Pharmacology Biochemistry & Behavior, Vol. 15, pp. 271–279, 1981. Printed in the U.S.A.

Genetic Influences on Cholinergic Drug Response[1]

MICHAEL J. MARKS, DOUGLAS M. PATINKIN, LINDA D. ARTMAN, JAMES B. BURCH AND ALLAN C. COLLINS[2]

Institute for Behavioral Genetics, School of Pharmacy, and Alcohol Research Center University of Colorado, Boulder, CO 80309

Received 8 April 1981

MARKS, M. J., D. M. PATINKIN, L. D. ARTMAN, J. B. BURCH AND A. C. COLLINS. *Genetic influences on cholinergic drug response.* PHARMAC. BIOCHEM. BEHAV. 15(2) 271–279, 1981.—Three mouse strains were tested for oxotremorine effects on open-field activity and body temperature. Open-field activity was depressed less severely in C3H mice than in C57BL, which were less affected than DBA. While no differences in the hypothermic effects of oxotremorine were observed 15 min after injection, the time courses of the drug effect on body temperature indicated that C3H were less affected than C57BL or DBA. No differences in the activities of acetylcholinesterase or choline acetyltransferase were found among the three strains in cortex, cerebellum, hindbrain (pons-medulla), or total midbrain. While no differences in muscarinic receptor levels were found in the four large brain areas, finer dissection of the midbrain revealed small differences in total receptor number in striatum, hippocampus, and remaining midbrain areas. C3H mice exhibited greater QNB binding than C57BL and DBA mice in striatum; DBA mice exhibited greater QNB binding in hippocampus than C57BL (C3H mice were not different from either strain); and C57BL had less QNB binding than the other two strains in midbrain. All of these differences were small (20% or less). No differences in K_D were observed. The inhibition of receptor binding by either oxotremorine or nicotine was the same in all strains, but the IC_{50} for oxotremorine varied from region to region. While behavioral differences in the effects of oxotremorine are clear, there is no obvious biochemical explanation for these differences.

Genetics Oxotremorine Open-field activity Hypothermia Muscarinic receptors

PREVIOUS studies from our laboratory have demonstrated genetic influences on behavioral responses to nicotine [14,15]. Specifically, three inbred mouse strains (C57BL/6Ibg, DBA/2Ibg, and C3H/2Ibg) have been compared with respect to initial sensitivity to the effects of nicotine on Y-maze and open-field measures of locomotor activity. In the Y-maze, both C57BL and DBA mice exhibit a marked depression in activity [14], while C3H mice are virtually unaffected by the dose of nicotine used [15]. In the open-field arena, C57BL and DBA mice exhibit behavioral depression over a relatively wide dose range, whereas C3H mice exhibit behavioral activation over the same range (Marks *et al.*, manuscript in preparation). All three strains appear to metabolize nicotine very similarly [15], suggesting that apparent strain differences in sensitivity to the effects of nicotine on locomotor activity are due to differential tissue sensitivity.

The actions of other agents which affect the cholinergic neuron also seem to be influenced by genetic factors. A number of studies have demonstrated that genetic factors influence the effects of scopolamine, a muscarinic cholinergic antagonist. For example, Oliverio *et al.* [25] reported a differential effect of scopolamine on avoidance conditioning in DBA and C3H/He mice. DBA mice showed a 10-fold increase in avoidance responses following a 2.7 mg/kg dose of scopolamine, while C3H mice exhibited only a 3-fold increase following this dose. Van Abeelen and Strijbosch [31] observed a decrease in exploratory activity in C57BL mice following scopolamine, whereas the activity of DBA mice increased. Similarly, Anisman [1, 2, 3] has repeatedly observed genetic influences on scopolamine effects. An initial study [2] noted that scopolamine enhanced active avoidance performance in mice of the A strain, but that DBA and C57BL mice were unaffected by the same dose. A subsequent study [3] utilized a diallel analysis wherein all of the possible F_1 hybrids derivable from DBA, C57, and A crosses were activity tested in an open-field arena with an electrified grid floor. Prior to shock administration, scopolamine affected activity minimally in the C57BL strain but increased activity in the DBA and A strains. In the A×C and A×D crosses, behavior resembled that of the A parent following scopolamine treatment; in the D×C cross, a D-like response to scopolamine was observed. Anisman [1] also observed a genotype×drug×behavioral task interaction.

All of these studies noted strain differences in control (no-drug) activity. Many investigators have found that in-

[1]Supported by a grant from The Council for Tobacco Research, Inc. (CTR 1204).

[2]Recipient of a Research Scientist Development Award from the National Institute on Alcoholism and Alcohol Abuse (AA-00029); reprint requests should be sent to Dr. Allan C. Collins, Institute for Behavioral Genetics, Box 447, University of Colorado, Boulder, CO 80309.

bred mouse strains differ in various activity measures. For example, when Thompson [28] measured exploratory behavior in 14 inbred strains and 1 hybrid strain of *Mus musculus* in 1953, large interstrain differences were observed. McClearn [23] measured the activity of six inbred strains and of the F_1 crosses derived from two of the most divergent strains in four different situations. Wide differences existed among the strains, but a high consistency of strain rank was found over the four tests. McClearn interpreted his results as suggesting that activity is under polygenic control.

The present study was initiated to ascertain whether inbred mouse strains that differ in sensitivity to the effects of nicotine on locomotor activity also differ in sensitivity to the effects of the muscarinic cholinergic agonist, oxotremorine. In addition, the effects of oxotremorine on body temperature were assessed. The activities of acetylcholinesterase (AChE) and choline acetyltransferase (ChAT), as well as the number and affinity of muscarinic receptors, were also measured in an attempt to provide a neurochemical explanation for the observed strain differences in oxotremorine response.

METHOD

Animals of the C57BL/6Ibg, DBA/2Ibg, and C3H/2Ibg inbred strains were bred and maintained in the colony of the Institute for Behavioral Genetics. Both sexes were used, and all animals were 70±10 days at time of testing. The animals were housed with like-sexed littermates (2 to 5 per group) with food (Wayne Lab Blox) and water available ad lib. The colony was maintained at 23±1 degrees and had a 12:12 hr light-dark cycle (lights on 0700 to 1900). All behavioral testing was carried out between 0900 and 1200.

