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Water-escape performance and water-escape learning ability in laboratory mice (*Mus musculus*) as dependent on different gonosomal constitution and paternal irradiation of spermatozoa with 600 R of gamma rays *)

von

Johannes Horst SCHRÖDER [+])

(Institut für Biologie der Gesellschaft für Strahlen- und Umweltforschung, Neuherberg bei München, Germany
and
Institut für Zoologie, Universität Innsbruck, Austria)

Die Leistungs- und Lernfähigkeit der Labormaus (*Mus musculus*) im WELA-Schwimmtest in Abhängigkeit von unterschiedlicher gonosomaler Konstitution und paternaler Gammabestrahlung der Spermatozoen mit 600 R

S y n o p s i s : In einer sehr einfachen Apparatur (ESSMAN und JARVIK, 1961) wurde die Zeit (sec) gemessen, welche verstreichen muß, bis die in ein Wasserbad gesetzten Mäuse eine am anderen Ende der Wanne fest angebrachte trockene Rampe schwimmend erreichen und erklimmen (WELA-Schwimmtest). Dabei wurden Mäuse verschiedenen Genotyps an fünf aufeinanderfolgenden Versuchstagen bei einem Zeitunterschied von jeweils 24 Stunden zwischen zwei aufeinanderfolgenden Versuchen untersucht. Die sogenannte "Entkommenszeit", gemittelt für die untersuchten Gruppen und Versuchstage, genügte in allen Fällen einer Regressionsgleichung zweiten Grades der allgemeinen Form $y = a + b \cdot x + c \cdot x^2$, wobei a ein Gesamtmaß für die Aktivität, b die lineare Neigung der Lernkurve und c den Krümmungsgrad der Kurve angibt, welcher anzeigt, mit welcher Geschwindigkeit sich die Kurve einer Asymptote nähert (FESTING, 1973). Die Gesamtaktivität a war immer mit der Lerngeschwindigkeit b korreliert, d.h. hochaktive Mäuse erwiesen sich als schneller lernend als schwachaktive Individuen. In einer ersten Serie von Experimenten wurden Leistungs- und Lernfähigkeit für Mäuse unterschiedlicher gonosomaler und autosomaler Konstitution im WELA-Schwimmtest ermittelt.

X/O-Mäuse (Ta/O und +/O) zeigten eine geringere Entkommenszeit am ersten Tag und eine geringere Gesamtzeit als X/X-Weibchen entsprechender gonosomaler Konstitution (Ta/Ta und +/+). Andererseits wurde bei den X/X-Mäusen eine höhere Lerngeschwindigkeit als bei ihren X/O-Pendants festgestellt. Das X-chromosomale Markierungsgen "Tabby" (Ta) beeinflußte nur im heterozygoten Zustand (Ta/+) die Entkommensleistung. Geschlechtsunterschiede fanden sich sowohl bei den normal gefärbten Agouti-Mäusen, deren Männchen eine geringere Gesamtzeit bei schlechterer Lernfähigkeit als die Agouti-Weibchen zeigten, als auch bei den NMRI-Albinomäusen, bei denen die Männchen schlechter lernten und höhere Gesamtentkommenszeiten aufwiesen als die NMRI-Albinoweibchen. Die zweite Versuchsserie bestand in der Untersuchung von Mäusen, die entweder von gammabestrahlten (Spermatozoen mit 600 R bestrahlt) oder unbestrahlten Männchen abstammten. Keine signifikanten Unterschiede ließen sich in der Nachkommenschaft von bestrahlten Männchen des Teststammes (+/Y, homozygot für sieben rezessive autosomale Gene), die mit unbehandelten Ta/O-Weibchen gekreuzt worden waren, im Vergleich zu den Kontrollen finden, obwohl die Lerngeschwindigkeit bei den weiblichen Nachkommen (Ta/+, +/O) bestrahlter Männchen niedriger war als bei den Kontrollen. In weiteren Versuchen wurden die Leistungsfähigkeit von Nachkommen bestrahlter und unbestrahlter NMRI-Albinomännchen, die mit unbestrahlten NMRI-Albinoweibchen gepaart worden waren, miteinander verglichen. Obwohl kein direkter Einfluß der Geschwisterzahl auf die Gesamtleistungsfähigkeit im WELA-Schwimmtest nachzuweisen war, wurde dann eine geringere Gesamtentkommenszeit bei den Nachkommen bestrahlter NMRI-Männchen gefunden, wenn die Zahl der Jungen pro Wurf bei Geburt bei den Nachkommen bestrahlter und unbestrahlter Männchen einander angeglichen worden war. Mit Ausnahme der weiblichen Nachkommen bestrahlter NMRI-Männchen, wurde dieser Befund auch bei einem anderen NMRI-Versuch mit ungleichen Wurfgrößen erhoben, wo die Wurfgrößen der Kontrollen nicht auf das niedrigere Niveau der bestrahlten Serie gebracht worden waren. Wegen der strahleninduzierten dominanten Letalmutationen erzeugten bestrahlte NMRI-Männchen Würfe, die nur halb so groß waren wie die Würfe unbestrahlter NMRI-Männchen. Die Lerngeschwindigkeit erwies sich in den NMRI-Versuchen als uneinheitlich und widersprüchlich. Im Versuch mit ungleichen Wurfgrößen zeigten die Nachkommen bestrahlter Männchen eine geringere Lerngeschwindigkeit als die entsprechenden Kontrolltiere. Der Versuch mit angeglichenen Wurfgrößen erbrachte jedoch F_1-Nachkommen, bei denen nur die weiblichen Nachkommen bestrahlter Männchen schlechter als die Kontrollen lernten, während die dazugehörigen männlichen Nachkommen bessere Lernergebnisse als die entsprechenden F_1-Kontrollmännchen zeigten. Da die Lerngeschwindigkeit ein Verhaltensmerkmal mit Fitnesseigenschaften ist (FESTING, 1973), ist anzunehmen, daß die Lernfähigkeit unter natürlichen Umweltbedingungen sehr eng mit der Überlebensfähigkeit in Beziehung steht, was bedeutet, daß die hier angeführten, vermutlich mutativen Veränderungen der Lernfähigkeit im Freiland einer starken Selektion unterliegen müssen.

Introduction:

As pointed out by BOVET *et al.* (1969), the problem of "nature and nurture" constitutes the target of interest in today's psychobiology, a relatively new branch of biology that affords a tentative bridge between neurophysiology and comparative psychology, and ethology. Psychobiology attempts to study whether, and to what extent, learning behaviour is genetically determined. One of the first extensive experiments in the field of psychogenetics (or ethogenetics) was done by TYRON (1934), who selected rats with the highest or lowest performance in a standardized maze and bred their offspring. Bright maze learners produced bright progeny, whereas dull maze learners produced dull progeny. TYRON's experiments established the inherited nature of this adaptive behavioural trait. NEWCOMBE and McGREGOR (1964) were then the first authors to demonstrate an increase of dull and a complementary decrease of bright maze learners in rats after

