MONOZYGOTIC VS. DIZYGOTIC TWIN BEHAVIOR IN ARTIFICIAL MOUSE TWINS

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Manuscript received June 6, 1983

Revised copy accepted October 25, 1983

ABSTRACT

Adult inbred mice of an isogenic strain (AKR/NHan or C57BL/6J Han) differ in social (sexual and agonistic), emotional and psychomotoric behavior, depending on the kind of manipulation to which they were subjected at an early ontogenetic stage. Monozygotic twins (MZT) from eight-cell stages halved and transferred to uterine foster mothers were compared with dizygotic twins (DZT) from nonreduced but transferred eight-cell stages and with naturally born animals (NBA). Generally, early embryonic conditions predict the behavioral characteristics of the adult animals to a high degree. The MZT are motorially less active, less emotional, less aggressive and less socially interested than DZT and NBA. In tests of spontaneous social behavior (allogrooming, anogenital licking, mounting, fighting), as well as in tests for emotionality (open field: crossed fields and defecation), these behavioral patterns occurred less frequently in MZT than in DZT; the NBA were mostly intermediate. The copulatory pattern of male MZT differs from that of male DZT by a shortage of intromission latency and duration; furthermore, MZT pairs do not build up a steady rank order in competitive copulation tests, as opposed to DZT and NBA pairs. In a test for psychomotoric behavior (swimming), the MZT prefer "floating" as a survival strategy, whereas the DZT and NBA prefer "adult swimming." Therefore, it can be concluded that these behavioral differences may be caused by the particular psychosocial environment in which the twins grow up or may be due to early prenatal peculiarities, such as inadequate synchronization of the developmental status of uterus and embryo.

INVESTIGATIONS following the classical twin method are numerous but none satisfactorily define the extent to which heredity, environment or an interaction of both determine the behavior of adults. The possible influence of third factors—ZAZZO (1978) says that heredity and "nongenetic factors" influence behavior, and VON VERSCHUER (1954) mentions an undefined "third factor" in addition to heredity and environment—is hardly taken into account in these investigations. Newer approaches to twin investigations (ZAZZO 1960) no longer regard the twin partners as two independent individuals but as parts of a pair, based on the principle that life in a pair has certain consequences. Such living conditions can fix nongenetic similarities (mutual imitation) and nonenvironmental differences (relationships of dominance and submissiveness).

Compared with investigations in human twins, the investigation of inbred mouse twins offers ideal conditions, *i.e.*, isogenic individuals in a standardized

postnatal environment. Our investigations using such genetic uniform animals, prepared artificially to be either dizygotic or monozygotic twins, showed remarkable behavioral differences.

MATERIALS AND METHODS

Animals: The experiments were carried out in AKR/NHan (AKR) and C57BL/6JHan (C57BL) mice derived from brother-sister matings over more than 130 generations. Isogenicity in these inbred strains was continuously verified by testing different immunogenetic and biochemical markers and other markers representing about 100 loci by the breeding unit at Zentralinstitut für Versuchstiere, Federal Republic of Germany; the results have been reported elsewhere (HEDRICH 1981).

The animals were caged in transparent polycarbonate cages (Makrolon) filled with wooden granula, at room temperature ($22 \pm 2^\circ$) and a relative humidity of $55 \pm 5\%$; they had free access to drinking water and were fed a commercial pelleted diet (Altromin 1320) *ad libitum*. They were continuously confirmed to be free from diseases recorded in GV-SOLAS (1972).

Preparation of the twins: The preparations were done according to GÄRTNER and BAUNACK (1981). Ova were collected by flushing the tuba uteri on day 3.5 postconception (p.c.) (plug = day 1) after spontaneous ovulation. The flushing fluid (NIEMIERKO and OPAS 1978) contained phosphate-buffered saline supplemented with 20% heat-inactivated (30 min at 56°) fetal calf serum. The zona pellucida was removed by incubation with 0.5% pronase (MCLAREN 1969). The denuded ova were cultivated in the flushing medium for 3 hr at 37° in an atmosphere of 95% air and 5% CO_2 (WHITTEN 1971; HOGAN and TILLY 1977).

