dence in humans of a relation between CNS noradrenergic activity and memory function.

There is some uncertainty as to whether lumbar spinal fluid MHPG directly reflects brain norepinephrine metabolism in humans. Insignificant amounts of labeled MHPG administered intravenously in three patients were detected in the CSF (*19*), indicating that MHPG found in lumbar CSF is derived from norepinephrine degradation in the CNS; however, the relative contributions of brain and spinal cord norepinephrine metabolism to lumbar MHPG are not known. Humans with spinal cord transection have diminished lumbar MHPG concentrations, suggesting that spinal cord norepinephrine catabolism does contribute to spinal fluid MHPG at this level (*20*). Spinal cord norepinephrine is found principally in descending tracts whose cell bodies are located in the same regions of the brainstem as those giving rise to ascending norepinephrine pathways (*5*). Apart from occasional mild pallor of the dorsal columns related to peripheral neuropathy, spinal cord pathology is not a feature of the Wernicke-Korsakoff syndrome (*2*); it therefore seems reasonable to attribute the decrease in spinal fluid MHPG observed in our patients to supraspinal lesions known to occur in this disease.

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Fractional Factorial Analysis of Growth and Weaning Success in *Peromyscus maniculatus*

Abstract. *Fractional factorial designs were used to explore simultaneously the effects of eight variables on survival and growth of neonatal deer mice,* Peromyscus maniculatus. *Two of the variables had significant effects on weaning success. The magnitudes of their effects are illustrated.*

We have used fractional factorial designs sequentially to identify variables affecting the ability of female mice to raise young to weaning. Two of the variables considered were significant and were used in full factorial designs to establish a response surface. Fractional factorial experimental designs, which require fewer experiments to estimate main effects of the variables, permit the screening of large numbers of variables.

Knowledge of survival, growth, and reproductive potential for any species in different climates may aid our understanding of aspects of animal distributions and the role of climate in population dynamics. Heat and mass transfer equations have been used to predict survival requirements of adults of several species (*1*), but prediction of growth and reproduction potential as influenced by climate has been done only for ectotherms (*2*). Growth and reproductive potential for small endotherms may depend on heat and mass transfer, and on other "black box" variables, whose effects must be empirically determined, such as photoperiod, amount of fresh green sprouts, presence of other animals, and so on. The problem is to sort out those variables that have a significant impact in the context of the environmental variables, such as air temperature and movement, that also affect potential for growth and reproduction.

For our analysis, we chose variables (Table 1) that might affect growth rates of the young and chose initial variable levels arbitrarily for the first experiment. The results of our initial experiment guided our selection of new levels for subsequent experiments. The plus, minus coding is for high, low levels for each variable.

Table 1. Data from experiments 1 to 4 on *Peromyscus maniculatus*. Survival was 55 ± 5 percent for all center replicates; *N*, variable number; *n*, number of litters.

Variable		Level		Main effect (g) in experiments:			
N	Description	−	+	1 and 4	1 only	2 and 3	2 only
1	Sprouts	None	Free access	2.34		1.24	
2	Frequency of weighing	Once per day	Once per 3 days	−1.44	0.96	2.01	0.62
3	Nest box	No	Yes	1.25	−0.46	0.86	1.29
4	Remove young	No	Leave 2	−1.79	−0.95	0.80	0.39
5	Male presence	Yes	No	0.16	−1.39		
5	Photoperiod	LD 10 : 14	LD 14 : 0			−1.19	0.08
6	Exercise wheel	Locked	Free	−0.56		0.73	
7	Available food	80 percent	Free access	4.24	5.73	0.44	0.65
8	Available water	40 percent	80 percent			2.54	2.04
		80 percent	Free access	1.15	.18		

Mean weight/run (g) for:	
Blocked experiments for 1 and 4	5.93 ± 3.25 (n = 23)
Blocked experiments for 2 and 3	4.62 ± 2.08 (n = 40)
Blocked experiments for 1	4.76 ± 3.67 (n = 16)
Blocked experiments for 2 with variables 1 and 6 confounded	3.46 ± 1.74 (n = 32)
Center replicates for experiments 1 and 4	5.21 ± 3.49 (n = 14)
Center replicates for experiments 2 and 3	3.32 ± 2.52 (n = 27)