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several generations of ancestral irradiation. This was the first investigation concerning the mutability of behavioural patterns in vertebrates. Such studies are not only important for theoretical reasons but also contribute to the question of how to evaluate the genetic risk of mental diseases to human beings due to increasing amounts of environmental mutagens. Otherwise, ionizing radiation is known to increase the frequency of sex-chromosome losses in mature female and male germ-cells of the mouse (RUSSELL, 1968; SCHRÖDER, 1978); thus giving rise to X/O females which exhibit the same gonosomal constitution as human patients with Turner's syndrome (short stature, skeleton abnormalities, webbing of the neck, coarctation of the aorta). As shown by several authors (ALEXANDER et al., 1964; ALEXANDER and MONEY, 1966; BEKKER, 1969; BEVER and CHIARELLO, 1974; MONEY, 1963, 1964; NEBES, 1971, 1972, 1974; SHAFFER, 1962), patients with Turner's syndrome performed significantly poorer than controls on tests of spatial ability, but only on spatial tests requiring the integration of isolated elements as synthetic wholes or the remembering of spatial configurations which could not be verbally mediated. Patients also performed less well than normal controls on tasks of serial processing when the tasks could not be mediated verbally (SILBERT et al., 1977), i.e. patients of normal intelligence with Turner's syndrome are deficient in one or several aspects of spatial visualization and spatial orientation. ALEXANDER and MONEY (1966) therefore concluded that humans with Turner's syndrome suffer from a variable developmental defect in the parietal functions of the cortex, and SILBERT et al. (1977) concretized this suggestion that patients with Turner's syndrome may have a selective deficiency in mental functions for which the right hemisphere is specialized. The study of MONEY and MITTENTHAL (1970) indicated another direct effect of genetics of behaviour in Turner's syndrome, namely with respect to personality, which might be identified as "inertia of emotional arousal". However, whereas human patients with Turner's syndrome cytogenetically detectable as deletion, translocation, or total absence of the second X chromosome with the presence of female external genitalia, suffer from primary amenorrhoea because of gonadal dysgenesis, X/O female mice are fertile, but revealing a pronounced reduction of their reproductive life span as compared to normal X/X sibs (LYON and HAWKER, 1973). Accordingly, X/O mice are able to produce X/O and X/X daughters and X/Y sons after mating with normal (X/Y) males. O/Y zygotes, also produced through fertilization of ova lacking the X chromosome by Y-carrying spermatozoa, are lethal at the pre-implantational stage (MORRIS, 1968; SCHRÖDER, unpublished results). Thus, X/O mice can be bred in sufficient numbers for different experimental purposes. Despite the difference in fecundity between human and murine subjects of the X/O constitution, a comparison of both species seems worthwhile in order to elicit the effects of sex differentiation and sex hormones on cognitive style in mammals.

ESSMAN and JARVIK suggested already in 1961 that water-escape ability could be used to study "the rapid acquisition of an orientation response". A very simple water-escape apparatus as a method of measuring learning ability in mice on repeated trials was then used by several authors (FESTING, 1973a, b; MEIER and FOSHAE, 1965; WINSTON, 1963). FESTING (1973a) showed that C3H inbred mice learned slowly in this apparatus, whereas C57BL/10 inbred mice learned rapidly in spite of initially poor

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performance. A replicated four-generation selection for faster learning speed resulted in the conclusion that learning speed has low additive genetic variance. Such a pattern of inheritance would be expected with a fitness trait (FESTING, 1973b).

The present paper deals with water-escape performance and learning ability in this apparatus (Fig. 2) as dependent on the gonosomal constitution (X/O *versus* X/X and X/Y), coat colour (agouti *versus* tabby and albino) or irradiation of paternal spermatozoa with 600 R of gamma rays. This report belongs to a series of forthcoming papers dealing with the mutational changes of learning ability and agonistic behaviour (SCHRÖDER, 1977) of the laboratory mouse.

Material and Methods:

I. Maintenance and Origin of the Mice

All the mice were bred and kept as specific pathogen-free mouse strains as already described alsewhere (SCHRÖDER, 1977). Water and ALTROMIN $^{®}$ food pellets were available *ad libitum*. The mice were counted and sexed at birth and at weaning. In one irradiation experiment with NMRI mice, the litter sizes of the control line and the irradiated line were equalized, *i.e.* the number of young per litter sired by unirradiated (control) males was reduced to the number of young per litter produced by irradiated males, hereafter designated "equalized litter sizes". In all the other irradiation experiments, the litter sizes of irradiated and control males remained "unequal" due to the reduction of litters derived from irradiated males. The X/O females, characterized by a diploid chromosome number of 39 instead of 40, originated from spontaneously arisen X/O exceptions 15 generations ago. Since then the X/O female mice were bred by alternating mating to either inbred C3H or tabby males (Fig. 1). Thus, X/O females could be recognized by the Tabby locus (Ta) or its wildtype allele (+), located on the X-chromosome. In other words, X/O-tabby females (Ta/O) were crossed with wildtype (agouti) C3H males (+/Y), and X/O-wildtype (agouti) females (+/O) with tabby males (Ta/Y), respectively. In the first cross (Ta/O x +/Y), all X/O offspring were expected to be wildtype (agouti) females (+/O), whereas X/O daughters of the second cross (+/O x Ta/Y) were recognizable by their tabby coat colour (Ta/O). This breeding scheme was possible because the semidominant X-chromosome marker "Tabby" (Ta) affects both the colour and structure of fur and skin (GRÜNEBERG, 1966). Homozygous and azygous tabby females (Ta/Ta and Ta/O, respectively) can be distinguished phenotypically by their sex from hemizygous tabby males (Ta/Y) and by their colour from the transversally striped heterozygous females (Ta/+). A phenotypic distinction between the former phenotypes and homozygous wildtype (agouti) females (+/+) and hemizygous wildtype (agouti) males (+/Y) is also feasible. For the purpose of behavioural comparisons, normal X/X and X/Y sibs, both wildtype and tabby mice, were bred by crossing heterozygous females (Ta/+) with tabby males (Ta/Y), producing tabby (Ta/Ta) and striped (Ta/+) females and wildtype males (+/Y). Wildtype females (+/+) were obtained by mating heterozygous females (Ta/+) to wildtype males (+/Y). This mating procedure ensured a similar genetic bachground for X/O (Ta/O and +/O), X/X (Ta/Ta, Ta/+, and +/+) females and

X/Y (Ta/Y and +/Y) males which then were used to determine the possible influence of the gonosomal constitution on water-escape performance and water-escape learning ability.
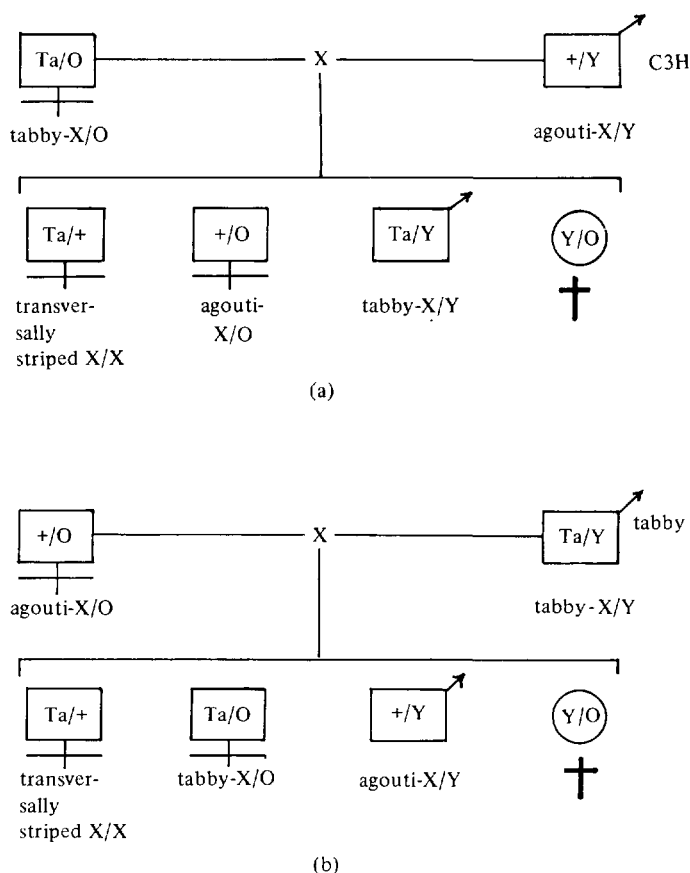

(a)

(b)

Fig. 1:   Breeding scheme for the production of X/O female mice.
        a)   Production of +/O females,
        b)   production of Ta/O females.