Preparation of monozygotic twins (MZT): Mechanical separation of the eight-cell stages in a micro-drop of the flushing medium under liquid paraffin was then carried out with a stiletto-like extended glass capillary tube, and both halves were incubated for 3 hr. The two halves, together with five to seven denuded eight-cell zygotes of a "strange" genotype, were then transferred into the uterus of 2.5-day pseudopregnant, uniparous recipients (MCLAREN and MICHIE 1956; MCLAREN 1969). Animals prepared in this way were called MZT.

Dizygotic twins (DZT) were produced in a similar way, including chemical treatments and incubations but without dividing the zygotes. Instead of two halves of one eight-cell zygote, however, two undivided, denuded eight-cell zygotes derived from one mother were transferred. Embryos of the AKR genotype were transferred to C57BL foster mothers together with five to seven C57BL strange eight-cell zygotes. Embryos of the C57BL genotype were transferred to C57BL foster mothers together with five to seven AKR strange eight-cell zygotes.

Naturally born animals (NBA) were bred by mongameous limited pairing and without any manipulation in the early embryonic stages.

Postnatal care: The animals were born on day 19 p.c. The litters with four to eight offspring were suckled by their uterine foster mothers and weaned at 25 days of age, when the males and females of each litter were placed in separate cages. All animals were examined daily for health and signs of maturation and were weighed every 10 days from 31 to 101 days. The two twins in the MZT or DZT preparations were identified among their strange littermates by their different hair color.

Behavioral tests: Ten days prior to the beginning of the behavioral tests, the adult mice of the AKR strain were caged in pairs (two randomly selected brothers or sisters of the NBA and the two twin mates of the MZT and DZT), whereas each mouse of the C57BL strain was caged individually. The AKR mice were tested between 200 and 300 days either from 9 to 11 a.m. (social behavior) or in the first 3 hr of the dark period (7 to 10 p.m.) (competitive copulation) daily over a period of 12 days for each investigated character. They started with competitive copulation followed by spontaneous social behavior after about 8 wk. The C57BL mice were tested starting on day 112. The tests were performed daily between 9 and 11 a.m. They started with swimming, followed by open field after about 2 wk, then spontaneous behavior, and ended with copulatory behavior.

All animals performed only one of the following tests per day:

Open field: According to the method of IVINSKIS (1966), we placed each mouse singly into the

center of the open field (round area, diameter 80 cm, gray walls, gray bottom divided into 19 squares of identical dimension by thin black lines). Over 5 min the squares crossed and the deposited feces were counted. The test was repeated 12 times.

Spontaneous social behavior: According to the method of MEYERSON and HÖGLUND (1981), we tested spontaneous social behavior against the twin mate or against a brother or sister in the NBA, respectively, and against a strange partner. The test was performed for 10 min daily for 12 days in a transparent polycarbonate cage (Makrolon I; $12 \times 20 \times 13$ cm), which was renewed for every single test. The numbers of the following behavior patterns were counted: allogrooming, anogenital licking, mounting, fighting and self-grooming. Points were given whenever the behavior pattern occurred, irrespective of its duration. Concerning mounting and fighting, only the dominant animal was considered, whereas in the other tests both animals were recorded.

Male sexual behavior, noncompetitive copulation: The C57BL males were confronted with castrated female C57BL mice in which estrus was induced artificially by injection of $12 \mu\text{g}$ of estradiolbenzoate and 0.6 mg of progesterone (FALK 1981). All male mice were confronted with the same female per day, and the female mice were changed every other day. During the 10 min of testing, the following characters were examined: latency until first intromission, number of intromissions during 10 min of observation and duration of each intromission. The order of the tested males changed daily, and the tests were done in the female's home cage.

Male sexual behavior, competitive copulation: GÄRTNER, WANKEL and GAUDSZUHN (1981) described a test for the estimation of relative Darwinian fitness in rats. It was adapted to mice in the following way: both twin mates living together in a transparent polycarbonate cage ($22 \times 17 \times 12$ cm) were adapted to the test situation by confronting them with one estrous female for 30 min/day. This was done for 7 days prior to the actual test. Then, on 12 consecutive days between 9 and 11 a.m., the male twin pairs were confronted with one estrous female for 15 min in their home cage. The intromissions were counted separately for each male. The daily follow-up of male pairs differed randomly. The females were changed every other day.

Swimming: According to the method of KLAUS and ERDMANN (1978), we dropped each mouse gently from about 10 cm into a glass jar with a diameter and height of 30 cm and containing warm (30°) water. The maximum swimming time was 10 min; exhaustion of the mouse caused test termination. Five criteria were used to qualify the mouse's swimming behavior: uncoordinated staggering, "adult" swimming in circles, floating, diving and exhaustion. Latency and duration of these patterns were recorded over 5 days.

