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Selective Breeding for Sensitivity to DFP: Generalization of Effects Beyond Criterion Variables

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RUSSELL, R. W., D. H. OVERSTREET, M. MESSENGER AND S. C. HELPS. *Selective breeding for sensitivity to DFP: Generalization of effects beyond criterion variables.* PHARMAC. BIOCHEM. BEHAV. 17(5) 885–891, 1982.—The degree of generalization to the effects of DFP, an organophosphate anticholinesterase, was studied in two lines of Sprague-Dawley derived rats selectively bred for varying sensitivities to DFP. In the S13, S14, S15, and S16 generations the Flinders S-line of rats were still more sensitive to the effects of DFP on the criterion variables upon which selection was based: core body temperature, body weight and a simple operant response for water reward. The flinders S-line were also more sensitive to the effects of DFP on locomotor activity, FR5 responding for a water reward, and analgesia, indicating some degree of generalization. However, diarrhea, a symptom of peripheral effects of DFP, occurred at a similar incidence in the two lines, although males of both lines had higher incidences than the females. Neither of the two lines was affected by DFP for variables in which aversive (i.e. shock) motivation was used: The number of discriminative escape responses and the escape times were similar. These findings indicate that while the effects of DFP do generalize beyond the criterion variables upon which selection was based, the generalization is relatively specific. The data are consistent with the hypothesis that the changes in sensitivity have arisen because of changes in the functioning of a central cholinergic system(s).

DFP sensitivity Selective breeding Generalization Temperature Locomotor activity
FR5 responding Diarrhea

RECENTLY we reported the development, by assortative mating, of a line (Flinders S-line) of Sprague-Dawley rats which were more sensitive to the acute effects of the irreversible anticholinesterase, DFP, on selected behavioral and physiological variables than were animals (Flinders R-line) similarly bred for resistance to such effects [7]. Although selection pressure in both directions was maintained, there was no apparent success in producing an R-line more resistant to DFP than randomly bred animals. These findings differ from results of research on insects which have shown the development of resistance to anticholinesterase agents as a result of exposures over several generations [16]. Our results, although comparable to a report of increased sensitivity to ethanol in a study also involving the selective breeding of rats [10], may have been constrained by the measures on which the assortative mating was based. The measures may be truncated at the resistant end, thus restricting observation of any changes that might occur in that direction. Continuation of the mating procedure appears to have led to a stable difference between the S- and R-lines on the three criterion variables (body weight, core body temperature and drinking behavior) following acute injections of DFP.

The present report describes observations of animals from the S13, S14, S15 and S16 generations. The series of experiments involved were designed to obtain information relating to three primary questions: Had the differences on criterion variables, i.e., those upon which selective breeding has been based, between lines following DFP treatment been maintained in continuing generations? If differences had been maintained, had they stabilized or were they continuing to diverge? Was the selective breeding reflected in non-criterion behavior, behaviors other than the criterion variables, i.e., were the DFP effects specific to particular behavior-cholinergic system relations or were they more generally non-specific?

METHOD

Animals

The animals in all experiments were Sprague-Dawley rats of both sexes from the S13, S14, S15 and S16 generations of the Flinders lines. They were housed in groups of ten in a temperature-controlled (22° ± 1° C) room under continuous lighting. Food and water were available ad lib, except in studies of operant drinking behavior when water was restricted to a 30 min session per day.

DFP

DFP, obtained from Sigma Chemical Co., was administered intramuscularly (IM) as acute doses of 1.0 mg/kg in Arachis oil, the latter serving as the vehicle for injection into

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control animals. Prior to its use in the experiments proper, the potency of the DFP was tested in other animals by observation of its symptomatological effects. The dose, 1.0 mg/kg, and route of entry, IM, were chosen on the basis of extensive prior research (e.g., [4,12]) which had demonstrated that these conditions lower AChE activity to less than approximately 40% of normal, the critical level when behavioral and physiological effects appear [5].

