

## Strain differences in radial arm maze performance of rats

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### Introduction

An ongoing discussion within the field of toxicology relates to the choice of animal species and strain for experimentation. Outbred stocks are often preferred because these animals represent a broad spectrum of individual phenotypes ranging from very sensitive to insensitive individuals. This means that the effects of the test compound will be revealed at least in some individuals. However, it also means that the responses of individuals will be variable – i.e. there is much biological "noise".

With a given group size, a difference between groups is more likely to be detected if the variability within groups is small.

As the purpose of most toxicological experiments is to detect a quantitative difference between different dose groups, the variability of responses of individuals given the same treatment should be minimized in order to allow clear identification of effects caused by the test compound. Further, a reduced variability may make it possible to reduce the number of experimental animals, which is desirable for ethical and economical reasons. Inbred animals are practically genetically identical. Therefore, one would expect much less variability in the responses of such individuals to the same treatment (Festing 1990). An argument against the use of inbred strains is that one may accidentally choose a strain that is either insensitive to the particular test compound, or abnormally sensitive.

One will rarely have the possibility to predict which strain will be optimal for a particular experiment. However, before initiating expensive and time-consuming experiments with an unfamiliar rat strain, the Institute of Toxicology finds it useful to conduct initial

studies of basic performance of these animals. The scope of the present experiment was to examine the behaviour of three different rat strains in a cognitive task, the eight-arm radial maze. The outbred Wistar rat was compared to the inbred strains Sprague-Dawley and Long-Evans.

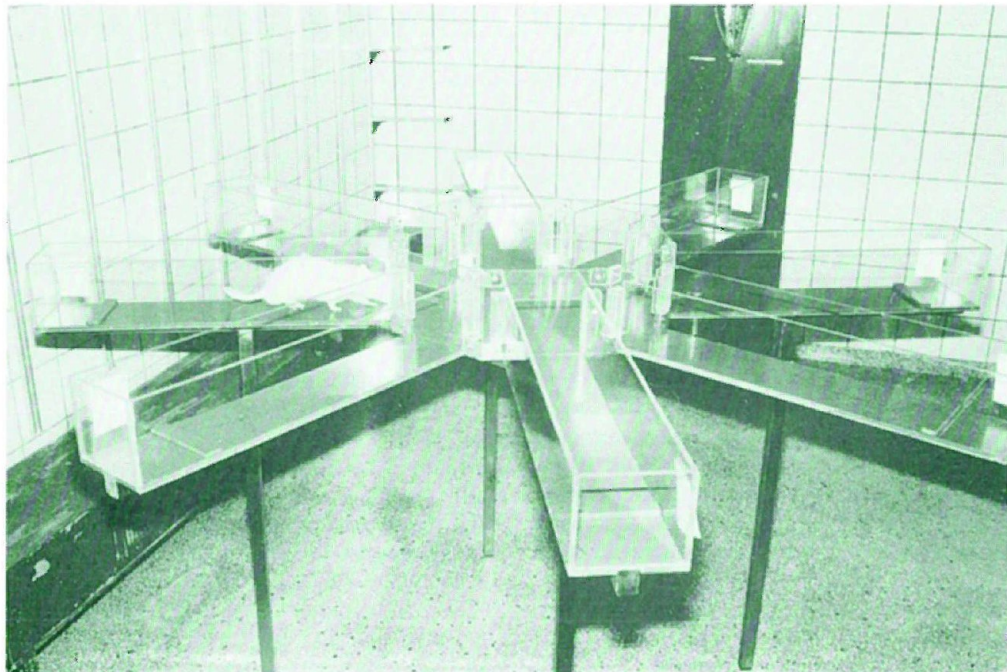
### Material and methods

#### Animals

Thirty experimentally naive male rats, three months of age, were obtained from Møllegaard Ltd., Ll. Skensved, Denmark. The strains were: Wistar (Mol:WIST); Long-Evans (LE/Mol); and Sprague-Dawley (SPRD/Mol); n = 10 in each group. The rats were kept singly in steelwire cages and fed a restricted amount of commercial pelleted diet (Altromin 1324, Brogård, Denmark). The amount of food was adjusted to keep the body weight of the rats at 85 % of ad libitum feeding weight. On test days the food was given after testing.