Statistical evaluations for comparing means were the X-test (VAN DER WAERDEN and NIEVERGELT 1956), the exact Fisher test (DOCUMENTA GEIGY 1968) and analysis of variance (WEBER 1972); for comparing distributions were the Kolmogoroff-Smirnoff test (SACHS 1969) and the χ^2 -test (HOFSTÄTTER and WENDT 1974) and for comparing intrapair correlation of ranks was the Kendall's τ -test (HOFSTÄTTER and WENDT 1974).

RESULTS

Behavioral patterns were compared among groups of adult animals identical in genotype, age, sex and environmental conditions but different in the manipulations to which the early embryos had been submitted. Body weight on day 101 (in grams) was C57BL: MZT δ , 27.98 ± 0.74 ; DZT δ , 25.45 ± 1.14 ; NBA δ , 26.87 ± 4.10 ; MZT η , 22.31 ± 0.39 ; DZT η , 21.15 ± 0.60 ; NBA η , 21.34 ± 1.04 ; AKR: MZT δ , 32.00 ± 0.74 ; DZT δ , 29.68 ± 1.30 ; NBA δ , 37.40 ± 0.72 (mean $\pm$ SE). MZT were derived from one denuded eight-cell zygote by dividing the total cell mass into two equal parts. DZT were animals whose zona pellucida was removed at the eight-cell stage but whose cell mass was not reduced. NBA were mice without any manipulation in early embryonic stages. MZT, DZT and NBA of the same sex and genotype were investigated simultaneously. A comparison of behavioral patterns between the strains is unrealistic because of additional differences (personal and environmental conditions, methods, seasons of the year, etc.).

Open field behavioral patterns in MZT, DZT and NBA: Two behavioral patterns (squares crossed and defecation) were recorded in 33 animals of the C57BL strain. Because there were no sex-dependent differences, the results for males and females were combined (Table 1). The number of *squares crossed* differed between the groups. A MZT crossed about half as many squares (87) as a DZT (167) during a residence of 5 min, whereas a NBA was intermediate (108). The differences between the groups were verified as significant ($P \leq 0.01$) by both parametric tests (analysis of variance) and the nonparametric x-T. The χ^2 tests proved that the three tested groups did not descend from the same basic group. Intrapair correlation was highest in MZT pairs (τ -test).

Social behavior against the twin mate or a brother or sister: The four spontaneous social patterns (allogrooming, anogenital licking, mounting and fighting) were counted in male and female MZT and DZT confronted with their twin mate on 12 consecutive days. The same observations were made using pairs of NBA confronted with a particular natural born brother or sister. Self-grooming was recorded simultaneously.

Tables 2 and 3 show the social patterns. The number of each performed pattern differed according to the early ontogenetic situation of the animals in all compared groups. In general, MZT performed significantly fewer spontaneous social activities than did DZT and NBA. Among 12 comparisons, only three did not support the general conclusion. A decreased occurrence of allogrooming and anogenital licking in the MZT of the C57BL males could not be proved statistically against an increased occurrence in DZT. In males of the AKR strain, the MZT performed more active fights than did the NBA. DZT differ frequently in all social patterns from the NBA ($P \leq 0.01$), but the tendency of deviation was irregular. Male DZT of the AKR strain were more active in all four social patterns than the NBA, whereas DZT of the C57BL strain were less active than the NBA in all four social patterns. Obviously, strain and sex differences interacted with differences between the early onto-

TABLE 1

Number of squares an animal crossed during a 5-min open field residence observed on 12 consecutive days in MZT, DZT and NBA of C57BL mice

Animals	Mean $\pm$ SE (n)	χ^2	τ
MZT ($\delta + \varphi$)	87.7 $\pm$ 14.19 (12)	***	0.47
	***	**	
DZT ($\delta + \varphi$)	167.7 $\pm$ 16.96 (11)	***	0.35
	***	**	
NBA ($\delta + \varphi$)	108.9 $\pm$ 11.36 (10)	***	0.40
	***	NS	

Asterisks on the lines indicate differences proven by analysis of variance (left) or by the X-test (right) between MZT and DZT (first line), DZT and NBA (second line), NBA and MZT (third line); NS, not significant; * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