For the first radiation experiment, test stock males (+/Y), homozygous for seven specific autosomal recessive loci (a, b, c^{ch}, d, se, s, p; SEARLE, 1975), were either irradiated with 600 R of gamma rays or remained untreated to serve as controls, and were then mated to unirradiated, virgin Ta/O females. The resulting F_1 generation consisted in Ta/+ and +/O females and Ta/Y males. Non-inbred NMRI albino males, homozygous for the autosomal recessive gene (c) causing lack of tyrosinase, were used for the second irradiation experiment. The NMRI males (+/Y, c/c) were also exposed to 600 R of gamma rays or remained unirradiated (controls) and were then mated to unirradiated virgin

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NMRI females (+/+, c/c). In order that only irradiated spermatozoa were used for the fertilization of the ova, irradiated and control males of both irradiation experiments were mated to untreated females during the first week after exposure (OAKBERG, 1957).

II. Irradiation

All males were 10 - 12 weeks old when irradiated with 600 R of Cs-gamma rays at a dose-rate of 30 R/min, measured by a Victoreen dosimeter. During the exposure, only the pelvic region of the body was irradiated directly while the head and the front of the trunk were shielded with lead 10 - 12 mm thick.

III. Water-Escape Apparatus

The apparatus was constructed according to the measures given by ESSMAN and JARVIK (1961) and FESTING (1973a). It consisted of a plastic bath measuring 600 mm long by 350 mm wide by 200 mm deep (Fig. 2), filled to a depth of about 100 mm with tap water. The water temperature was adjusted to $(26 \pm 2)^{\circ}C$ at the beginning of each experimental session. An escape ramp consisting of a strip of rust-resistant wire mesh 50 mm wide and bent into the shape of a bridge 80 mm across was situated 90 mm from one end of the bath. Moderately uniform illumination was provided by the neon lights fixed at the ceiling of the stable room where daylight was excluded.
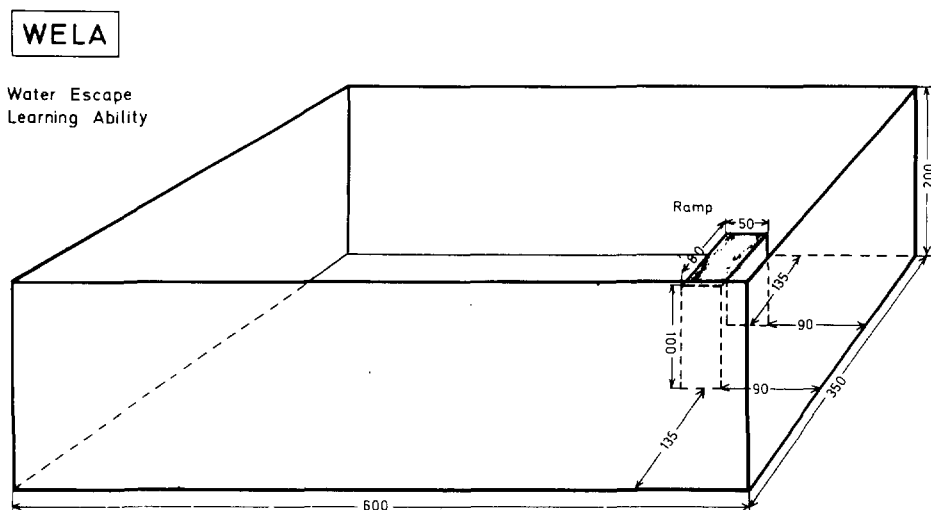
WATER ESCAPE APPARATUS, according to W. B. ESSMAN & M. E. JARVIK, 1961 ("A water escape test for mice." Psychol Rep. 8: 58).

Fig. 2

IV. Measurement of Water-Escape Time

Testing was carried out in an animal room adjacent to the mouse breeding rooms with normal background noise. Five trials were performed within five consecutive days, each trial beginning 24 hours after the preceding one, thus giving an indication of long-term memory. In the beginnung of the first trial, the mice were 10 - 12 weeks old and inexperienced in swimming or in any other test system. At weaning (21 - 28 days old) the sexes were separated and housed in groups, about five or less per standard plastic mouse cage (25 cm x 20 cm x 14 cm) with free access to water and dry food.

The experimental subjects were placed in the water at one end of the bath opposite to the escape ramp. The time taken to find and climb onto the escape ramp was recorded by a stopwatch. In the first experimental set concerning the comparison of mice with different gonosomal constitution, all animals which did not climb onto the escape ramp within 60 sec or which almost drowned because of exhaustion were recorded as unsuccessful. This distinction between successful and unsuccessful subjects and trials was not necessary in the second set of experiments because of the better physical condition of these mice.

V. Statistical Analysis

TYLER and McCLEARN (1970) analyzed the learning curves obtained in an "appetitively rewarded runway rask" in terms of a polynomial regression equation as follows:

$$y = a + b \cdot x + c \cdot x^2,$$

where a is an overall measure of activity; b is the linear slope of the learning curve, representing rate of learning; and c is the degree of curvature of the learning curve, indicating the speed of approach to an asymptote. FESTING (1973a, b) used this equation to determine the learning curve for his water-escape experiments. Accordingly, the mean values for each group of animals in the present study were fitted in the same way to this second degree polynomial regression equation. The best analysis that can be applied to such kind of data reported here is the multivariate method proposed by GRIZZLE and ALLEN (1969) which takes into account the correlations between trials and tests the goodness of fit of linear, quadratic, cubic, etc. models as well as tests for group and treatment effects on the intercept (a), slope (b), and curvature (c) of the learning curve. This method was not used for the present report, but its application to the computerized data is now in progress.

The index of learning ability was the linear slope of the learning curve based either on four (L_4) or five (L_5) trials. According to FESTING (1973a, b), two learning indices were used, *viz.*

$$L_4 = \frac{3 S_1 + S_2 - S_3 - 3 S_4}{10}$$

and

$$L_5 = \frac{2 S_1 + S_2 - S_4 - 2 S_5}{10}$$

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where S_i is log mean escape time on the i-th trial.

However, the question whether the differences of L_4's and L_5's between the mice of different gonosomal constitution or of different paternal treatment are really statistically significant cannot be answered before the univariate analysis of variance has been applied to the present data.

The mean values with their standard deviations and standard errors (S.E.) were calculated for each treatment group or genotype separately from the individual escape times by the use of a HP 65-calculator programme. To compare the mean escape times and the frequency of unsuccessful trials between the different groups, the t-test and the standard chi-square method were applied, respectively.

Results:

1. Comparison of Mice with Different Gonosomal Constitution

a. M e a n   O v e r a l l   E s c a p e   T i m e

The performance of mice in the water-escape test was expressed as mean overall escape time obtained by summing up the escape times for the five successive trials (Table 1). The different genotypes can be ordered in the following rank of increasing mean overall escape time:

$$+/Y \text{ (agouti)} \leqslant +/O \leqslant Ta/+ \leqslant Ta/O < +/+ \text{ (albino)} < +/+ \text{ (agouti)} \leqslant Ta/Ta =$$
$$= +/Y \text{ (albino)} \leqslant Ta/Y.$$

Significant differences (Table 1) were found between the genotypes as follows:
+/O *versus* +/+ (agouti), Ta/O, +/+ (albino), and +/Y (albino);
+/+ (agouti) *versus* +/Y (agouti), Ta/O, Ta/+, and +/+ (albino);
+/Y (agouti) *versus* Ta/O, Ta/Ta, Ta/Y, Ta/+, +/+ (albino), and +/Y (albino);
Ta/O *versus* Ta/Ta, Ta/Y, Ta/+, +/+ (albino), and +/Y (albino);
Ta/Ta *versus* Ta/+;
Ta/Y *versus* Ta/+;
Ta/+ *versus* +/+ (albino), and +/Y (albino), and
+/+ (albino) *versus* +/Y (albino).