*Apparatus.* The radial maze was similar to that described by Olton & Samuelson 1976. Eight maze arms extended outward from a central maze area (see photo). The rat's movements were monitored via photo cells placed at the entrance and at the end of each maze arm; coupled to a computer. "Choice" was defined as the rat's passing the entrance photo cell. "Reward" was defined as the rat's passing the distal photo cell.

*Testing.* The rats were tested on weekdays only. In a one-week pretesting period, the animals were acclimatized to the experimental conditions. Bait (peanut chops) was strewn around the maze, and each rat was allowed five minutes' exploration of the maze. No data were collected during this week.



The radial maze. Eight arms extend outwards from a central area. Bait is placed distally in the maze arms, invisibly to the rat.

The following four weeks consisted of standard radial maze testing (*Olton & Samuelson 1976*). All eight arms were baited. In the daily test session, each rat was placed in the central maze area and allowed to explore the maze until all chops were collected, or 10 minutes had passed.

The remaining two weeks' testing consisted of two daily sessions per rat. In the first session (pre-delay trial or sample trial), four maze arms were shut off by insertion of a clear plastic barrier across the entrance. The remaining four open arms were baited. The rat was placed in the central area and was allowed to collect the bait. The rat was then removed from the maze and kept in its home cage for one hour. In the second session (delayed non-match-to-sample trial) all arms were left open, with bait only in those arms that had previously been shut off. The correct response in this session was to visit those

arms which had not been visited in the first session one hour earlier.

During the entire test period, the order of testing was randomized with respect to strain; and the rats were tested in the same sequence every day. In each session, the following measures were collected: **a.** Number of correct choices (first-time visits) among the open arms. **b.** Number of correct choices with reward among the open arms. **c.** Total number of choices. **d.** Total number of rewards. **e.** Number of different choices. **f.** Number of rewards obtained in first choice of arm. **g.** Total time to completion. **h.** Maximum number of sequential choices (choices of adjoining arms) **i.** Maximum number of sequential rewards.

#### Statistical analysis

Separate statistical analysis was performed on the "standard" and on the "delayed non-



match-to-sample" part (divided into pre-delay and post-delay session) of the experiment. All measures were subjected to repeated measures GLM (general linear model) analysis with strain as the independent variable, using the SAS PC version software packet. The frequency of tests being terminated by timeout was calculated for each strain. Further, the coefficient of variance was calculated for each strain and session. No statistical comparison of frequencies and coefficients of variance was performed.

#### Results and discussion

The observer noted several differences in behaviour between Long-Evans rats and the other two strains. Long-Evans rats were very docile and unresisting to being picked up. During handling, the muscle tone of the Long-Evans rats appeared much weaker than that in rats of the other two strains. When Long-Evans rats were placed in the radial maze, they did not show the exploratory activity which is usually observed in a novel environment, and often remained immobile for long stretches of time. None of these observations were quantitated.

Results from statistical analysis of radial maze measures from standard testing and post-delay sessions of delayed non-match-to-sample testing are shown in tables 1-3.

*Standard testing.* (Table 1). A significant time effect was found on all examined para-

eters except one (maximum no. of sequential choices); indicating that these measures are probably all relevant for the assessment of the learning process; and statistically coupled to each other. *Strain* effects were found on several measures, but not on the principal measure of performance, the no. of correct choices among the eight first choices (Fig. 1). The improvement in mean performance was from 6.2-6.3 correct choices to 7.5-7.9. The no. of correct choices among the eight first choices with reward (and the total number of rewards) was significantly lower in Long-Evans rats; indicating that these rats initially were reluctant to enter the maze arms beyond the entrance - this was evident during the initial week of training where Long-Evans rats obtained a score of 4 to 5 while the other two strains scored 6 to 7 (Fig. 2). The most striking difference between strains was found in the amount of time to completion, where Long-Evans rats required much longer time throughout the entire test period. The time consumption of Long-Evans rats was down to 217 seconds, while Sprague-Dawley used 95 seconds and Wistar 120 seconds (Fig. 3). This is not evidence of impaired learning, as the number of correct choices was not lower in Long-Evans rats. Nor was the total number of choices lower in this strain. The maximum number of sequential choices (and rewards), a measure of the tendency of the rats to employ non-working memory strategies (choosing

Table 1. Repeated measures GLM analysis. P values. Training series 1-20 (Standard training).

