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The aged mouse as a model of cognitive decline with special emphasis on studies in NMRI mice

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The use of the aged mouse as an integrated model of age-related cognitive decline is reviewed, with special emphasis on experiments covering the life span of NMRI mice, using different age-groups ranging from 3 through to 22 months. Age-related changes in the sensorimotor profile, spontaneous behaviour and performance in learning and memory tasks are considered. The data provide evidence for cognitive impairment and decreases in spontaneous activity and exploration from middle age onwards. Chronologically, this age depends on the longevity of the strain selected; in NMRI mice, middle age corresponds to 11–12 months. Complex learning tasks, such as the Morris water maze for spatial learning, appear to be the most sensitive to age-related changes, as are tests requiring prolonged retention of acquired information, for example, using passive avoidance. Cued and simple discrimination learning are only impaired in the oldest animals. Age-related changes in non-cognitive variables, including sensorimotor capacity, pain sensitivity, emotionality, or locomotor activity, do not account for the learning impairments, although deficits in visual acuity cannot be excluded in the very old animals. Detailed analysis of the individual data for middle aged and old mice, using discriminant and correlation studies highlight a marked heterogeneity between animals of any given chronological age. Furthermore, individual aged mice do not exhibit similar degrees of impairment across all the behavioural variables, showing that aging is not a uniform process. The possible relationship between age-related behavioural decline and neurochemical changes is an area as yet unexplored apart from a few isolated investigations, including a study on ChAT and AChE in NMRI mice. The studies in the NMRI mice illustrate the value of investigating the full age-range to detect an age group which shows cognitive decline dissociable from physical or emotional changes and which is representative of the population as a whole.

INTRODUCTION

Alzheimer's disease (AD), including both early onset Alzheimer's dementia and senile dementia of the Alzheimer's type, accounts for the major proportion of neurodegenerative diseases. In the United States, according to a recent analysis[1], the incidence of AD now stands at more than 100,000 new cases per year. This is approximately double the incidence of the other major neurodegenerative diseases (Parkinson's disease, Huntingdon's disease and amyotrophic lateral sclerosis) taken together. With an ever increasing population of aged persons, it is clear that AD represents a growing socio-economic problem. It is therefore not surprising that a great deal of research effort is being directed at investigating animal models for AD, both for better understanding of the degenerative processes and in the search for means, including drugs, to alleviate the condition.

Any animal model aims to incorporate at least some of the characteristics of the disease in question. Although, in the case of AD, there is no single animal model which reproduces the disease's primary pathological markers, the study of AD involves numerous interdependent levels of analysis, from molecular biology to behaviour. This gives rise to the development of different types of models which together contribute towards advancing understanding of the disease state. For example, models involving lesions or pharmacological manipulation, generally in young animals, are used to mimic certain of the known biochemical changes, such as reduced cholinergic transmission[32,47,49]. In this type of model, the technique of the transgenic mouse (see Lannfelt et al., this issue) seems promising.

An additional, more integrative approach is that of using aged animals. This approach supposes an over-

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lap between AD and normal brain aging in humans, a debate of long standing[19] but supported by data indicating parallel but more marked biochemical changes in AD as occur in the normal aged brain. Further, this approach supposes that the aged animal is relevant to the aged human. In effect, aged animals share certain biochemical deficiencies with AD as well as showing neuronal degeneration and declining cognitive functioning such as memory impairment and disorientation, characteristic of the early stages of the disease[2,3,42]. On such a basis, most studies featuring aged animals for modelling AD, have involved rats or where possible, aged nonhuman primates. Although, in terms of evolution, the non-human primate may be most relevant[1], for practical and economic reasons, the rat is generally the animal of choice.

An alternative to the rat is the mouse. However, to date, in the context of AD, the aged mouse has generally been overlooked, an oversight partly dictated by the size of the brain limiting biochemical and histological analyses and hence behavioural neurochemical–pathological correlates. On the other hand, precisely because of its small size, the aged mouse offers an even more practical and economic advantage over the rat. A lot of information is available relating to life spans and the mouse has been widely used for assessment of age-related physiological changes such as changes in immune responses, reproduction and neoplasms[41]. However, among the 250 or so recognised inbred strains of mice, few have been examined as models of AD. Notable exceptions which are receiving considerable attention are firstly, the Senescent Accelerated mouse, developed by inbreeding from the short-lived AKR strain[51]. This strain exhibits early onset of impaired learning and memory as well as certain neurochemical changes associated with the aging process[39,52]. Secondly, as recently reviewed[13,25], specific short-lived strains of mice prone to autoimmune disorders likewise present cognitive deficits and are being investigated as models of age-related dementia.