Open-Field Activity

The effect of oxotremorine on locomotor activity was measured in the automated open-field arena developed by DeFries and Hegmann [10]. The arena is constructed of white Plexiglas, 91.4 cm on each side, and the floor is marked off into 36 equal squares. Movements between squares interrupt photocell beams and activate electronic counters. Animals were tested under red light in order to minimize any artifactual decrease in activity that might arise from photophobia [9,24]. The C3H and C57BL strains were challenged with 0.01, 0.02, 0.04, 0.08, 0.10, or 0.2 mg/kg of oxotremorine. Due to the sensitivity of DBA mice to oxotremorine's effects on open-field activity, their challenge doses were 0.0025, 0.005, 0.01, 0.02, 0.04, or 0.08 mg/kg. All doses were prepared by making an appropriate dilution of a 1 mg/ml solution of oxotremorine in sterile saline (0.9% NaCl) such that the required dose was injected in a volume of 0.01 ml/g body weight. Control animals received an equivalent volume of saline. Immediately after intraperitoneal injection of oxotremorine or saline, each animal was placed in a cylinder in a corner of the open-field arena for 3 min. The cylinder was then removed and activity was measured for a 5-min period.

Hypothermia

Rectal temperature was measured 15 min after oxotremorine or saline injection. Temperatures were recorded with a Digitec HT-5810 digital thermometer using a thermistor probe inserted 2.5 cm rectally. Two additional doses of oxotremorine, 0.1 and 0.2 mg/kg, were administered to groups of DBA mice that were not open-field tested.

The time course of oxotremorine's effect on body temperature was also determined in the three strains. Rectal temperature of animals injected with 0.025, 0.05, 0.1, or 0.2 doses of oxotremorine was measured every 10 min until normothermia was reattained.

Biochemical Assays

Animals which had not been treated with oxotremorine were used for the neurochemical analyses. The mice were sacrificed by cervical dislocation, and the brain was removed and rinsed with ice-cold 50 mM potassium phosphate buffer. In our initial study, the brain tissue was placed on ice and dissected into four "regions": cortex, cerebellum, hindbrain (pons-medulla), and midbrain (the tissue remaining after the other regions had been removed). In a subsequent study, the striatum, hippocampus, and hypothalamus were dissected out of the midbrain. The tissue left after this dissection was labelled "midbrain."

AChE activity was measured using a modification of the method of Ellman *et al.* [12]. After the animals were killed by cervical dislocation, the brain was quickly removed; placed on ice; and dissected into cortex, cerebellum, midbrain, hindbrain, hippocampus, hypothalamus, and corpus striatum. The tissue was homogenized in 25 volumes of 100 mM potassium phosphate, pH 7.4, except for hypothalamus, which was homogenized in 40 volumes of buffer. The following dilutions of tissue homogenates were prepared in 100 mM potassium phosphate, pH 7.4, containing 0.5% (v/v) Triton X-100 [16]: cortex, midbrain, and hindbrain, 1:20; hippocampus and hypothalamus, 1:10; cerebellum 1:5; and corpus striatum, 1:60. A typical assay contained 40–300 μM (final concentration) acetylthiocholine iodide (Sigma) (ASCh) and 10 μl of diluted tissue homogenate in 100 mM potassium phosphate, pH 7.4, in a total volume of 200 μl. Reactions were run in disposable 10- × 75-mm test tubes. Blanks contained 5 μM of the specific true cholinesterase inhibitor, 1,5-bis-(4-allyldimethyl ammonium phenyl)-pentan-3-one dibromide (B.W. 284 C51) (a generous gift of Wellcome Research Laboratories, Beckenham, England) to control for both non-specific hydrolysis of ASCh and pseudocholinesterase activity in the samples [5]. The reaction was initiated by addition of 40 μl of 5-fold concentrated substrate and was incubated at 37° for 30 min. At the end of this time, the reaction was terminated by addition of 20 μl of 100 mM neostigmine bromide (final concentration, 9.09 mM). This concentration of neostigmine terminates the reaction immediately and the samples are stable for several hours in the cold. Color was developed by adding 300 μl of 425 μM 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) dissolved in 200 mM sodium pyrophosphate, pH 8.0, to each sample. Final DTNB concentration was 245 μM. Absorbance was determined at 412 nm against water in a Gilford 240 spectrophotometer. Activity of AChE was calculated from the extinction coefficient of the 5-thio-2-nitrobenzoic acid anion produced: 13,600 liter/mole/cm. This assay will be referred to as the batch assay in the remainder of the text.

AChE kinetic constants were also determined by the radioactive assay of Blume *et al.* [7]. The radioactive assay used acetylcholine iodide (ACh) and [1-^{14}C] ACh (0.01 μCi/assay) in place of ASCh. The protocol was exactly the same as described for the batch assay until the time the reaction was terminated. The radioactive assay was terminated by rapid dilution with 1.0 ml of H_2O, and the unreacted [1-^{14}C] ACh was separated from released [1-^{14}C] acetate on

TABLE 1

COMPARISON OF BATCH AND RADIOMETRIC ASSAYS FOR ACETYLCHOLINESTERASE ACTIVITY IN SEVEN BRAIN REGIONS

Brain region	Batch assay		Radiometric assay	
	K_m	V_{max}	K_m	V_{max}
Cortex	69.0 ± 8.2	9.75 ± 0.64	101.1 ± 13.8	8.85 ± 0.89
Cerebellum	65.5 ± 6.3	2.20 ± 0.12	98.7 ± 10.2	2.08 ± 0.17
Midbrain	65.8 ± 5.3	13.65 ± 1.08	101.0 ± 9.9	11.61 ± 0.70
Hindbrain	57.7 ± 8.1	7.65 ± 0.37	95.3 ± 12.8	7.60 ± 0.90
Hippocampus	65.0 ± 7.1	6.47 ± 0.34	113.5 ± 20.6	6.72 ± 0.90
Hypothalamus	64.0 ± 8.9	5.67 ± 0.34	129.8 ± 13.8	6.55 ± 0.55
Striatum	76.7 ± 9.7	37.28 ± 2.39	128.7 ± 25.0	37.25 ± 3.64

Each tabled value is mean ± SEM of six separate determinations for regions obtained from male BALB/c mice. K_m is expressed as μM; V_{max} for the batch assay is expressed as μmoles acetylthiocholine hydrolyzed per hr per mg protein; and V_{max} for the radiometric assay is expressed as μmoles acetylcholine hydrolyzed per hr per mg protein.

0.5- × 3.0-cm columns of AG 50W-X8 in the sodium form. Tubes were washed with an additional 1.0 ml of H_2O and this was also added to the column. The entire 2.0 ml was collected from the column in Nalgene filmware scintillation bags (Nalge Co.) and counted in a Beckman 7000 liquid scintillation counter at 80% efficiency.