	Strain	Time	Strain > < Time
No. of correct choices in 8 first	n.s.	***	n.s.
No. of correct choices with reward in 8 first	**	***	*
Total no. of choices	n.s.	***	n.s.
Total no. of rewards	***	***	***
No. of different choices	**	***	**
No. of rewards obtained in first choice of arm	***	***	***
Total time to completion	***	***	***
Maximum number of sequential choices	n.s.	n.s.	**
Maximum number of sequential rewards	n.s.	***	n.s.

n.s.: not significant,  $p > 0.05$ . \*:  $p < 0.05$ . \*\*:  $p < 0.01$ . \*\*\*:  $p < 0.001$ .

adjoining arms instead of remembering specific arms), did not differ between strains. The within-strain variability with respect to the number of correct choices was generally similar in the three strains. Wistar rats showed greater variability with respect to time to completion (Fig. 3), while Long-Evans rats performed more variably with respect to the number of correct choices with reward (Fig. 2). The frequency of tests terminated by timeout (Table 2) was initially much higher in Long-Evans rats compared to the other two strains. Sprague-Dawley rats only showed a single time-out in the first session. Wistar time-outs beyond the first sessions could be ascribed to a single individual.

Table 2. The number of animals where testing was terminated by time-out (10 minutes) during standard radial maze testing.

Test	Long-Evans	Sprague-Dawley	Wistar
1	5	1	4
2	4	0	1
3	3	0	1
4	6	0	0
5	4	0	0
6	2	0	0
7	1	0	1
8	0	0	0
9	1	0	1
10	1	0	1
11	0	0	1
12	1	0	0
13	0	0	0
14	1	0	0
15	0	0	1
16	1	0	0
17	1	0	0
18	1	0	0
19	0	0	0
20	0	0	0

