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Motor Responses of Autoimmune NZB/BlNJ and C57BL/6Nnia Mice to Arecoline and Nicotine

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RETZ, K. C., C. K. TRIMMER, M. J. FORSTER AND H. LAL. *Motor responses of autoimmune NZB/BlNJ and C57BL/6Nnia mice to arecoline and nicotine.* PHARMACOL BIOCHEM BEHAV **28**(2) 275–282, 1987.—In 11–13 month C57BL/6Nnia mice, arecoline produced a dose-dependent decrease in motor activity at doses of 0.64–2.5 mg/kg, whereas at doses of 5.0–20.0 mg/kg arecoline produced a dose-dependent increase in motor activity. In marked contrast, age-matched NZB/BlNJ (New Zealand Black) mice failed to exhibit the first phase of the response, but showed a greater dose-dependent increase in motor activity following the doses of 10 and 20 mg/kg. Nicotine, 0.64–2.5 mg/kg, produced a dose-dependent decrease in motor activity in both strains. The effects of arecoline and nicotine were antagonized by scopolamine (2.5 mg/kg) and mecamylamine (1.0 mg/kg). respectively. These findings suggest that muscarinic neurotransmission may be altered in NZB/BlNJ mice, which produce brain-reactive autoantibodies, exhibit learning/memory dysfunctions, and also exhibit a loss of neurons staining positive for choline acetyltransferase.

Autoimmunity NZB/BlNJ mice C57BL/6Nnia mice Motor activity Arecoline Scopolamine Nicotine Mecamylamine

RECENTLY, studies in our laboratories have suggested a correlation between the appearance of brain-reactive antibodies "BRA" and deficits in learning in normally aging outbred mice [27,28] and NZB/BlNJ (New Zealand Black) mice, a genetically autoimmune-prone strain known to develop serum brain-reactive antibodies at much earlier chronological ages than nonautoimmune outbred or inbred strains [18, 27, 28, 40]. The formation of brain-reactive antibodies is a concomitant of normal aging in several species, including humans, primates, rats and mice [6, 11, 12, 25, 36–38, 40, 50]. Moreover, a higher occurrence of brain-reactive antibodies is also found in patients with Alzheimer's disease and other states involving neuropsychopathology [1, 2, 5, 37, 47]. Therefore, it is possible that immune mechanisms may have a role in these and other ageing associated CNS dysfunctions [14, 27, 28].

One such age-dependent dysfunction that rodents exhibit is altered motor responses to cholinomimetics [15, 26, 29]. Because the biphasic effects of muscarinic agonists on locomotor performance in rodents are well-characterized [13, 20, 24, 34, 46], this behavior can be used for pharmacological studies of the intactness of the muscarinic system in rodents. As a first test of the hypothesis that NZB/BlNJ mice have altered cholinergic neurotransmission, we have studied the effects of administration of either oxotremorine, a muscarinic agonist, or physostigmine, a cholinesterase inhibitor, to 8–10 month NZB/BlNJ and C57BL/6J mice [43]. In C57BL/6J mice, both drugs produced a characteristic biphasic response [43]. The first phase occurs at lower doses and consists of a dose-dependent decrease in motor activity. In the second phase occurring at higher doses, a dose-dependent increase in motor activity accompanied by tremors is observed. By contrast, age-matched NZB/BlNJ mice exhibited little, if any, decrease in motor activity at the lower doses, but had much greater increases in activity following the higher doses.

In addition to exhibiting altered locomotor effects after administration of cholinomimetics, NZB/BlNJ mice also exhibit deficits in learning and memory abilities [27,28]. Already there is much evidence to indicate that learning and memory processes in rodents are: (a) dependent on intact cholinergic innervation; (b) disrupted by muscarinic antagonists; and (c) facilitated by muscarinic agonists [3, 4, 10, 31, 45]. Taken together, our findings are consistent with the hypothesis that, as a consequence of rapidly developing aged-like dysfunctions, NZB/BlNJ mice also exhibit a learning/memory deficit that is accompanied by cholinergic dysfunction. If this were so, then one would predict that the cholinergic alteration in NZB/BlNJ mice is due more to changes in muscarinic neurotransmission, and less to changes in nicotinic neurotransmission. In the present study, we have sought to pharmacologically investigate the impor-

tance of muscarinic- vs. nicotinic-mediated components of the altered motor response to cholinomimetics exhibited by NZB/BINJ mice.

METHOD

Male C57BL/6Nnia mice were obtained from the NIA at 3 months of age and housed in the College vivarium until use at 11–13 months. NZB/BINJ mice were obtained from the Jackson Laboratories (Bar Harbor, ME) at 3 months of age and housed in a similar manner. The colony room was maintained at 23±1°C under a normal 12 hour light-dark cycle from 0800 through 1959 hours. The mice had unlimited access to food and water.

All drugs were dissolved in normal saline solution and administered by IP injection (10 ml/kg) in doses indicated in the text. Arecoline hydrobromide, mecamylamine hydrochloride, nicotine bitartrate, and scopolamine hydrobromide (Sigma Chemical Co., St. Louis, MO) were administered as their respective salt forms with doses calculated and expressed as the amount of drug present in its pharmacologically active ("free" base) molecular species.

Motor activity was assessed in four, clear, polyacrylate chambers (27 cm L × 19 cm W × 16 cm H, i.d.) with polyacrylate bases and tops, each located above one sensor of an Electronic Activity Monitor "EAM" (Stoelting Co., Chicago, IL). Each monitor was placed in a mechanically ventilated, partially sound-attenuated chamber (46 cm L × 34 cm W × 33 cm H, i.d.) which had a polyacrylate window (0.5 cm thick, 12 cm W × 12 cm H) on the door to serve as a low intensity light source and to permit observation of the animal while inside the chamber. The chambers were placed in a room illuminated by fluorescent lamps under normal laboratory intensity. The apparatus as employed measured activity as numbers of movements above a 3 Hz frequency cutoff, detected through disruption of a radio frequency capacitance field by the mouse. The activity ≥3 Hz on each of the two channels was monitored over 5-min intervals for a total time of 30 min using a sensitivity of 15% full scale on both channels. This sensitivity setting resulted in zero background activity counts when no animals were on the activity monitors. During the observation period, only one experimenter/observer was present in the room. The presence or absence of tremors occurring during the total 30 min session was noted and recorded. When tremors were present, the time of their onset and cessation was noted and recorded to the nearest minute.