Kinetic constants were determined by linear regression analysis of Eadie-Hofstee plots of the data using the average substrate concentration over the time of the incubation [19]. Initial substrate concentrations for the colorimetric batch assay ranged from 20 to 500 μM. For the radiometric assays, the substrate concentrations were adjusted with cold ACh to be in the same range as the batch assay. The concentration of carrier-free [1-^{14}C] ACh was approximately 20 μM per 0.01 μCi.

The batch assay is linear with both time and protein concentration. For routine use, a 30-min incubation was chosen for convenience, and the protein concentration was adjusted to lie within the linear range of the assay (1–10 μg/assay). It was of interest to know if the batch assay would be suitable for use in the routine determination of K_m and V_{max} in mouse brain regions. These values are presented in Table 1 along with results from the radiometric assay for comparison. In general, the radiometric assay with ACh gives K_m values which average 40% greater than the batch assay with ASCh, while both assays yield approximately the same V_{max} values.

The activity of ChAT was measured using a modification of the method of Schrier and Schuster [26]. An aliquot of the original tissue homogenate prepared for the AChE assay was diluted with 50 mM potassium phosphate buffer, pH 7.4, containing 0.5% Triton X-100 (Research Products International). The dilutions were adjusted to provide a workable amount of enzyme activity (cerebellum dilution was 1:2; other regions, 1:4). The assay mixture contained: choline Br (Sigma), 5 mM; neostigmine Br (Sigma), 10 mM; NaCl, 100 mM; potassium phosphate, 50 mM, pH 7.4; in a final volume of 200 μl. The reaction was initiated by the addition of (^{14}C) acetyl coenzyme A (AcCoA) (Boehringer-Mannheim). Final AcCoA concentrates were 20 mM, 50 mM, 100 mM, and 400 mM. Each assay tube contained 5 nCi (^{14}C) AcCoA (New England Nuclear, s.a.=58 mCi/mmol). The reaction was incubated for 60 min at 37° and was terminated by the addition of 1 ml ice-cold H_2O. This mixture was then immediately applied to a 0.5- × 3-cm Dowex-1 (Sigma) anion exchange column. The effluent was collected directly in Nalgene filmware bags. The columns were washed with a second 1-ml volume of ice-cold H_2O which was also collected in the filmware bags. Four milliliters of scintillation cocktail (Toluene, 1250 ml; Triton X-100, 900 ml; PPO, 10.6 g) was added to each bag, and the samples were counted at 80% efficiency. Kinetic constants were determined by linear regression analysis of Eadie-Hofstee plots of the data.

Muscarinic receptor assays were carried out using a slight modification of the method of Yamamura and Snyder [34]. Homogenate was added to 10 ml of 20 mM potassium phosphate buffer, pH 7.4. Incubation was initiated by addition of (^{3}H) dl quinuclidinyl-N-benzilate (QNB) (New England Nuclear, 29.4 Ci/mmol). Four (^{3}H) QNB concentrations between 20 pM and 250 pM were used in each experiment. The actual concentrations varied somewhat from experiment to experiment. The receptor concentration never exceeded 15 pM, which is approximately one-third the dissociation constant (K_D). Samples were incubated for 40 min at 37°, at which time the particulate protein and bound QNB were collected by filtration on Whatman GFC glass fiber filters. The filters were washed with four 5-ml portions of ice-cold 10 mM potassium phosphate buffer, pH 7.4, and were then placed in Nalgene filmware bags to which 3 ml of scintillation cocktail was added. The filters were mechanically crushed and counted at 20% efficiency in a Beckman 7000 liquid scintillation counter. Kinetic constants were calculated using the method of least squares on data represented as Scatchard plots.

The relative inhibition of QNB binding by oxotremorine and nicotine was determined in the seven brain regions (cortex, cerebellum, midbrain, hindbrain, hippocampus, hypothalamus, and striatum) in the three strains. Binding assays were carried out essentially as described above using 120 pM (^{3}H) QNB as the ligand. In those assays in which oxotremorine inhibition was evaluated, five different concentrations of oxotremorine (10^{-7} to 10^{-5} M) were added to the incubation immediately before addition of the QNB. Nicotine inhibition was determined in the same fashion ex-

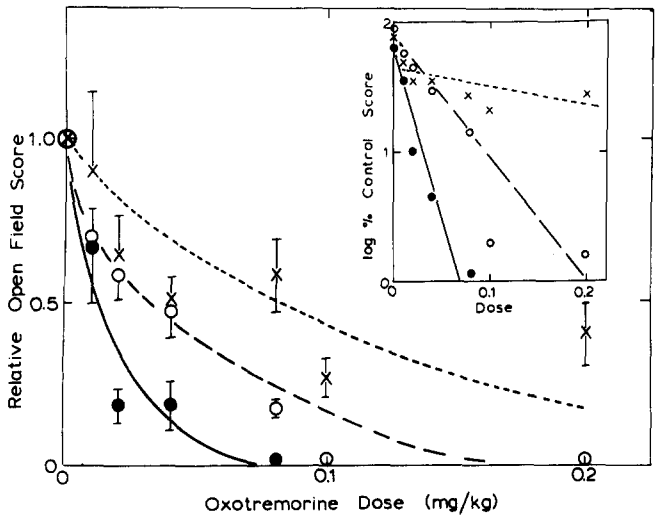

FIG. 1. Effect of oxotremorine on open-field activity in three mouse strains. Normalized open-field scores for C3H (X), DBA (●), and C57BL (○) mice represent the mean±SEM of 12–20 determinations at each dose. Scores for both sexes have been combined. Control open-field counts±SEM are: 248±20 for C57BL; 126±20 for DBA; and 120±29 for C3H. The inset presents logs of the normalized scores as a function of oxotremorine dose.

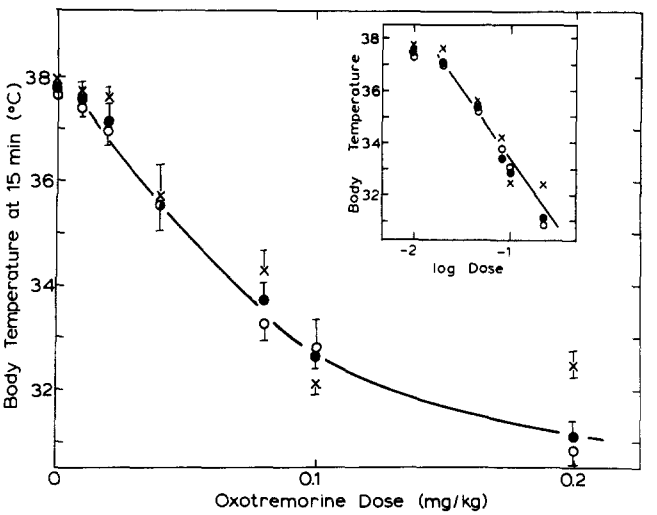

FIG. 2. Effect of oxotremorine on body temperature in three mouse strains. Body temperature was measured in C3H (X), DBA (●), and C57BL (○) mice 15 min after administration of the indicated doses of oxotremorine. The data are expressed as mean±SEM for 12–20 measurements at each dose. Data for both sexes have been combined. The inset displays the temperature as a function of log oxotremorine dose.

cept the five nicotine concentrations ranged from 10^{-5} to 10^{-3} M. Log dose-response curves were constructed, and that concentration which inhibited QNB binding by 50% (the IC_{50} value) was determined. Four estimates of the IC_{50} value were determined for both oxotremorine and nicotine in each brain region in each strain.

