An example of an invertebrate self-recognition system characterized by these conditions is found in the colonial tunicate, *Botrylus* (*12*). However, in *Aplysina* and other sponges, a requirement for complete rather than partial sharing of histocompatibility determinants in graft acceptances is indicated by the observation that histocompatibility identity is always transitive (*3, 11*). Therefore, unless both gamete and larval dispersal are extremely limited, it is unlikely that histocompatibility-defined clones of *A. fistularis* are often multiple-related clones. Phenotypic variation within histocompatibility-defined clones of *A. fistularis* can be attributed to nongenetic influences. Aside from the taxonomic significance of such findings, the recognition of phenotypic variation as nongenetic carries basic evolutionary implications. Phenotypic plasticity may imply differential response to environmental conditions or, as we suggest for *A. fistularis*, may represent developmental alternation of specialized morphologies. For *A. cauliformis*, the complete association between a visible polymorphism and individual clones implies a genetic basis for the polymorphism, although somatic inheritance of nongenetic determinants cannot be ruled out. The three forms of *A. cauliformis* may represent either a polymorphism within a single species or distinct sympatric species.

Self-recognition bioassays provide a new approach for the study of variation. Exploitation of the natural replication of genotypes present in many invertebrate populations may lead to isolation of nongenetic components of variation.

JOSEPH E. NEIGEL
Department of Molecular and Population Genetics, University of Georgia, Athens 30602
GEORGE P. SCHMAHL
Department of Zoology, University of Georgia

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10. The variance to mean ratio of the quadrat counts was significantly greater than one, and the Morisita index of dispersion indicated a contagious distribution that was significantly different from random ($\chi^2 = 1929.8$, $Z = 27.5$, d.f. = 599, $P < 0.005$); J. M. Elliot, *Freshwater Biol. Assoc. Sci. Publ.* 25 (1977).
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Ingestive Behavior Evoked by Hypothalamic Stimulation and Schedule-Induced Polydipsia Are Related

Abstract. *Some, but not all, rats eat or drink in response to electrical stimulation of the lateral hypothalamus. Similarly, some, but not all, rats given food intermittently display schedule-induced polydipsia. In this experiment, animals that ate or drank during electrical stimulation tended also to be those displaying polydipsia. Thus, individual differences in predisposition to engage in ingestive behavior are consistent under two very different conditions.*

Rats become more active during electrical stimulation of the lateral hypothalamus (ESLH), but only some of them eat, drink, or exhibit other specific behaviors (*1, 2*). The explanation of why animals should eat or drink during ESLH has been disputed. Some investigators have suggested that ESLH activates specific motivational states such as hunger and thirst when electrodes are in the appropriate location (*3*). Others have maintained that stimulation evokes an increase in activity that may be channeled into eating, drinking, or other behaviors, depending on environmental factors and the predisposition of the animal (*1*). The evidence supporting these competing interpretations has been reviewed (*4, 5*).

We have addressed this question by trying to understand why only some animals eat or drink during ESLH even though electrodes seem to be in identical locations. The most obvious explanation is small, but critical, differences in electrode placements between animals. This explanation, however, seems to have been ruled out by a number of experiments. Many animals, for example, engage in the same behavior in response to stimulation at very different brain sites (*2*), and rats that eat or drink in response to ESLH continue to display the same behaviors after the electrode position has been changed (*6*). The same behavior is also evoked after the neural tissue surrounding the electrode tip has been destroyed and higher currents are used to stimulate more distal areas (*7*). Moreover, strains that normally consume equal amounts of food and water differ significantly in the probability that ESLH will evoke eating or drinking. This suggests that factors other than hunger and thirst must be considered (*8*). Thus, the earlier idea that individuals differ in predisposition to display specific behaviors during ESLH has gained support (*1, 2*).

