures were sep-arated at the 5 percent level of significance by Duncan's multiple range test. L. B. Smith and C. H. Hadley, "The Japanese Beetle," U.S. Dep. Agric. Circ. No. 363 (1926).
- 4.
- T. L. Ladd, Jr., J. Econ. Entomol. 63, 905 (1970). 5. T.
- Up to 250 live virgin female beetles were held in a 2800-ml Erlenmeyer flask. When additional beetles were available, 2150 to 3000 females 6. were held in a glass chromatography jar (31 by 31 by 62 cm). Each morning, beetles were transferred to a clean vessel, and the vessel that had contained them overnight was rinsed three times with 15- or 30-ml portions of benzene for the flasks and chromatography jars, respectively. Each evening, the beetles were transferred to a clean vessel containing apple slices for food, and the vessel that had contained them during the day was rinsed three times. Neither benzene alone nor the benzene washings of flasks used to hold apples or male beetles plus apples were ac-tive in field bioassays.
- The bioassay was similar to that used with the introduced pine sawfly by J. E. Casida, H. C. Coppel, and T. Watanabe [J. Econ. Entomol. 56, 18 (1963)] and with the lesser peachtree bor-er by C. E. Yonce, J. H. Tumlinson, C. R. Gen-try, and E. R. Mitchell [Environ. Entomol. 3, 569 (1974)]; J. H. Tumlinson, C. E. Yonce, R. E. Doolittle, R. R. Heath, C. R. Gentry, E. R. Mitchell, Science 185, 614 (1974). A glass column (0.5-inch inside diameter) was packed to a height of 100 cm with a hexane slurry of Styragel (Waters Associates) and eluted with hexane at a flow rate of 5 ml/min. The active material eluted from this column in the 250- to 350-ml fraction. The column effluent was monitored with a refractive index detector.
- was monitored with a refractive index detector.
- Gas chromatography was performed on a gas chromatograph (Varian model 1400) equipped with a flame ionization detector. The column ef-fluent was split so as to send 5 percent to the detector and 95 percent to a glass capillary col-lector [R. G. Brownlee and R. M. Silverstein, *Anal. Chem.* **40**, 2077 (1968)]. Stainless steel col-

umns (2 m by 2.3 mm inside diameter) with a flow (N_2) rate of 20 ml/min were used with the now (12) rate of 20 ml/min were used with the other conditions noted; silanized Chromosorb W (120 to 140 mesh, acid washed) was used as the support in each column; the OV-101 (4.5 per-cent) column temperature was held at 150°C for 30 minutes and the temperature was then pro-grammed at 8%/min to 225°C; the Carbowax 20M, (5.2 percent) column temperature was 100°C (5.2 percent) column temperature was 190°C; the SP 2300 (7.6 percent) column temperature was 200°C; the SP 2340 (10.4 percent) column temperature was 200°C; and the Carbowax 20M

- (4.6 percent) column temperature was 190°C. The infrared spectrum was obtained by washing 10. The pheromone sample from the glass capillary collection tube into a NaCl microcavity cell (Barnes Engineering) with about 5 μ l of carbon tetrachloride. The cell (0.5-mm path length) was placed in a 3X beam condenser (Barnes Engineering) in a Perkin-Elmer model 467 infrared spectrometer. The reference was CCl₄.
- M. Beroza and B. Bierl, Anal. Chem. 38, 1976 (1966); *ibid.* 39, 1131 (1967).
 The methyl 4-oxobutyrate was prepared as de-
- scribed [Org. Synth. Collect. Vol. III (1955), p. 630]. The acetylenic lactone was reduced to the olefinic lactones with H_2 over Pd/BaSO₄ (Lindlar catalyst) or to the saturated lactone with H_2 over Pd on charcoal.
- 13. The pure enantiomers of glutamic acid (Sigma) The pure enantiomers of glutamic acid (Sigma) were deaminated either by the method of A. T. Austin and J. Howard [J. Chem. Soc. (1961), p. 3593] or by a modification of the method of M. Winitz, L. Bloch-Frankenthal, N. Izumiya, S. M. Birnbaum, C. G. Baker, and J. P. Greenstein [J. Am. Chem. Soc. 78, 2423 (1956)]. This reac-tion is considered to proceed with full retention of configuration [K. Koga, M. Taniguchi, S. Yamada, *Tetrahedron Lett.* (1971), p. 263]. The conversion of the acid to the acid chloride with conversion of the acid to the acid chloride with either thionyl or oxalyl chloride is considered to