Since high mean escape time expresses bad water-escape performance and low mean escape time means good performance, tabby males (Ta/Y) did worst and agouti (wild-type) males (+/Y) best. The significant differences between +/O and +/+ on the one hand and between Ta/O and Ta/Ta on the other hand are of particular interest because of the comparison between X/O and X/X females. In other words, both types of X/O females (+/O and Ta/O) did better than their gonosomally normal X/X pendants (+/+ and Ta/Ta, respectively). The tabby phenotype seems to increase the mean overall escape time, especially in the male, which is also true for albinism (c/c). Heterozygous striped females (Ta/+) performed better than both agouti (+/+) and tabby (Ta/Ta) females. Sex differences were found to be present between agouti females and agouti males, between albino females and albino males, and between Ta/+ females and all types of males. However, no significant difference could be detected between tabby females and tabby males.

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The highest value of the standard deviation within the series was established in tabby males, while it was lowest in agouti males.

b. F r e q u e n c y   o f   U n s u c c e s s f u l   T r i a l s

If one compares the frequency of unsuccessful trials for mice of different gonoso- mal constitution (Table 2), the highest number of unsuccessful trials was found in Ta/O females (Table 1). Thus, the frequency of unsuccessful trials for this gonosomal consti- tution is significantly different from that of Ta/Ta, Ta/Y, Ta/+, +/+ (albino), and +/Y (albino). Further significant differences were obtained by comparisons made between +/O *versus* +/+ (agouti) and Ta/O, between +/+ (agouti) *versus* +/Y (agouti), Ta/O and Ta/+ as well as between +/Y (agouti) *versus* Ta/O and Ta/Y (Table 2). No unsuccessful trials were found in + /Y males (both agouti and albino) and in homo- and heterozygous tabby females (Ta/Ta and Ta/+).

c. M e a n   E s c a p e   T i m e   f o r   t h e   F i v e   S u c c e s s i v e   T r i a l s

Significant differences for all five succeeding trials were detected between +/O and +/+ (agouti), and between Ta/O and Ta/Ta for the days (= trial number) 1, 3 und 4 (Table 3). Comparing agouti females with agouti males, statistically confirmed differences were observed for the days 1, 2, 3, and 5. This was not the case for the comparison between tabby females and tabby males, where all differences proved to be insignificant. Further significant differences were obtained between agouti males (+/Y, c^+/c^+) and albino males (+/Y, c/c) for all five trials, between heterozygous (Ta/+) and agouti females (+/+) for the days 1, 2, 3, and 5, and between heterozygous and homozygous tabby females for the days 1 through 4. Thus, most of the differences found for the mean overall esca- pe time are also reflected by differences between the single successive trials indicating that these differences sometimes remain consistent over the whole lapse of the learning curve.

d. W a t e r - E s c a p e   L e a r n i n g

If a is an overall measure of activity and b is the linear slope of the learning curve, $y = a + b \cdot x + c \cdot x^2$, the different values of a and b in the equations of Fig. 3 represent different activity and learning rate, respectively. For all genotypes tested, overall activity (a) and learning rate (b) were found to be correlated to each other (/r/ = 0.83; 0.001 < p < 0.01) in the sense that genotypes with high activity also exhibit a high learning rate and *vice versa*. Thus, the highest values of both activity and learning rate was found in agouti-X/X females (+/+), the lowest ones in the corresponding agouti-X/Y males (+/Y). The highest initial escape times were also found in +/+ (agouti) females and Ta/Y males, the lowest one in agouti males (Fig. 3). As far as learning rate is concerned, NMRI albino males behaved exceptionally because they did not show any learning success from the first through fourth trial. On the contrary, there was an increase of mean escape time from the first to the following trials as expressed by a convex instead of a concave learn- ing curve and by a positive sign of b in the learning equation (Fig. 3). Comparing X/O with X/X females of the corresponding genotypes, the learning rate was considerably

Table 1: Mean overall escape time and frequency of unsuccessful trials of mice of different gonosomal and autosomal constitution.

Strain	Phenotype	Gonosomal constitution	No. of successful trials	Unsuccessful trials		Mean overall escape time (sec)		Standard deviation within the series
				No.	%	Mean ± S. E.	95 %-Confidence limits	
Outcross	agouti, ♀	+/O	898	2	0.22	14.92 ± 0.44 a)b)c)d)	14.06 - 15.78	12.9
Outcross	agouti, ♀	+/+	566	4	0.70	24.11 ± 1.54 a)e)f)g)h)	21.11 - 27.10	36.3
Outcross	agouti, ♂	+/Y	620	0	0.00	13.59 ± 0.43 e)i)j)k)l)m)n)	12.76 - 14.42	10.5
Outcross	tabby, ♀	Ta/O	893	22	2.40	16.84 ± 0.50 b)f)i)o)p)q)r)s)	15.86 - 17.81	14.8
Outcross	tabby, ♀	Ta/Ta	215	0	0.00	24.25 ± 2.01 j)o)t)	20.33 - 28.16	29.3
Outcross	tabby, ♂	Ta/Y	561	4	0.71	26.01 ± 1.81 k)p)u)	22.47 - 29.54	42.6
Outcross	striped, ♀	Ta/+	1420	0	0.00	15.61 ± 0.38 g)l)q)t)u)v)w)	14.87 - 16.35	14.2
NMRI	albino, ♀	+/+	474	1	0.21	19.52 ± 0.74 c)h)m)r)v)x)	18.08 - 20.97	16.1
NMRI	albino, ♂	+/Y	650	0	0.00	25.23 ± 1.28 d)n)s)w)x)	22.74 - 27.73	32.5

a) - x): Significant differences ($p < 0.05$)

Table 2: Significance of differences in unsuccessful trials.

Comparison made between	d.f.	χ^2	p
all phenotypes examined (cf. Table 1))	8	80.6	10^{-10}
+/O (agouti) *versus* +/+ (agouti)	1	7.0	0.008
+/O (agouti) *versus* Ta/O (tabby)	1	14.5	0.0002
+/O (agouti) *versus* +/+ (albino)	1	0.002	0.95
+/O (agouti) *versus* +/Y (albino)	1	1.4	0.24
+/+ (agouti) *versus* +/Y (agouti)	1	4.4	0.037
+/+ (agouti) *versus* Ta/O (tabby)	1	7.6	0.0055
+/+ (agouti) *versus* Ta/+ (striped)	1	9.96	0.0016
+/+ (agouti) *versus* +/+ (albino)	1	1.31	0.19
+/Y (agouti) *versus* Ta/O (tabby)	1	14.9	0.0001
+/Y (agouti) *versus* Ta/Ta (tabby)	1	0.0	1.0
+/Y (agouti) *versus* Ta/Y (tabby)	1	4.4	0.035
+/Y (agouti) *versus* Ta/+ (striped)	1	0.0	1.0
+/Y (agouti) *versus* +/+ (albino)	1	1.3	0.25
+/Y (agouti) *versus* +/Y (albino)	1	0.0	1.0
Ta/O (tabby) *versus* Ta/Ta (tabby)	1	5.2	0.023
Ta/O (tabby) *versus* Ta/Y (tabby)	1	5.7	0.017
Ta/O (tabby) *versus* Ta/+ (striped)	1	34.2	$8 \cdot 10^{-9}$
Ta/O (tabby) *versus* +/+ (albino)	1	9.1	0.0025
Ta/O (tabby) *versus* +/Y (albino)	1	15.6	$8 \cdot 10^{-5}$
Ta/Ta (tabby) *versus* Ta/+ (striped)	1	0.0	1.0
Ta/Y (tabby) *versus* Ta/+ (striped)	1	10.0	0.0015
Ta/+ (striped) *versus* +/+ (albino)	1	2.98	0.085
Ta/+ (striped) *versus* +/Y (albino)	1	0.0	1.0
+/+ (albino) *versus* +/Y (albino)	1	1.37	0.24

Table 3: Mean escape time ± standard error for five successive trials of mice of different gonosomal and autosomal constitution.