Behavioral and Physiological Measures

Eleven dependent variables were measured. Three of these were the criterion variables upon which the assortative mating was based: core body temperature, body weight and operant drinking behavior [7]. Temperature was recorded in °C using a thermister probe inserted 6–8 cm into the rectum, the output of the probe being registered on a CRL digital recorder. Weight was taken as the means of five successive independent recordings from a Sartorius 1203 balance programmed by a 7080 printer. The method for measuring drinking behavior has been described, in detail, earlier [8]: it involved the simple operant response of licking water from a drinking tube during 30 min sessions under conditions of 23.5 hr water deprivation, drinking behavior being measured in ml of water intake.

The presence or absence of three indices of general symptomatology were noted. These were symptoms associated with manipulations of the cholinergic system involving direct or indirect agonists: diarrhea, tremor and analgesia.

Locomotor activity was assessed in an open field, 60 × 30 cm, with 10 × 10 cm squares marked on its base. All observations were carried out in a special experimental room under dim red illumination. The number of squares entered during a 2 min trial were recorded.

Operant lever-press responding for water reinforcement was observed under conditions of an FR5 schedule. Animals, water deprived for 23.5 hr, were given daily trials of 30 min each. A battery of operant chambers made it possible to observe the performances of eight animals during any one session. Schedules for each chamber and records of performance (lever press) were controlled by a TRS-80 microprocessor with a Lehigh-Valley interface circuit. Counts of the number of reinforcements (5 presses) were recorded automatically every 5 min during a session.

Three other measures of behavior were obtained from trials in a visual discrimination situation, consisting of a Y-shaped apparatus in which responses to visual stimuli could lead to escape or avoidance of an electric foot shock (1.00 mA). The animal was placed in a lighted compartment at the end of one alley of the Y. Shifting of the light to the end of another alley signalled the start of a trial and the position of the non-shock portion of the apparatus. Five sec later the shock was presented. Avoidance responses could occur during that interval; escape responses thereafter. Which type of response and the time taken to make it were recorded, together with notation as to whether or not a correct discrimination (i.e., movement to the lighted alley without entry or re-entry into either of the other alleys) had been made.

Procedure

The basic research design in all experiments called for a comparison of behavioral and physiological variables between two groups of animals: the Flinders S- and R- lines of both sexes following IM administration of DFP. The general procedure for obtaining the data for comparison involved three major phases: preliminary training, determination of pretreatment baseline performance, and measurement of treatment effects. Preliminary training consisted of systematic daily trials designed either to adapt the animal to conditions of measurement, e.g., weighing, temperature recording, or to develop the particular behavioral pattern under study, e.g., operant drinking, FR5 operant responding, visual discrimination. Locomotor activity was an exception because adaptation occurs so rapidly to a very low level; therefore it would not have been possible to have measured any depressant effects of DFP had the preliminary adaptation procedure been applied. When the behavior had stabilized, measures of pretreatment performance were taken to be used during later analyses for the primary purpose of analyzing DFP effects using each animal as its own control. The third phase, acute DFP treatment, then followed.

Times after treatment at which measures were taken varied with the particular dependent variable involved. Core body temperature was recorded 4 hr after injection when the effects are maximal [5], at which time the presence or absence of symptoms was also noted. Measures of the performance of other behaviors were taken 24 hr after DFP or Arachis oil administration, earlier studies having shown that by that time incapacitating motor effects had disappeared.

RESULTS

Results of the experiments are presented in terms of the several dependent variables (behavioral and physiological) measured under the experimental conditions. Separate tables for criterion and for non-criterion variables summarize the data for central tendencies, variabilities, sample sizes and tests for significances of differences. Statistical analyses of DFP effects have been carried out using 2- or 3-way ANOVAs, depending upon availability of data across generations.

Pretreatment Baseline Measures

Criterion variables. Table 1 presents a summary of results of pretreatment baseline measures for the three criterion variables. Three-way ANOVAs established that there were significant sex (S), line (L) and generation (G) differences in body weight: female and S line animals weighed less than their male and R line counterparts; animals of the S14 and S15 generations weighed more than those of the S13 and S16. Two significant 2-way interactions occurred, both involving the generation variable: an S × G interaction depended upon the fact that differences between sexes of the same lines were greater for the S14 and S15 generations than for the S13 and S16; differences between lines of the same sexes tended to increase with increasing generations.

