sex linkages or maternal cytoplasmic effects. Yet, in the light of the relatively simple genetic determination indicated by F_2 data, they may reflect nothing more than random error in drawing the small parental groups. This issue, together with the task of specifying the source of segregation in F_1 and isolating genes that segregate in F_2, remain matters for further investigation.

The target of genetically selecting quail for color preferences is not peripheral but central mediation (*1*, *6*). Therefore, these genetic preparations may facilitate the search for physiological events of processing visual information by the vertebrate brain. Because the quails' early approach preferences are readily modified by exposure to stimuli, gene-environment interactions may be tested in choices between composite discriminanda that combine genetically and environmentally labeled elements of this behavior (*7*). Particularly promising is a six-way comparison (Fig. 2).

These comparisons are based on the assumption that individuals exhibiting a behavior solely for reasons of genotype and individuals of different genotypes exhibiting the same behavior for reasons of prior experience should exhibit differences in the mediation of that behavior. Examining anticipated behavioral and neurophysiological differences (such as, for example, in stimulus generalization or in neuroelectrical and neurochemical indicators) may lead to inferences on how gene effects, environment effects, and gene-environment interactions are mediated in behavior.

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2. Mass-screening procedures were first applied to geotactic and phototactic responses in *Drosophila* [J. Hirsch and R. C. Tryon, *Psychol. Bull.* **53**, 402 (1956); N. M. Hadler, *Biol. Bull. (Woods Hole, Mass.)* **126**, 264 (1964); Th. Dobzhansky and B. Spassky, *Proc. R. Soc. London Ser. B* **168**, 27 (1967)]. The mass-screening apparatus developed for the present study of the quail was built of 28 discrimination compartments, arranged in the manner of a Galton board so that a single compartment was on top, two compartments were in the second row, three in the third row, and so on through eight collection boxes on the ground floor. The target end of each compartment offered choice between two stimuli, one blue and another red. Subjects could proceed from one to the next compartment through two trapdoors in each compartment, one in front of each stimulus, which opened under the weight of the subject as it approached within 7.5 cm of the stimulus. Arrival in a collection box indicated the number of times one over another stimulus was chosen in seven trials. Stimuli backlighted with fluorescent light sources flashed at the rate of 3 Hz of equal light-dark cycles. Size (2.5 cm^2) and luminance of stimuli (10.1 lux) were kept identical; only colors were different. Stimulus-on intensities were measured at the source with a photometer (Tektronix J16) and probe (Tektronix J6501). Spectral characteristics corresponded to Wratten gelatin filters (No. 45 for blue and No. 29 for red). Subjects were light-adapted to background illumination of 1- to 2-lux scattered light before testing. They were incubated and reared in the dark, received no prior experiences with colors, and were placed into the starting compartment of the apparatus in successive groups of 25, accumulating to not more than 250 in a testing session. Although tested in groups, each subject was individually identified and scored for two consecutive runs through the apparatus. Average age at testing was 27 hours after hatching, with 1 standard deviation of age variation being approximately 5 hours.
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4. Parental subjects used in this selection experiment were mated and housed in individual pairs. Because the quail is unusually sensitive to inbreeding depression [H. Abplanalp, *Zuecht. Z. Theor. Angen. Gen.* **37**, 99 (1967); L. E. Iton, *Poultry Sci.* **46**, 1275 (1967); A. W. Kulenkamp, thesis, Washington State University (1967)], only subjects that shared no ancestors within three preceding generations were mated. Approximately 20 percent of subjects were selected from appropriate ends of preference distributions. A genetic control line was maintained without selection. Selected lines were hybridized for F_1 and F_2 at S_8 and again at S_{12}. Data were examined by reciprocal crosses at S_{12}. At this generation, backcrosses of selected subjects to genetic controls were also made.
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7. J. K. Kovach, paper presented at a meeting of the Animal Behavior Society, New Orleans, 10 to 15 June 1979.
8. I thank L. Coyne for help with statistical analysis and G. Wilson, L. Baker, and C. Neal for assistance in conducting this experiment. Supported by NICHD grant HD-06770-07, NIH Research Career Development award 5-K02-MH-20140-10, and The Menninger Foundation. I would be glad to provide subjects from the described genetic populations to investigators who could profitably use them in related research.