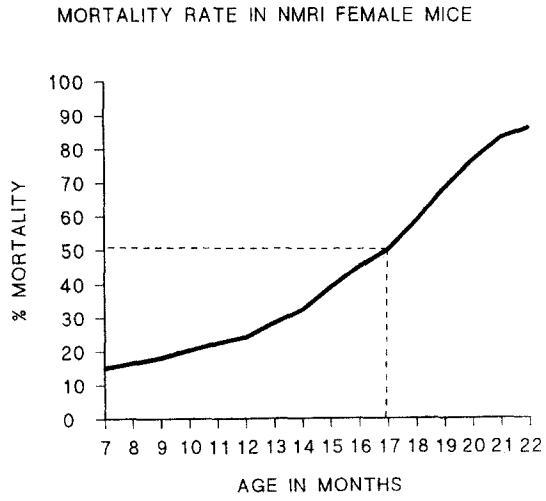

Fig. 1. Cumulated mortality in female NMRI mice (bred at UCB, Braine L'Alleud, Belgium) as a function of age. The 50% mortality rate coincides with the age group of 17 months. The mortality rate was calculated on a sample of 1023 subjects over 4 years.

Aside from these specific inbred strains, there is sufficient information supporting consideration of aged mice in general for modelling specific aspects of AD. In particular, we have carried out studies with the NMRI strain, showing age-related changes in behaviour, including impaired performance in learning and memory tasks[28,29,30,31]. These studies, which include consideration of additional factors, such as age-related physical disabilities, will form the basis of this review, supplemented by other published data on aged mice.

HOW OLD IS AN OLD MOUSE?

A basic problem encountered in any research into age-related changes in animals or humans, lies in defining biological or functional aging[20]. Chronological aging is generally used as an indicator of the age state of the animal and reference to the survival curve constitutes a good experimental starting point. Since the longevity is strain-dependent, it is important to obtain survival curve data relating to the particular strain under investigation. If possible, it is advisable to establish the survival curve under the laboratory conditions routinely employed, because of the known influence of the husbandry conditions on the survival rate. The survival curve of the NMRI mice used in our studies is given in Fig. 1 and shows a 50% (median) survival age of 17 months. This contrasts to, as an example, the median life span of 27 to 31 months of certain C57BL strains[41].

A common practice in age-related studies is to select and compare performance of one group of aged animals with one group of young animals, often without reference to the expected survival. This practice assumes that aging follows a linear function and can be readily criticized[20], raising questions such as the age of onset of the change, or whether the phenomenon is truly age-related or relevant only to a small percentage of survivors. To answer such questions, it is desirable to compare a selection of different age-groups, in parallel. Accordingly, our basic studies were undertaken using mice aged 3, 6, 9, 11–12, 17 and 22 months respectively. These age groups represent 87, 85, 80, 77, 50 and 10% of the original population. Thus, in terms of the life expectancy, this spanned from young mature (3–6 months), middle-aged (9–12 months) to old age (17 to 22 months). Within these age groups, a preselection

was carried out in order to minimize the influence of age-related physical incapacitation on behavioural performance. To this end, animals with gross defects such as tumours, spontaneous motor incapacitation and overt blindness were excluded prior to experimentation. In practice this amounted to 12% of the 11–12 month age group, 25% of the 17 month age group and 30% of the 22 month age group. The age-groups of mice selected were subjected to a range of tests, including sensorimotor evaluation, spontaneous activity measurements and learning and memory tasks.

Sensorimotor considerations

Among the potential non-cognitive factors which may influence complex behaviours such as performance in learning and memory tasks are sensory and motor changes. While such changes are integral to the aging process, it is important that they be identified, in order to more fully interpret age-related changes in performance.

We evaluated sensorimotor capacity over the life span of the NMRI mouse in a battery of tests to assess the following: visual acuity using the visual placing reflex; muscle strength and traction in terms of ability to grasp and remain suspended from a taut, horizontal wire and psychomotor integration and equilibrium in walking along an elevated narrow bar. The results showed that the oldest mice, aged 17 and 22 months, were impaired only in those tasks with a high demand on muscular strength, motor coordination and maintenance of equilibrium, i.e. traction and walking along a narrow rod.

The tests used in our battery were based on studies described by Dean et al.[8] and Ingram[20], both investigating motor performance in cross-sectional age samples of C57BL/6J. Our results corroborate their findings of most marked age-related deficits in tasks with high physical demand. However, there is a clear difference in the chronological age at which severe deficiencies were observed, this being at least 25 months in C57BL/6J mice compared to 17 months in NMRI mice, consistent with the extended expected life span of the former.

Depending on the particular behaviours under investigation, it is sometimes necessary to investigate specific sensory or motor functioning as distinct from a general battery of tests for physical capacity. For example, in our studies[30], since the mice were subjected to a Morris-type water maze, we also assessed the swimming ability and speed to swim along a straight alley directly to a visible platform to escape from the water. Under these conditions, no statistically significant deterioration was detected in mice aged up to 22 months. It can therefore be concluded that neither motivation to escape from water, nor swimming ability nor swimming speed per se deteriorate with age.

Another specific and well recognised example of possible age-related changes in sensory perception influencing performance is the detection of aversive or painful stimuli used to motivate behaviours, such as escape or avoidance. However, although age-related impaired acquisition of passive avoidance has been reported (see below), the deficits do not appear to be attributable to decreases in sensitivity to electric shock[8,24,28,45].