Gonosomal constitution	Trial number (24 hs. apart)				
	1	2	3	4	5
+/O (n = 179)	a) 19.82 ± 1.03	b) 15.13 ± 0.96	c) 14.12 ± 1.18	d) 11.98 ± 0.72	e) 13.60 ± 0.88
+/+ (n = 114)	a) 32.82 ± 4.22 q) i)	b) 27.69 ± 2.91 r) j)	c) 21.95 ± 3.39 s) k)	d) 17.14 ± 2.46	e) 20.87 ± 3.76 t) l)
+/Y (n = 124)	14.73 ± 1.13 u) i)	15.65 ± 1.06 v) j)	11.63 ± 0.85 w) k)	12.23 ± 0.91 x)	13.69 ± 0.73 y) l)
Ta/O (n = 174)	f) 20.77 ± 1.17	19.15 ± 1.24	g) 15.14 ± 1.18	h) 15.86 ± 1.07	13.47 ± 0.85
Ta/Ta (n = 43)	f) 32.00 ± 6.25 m)	24.86 ± 5.19 n)	g) 24.42 ± 4.04 o)	h) 23.14 ± 3.50 p)	16.81 ± 2.29
Ta/Y (n = 112)	34.32 ± 5.22	30.29 ± 4.39	17.88 ± 1.77	28.38 ± 4.02	19.24 ± 3.94
Ta/+ (n = 284)	19.12 ± 0.88 q) m)	16.69 ± 0.93 r) n)	14.53 ± 0.80 s) o)	13.44 ± 0.92 p)	14.27 ± 0.66 t)
+/+ NMRI, albino (n = 95)	22.62 ± 1.92	21.53 ± 1.60	19.46 ± 1.67	16.29 ± 1.33	17.74 ± 1.66
+/Y NMRI, albino (n = 130)	20.32 ± 1.41 u)	22.48 ± 2.25 v)	26.87 ± 2.88 w)	30.13 ± 3.76 x)	26.37 ± 3.36 y)

a) - y): Significant differences (t-test; $p < 0.05$)

smaller in +/O than in +/+ females but almost identical for Ta/O and Ta/Ta. One must remember, however, that Ta/O females differed significantly from Ta/Ta females with respect to their high frequency of unsuccessful trials (Table 2). Comparisons of the water-escape learning indices L_4 and L_5 were performed by dividing the corresponding indices of those geriotypes for which a comparison seemed to be desirable. Accordingly, the·comparison between X/O and X/X agouti females was carried out by calculating the quotients of

$$\frac{L_4 \text{ of } +/O}{L_4 \text{ of } +/+} \quad \text{and} \quad \frac{L_5 \text{ of } +/O}{L_5 \text{ of } +/+}, \quad \text{respectively.}$$

The differences between the L_4 quotients and L_5 quotients reflect the observation that

the mean escape time often increased from the fourth to the fifth trial indicating an impairment of water-escape performance on day 5. A quotient near or equal to unity means no difference between the two genotypes compared. This was found to be the case for Ta/O *versus* Ta/Ta, Ta/Y *versus* Ta/Ta, and albino male *versus* albino female. A ratio near unity was found only for one quotient in the case of Ta/Y compared with Ta/O (L_4) and agouti female relative to albino female (L_5). The differences between both kinds of quotient were more pronounced for +/O *versus* +/+, agouti male *versus* agouti female, agouti male *versus* +/O, Ta/+ *versus* agouti female, Ta/+ *versus* Ta/Ta, and agouti male *versus* albino male (Table 4). Larger differences were established only for one quotient, namely for L_5 in the case of Ta/Y *versus* Ta/O and for L_4 in the comparison of agouti female *versus* albino female. However, more information on the reliability of these differences requires the application of the GRIZZLE and ALLEN analysis to the present data which is still under investigation.

II. Irradiation Experiments

Two radiation experiments were performed with 600 R of gamma rays to paternal spermatozoa. The F_1 progeny were then checked for water-escape performance and water-escape learning ability (WELA). While Ta/O females were mated to irradiated or control test-stock males in the first experimental set, only NMRI mice were used for the second set of experiments.

a. X/O Experiments

Ta/+ and +/O females performed a higher and Ta/Y males a lower mean overall escape time in the irradiated F_1 (I-F_1) than in the control-F_1 (C-F_1) generation (Table 5). This also holds true for the mean escape time for the five succeeding trials (Table 6). However, none of these differences reached a significant level, perhaps because of the small sample size of animals examined. The same situation is also reflected by the overlapping learning curves (Fig. 4). They are close together with a higher learning speed (b) in the control-F_1. There was again a correlation between overall activity, a, and the learning rate, b, as calculated from the equation given in Fig. 4 ($/r/$ = 0.94; $0.001 < p < 0.01$). The learning curves of C-F_1 are partly different from those obtained from subjects of the same gonosomal constitution in the X/O *versus* X/X comparison reported above. These differences may be the result of the small sample size in the X/O irradiation experiment.

The I/C ratios for L_4's and L_5's (Table 7) were determined as follows:

$$\frac{L_4 \text{ of I-}F_1}{L_4 \text{ of C-}F_1} \quad \text{and} \quad \frac{L_5 \text{ of I-}F_1}{L_5 \text{ of C-}F_1} \ , \quad \text{respectively.}$$

Except for L_5 of Ta/+ females, these quotients express the better learning rate of controls up to the fifth trial. The shape of the learning curve of heterozygous (Ta/+) females is convex instead of concave with a positively signed b (Fig. 4). The standard deviation within the series was highest for Ta/+ of I-F_1 (Table 5).

Table. 4: Comparison of the water-escape learning indices L_4 and L_5 between mice of different gonosomal and autosomal constitution.

Comparison made between		Ratio of	
phenotypes	gonosomal constitutions	L_4's	L_5's
$\dfrac{\text{agouti-}♀}{\text{agouti-}♀}$	$\dfrac{+/O}{+/+}$	0.73	0.72
$\dfrac{\text{agouti - }♂}{\text{agouti - }♀}$	$\dfrac{+/Y}{+/+}$	0.39	0.28
$\dfrac{\text{agouti-}♂}{\text{agouti-}♀}$	$\dfrac{+/Y}{+/O}$	0.54	0.40
$\dfrac{\text{striped }♀}{\text{agouti-}♀}$	$\dfrac{Ta/+}{+/+}$	0.55	0.58
$\dfrac{\text{tabby-}♀}{\text{tabby-}♀}$	$\dfrac{Ta/O}{Ta/Ta}$	1.05	1.15
$\dfrac{\text{tabby-}♂}{\text{tabby-}♀}$	$\dfrac{Ta/Y}{Ta/Ta}$	1.12	0.90
$\dfrac{\text{tabby-}♂}{\text{tabby-}♀}$	$\dfrac{Ta/Y}{Ta/O}$	1.07	0.78
$\dfrac{\text{striped }♀}{\text{tabby-}♀}$	$\dfrac{Ta/+}{Ta/Ta}$	1.21	0.59
$\dfrac{\text{agouti-}♀}{\text{albino-}♀}$	$\dfrac{+/+}{+/+}$	1.38	1.03
$\dfrac{\text{agouti-}♂}{\text{albino-}♂}$	$\dfrac{+/Y}{+/Y}$	0.48	0.29
$\dfrac{\text{albino-}♂}{\text{albino-}♀}$	$\dfrac{+/Y}{+/+}$	1.12	1.02

Table 5: Mean overall escape time of different irradiated (I) and control (C) F_1 genotypes from the cross test-stock male (+/Y) x X/O (Ta/O) female.