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Cigarette Smoking Associated with Sleep Difficulty

Abstract. *A group of 50 smokers experienced greater sleep difficulty than a group of 50 nonsmokers matched by age and sex. The two groups did not differ in personality patterns or drug consumption. Also, sleep patterns significantly improved in a group of eight chronic smokers when they abstained from cigarette smoking. These findings are consistent with reports on the stimulant effects of nicotine.*

The findings of two studies in this report suggest that chronic cigarette smoking is associated with sleep difficulty and that the abrupt withdrawal of cigarette smoking in chronic smokers results in improved sleep. In the first study we used sleep laboratory recordings and data from a questionnaire given to 50 cigarette smokers (31 males and 19 females, with a mean age of 39.8 years) and 50 nonsmokers matched by sex and age. All of the smokers had been smoking for more than 3 years, and at the time of the study they smoked a mean of 1.25 packs of cigarettes per day. Each of the 100 subjects was evaluated in the sleep laboratory for four consecutive nights, the first night allowing for adaptation of the subjects to the laboratory. Standardized methodology was used for recording electroencephalograms (EEG), electromyograms, and electrooculograms. The records were scored independent of any knowledge of the experimental conditions (*1*). In addition, each subject completed a sleep history questionnaire and the Minnesota Multiphasic Personality Inventory (MMPI).

Table 1. Comparison of sleep efficiency and sleep stage variables between smokers and nonsmokers monitored for four consecutive nights in a sleep laboratory. The values are means and standard errors of nights 2 to 4 for both groups. The length of the recordings each night was 8 hours. The Student's *t*-test was used for all comparisons. Sleep efficiency is measured in minutes; stages are in percentages.

Parameter	Smokers	Nonsmokers
Sleep efficiency		
Sleep latency	43.7 ± 5.5	29.8 ± 2.8*
Time awake after sleep onset	49.0 ± 4.2	44.1 ± 3.3
Total time awake	92.7 ± 6.8	73.9 ± 4.5*
Sleep stage		
1	5.7 ± 0.4	5.1 ± 0.4
2	64.4 ± 1.0	63.3 ± 0.9
3	4.8 ± 0.8	6.0 ± 0.7
4	0.7 ± 0.3	1.1 ± 0.4
REM	24.4 ± 0.6	24.5 ± 0.5

*$P < .05$.

The results of the sleep laboratory recordings (on nights 2 to 4) comparing smokers with nonsmokers suggest that cigarette smoking contributes to sleep difficulty (Table 1). The smokers were awake for a significantly longer time than the nonsmokers (92.7 versus 73.9 minutes, $P < .05$), primarily because they had greater difficulty in falling asleep (43.7 versus 29.8 minutes, $P < .05$). The two groups did not differ significantly in sleep stage parameters.

Analyses of the MMPI revealed no significant differences between the smokers and nonsmokers on any of the eight major clinical scales, and the two groups showed no differences in the use of

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Table 2. Effects of withdrawal from cigarette smoking in eight subjects. The values are means and standard errors. The length of recording each night was 8 hours. All statistical comparisons are with the baseline data; the Dunn multiple comparison t-test was used for the analysis. Sleep efficiency is measured in minutes; stages are in percentages.