proceed with retention of configuration [C. Eguchi and A. Kakuta, *Bull. Chem. Soc. Jpn*, (1974), p. 1704]. The acid chlorides were reduced to the aldehydes in dry toluene at 65° to 70°C. The amount of *E* isomer produced in the Wittig reaction ranged from 10 percent when a mixture of tetrahydrofuran and hexamethyl phosphoramide (THF/HMPA) was used as the solvent to about 25 percent when a mixture of diethyl ether and methylene chloride was used. Undiluted THF gave about 15 to 20 percent of E isomer; THF/HMPA gave a higher overall yield

- and fewer by-products. All concentrations given are in grams per 100 ml of chloroform. The rotations of the R,Z and S,Zenantiomers were measured at concentrations of 5.0 and 5.1, respectively. The rotations of the R_E and S_rE enantiomers were $[\alpha]_{B}^{9e^{\circ}c} = -31.2^{\circ}$ (concentration, 2.237) and $[\alpha]_{B}^{9e^{\circ}c} = +30.2^{\circ}$ (concentration, 2.112), respectively. The R- and S-5-(1-decy)/dihydro-2(3H)-furance
- The *R* and *S*-5-(1-decyl)dihydro-2(3*H*)-furanone enantiomers gave rotations of $[\alpha]_{\beta}^{8^{\circ}C} = -31.8^{\circ}$ (concentration, 2.387) and $[\alpha]_{\beta}^{2^{\circ}C} = +30.0^{\circ}$ (concentration, 2.739), respectively. In cooperation with the Ohio Agricultural Re-search and Development Center, Wooster 44691. Approved for publication as Journal ar-ticle No. 197-76. We thank the Animal and Plant Health Inspection Service (APHIS), U.S. De-partment of Agriculture, for locating sources of larvae and populations for bioassay. K. O. Law-rence and C. R. Buriff gave assistance in obtain-ing enough female beetles to complete this proj-ect. We thank R. R. Heath for NMR spectra. 15 ect. We thank R. R. Heath for NMR spectra, and J. M. DeVore, K. Allen, C. H. Johnson, C. R. Clark, M. Roth, K. P. Callahan, and B. Gold for technical assistance, and the management and members of the Buccaneer Country Club, Burgaw, N.C., for cooperation.

22 November 1976; revised 22 March 1977

Augmenting Mental Chronometry: The P300 as a **Measure of Stimulus Evaluation Time**

Abstract. A technique for measuring the latency of the P300 component of eventrelated brain potentials on individual trials is described. Choice reaction times and the latency of the P300 were compared under speed-maximizing and under accuracymaximizing instructions. The choice stimuli required different levels of semantic categorization. The data support the proposition that the latency of P300 corresponds to stimulus evaluation time and is independent of response selection.

In his 1938 survey of experimental psychology, Woodworth (1) ventured the hope that "brain waves" might be used in the timing of mental events: "the speed of thought' we say; but as soon as we set about measuring the time occupied by a thought we find that the beginning and end of any measurable time must be external events. We may in the future use 'brain waves' as indicators of the beginning and end of a mental process . . . but in general it has seemed necessary to let the timed process start with a sensory stimulus and terminate with a muscular response." In the decades that followed, it became clear that while the electroencephalogram (EEG) can be a useful index of neural pathology and global changes in a subject's state, it cannot support studies of the timing of specific mental events; the suggestion that brain waves may play such a role is absent from the second edition of Woodworth's book (2).

Yet, the need for an index of the timing of mental processes, independent of response selection and execution time, is as acute now as in the earliest days of mental chronometry (1). Much of contemporary cognitive psychology (3) is concerned with the analysis of mental events into their presumed stages. The traditional approach to this problem using reaction time (RT) could be complemented by a measure of stimulus processing that is independent of overt motor responses. In this report we present evidence that the P300 component of the human event-related brain potential (ERP) can serve as such an index for measuring stimulus evaluation time.

The P300 is elicited by a class of taskrelevant events (4, 5). Its amplitude has been shown to be directly proportional to the "surprise value" (the reciprocal of expectancy) of a stimulus (6). However, before a stimulus can surprise it must be identified. As P300 commonly appears as a discriminative response to specific stimuli within a series, its elicitation must be preceded by an adequate evaluation of the stimulus at some level of pro-SCIENCE, VOL. 197