Significant S, L and G differences also appeared in measures of core body temperature. However, in this case they were dependent upon the fact that measures for the S13 generations were consistently lower than for other generations. A significant L × G interaction appeared to arise from a decrease across generations of differences between S and R lines.

Significant S and S × G effects characterized measures of water intake. Female subjects drank consistently less than males. Differences between sexes of the same lines tended to be greater for generations S14 and S15.

Non-criterion variables. Results of pretreatment baseline measurements of five non-criterion variables are presented in Table 2. No significant main effects of sex appeared with

TABLE 1

PRETREATMENT BASELINE MEASURES OF CRITERION VARIABLES

A. Means, standard errors of means, sample sizes

		SM			RM			SF			RF		
Generation		Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n
Body	13	336	7.2	22	343	5.7	28	224	2.5	20	243	3.4	22
Weight	14	363	6.5	15	377	11.8	14	230	3.3	12	259	8.9	11
	15	355	6.6	15	379	5.1	15	225	3.8	15	247	5.0	15
	16	312	6.5	25	348	6.0	25	214	2.9	25	255	4.3	25
Core	13	37.7	0.1	22	38.1	0.1	28	38.1	0.1	20	38.5	0.1	22
Temp.	14	38.4	0.1	15	38.4	0.1	14	38.9	0.1	12	38.7	0.1	11
	15	38.2	0.1	15	38.3	0.1	15	38.5	0.1	15	38.5	0.1	15
	16	38.3	0.1	25	38.3	0.1	25	38.4	0.1	25	38.6	0.1	25
Water	13	22.3	0.8	22	22.6	0.4	28	16.9	0.5	20	16.7	0.6	22
Intake	14	22.7	0.9	15	22.2	0.9	14	16.6	0.4	12	16.4	0.9	11
	15	24.8	0.7	15	23.5	0.7	15	16.3	0.6	15	16.7	0.6	15
	16	22.9	0.5	25	20.8	0.4	25	18.1	0.7	25	17.6	0.6	25

B. 3-Way ANOVAs: F-values

	Sex	Line	Generation	SXL	SXG	LXG	SXLXG
Body Weight	1430.53*	71.35*	16.25*	1.59	8.09*	4.19†	0.35
Core Temp.	35.05*	4.87‡	13.51*	0.03	1.39	2.77‡	0.40
Water Intake	301.14*	3.58	1.10	1.28	5.68*	1.15	0.78

*$p<0.001$.
†$p<0.01$.
‡$p<0.05$.

TABLE 2

PRETREATMENT BASELINE MEASURES OF NONCRITERION VARIABLES

A. Means, standard errors of means, sample sizes

		SM			RM			SF			RF		
Generation		Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n
Activity	15	55.9	3.4	8	74.6	3.1	8	47.6	3.6	8	87.8	2.9	8
	16	47.1	1.7	8	64.1	2.3	8	44.0	2.2	8	60.9	2.3	8
FR5													
Operant	R15	58.5	3.0	8	96.4	8.6	8	64.9	7.2	8	79.3	6.4	8
Discrim	R16	5.6	1.0	7	2.9	1.5	7	7.6	1.3	7	5.0	1.2	7
Stereo	R16	4.4	1.0	7	7.1	1.5	7	2.4	1.3	7	5.0	1.2	7
Escape time	16	5.7	0.7	7	6.0	0.6	3	4.1	1.2	7	8.3	1.8	7

B. 2-Way ANOVAs: F-values

		Sex	Line	SXL
Activity	15	0.22	31.84*	4.20†
	16	0.77	21.84*	0.00
FR5				
Operant	15	0.65	15.46*	3.13
Discrim	R16	0.68	0.06	0.72
Stereo	R16	0.68	0.06	0.72
Escape time	16	0.03	3.62	2.08

*$p<0.001$.
†$p<0.01$.
‡$p<0.05$.