The factors referred to above serve as examples to draw attention to possible non-cognitive variables which may account for age-related behavioural changes. Clearly, it is important that each experimental design considers the possible influence of such variables, to include the necessary control evaluations.

Spontaneous locomotor activity and exploration

In the previous section, we discussed possible specific sensory and neuromotor deficits occurring in aged animals. Complementary to this are assessments of age-related changes in spontaneous locomotor and exploratory behaviour. Consequently, we examined the behaviour of different age groups of NMRI mice in an open field; hole board exploration; plus-maze activity and Y-maze exploration; methodological details are given elsewhere[28,30]. Evaluation of the NMRI mice on locomotor activity in a square open field showed reduced activity from middle age (9–12 months) onwards. Exploration of a simple hole board was likewise reduced. However, no statistical differences were apparent between groups aged 11, 17 or 22 months, in either test, indicating declines between maturity and middle age, but remaining stable thereafter.

A similar age-related pattern in spontaneous open field activity was reported by Dean et al.[8] for C57BL/6J mice; marked decreases were observed in 9-month-old mice compared to the 3-month-old group, with little decline with age up to 23 months and only slight further decreases at 31 months. Lhotellier and Cohen-Salmon[35] obtained similar findings for both wheel running activity and nose-pokes in a hole board in 3 strains of mice, namely, C57BL/6J, BALB/C and DBA/2. All 3 strains had a similar life expectancy and all three showed declines in activity at 400 days compared to 150 days with little further change at 750 days. However, whereas declines in ambulatory activity occurred at 400 days for the C57 mice, confirming the findings by Dean et al.[8] in this strain, activity at 400 days in BALB/C and DBA/2 mice remained elevated, but had declined by 750 days.

Thus, it seems that decreases in general locomotor and exploratory activity, once established in late adulthood to middle age, do not become progressively more marked into old age. This observation is important since it eliminates an obvious variable accounting for age-related changes in more complex behavioural situations, not only ones designed to assess aspects of learning and memory but also ones for evaluating emotionality, for example, the elevated plus-maze.

The elevated plus-maze test is applied as an anxiolytic test, based on the natural aversion of rodents for open, exposed spaces. The maze consists of a square cross made up of two open and two closed arms, raised above floor level. The dimensions and design appropriate for mice have been described by Lister[36], although, according to our experience, it may be necessary to enlarge the width of the arms to take into account the larger size of the aged mouse. When NMRI mice were tested in this paradigm, changes in the indices considered to reflect the emotional state, i.e. fear, fearfulness, only occurred in the oldest mice (17–22 months) and not for mice aged 12 months or younger. Such indices include the ratio of exploration of the open arms to closed arms, hence independent of the total activity. These results could be interpreted as indicating greater emotionality in old mice placed in a stressful situation. Although a test based on spontaneous behaviour, the age of onset of effects in the plus-maze was superior to the age at which changes in general exploratory activity may be detected, hence illustrating a dissociation from the latter.

A similar dissociation from general activity decreases may be inferred from results obtained in a Y-maze, a simple test for evaluating spontaneous spatial orientation. This test consists of a symmetrical Y-maze and is based on the spontaneous ability of mice and rats to alternate places and thus to explore that part of the maze visited least recently. Spontaneous alternation in NMRI mice aged 11 months and more, decreased to chance level compared to 65 to 75% alternation observed in young animals. Moreover, an additional measure of position bias, based on the preferred sequence of responses indicated evidence of a rotational tendency in the 17- and 22-month-old mice, a result interpretable in terms of a switch from allocentric orientation influenced by the environment, to egocentric responding. A similar phenomenon is obtained following cholinergic blockade or hippocampal dysfunction[37,55].

Thus, even under relatively simple testing conditions, it is possible to detect age-related changes in spontaneous behaviour, independent of changes in general activity and exploration.

Age-related changes in learning and memory paradigms

The assessment of learning ability and memory is fundamental to any behavioural model for AD. Age-related deficits have been reported in several situations although, as noted earlier, demonstration of the age-related effect often depends on a comparison of one age-group of old mice with one group of young adults, thereby preventing any conclusions as to its progressive nature. The situations investigated range from the straightforward passive avoidance test through to complicated discrimination and spatial learning tasks. Motivation to support acquisition and performance of learned behaviour depends on standard procedures such as escape from electric shock, food or water reinforcement in deprived animals or escape from water, with the possible introduction of particular conditions either to equilibrate the motivation levels or to take into account special needs of the old animals.

Passive avoidance learning

By virtue of its simplicity and rapidity, the passive avoidance test is conveniently used to assess learning and memory in rodents and is often incorporated into routine screening programmes. We therefore evaluated the performance of different age-groups of NMRI mice, namely groups aged 3, 6, 9 and 12 months, in a single-trial, stepthrough paradigm[28]. A retention deficit could be demonstrated 24 h after acquisition in the 9- and 12-month age-groups.