Two separate groups of each strain (N=10 in each) were used for: (a) studies with arecoline and its antagonism by scopolamine; and (b) studies with nicotine and its antagonism by mecamylamine. Each drug treatment was given on 2 days in a counterbalanced design such that for each dose level of drug administered, each animal received a "paired" saline session. For example, half of the mice in each group received saline vehicle and half received drug on a single day. In a subsequent session 3–4 days later, this order of presentation was reversed to complete one dose level. One week elapsed between administrations of drug (in each dose level) to each animal.

In antagonism studies, scopolamine or mecamylamine were injected 10 min prior to a saline, arecoline, or nicotine injection, at which time the mice were placed in the monitoring chambers for a 30 min observation period as previously described. The order of dose presentation for arecoline was 5.0, 2.5, 1.25, 10, 20, and 0.64 mg/kg. These treatments were followed by the treatments with scopolamine (2.5 mg/kg) alone, scopolamine + arecoline-5.0 mg/kg, scopolamine + arecoline-20 mg/kg. In the group of mice receiving nicotine, the doses of nicotine were presented in ascending order followed by mecamylamine (1.0 mg/kg), and mecamylamine + nicotine-2.5 mg/kg. In order to control for any slight sensitivity differences between monitors, each animal was observed in the same chamber during vehicle and drug session pairs for a given drug and dosage.

Statistical analyses of the parametric data were made using a multifactor ANOVA and individual comparisons as appropriate for each analysis [51].

RESULTS

Arecoline Dose Effect

Basal activity. Previous studies using this apparatus and a similar experimental design suggested that activity of mice declined within individual saline sessions, but that there was little change in total activity as a function of repeated saline sessions throughout the course of experimentation [43]. In the current study, the validity of using dose group as a repeated measure was more critically examined. In the C57BL/6Nnia mice (N=10), the total mean activity during the saline sessions of each of the six counterbalanced saline-arecoline presentations ranged from 2,307±337 to 3,000±346 counts for the entire 30 minute observation period. Activity counts during the first 5 minute observation period ranged from 506±52 to 667±46 counts and declined to a range of 255±57 to 441±62 counts during the sixth 5 minute observation period. Visual inspection of these data suggested that neither repeated test exposure nor intervening drug sessions altered basal activity.

A similar lack of effect of presentation order was observed in the NZB/BINJ mice (N=10), where the total mean activity during the saline sessions of each of the six counterbalanced saline-arecoline presentations ranged from 1,300±113 to 1,707±174 counts for the entire 30 minute observation period. Activity counts during the first 5 minute period ranged from 232±46 to 416±37 counts and declined to a range of 109±23 to 297±40 counts during the sixth 5 minute period. Although the absolute number of activity counts was less in NZB/BINJ mice, again visual inspection of these data suggested that neither repeated test exposure nor intervening drug sessions altered basal activity.

A 2×6×6 analysis of variance on 3 Hz activity during saline sessions (with Strain, Session, and 5-min periods as factors) supported these observations, yielding neither a significant main effect of Session, $F(5,90)$, $p<1.7$, nor interactions of Session with Strain or 5-min periods (all higher order interactions were nonsignificant, $Fs<2.1$, $ps>0.05$). A significant main effect of Strain, $F(1,18)=23.1$, $p<0.001$, reflected the overall greater activity of C57BL/6Nnia mice, while a significant effect of 5-min periods, $F(5,90)=49.1$, $p<0.001$, reflected the overall decline in activity as a function of 5-min periods.

Time course of effects on motor activity. The major trends in activity under drug are shown in Table 1 as a function of dose, strain, and sampling periods. To facilitate interpretation, activity under drug is expressed as percent of the mean control response during the appropriate period of the saline session corresponding to each dose. Examination of the time-courses for both NZB/BINJ and C57BL/6Nnia mice suggested that peak drug response occurred during the first 5 minutes of testing. For C57BL/6Nnia mice, the expected

TABLE 1

MOTOR ACTIVITY (% CONTROL) OF ARECOLINE-TREATED C57BL/6NniA AND NZB/BINJ MICE*

AREC dose (mg/kg)	% Control Activity/5-Min Period 0–5	5–10	10–15	15–20	20–25	25–30	Total
			C57BL/6Nnia Mice				
0.64	76	90	89	67	89	91	83
1.25	37	104	83	118	104	105	85
2.50	22	43	73	78	74	87	60
5.0	48	13	42	52	60	86	46
10.0	84	50	28	74	60	52	59
20.0	149	106	43	59	46	33	83
			NZB/BINJ Mice				
0.64	109	99	71	73	95	98	92
1.25	108	110	123	89	128	116	112
2.5	126	84	127	72	88	158	103
5.0	134	86	84	59	80	57	91
10.0	225	76	84	88	68	76	117
20.0	283	240	70	58	68	39	144

*The data from each arecoline (AREC) drug treatment session are expressed as percentage of the mean control activity (≥3 Hz) from the corresponding interval of the matching saline session; N=10. Statistical analyses of the data are described in the text.