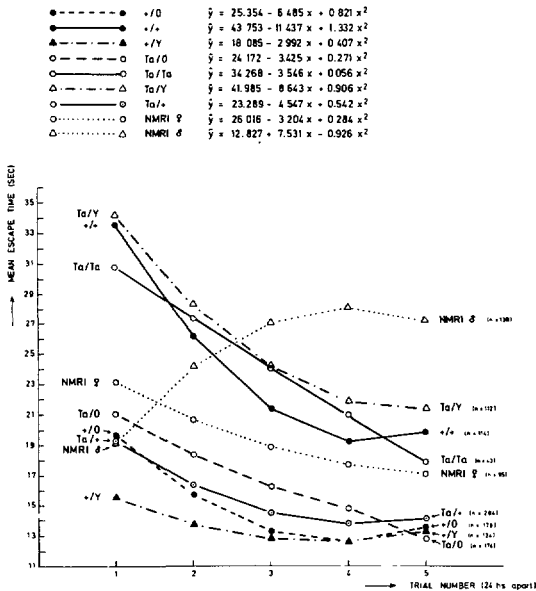| Phenotype	Gonosomal constitution	Series	Mean overall escape time (sec)		Standard deviation within the series	Comparison between I-F_1 and C-F_1		
			Mean ± S.E.	95%-confidence limits		$\frac{I}{C}$	t	p
striped ♀	Ta/+	I-F_1	12.65 ± 1.20	10.25 – 15.05	± 12.05	1.35	1.93	0.055
		C-F_1	9.34 ± 1.94	7.40 – 11.28	± 7.20			
agouti-♀	+/O	I-F_1	11.71 ± 2.78	8.93 – 14.48	± 8.51	1.27	1.22	0.23
		C-F_1	9.20 ± 1.60	5.83 – 12.57	± 8.80			
tabby-♂	Ta/Y	I-F_1	10.04 ± 1.43	7.18 – 12.89	± 10.59	0.93	0.66	0.52
		C-F_1	11.36 ± 1.45	8.46 – 14.26	± 9.82			

Fig. 3

Table 6: Mean escape time ± standard error for five successive trials of different irradiated (I) and control (C) F_1 genotypes from the cross test-stock male (+/Y) × X/O (Ta/O) female.

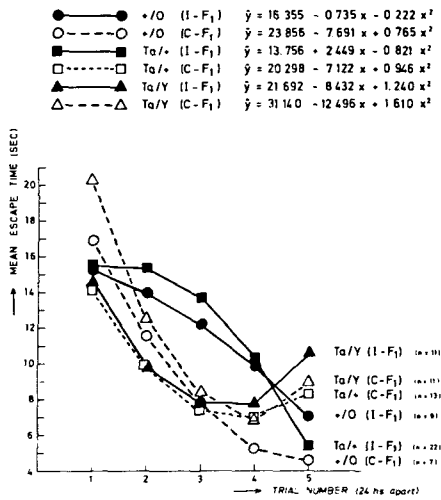| Gonosomal constitution	Ta/+					+/O					Ta/Y				
Trial number	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
600 R to spermatozoa (I) (22 Ta/+; 9 +/O; 11 Ta/Y)	16.32 ± 3.22	12.45 ± 2.51	16.86 ± 3.48	9.14 ± 1.83	5.59 ± 0.90	16.22 ± 3.84	11.33 ± 2.22	15.22 ± 2.99	8.44 ± 2.52	7.33 ± 2.29	13.73 ± 3.72	11.18 ± 2.99	8.00 ± 1.65	5.82 ± 1.26	11.45 ± 4.89
Control spermatozoa (C) (13 Ta/+; 7 +/O; 11 Ta/Y)	16.23 ± 2.86	8.31 ± 1.52	7.38 ± 1.18	6.23 ± 1.26	8.54 ± 2.55	17.00 ± 4.98	11.14 ± 4.51	8.43 ± 2.89	4.71 ± 0.92	4.71 ± 0.99	19.45 ± 4.74	13.82 ± 3.20	9.27 ± 2.08	4.18 ± 0.99	10.09 ± 2.43
$\frac{I}{C}$	1.01	1.50	2.29	1.47	0.66	0.95	1.02	1.81	1.79	1.56	0.71	0.81	0.86	1.39	1.14

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Fig. 4

Table 7: Comparison of the water-escape learning indices L_4 and L_5 between irradiated (I) and control (C) F_1 mice of different gonosomal and autosomal constitution.

	Comparison made between		$\frac{I}{C}$ ratio of	
litter sizes	phenotype	gonosomal constitution	L_4's	L_5's
	striped ♀	Ta/+	0.48	1.56
	agouti-♀	+/O	0.40	0.55
unequal	tabby-♂	Ta/Y	0.58	0.40
	albino-♀	+/+	0.62	1.01
	albino-♂	+/Y	1.05	0.68
equalized	albino-♀	+/+	−2.37	−1.31
	albino-♂	+/Y	3.18	3.20

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WATER ESCAPE LEARNING ABILITY

NMRI mice

I − F$_1$:   600 R of gamma - rays to paternal spermatozoa ⎫ unequal
C − F$_1$:   Control - F$_1$ generation   ⎬ litter
                                                                  ⎭ sizes

●━━━━━● I − F$_1$ ♀   ŷ = 29.334 − 2.432 x + 0.106 x^2
○------○ C − F$_1$ ♀   ŷ = 26.016 − 3.204 x + 0.284 x^2
▲━━━━━▲ I − F$_1$ ♂   ŷ = 7.230 + 10.597 x − 1.579 x^2
△----△ C − F$_1$ ♂   ŷ = 25.944 − 3.755 x + 0.955 x^2
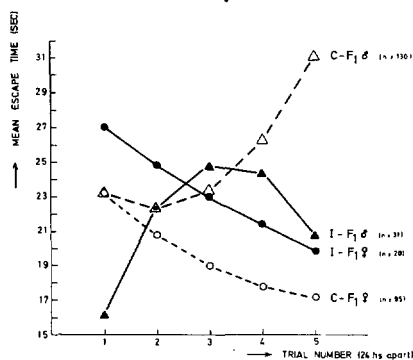
Fig. 5

WATER ESCAPE LEARNING ABILITY

NMRI mice

I − F$_1$ 600 R of gamma - rays to paternal spermatozoa ⎫ equalized
C − F$_1$ Control - F$_1$ generation   ⎬ litter
                                                              ⎭ sizes

●━━━━━● I − F$_1$ ♀   ŷ = 14.742 + 4.987 x − 0.711 x^2
○------○ C − F$_1$ ♀   ŷ = 32.369 − 1.193 x + 0.124 x^2
▲━━━━━▲ I − F$_1$ ♂   ŷ = 42.357 − 15.220 x + 1.831 x^2
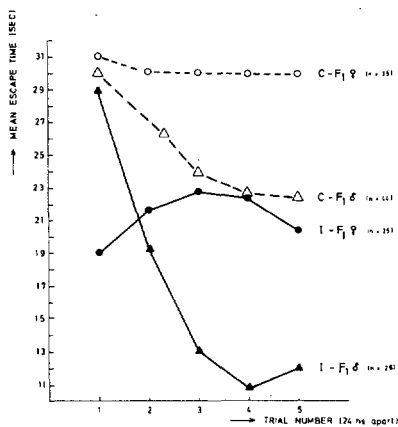△----△ C − F$_1$ ♂   ŷ = 34.292 − 5.066 x + 0.536 x^2