TABLE 3

EFFECTS OF ACUTE DFP TREATMENT ON CRITERION VARIABLES: PERCENTS OF PRETREATMENT BASELINES

A. Means, standard errors of means, sample sizes

		SM			RM			SF			RF		
Generation		Mean	SEM	n	Mean	SEM	N	Mean	SEM	n	Mean	SEM	n
Body	13	92.4	0.6	22	95.0	0.5	24	93.0	0.5	20	96.9	0.4	22
Weight	14	91.7	0.6	15	94.9	0.5	14	94.4	0.7	12	97.3	0.4	11
	15	94.0	0.6	13	95.9	0.5	13	93.9	0.5	13	98.2	0.4	13
	16	94.6	0.6	12	98.2	0.5	12	94.4	0.5	12	97.9	0.4	6
Core	13	88.1	0.5	22	91.6	0.6	24	90.3	0.6	20	94.3	0.6	22
Temp	14	86.2	0.4	15	90.5	0.6	14	90.6	0.7	12	94.7	0.9	11
	15	87.7	0.7	13	92.4	0.6	13	88.7	0.5	13	95.3	0.6	13
	16	89.6	0.8	12	96.2	0.6	12	90.9	0.9	12	96.7	0.5	12
Water	13	32.2	5.5	22	48.8	4.8	24	35.4	6.5	20	70.1	4.2	22
Intake	14	21.1	5.0	15	48.3	5.4	14	38.4	7.2	12	76.1	4.4	11
	15	38.3	5.6	13	68.6	3.7	13	33.2	5.6	13	81.0	2.8	13
	16	51.3	11.9	6	76.0	4.0	6	51.0	7.1	6	81.8	5.6	6

B. 3-Way ANOVAs: F-values

	Sex	Line	Generation	SXL	SXG	LXG	SXLXG
Body Weight	27.81*	124.96*	11.74*	2.01	1.32	0.04	1.39
Core Temp.	66.41*	225.64*	3.51‡	0.20	2.95‡	7.14*	1.29
Water Intake	19.62*	133.89*	8.10*	7.90†	2.56	1.42	0.23

*$p<0.001$.
†$p<0.01$.
‡$p<0.05$.

the application of 2-way ANOVAs, but line differences were found for two of the variables, i.e., activity and operant responding. S line animals were less active and gave fewer operant responses than their R line counterparts. In the one significant interaction R line females showed greater general activity than other groups.

Effects of Acute DFP Treatment

Because of the various baseline differences described above it was important to use each animal as its own control in analyses of the effects of acute DFP treatment. Therefore, measures of criterion and non-criterion variables after treatment are expressed in the following Tables 3 and 5 as percents of pretreatment baselines. Table 4 summarizes observations of DFP-induced symptomatology, for which no pretreatment measures could be obtained.

Criterion variables. Table 3 summarizes results for the three criterion variables. There were significant main effects and some significant interactions.

There were significant differences in body weight between lines, sexes and generations after acute DFP treatment, but there were no significant interactions between these three variables (Table 3). The effects of DFP were greater in the S-line rats of both sexes, in the males of both lines and in the S13 and S14 generations.

Effects of acute DFP on core body temperature were greater for male than female animals and greater for S than R lines. There were significant differences but no consistent general trends over generations. A significant S × G interaction appeared to eb dependent upon a systematic decrease in effects with increasing generations for R line females and a significant L × G interaction upon systematically increasing differences between the two lines across generations.

TABLE 4

SYMPTOMATOLOGICAL EFFECTS OF ACUTE DFP TREATMENT: PERCENT INCIDENCE

Symptom	SM (15)	RM (15)	SF (11)	RF (15)
Diarrhea	80.0*	66.7*	27.3	6.7
Analgesia	73.3†	26.7	18.1	13.3
Tremor	13.3	0.0	18.2	0.0

*Significantly different from females of the same line $p<0.01$.
†Significantly greater than all other groups $p<0.01$.