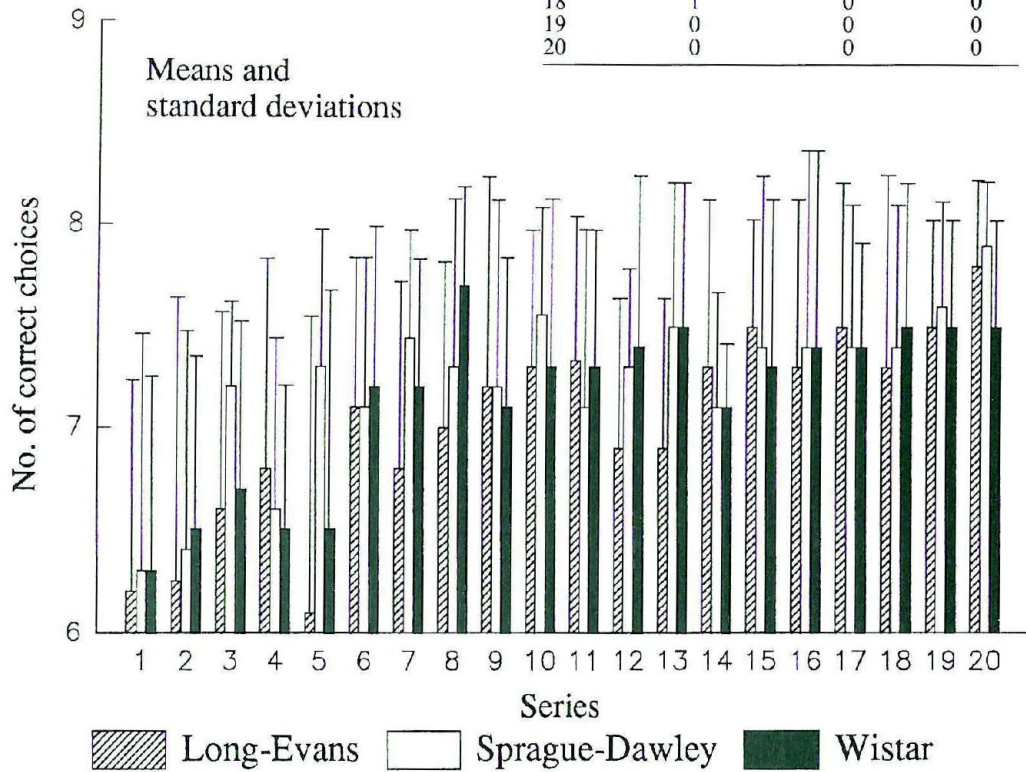


Fig. 1. Standard radial maze test. The no. of correct choices among the eight first choices. This is the principal measure of performance in the radial maze.



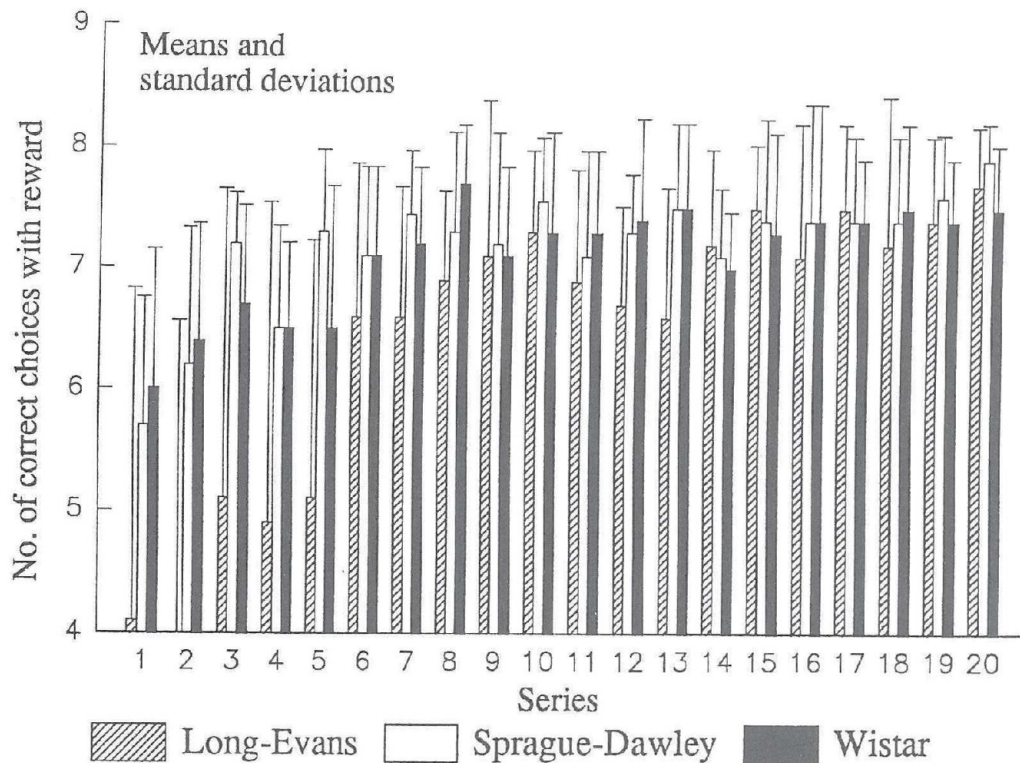


Fig. 2. Standard radial maze test. The no. of correct choices among the eight first choices with reward.

*Delayed non-match-to-sample testing.* (Table 3). Although the duration of this part of the radial maze test was very short (normally six weeks' testing is performed) a significant *time* effect was apparent on most mea-

asures. The only *strain* difference was found in the total time to completion; again, Long-Evans rats took much longer to complete the task. If the testing had been continued, further strain differences may have appeared.

Table 3. Repeated measures GLM analysis. P values. Training series 22-44 (Delayed non-match to sample, second session).