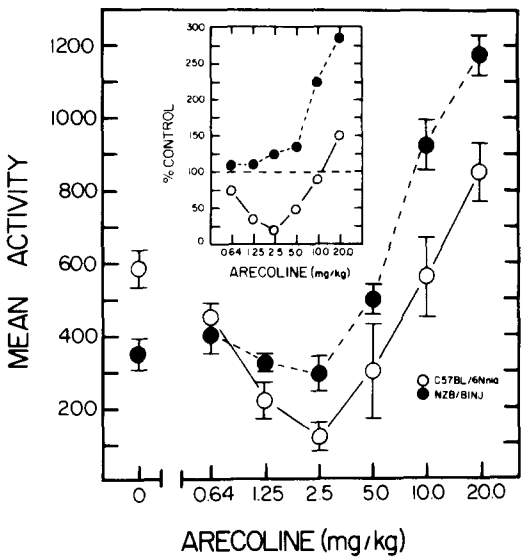

FIG. 1. Motor activity (≥3 Hz) of 10–12 month. C57BL/6Nnia and NZB/BINJ mice during the 0–5 min interval following administration of arecoline. Mice were administered arecoline HBr immediately prior to placement into the activity monitors. The doses represent the amount of the pharmacologically active molecular species ("free" base) of arecoline. The plotted values for saline (i.e., 0 mg/kg) represent the averages of the mean and SE for each of the 6 saline sessions. The plotted values for arecoline represent the mean (±SE) of 10 mice. The inset shows the data expressed as mean percent of the corresponding saline session. Statistical analyses of the data are presented in the text.

U-shaped dose effect relationship was most pronounced during this period. A phase in which activity decreased as a function of dose occurred within the 0.64–2.5 mg/kg dose range, whereas a different phase, in which activity increased as a function of dose, occurred between 5.0 and 20.0 mg/kg (only the 20 mg/kg dose resulted in activity above baseline). This same trend was evident during the 5–10 min time period, although the activity-increasing and activity-decreasing phases were of lesser magnitude. NZB/BINJ mice failed to show substantial activity decrements at any time point or dose of arecoline, although activity increased as a function of dose within the 5.0 to 20 mg/kg range within the first 5-min of testing.

Arecoline-induced tremors. Tremors were produced by the 10 and 20 mg/kg doses in C57BL/6Nnia mice, occurring in 5/10 and 9/10 mice, for each dose respectively. Tremors were also produced by these same two doses in NZB/BINJ mice, occurring in 8/10 and 10/10 mice, for each dose respectively. During the tremors, the animals continued locomotion within the horizontal plane of the observational chambers. The tremors had a rapid onset, beginning by 3–4 min post-injection, and short duration which was completed by 6–7 minutes. This time of occurrence was coincident with the most rapid accumulation of activity counts during the 0–5 min and 5–10 min observation periods (Table 1).

Strain differences. In view of the clear peak effect at 0–5 min in both strains, activity from only the 0–5 min time interval was considered in comparisons of dose-effect relationships between strains (Fig. 1). An analysis of variance (with Strain, Dose, and Treatment (drug vs. saline) as factors) conducted on the dose-effect data revealed a significant Strain × Treatment interaction, $F(1,18)=55.1$, $p<0.001$, and a 3-way interaction of Strain, Dose, and Treatment, $F(5,90)=2.5$, $p<0.05$. These interactions reflected the fact

TABLE 2

EFFECTS OF SCOPOLAMINE PREADMINISTRATION ON MOTOR ACTIVITY CHANGES INDUCED BY ARECOLINE*

Dose (mg/kg)		C57BL/6Nnia Mice			NZB/BINJ Mice		
SCOP	AREC	Saline	Drug	%C	Saline	Drug	%C
0	5.0	629 ± 22	299 ± 133	48	374 ± 52	501 ± 39	134
0	20.0	569 ± 66	845 ± 87	149	416 ± 37	1,175 ± 174	283
2.5	0	475 ± 75	818 ± 45	172	304 ± 48	522 ± 50	172
2.5	5.0	555 ± 66	763 ± 41	137	331 ± 47	530 ± 59	160
2.5	20.0	526 ± 73	564 ± 69	107	287 ± 49	569 ± 54	198

*The activity data (≥3 Hz) obtained during the 0–5 min interval following administration of saline and of [scopolamine (SCOP) and/or arecoline (AREC)] in each of the saline/drug pairs are expressed as mean ± SE, and as the percentage (%C) of the mean from the matching saline session; N=10. Statistical analyses of the data are described in the text.

TABLE 3

MOTOR ACTIVITY (% CONTROL) OF NICOTINE-TREATED C57BL/6Nnia AND NZB/BINJ MICE*

NIC Dose (mg/kg)	% Control Activity/5-Min Period						
	0–5	5–10	10–15	15–20	20–25	25–30	Total
	C57BL/6Nnia Mice						
0.16	88	108	111	89	107	100	100
0.32	103	136	113	112	88	97	106
0.64	56	68	71	87	74	83	72
1.25	58	36	62	114	96	140	77
2.50	50	3	6	17	26	42	24
	NZB/BINJ Mice						
0.16	122	117	107	90	114	95	106
0.32	142	149	103	214	112	172	139
0.64	86	82	109	101	88	71	90
1.25	107	45	79	115	93	92	86
2.50	48	1	13	33	79	79	42

*The data from each nicotine (NIC) drug treatment session are expressed as percentage of the mean control activity (≥3 Hz) from the corresponding interval of the matching saline session; N=10). Statistical analyses of the data are described in the text.

that NZB/BINJ mice failed to exhibit activity decreases following low doses (0.64–2.5 mg/kg) of arecoline and showed greater activity following 5.0–20.0 mg/kg, when compared to the C57BL/6Nnia mice. Individual comparisons [51] within the Strain × Dose × Treatment interaction verified that the activity of NZB/BINJ mice during drug sessions with 0.64, 1.25, 2.5 and 5.0 mg/kg arecoline failed to differ from corresponding saline sessions (all Fs<3.12). In contrast, C57BL/6Nnia mice showed a significant activity decrements after each of these doses [smallest $F(1,90)=4.1$, $p<0.05$]. Activity during drug sessions was higher than during the corresponding saline session for C57BL/6Nnia mice following 20.0 mg/kg, $F(1,90)=14.8$, $p<0.001$, and for NZB/BINJ mice following 10 and 20 mg/kg (Fs>51.4, ps<0.001).