Parameter	Baseline nights 2 to 4	Withdrawal	
		Nights 5 to 7	Nights 8 and 9
Sleep efficiency			
Sleep latency	51.7 ± 9.7	18.1 ± 4.0*	34.3 ± 8.1
Time awake after sleep onset	24.2 ± 4.7	23.9 ± 5.3	16.7 ± 2.4
Total time awake	75.9 ± 10.5	42.0 ± 6.7*	51.0 ± 7.9†
Sleep stage			
1	5.6 ± 0.5	5.7 ± 0.6	5.5 ± 0.5
2	66.5 ± 1.6	65.0 ± 1.1	64.4 ± 1.7
3	5.0 ± 1.0	4.0 ± 0.9	5.5 ± 1.4
4	0.7 ± 0.3	0.5 ± 0.2	1.1 ± 0.4
REM	22.2 ± 1.0	24.8 ± 0.9	23.5 ± 0.7

*$P < .01$. †$P < .05$.

drugs, including alcohol consumption. However, the smokers drank significantly more coffee than the nonsmokers. To determine the contribution of coffee consumption to the sleep differences between smokers and nonsmokers, we reanalyzed the data and compared groups matched by age and sex for coffee consumption above and below the median level of 3 cups per day. The group consuming more than the median amount drank an average of 4.7 cups of coffee per day; the group consuming less than the median amount drank an average of 1.2 cups. In spite of the large differences regarding coffee consumption ($P < .001$), these groups showed no differences in sleep parameters. This finding suggests that coffee consumption in the smokers was not a primary contributing factor to their sleep difficulty.

In the second study conducted in the sleep laboratory, we evaluated the effects of abrupt smoking withdrawal on the sleep of eight male smokers (mean age, 30 years). All of the men had consistently smoked between 1.5 and 3 packs of cigarettes a day (mean, 2.0) for at least 2 years before the study. The subjects were studied on several consecutive nights, including an initial adaptation night allowing for adjustment to the laboratory, three nights during which baseline data were collected, and for at least five nights after the subjects had stopped smoking. During days 1 to 4, the subjects continued to smoke at their usual frequency. On the morning of day 5, subjects were asked to refrain completely from smoking. They were instructed to maintain their normal level of daily activity and to report any unusual feelings or events occurring during the day. The sleep patterns of the eight subjects were recorded in the laboratory for the first five nights of abstinence (study nights 5 to 9). For four of the subjects, the abstinence period was extended to 12 days, and they were again monitored in the sleep laboratory on nights 15 and 16.

Data showing the effects of abrupt cigarette withdrawal on sleep induction and maintenance are listed in Table 2. Total time spent awake decreased by 45 percent on the first three nights of abstinence, from 75.9 minutes to 42.0 minutes ($P < .01$); sleep latency decreased notably, but there was little change in time spent awake after sleep onset. On nights 8 and 9 of withdrawal, the total time the subjects were awake continued to be less than during the baseline nights (51.0 versus 75.9 minutes; a 33 percent decrease, $P < .05$). Both sleep latency and time spent awake after sleep onset decreased on these nights. The four subjects who were evaluated after the extended abstinence period continued to show a decrease in the total time they were awake, from 100.2 minutes during the baseline nights to 71.4 minutes during the extended abstinence period (not statistically significant).

Data showing the effects of abstinence from cigarette smoking on sleep stages are listed in Table 2. None of the sleep stage variables changed significantly from baseline levels. Although the amount of rapid eye movement (REM) sleep increased from 90.5 minutes during baseline to 113.1 minutes on nights 5 to 7 (not significant), the percentage of total sleep time spent in the REM stage only slightly increased.

The results of these two studies suggest that cigarette smoking is associated with sleep difficulty. The smoking and nonsmoking groups did not differ in terms of their degree of psychopathology or use of alcohol or other drugs that affect the central nervous system. We found that coffee drinking did not significantly influence the degree of sleep difficulty. This is probably because coffee drinkers develop a tolerance to caffeine (*2*). Our findings suggest that the stimulant effects of cigarette smoking directly influence sleep efficiency and that tolerance does not develop to the stimulant effect of nicotine.

