

The change of the population EPSP is consonant with the report by Hesse and Teyler⁸ who found complete postictal depression lasting up to 10 min followed by gradual recovery lasting 15–25 min. No population spike was recorded in the latter study, however, which examined a different part of hippocampus (CA1) in deeply anesthetized rats. The different recovery rates of the population spike and population EPSP indicate that ECS interferes not only with synaptic transmission but that it elicits an even more prolonged decrease of postsynaptic excitability.

Comparison of the above electrophysiological findings with the behaviorally established anterograde effects of ECS brought controversial results. Electrically elicited hippocampal after-discharge caused only a short-lasting (10 min or less) anterograde impairment of spatial working memory of rats^{12–14}.

Since these tasks are all critically dependent on hippocampal integrity¹⁵, it is obvious that the 30–40% reduction of the fascia dentata response does not impair this memory function. On the other hand the recovery of the population spike amplitude has a similar time course to the ECS-induced anterograde impairment of the acquisition of passive avoidance¹⁶ or of conditioned taste aversion¹⁷ and of the retrieval of conditioned emotional reactions^{18,19}. The significance of these positive correlation is diminished, however, by the fact that neither of the above conditioned reactions is impaired by hippocampectomy³.

The above evidence indicates that the postictal behavioral deficit cannot be simply accounted for by interference with the transhippocampal information flow.

- 1 Visiting scientist from the National Center for Scientific Investigations, Havana, Cuba.
- 2 Address for correspondence: Dr J. Bureš, Institute of Physiology, Czechoslovak Academy of Sciences, Vídeňská 1083, CS-142 20 Prague 4, Czechoslovakia.
- 3 O'Keefe, J., and Nadel, L., *The Hippocampus as a Cognitive Map*. Clarendon Press, Oxford 1978.
- 4 Deadwyler, S.A., West, M.O., and Christian, E.P., in: *Conditioning: Representation of Involved Neural Functions*, p. 63. Ed. C.D. Woody. Plenum Press, New York and London 1982.
- 5 Weisz, D.J., Clark, G.A., Yang, Bo-yi, Thompson, R.F., and Solomon, P.R., in: *Conditioning: Representation of Involved Neural Functions*, p. 131. Ed. C.D. Woody. Plenum Press, New York and London 1982.
- 6 McGaugh, J.L., and Herz, M.J., *Memory Consolidation*. Albion Publishing Corp., San Francisco, Calif. 1972.
- 7 Gibbs, M.E., and Mark, R.F., *Inhibition of Memory Formation*. Plenum Press, New York and London 1973.
- 8 Hesse, G.W., and Teyler, T.J., *Nature* 254 (1976) 562.
- 9 Fifková, E., and Maršala, J., in: *Electrophysiological Methods in Biological Research*, p. 653. Eds J. Bureš, M. Petrůň and J. Zachar. Publ. House Czech. Acad. Sci., Prague 1967.
- 10 Douglas, R.M., and Goddard, G.V., *Brain Res.* 86 (1975) 205.
- 11 Swinyard, E.A., in: *Experimental Models of Epilepsy*, p. 433. Eds D.P. Purpura, J.K. Penry, D.B. Tower, D.M. Woodbury and R.D. Walter. Raven Press, New York 1972.
- 12 Kaye, H., Burešová, O., and Bureš, J., *Physiol. bohemosl.* 33 (1984) 205.
- 13 Collier, T.J., Miller, J.S., Travis, J., and Routtenberg, A., *Behav. neural Biol.* 34 (1982) 227.
- 14 Olton, D.S., and Wolf, W.A., *Behav. neural Biol.* 33 (1981) 437.
- 15 Olton, D.S., Becker, J.T., and Handelman, G.E., *Behav. Brain Sci.* 2 (1979) 313.
- 16 Kopp, R., Bohdanecký, Z., and Jarvik, M.E., *J. comp. Physiol. Psychol.* 64 (1967) 22.
- 17 Burešová, O., and Bureš, J., *Physiol. Behav.* 22 (1979) 641.
- 18 Nachman, M., Meinecke, O.R., and Baumbach, H.D., *Psychon. Sci.* 17 (1969) 137.
- 19 D'Andrea, J.A., and Kesner, R.P., *Physiol. Behav.* 11 (1973) 747.