TABLE 2

Differences in social behavioral patterns toward the twin mate in MZT and DZT and a brother or sister in NBA on 12 consecutive days

Animals	Allogrooming							
	AKR ♂				C57BL ♂			C57BL ♀
	Mean ± SE (n)	χ ²	τ		Mean ± SE (n)	χ ²	τ	Mean ± SE (n) χ ²
MZT	0.77 ± 0.38 (12)	***	0.60		3.10 ± 1.41 (8)	NS	0.33 ^a	2.55 ± 0.88 (4) ***
	***	***			—NS—	NS—		—***—**
DZT	3.43 ± 0.68 (10)	***	0		3.92 ± 2.43 4	NS	0 ^a	10.6 ± 3.57 (6) ***
	***	*			—NS—	*		—**—NS—
NBA	1.90 ± 0.53 (10)	NS	0		6.60 ± 1.91 (6)	NS	−0.40 ^a	8.0 ± 1.35 (4) NS
	***	NS			—***—	NS—		—***—**
Anogenital licking								
MZT	0.50 ± 0.29 (12)	***	0.60		2.50 ± 1.22 (8)	NS	0.40 ^a	2.25 ± 0.78 (4) ***
	***	***			—NS—	NS—		—***—**
DZT	2.22 ± 0.53 (10)	**	−0.20		1.96 ± 1.55 (6)	**	−0.20 ^a	6.90 ± 2.53 (4) *
	**	NS			—***—**			—NS—*
NBA	1.48 ± 0.68 (10)	NS	0.20		6.64 ± 2.34 (6)	***	−0.30 ^a	8.02 ± 3.27 (4) NS
	***	NS			—***—**			—***—***

See legend to Table 1 concerning levels of significance indicated in horizontal lines.

^a The τ is proven for C57BL males and females combined.

genetic situations. Intrapair correlations among the tested pairs always showed the highest τ values in the MZT pairs.

Behavioral patterns with a strange partner: These investigations were performed using males of the C57BL strain. Each animal was confronted with an adult strange C57BL male for 10 min daily in a separate cage. These strange partners were either dominant (A) or submissive (O) mice. They were used every other day, so that each tested male was confronted six times with an A partner and six times with an O partner. The tested mice did not show significant differences in the number of performed patterns against A or O partners; therefore, the calculations were combined. Table 4 shows the results that support this conclusion. The MZT were less active toward a strange partner than the DZT and the NBA. The NBA partly resembled the MZT (allogrooming 4.9, mounting 0.4), sometimes exceeded both MZT and DZT (anogenital licking 6.0) and were sometimes intermediate (fighting 5.8). The χ^2 test shows varying results for the different behavioral patterns: in allogrooming and mounting only the DZT differed significantly, anogenital licking showed a reverse result and in fighting all three groups differed significantly from each other. In three of four observed behavioral patterns the MZT showed higher concordance than the DZT and the NBA (τ -test).

TABLE 3

Differences in social behavioral patterns toward the twin mate in MZT and DZT and a brother or sister in NBA on 12 consecutive days

Animals	Mounting							
	AKR ♂				C57BL ♂			C57BL ♀
	Mean ± SE (n)	χ ²	τ		Mean ± SE (n)	χ ²	τ	Mean ± SE (n)
MZT	0.13 ± 0.21 (12)	***	0.47		0.25 ± 0.44 (8)	***	0.33 ^a	0.00 ± 0.0 (4)
DZT	1.48 ± 0.66 (10)	***	-0.60		1.90 ± 1.91 (4)	NS	-0.70 ^a	2.25 ± 1.53 (6)
NBA	0.54 ± 0.38 (10)	NS	0		2.17 ± 1.52 (6)	NS	0.30 ^a	4.29 ± 2.69 (4)
	***	NS			***	**		***
Fighting								
MZT	0.25 ± 0.04 (12)	***	0.47		0.18 ± 0.29 (8)	***	0.60 ^a	0.00 ± 0.0 (4)
DZT	0.74 ± 0.40 (10)	***	-0.60		4.67 ± 4.58 (4)	*	0.30 ^a	0.46 ± 0.62 (6)
NBA	0.15 ± 0.15 (10)	NS	0		5.0 ± 2.35 (6)	***	1 ^a	0.31 ± 0.72 (4)
	**	NS			***	**		NS

See legend to Table 1 concerning levels of significance indicated in horizontal lines.