Fig. 6

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b. NMRI Experiments

Two NMRI experiments were carried out, one with unequal litter-sizes between I-F_1 and C-F_1 due to the induction of dominant lethal mutations in I-F_1, the other with litter sizes equalized to the I-F_1 level. In both experiments the mean overall escape time (Table 8) of C-F_1 exceeded that of I-F_1 significantly with the exception of females in the first experiment with unequal litter sizes. The differences found for the five successive trials, however, proved to be statistically significant only for the trials 3, 4, and 5 in the second experiment with equalized litter sizes (Table 9). Considering the learning curves for the two NMRI experiments, different slopes were found for unequal (Fig. 5) and equalized (Fig. 6) litter sizes. An increasing mean escape time for C-F_1 males, as already described for NMRI males (cf. Fig. 3), was detected in the first experiment. In this group, the standard deviation within the series (Table 8) reached a value twice as high as that of the corresponding females, indicating that some subjects of this group behaved quite unusually. A similar value for the within-series standard deviation was found for C-F_1 females of the second experiment. Thus, roughly spoken, the water-escape learning of I-F_1 females exceeded that of C-F_1 females with the exception of the females in the first experiment. The learning rate (b) was slowest for I-F_1 males of the first experiment with unequal litter-sizes, while it was worst for I-F_1 females of the second experiment with equalized litter-sizes (Figs. 5 and 6). Overall activity (a) and learning rate (b) were again strictly correlated to each other ($/r/ = 0.94$; $p = 0.001$). This situation was also expressed by the I/C quotients for L_4 and L_5 (Table 7). As in the first experiment with X/O females, the quotient for the learning speed was different for L_4's and L_5's. Control males did better up to the fourth day and control females up to the fifth day, whereas no difference between C-F_1 and I-F_1 was found for L_5 of females and L_4 of males in the first NMRI experiment. In the second experiment, however, the values for both L_4 und L_5 indicated a higher learning index of irradiated F_1 mice. Because of the different litter sizes in C-F_1 and I-F_1, the different number of siblings may be assumed to be responsible for the different results in the two NMRI experiments. There could have been a stronger competition among the young of the control litters, perhaps associated with more social contacts and emotional arousal in larger than in smaller litters. Since irradiated NMRI males produced only half the number of young per litter of control NMRI males, I-F_1 mice of the first experiment eventually had less chance to learn by competition than had C-F_1 mice. However, no correlation could be found between the mean water-escape performance of 178 NMRI mice and their number of siblings (Fig. 7).
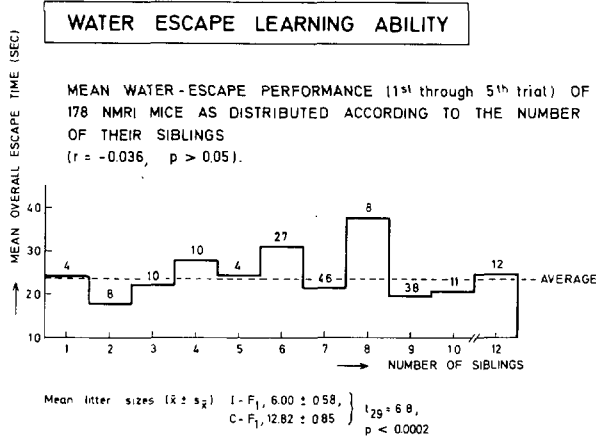
Fig. 7

Table 8: Mean overall escape time of irradiated (I) and control (C) F_1 NMRI mice.

Litter sizes	Sex	Series	Mean overall escape time (sec)		Standard deviation within the series	Comparison between I-F_1 and C-F_1		
			Mean ± S.E.	95%-confidence limits		$\frac{I}{C}$	t	p
Unequal	Females	I-F_1	23.25 ± 1.40	20.45 – 26.04	± 14.25	1.19	2.15	0.035
		C-F_1	19.52 ± 0.74	18.04 – 21.01	± 16.06			
	Males	I-F_1	21.64 ± 1.00	19.64 – 23.64	± 12.20	0.86	2.67	0.007
		C-F_1	25.23 ± 1.28	22.68 – 27.79	± 32.53			
Equalized	Females	I-F_1	21.21 ± 1.50	18.22 – 24.20	± 16.87	0.70	2.66	0.007
		C-F_1	30.15 ± 2.60	24.96 – 35.35	± 34.71			
	Males	I-F_1	16.84 ± 1.48	13.83 – 19.79	± 15.62	0.67	2.91	0.0035
		C-F_1	24.99 ± 1.90	21.19 – 28.79	± 28.24			

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Table 9: Mean escape time ± standard error for five successive trials of irradiated (I) and control (C) F_1 NMRI mice.

Litter sizes	Series	Females					Males				
		Trial number					Trial number				
		1	2	3	4	5	1	2	3	4	5
Unequal	I-F_1	27.91 ± 3.85	22.65 ± 2.80	24.31 ± 2.71	21.79 ± 3.47	19.36 ± 2.58	17.23 ± 1.98	19.87 ± 2.11	25.63 ± 2.54	25.50 ± 2.37	20.03 ± 1.86
	C-F_1	22.62 ± 1.92	21.53 ± 1.60	19.46 ± 1.67	16.29 ± 1.33	17.74 ± 1.66	20.32 ± 1.41	22.48 ± 2.25	26.87 ± 2.88	30.13 ± 3.76	26.37 ± 3.36
Equalized	I-F_1	18.56 ± 2.96	22.71 ± 2.91	21.92 ± 3.71	22.40 ± 3.52	20.52 ± 3.71	28.96 ± 5.34	19.88 ± 3.18	11.31 ± 1.39 a)	12.62 ± 1.86 b)	11.42 ± 1.71 c)
	C-F_1	30.20 ± 3.33	33.57 ± 5.61	27.23 ± 5.84	30.06 ± 8.03	29.71 ± 9.44	28.75 ± 5.45	28.95 ± 5.26	21.80 ± 2.63 a)	22.95 ± 3.66 b)	22.50 ± 3.61 c)

a) - c): Significant differences (t-test; p $<$ 0.05)

Discussion:

I. General Remarks

The main objective of this investigation was to determine the possible influence of the gonosomal constitution (X/O *versus* X/X *versus* X/Y), autosomal constitution (agouti *versus* albino), and of the mutational treatment of parental spermatozoa on the water-escape performance and learning ability in mice. As already emphasized by FESTING (1973a), the water-escape apparatus of ESSMAN and JARVIK (1961) approaches an ideal test system which does not require individual pretreatment of animals, such as the starving necessary for an appetitively rewarded task. Furthermore, an ideal test apparatus would be quick and easy to use, requiring only a few minutes for each trial per subject. In addition, it is inexpensive, and it gives precise results which can be analyzed by normal statistic procedures. The shortcomings and advantages of this apparatus were discussed in detail by FESTING (1973a), so it is not necessary to repeat the arguments favouring this type of test system. However, it seems worthwhile to mention that the water-escape apparatus as used here fits the requirements of determining learning speed according to the

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definition of learning "in the biological sense" as "the re-organization of behaviour as a result of individual experience" (RICHTER, 1966). As FESTING (1973b) pointed out, learning speed determined by this apparatus could be closely related to the ability to survive in the wild and therefore may be a fitness character as well.

II. X/O *versus* X/X

X/O mice of both genotypes (+/O and Ta/O) exhibited a shorter mean overall escape time than the corresponding X/X females (+/+ and Ta/Ta). At first view, this seems to be at variance with the reduced spatial ability of human patients with Turner's syndrome (X/O), because the water-escape task requires the ability to orient spatially. However, if one regards the learning rate (b in the learning equation), which always was correlated with the overall activity (a), a reduction of learning rate was characteristic for +/O females as compared to +/+ mice. There was also a slight, but non-significant reduction of b in Ta/O relative to Ta/Ta. In the latter case, the frequency of unsuccessful trials of Ta/O mice was significantly greater in comparison with Ta/Ta females and other genotypes. Thus, despite the better overall performance of X/O females, their learning ability was reduced as compared to that of X/X females, *i.e.* water-escape performance and learning rate are different in X/O mice as shown by the slope of the learning curve. Consequently, some accordance between human and murine X/O individuals seems to exist with respect to the acquisition of an orientation response.