0014-4754/84/070761-03\$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1984

Habitat marking: males attracted to residual odors of two *Drosophila* species

G.E. Spence, A.A. Hoffmann and P.A. Parsons

Department of Genetics and Human Variation, La Trobe University, Bundoora (Victoria 3083, Australia), 12 August 1983

Summary. We have found that males of the sibling species *D. melanogaster* and *D. simulans* are preferentially attracted to sites marked by the residual odors of conspecific females, especially if non-virgin. In natural populations, this could enhance sexual selection among males, and cause some isolation at the microhabitat level.

The potential importance of habitat selection is being increasingly debated¹. Many studies are based upon gene (in particular electrophoretic) and chromosomal polymorphisms. Since these genotypic assessments are not directly relatable to the field situation interpretations are often difficult, and stress the need to study ecobehavioral traits important in determining the distribution and abundance of organisms in habitats. In insects, such traits can be related to abiotic and biotic (especially resources) features of the environment². In *Drosophila*, there are recent indications of the feasibility of this approach in habitat selection studies for phototaxis³.

Cosmopolitan *Drosophila* species typically utilize resources and court in the same habitats. The attraction of flies to resources is therefore an important ecobehavioral component of habitat selection. While there is good evidence that certain chemicals act as attractants^{4,5}, it has not been explored whether flies themselves mark habitats. Here we present experimental evi-

dence at the interspecific level for 2 cosmopolitan sibling species, *D. melanogaster* and *D. simulans*, which predominantly involves 'marking' of habitats by females.

Adults aged 2–3 days were placed into 470-ml glass cylinders covered with gauze at both ends; 600 virgin females, 600 non-virgin females or 700 males of each species were separately used as 'marker' populations. The higher number of males compensated by volume for the size difference between sexes. After 8 h, the flies were discarded, and the cylinders placed in a wind tunnel (figure). Light was provided by a single fluorescent tube (40 W) placed vertically behind the tunnel. After starving for 8 h exposed to water-saturated vapour at 20°C, 300 males and females of each species were then simultaneously released into the observation chamber (B). Each species was marked with a different fluorescent dust and colors were alternated between experiments. Odors were drawn from the marked glass cylinders (C) into the observation chamber

by means of an exhaust fan (A). The 'marked' cylinders served as traps, and after 16 h overnight trapped flies were immobilized at 4°C for scoring.

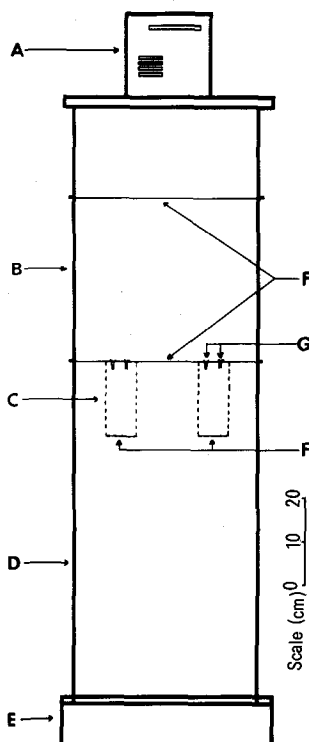
Even though the proportions of the released flies attracted were low, the residual odours from all 3 'marker' populations attracted more males than females (table). For females as 'markers', there was a significant tendency of males to select traps marked with the residual odor of their own species. Contingency coefficients⁶, *c*, suggest that this tendency is somewhat higher for non-virgin females perhaps because of the deposition of eggs. Males tend to select traps marked with the residual odors of males of their own species to a lesser extent than females as markers, however a composite X^2_3 is highly significant ($X^2_3 = 12.19$, $p < 0.01$).