Protein concentration was determined usng the method of Lowry *et al.* [20].

Statistical Analyses

Data obtained in the experiments in which the effects of oxotremorine on open-field activity and body temperature were measured were analyzed in two ways. Open-field activity was analyzed using normalized scores because strain differences in control activity were detected. An initial three way analysis of variance (strain×sex×dose) revealed significant strain×dose interactions. These data were also subjected to a linear transformation by fitting the dose-response data to a line using the method of least squares. The slopes and 95% confidence limits of the lines were estimated.

Data obtained in the AChE and ChAT enzyme assays and in the QNB binding assays were subjected to two-way analyses of variance. Post hoc comparisons of individual sample means were made using the Tukey B test for critical differences. Differences between group means were not considered significant unless $p<0.05$. One-way analyses of variance were used to analyze the data obtained in the experiments in which the IC_{50} values for inhibition of QNB binding by oxotremorine or nicotine were determined. Post hoc comparisons were made using the Tukey B test for critical differences.

RESULTS

Figure 1 presents the dose-response curves for the effect of oxotremorine on open-field activity in the three mouse strains. A three-way analysis of variance revealed no sex difference in the effects of oxotremorine on open-field activity in any of the strains. A significant main effect for dose was observed, i.e., oxotremorine decreased open-field activity in all three strains, and a significant line×dose interaction suggested a differential sensitivity to oxotremorine's effects on open-field activity.

Since the effects of oxotremorine on open-field activity resulted in a curve which has the appearance of a first order decay process, a linear transformation was attempted (see Fig. 1). In this transformation, the logarithm of activity was plotted against oxotremorine dose. The slope of the resulting line was calculated, as was the oxotremorine dose required to reduce open-field activity by 50%, i.e., an estimate of the ED_{50}. The ED_{50} values obtained by this method were: 0.014 mg/kg (0.01, 0.018) for DBA mice; 0.032 mg/kg (0.027, 0.041) for C57BL mice; and 0.082 (0.045, 0.444) for C3H mice. Numbers in parentheses represent the lower and upper values for the 95% confidence limits. The slopes of the log activity vs dose lines are −21.96 for the DBA strain, −9.20 for the C57BL strain, and −0.94 for the C3H strain. Thus, the rank order for sensitivity to oxotremorine's depressant effects on open-field activity is DBA > C57BL > C3H. The C3H strain appeared to be uniquely insensitive to oxotremorine's effects on open-field activity.

The effects of oxotremorine on body temperature measured 15 min after injection are depicted in Fig. 2. A three-way analysis of variance of the data failed to reveal any

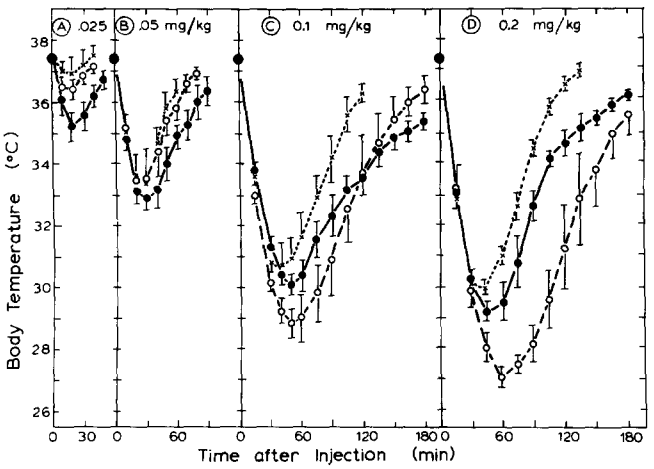

FIG. 3. Time course of oxotremorine-induced hypothermia in three mouse strains. Body temperature of C3H (X), DBA (●), and C57BL (○) mice was measured at various times after injection of 0.025, 0.05, 0.1 and 0.2 mg/kg of oxotremorine. Data represent mean±SEM for both males and females, N=6. Areas under the curves were calculated for C3H, C57BL, and DBA mice at each dose. The mean±SEM obtained from the individual curves were: 0.025 mg/kg—19.2±12.3, 17.7±9.0, and 89.6±25.9; 0.05 mg/kg—135±21, 163±37, and 211±38; 0.1 mg/kg—468±85, 864±82, and 836±45; and 0.2 mg/kg—576±49, 1583±111, and 751±49, respectively. Units of the area are degree×min.

significant strain difference in the hypothermic effect elicited by oxotremorine. The inset to Fig. 2 indicates that the log dose-response curves for the hypothermic effect are linear between doses of 0.02 and 0.2 mg/kg. Thus, the results of this experiment suggested that the three strains do not differ in sensitivity to the effects of oxotremorine on body temperature.

However, the experiment which assessed the time course of oxotremorine's effects on body temperature demonstrated that strain differences in the hypothermia-producing effects of oxotremorine exist (Fig. 3). The C3H mice appeared to be less sensitive than the C57BL or the DBA. In general, maximum hypothermia was less in the C3H mice, and it occurred sooner after injection. The areas under the time-temperature curves were significantly less for C3H mice than for either the C57BL or DBA strains at the two highest oxotremorine doses. At the lowest dose (0.025 mg/kg), the C57BL mice were significantly less affected than were the DBA, while at 0.2 mg/kg this relationship was reversed. The rate of return to normothermia was estimated for each strain at the three higher doses by fitting the body temperature data to a linear function of time. The temperatures between the point after the greatest depression and 36° were used to construct these lines. Six estimates were made for each strain (three doses×two sexes). Values obtained were 0.089±0.003 degrees×min^{-1} for C3H mice, 0.089±0.008 degrees×min^{-1} for C57BL, and 0.068±0.005 degrees×min^{-1} for DBA. A one-way analysis of variance showed a significant ($p<0.05$) effect, and a Tukey's B post hoc test detected significant differences between strains in rate of return to normothermia.