	Strain	Time	Strain > < Time
No. of correct choices in 8 first	n.s.	***	n.s.
No. of correct choices with reward in 8 first	n.s.	***	n.s.
Total no. of choices	n.s.	***	*
Total no. of rewards	n.s.	n.s.	n.s.
No. of different choices	n.s.	***	n.s.
No. of rewards obtained in first choice of arm	n.s.	n.s.	n.s.
Total time to completion	***	*	n.s.
Maximum number of sequential choices	n.s.	*	n.s.
Maximum number of sequential rewards	n.s.	n.s.	n.s.

n.s.: not significant,  $p > 0.05$ . \*:  $p < 0.05$ . \*\*:  $p < 0.01$ . \*\*\*:  $p < 0.001$ .

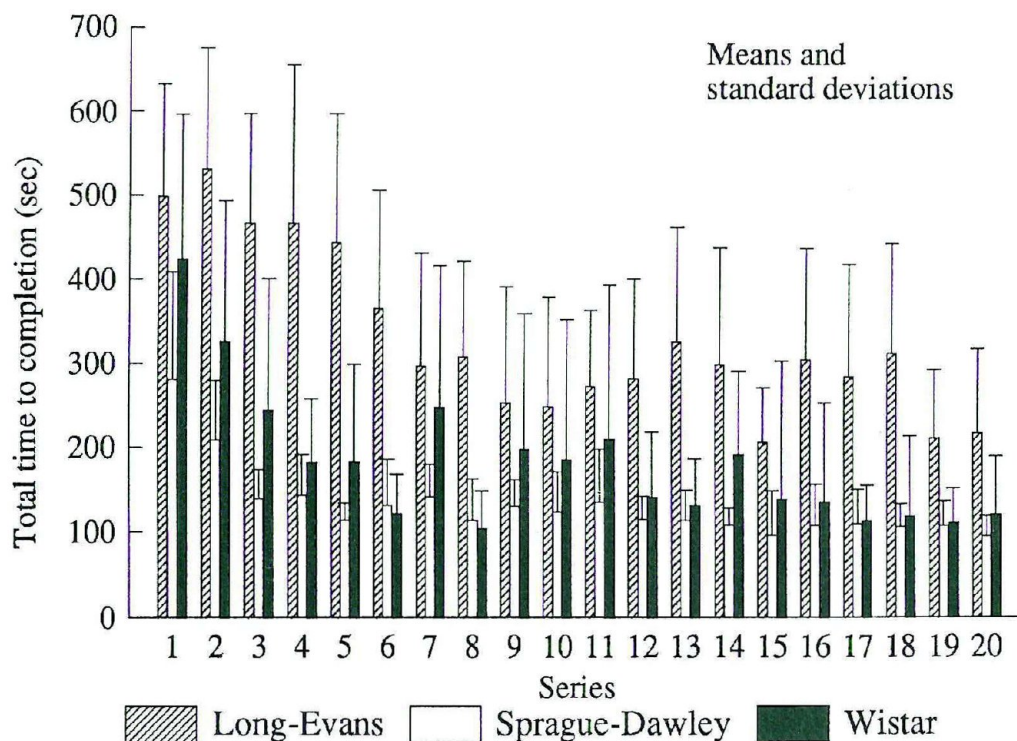


Fig. 3. Standard radial maze test. The total time to completion. The maximum time allowed was 10 minutes (600 sec).

**Conclusion**

Strain differences were found in several parameters. The most striking difference was in the time required to complete the task, where Long-Evans rats clearly required much more time than the other two strains. This also meant that Long-Evans rats were more often removed from the maze because of time-out, and thus were not allowed the same learning opportunity as the other rats. In spite of this, all strains learned the task equally well as measured by the principal performance parameter, the number of correct choices. Wistar rats did not in general perform with greater variability than the two inbred strains. However, outbred rat stocks are subject to inbreeding, random genetic drift, and the selection of particular phenotypes which lead to a certain degree of genetic similarity of

outbred individuals from a particular breeder (Festing 1990), so the Mol:WIST rats of the present experiment may in fact not be truly outbred. The results of the experiment, of course, cannot be used to predict the response of each strain to a test chemical. The experiment clearly demonstrates strain differences in basic behavioural parameters.