^a The τ is proven for C57BL males and females combined.

Self-grooming was observed under both conditions: when the test animals were confronted either with their twin mates or with a strange partner (Table 5). Self-grooming behavior differed among the MZT, DZT and NBA. In all four compared groups differing in genotype and sex, the MZT performed less self-grooming than the DZT. The NBA were mostly intermediate. These differences could not always be verified statistically. The within-pair correlations (τ-test) of the MZT or DZT was no greater among them or against the NBA.

Male sexual behavior: Male mice confronted with an estrous female mouse in an observation box showed only a short period of courting behavior, characterized by patterns of social behavior like allogrooming and anogenital licking. This was followed by repeated cycles of mounting, intromission, dismounting, licking the penis and resting. About 15–30 of these intromission cycles occurred during a period of 10–20 min. The last cycle was finished by ejaculation, characterized by a cautious dismounting and a long resting period. Male copulatory behavior was investigated under two conditions: confrontation of single males with one estrous female (noncompetitive copulatory behavior) and confrontation of the two males housed together with one estrous female in the home cage of the males (competitive copulatory behavior).

TABLE 4
Social behavioral patterns of MZT, DZT and NBA of C57BL males against dominant and submissive strange C57BL males
on 12 consecutive days

Animals	Allogrooming			Anogenital licking			Mounting			Fighting		
	Mean $\pm$ SE (n)	χ^2	τ	Mean $\pm$ SE (n)	χ^2	τ	Mean $\pm$ SE (n)	χ^2	τ	Mean $\pm$ SE (n)	χ^2	τ
MZT	4.14 $\pm$ 2.01 (8)	NS	0.33	2.15 $\pm$ 0.80 (8)	**	0.67	0.3 $\pm$ 0.35 (8)	NS	-0.17	1.24 $\pm$ 0.86 (8)	***	0.33
DZT	10.8 $\pm$ 4.01 (5)	***	-1	4.07 $\pm$ 2.19 (5)	NS	-1	2.3 $\pm$ 2.05 (5)	***	-1	15.5 $\pm$ 8.48 (5)	***	-1
NBA	4.89 $\pm$ 2.03 (6)	NS	-1	6.01 $\pm$ 2.0 (6)	**	-1	0.4 $\pm$ 0.41 (6)	NS	0	5.8 $\pm$ 3.70 (6)	**	-0.33
	NS	NS		***	***		NS	NS		***	***	

See legend to Table 1 concerning levels of significance indicated in horizontal lines.

TABLE 5
Self-grooming during different social contact in MZT, DZT and NBA on 12 consecutive days

Animals	AKR ♂			C57BL ♂			C57BL ♀			C57BL ♂ (strange)		
	Mean $\pm$ SE (n)	χ^2	τ	Mean $\pm$ SE (n)	χ^2		Mean $\pm$ SE (n)	τ		Mean $\pm$ SE (n)	χ^2	τ
MZT	1.25 $\pm$ 0.29 (12)	NS		1.0 $\pm$ 0.41 (8)	*** ^a		0.85 $\pm$ 0.55 (4)	-0.15 ^a		1.56 $\pm$ 0.52 (8)	NS	0.33
DZT	1.62 $\pm$ 0.45 (10)	NS		3.35 $\pm$ 3.10 (4)	*** ^a		2.21 $\pm$ 0.72 (6)	0.20 ^a		1.83 $\pm$ 1.20 (4)	NS	-1
NBA	1.44 $\pm$ 0.31 (10)	NS		2.78 $\pm$ 1.0 (6)	NS ^a		1.44 $\pm$ 0.85 (4)	0.20 ^a		2.50 $\pm$ 1.02 (6)	NS	-0.33
	NS	NS		NS	NS		NS	NS		NS	NS	

See legend to Table 1 concerning levels of significance indicated in horizontal lines.

^a The χ^2 and τ is proven for C57BL males and females combined.

Noncompetitive copulatory behavior: In these investigations three characteristics of male sexual behavior were recorded: intromission latency (the time between confrontation with the female and first intromission), number of intromissions during 10 min of observation and duration of each intromission. During a 10-min observation time no ejaculation was observed. Each male was tested on 12 consecutive days. The results are shown in Table 6. Only male MZT and DZT of C57BL strain were compared. Both groups showed nearly the same number of intromissions (8.7 and 9.4). The MZT had a shorter intromission latency (241 sec) than the DZT (289 sec), and each intromission in the MZT lasted about half as long as in the DZT (5.1 sec).