The significant differences between the strains in dose-effect were more obvious when activity during drug sessions was expressed as a precentage of the mean of each corresponding saline session, as shown in the inset to Fig. 1. A 2-way analysis of variance conducted on the percent control data (subjected to a 2 arcsin $X^{0.5}$ transformation) indicated a significant Strain × Dose interaction, $F(5,90)=3.8$, $p<0.005$, further confirming the evident strain differences in dose-effect.

Antagonism. Although arecoline is traditionally considered to be a muscarinic agonist, the drug also has some nicotinic actions at high doses [17,39]. To ascertain the relative importance of muscarinic activation in the activity responses following arecoline, antagonism studies with scopolamine were attempted. The effects of scopolamine pre-treatment (2.5 mg/kg) upon subsequent activity response to 5.0 and 20.0 mg/kg arecoline are shown in Table 2. Because scopolamine alone increased motor activity of both strains by approximately 70%, antagonism by this single dose of scopolamine can only be estimated. For example, a complete antagonism could only be demonstrated given stoichiometric amounts of each drug at the receptor sites and a return of the motor activity value to control. A less than complete antagonism would be indicated if the motor activity increasing effects of arecoline yielded a value intermediate between that of control and either scopolamine alone or arecoline alone.

The prediction that arecoline's effects were due to muscarinic stimulation was confirmed with a dose of 5 mg/kg, an activity-decreasing dose in C57BL/6Nnia mice, and a dose of 20 mg/kg, an activity-increasing dose in both C57BL/6Nnia and NZB/BINJ mice. The effect of 5 mg/kg arecoline was clearly reversed when given in combination with 2.5 mg/kg scopolamine in C57BL/6Nnia mice (Table 2). To verify this effect, individual comparisons were conducted within the treatment effect, $F(2,18)=25.1$, $p<0.001$, of a 1-way repeated measures analysis of variance on 2 arcsin $X^{0.5}$-transformed percent control data (with arecoline alone, scopolamine alone, and the combination as treatments). The effects of scopolamine alone and the scopolamine-5 mg/kg arecoline combination failed to differ, $F(1,18)<1.8$, whereas each differed significantly from the effect of arecoline alone (Fs>28.7, ps<0.001). There was no significant effect of 5 mg/kg arecoline in NZB/BINJ mice, and the effect of the

TABLE 4

EFFECTS OF MECAMYLAMINE PREADMINISTRATION ON MOTOR ACTIVITY CHANGES INDUCED BY NICOTINE*

Dose (mg/kg)		C57BL/6Nnia Mice			NZB/BINJ Mice		
MEC	NIC	Saline	Drug	%C	Saline	Drug	%C
0	2.5	511 ± 49	14 ± 6	3	238 ± 49	2 ± 1	1
1.0	0	583 ± 69	589 ± 60	101	240 ± 36	219 ± 44	91
1.0	2.5	478 ± 67	391 ± 92	82	203 ± 51	167 ± 55	82

*The activity data (≥3 Hz) obtained during the 0–5 min interval following administration of saline and of mecamylamine (MEC) and/or nicotine (NIC) in each of the saline/drug pairs are expressed as mean ± SE, and as the percentage (%C) of the mean from the matching control saline session; N=10. Statistical analyses of the data are described in the text.

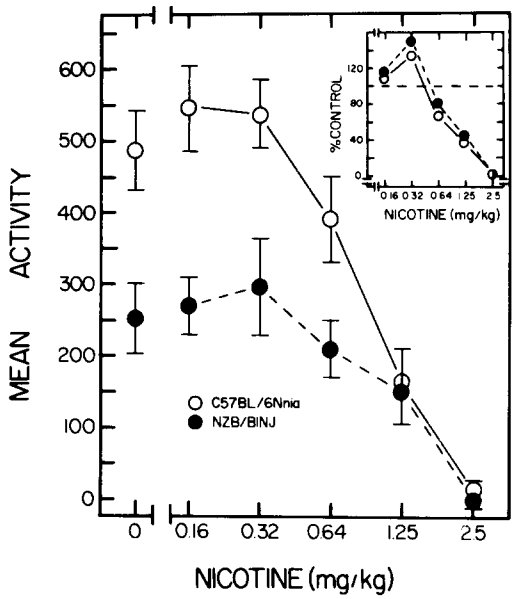

FIG. 2. Motor activity (≥3 Hz) of 10–12 month C57BL/6Nnia and NZB/BINJ mice during the 5–10 min interval following administration of nicotine. Mice were administered nicotine bitartrate immediately prior to placement into the activity monitors. The doses represent the amount of the pharmacologically active molecular species ("free" base) of nicotine. The plotted values for saline (i.e., 0 mg/kg) represent the averages of the mean and SE for each of the 7 saline sessions. The plotted values for arecoline represent the mean (±SE) of 10 mice. The inset shows the data expressed as mean percent of the corresponding saline session. Statistical analyses of the data are presented in the text.

scopolamine-arecoline combination in this strain differed little from the effect of scopolamine alone.

The effect of scopolamine pre-treatment on activity response to 20 mg/kg arecoline was considered in a separate analysis of variance on transformed percent control, with Strain and Treatment as the factors. This analysis indicated significant main effects of both Strain and Treatment (Fs>11.7, $ps<0.001$), as well as a Strain × Treatment interaction, $F(2,36)=13.7$, $p<0.001$. The greater absolute magnitude of the difference between arecoline alone and arecoline in combination with scopolamine in NZB/BINJ mice, when compared to C57BL/6Nnia mice, contributed to this interaction. However, relative to the response to arecoline alone within each strain, the antagonism was marked in both NZB/BINJ and C57BL/6Nnia mice. Individual comparisons within the Strain × Treatment interaction verified that the scopolamine-arecoline combination yielded less activity when compared to arecoline alone, for each strain, Fs(1,36)>6.8, $ps<0.025$.