Considering the attraction of females, Fisher's exact test⁶ was significant once only (attraction to males, trial 2, $p < 0.01$) in the same direction as the comparable male data, but the numbers attracted are generally inadequate for the detection of trends.

Chemical stimuli from the surface of the cuticle of mature females are important in initiating courtship of males⁷⁻⁹. Here, residual odors provided by non-virgin females provide the greatest attraction of males followed by virgin females, and then males. Refinements of experimental procedure could increase the proportions attracted. In any case, in nature, this would mean that flies at a feeding and oviposition site would tend to attract males to that site. A likely consequence would be substantial competition among males for mates enhancing levels of sexual selection, and presumably helping to explain the high levels of multiple insemination usual in *D. melano-*

gaster populations¹⁰. Interestingly, unpublished field collection data in Melbourne, Victoria, reveals a frequent excess of males when flies are collected by sweeping, especially when physical conditions are optimal. Given the extremely high fecundity of *Drosophila* females in natural populations, a corresponding attraction of females could well be disadvantageous since it would lead to excessive competition for larval resources.

Attraction to 'marked' microhabitats is a dimension to habitat selection additional to that based upon the abiotic and biotic factors of the environment. A prerequisite is obviously permissive physical conditions and available resources¹¹. While the chemicals in resources may act as attractants for both sexes of several species attracted to fermenting fruit and vegetable matter, the marking of habitats presumably ensures the attraction of males of the appropriate species to highly favorable habitats. In addition, a degree of isolation may occur at the microhabitat level unforeseen at the present time, and calls for studies of species distributions at this level. Extrapolations to the field are of course difficult with *Drosophila* although flies can be assumed to manifest their normal behaviors in responding to odors in this olfactometer⁵. The lek species *D. mycetophaga* and *D. polypori* that utilize hard bracket fungi as leks and soft forest fungi as resources may offer interesting possibilities for further studies, especially as the sex ratio at the leks is heavily biased in favor of males¹². In any case a need to study sexual selection at the ecobehavioral level as recommended elsewhere¹³ is confirmed.



Flies were tested in a wind tunnel modified from a design by Wright⁵. The apparatus was constructed from white perspex, and comprised 3 sections: 1. an upper section supporting the exhaust fan (A), 2. an observation chamber (B) with clear perspex on one side and gauze at both ends (F), and 3. a lower section (D) on a stand (E) into which 470 ml cylinders (C) were suspended; these cylinders were covered at the lower end with gauze, the entry of flies being permitted through 2 small plastic funnels (G) (4 mm at entrance reducing to 2 mm) extending through the lower gauze of the observation chamber into each glass cylinder.