Three-way ANOVAs of measures of water intake also yielded significant main effects. There were general trends for lower sensitivities to DFP in females than in males and in R line than in S line animals. Effects of DFP diminished over generations. A significant S × L interaction appeared to arise from the fact that R line female subjects were much more resistant to DFP than were the other three groups.

Symptomatology. Records kept of the occurrence of three basic symptoms following acute administration of DFP (diarrhea, analgesia and tremor) provided the basis for the summary of percent incidences in Table 4. Chi square tests established that significantly more male than female animals suffered from diarrhea; there were no significant line differ-

TABLE 5

EFFECTS OF ACUTE DFP TREATMENT ON NON-CRITERION VARIABLES: PERCENTS OF PRETREATMENT BASELINES

A. Means, standard errors of means, sample sizes

		SM			RM			SF			RF		
Generation		Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n
Activity	15	5.8	1.2	7	22.4	2.8	7	6.8	1.6	6	46.7	4.9	7
	16	13.6	2.4	7	35.0	3.4	7	18.5	2.4	6	38.3	4.0	7
Operant	15	51.4	8.6	7	74.1	5.8	7	47.8	12.8	6	99.0	11.0	7
Discrim	R16	136.9	20.0	7	107.7	13.0	7	95.2	22.6	7	109.6	22.5	7
Stereo	R16	75.8	19.4	7	79.5	9.9	6	81.0	34.2	3	72.4	24.1	6
Escape time	16	127.0	20.4	7	126.3	14.5	3	89.8	25.5	6	77.5	24.3	6

B. 2-Way ANOVAs: F-values

		Sex	Line	SXL
Activity	15	12.76	56.17*	9.21†
	16	0.64	16.40*	0.26
Operant	15	1.34	14.17*	2.17
Discrim	R16	1.38	0.03	0.85
Stereo	R16	1.38	0.03	0.85
Escape time	16	2.92	0.09	0.05

*$p<0.001$.
†$p<0.01$.
‡$p<0.05$.

ences. Assays for analgesia found a sex-line interaction, i.e., males of the S line were significantly more affected than were the other three groups. Relatively few tremorogenic effects appeared at the dose level of DFP used, the dose having been chosen in order that such motor effects not confound other behaviors being studied. When tremors were observed, they always occurred in S line animals.

Non-criterion variables. Data for effects of the acute DFP injections on non-criterion variables are summarized in Table 5. Two-way ANOVAs established both significant main effects and their interaction for the dependent variable, general activity. Female subjects were less affected, i.e., more active than males; R line animals showed less effects of DFP than the S line subjects. An interaction between sex and line appeared in the S15, but not in the S16 generation; R line females were less affected than R line males, while there was no difference between sexes in the S lines.

Only line effects were found to be significant in operant responding. R line animals were less affected than S line, i.e., gave greater numbers of responses during the assay period.

There were no significant effects of DFP treatment on any of the other three dependent variables. It should be pointed out that performance of the active discrimination response had been so well learned by the time of assay for DFP effects that only two types of response occurred: either an animal discriminated correctly or engaged in a stereotyped response, e.g., "freezing."

The possibility that effects of acute DFP on the S and R lines might include differential changes in foot shock thresholds was investigated in a non-contingent shock situation. Behavior under these conditions constitutes unconditioned responses to inescapable electric shock. Low shock intensities produce a flinching response, followed at higher intensities by skeletal activity which is intensity dependent [6]. Ten animals from each of the four line-sex groups were exposed approximately 24 hours after administration of DFP to three shock intensities (0.25, 0.50 and 1.00 mA) in a counterbalanced order, each intensity being administered twice. Occurence of the flinch response was noted. No responses were observed at the lowest shock intensity; all animals responded on all presentations of the 1.0 mA stimulus. Median effective intensities (EI_{50}) in mA for the four groups were: SM, 0.46; RM, 0.55; SF, 0.44; RF, 0.41. The close similarities of these EI_{50}s and the fact that they were all well below the intensity, 1.0 mA, used in the present assays, strongly suggest that effects of the DFP treatment cannot be accounted for in terms of differential changes in foot shock thresholds.