A strain comparison of AChE activity in four brain regions is presented in Table 2. The regions examined (cortex, cerebellum, midbrain, and hindbrain) appeared to have similar enzyme activities in the three strains. Although regional differences were detected, the activity profiles for both substrate affinity (K_m) and maximal velocity (V_{max}) were virtually identical among strains. Small, but statistically significant, sex differences in V_{max} (females less than males) were observed in cortex, midbrain, and hindbrain.

Table 3 presents the results of the analyses of ChAT activity. Once again, regional differences in enzyme activity were detected, but virtually identical K_m and V_{max} values were observed in all three strains. Small sex differences were seen, with the K_m for AcCoA being less in females in all brain regions. Thus, it does not seem likely that the difference in locomotor response to oxotremorine is related to a strain difference in the ability to synthesize or metabolize acetylcholine.

A strain comparison of muscarinic receptors in the four brain regions is presented in Table 4. The dissociation constant for QNB (K_D) and the total number of QNB binding sites (B_{max}) were estimated in cerebral cortex, cerebellum, midbrain, and hindbrain. Within each strain, no significant regional differences in K_D were detected, but sizable differ-

TABLE 2

ACETYLCHOLINESTERASE ACTIVITY IN THREE INBRED MOUSE STRAINS

		C57BL		DBA		C3H	
Brain region	Sex	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
Cortex	Male	72 ± 2	6.1 ± 0.5	56 ± 11	6.7 ± 0.7	56 ± 5	6.8 ± 0.7
	Female	60 ± 9	5.4 ± 1.4	77 ± 21	5.4 ± 0.5	59 ± 10	5.9 ± 0.3
Cerebellum	Male	61 ± 12	1.6 ± 0.2	61 ± 8	1.4 ± 0.3	62 ± 9	1.5 ± 0.2
	Female	57 ± 2	1.6 ± 0.2	64 ± 19	1.3 ± 0.2	67 ± 12	1.3 ± 0.3
Midbrain	Male	49 ± 3	8.2 ± 0.7	60 ± 5	9.3 ± 0.6	57 ± 10	8.1 ± 0.6
	Female	62 ± 3	6.7 ± 0.5	67 ± 11	8.0 ± 0.1	62 ± 9	7.9 ± 1.4
Hindbrain	Male	54 ± 3	5.5 ± 0.6	57 ± 6	6.9 ± 0.7	63 ± 10	6.3 ± 0.2
	Female	57 ± 7	4.9 ± 0.3	64 ± 10	5.6 ± 0.5	58 ± 7	5.8 ± 0.6

Each K_m (μM) or V_{max} (μmol/mg protein/hr) value is mean ± SEM for four determinations. V_{max} for females, collapsed across strains, was less than V_{max} for males ($p<0.05$) in cortex, midbrain, and hindbrain.

TABLE 3

CHOLINE ACETYLTRANSFERASE ACTIVITY IN THREE INBRED MOUSE STRAINS

Brain region	Sex	C57BL K_m	C57BL V_{max}	DBA K_m	DBA V_{max}	C3H K_m	C3H V_{max}
Cortex	Male	55 ± 4	1.8 ± 0.1	53 ± 4	2.1 ± 0.2	53 ± 5	1.8 ± 0.2
	Female	44 ± 5	1.5 ± 0.1	44 ± 3	1.7 ± 0.1	44 ± 5	1.7 ± 0.1
Cerebellum	Male	38 ± 2	0.4 ± 0.03	39 ± 4	0.3 ± 0.04	34 ± 5	0.2 ± 0.03
	Female	30 ± 3	0.2 ± 0.05	32 ± 4	0.2 ± 0.03	32 ± 6	0.2 ± 0.03
Midbrain	Male	56 ± 3	2.4 ± 0.1	57 ± 6	2.8 ± 0.2	59 ± 2	2.8 ± 0.1
	Female	52 ± 2	2.3 ± 0.2	52 ± 4	2.5 ± 0.2	49 ± 3	2.2 ± 0.1
Hindbrain	Male	52 ± 3	1.8 ± 0.1	50 ± 4	1.6 ± 0.1	50 ± 3	1.6 ± 0.1
	Female	42 ± 4	1.5 ± 0.1	44 ± 2	1.5 ± 0.1	42 ± 3	1.4 ± 0.02

Each K_m (μM) or V_{max} (μmol/mg protein/hr) value is mean ± SEM of four determinations. K_m for females, collapsed across strains, was less than K_m for males ($p<0.05$) in all brain regions.

TABLE 4

QNB BINDING IN THREE INBRED MOUSE STRAINS

Brain region	Sex	C57BL K_D	C57BL B_{max}	DBA K_D	DBA B_{max}	C3H K_D	C3H B_{max}
Cortex	Male	66 ± 22	1.48 ± 0.04	61 ± 9	1.38 ± 0.11	38 ± 10	1.30 ± 0.14
	Female	60 ± 20	1.42 ± 0.16	75 ± 17	1.36 ± 0.10	44 ± 11	1.42 ± 0.08
Cerebellum	Male	43 ± 12	0.077 ± 0.012	39 ± 4	0.088 ± 0.006	36 ± 3	0.094 ± 0.003
	Female	49 ± 10	0.082 ± 0.008	60 ± 10	0.087 ± 0.003	32 ± 7	0.091 ± 0.008
Midbrain	Male	52 ± 19	1.15 ± 0.08	57 ± 8	1.26 ± 0.08	34 ± 1	1.17 ± 0.08
	Female	81 ± 39	1.12 ± 0.12	74 ± 12	0.98 ± 0.15	38 ± 6	1.14 ± 0.03
Hindbrain	Male	62 ± 32	0.60 ± 0.10	69 ± 22	0.50 ± 0.05	34 ± 4	0.52 ± 0.05
	Female	62 ± 25	0.50 ± 0.08	54 ± 10	0.51 ± 0.11	33 ± 8	0.49 ± 0.03

Each K_D (pM) or B_{max} (pmol/mg protein) value is mean ± SEM of four determinations.

ences in B_{max} were obtained. Total number of QNB binding sites in cortex or midbrain considerably exceeded the number observed in cerebellum or hindbrain. B_{max} did not differ significantly among the strains in any of the four brain regions examined.

A considerable literature suggests that nigrostriatal pathways are involved in locomoter activity [18, 30, 33], and it has been reported that genetic factors influence the effect of intrahippocampal injection of methylscopolamine on exploratory behavior [32]. A finer analysis of midbrain QNB binding was therefore undertaken. Table 5 presents the K_D and B_{max} values for striatum, hippocampus, hypothalamus, and the remaining midbrain tissue. While no strain differences in K_D were apparent, an analysis of variance did reveal statistically significant strain differences in B_{max} in striatum, with C3H mice exhibiting 15–20% greater QNB binding than C57BL or DBA; in midbrain, where 17% less QNB binding was detected for C57BL mice than for the other two strains; and in hippocampus, where DBA mice exhibited 13.8% greater QNB binding than did C57BL mice. Significant differences were not detected in hypothalamus.