Results of studies on the effects of cigarette smoking and its withdrawal generally indicate that smoking stimulates catecholaminergic systems. Reports on the physiological effects of smoking have suggested that it produces increases in catecholamine concentrations in the blood and urine (*3-5*). Smoking also increases blood pressure (*5-7*) and heart rate (*4-6*); in addition, increased concentrations of free fatty acids (*8, 9*), plasma corticosteroids (*5, 9*), and growth hormone (*5, 10*) are found in smokers. Smoking decreases the amount and increases the frequency of alpha activity and produces more high-amplitude beta activity in the EEG (*11*). All of these effects suggest physiological activation or arousal and are consistent with the finding of our first study that chronic smoking is associated with sleep difficulty.

Studies of withdrawal from cigarette smoking have shown decreases in the amount of catecholamine excreted (*12*), decreases in heart rate (*12-14*) and blood pressure (*13, 14*), and increases in slow EEG frequencies (*15*). These physiological effects are accompanied by mood and behavioral changes such as depression, lack of concentration, irritation, anxiety, tension, restlessness, and drowsiness (*12, 15, 16*). In our second study, sleep improved during abstinence from cigarette smoking, in spite of the daytime discomfort known to be associated with cigarette withdrawal. This improvement in sleep can be explained by a decrease in catecholamine concentrations after withdrawal from cigarette smoking.

Many reports have documented the risks of smoking for a wide range of health variables (*16*). One study evaluated the frequency of physical complaints in relation to smoking habits (*17*). These data, based on almost 50,000 questionnaires, included insomnia in a large number of complaints related to chronic smoking habits, such as cough, hoarseness, shortness of breath, loss of appetite, nausea, pain in the abdomen, stomach, or chest, and fatigue. The results of our studies document sleep difficulty in chronic cigarette smokers and sleep improvement during abstinence from cigarette smoking.

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16 July 1979

Auditory and Vocal Nuclei in the Frog Brain Concentrate Sex Hormones

Abstract. *Mate calling by South African clawed frogs,* Xenopus laevis, *is under the control of androgens. Autoradiographic studies demonstrate androgen-concentrating neurons in a motor nucleus that controls mate calling and a midbrain nucleus that is stimulated by sound. Hormone concentration by laryngeal motor neurons suggests that steroids regulate the final common path for vocal behavior. Modulation of auditory sensitivity by hormones could explain seasonal variations in behavioral responsiveness to conspecific vocalizations.*

Sex hormones are powerful modulators of reproductive behaviors. Gonadectomy eliminates and steroid hormones restore sexual responses in most vertebrates (*1*). Hormone accumulation by target cells in specific brain nuclei is believed to regulate the activity of neural circuits that mediate sexual behaviors. Most steroid-concentrating cells are found in the hypothalamus and infundibulum (*1*). Recent autoradiographic evidence has shown that motor neurons in nuclei of cranial nerves and in the spinal cord concentrate androgen (*2–4*) and that some nuclei in the central nervous system (CNS) that receive sensory information are labeled by estrogen (*5*). I have been investigating mate calling, an androgen-dependent male sexual behavior in the South African clawed frog, *Xenopus laevis*, and now report that a CNS nucleus that receives auditory information and one that controls vocal behavior contain androgen-concentrating cells.

Male mate calling is stimulated by injection with human chorionic gonadotropin, abolished by castration, and reinstated by treatment with the androgens testosterone or dihydrotestosterone (DHT), but not with estradiol (*6*). Both DHT and testosterone are present in *X. laevis* blood; treatment with chorionic gonadotropin increases concentrations of both androgens to five times those seen in untreated males (*7*). Since testosterone can be metabolized to estradiol by CNS target cells (*8*), I investigated androgen-specific hormone accumulation in the frog brain with the nonaromatizable hormone DHT. The gonads of five adult male *X. laevis* were removed. One week later, 200 μCi of octalabeled, tritiated DHT (*9*) was injected into the dorsal lymph sac; the frogs were allowed to survive for 2 hours, after which the brains (*3*) and spinal cords were removed, frozen, and processed for steroid autoradiography. Slides were exposed for 4, 6, or 8 weeks, developed, lightly stained with cresyl violet acetate, and microscopically examined for the presence of labeled cells (*10*). The locations of such cells were plotted on enlarged microprojector drawings of the entire section with the aid of an x-y plotter coupled to linear potentiometers on the microscope stage.