These results confirmed previous findings of age-related deficits, in different mouse strains. For example, using a like paradigm, Bartus et al.[5] examined passive avoidance retention in C57BL/6J mice across an age-span from 2 to 31 months and observed a decline from 13 months, attaining significant levels at 23 months and older, both at 24 h and 5 days post-acquisition. In a later study, Puglisi-Allegra et al.[45] were unable to substantiate an age-related difference in C57BL/6 mice, comparing 6, 12 and 24 month age-groups but obtained a clear-cut deficit in retention at 48 h in 24-month-old BALB/C mice. Leslie et al.[34] reported age-related deficits in 24 h retention of stepthrough passive avoidance in aged CFW mice. Using a stepdown passive avoidance procedure, Kubanis et al.[24] reported a significant deficit in 24 h retention but no effect at 2 h post-acquisition, for 20–21-month-old Swiss mice, compared to ones aged 3–5 months.

Thus, it can be concluded that retention of passive avoidance of electric shock declines progressively with age in mice, with the chronological age of onset depending on the mouse strain but approximating to middle age and above. Since sensitivity to electric shock appears unaffected by age, the effects on passive avoid-

ance can be attributed to age-related deterioration of retention.

Active avoidance/escape learning

The independence from age of shock sensitivity, the ease of provoking responding and the independence from deprivation schedules makes electric shock an attractive motivating agent to use in learning experiments, especially in aged animals. In addition to passive avoidance, escape or avoidance of electric shock has been applied to determine age-related changes in active learning paradigms in mice. Forster and Lal[12] reported a progressive increase from 6 months onwards in the number of trials to attain criterion in C57BL/6Nnia mice, in a step-up active avoidance test, indicative of age-related declines in rate of acquisition. Age-dependent impairment of retention at 48 h was observed from the age of 12 months.

Using a simple T-maze avoidance-escape test, in which a buzzer, paired with the raising of the start box door, served as the conditioned stimulus (CS) for the presentation 5 s later of electric shock, the unconditional stimulus (UCS), Flood and Cherkin[11] likewise showed an apparent deficit in the rate of learning in 24-month-old animals. The old mice required more trials to reach the criterion of 1 avoidance response, i.e. entering the correct goal box before the onset of the UCS. The old mice also showed impaired retention when tested 7 days later, taking more trials to reach the criterion of the first avoidance response and more trials to achieve the criterion of 5 out of 6 consecutive avoidances. Unfortunately, the authors only reported on avoidance responses as the dependent variable without mentioning escape responses as possible learning parameters. The 5 s CS-UCS was perhaps too short to allow for possible slowed responding in the aged mouse.

However, such a possibility of physical impairment was taken into account by Crady and Quinton[7], employing a more complicated avoidance-escape procedure. These authors similarly concluded an age-related decrease in the rate of learning, in a study which considered both avoidance and escape reponses. C57BL/6J mice, either 7–10 or 27–30 months were required to learn to escape from electric shock by selecting the correct goal box out of a choice of 5, identified by light. The older mice needed more sessions to achieve the same escape success rate as their young counterparts, making consistently more errors during the early sessions. This illustrates that the association function was more difficult for the old mice to learn, although Crady and Quinton were careful to show that this was not attributable to age-related visual impairment. In contrast, indices of physical performance in the old mice, such as response latencies and freezing, remained inferior throughout, indicating a dissociation from the learning impairment. Such factors would have contributed to the lower rate of avoidance responses observed in the old animals.

Finally, Ingram[22] reviewed studies supporting age-related impairments in reference memory. Various age-groups of 3 different strains of mice, namely, C57BL/6J, A/J and C3B1ORF$_1$ mice, were trained in a 14-unit Stone maze, motivated by shock avoidance or escape. In essence, the Stone maze consists of a series of T-maze choices. At each choice point, the mouse had 10 s to make an avoidance response. Appropriate consideration was given to non-cognitive influences. Age-related impairment, assessed in terms of number of errors, was most marked in 24-month-old A/J mice, contrasting with C57BL/6J mice which showed only modest, age-dependent, error increases from 6 months through to 30 months.

Morris maze learning

There are several studies demonstrating age-related deficits in spatial learning in mice. The Morris water maze[40] in which rodents have to escape from water, is of particular interest as a test of spatial learning since it obviates the need for food or water deprivation or electric shock to motivate behaviour. We have carried out detailed studies[28,29,30,31] with NMRI mice, using a Morris type water maze scaled down for mice. The maze consisted of a circular plastic pool, 90 cm in diameter, filled with opaque water and having a platform either concealed below the level of the water at a fixed position in the pool for place learning or rendered "visible" by attaching a cue, consisting of a black and white striped flag, to signal its position for cued learning. Under place learning, only extra maze cues can serve to locate the platform. We have shown elsewhere[26,27] that young mice rely exclusively on fixed environmental cues to find the platform, even when given the opportunity of adopting a response strategy, such as might be expected by always starting from the same place in the pool. Thus, place learning in young mice appears to depend on allocentric orientation.

In the place learning situation, each animal was tested for 4 consecutive days, receiving 3 trials per day, each trial starting from a different position at the perimeter of the pool, with a set time on the platform after each trial. The time (latency) taken to reach the platform was recorded as an index of learning and memory. A probe trial of 100 s was given at the end of training to assess memory, and consisted of removing the platform and recording the time spent in the quadrant (quadrant bias) in which the platform had previously been located.