Tables 6 and 7 present the results of the experiments in which the inhibition of QNB binding by oxotremorine (Table 6) and nicotine (Table 7) was determined. No strain differences in IC_{50} value were obtained for either drug. Regional differences were seen with oxotremorine, but the profiles of inhibition were identical for the three strains. No regional differences wers observed for nicotine. The IC_{50} values obtained for oxotremorine were approximately two orders of magnitude lower than those obtained for nicotine. These data argue that the strain differences in sensitivity to oxotremorine or nicotine are not due to differential affinity of oxotremorine or nicotine for the muscarinic receptor.

DISCUSSION

The strain differences in sensitivity to the effects of oxotremorine on activity in the open-field arena are similar to those detected for the effects of nicotine on activity in the Y-maze [15] and the open field (Marks *et al.*, manuscript in preparation). C3H mice appear to be uniquely insensitive to the depressant effect of both drugs on open-field activity. The rank order for sensitivity to oxotremorine is C3H < C57BL < DBA.

TABLE 5

QNB BINDING IN MIDBRAIN OF THREE INBRED MOUSE STRAINS

Brain region	Sex	C57BL K_D	C57BL B_{max}	DBA K_D	DBA B_{max}	C3H K_D	C3H B_{max}
Midbrain	Male	39 ± 9	1.02 ± 0.12*	40 ± 3	1.23 ± 0.08	40 ± 2	1.21 ± 0.05
	Female	42 ± 4	1.14 ± 0.09*	40 ± 5	1.30 ± 0.09	44 ± 4	1.29 ± 0.05
Hippocampus	Male	31 ± 4	1.27 ± 0.09†	36 ± 2	1.53 ± 0.03	32 ± 5	1.42 ± 0.10
	Female	35 ± 6	1.47 ± 0.10†	38 ± 4	1.66 ± 0.12	38 ± 3	1.57 ± 0.06
Hypothalamus	Male	42 ± 4	0.57 ± 0.05	44 ± 8	0.57 ± 0.05	44 ± 2	0.61 ± 0.05
	Female	34 ± 5	0.62 ± 0.10	44 ± 5	0.66 ± 0.07	43 ± 8	0.68 ± 0.03
Striatum	Male	44 ± 4	2.72 ± 0.14	44 ± 4	2.98 ± 0.09	49 ± 4	3.21 ± 0.20‡
	Female	53 ± 1	2.86 ± 0.19	43 ± 3	2.75 ± 0.14	49 ± 5	3.41 ± 0.20‡

Each K_D(pM) or B_{max} (pmol/mg protein) value is mean ± SEM at six determinations.

Significance levels for differences among strains (combined sexes) are as follows: *$p<0.05$, C57BL less than DBA and C3H; †$p<0.05$, C57BL less than DBA; ‡$p<0.05$, C3H greater than DBA and C57BL.

TABLE 6

OXOTREMORINE INHIBITION OF QNB BINDING IN BRAIN REGIONS

Strain	Cortex	Cerebellum	Midbrain	Hindbrain	Hippocampus	Hypothalamus	Striatum
C3H	1.5 ± 0.3	0.42 ± 0.08	1.1 ± 0.3	0.57 ± 0.17	2.5 ± 0.5	0.49 ± 0.05	1.7 ± 0.6
DBA	1.7 ± 0.3	0.36 ± 0.05	1.3 ± 0.6	0.29 ± 0.01	2.6 ± 0.4	0.70 ± 0.19	1.7 ± 0.3
C57BL	1.6 ± 0.3	0.56 ± 0.07	1.2 ± 0.2	0.38 ± 0.07	2.5 ± 0.5	0.79 ± 0.08	1.7 ± 0.3

IC_{50} values (μM) for oxotremorine were measured using a QNB concentration of 145 pM. Each value is mean ± SEM of four determinations.

TABLE 7

NICOTINE INHIBITION OF QNB BINDING IN BRAIN REGIONS

Strain	Cortex	Cerebellum	Midbrain	Hindbrain	Hippocampus	Hypothalamus	Striatum
C3H	350 ± 60	410 ± 10	370 ± 20	370 ± 40	470 ± 40	340 ± 30	320 ± 30
DBA	300 ± 30	370 ± 30	300 ± 50	320 ± 30	400 ± 30	390 ± 30	360 ± 40
C57BL	350 ± 50	390 ± 40	400 ± 40	420 ± 40	450 ± 20	390 ± 40	360 ± 60

IC_{50} values (μM) for nicotine were measured using a QNB concentration of 125 pM. Each value is mean ± SEM of four determinations.

Although dose-response curves for the effect of oxotremorine on body temperature constructed 15 min after oxotremorine treatment failed to detect strain differences in hypothermia-producing effects, it seems likely that strain differences do exist. The time-course experiments revealed differences among strains in both maximal degree of hypothermia induced by oxotremorine and in the time post injection at which the maximal depression of body temperature occurred. Once again, the C3H mice were less affected than were the DBA and C57BL. C3H mice are also less sensitive to the hypothermia-producing effects of nicotine (Marks *et al.*, manuscript in preparation). Since the open-field activity of DBA mice is depressed to a greater degree than is C57BL activity by oxotremorine, while body temperature is decreased more in C57BL mice at the same doses, it does not seem likely that the hypothermia induced by oxotremorine is caused simply by decreased motor activity. It seems probable that the mechanisms underlying these two effects of oxotremorine are not directly related.

The observation that mice of the three strains also differ in sensitivity to the effects of nicotine on locomotor activity suggests that nicotine and oxotremorine may elicit some of their actions via similar mechanisms. Perphaps the simplest explanation would be that the two drugs act at the same receptor. Such an explanation, if proposed for the peripheral autonomic nervous system, would seem totally unacceptable. However, the identity of the receptor at which nicotine acts in the central nervous system remains uncertain. Several recent studies have demonstrated that the binding properties of brain nicotine receptors are quite different from

those found in the peripheral nervous system [8,21]. While our observation that C57BL, DBA, and C3H mice differ in sensitivity to classic nicotinic and muscarinic receptor agonists in a similar fashion suggests that nicotine and oxotremorine may act at the same cholinergic receptor in the brain, the high IC_{50} value for nicotine inhibition of QNB binding makes this unlikely.