DISCUSSION

Behavioural and physiological phenotypes are results of interactions between genetic and environmental factors: $P = G + E + f(G \cdot E)$. The assortative mating program of which the present experiments are parts was designed to select for interactions between a specific manipulation of the cholinergic neurohumoral transmitter system (decreased activity of cholinesterase following acute administration of DFP) and three specific criterion variables (drinking behav-

ior, core body temperature and body weight). The matings successfully established two lines of rats with significantly different reactions to DFP, when environmental conditions were the same for all subjects [7]. Questions then arose as to whether genetic selection had been specific to the particular criterion variables or whether it had also affected other behavioral and physiological capacities of the two lines. Results of the present series of experiments show that generalization to other variables has in fact occurred but that generalization has not extended to all non-criterion variables measured, i.e., selection has not been completely nonspecific.

Pretreatment Differences

The present results show some significant line differences in pretreatment criterion variables which can be attributed to our selective breeding procedure. Pretreatment line differences in non-criterion variables, where S line animals were less active and made fewer operant responses, must also be considered as a result of the selective breeding procedure. That sex differences appeared in pretreatment measures of criterion variables is not surprising: female rats are innately smaller structurally and, hence, weigh less and consume smaller amounts of water than males [1]. There were no significant sex differences in the non-criterion variables. Because of such variations in relations of line and sex to the measures being studied it was important to compare the effects of acute DFP on the several groups of subjects by changes from pretreatment baselines rather than in absolute terms, i.e., to use each animal as its own control.

Effects of DFP: Differences Between Lines

One of the major questions which the present experiments were designed to answer concerned whether differences between lines following DFP treatment had been maintained in continuing generations. Earlier in the process of selective breeding we reported [7] that significant differences between Flinders S and R lines had appeared by the S5 generation and had been maintained through S9. The present measures of criterion variables for the S13, S14, S15 and S16 generations show quite clearly that differences between lines have continued to be significant. S line animals were more affected than their R line counterparts in all three variables: effects of acute administration of DFP on body weight, core body temperature and operant drinking behavior were greater for S than for R line animals.

Divergency of Lines

More detailed examination of data for the criterion variables shows that there were trends in body weight and operant drinking behavior for effects of DFP in absolute terms to decrease systematically in all groups as generations increased. Such a relationship was especially apparent in the R line female groups for all three criterion variables. This finding suggests that our previous report [7] of failure to produce an R line more resistant to DFP than randomly bred animals should be modified. We had pointed out that the divergence of lines was due primarily to increased sensitivity of S line animals. It now appears that changes at the resistant end of the scale may be truncated because of restrictions of the measuring instruments.

Generalization of Effects

Another major question for the present experiments was to determine how specific the selective breeding program had limited effects of DFP to the criterion variables. Earlier studies have shown that effects of selective breeding can be very specific [19]. Within limits imposed by the choices of non-criterion variables, the results are quite clear. Differential effects of the acute DFP treatment generalized to some, but not all variables measured. R line animals were less than S line subjects in general activity and in operant responding. There were no significant line differences in the accuracy nor time of discrimination responding nor in the amount of stereotyped behavior.

The latter three variables had in common an aversive source of motivation, i.e., foot shock. Other investigators [17] have reported that effects on behavioral and physiological variables of manipulating the cholinergic system are task dependent. Our present results show that line differences which appear under appetitive motivation are not observable under aversive motivation. Effects of acute DFP on the S and R lines appears not to generalize across all forms of motivation.

Symptomatological effects of the acute DFP treatment also provided some evidence for specificity of line differences. The incidence of diarrhea, associated with the peripheral actions of cholinergic agonists, showed significant sex but not line differences. In contrast, effects of DFP on analgesia and tremor, taken as indicators of central effects of cholinergic agonists, were suggestive of a difference between lines. These observations are consistent with the hypothesis that effects of our selective breeding program are mediated by central cholinergic mechanisms, although the possibility of interactions with other neurotransmitter systems must also be considered.