Quadrant bias has been shown to depend on the number of training trials[26] and can therefore be used as a measure of degree of learning. Mice aged 3 months acquired the spatial learning efficiently with latencies progressively declining during training, and a significant quadrant bias recorded in the probe trial (Figs. 2 and 3). Mice aged 11, 17 or 22 months acquired this learning more slowly, although there was little difference between the different age groups. This observation, plus the selection procedure, plus the lack of effect on swimming speed commented on earlier, indicate that the impairment in learning is unlikely to be attributable to physical incapacitation.

In contrast to the place learning, when the mice were tested for cued learning after a 72-h break, during 3 further sessions (days 7–9 in Fig. 2), the mice aged 11 and 17 months were able to achieve a final level of performance equivalent to the young mice, despite being inferior at the start (day 7). However, performance remained inferior in the mice aged 22 months. These results suggest that the cognitive ability to orientate in space is affected in middle aged and old mice.

However, this claim is made with some reservation, since while cued learning depends on a proximal well defined stimulus, spatial learning depends on more distal diffuse stimuli. The possibility that the age-related deficits in spatial learning may be due to age-related reductions in visual acuity or perceptual sensitivity should be considered. We approached this problem using an investigational procedure inspired by one described by Pelleymounter et al.[43] for rats. In a first experiment, the spatial learning of 17- and 3-month-old mice were compared in a Morris maze equipped with a black panel subtending an arc of 110° around the inside wall of the maze. Other extra maze cues were excluded by a high circular screen enclosing the pool. Under these conditions, it is rationalized that the salience of the environmental stimulus for place learning approaches that provided by the cue in cued learning or that provided by a simple visual discrimination task where old animals are generally not impaired[7]. The aged mice acquired this learning more slowly than the young mice but by the fourth training session, performances of the two age groups were very similar, confirmed by similar quadrant biases in the two groups during the probe trial (Figs. 4 and 5). However, when retested after an interval of 72 h (day 7), the aged mice showed retention deficits although performance was quickly re-established on a second and third day of retesting (days 8 and 9). The salience of the black cue in supporting the spatial learning was demonstrated in a final probe trial during which the black arc was removed; the quadrant bias of both age groups fell to chance level (Fig. 5).

In a second experiment, the mice from experiment 1 were subjected to prolonged training under conditions of classical spatial learning, i.e. depending on distal extra maze cues. Despite continued practice, the mice aged 17 months continued to present learning deficits, with latencies plateauing at a consistently higher level, suggesting that the old mice had reached their optimal level of performance in this test situation. When tested subsequently for cued learning, these old mice could perform as well as the young mice.

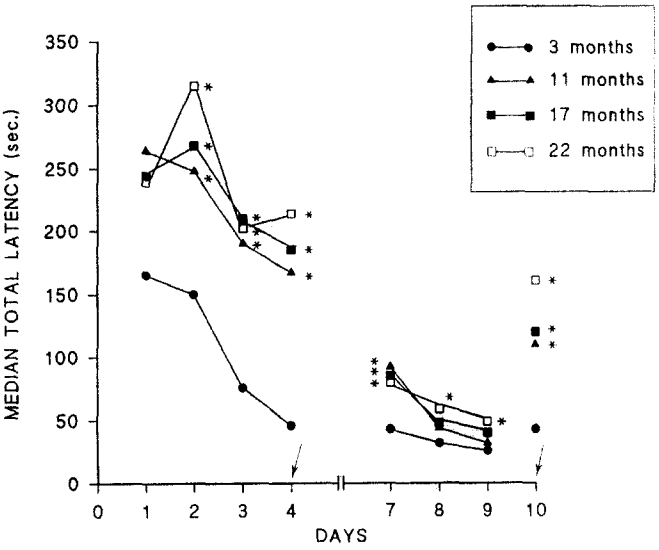

Fig. 2. Median escape latencies for 3, 11, 17 and 22 month age groups during place learning (days 1 to 4), cue learning (days 7 to 9) and retest place learning (day 10) in the Morris maze. Arrows indicate probe trials given at the end of sessions. Asterisks indicate a significant difference in comparison to the mice aged 3 months, $P < 0.05$ (adapted from Lamberty and Gower[30]).

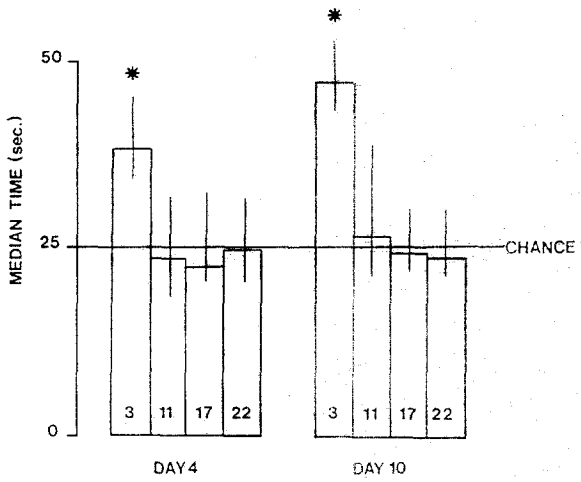

Fig. 3. Median time (with interquartile values) spent in the platform quadrant for 3-, 11-, 17- and 22-month-old mice during probe trials given at the end of the place learning test and retest (days 4 and 10). Asterisks indicate a significant difference with respect to chance level, $P < 0.01$ (adapted from Lamberty and Gower[30]).