Another possible explanation for the strain difference in sensitivity to oxotremorine is a difference in rate of oxotremorine metabolism. We have observed that C57BL, DBA, and C3H mice do not appear to differ in rate of nicotine metabolism [15], but we have not directly measured their oxotremorine metabolism rates. However, the hypothermia time-course experiments do provide some information. If metabolism rates differ among the strains, the rates of return of body temperature to normal should differ in a similar fashion, assuming metabolism of oxotremorine is the rate-limiting step for reversal of hypothermia. If this assumption is valid, the fact that the slopes of the temperature recovery curves are not identical suggests that metabolism differences may be responsible for part of the DBA's pronounced sensitivity to oxotremorine.

The rates of return to normothermia for C57BL and C3H mice are nearly identical, while the DBA rate is approximately 25% lower. Since the rates of return to normothermia for C3H and C57BL are nearly identical, it would seem that metabolic differences do not account for the differences in oxotremorine depression of temperature in these two strains. The onset of oxotremorine effect on open-field activity is rapid, and the effect is measured 3 min after drug administration. Metabolic differences would have virtually no effect on the behavioral responses in this short time.

The neurochemical analyses carried out in the present study fail to provide a straightforward explanation for the strain differences in sensitivity to oxotremorine. Since no strain differences were detected in AChE and ChAT activities, it does not seem likely that differential synthesis or metabolism of acetylcholine is related to the strain differences in sensitivity to oxotremorine. No sex differences were observed within strains, but sex differences in AChE and ChAT activity were found when the data for all strains were combined. The magnitude of the sex differences were small and difficult to interpret in view of the lack of difference between the sexes in sensitivity to oxotremorine.

Although strain differences in QNB binding were detected in our studies, these findings also are difficult to interpret. C3H mice exhibited greater QNB binding (B_{max}) than did the C57BL or DBA strains in striatum. Such an observation would suggest that a greater number of receptors leads to lesser sensitivity to the effects of a muscarinic agonist. However, several recent studies have shown that chronic inhibition of AChE leads to a decrease in the number of muscarinic receptors [11, 13, 29]. Uchida *et al.* [29] noted that chronic inhibition of AChE resulted in a decrease in the number of muscarinic receptors in brain and ileum which was accompanied by tolerance to oxotremorine as assessed by a decrease in the maximal contractions of ileum and an increase in the ED_{50}. More recently, we [22] have observed that chronic oxotremorine infusion in C3H mice leads to a reduction in muscarinic receptors in a number of brain regions, including striatum. This reduction is preceded by tolerance development, i.e., tolerance developed before a detectable reduction in receptor number occurred. These observations are difficult to reconcile with the finding of a greater number of QNB binding sites in C3H striatum. We would have expected C3H mice, which are less sensitive (more tolerant) to the effects of oxotremorine on open-field activity, to have fewer muscarinic binding sites than mice of the DBA and C57BL strains. Our studies on acquired tolerance to chronic oxotremorine treatment demonstrated the development of considerable tolerance before decreases in the number of muscarinic receptors were detected. As a result of this observation, we suggested the possibility that acquired tolerance to oxotremorine results from changes in some other parameter related to receptor function such as receptor coupling. This possible explanation was suggested because Su *et al.* [27] have demonstrated that the beta adrenergic receptor is uncoupled from adenylate cyclase before beta adrenergic receptor number decreases are observed when astrocytoma cells are treated chronically with beta agonists. Perhaps the dramatic difference between naive C3H mice and naive DBA and C57BL animals with respect to initial sensitivity to oxotremorine is attributable to a genetically determined difference in the mechanism which is altered by chronic oxotremorine treatment, i.e., initial strain differences may be due to a factor which is more labile and underlies the initial stages of tolerance development.

Aronstam *et al.* [4] have reported that C57BL and DBA mice show an increase in the number of muscarinic receptors with age. Adult levels were achieved when the mice were between 20 and 40 days old. As was the case in our study, Aronstam *et al.* [4] detected a greater number of QNB binding sites in the hippocampus of DBA mice. These authors suggest that the greater number of muscarinic receptors may be related to the difference between C57BL and DBA mice in audiogenic seizure sensitivity.

No differences among strains were detected in the IC_{50} value for inhibition by oxotremorine of QNB binding. This observation argues that the strain differences in sensitivity to oxotremorine are not due to a differential affinity of muscarinic receptors for the drug. Birdsall *et al.* [6] have reported that brain muscarinic receptors may have high- and low-affinity forms that are differentiable on the basis of agonist binding. Oxotremorine is not the agent of choice for estimating high- and low-affinity forms (an agent with a larger difference in affinity for the two forms, such as carbamylcholine, would be better). However, inspection of the data obtained in the IC_{50} determination suggests that the three strains have similar ratios of high- to low-affinity receptor forms in the brain regions we examined.

The differences in IC_{50} values in the various brain regions may be related to regional differences in the relative numbers of the high- and low-affinity receptor forms. Aronstam *et al.* [4] noted that C57BL and DBA mice demonstrate similar regional variations in the ratio of these two receptor forms. These authors reported that over 60% of brainstem (hindbrain) and hypothalamic muscarinic receptors are of the high-affinity form, while only 30–40% of cortical, thalamic, and striatal receptors are of this type. Such differences could account for the regional variation in IC_{50} values for oxotremorine seen in our study. Further studies using more appropriate ligands are necessary to determine whether the strains do or do not have differing ratios of high- to low-affinity receptors.

Unlike the results obtained with oxotremorine, which suggested two different binding sites, the dose-response curves for nicotine inhibition of QNB binding fit a one-site model. The observation that competitive binding assays between QNB and muscarinic antagonists are not biphasic [17]

suggests that nicotine may be interacting with the muscarinic receptor in a fashion similar to the interactions shown by antagonists or by an agonist with equal affinity for both binding sites.

The present study demonstrates that the inbred strains of mice previously found to differ in sensitivity to nicotine also differ in a similar fashion to the muscarinic agonist, oxotremorine. Neurochemical analyses failed to provide a satisfactory explanation for this phenomenon. We hope that further studies will provide insight into the mechanisms underlying strain differences in sensitivity to cholinergic drugs.

ACKNOWLEDGEMENTS

We are grateful to Judith Ciero, Barbi Kirwin, and Rebecca Miles for their assistance in the preparation of this manuscript.