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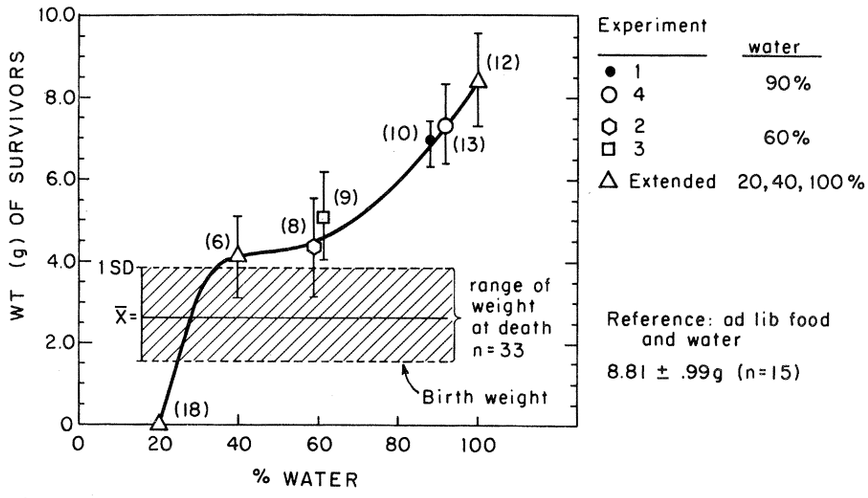

Fig. 1. The center replicate experiments were all done at air temperatures of 22°C, 50 percent relative humidity, and an air movement of about 10 cm/sec (typical room air circulation). In all experiments center replicates were given 90 percent of their free access food consumption. The means and standard deviations are represented by the symbols and vertical bars, respectively, The numbers in parentheses are number of individuals.

The advantages of full and fractional factorial experimental designs as compared to designs depending on one variable at a time have been described (*3*). In our experimental design, each "run" is defined as one row (Table 2). The design was broken into eight groups of two runs which were started sequentially. It was blocked (*3*) into eight groups enabling estimation of the effects of uncontrolled variables, for example, time of year, over the duration of the experiment. The blocks were randomized for each of the four executions of the experimental design to protect against possible effects of running the same block sequence exclusively. Most of the replication was concentrated in the central point of the design, where each variable was intermediate between the high and low levels. In Table 2, a center replicate female was started after the first run and before the second was begun in each successive block. In experiments 1 and 2 there was one female per run. In experiments 3 and 4 there were two females per run and per block center replicate. Central point data for each block illuminate time trends and provide information for estimating the mean and variance.

The colony of deer mice was from wild stock (*4*). A run was started on the day a female's young were born, and terminated either when all her young had died or otherwise at 21 days (if some young survived). The young surviving were then given free access to food and water and their recovery from the experiment was measured for an additional week. The dependent variables were weight and survivorship of young.

The effect of each independent variable on weaning weight was estimated (Table 2) by assigning each run's plus or minus sign for the variable to the run's dependent-variable value in the right-hand column. For example, the summation for variable 1 would be +9, 0, −9.25, and so on, yielding a sum of 18.71. The summation of the right column for each variable with the different sequence of signs under each variable number yields an estimate of the main effect when divided by the number of pairs of runs (eight in this case). Only the column sums are illustrated in Table 2.

Results of the fractional factorial experiments are in Table 1. The four fractional factorial experiments were run in the sequence numbered. In experiments 1 and 4 combined, presence of the male (variable 5) seemed to have no effect (0.16) on the size of the young weaned. Accordingly, in experiments 2 and 3, photoperiod duration was used as variable 5.

Initially, in experiments 1 and 2, there was an error in the signs for four of the eight blocks for variable 6. The signs had