Nicotine Dose-Effect

Basal activity. A separate group of mice of each strain was used in studies with nicotine. As with the arecoline studies, the validity of using dose group as a repeated measure was critically examined. In the C57BL/6Nnia mice (N=10), the total mean activity during the saline sessions of each of the six counterbalanced saline-nicotine presentations ranged from 2,437±363 to 3,191±372 counts for the entire 30 minute observation period. Activity counts during the first 5 minute observation period ranged from 519±84 to 700±66 counts and declined to a range of 287±62 to 432±77 counts during the sixth 5 minute observation period. Visual inspection of these data suggested that neither repeated test exposure nor intervening drug sessions altered basal activity.

A similar lack of effect of presentation order was observed in the NZB/BINJ mice (N=10), where the total mean activity during the saline session of each of the six counterbalanced saline-nicotine presentations ranged from 1,187±172 to 1,795±307 counts for the entire 30 minute observation period. Activity counts during the first 5 minute period ranged from 242±52 to 423±82 counts and declined to a range of 131±39 to 236±61 counts during the sixth 5 minute period. Although the absolute number of activity counts was less in NZB/BINJ mice, again visual inspection of these data suggested that neither repeated test exposure nor intervening drug sessions altered basal activity.

Upon comparison, it is self-evident that the absolute activity of the mice used in the studies with nicotine was nearly equal to that of the mice used in the studies with arecoline. To test for changes in activity during saline sessions as a function of repeated testing, activity counts for these sessions were subjected to an anlysis of variance in the same format as for the arecoline studies. As in the arecoline studies, there was little change in total activity as a function of repeated saline sessions. There was a complete lack of significant main and interaction effects involving the Session factor (all Fs<2.2). The analysis indicated significant main

effects of strain, $F(1,18)=17.1$, $p<0.001$, and 5-minute periods, $F(5,90)=20.0$, $p<0.001$.

Time course. The major trends in activity under nicotine are shown in Table 3 as percent of control as a function of dose, strain, and sampling periods. Examination of the time-course for both NZB/BlNJ and C57BL/6Nnia mice suggested that peak drug response occured during the 5–10 min time period, with values returning to baseline over the 30-min session at most doses.

Strain effects. In view of the peak effects at the 5–10 min period, analyses of strain differences in nicotine dose-effect were conducted using this time sample. Analyses of variance were conducted as outlined for the arecoline experiment. Nicotine dose-effect curves for each strain for the 5–10 min observation period are shown in Fig. 2. Inspection of the data suggested monophasic reductions in activity as a function of dose within each strain, which were reflected in significant main effects of Dose, $F(4,72)=8.8$, $p<0.001$, Treatment, $F(1,18)=15.9$, $p<0.001$, and a Dose × Treatment interaction, $F(4,72)=26.9$, $p<0.001$. The overall differences in magnitude of activity in C57BL/6Nnia and NZB/BlNJ mice during saline and drug sessions resulted in a significant main effect of Strain, $F(1,18)=44.5$, $p<0.001$.

Relative to baseline activity, the dose-dependent reductions in activity within each strain appeared to be equivalent. Nevertheless, the magnitude of strain differences in activity during drug sessions became smaller as a function of dose, leading to significant Strain × Drug, $F(4,72)=2.6$, $p<0.05$, and Strain × Dose × Treatment, $F(4,72)=2.5$, $p<0.05$, interactions. When activity data were considered as percent control (to correct for differences in baseline activity), a 2×6 analysis of variance indicated only a main effect of Dose, $F(4,18)=47.4$, $p<0.001$, with Strain and Dose × Strain effects being nonsignificant ($Fs<1.0$). The dose effect curves for each strain are shown as percent control in the inset to Fig. 1.

Antagonism. To examine the specificity of the motor activity depressing effects of nicotine, antagonism with mecamylamine was attempted. In both strains, mecamylamine (1.0 mg/kg) had no obvious effect upon motor activity (Table 4), but conferred appreciable reduction in the effects of the 2.5 mg/kg nicotine dose. An analysis of variance on percent control (with Strain and Treatment, 2.5 mg/kg nicotine, 1.0 mg/kg mecamylamine, or the combination) verified equivalent antagonism within each strain. The analysis indicated a significant effect of Treatment, $F(2,36)=44.8$, $p<0.001$, but no effects of Strain or Strain × Treatment ($Fs<1.0$).

DISCUSSION

In the C57BL/6Nnia mice, low doses of arecoline (0.64–2.50 mg/kg) produced a reduction in locomotor activity similar to that previously observed by other investigators [42]. This effect was also observed following low doses of other cholinomimetics [13, 24, 32, 43]. By contrast, the higher doses (10.0–20.0 mg/kg) produced stimulation of locomotor activity accompanied by tremor, findings also in agreement with investigations of other cholinomimetics [20, 42, 45]. The current observations are consistent with the possibility that tremors could have accounted for much of the dose-related increases in activity observed within the 0–5 min observation interval following the 10 and 20 mg/kg doses of arecoline. The NZB/BlNJ mice differed from C57BL/6Nnia mice in being sensitive to the motor activity increasing effects of the higher doses of arecoline, while lacking the motor activity decreasing effects of the lower doses. This latter finding is in agreement with our previous investigations using oxotremorine, a muscarinic agonist, and physostigmine, an acetylcholinesterase inhibitor [43]. The studies with scopolamine suggest that the effects of arecoline, at least at the 20 mg/kg tremorogenic dose, are due to interaction with muscarinic receptors. Other investigators have observed that the motor effects of 2.0 mg/kg arecoline [42] and of 20 mg/kg arecoline [21] are antagonized by scopolamine.