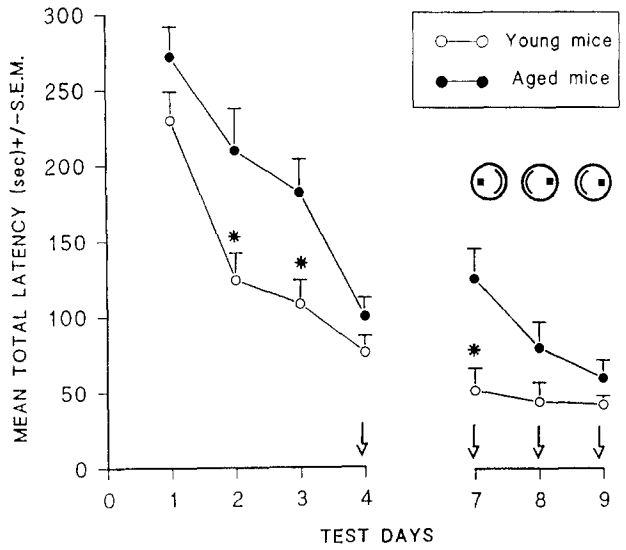

Fig. 4. Mean total escape latencies per day of young (3 months) and old (17 months) mice in the simplified place learning task. Arrows indicate probe trials given at the end of sessions. The circular diagrams above days 7, 8 and 9 indicate the position of the arc relative to the platform. The configuration on day 7 was also used during the first 4 days. On days 8 and 9, both arc and platform were rotated through 180°. Asterisks indicate a significant difference, $P<0.05$ (adapted from Lamberty and Gower[29]).

From the results of this investigation, it is evident that the salient configuration of a simple distal cue has a beneficial effect on place learning in NMRI mice, resulting in a level of performance equal to young mice, despite a slower rate of acquisition. This, together with the persistent impairments under classical conditions despite prolonged learning, suggest that old animals are

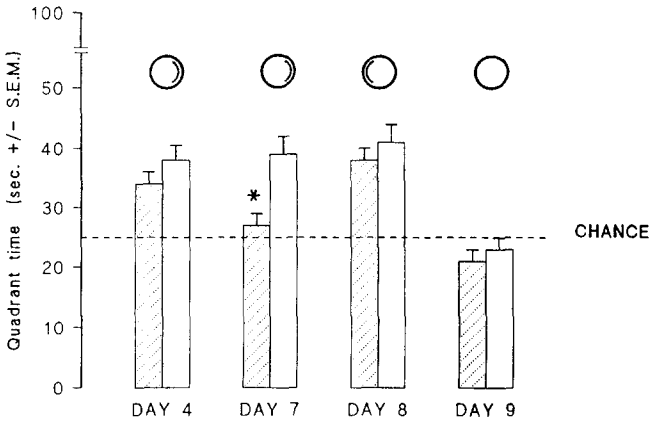

Fig. 5. Mean time spent in the platform quadrant for young (open columns) and aged mice (hatched columns) during probe trials given at the end of days 4, 7, 8, 9. The circular diagrams above each histogram represent the position of the black arc relative to the platform for each probe trial. The arc remained in the same position on days 4 and 7, was rotated 180° on day 8, and was removed on day 9. Asterisks indicate a significant difference, $P<0.05$ (adapted from Lamberty and Gower[29]).

impaired in their ability to select and integrate relevant information to guide them to the goal. However the facilitative effect of the simple black cue, imposing very little demand on visual acuity, also points to impaired visual ability as a contributing factor to the impaired spatial learning in aged mice.

Appetitive learning

Appetitive learning in aged mice has been less investigated, probably because of practical problems encountered such as enhanced mortality due to deprivation schedules and difficulty in sustaining motivation. In general, the findings support age-related disturbances although it is difficult to ascertain whether or not the deficits are attributable to cognitive deterioration or to confounding non-cognitive variables. Warren[54] carried out a detailed analysis, involving different experimental paradigms, in C57BL/6J and DBA/2J mice, aged 100, 200, 400, 600 or 700 days. Learning was reinforced mainly by water, with food used on one occasion, with appropriate concomitant deprivation schedules. Old mice were not impaired in learning simple spatial discriminations such as the Lashley maze, but were deficient in learning visual discriminations and discrimination reversal problems and complex mazes. However, the high mortality of the aged mice, possibly linked to starvation in water deprived animals, led Warren to conclude that appetitive learning is contraindicated as a method of investigating learning in old mice.