REFERENCES

1. Anisman, H. Dissociation of disinhibitory effects of scopolamine: Strain and task factors. *Pharmac. Biochem. Behav.* **3:** 613–618, 1975.
2. Anisman, H. Differential effects of scopolamine and D-amphetamine on avoidance: Strain interactions. *Pharmac. Biochem. Behav.* **3:** 809–817, 1975.
3. Anisman, H. Effects of scopolamine and D-amphetamine on one-way, shuttle and inhibitory avoidance: A diallel analysis in mice. *Pharmac. Biochem. Behav.* **3:** 1037–1042, 1975.
4. Aronstam, R. S., C. Kellogg and L. G. Abood. Development of muscarinic cholinergic receptors in inbred strains of mice: Identification of receptor heterogeneity and relation to audiogenic seizure susceptibility. *Brain Res.* **162:** 231–241, 1979.
5. Austin, L. and W. K. Berry. Two selective inhibitors of cholinesterase. *Biochem. J.* **54:** 695–700, 1953.
6. Birdsall, N. J. M., A. S. V. Burgen and E. C. Hulme. The binding of agonists to brain muscarinic receptors. *Molec. Pharmac.* **14:** 723–736, 1978.
7. Blume, A., F. Gilbert, S. Wilson, J. Farber, R. Rosenberg and M. Nirenberg. Regulation of acetylcholinesterase in neuroblastoma cells. *Proc. natn. Acad. Sci. U.S.A.* **67:** 786–792, 1970.
8. Carbonetto, S. T., D. M. Fambrough and K. J. Muller. Nonequivalence of α-bungarotoxin receptors and acetylcholine receptors in chick sympathetic neurons. *Proc. natn. Acad. Sci. U.S.A.* **75:** 1016–1020, 1978.
9. DeFries, J. C., J. P. Hegmann and M. W. Weir. Open-field behavior in mice: Evidence for a major gene effect mediated by the visual system. *Science* **154:** 1577–1579, 1966.
10. DeFries, J. C. and J. P. Hegmann. Genetic analysis of open-field behavior. In: *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype*, edited by G. Lindzey and D. D. Thiessen. New York: Appleton-Century-Crofts, 1970, pp. 23–56.
11. Ehlert, F. J., N. Kokka and A. S. Fairhurst. Altered [^{3}H]quinuclidinyl benzilate binding in the striatum of rats following chronic cholinesterase inhibition with diisoproplyfluorophosphate. *Molec. Pharmac.* **17:** 24–30, 1980.
12. Ellman, G. L., K. D. Courtney, V. Andres, Jr. and R. M. Featherstone. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmac.* **7:** 88–95, 1961.
13. Gazit, H., I. Silman and Y. Dudai. Administration of an organophosphate causes a decrease in muscarinic receptor levels in rat brain. *Brain Res.* **174:** 351–356, 1979.
14. Hatchell, P. C. and A. C. Collins. Influences of genotype and sex on behavioral tolerance to nicotine in mice. *Pharmac. Biochem. Behav.* **6:** 25–30, 1977.
15. Hatchell, P. C. and A. C. Collins. The influence of genotype and sex on behavioral sensitivity to nicotine in mice. *Psychopharmacology* **71:** 45–49, 1980.
16. Ho, I. K. and G. L. Ellman. Triton solubilized acetylcholinesterase of brain. *J. Neurochem.* **16:** 1505–1513, 1969.
17. Hulme, E. C., N. J. M. Birdsall, A. S. V. Burgen and P. Mehta. The binding of antagonists to brain muscarinic receptors. *Molec. Pharmac.* **14:** 737–750, 1978.
18. Kelly, P. H., P. W. Seviour and S. D. Iverson. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res.* **94:** 507–522, 1975.
19. Lee, H.-J. and I. B. Wilson. Enzymic parameters: Measurement of V and K_m. *Biochim. biophys. Acta* **242:** 519–522, 1971.
20. Lowry, O. H., N. H. Rosebrough, A. C. Farr and R. J. Randall. Protein measurement with Folin phenol reagent. *J. biol. Chem.* **193:** 265–275, 1951.
21. Lukasiewicz, R. J., M. R. Hanley and E. L. Bennett. Properties of radiolabelled α-bungarotoxin derivatives and their interaction with nicotinic acetylcholine receptors. *Biochemistry* **17:** 2308–2313, 1978.
22. Marks, M. J., L. D. Artman, D. M. Patinkin and A. C. Collins. Cholinergic adaptations to chronic oxotremorine infusion. *J. Pharmac. exp. Ther.*, in press.
23. McClearn, G. E. The genetics of mouse behavior in novel situations. *J. comp. physiol. Psychol.* **52:** 62–67, 1959.
24. McReynolds, W. E., M. W. Weir and J. C. DeFries. Open-field behavior in mice: Effect of test illumination. *Psychon. Sci.* **9:** 277–278, 1967.
25. Oliverio, A., B. E. Eleftheriou and D. W. Bailey. Exploratory activity: Genetic analyses of its modification by scopolamine and amphetamine. *Physiol. Behav.* **10:** 893–899, 1973.
26. Schrier, B. K. and L. Shuster. A simplified radiochemical assay for choline acetyltransferase. *J. Neurochem.* **14:** 977–985, 1967.
27. Su, Y. F., T. K. Harden and J. P. Perkins. Isoproterenol-induced desensitization of adenylate cyclase in human astrocytoma cells: Relation of loss of hormonal responsiveness and decrement in β-adrenergic receptors. *J. biol. Chem.* **254:** 38–41, 1979.
28. Thompson, W. R. The inheritance of behaviour: Behavioural differences in fifteen mouse strains. *Can. J. Physiol.* **7:** 145–155, 1953.
29. Uchida, S., K. Takeyasu, T. Matsuda and H. Yoshida. Changes in muscarinic acetylcholine receptors in mice by chronic administrations of diisopropylfluorophosphate and papaverine. *Life Sci.* **24:** 1805–1812, 1979.
30. Ungerstedt, V. and G. W. Arbuthnott. Quantitative recording of rotational behaviour in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system. *Brain Res.* **24:** 485–493, 1970.
31. Van Abeelen, J. H. F. and H. Strijbosch. Genotype-dependent effects of scopolamine and eserine on exploratory behavior in mice. *Psychopharmacologia* **16:** 81–88, 1969.
32. Van Abeelen, J. H. F., G. A. Ellenbrook and H. G. A. J. Wigman. Exploratory behaviour in two selectively-bred lines of mice after intrahippocampal injection of methylscopolamine. *Psychopharmacologia* **41:** 111–112, 1975.
33. Vanderwolf, C. H. Limbic-diencephalic mechanisms of voluntary movement. *Psychol. Rev.* **78:** 83–113, 1971.
34. Yamamura, H. I. and S. H. Snyder. Muscarinic cholinergic binding in rat brain. *Proc. natn. Acad. Sci. U.S.A.* **71:** 1725–1729, 1974.