In contrast to the strain differences obtained in response to arecoline, nicotine produced similar effects in both strains, with a reduction of motor activity occurring following the 0.64–2.50 mg/kg doses. The maximal reduction in motor activity following the 2.5 mg/kg dose was antagonized by mecamylamine, 1.0 mg/kg. Although high doses of nicotine, e.g., 30 mg/kg, are known to produce tremors [21], other workers have noted that lower doses of nicotine (0.4–1.0) depress motor activity [7,48] and that this depressive effect is antagonized by mecamylamine, but not by atropine [48].

When comparing the differences in response of NZB/BlNJ and C57BL/6Nnia mice to arecoline, one cannot fail to note the differences in basal levels of activity in these two mice. As we have previously reported [43] and now confirm, the basal activity rate of the NZB/BlNJ mice is approximately 50–60% of that of C57BL/6Nnia mice. We believe that this difference is not alone sufficient to account for the difference in response to low doses of arecoline, oxotremorine, and physostigmine. First, the present studies show that nicotine will lower the activity in both strains. Second, both strains exhibited an increase in motor activity after higher doses of arecoline, oxotremorine [43] and physostigmine [43]. Third, both strains were alike in that neither showed any evidence of habituation to the test apparatus when saline data were examined.

It is apparent that NZB/BlNJ mice lack the motor depressive component of the response to arecoline, oxotremorine and physostigmine, cholinomimetics which produce direct and indirect stimulation of muscarinic receptors. Aged C57BL/6J mice have been reported to be less sensitive to the cataleptic effects of the cholinergic agonist, pilocarpine [15,26], and to exhibit altered responses to other muscarinic agonists and antagonists [29]. Although catalepsy was not measured in the current studies, our findings suggest that the NZB/BlNJ mice may respond to lower levels of muscarinic stimulation in a manner that is possibly more like that of older C57BL/6 mice. It is also apparent that, with higher doses, the response of NZB/BlNJ mice to the motor activity increasing doses of arecoline, as well as to both oxotremorine and physostigmine [43] is equal to or greater than that of C57BL/6 mice. One interpretation of these findings is that different muscarinic processes may be mediating motor activity in each phase of the total dose-response to cholinomimetics.

When considering the effects of arecoline, oxotremorine and physostigmine on locomotor activity, it is tempting to speculate on the cellular events which may mediate these processes. Studies of the interaction of antagonists with muscarinic receptors have revealed the existance of two distinct receptor populations in both rats [8, 16, 23, 33, 41] and humans [9,33]. One class, designated M_1, is distinguished by the high specific affinity of binding of the antagonist pirenzepine and is located in the striatum, hippocampus and cortex of rats [8, 23, 41] and in the forebrain basal ganglia and

limbic system areas of humans [9]. By contrast, those receptors designated as M_2 are characterized by a high specific affinity binding of gallamine, a low specific affinity binding of pirenzepine and are found in the brainstem and thalamus of rats [8, 23, 41] and in the caudal brainstem areas of humans [9]. It has been suggested that M_1 receptors are postsynaptic [23,41] in contrast to M_2 receptors which may be presynaptic and postsynaptic and play a role in neuromodulation of the postsynaptic receptors [23,41]. Radioligand binding studies in the rat demonstrate the regional binding of the agonists carbachol, oxotremorine, and 2-ethyl-8-methyl-2,8-diazaspiro-[4,5]-decan-1,3-dion-hydrobromide (RS86) includes those areas containing M_1 receptors as well as M_2 receptors [8], making it premature to assign different roles to muscarinic agonists based solely upon their ability to bind to M_1 or M_2 sites.

Better evidence in support of a functional difference between muscarinic agonists comes from studies of acetylcholine release [35] and turnover of inositol phospholipids [16, 17, 30, 31]. For example, in rat cortical synaptosomes, carbachol, acetylcholine, methacholine, oxotremorine and bethanechol all inhibit basal rates of [^{3}H]acetylcholine release, an effect which can be blocked by atropine, scopolamine and gallamine, but not by pirenzepine. In this system, the muscarinic agonist pilocarpine had no effect. These studies suggest that the former four agonists mentioned may be modulating release of endogenous acetylcholine via interaction with M_2 receptors, and that pilocarpine is not active at those sites. With regard to phosphoinositide turnover, it is known that class A agonists including acetylcholine, carbachol, methacholine, and muscarine will stimulate turnover, whereas class B agonists including bethanechol, pilocarpine, arecoline and oxotremorine are much less effective [16, 17, 30, 31].

Based upon the evidence that is available, it is very probable that the molecular mechanisms underlying the abnormal motor responses to oxotremorine, physostigmine and arecoline in NZB/BINJ mice are complex, involving changes in both M_1 and M_2 receptors. By contrast, the role of phosphoinositide turnover is less easily predicted. For example, the responses to oxotremorine and arecoline, class B agonists, were qualitatively similar to those elicited by physostigmine, implying that phosphoinositide turnover may not be required for the behaviors under study. Further experimentation will be needed to address this question in a definitive manner.

Whether autoimmune processes in NZB/BINJ mice are causally related to or merely concomitant with strain differences in response to muscarinic agents will require a multifactor design to establish the ages of onset and maximal demonstration of this phenomenon as well as the immune status of the animals at the ages of difference. However, it should be noted that our previous studies have revealed that NZB/BINJ mice have an accelerated age-dependent difference in sensitivity to diazepam [19]. Independent of whether immune processes cause changes in sensitivity to muscarinic agents in NZB/BINJ mice, it is clear that these mice have an altered response to muscarinic agents. One possible source of this alteration lies in the finding that NZB/BINJ mice have lower densities of cholinergic cells in several basal forebrain regions [52]. While this finding would support the hypothesis that aging of muscarinic pathways is accelerated in the CNS of NZB/BINJ mice, other workers have reported that old C57BL/6 mice have no loss of cholinergic neurons in the basal forebrain [22].