Increased mortality in water-deprived old mice did not appear to be a confounding variable in several other mouse studies. Thus, Ingram[21] reported a study comparing the ability of 6–8-month- and 26–27-month-old C57BL/6J mice to learn a 14 unit Stone maze for water reinforcement. The old mice made more errors per trial and showed higher error performance at the asymptote level compared to young animals. Ingram presented data arguing against age-related differences in motivation. In contrast, Bernstein et al.[6] failed to find evidence of age-related differences between young (8 months) and old (27–28 months) C57BL/6J mice trained in a spatial discrimination task requiring working memory.The task consisted of an 8 arm radial maze in which choice accuracy was reinforced by isotonic saline. No significant age-related difference in learning rate or performance was observed. However, since all 8 arms were baited, the task is not actually too difficult and can be solved by adopting an egocentric procedure, such as discussed above in the context of Y-maze performance. If the working memory task is rendered more difficult by introducing a delayed non-matching element into the radial maze, 24-month-old food deprived C57BL/6J mice exhibit learning difficulties compared to mice aged

9 weeks[33]. A possible factor contributing to the age-related impairment in this last procedure is the interval before the second choice response following the initial response. Ritzmann et al.[46], reported reduced choice accuracy in a straightforward win–stay paradigm using a T-maze, with increasing delays between the pairs of responses, in 11-month-old Swiss Webster mice compared to 6-month-old mice. Retention deficits, even at relatively short intervals, are therefore likely to contribute towards poorer learning in aged mice.

Neurochemical changes in aging mice

There is ample evidence of age-related changes in parameters associated with neurotransmitter functioning in the brain. As far as rodents are concerned, most data has been obtained in rats, although there are several published studies on mice[10,17,34,38,44,48,50,53]. The majority of rat or mouse studies were carried out independently of behavioural analyses.

The apparent association between cognitive ability and brain cholinergic activity, summarised as the cholinergic hypothesis of geriatric memory dysfunction[4] predicts that animals most deficient in cognitive functioning will show most marked changes in cholinergic parameters. Accordingly, we determined ChAT and AChE levels in the frontal cortex and hippocampus of 3- and 17-month-old NMRI mice which had completed both spatial and cued learning in the Morris maze. The results (Table I) show that ChAT levels were increased in the frontal cortex of the old mice but unchanged in the hippocampus. Conversely, age-related decreases in AChE levels were obtained in the hippocampus but not frontal cortex. The finding of increased ChAT activity is at variance with several other studies both in rats and mice[9] although Waller and London[53] obtained a similar increase in C57 Black mice. Although statistically significant, the cholinergic changes were quite subtle and did not correlate with performance in the learning and memory tasks. Such a result argues against a simple link between brain levels of cholinergic markers and cognitive impairment. However, the markers were measured a few days after completion of the learning tests and can be considered to reflect basal activity which may be irrelevant to the dynamic state of the cholinergic system during learning. LeBrun et al.[33], for example, showed that while basal choline uptake was unaffected by age in C57BL/6J mice, the activation observed in young mice 30 s after the learning test was absent in aged mice in the hippocampus and attenuated in the cortex. Whether or not the individual results correlated with performance was unfortunately not reported.

TABLE I

ChAT and AChE levels in frontal cortex and hippocampus of young (3 months) and old (17 months) mice

Values given are means with S.E.M. between brackets, expressed in nmole/mg protein/h. Asterisks indicate a significant difference, $P < 0.05$, from young mice.

	Frontal cortex		*Hippocampus*	
	Young mice	*Old mice*	*Young mice*	*Old mice*
ChAT	75.5 (1.2)	92.7 (5.2)*	63.1 (0.9)	62.3 (1.8)
AChE	3217 (71)	3497 (212)	3001 (55)	2740 (57)*

Correlational analysis between different behaviours measured in old NMRI mice

A striking general observation arising from the behavioural studies in NMRI mice referred to in this review, was the marked heterogeneity of performances in the older mice and, generally, a lack of correlation between performances in the various tasks for a given individual. In addition, there was often no difference in the mean response values obtained between middle aged (11–12 months) and old mice (17–22 months). This overlap is illustrated clearly in Fig. 6. These observations support a conclusion that functional aging is not necessarily proportional to chronological aging, after a certain point. The findings also raise the question as to what extent the changes are interrelated and whether there exist elements common to the different overt behavioural effects. To try to answer these questions, we performed a multivariate analysis on a group of 31 mice aged 17 months on data obtained in the different behavioural test situations, including open field activity, Y-maze alternation, plus-maze behaviour and spatial and cued learning in the Morris-type swimming maze[31]. The results confirmed that age-related changes were mostly independent of each other. The exceptions were firstly an inverse relationship between total open field activity and spatial learning indices in the Morris water maze, in that mice performing poorly in the latter were more active in the open field. Secondly, there was a positive correlation between the ratio open/total arms visited in the plus-maze, considered as an index of fear or anxiety, and locomotor activity during the first 2 min of testing in the open field. The independence of the plus-maze index of emotionality and Morris maze performances is further interesting, since the latter task could also be considered to be stressful. This therefore argues against an interpretation of age-related deficits in water maze spatial navigation in terms of emotionality rather than cognitive and/or perceptive disturbances. Similarly, the independence of swimming maze learning and spontaneous alternation in the Y-maze