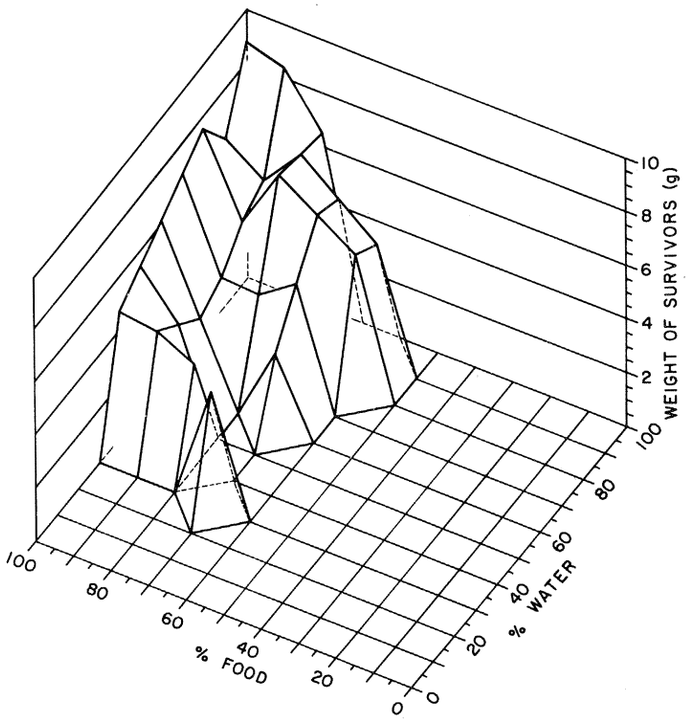

Fig. 2. The three-dimensional response surface, with center replicate variable levels except for food and water, shows that sensitivity to food deprivation is about twice as great as water deprivation as judged by the minimum percentage of the "free access" ration that will still result in some young surviving. The data from experiments 1 through 4 are also included. The food and water value (100 percent) is taken from Fig. 1 (as a reference). Each point on the surface represents the mean of a minimum of four litters and in one case 27 litters. Standard deviations were omitted for viewing clarity, but they ranged from ± 0.28 g to as high as ± 3.5 g in the "valley" area representing 90 percent of the "free access" food consumption. Typical standard deviations were about ± 1.0 g.

been reversed, leading to a confounding pattern (*3*) with variable 1. Confounding made it impossible to separate main effects of variable 1 from those of variable 6. The design was corrected and rerun (experiments 3 and 4) and included replicate runs of two of the four correct blocks in the original experiments. In all four experiments only variables 7 and 8 (available food or available water) had main effects greater than the center replicate standard deviations. The standard deviation of center replicates, rather than the pooled estimate from those runs replicated in experiments 1 and 4 and in experiments 2 and 3, was used as a measure of significant effect because the sample size was much larger. Sample size for pooled estimates for experiments 1 and 4 was six; for experiments 2 and 3 it was 16.

The main-effect calculations in Table 1 show that the confounding of variables 1 and 6 in experiments 1 and 2 do not affect the conclusions as to the calculated lack of effects of variables other than 7 and 8. This suggests that there are no three-factor or higher interactions large enough to affect the estimates of main effects appreciably. More support for this inference was obtained by ignoring the four least significant variables with the exception of water and food, thus the design was regarded as a complete factorial (*3*). All interactions were then calculated for the remaining four variables. No interaction estimates exceeded the standard deviations of the center replicates. The interaction between variables 4 and 7 in experiments 1 and 4 was 2.55, which was the only interaction in any experiment to exceed the standard deviation of the pooled experiments. Full factorial experiments with the five most significant of the eight variables also show (*5*) that higher order interactions are small relative to the standard deviations. It was also found that, when identical blocks of two identical experiments, such as experiments 1 and 4, were overlaid, the trend of results in each block was consistent and never reversed for any of the experiments.

Evidence of the repeatability of the experiments is the close agreement (Fig. 1) of center replicate mean and standard deviation from identical experiments (experiments 1 and 4) separated in time by up to 8 months. The means and standard deviations are nearly identical for experiments 1 and 4 and for experiments 2 and 3. The standard deviations for different experiments (1 and 4 compared to 2 and 3) do not overlap and are significantly different for the 60 and 90 percent water levels.

Table 2. Experiments 1 and 4 with *P. maniculatus*. The variable numbers refer to those given in Table 1.