To pursue the question of individual differences, we are exploring other experimental procedures in which animals consume excessive amounts of food or water. The experiment reported here was undertaken because of the many similarities between ESLH-evoked ingestive behaviors and the phenomenon of schedule-induced polydipsia (SIP). When food-deprived animals are given small amounts of food reward, intermittently, they consume excessive amounts of water—under some conditions, as much as half their body weight in 3 hours (*9*). In common with ELSH, intermittent delivery of food to hungry animals produces an increased activation that can be channeled into other responses besides drinking, including aggression, wheel running, and ingestion of nonnutritive substances such as sawdust (*10*). Moreover, the behaviors evoked by ESLH and SIP normally get stronger over repeated trials (*10, 11*). Finally, the behaviors evoked under both conditions may depend on dopamine pathways (*5, 12, 13*).

Schedule-induced polydipsia has been called an "adjunctive" or "displacement" behavior because it occurs when a desired goal cannot be obtained (*10, 14*). The increases in general activity may be accompanied by stress, as ele-

vated corticosterone levels have been shown in animals subjected to intermittent feeding schedules (*13*). Because of the many similarities between ESLH-evoked eating and drinking and SIP, we have studied the behavior of animals tested under both conditions and now report striking parallels in the responses of individuals.

The subjects were 42 adult (366 to 480 g), male Long-Evans hooded rats (Simonsen), with bipolar stainless steel electrodes (Plastic Products, MS 303/1, 0.25 mm in diameter) bilaterally implanted in the lateral hypothalamus (*15*). After recovering from surgery, animals were habituated for at least 15 minutes in a Plexiglas chamber and tested for the behavior evoked by ESLH. Stimulation consisted of 20-second trains of 60-Hz sine waves alternating with 15-second intertrial intervals. During testing, 75-mg food pellets (P. J. Noyes) were distributed evenly over the floor, and a standard water bottle with a metal drinking tube was attached to one wall. Stimulation intensity was increased by 1-μA steps until the animal either ate or drank or became excessively agitated or displayed "forced" motor responses, either of which precluded eating or drinking. Animals that ate or drank were given additional ESLH at a current intensity just above the threshold until they ate or drank on five stimulations. The number of food pellets eaten, the duration of drinking, and the current intensity threshold were recorded. All rats were retested within 48 to 72 hours according to the same procedure. After screening with the right hypothalamic electrode, animals were tested for their responses to stimulation at the left electrode. Rats that ate, drank, or both during stimulation at either electrode, were designated ESLH-pos; those that did not eat or drink were classified ESLH-neg.

The 24 ESLH-pos and 18 ESLH-neg animals were divided into weight-matched experimental (19 positive, 14 negative) and control (5 positive, 4 negative) groups. All animals were deprived of food until they reached 85 percent of their baseline weight. Experimental animals were given ten daily tests for schedule-induced drinking in a Plexiglas chamber equipped with a food dispenser and two water-filled Richter tubes located 5 cm on either side of the dispenser. A food pellet was automatically dispensed every 60 seconds during the 30-minute test. All animals ate the food pellets almost immediately. Behavior was continuously monitored on closed-circuit television, and the amount of water consumed was recorded at the end of the 30-minute session. After each test the experimental and control animals were weighed and given sufficient food to ensure that they would be at 85 percent of their free-feeding weight 24 hours later.

Table 1. Retest results of initially ESLH-neg animals after different schedule-induced polydipsia (SIP) experiences.

SIP experience	Retest results	
	ESLH-pos	ESLH-neg
Positive*	7	0
Negative	2(1)†	4(5)†
Control (no SIP test)	0	4

*One animal could not be retested because its electrode had become defective. †One animal was ESLH-pos on the first retest but subsequently became ESLH-neg.

After the rats had finished ten daily 30-minute SIP sessions, they were given free access to food for 14 days and then retested for ESLH-evoked behavior as described above. At this time, all groups were above their predeprivation weight by an average of 11.8 g.

The behavior of the ESLH-pos and ESLH-neg animals differed during the SIP tests. In contrast to the ESLH-neg animals, the average amount of water consumed by the ESLH-pos animals increased at a significantly higher rate (Fig. 1A) (*16*). Moreover, of the ESLH-pos animals, 74 percent exhibited SIP on test session 1 in contrast to only 36 percent of the ESLH-neg animals (Fig. 1B). The percentage of ESLH-neg animals that drank did not increase until session 9 and never exceeded 57 percent. The differences between the groups were statistically significant for each of the ten daily sessions (*16*).