FESTING's results (1973a) showed clearly that the slope of the learning curve for interstrain comparisons and their F_1 hybrids is inherited to a substantial degree. The ratio of the learning indices L_4 and L_5 for X/O relative to X/X mice as well as the slope of the learning curves for X/O and X/X females support the assumption that genetic differences between X/O and X/X mice may be responsible for the differences obtained in learning speed.

III. Females *versus* Males

FESTING (1973a) suggested that sex differences for the mouse strains examined (ICFW, C3H, NMRI, C57L, CBA-T6, C57BL/10) were not of great importance for the water-escape learning ability. This result disagrees with the present finding that agouti females (+/+) exhibit a better learning rate (b) but a worse overall escape time than the corresponding agouti (+/Y) males. Otherwise, NMRI (albino) males did worse than the corresponding females both with respect to overall mean escape time and learning rate. Thus, from the present mouse experiments no general conclusion can be drawn on a possibly lower spatial ability of females as it was shown to be realized in rats and men influenced by estrogen, endocrine dysfunction or castration (DAWSON, 1972).

IV. Coat Colour Differences

According to several authors (DeFRIES *et al.*, 1966; FESTING, 1973b; OLIVERIO and MESSERI, 1973; TYLER, 1970; WILCOX, 1969; WINSTON and LINDZEY, 1964), it is well established that albinism (*i.e.* lack of tyrosinase due to homozygosity for the autosomal recessive, c) can considerably affect different types of behaviour, including

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open-field acticity (DeFRIES *et al.,* 1966) and water escape (WINSTON and LINDZEY, 1964). FESTING (1973b) demonstrated that albino mice in a segregating population escape as well as pigmented mice initially but fail to improve their performance on repeated trials. These results were in striking agreement with the findings of TYLER (1970), who found that the rate of learning was less in albino mice than in pigmented ones in an appetitive situation, which could not be explained in terms of a lower overall activity of the albinos. The present study revealed an initial escape time of albino females lying between that of striped (Ta/+) and agouti (+/+) females. The overall activity of the albino females as expressed by the term a of the learning equation was similar to that of X/O and striped X/X females, but considerably lower than that of agouti and tabby X/X females. As expressed by b in the learning equation, the learning rate of albino females was even lower than that of X/O and of pigmented X/X females. This holds true also for the learning speed as compared to that of agouti females, but almost no difference was found relative to Ta/+ females.

The NMRI (albino) males behaved quite ununsually because their mean escape time increased from the fist to the fourth trial. Consequently, the present results confirmed to some degree the previous findings on water escape of albino mice.

The X-chromosomal marker gene ”Tabby” (Ta) seems to have only minor influence on water-escape performance and learning ability. Homozygous tabby females and tabby males exhibited learning curves similar to that of agouti (+/+) females. Ta/O females were characterized by a learning curve very close to that of +/O females indicating that the X/O or X/X constitution affects the learning curve and water-escape performance to a much greater extent than the presence of the Ta gene. However, there was one exception with tabby males as compared to agouti males; whereas the former showed the highest initial escape time of all genotypes with a good learning speed, the latter had the lowest escape time with an extremely bad learning rate. The learning curve of heterozygous Ta/+ females was intermediate between the learning curves of Ta/O and +/O females, and they also exhibited a medium learning rate. Thus, the tabby gene (Ta) has some influence only in the heterozygous state, while the difference between agouti-X/X females and agouti males should be due to the different water-escape ability of the sexes discussed above.

V. Irradiation Experiments

The different litter sizes between the offspring sired from irradiated (I) or control (C)   males seemed to have no influence on the overall water-escape performance, thus confirming the finding of FESTING (1973b) that ”the common environment within a litter is of little or no importance in determining learning speed”. Despite this, unequal and equalized litter-sizes gave different results in the NMRI experiments.

In the experiment with unequal litter-sizes, I-F_1 females revealed a higher mean overall escape time than C-F_1 females, and I-F_1 males a lower escape time than C-F_1 males, whereas in the experiment with equalized litter sizes both females and males of C-F_1 exerted a higher overall escape time than the individuals of I-F_1. Thus, in the NMRI experiments the mean overall escape time tends to decrease rather then increase in the irradiated series. The other irradiation experiment carried out with the offspring of irradia-

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ted or control test-stock males mated to Ta/O females gave only non-significant changes in overall escape time. As to the learning rate (b), both females and males of the first NMRI experiment (unequal litter sizes) of I-F_1 did worse than the controls, whereas I-F_1 males of the second NMRI experiment (equalized litter sizes) showed a better learning rate than their controls. The I / C ratio of learning indices reflect this situation with some differences between L_4 and L_5, due to the fact that in somes cases the mean escape time increased from the fourth to the fifth trial. Thus, the irradiation experiments gave indeed significant differences between irradiated and control F_1 generations, but the findings remain inconsistent and difficult to interpret. It can be suggested that radiation-induced chromosome breaks affect the overall ectivity, and thus the learning rate which then might be responsible for the differences observed between the progeny of paternally irradiated and control mice. However, further investigations dealing with possible interactions of mutationally changed aggressiveness (SCHRÖDER, 1977), learning ability and chromosomal rearrangements are necessary to clarify this important assumption.

Summary:

In a very simple water-escape apparatus the escape time of mice of different genotypes was measured for five successive trials with a time interval of 24 hours. In all cases examined, the mean escape time for the five trials was found to fit a second degree polynomial regression equation of the form, $y = a + bx + cx^2$, where a is an overall measure of activity; b is the linear slope of the learning curve, representing rate of learning; and c is the degree of curvature of the learning curve, indicating the speed of approach to an asymptote. The overall activity (a) was always correlated to the learning rate (b), *i.e.* high active mice were faster learner than low active subjects. Since the learning speed has already been shown to be a fitness character (FESTING, 1973), it seems reasonable to assume that learning ability could be closely related to ability to survice under natural conditions.

In the first experimental set, the water-escape performance and water-escape learning ability was determined for mice of different gonosomal and autosomal constitutions. X/O (Ta/O and +/O) female mice were found to have a lower initial escape time and a lower overall escape time than X/X females of the corresponding gonosomal constitution (Ta/Ta and +/+). However, X/X females showed a better learning rate than X/O females. The X-chromosomal marker gene "Tabby" (Ta) affected the escape performance only when present in the heterozygous state (Ta/+). Sexual differences were detected in normally pigmented agouti mice the males of which revealed a lower overall escape time but learned more slowly than agouti females, and in NMRI albino mice the males of which learned more slowly and had a higher overall escape time than NMRI albino females.

The second set of experiments consisted in the examination of mice either sired from irradiated males (600 R of gamma rays to spermatozoa) or control males. No significant differences between the progeny of irradiated and control males were found after crossing test-stock males (+/Y; homozygous for seven autosomal recessives) with untreated

Ta/O females, though the learning rate in female offspring of irradiated males was slightly lower. Further experiments with irradiated and control males were carried out with NMRI albino mice. In this case, NMRI albino males were mated to untreated NMRI albino females. Though no direct influence of the number of siblings on the overall water-escape performance was established, offspring of irradiated NMRI males had a lower mean escape time than their controls when litters were equalized at birth to the number of young per litter as found in the irradiated series. Except for female offspring of irradiated NMRI males, this also held true for another experiment with unequal litter sizes where the control litter sizes were not adjusted to the level of the irradiated F_1. Because of radiation-induced dominant lethal mutations, irradiated NMRI males produced litters of only half the size of that of control males. The irradiated F_1 showed a slower learning rate than the corresponding controls, but in the NMRI experiment with equalized litter-sizes only female offspring of irradiated males learned more slowly than the controls, while irradiated F_1 males did even better than the corresponding control males.

Acknowledgements:

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