Block	Variable number								Run	Weight/ run (g)
	1	2	3	4	5	6	7	8		
1	+	−	−	−	−	+	+	+	2	9.0
	−	+	+	+	+	−	−	−	10	0
2	−	−	+	+	−	−	+	+	5	9.25
	+	+	−	−	+	+	−	−	13	4.90
3	+	−	+	−	+	−	−	+	6	8.80
	−	+	−	+	−	+	+	−	14	4.35
4	−	−	−	+	+	+	−	+	1	0
	+	+	+	−	−	−	+	−	9	7.43
5	−	+	+	−	−	+	−	+	7	5.35
	+	−	−	+	+	−	+	−	15	9.90
6	+	+	−	+	−	−	−	+	4	2.60
	−	−	+	−	+	+	+	−	12	7.43
7	−	+	−	−	+	−	+	+	3	6.80
	+	−	+	+	−	+	−	−	11	3.93
8	+	+	+	+	+	+	+	+	8	10.20
	−	−	−	−	−	−	−	−	16	4.87
	18.71	− 11.55	9.97	− 14.35	1.25	− 4.49	33.91	9.19		94.81

The experiments show a capacity of females to nurse young successfully when water deprivation was as low as 40 percent of the "free access" consumption. It is possible that there is a very wide range within the population of adult females for capacity to raise young when food or water (or both) is limited. We could find no overt correlation either with age or size of female that would predict the success of the mother's raising young to weaning. Neither could we find significant effects of prior experimental experience, either by the animals' exposure to severe stress or their having previously been in an experiment in which there was free access to food and water (*6*).

Table 1 seems to show that the level of the variables determines which variable or variables may predominate. For example, in experiments 1 and 4 only available food had a main effect greater than the standard deviation of the center replicates (±3.49). In contrast, experiments 2 and 3 showed that only available water had a main effect greater than the center replicate standard deviation (±2.52). The explanation for this "hierarchy" of effects may be seen on the response surface in Fig. 2. Here additional center replicates with different levels of food and water from those of experiments 1 through 4 have been run. The surface bounded by 80 and 100 percent for free access to food and 80 and 100 percent for free access to water changes more for changing food than for changing water (experiments 1 and 4). However, the surface bounded by 80 and 100 percent for free access to food and 40 and 80 percent for free access to water (experiments 2 and 3) changes more with water change than with food change.

In summary, over the ranges of variables studied at room temperature and 50 percent relative humidity, available food and water are much more effective than seven other potential variables in their effect on capacity to bring pups from birth to weaning in the deer mouse, *Peromyscus maniculatus*. There is great variability in the population to effects of food and water stress with respect to weaning young. The physiological basis for these wide differences remains unknown. Effects of environmental and other variables remain to be explored.

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6. Food and water consumption were determined empirically by monitoring food and water consumed by six or more lactating females with young. At 22°C and 50 percent relative humidity consumption (grams of Purina rat chow) was 0.21 times the body weight (grams) of the mother plus the young. Consumption (milliliters of water) was 0.29 times the body weight (grams) of the mother plus the young. Food and water rations were always based on the total biomass in the cage.
7. We thank H. W. Norton, W. G. Hunter, P. A. Randolph, and G. E. P. Box for critical reviews. Supported by DOE contract EY-76-S-02-2270.

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Detection of an Auditory Nerve–Activating Substance

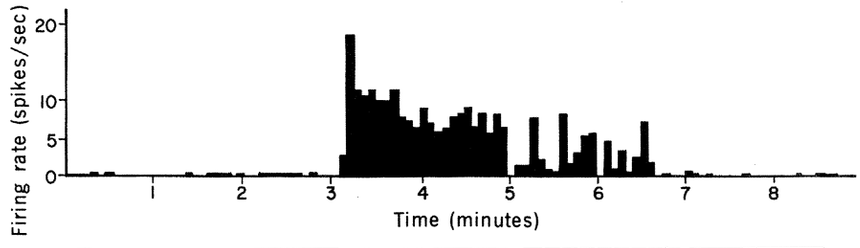
Abstract. *A substance or substances capable of increasing the firing rate of primary auditory fibers is detectable in the perilymph of frogs and guinea pigs subjected to sound stimulation. The increase in firing rate occurs in single units of the frog auditory nerve after perilymph obtained from frogs or guinea pigs during sound stimulation is infused into the frog perilymphatic sac. Perilymph collected from animals maintained in silence failed to cause an increase in firing rate of primary auditory fibers of the frog.*

The primary afferent transmitters of most sensory systems, including audition, remain unidentified. This report presents evidence of the presence of an auditory nerve-activating substance (ANAS) in the perilymph of animals stimulated with sound. Although we are aware of other possibilities, our working hypothesis is that ANAS is the primary afferent transmitter of audition.