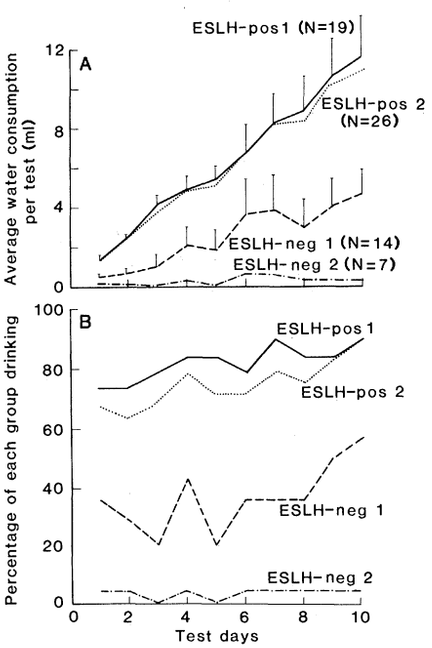

Fig. 1. Schedule-induced polydipsia. (A) Mean (± standard error) amount of water consumed in each SIP test. The designations ESLH-pos 1 and ESLH-neg 1 refer to initial tests prior to SIP experience; ELSH-pos 2 and ESLH-neg 2 refer to performance during retests after SIP experience. After reclassification, the standard errors were reduced. (B) Percentage of animals that drank during each SIP test.

Two weeks after completion of the SIP tests, the experimental and control animals were retested for ESLH-induced eating and drinking. Not surprisingly, all of the original ESLH-pos animals continued to eat and drink in response to brain stimulation. There was a nonsignificant decline in the current threshold for evoking eating or drinking between the tests before and after the SIP experience. Originally, nine animals had displayed drinking, four eating, and six eating and drinking in response to ESLH. On retest, this distribution had changed to four, five, and ten. These retest results are consistent with our observations of the changes that commonly occur over successive tests with ESLH.

All seven of the original ESLH-neg animals that had displayed SIP drinking became ESLH-pos when retested (Table 1). During the retests with brain stimulation, the behavior displayed, the amount consumed, and the current threshold for evoking eating or drinking did not differ from those that were originally ESLH-pos. The evoked behavior of the converted ESLH-pos animals did not decline during three retests given as long as 1 month after completion of the SIP experience. Therefore, the change seems to have been permanent. These results contrast sharply with the original ESLH-neg animals that were in the control group or those in the experimental group that had not exhibited SIP drinking. None of the ESLH-neg control animals ($N = 4$) became positive when retested; only two of seven experimental animals did, and one of these did not remain positive when retested. These differences in the ESLH retest results (Table 1) are statistically significant [$\chi^2(2) = 11.65$, $P < 0.01$]. When the SIP data were reclassified on the basis of retest performance, the difference between the ESLH-pos and ESLH-neg groups was even more striking (Fig. 1).

These results support the conclusion that the similarities in the ingestive behavior displayed during ESLH and SIP may be due to common mechanisms.

Animals that eat or drink in response to ESLH are much more likely to display SIP. The finding that all ESLH-neg animals that drank water during the SIP testing sessions became ESLH-pos suggests that a tendency to display ingestive behavior when active may transfer from one situation to another.

A great many nonspecific stimuli, such as noise, social facilitation, and tail pinch have been reported to initiate eating in animals or humans (*17, 18*). What seems to be common to all these stimuli is that they cause animals to become more active. When hungry animals are given small amounts of food at regular intervals they become highly active and possibly more responsive to environmental stimuli. Several investigators have observed that animals from a variety of species tend to display attack or escape behaviors during SIP sessions (*10*). We observed that during the SIP testing animals frequently jumped vigorously toward the top of the chamber in an apparent attempt to escape, and at the end of the session they were often difficult to handle (on one occasion biting the experimenter). Although animals do not normally become difficult to handle after ESLH, they are consistently highly active during the stimulation.