Competitive copulation: Under competitive conditions only one characteristic, namely, the frequency of intromissions, was recorded during 15 min and compared between MZT, DZT and NBA of the AKR strain (Table 7). The number of intromissions differed significantly between the two competing twin partners; the Rollenverteilung (animal with many intromissions = A male, animal with few intromissions = O male) remained constant over the 12 observation days. These consistent differences necessitated performing three different examinations of the results in order to neutralize these differences and to compare the results separately for A and O males. The number of intromissions for each pair and day was calculated (Table 7, left column), and the totals were compared for the frequency of intromission between the groups of MZT, DZT and NBA (Table 7). The MZT pairs showed more intromissions than the DZT and the NBA, but the difference could not be verified statistically. Following this, the number of intromissions between MZT-A, DZT-A, NBA-A and MZT-O, DZT-O, NBA-O were compared (Table 7, center columns). The intromission rate of the A animals did not differ between MZT, DZT and NBA. The O animals showed an unexpectedly high number of intromissions in MZT. The O animals of NBA and DZT showed the expected submissive behavior of an O partner in a constant rank order; they hardly ever copulated. The intrapair differences between the MZT, DZT and NBA were quantitatively compared by estimating the relative frequency of intromissions performed by O animals as a percentage of the intromissions the pair performed together (Table 7, right column). The MZT partners showed the highest intrapair concordance of intromissions.

Survival test (swimming): If a mouse was dropped into a basin filled to the edge with water, it looked for a way out as soon as it reached the surface by moving to the wall of the basin and trying to climb out. To test the swimming behavior of mice it was necessary to prevent the animals from escaping from the water by not filling the jar entirely. Under such conditions a mouse changed its behavior in the following way. After reaching the surface the mouse moved its extremities uncoordinatedly, which led to a staggering or spinning motion (uncoordinated swimming). It then showed well-coordinated swimming movements (adult swimming) with the dorsal part of the body above the surface. It then hardly moved except for a few coordinated movements that stabilized a position where nostrils, eyes, ears and back were above the surface (floating). How long the animal continued this kind of swimming de-

TABLE 6

Noncompetitive copulatory behavior of C57BL males on 12 consecutive days

Animals	Latency		No. of intromissions		Duration of one intromission (sec)	
	Mean $\pm$ SE (n)	τ	Mean $\pm$ SE (n)	τ	Mean $\pm$ SE (n)	τ
MZT	241 $\pm$ 43.82 (8)	0	8.7 $\pm$ 1.84 (8)	-0.67	2.9 $\pm$ 0.42 (8)	-0.33
	***	**	—NS—NS—		***	*
DZT	289 $\pm$ 50.89 (5)	1	9.4 $\pm$ 1.88 (5)	1	5.1 $\pm$ 1.16 (5)	1

See legend to Table 1 concerning levels of significance indicated in horizontal lines.

TABLE 7

Number of intromissions under competitive conditions of AKR males on 12 consecutive days

Animals	No. of intromissions of both competing males			Dominant male (A)		Submissive male (O)		% Intromissions of O animal ^a
	Mean $\pm$ SE (n)	χ^2	τ	Mean $\pm$ SE (n)	χ^2	Mean $\pm$ SE (n)	χ^2	(n)
MZT	4.25 $\pm$ 1.33 (6)	NS	-0.33	6.2 $\pm$ 1.68 (3)	NS	2.7 $\pm$ 1.21 (3)	***	30 (3)
	NS			—NS—		—**—		
DZT	3.65 $\pm$ 1.88 (8)	NS	0	7.1 $\pm$ 2.85 (4)	NS	0.2 $\pm$ 0.25 (4)	*	3 (4)
	**			—NS—		—**—		
NBA	2.70 $\pm$ 1.32 (8)	NS	-0.17	5.3 $\pm$ 1.90 (4)	NS	0.1 $\pm$ 0.20 (4)	**	2 (4)
	NS			—NS—		—*—		

See legend to Table 1 concerning levels of significance indicated in horizontal lines.