Attraction of *Drosophila melanogaster* and *D. simulans* to residual odors

	Males		Females	
	<i>D. simulans</i>	<i>D. melanogaster</i>	<i>D. simulans</i>	<i>D. melanogaster</i>
a) Attraction to odor of non-virgin females				
1. <i>D. simulans</i>	25 (16.4)	12 (20.6)	10	6
<i>D. melanogaster</i>	6 (14.6)	27 (18.4)	4	9
	$\chi^2_1 = 17.24^{***}$, c = 0.44			
2. <i>D. simulans</i>	15 (9.1)	6 (11.9)	5	2
<i>D. melanogaster</i>	8 (13.9)	24 (18.1)	6	11
	$\chi^2_1 = 11.13^{***}$, c = 0.42			
3. <i>D. simulans</i>	16 (11.2)	7 (11.8)	2	2
<i>D. melanogaster</i>	2 (6.8)	12 (7.2)	0	1
	$\chi^2_1 = 10.64^{**}$, c = 0.47			
b) Attraction to odor of virgin females				
1. <i>D. simulans</i>	14 (9.5)	7 (11.5)	9	7
<i>D. melanogaster</i>	6 (10.5)	17 (12.5)	5	2
	$\chi^2_1 = 7.29^{**}$, c = 0.38			
2. <i>D. simulans</i>	19 (14.8)	8 (12.2)	7	1
<i>D. melanogaster</i>	9 (13.2)	15 (10.8)	5	2
	$\chi^2_1 = 5.54^*$, c = 0.31			
3. <i>D. simulans</i>	10 (6.7)	3 (6.3)	1	3
<i>D. melanogaster</i>	6 (9.3)	12 (8.7)	0	1
	$\chi^2_1 = 5.74^*$, c = 0.39			
c) Attraction to odor of males				
1. <i>D. simulans</i>	14 (11.4)	5 (7.6)	4	6
<i>D. melanogaster</i>	10 (12.6)	11 (8.4)	8	6
	$\chi^2_1 = 2.82$, c = 0.26			
2. <i>D. simulans</i>	26 (2.6)	20 (23.4)	12	2
<i>D. melanogaster</i>	5 (8.4)	12 (8.6)	4	8
	$\chi^2_1 = 3.65$, c = 0.23			
3. <i>D. simulans</i>	8 (4.8)	6 (9.2)	9	4
<i>D. melanogaster</i>	3 (6.2)	15 (11.8)	3	4
	$\chi^2_1 = 5.72^*$, c = 0.39			

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Numbers refer to flies caught in trap cylinders containing the residual odors of either *D. melanogaster* or *D. simulans*. χ^2 values refer to heterogeneity tests ($df = 1$) with expected numbers given in brackets. Contingency coefficients (*c*) are also given.

- 1 Parsons, P.A., The Evolutionary Biology of Colonizing Species. Cambridge University Press, New York 1983.
- 2 Andrewartha, H.G., and Birch, L.C., The Distribution and Abundance of Animals. University of Chicago Press, Chicago 1954.
- 3 Kekić, V., Taylor, C.E., and Andjelković, M., Genetika 12 (1980) 219.
- 4 Fuyama, Y., Behav. Genet. 6 (1976) 407.
- 5 Wright, R.H. Can. Ent. 98 (1966) 282.
- 6 Siegel, S., Non-parametric Statistics. McGraw-Hill, Kogakusha, Tokyo 1956.
- 7 Tompkins, L., Hall, J.C., and Hall, L.M., J. Insect Physiol. 26 (1980) 689.
- 8 Venard, R., and Jallon, J.M., Experientia 36 (1980) 211.
- 9 Antony, C., and Jallon, J.M., J. Insect Physiol. 28 (1982) 873.
- 10 Milkman, R.D., and Zeitler, R.R., Genetics 78 (1974) 1191.
- 11 Parsons, P.A., Evol. Biol. 14 (1981) 297.
- 12 Parsons, P.A., Behav. Genet. 8 (1978) 511.
- 13 Parsons, P.A., A. Rev. ecol. Syst. 14 (1983) 35.

0014-4754/84/070763-03\$1.50 + 0.20/0

© Birkhäuser Verlag Basel, 1984

Neuropeptide Y in the guinea-pig biliary tract

J.M. Allen, J. Gu, T.E. Adrian, J.M. Polak and S.R. Bloom^{1,2}

Departments of Medicine and Histochemistry, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS (England), 17 August 1983

Summary. High concentrations of neuropeptide Y (NPY) have been demonstrated in the gall bladder (16.7 ± 5.4 pmol/g), cystic duct (25.4 ± 9.2 pmol/g) and common bile duct (54.7 ± 11.5 pmol/g) of the guinea-pig using a recently developed radioimmunoassay. Immunoreactive NPY containing nerves were demonstrated in all layers of the biliary tree using immunocytochemistry, being particularly dense in the myenteric and mucosal plexuses.

Neuropeptide Y (NPY) has recently been isolated from porcine brain. It consists of 36 amino acids and is characterized by an N-terminal tyrosine (Y) and a C terminal tyrosine amide^{3,4}. This peptide has been identified within neurones in the brain⁵ and gastrointestinal tract^{6,7}. The presence of this peptide has been determined in the biliary tract of the guinea pig using radioimmunoassay and immunocytochemistry.