**Summary**

Rats of three different strains, one outbred and two inbred (Wistar, Mol/WIST; Long-Evans, LE/Mol; Sprague-Dawley, SPRD/Mol; n = 10 in each group) were tested in a radial maze training procedure. Throughout the entire test period, the rats were fed a restricted amount of food. Testing was initiated with one week's adaptation where rats were placed in the radial maze for five minutes each. This was followed by four weeks of testing consisting of one daily training session per rat with all eight arms open and baited (classical



procedure). During this period, a stable performance was established. The remaining two weeks' training consisted of two daily sessions: In the first, four arms were shut, and the remaining four arms were baited. The rat was allowed to collect the bait, after which it was removed from the maze to the home cage for one hour. In the second session all arms were open and bait was placed in those arms only that had previously been shut (delayed non-match-to-sample procedure). Analysis of various aspects of performance in the maze showed comparable learning ability of all strains. However, certain strain differences were clearly demonstrated.

#### *Sammendrag*

Tre forskellige rottestammer, en udavlet og to indavlede (Wistar, Mol:WIST; Long-Evans, LE/Mol; Sprague-Dawley, SPRD/Mol; n = 10 i hver gruppe) blev testet i ottearmet labyrint. Igennem hele forsøgsperioden blev dyrene fodret restriktivt. Testning blev indledt med en tilvænningsperiode på en uge, hvor hver rotte blev anbragt i labyrinten fem minutter. I de næste fire uger blev hver rotte testet i det klassiske paradigme, hvor alle otte arme er åbne og forsynet med godbidder. I løbet af disse fire uger opnåedes en stabil præstation. De sidste to uger af forsøget kørtes den daglige testning i to sessioner: I den første session blev fire arme spærret, mens de øvrige blev forsynet med godbidder. Rotten fik lov til at tage godbidderne, hvorefter den blev flyttet i sit bur igen i én time. I den anden session stod alle otte arme åbne, men der blev kun anbragt godbidder i de fire arme, der var spærret i første omgang ("delayed non-match-to-sample" procedure). Analyse af forskellige testparametre viste, at indlæringscurven overordnet set var den samme hos alle tre stammer, men at der var klare forskelle imellem de testede stammer på visse parametre.

#### *Yhteenveto*

Kolmeen eri kantaan kuuluvia rottia, yksi ulkosiittoinen ja kaksi sisäsiittoista (Wistar, Mol/WIST; Long-Evans, LE/Mol; Sprague-Dawley, SPRD/Mol; n = 10 kussakin ryhmässä), testattiin säteettäislabyrintiharjoituksessa. Rotat olivat kokeen aikana rajoitetulla ruokinnalla. Testaus aloitettiin viikon totuttautumisjaksolla, jolloin rotat viettivät kukin labyrintissä yksittäin viisi minuuttia kerrallaan. Tämän jälkeen seurasi neljän viikon testijakso, jolloin rottia harjoitettiin kerran päivässä kaikki kahdeksan säteettäistä käytävää avoimena ja kussakin syöti. Tänä aikana voitiin havaita säännöllinen oppimissuoritus. Jäljelläolevan kahden viikon harjoitus koostui kaksiosaisesta harjoituksesta: ensimmäisessä osassa neljä käytävää oli suljettuna ja lopuissa neljässä oli syötit. Rottien annettiin kerätä syötit, jonka jälkeen rotat palautettiin häkkiinsä tunniksi. Toisessa osassa harjoitusta kaikki käytävät olivat avoimina ja syötit pantiin vain ensimmäisellä kerralla suljettuina olleisiin käytäviin. Suoritusten osien analysointi osoitti yleisesti ottaen samanlaista oppimiskykyä kaikissa kolmessa kannassa. Kantojen välillä voitiin kuitenkin selvästi osoittaa myös joitakin eroavaisuuksia.

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