^a Total intromissions of both competing males.

pended on the amount of air bubbles in the coat, which diminished as soon as the skin came into contact with water. Finally, the animal became unable to hold its eyes and nostrils continuously above the surface and dove in a vertical position until its hind legs touched the bottom, then pushed off the bottom, paddled to the surface to breathe and sunk again. After some time the mouse became exhausted and drowned unless taken out of the water.

The latency and the duration of each of the four swimming patterns varied individually. After surfacing, most animals started with uncoordinated staggering, some changed to adult swimming, others to floating and a few changed to adult swimming followed by floating. Only a very few animals were unable to hold their eyes and nostrils continuously above the surface and continued to dive.

Figure 1 shows how daily repetition of the test influenced the time an animal performed a particular swimming pattern. Table 8 compares the results on

FIGURE 1.—Swimming behavior of adult MZT, DZT and NBA mice of the inbred C57BL strain during 10 min on 5 consecutive days. Each line represents one animal of the group in the same succession every day. uncoordinated; **** adult; — floating.

TABLE 8

Time (seconds) the MZT, DZT and NBA perform adult swimming or floating during 10-min residence in a water jar on days 4 and 5 (C57BL animals)

Animals	Day 4		Day 5	
	Adult swimming	Floating	Adult swimming	Floating
	Mean $\pm$ SE (n)	Mean $\pm$ SE (n)	Mean $\pm$ SE (n)	Mean $\pm$ SE (n)
MZT	22 $\pm$ 21.10 (12)	528 $\pm$ 51.45 (12)	11 $\pm$ 8.96 (12)	488 $\pm$ 66.47 (12)
	—**—	—NS—	—NS—	—NS—
DZT	330 $\pm$ 88.55 (11)	270 $\pm$ 88.25 (11)	169 $\pm$ 83.43 (11)	430 $\pm$ 83.43 (11)
	—NS—	—NS—	—NS—	—*—
NBA	388 $\pm$ 72.46 (14)	172 $\pm$ 69.52 (14)	301 $\pm$ 78.61 (14)	214 $\pm$ 79.68 (14)
	—***—	—**—	—**—	—**—

See legend to Table 1 concerning levels of significance indicated in horizontal lines.

days 4 and 5. The DZT, MZT and NBA differed remarkably in their choice of adult swimming or floating. DZT and NBA showed a similar survival strategy. About 50% of each group preferred adult swimming and the other half floating after the third repetition. Toward the 5th day, DZT exhibited a growing preference for floating. The general strategy of the MZT was quite different. They tried adult swimming instead of uncoordinated staggering only in the first 2 days. In the following days they homogeneously preferred floating, a rather inactive behavioral pattern. The different frequencies preferring

a particular strategy of MZT (11 of 11) against NBA (three of eight) could be verified statistically on day 5 with the exact Fisher test; the numbers of animals that preferred floating (first number) as a fraction of the number of all animals that continued above the water surface (second number) differed significantly ($P \leq 0.01$). The DZT were intermediate, but these differences could not be verified. The differences also were verified statistically by comparing the times of adult swimming and floating between MZT, DZT and NBA on the 4th and 5th days by analysis of variance (Table 8).

DISCUSSION

Methods: Due to the considerable difficulties of preparing artificial MZT in mice, the animals investigated here were not prepared for a prospectively planned behavioral study. Before this study they had been subjected to several developmental studies (GÄRTNER and BAUNACK 1981), which were first designed for AKR and then continued with C57BL mice. Because of the technical difficulties the investigated groups were small and only a few patterns were recorded in both genotypes with identical methods. These restrictions prohibit comparisons of the results between genotypes. Only differences between MZT, DZT and NBA within the same genotype are reliable.

There are two different ways of observing and judging behavior: either to watch one individual thoroughly over a long period in its natural environment without disturbing it in any way and then to describe its behavior ("ethnomethodology") or to submit several individuals repeatedly to predetermined behavioral tests and then to analyze the test results quantitatively. The first method is rather time-consuming, and its results are difficult to compare between individuals. The second method counts and finally compares the numbers of certain motoric activities and disregards the fact that the same behavioral pattern may occur with completely different motivations. On the other hand, the subjective error of the person conducting the tests can be almost disregarded, and a change of observers should not necessarily lead to different results. We decided upon the second method. The chosen tests were considered to correlate to psychomotoric (swimming test), social (spontaneous social and male sexual behavior) and emotional (open field) aspects of behavior.