Although these statements about the generalization of effects correctly summarize the findings in the experiments now being reported, it is essential to consider the possibility that the behaviors which are related to the variables for which selection has been made may reflect an "accident of selection" [20], a spurious or fortuitous nongenetic correlation. The latter differs from "directly conjointed traits" in that they do not "fall directly in the causative pathway" "from genes to brain to behavior" [11]. Surveys of various selection lines have considered the matter of specificity of the selection, pointing to problems involved in establishing the nature and extent of direct genetic contributions [2]. Adventitiously associated traits may result, for example, from genetic drift in a relatively small population. The probability of a chance finding for a given trait may be increased as a function of the number of dependent variables being studied in parallel. Procedures for avoiding misinterpretation of what may, in fact, be fortuitous associations have been suggested by several investigators [3, 11, 20]. These include repeated selection experiments (simultaneous replications); a generational-developmental approach by which a correlated trait is observed over a number of generations; and analysis of segregating populations, i.e., cross-breeding as well as inter-breeding. For some of the variables presented in the present experiments the results have been similar in two generations. Further generations are currently being examined and the results of these and those of cross-breeding experiments will be reported later. In the meantime, "correlated responses are important to ascertain, for they provide cues about other traits which are in the same causative

pathway (developmental or physiological) as the selected trait" [11].

Mechanism(s)

In the "systems" approach to the study of ways in which living organisms cope with their environments the total system is taken to be the organism in its biosphere [15]. Within the total system the organism receives inputs from the environment and produces outputs to the environment. Within the organism intervening mechanisms, biochemical and electrophysiological events occuring within various morphological sites, are involved in receiving inputs and affecting the nature of outputs, which may be behavioral or physiological. Presumably selective breeding alters intervening events, leading to changes in behavioral phenotypes. What intervening mechanism(s) may be involved in selective breeding for interactions between the cholinergic neurotransmitter system and behavior as we have been studying them?

It has been pointed out that DFP is ". . . particularly valuable as an investigative tool . . . (because of) . . . the virtually irreversible inactivation it produces by alkylphosphorylation of AChE and certain other esterases; its high lipid solubility, results in penetration into the CNS and its relative specificity" [18]. Its effect is to decrease the activity of acetylcholinesterase, AChE, thereby elevating the level of the neurotransmitter, ACh [14]. In the search for mechanisms affected by our selective breeding program, hypotheses about changes in the cholinergic system have priority, although it is appreciated that this neurotransmitter system interacts with other intervening biochemical events in the overall processes underlying behavioral effects.

One likely hypothesis is that selective breeding may increase sensitivity of the target enzymes. This hypothesis gains credence from evidence that increased resistance to anticholinesterases in insects is related to a resistant form of AChE [16]. However, results of our earlier experiments [7] cannot be interpreted as consistent with the involvement of such a mechanism in the differences between the Flinders S and R lines.

The fact that R line animals showed less sensitivity to DFP suggests an analogy with the process of tolerance development to chronic exposure to that anticholinesterase [9, 12, 15]. Animals chronically exposed show no significant differences from control animals on a number of different behavioral and physiological variables. The search for mechanisms underlying tolerance development has excluded several possibilities [12,14] and has clearly indicated the involvement of changes in muscarinic receptor (mAChR) sensitivity [9]. It is a reasonable hypothesis, worthy of testing, that our selective breeding program has led to a subsensitivity of mAChRs in R line animals and/or a supersensitivity in the S line.