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REFERENCES

1. Bahmanyar, S., M.-C. Moreau-Dubois, P. Brown, F. Cathala and D. C. Gajdusek. Serum antibodies to neurofilament antigens in patients with neurological and other diseases and in healthy controls. *J Neuroimmunol* **5:** 191–196, 1983.
2. Baldinger, A. and H. T. Blumenthal. Neuroimmunology of the aging brain. In: *Geriatrics*, edited by D. Platt. Berlin: Springer Verlag, 1982, pp. 283–299.
3. Bartus, R. T., R. L. Dean, III, B. Beer and A. S. Lippa. The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217:** 408–417, 1982.
4. Bartus, R. T., R. L. Dean, M. J. Pontecorvo and C. Flicker. The cholinergic hypothesis: A historical overview, current perspective, and future directions. *Ann NY Acad Sci* **444:** 332–358, 1985.
5. Bergsma, D. and A. L. Goldstein. *Neurochemical and Immunologic Components in Schizophrenia*. New York: Alan R. Liss, 1978.
6. Blumenthal, H. T., D. Young, D. Wozniak and S. Finger. Effects of age, brain damage and stress on antibody binding to the brain. *J Gerontol* **39:** 552–560, 1984.
7. Clarke, P. B. S. and R. Kumar. The effects of nicotine on locomotor activity in nontolerant and tolerant rats. *Br J Pharmacol* **78:** 329–337, 1983.
8. Cortes, R. and J. M. Palacios. Muscarinic cholinergic receptor subtypes in the rat brain. I. Quantitative autoradiographic studies. *Brain Res* **362:** 227–238, 1986.
9. Cortes, R., A. Probst, H.-J. Tobler and J. M. Palacios. Muscarinic cholinergic receptor subtypes in the human brain. *Brain Res* **362:** 239–253, 1986.
10. Coyle, J. T., D. L. Price and M. R. DeLong. Alzheimer's disease: A disorder of cortical cholinergic innervation. *Science* **219:** 1184–1190, 1983.
11. Elizan, T. S., J. Casals and M. D. Yahr. Antineurofilament antibodies in postencephalitic and idiopathic Parkinson's disease. *J Neurol Sci* **59:** 341–347, 1983.
12. Feden, G., A. Baldinger, D. Miller-Soule and H. T. Blumenthal. An *in vivo* and *in vitro* study of an aging-related neuron cytoplasmic binding antibody in male Fischer rats. *J Gerontol* **34:** 651–660, 1979.
13. Fibiger, H. C., G. S. Lynch and H. P. Cooper. A biphasic action of central cholinergic stimulation on behavioral arousal in the rat. *Psychopharmacologia* **20:** 366–382, 1971.
14. Fillit, H., V. N. Luine, B. Reisberg, R. Amador, B. McEwen and J. B. Zabriskie. Studies of the specificity of antibrain antibodies in Alzeheimer's disease. In: *Senile Dementia of the Alzheimer Type*, edited by J. T. Hutton and A. D. Kenny. New York: Alan R. Liss, 1985, pp. 307–318.
15. Finch, C. E., J. F. Marshall and P. K. Randall. Aging and basal ganglia function. *Annu Rev Gerontol Geriatr* **2:** 49–90, 1981.
16. Fisher, S. K., J. C. Figueiredo and R. T. Bartus. Differential stimulation of inositol phospholipid turnover in brain by analogs of oxotremorine. *J Neurochem* **43:** 1171–1179, 1984.
17. Fisher, S. K., P. D. Klinger and B. W. Agranoff. Muscarinic agonist binding and phospholipid turnover in brain. *J Biol Chem* **258:** 7358–7363, 1983.
18. Forster, M. J., K. C. Retz, M. D. Popper and H. Lal. Age-related deterioration of learning ability is accelerated in autoimmune mouse strains. *Soc Neurosci Abstr* **11:** 722, 1985.