Behavioral differences between MZT, DZT and NBA: Altogether, 16 patterns were observed. In all tests the MZT differed quantitatively from the DZT and the NBA. The most important general differences were that, first, throughout all studies the MZT showed less motoric activity in psychomotoric as well as in emotional tests than both DZT and NBA, and, second, that the MZT lacked almost any rank order in competitive copulation. This fact is striking, because male mice living together in one cage usually established a consistent rank order with one absolutely dominant and one submissive animal (EIBL-EIBESFELDT and KRAMER 1958). The lack of submissiveness of MZT in competitive copulation observed here fits well with the observations of the spontaneous social behavior of the MZT toward the co-twin; the two males interacted socially more like "togetherness" (SEWARD 1945) in a promiscuous manner, like rats.

The different swimming (survival) strategy of the MZT compared with the other two groups (MZT preferring floating and DZT adult swimming) could be explained either by the MZT tendency to submit passively to their fate or by the inability of the MZT to properly coordinate their extremities. The second explanation might be partly valid, but the results of the open field tests tend to support the first assumption: the emotionality of the MZT in social encounters is also reduced compared with that of the DZT and the NBA. The question is: why are the MZT motorially more passive, less emotional, less aggressive and less interested in social contacts?

Possible "psychic" reasons for the behavioral differences between MZT, DZT and NBA: VOGEL (1981) discussed behavioral peculiarities in human twins as secondarily caused by intrauterine somatic conditions. HUSÉN (1960) described subnormal IQ values in human twins in comparison with singletons and observed more frequent somatic and mental retardation in twins than in singletons.

The observed behavioral differences in mouse twins may be caused by the particular social environment in which the MZT and DZT live, an environment that consists of a genotypically strange "mother" and littermates from which they differ by well-known somatic characteristics such as growth rate, body weight and maturation ages (ear and eye opening, first appearance of hair; BAUNACK and GÁRTNER 1981) and by strain-specific behavioral peculiarities. This difference in genotype allots to them an outcast position, a conflict that MZT and DZT master in different ways. The MZT retire to their "twin-microcosmos" and become excessively conformist (HEIGL-EVERS 1970). They are not integrated into the social cage-group and so remain socially incompetent. The DZT become very aggressive toward each other as well as toward strange mice and act more emotionally in general.

Possible "somatic" reasons for the behavioral differences between MZT, DZT and NBA: VOGEL (1981) attributes the retardation of human twins to the limited intrauterine space. The validity of this explanation for mouse twins could be doubted, but we have no measurements of the newborn mouse twins.

Another somatic explanation for the observed behavioral differences between MZT, DZT and NBA might be the fact that the denuded embryos were transferred asynchronously (the foster mother being at day 2.5 of pseudopregnancy, the embryos being 3.5 days old). Thus, later endocrine signals, especially those determining postnatal masculine or feminine behavior (DÖHLER *et al.* 1982; DENENBERG 1962), reach the embryo at inappropriate developmental stages and fail to act on it properly. The animals were born on the 19th day of the foster mother's pseudopregnancy. Thus far, it is still a matter of speculation to consider whether the observed behavioral differences may be primarily caused by the reduction of the embryonic cell mass at the eight-cell stage. Such speculation is inappropriate because the cells of mouse embryos at the eight-cell stage are polypotent. The two halves, consisting of four blastomeres each, can develop into two somatically normal mice. It should be expected that both also have polypotency for the normal behavioral pattern of adult animals. This polypotency is known for functions of the nucleus. How-

ever, there are some reports that cytoplasm loses its polypotency at the eight-cell stage, whereas the polypotency of the nucleus remains. This was shown in early *Drosophila* embryos (ILLMENSEE, HOPPE and CROCE 1978) but is surmised to a smaller degree also for mice (ILLMENSEE and HOPPE 1981). The reduction of cytoplasmic polypotency in the eight-cell stage of mouse embryos may interrupt the exchange of cytoplasmic information among the blastomeres of one embryo. Such an interruption does not impair the somatic development of a mouse in general but may cause particular developmental deviations in such embryos.

Supported by Deutsche Forschungsgemeinschaft SFB 146, B 9. We are very much obliged to Prof. U. TEWES, Hannover, for his constructive remarks concerning the manuscript and to B. DREYER, B. LANGER and E. NEUMANN for their assistance on many occasions.

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Corresponding editor: J. W. DRAKE