After DHT injection, labeled cells were found in the anterior pituitary, the posterior thalamus, the laminar nucleus of the torus semicircularis, the dorsal tegmental area of the medulla, the principal nucleus of cranial nerve V, the motor nucleus of cranial nerves IX and X (N IX-X), the medullary tegmentum, and in large and small neurons in the ventral portion of the anterior spinal cord [for neuroanatomical nomenclature see (*3*)] (Fig. 1). Autoradiograms of cells in N IX-X are shown in Fig. 2, A and B. No DHT-labeled cells were ever seen in the anterior preoptic area or the ventral infundibular nucleus, the two regions with the greatest number of heavily labeled neurons after injection of tritiated estradiol or testosterone (*3*); this absence supports the hypothesis that testosterone-labeled cells in these nuclei are attribut-

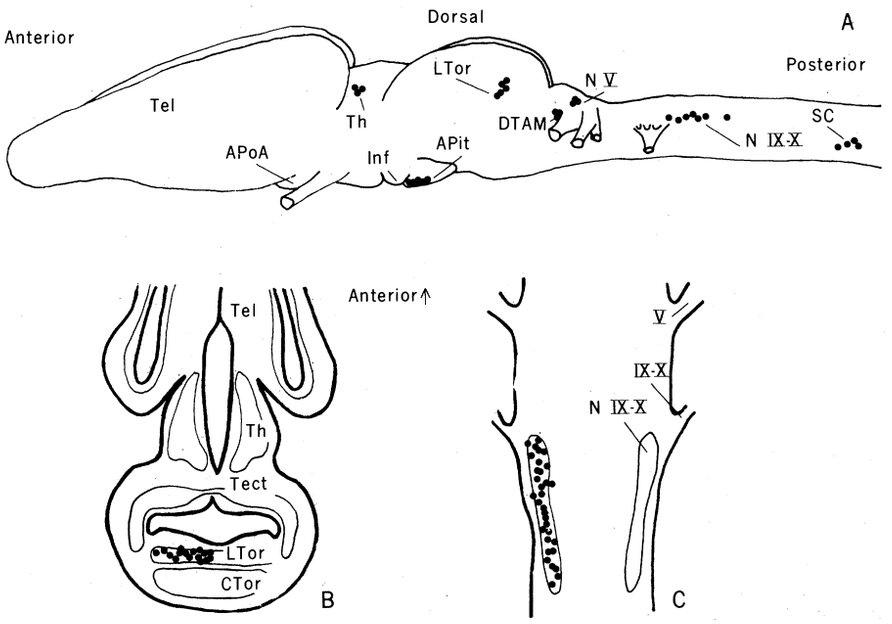

Fig. 1. (A) A lateral view of the brain and anterior spinal chord of *X. laevis*. Locations of DHT-concentrating cells (•) are indicated schematically. (B) A representative horizontal section through the torus semicircularis. Locations of labeled cells (•) are shown on the left side; major brain nuclei are identified on the right. The figure represents all labeled cells from one 10-μm autoradiogram projected onto a standard reference section. (C) A representative horizontal section through the nucleus of cranial nerves IX and X. Abbreviations: *APit*, anterior pituitary; *ApoA*, anterior preoptic area; *CTor*, caudal nucleus of the torus semicircularis; *DTAM*, dorsal tegmental area of the medulla; *Inf*, infundibulum; *LTor*, laminar nucleus of the torus semicircularis; *NV*, sensory nucleus of the fifth cranial nerve; *N IX-X*, motor nucleus of the ninth and tenth cranial nerves; *SC*, ventral horn of the anterior spinal cord; *Tect*, optic tectum; *Tel*, telencephalon; *Th*, thalamus; *V*, fifth cranial nerve; and *IX-X*, ninth and tenth cranial nerves.

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