19. Forster, M. J., K. C. Retz, M. D. Popper and H. Lal. Age-dependent enhancement of diazepam sensitivity is accelerated in New Zealand Black mice. *Life Sci* **38:** 1433–1439, 1986.
20. Gonzalez, L. P. Quantitative analysis of physostigmine-induced changes in behavior. *Pharmacol Biochem Behav* **21:** 551–554, 1984.
21. Holmstedt, B. and G. Lundgren. Arecoline, nicotine and related compounds. Tremorogenic activity and effect upon brain acetylcholine. *Ann NY Acad Sci* **142:** 126–142, 1967.
22. Hornberger, J. C., S. J. Buell, D. G. Flood, T. H. McNeill and P. D. Coleman. Stability of numbers but not size of mouse forebrain cholinergic neurons to 53 months. *Neurobiol Aging* **6:** 269–275, 1985.
23. Hoss, W. and W. Messer. Multiple muscarinic receptors in the CNS: Significance and prospects for future research. *Biochem Pharmacol* **35:** 3895–3901, 1986.
24. Hughes, R. N. and R. Trowland. Physostigmine effects on activity and reactions to novelty. *Life Sci* **19:** 793–796, 1976.
25. Ingram, C. R., K. J. Phegan and H. T. Blumenthal. Significance of an aging-linked neuron binding gamma globulin fraction of human sera. *J Gerontol* **29:** 20–27, 1974.
26. Ingram, D. K. and M. J. Brennan. Animal behavior models in biogerontology. *Neurobiol Aging* **5:** 63–66, 1984.
27. Lal, H., M. J. Forster and K. Nandy. Immunologic factors related to cognitive/behavioral dysfunctions in aging. In: *Senile Dementia of the Alzheimer Type: Early Diagnosis, Neuropathology and Animal Models (Series: Advances in Applied Neurological Sciences, Vol 2)*, edited by W. H. Gispen and J. Traber. Berlin: Springer Verlag, 1985, pp. 343–354.
28. Lal, H. and M. J. Forster. Cognitive disorders related to immune dysfunction: Novel animal models for drug development. *Drug Dev Res* **7:** 195–208, 1986.
29. Lal, H., R. Gianforcaro and K. Nandy. Marked alterations in responsivity to psychostimulant and cholinergic drugs associated with senescence in the female mouse. *Soc Neurosci Abstr* **5:** 7, 1979.
30. Lippa, A. S., D. J. Critchett and J. A. Joseph. Desensitization of muscarinic acetylcholine receptors: Possible relation to receptor heterogeneity and phosphoinositides. *Brain Res* **366:** 98–105, 1986.
31. Lippa, A. S., C. C. Loullis, J. Rotrosen, D. M. Cordasco, D. J. Critchett and J. A. Joseph. Conformational changes in muscarinic receptors may produce diminished cholinergic neurotransmission and memory deficits in aged rats. *Neurobiol Aging* **6:** 317–323, 1985.
32. Maayani, S., Y. Egozi, I. Pinchasi and M. Sokolovsky. On the interaction of drugs with the cholinergic nervous system—V. Characterization of some effects induced by physostigmine in mice: *in vivo* and *in vitro* studies. *Biochem Pharmacol* **27:** 203–211, 1978.
33. Mash, D. C., D. D. Flynn and L. T. Potter. Loss of M_2 muscarine receptors in the cerebral cortex in Alzheimer's disease and experimental cholinergic denervation. *Science* **228:** 1115–1117, 1985.
34. Mason, S. T., D. C. S. Roberts and H. C. Fibiger. Noradrenergic influences in catalepsy. *Psychopharmacology (Berlin)* **60:** 53–57, 1978.
35. Meyer, E. M. and D. H. Otero. Pharmacological and ionic characterizations of the muscarinic receptors modulating [^{3}H]acetylcholine release from rat cortical synaptosomes. *J Neurosci* **5:** 1202–1207, 1985.
36. Nandy, K. Brain-reactive antibodies in mouse serum as a function of age. *J Gerontol* **27:** 173–177, 1972.
37. Nandy, K. Brain-reactive antibodies in aging and senile dementia. In: *Alzheimer's Disease–Senile Dementia and Related Disorders*, edited by R. Katzman, R. Terry and K. Bick. New York: Raven Press, 1978, pp. 503–512.
38. Nandy, K. Brain-reactive antibodies in aging non-human primates. *Mech Ageing Dev* **16:** 141–146, 1981.
39. Nandy, K. Immunopathology of aging and dementia. In: *Senile Dementia of the Alzheimer Type*, edited by J. T. Hutton and A. D. Kenny. New York: Alan R. Liss, 1985, pp. 293–305.
40. Nandy, K., H. Lal, M. Bennett and D. Bennett. Correlation between a learning disorder and elevated brain-reactive antibodies in aged C57BL/6 and young NZB mice. *Life Sci* **33:** 1499–1503, 1983.
41. Potter, L. T., D. D. Flynn, H. E. Hanchett, D. L. Kalinoski, J. Luber-Narod, and D. C. Mash. Independent M_1 and M_2 receptors: ligands, autoradiography and functions. *Trends Pharmacol Sci* Suppl, 22–31, 1984.
42. Pradhan, S. N. and S. N. Dutta. Behavioral effects of arecoline in rats. *Psychopharmacologia* **17:** 49–58, 1970.
43. Retz, K. C., M. J. Forster, N. Frantz and H. Lal. Differences in behavioral responses to oxotremorine and physostigmine in New Zealand Black (NZB/BlNJ) and C57BL/6 mice. *Neuropharmacology* **26:** 445–452, 1987.
44. Retz, K. C., M. J. Forster, M. D. Popper and H. Lal. Neurochemical and pharmacological studies suggest potential mechanisms for impaired task performance in the aging NZB mouse. *J Neurochem* **44:** S124, 1985.
45. Retz, K. C. and H. Lal. Cholinergic neuropsychopharmacology and neuropathology of dementias. In: *Central Cholinergic Mechanisms and Adaptive Dysfunctions*, edited by M. M. Singh, D. M. Warburton and H. Lal. New York: Plenum Press, 1984, pp. 335–352.
46. Ringdahl, B., F. J. Ehler and D. J. Jenden. Muscarinic activity and receptor binding of the enantiomers of aceclidine and its methiodide. *Mol Pharmacol* **21:** 594–599, 1982.
47. Sotelo, J., C. J. Gibbs, Jr. and D. C. Gajdusek. Autoantibodies against axonal neurofilaments in patients with kuru and Creutzfeldt-Jakob disease. *Science* **210:** 190–193, 1980.
48. Stolerman, I. P., R. Fink and M. E. Jarvik. Acute and chronic tolerance to nicotine measured by activity in rats. *Psychopharmacologia* **30:** 329–342, 1973.
49. Taylor, P. Cholinergic agonists. In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics, 7th Edition*, edited by A. G. Gilman, L. S. Goodman, T. W. Rall and F. Murad. New York: Macmillan Publishing Co., 1985, pp. 100–109.
50. Threatt, J., K. Nandy and F. Fritz. Brain-reactive antibodies in serum of old mice demonstrated by immunofluorescence. *J Gerontol* **26:** 316–323, 1972.
51. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill Inc., 1971.
52. Zilles, K. Morphological studies on brainstructures of the NZB mouse: An animal model for the aging human brain? In: *Senile Dementia of the Alzheimer Type: Early Diagnosis, Neuropathology and Animal Models (Series: Advances in Applied Neurological Sciences, Vol 2)*, edited by W. H. Gispen and J. Traber. Berlin: Springer Verlag, 1985, pp. 355–365.