REFERENCES

1. Beatty, W. Gonadal hormones and sex differences in nonreproductive behavior in rodents: Organizational and activational influences. *Hormones Behav.* **12:** 112–163, 1979.
2. Broadhurst, P. L. *Drugs and the Inheritance of Behaviour.* New York: 1978, p. 206.
3. Elias, M. F. Some contributions of *mus musculus* to the study of hypertension and behavior over the life-span: Methodological considerations and useful directions. In: *Genetic Effects on Aging,* edited by D. Borgarva and D. E. Harrison. White Plains, NY: March of Dimes, 1978, pp. 121–156.
4. Glow, P. H., S. Rose and A. Richardson. The effects of acute and chronic treatment with diisopropyl fluorophosphate on cholinesterase activities of some tissues of the rat. *Aust. J. exp. biol. med. Sci.* **44:** 73–86, 1966.
5. Kozar, M., D. H. Overstreet, T. C. Chippendale and R. W. Russell. Changes in acetylcholinesterase activity in three major brain areas and related changes in behavior following acute treatment with diisopropyl fluorophosphate. *Neuropharmacology* **15:** 291–298, 1976.
6. Myer, J. S. Some effects of noncontingent aversive stimulation. In: *Aversive Conditioning and Learning,* edited by F. R. Brush. New York: Academic Press, 1971, pp. 464–536.
7. Overstreet, D. H., R. W. Russell, S. C. Helps and M. Messenger. Selective breeding for sensitivity to the anticholinesterase, DFP. *Psychopharmacology* **65:** 15–20, 1979.
8. Overstreet, D. H., R. W. Russell, S. C. Helps, P. Runge and A. Prescott. Sex differences following pharmacological manipulation of the cholinergic system by DFP and pilocarpine. *Psychopharmacology* **61:** 49–58, 1979.
9. Overstreet, D. H. and H. I. Yamamura. Receptor alterations and drug tolerance. *Life Sci.* **25:** 1865–1878, 1979.
10. Riley, E. P., E. X. Freed and D. Lester. Selective breeding of rats for differences in reactivity to alcohol: An approach to an animal model of alcoholism I. General Procedures *J. Stud. Alcohol* **37:** 1535–1547, 1976.
11. Roderick, T. H., R. E. Wimer and C. C. Wimer. Genetic manipulation of neuroanatomical traits. In: *Knowing, Thinking and Believing: A Festschrift for Professor David Krech,* edited by L. Petrinovich and J. L. McGaugh. New York: Plenum Press, 1976, pp. 143–178.
12. Russell, R. W., D. M. Warburton and D. S. Segal. Behavioral tolerance during chronic changes in the cholinergic system. *Communs Behav. Biol.* **4:** 121–128, 1969.
13. Russell, R. W., D. H. Overstreet, C. W. Cotman, V. G. Carson, L. Doyle, R. W. Dalglish and B. J. Vasquez. Experimental tests of hypotheses about neurochemical mechanisms underlying behavioral tolerance to the anticholinesterase, DFP. *J. Pharmac. exp. Ther.* **192:** 73–85, 1975.
14. Russell, R. W., V. G. Carson, R. A. Booth and D. J. Jenden. Mechanisms of tolerance to the anticholinesterase, DFP: Acetylcholine levels and dynamics in rat brain. *Neuropharmacology* **20:** 1197–1201, 1981.
15. Russell, R. W. The cholinergic system in behavior: the search for mechanisms of action. *A. Rev. Pharmac. Toxicol.* **22:** 435–463, 1982.
16. Shaw, R. D. and H. A. Malcolm. Resistance of *Boophilus microplus* to organophosphoric insecticides. *Vet. Res.* **76:** 210–211, 1964.
17. Squire, L. R. and H. P. Davis. The pharmacology of memory: a neurobiological perspective. *A. Rev. Pharmac. Toxicol.* **21:** 323–356, 1981.
18. Taylor, P. Anticholinesterase agents. In: *The Pharmacological Basis of Therapeutics,* edited by A. G. Gilman, L. S. Goodman and A. Gilman. New York: MacMillan, 1980, pp. 100–119.
19. Tryon, R. C. Genetic differences in maze-learning ability in rats. *Yb. Natn Soc. Stud. Educ.* **39:** 111–119, 1940.
20. Wood, W. G., M. F. Elias and G. A. Pentz. Ethanol concumption in genetically selected hypertensive and hypotensive mice. *J. Stud. Alcohol* **39:** 820–827, 1978.