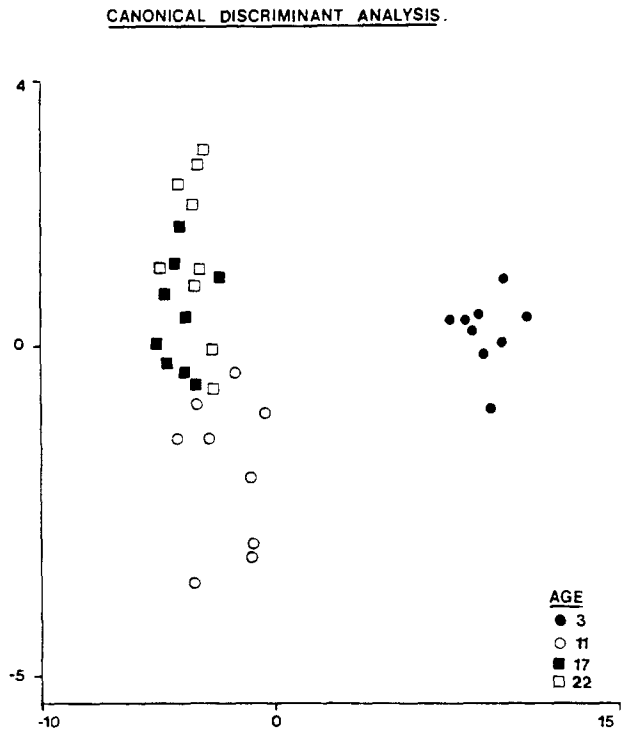

Fig. 6. Plot of results of a discriminant analysis classifying performances of mice aged 3, 11, 17 and 22 months on the basis of their scores obtained in different tasks aimed at evaluating spontaneous and learning changes. Each symbol represents one individual. While mice aged 3 months are clearly "discriminated" by the analysis, aged mice are not so readily categorised on the basis of their performances. The groups aged 11, 17 and 22 months could not be statistically differentiated.

suggests that the concept of deterioration of an allocentric system with aging cannot be applied as a general construct to explain the deficits in both tasks.

SUMMARY AND CONCLUSIONS

The results of a series of studies involving different age groups of NMRI mice, clearly demonstrate progressive age-dependent declines in behavioural performance and cognitive functioning and substantiate published data in the aged mouse. In common with other similar studies in the rat[14–16], the NMRI mouse studies revealed that aging is not a unitary process and for a given chronological age, the degree of impairment is heterogeneous. In this respect, an important conclusion was that impaired cognitive functioning is not secondary to age-related changes in activity or emotional reactivity, or to gross sensorimotor effects, albeit that less obvious changes in sensory perception or visual acuity cannot be excluded.

In the NMRI mouse, the onset of decline is between 9 and 12 months, which, in terms of the median life expectancy in this strain, approximates to middle age. On the basis of the limited data available, a decline in performance from middle age appears to generalize to other mouse strains although more data are required. Since the expected life span in normal laboratory mice can vary by a factor of 2, according to the strain, it follows that the chronological age corresponding to middle age will be strain-dependent. Although the life expectancy of the NMRI mice lies towards the lower end of the range, this strain does not present as a particularly short-lived mutant animal. We selected this strain simply because it is the strain bred in our research laboratories for routine use.

In this context, it is interesting to note that our preliminary observations of age-related changes were, in fact, made in ex-breeder females, then confirmed in virgin female mice[18] and the age range expanded. Our findings that ex-breeders and non-breeders behave similarly, at least in spatial learning and general activity, supports a similar conclusion by Ingram et al.[23] that the breeding history is of little consequence to age-related behavioural decline. Another factor to take into consideration relates to the sex of the animal. For practical reasons related to high aggressivity in grouped male mice, we elected to use female mice. It is unlikely, however, that differences in the phase of the oestrous cycle could have accounted for the heterogeneity since it is recognised that female rodents, housed together over a long period, cycle in harmony.

It can be concluded from this review, that for any research group interested in investigating age-related cognitive changes, the mouse provides an attractive alternative to the rat. The NMRI mice studies illustrate that it is possible to identify a standard laboratory strain which shows age-related impairments at a relatively young chronological age, thereby representing a similar practical advantage as the SAM strain or autoimmune mice. Moreover, it is well worthwhile spending time to investigate a full age-range rather than opting, arbitrarily, for a specific age-group. The extensive studies carried out over the life span of the NMRI mouse, indicate the usefulness of such detailed analyses. Studies spanning the full age-range enable the age of onset to be established, thereby favouring the selection of a chronologically younger mouse with the incumbent advantages of minimal gross physical defects and sensory impairment. In addition, selection can be made of an age-group which is representative of the population as a whole rather than a minority of survivors. Furthermore, choosing the mouse as opposed to the rat, does not necessarily exclude neurochemical analysis, particularly with continually improving techniques. Finally, although to date, there has been much less research in

the aged mouse compared with the rat, it is hoped that the available data will stimulate further interest in this species as a model of cognitive decline.

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