Ample evidence indicates that transmission between the labyrinthine hair cells and the auditory (eighth) nerve is chemically mediated. For example, synaptic bodies and synaptic vesicles are present in many cochlear hair cells (*1*), and Furukawa *et al.* have demonstrated the presence of miniature excitatory postsynaptic potentials in the eighth nerve of the goldfish (*2*). The identity of the neurotransmitter released by the hair cells, however, remains unknown. There is evidence that the transmitter is not acetylcholine (*3, 4*), γ-aminobutyric acid (*3, 5-7*), 5-hydroxytryptamine (*8*), glycine (*7*), or a catecholamine (*9, 10*). Although aspartate and glutamate have been suggested as possible candidates (*7, 11, 12*), preliminary evidence from our laboratory is not consistent with that hypothesis (*13*). Transmission across the afferent synapse is resistant to the action of many known pharmacological blocking agents (*3, 5, 9, 12*). Therefore, the transmitter may be a substance not currently listed among the putative transmitters. If it is not, the standard approaches to transmitter identification, such as screening by applying agents from the list of transmitters or standard blocking agents, or the application of radioactively labeled precursors, may not prove fruitful. What is required is a method that will detect the presence of the transmitter regardless of its chemical nature. One answer to this methodological problem is to use the animal as the detector, as Otto Loewi did in his classical experiments with Vagusstoff (*14*). Such a bioassay would permit the detection of the transmitter as well as the monitoring of the progress of efforts to concentrate and to purify it. We have developed such an assay by using the change in firing rate of single nerve fibers of the eighth nerve of the bullfrog (*Rana catesbeiana*) as an indicator of the presence of ANAS in fluid infused into the frog perilymphatic space.

The perilymphatic space of the frog was perfused during acoustic stimulation. The collected perfusate was reinfused into the perilymphatic space during silence while we monitored the firing rate of a single auditory fiber of the eighth nerve. As a control, the same unit was monitored during perfusion in silence and infusion of this perfusate into the perilymphatic space during silence. The firing rate during the infusion of perfusate collected in sound was compared to the firing rate during the infusion of perfusate collected in silence. This procedure was based on the assumption that the transmitter, if released into the perilymphatic space during acoustic stimulation, would, when administered during the infusion procedure, produce an increased firing rate when compared to the control procedure.

The frogs were immobilized by the intramuscular injection of 2 to 3 mg of *d*-tubocurarine hydrochloride in 1 ml of saline. The eighth nerve and the perilymphatic sac were exposed through the roof of the mouth. The frogs were subjected to a free-field search stimulus of 60-dB, 300-msec burst of white noise once per second. This stimulus was designed to induce auditory nerve fibers to fire. Single-unit recordings were obtained by using a motorized microdrive to advance a 3*M* KCl-filled glass microelectrode (resistance, 20 to 80 megohms) through the posterior branch of the eighth nerve. Once an auditory fiber was found (as indicated by action potentials elicited in synchrony with the search stimulus), its characteristic frequency was determined by sweeping across frequencies while noting changes in firing rate. The characteristic frequency was used to ascertain its origin in the inner ear (*15*). Only those units originating in the amphibian papilla and exhibiting low spontaneous activity (0 to 5 spikes per second) were used as biodetectors of transmitter-induced activity. The firing rate of the unit during the procedure was recorded on tape and later analyzed with a window discriminator and rate-interval analyzer. The perilymphatic space was perfused with modified Ringer solution (*16*) by means of a double-barreled coaxial pipette inserted into the perilymphatic sac. During the perfusion procedure, perilymph was withdrawn into the inner barrel (diameter, 75 μm). The duration of the perfusion procedure ranged from 60 to 120 seconds. The collected perfusate was then infused back into the perilymphatic space. The rate of perfusion and infusion



	Withdrawal in silence	Reperfusion in silence	Withdrawal in sound	Reperfusion in silence	Pump off
Mean rate	0.03	0.11	8.1	2.7	0.13
Standard error	±0.01	±0.10	±0.4	±0.5	±0.04

Fig. 1. The results of a typical experiment designed to collect and detect the afferent transmitter. As a control, perfusate was collected in silence. The pumps were reversed, and the collected perfusate was reperfused in silence while the firing rate was being monitored. In order to collect the transmitter, perfusate was collected during sound stimulation. The pumps were reversed, reperfusing the collected perfusate during silence.

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