Methods. For radioimmunoassay four Dunkin-Hartley guinea-pigs were sacrificed by cervical dislocation. The gall bladder, cystic duct and common bile duct were dissected and the peptides extracted from these tissues by boiling in 0.5 M acetic acid (10% weight/volume) for 10 min.

NPY concentrations in these extracts were measured using an antiserum raised in a rabbit by conjugating porcine NPY to bovine serum albumin with carbodiimide. The third and subsequent monthly boosts of the conjugate used keyhole limpet hemocyanin as carrier. Natural porcine NPY was iodinated using chloramine T and purified on a G-50 superfine column providing a tracer with a specific activity of 70 Bq/fmol. The antiserum was used in a final dilution of 1:10,000 in an assay volume of 600 μ l, 0.06 M sodium phosphate buffer, pH 7.2 containing 0.05 M EDTA and 1% bovine serum albumin. The assay could detect with 95% confidence, a minimum of 2 fmol of NPY per assay tube, and 20 fmol of peptide YY per assay tube. No crossreaction was observed with human or porcine pancreatic polypeptide up to 100 pmol/tube. Pure porcine NPY was used as standard. Results are expressed as mean and SEM.

Chromatography. Tissue extracts were fractionated on a HPLC Bondapak C18 reverse phase column (3.9 \times 300 mm) using a linear gradient elution system from 35% to 45% acetonitrile in water containing 0.2% trifluoroacetic acid over 10 min at a flow rate of 1 ml per min. 1-ml fractions were collected for subsequent radioimmunoassay. Pure porcine NPY and PYY were used as standards.

Immunocytochemistry. 6 adult Dunkin-Hartley guinea-pigs of both sexes were sacrificed by cervical dislocation. The gall bladders, cystic ducts, hepatic ducts, common bile ducts and the sphincters were removed and fixed in 0.4% benzoquinone in 0.01 M phosphate buffered saline (pH 7.2) (PBS) for 1 h at room temperature⁸. The samples were then washed in 7% sucrose in PBS overnight. 5- μ m-thick cryostat sections were cut

and mounted on poly-L-lysine-coated⁹ glass slides. To investigate the complete distribution pattern of the NPY-containing nerves, whole-mount stretch preparations of the gall bladder were made according to a procedure described previously¹⁰. Immunocytochemistry was carried out on both cryostat sections and whole mount stretch preparations following modified indirect immunofluorescence procedures described previously^{11,12}. Antiserum to NPY was raised in rabbit against natural porcine NPY conjugated to bovine serum albumin with bis-diazobenzidine. The antiserum was used in a dilution of 1:800 in PBS. The controls included pre-absorption of the first layer antiserum with pure natural NPY (5.0 nmol/ml of

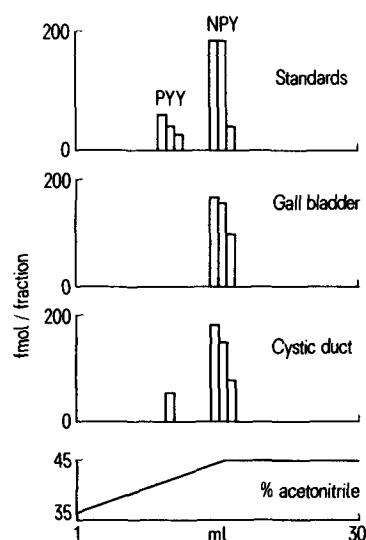


Figure 1. Fractionation of extracts of gallbladder and cystic duct on an HPLC C18 Bondapak reverse phase column using a linear gradient elution of 35–45% acetonitrile in water containing 0.2% trifluoroacetic acid. Fractions of 1 ml were collected for subsequent radioimmunoassay. The positions of porcine NPY and PYY standards are shown in the top panel for comparison. Recoveries from the columns ranged from 85 to 98%.