What needs to be explained is why some animals more readily direct this increased activity into eating and drinking. Any answer must take into consideration the fact that the response to ESLH is not correlated with unrestricted food and water consumption and presumably not with differences in hunger or thirst states that are regulated by nutritional needs. To our knowledge, this investigation represents the first attempt to study the consistency of individual differences in readiness to ingest food or water in response to different arousing stimuli. The results suggest that this approach may help in searching for underlying mechanisms by more fully describing the behavior to be explained.

GUY MITTLEMAN
ELLIOT S. VALENSTEIN
Department of Psychology,
and *Neuroscience Laboratory,*
University of Michigan,
Ann Arbor 48109

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15. Stereotaxic coordinates: skull level between lambda and bregma; 3.50 mm posterior to bregma, 1.50 mm lateral to the midline, and 8.40 mm below the dorsal surface of the skull.
16. Data were analyzed by a two-factor analysis of variance for repeated measures. The ESLH-pos rats consumed significantly more water than did ESLH-neg rats during each of the ten SIP sessions [$F(1, 31) = 5.6$, $P < 0.025$] and increased the amount consumed over sessions at a significantly greater rate [$F(9, 279) = 3.18$, $P < 0.001$]. Similarly, the percentage of ESLH-pos animals that drank was significantly greater on each session [$F(1, 31) = 15.9$, $P < 0.001$].
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Amplification and Enhanced Expression of the Epidermal Growth Factor Receptor Gene in A431 Human Carcinoma Cells

Abstract. *The sequence of the human epidermal growth factor (EGF) receptor shows great homology with the avian erythroblastosis virus v-*erb *B oncogene, raising the possibility that the receptor gene is identical to the c-*erb *B protooncogene. Human A431 epidermoid carcinoma cells, which have an unusually high number of EGF receptors, were examined to determine whether elevated EGF receptor levels correlate with gene amplification. Southern blots of genomic DNA's from A431 and other human cell lines were probed with either a v-*erb *B gene fragment or a human EGF receptor complementary DNA clone (pE7), previously isolated from an A431 complementary DNA library. When either probe was used to analyze Eco RI– or Hind III–generated DNA fragments, EGF receptor DNA sequences were amplified about 30-fold in A431. Differences in the banding pattern of A431 DNA fragments relative to normal fibroblast DNA indicate the occurrence of a rearrangement in the region of the receptor gene. Furthermore, A431 cells contain a characteristic, prominent 2.9-kilobase RNA. These results are consistent with the hypothesis that, in A431 cells, gene amplification, possibly associated with a translocation event, may result in the overproduction of EGF receptor protein or the appearance of the transformed phenotype (or both).*

Many retroviruses induce malignant transformation in cells via expression of specific proviral DNA sequences (v-onc). Mammalian cells also contain evolutionarily conserved homologous counterparts to these transforming genes (c-onc) (*1, 2*). It has been proposed that enhanced expression of cellular oncogenes may result in the manifestation of the malignant phenotype (*3–5*).

One retrovirus, avian erythroblastosis virus (AEV), induces both erythroblastosis and sarcomas in infected chickens (*6, 7*). The AEV is replication defective; portions of the replication genes are replaced by a region implicated in cellular oncogenesis (*erb*) (*8–11*). The AEV *erb* region consists of two putative oncogenes, A and B, whose human cellular homologs (c-*erb*) are located on separate chromosomes (*12, 13*). The main protein product of the v-*erb* B gene is approximately 62,000 daltons before modification (*14*) and is the cause of erythroblastosis and sarcomas (*15, 16*).

Recently, the amino acid sequence of six distinct peptides from the human epidermal growth factor (EGF) receptor have been shown to be strikingly similar to that of the v-*erb* B transforming protein (*17*). It is therefore possible that the EGF receptor and the c-*erb* B gene product are derived from the same cellular gene.

The human epidermoid carcinoma cell line A431 has an unusually high number of EGF receptors (approximately 3×10^6 sites per cell) (*18, 19*) when compared with normal human fibroblasts and other cell types (about 1×10^5 sites per cell) (*20*). This has been shown by measuring the number of binding sites for ^{125}I-labeled EGF or by immunoprecipitation of the receptor from [^{35}S]meth-