Mild cognitive impairment: animal models

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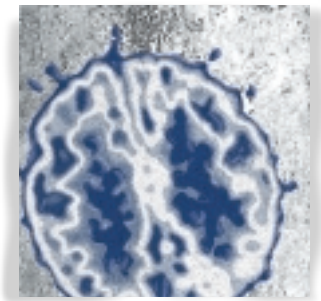

Mild cognitive impairment (MCI) is an aspect of cognitive aging that is considered to be a transitional state between normal aging and the dementia into which it may convert. Appropriate animal models are necessary in order to understand the pathogenic mechanisms of MCI and develop drugs for its treatment. In this review, we identify the features that should characterize an animal model of MCI, namely old age, subtle memory impairment, mild neuropathological changes, and changes in the cholinergic system, and the age at which these features can be detected in laboratory animals. These features should occur in aging animals with normal motor activity and feeding behavior. The animal models may be middle-aged rats and mice, rats with brain ischemia, transgenic mice overexpressing amyloid precursor protein and presenilin 1 (tested at an early stage), or aging monkeys. Memory deficits can be detected by selecting appropriately difficult behavioral tasks, and the deficits can be associated with neuropathological alterations. The reviewed literature demonstrates that, under certain conditions, these animal species can be considered to be MCI models, and that cognitive impairment in these models responds to drug treatment.

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On the basis of the descriptions presented elsewhere in this issue, it is clear that it is difficult to identify or develop an animal model reproducing most, if not all, the features of human mild cognitive impairment (MCI). To begin with, an animal cannot complain about memory, and it is difficult to assess whether its daily life is affected. However, correspondence between animal models and human pathology is only partial in all neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease. Nevertheless, even if they only partially reproduce the disease, animal models are quite useful for at least two purposes: understanding the pathogenic mechanisms of a disease; and testing the activity of new drugs to assess their potential activity prior to clinical trials.

General features of MCI animal models

If the purpose is to understand pathogenic mechanisms, the animal model should mimic as closely as possible the symptoms, neuropathology, and mechanisms of the disease. Conversely, if the purpose is to demonstrate the potential efficacy of a drug, the animal model could be less complex, but should be easily available in large quantities and reasonably priced.

On the basis of the clinical description of MCI and other considerations, the ideal features of an MCI animal model are listed in *Table I*. The number of these features actually present in the models may vary according to the animal species used. Cerebrovascular alterations should be present only in models reproducing MCI occurring in patients affected by cerebrovascular diseases.[1]

In attempting to identify MCI animal models, the problem arises of how to distinguish them from AD animal models. This problem parallels the situation facing the clinician having to distinguish between MCI and the ini-

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Selected abbreviations and acronyms

Aβ *β-amyloid*
AD *Alzheimer's disease*
APP *amyloid precursor protein*
MCI *mild cognitive impairment*
SHR *spontaneously hypertensive rat*

tial stages of AD.[2] Since a characteristic of AD is the degeneration of forebrain cholinergic neurons, animal models of AD were obtained by destroying the forebrain cholinergic nuclei, namely the nucleus basalis,[3] in the rat through the use of neurotoxins.

Rats with small lesions in the nucleus basalis show only limited cognitive impairment associated with a modest cholinergic deficit and present at least two of the features listed in *Table I*, namely subtle memory impairment and mild neuropathological lesions. They could reasonably be considered a model of MCI or of the prodromal phase in AD. In an extensive review of animal models of the mnemonic impairment in AD, McDonald and Overmier[4] conclude that those with a lesion in the medial septal nucleus show behavioral deficits that are most similar to the memory impairment observed in the earliest stage of AD. Transgenic mice overexpressing β-amyloid (Aβ) and presenilin 1, and aging animals in general, including aging monkeys, are commonly used as animal models in AD research. In all these models, the discriminating criteria between MCI and AD models are the severity of damage induced by the lesions, and the age at onset and severity of the cognitive impairment in the rats, monkeys, and transgenic mice under study.

Memory loss complaints are the first and most important symptom of MCI[5] and the most obvious expression of the cognitive impairment. Cognitive impairment can be easily induced pharmacologically in animals by administering anticholinergic agents, such as scopolamine. However, according to Sarter et al,[6] this creates an "indiscriminate" model, since too many of the drugs tested on it gave positive results, but then failed to pass further, more specific testing. Other pharmacological models of memory impairment can be created by blocking the glutamate *N*-methyl-D-aspartate (NMDA)–type receptors,[7] and by administering benzodiazepines.[8] However, the animals showing drug-induced memory impairment cannot be considered to be models of MCI, as they only show one of the features listed in *Table I*, ie, memory impairment. For this reason, they will not be discussed further in this review. They are, however, useful tools in the investigation of cognitive mechanisms and their neurotransmitter systems, and for rapid and inexpensive screening of new molecules that are potentially active on cognitive deficits. Animals with cognitive impairment resulting from lesions in the forebrain cholinergic system, induced by neurotoxin administration, will not be included in this overview either, since they are considered a model of AD,[9] and their deficit in learning and memory is often too severe.[10] The animal models described above will be examined in detail in the following sections.

Old age
Subtle memory impairment
Mild neuropathological changes
Changes in the cholinergic system
The above features are associated with:
• Normal motor activity
• Normal feeding behavior
Cerebrovascular alterations

Table I. Features that characterize mild cognitive impairment (MCI) animal models.

Aging rats

Aging rats have been used extensively for investigating age-dependent memory impairment, and the underlying neurochemical changes, and for studying drugs that are potentially active on the aging process. Out of the extensive literature on the learning and memory impairment in aging rats, we can select studies comparing the cognitive behavior of rats of different ages (young, middle-aged, and old) and those in which middle-aged rats were used. After analyzing the collected data, an attempt has been made, in the following paragraph, to answer two questions: (i) to what extent can aging be considered a model of MCI; and (ii) what is the earliest age at which a decline in learning and memory can be detected in the rat?

In male Wistar rats, Pepeu et al[11] demonstrated that a statistically significant impairment in the acquisition and retention of a passive avoidance conditioned response can be detected at 16 months of age, and the impairment severity gradually increases in the following months. In the same rat strain, a statistically significant impairment in object recognition was detected at 20 to 22 months of age, using a 60-min intertrial time, while at 16 to 18 months there was only a slight reduction of the discrimination index in comparison with the 3-month-old rats.[12]

Thus, it can be assumed that, if the intertrial time is longer, impairment could also be detected in younger rats. In a social memory/recognition task in which 3-, 15-, and 22-month-old Fischer-344 rats were exposed to a novel female stimulus, a significant shortening in the exploration time had already occurred in the 15-month-old rats, in comparison with the 3-month-old ones, when a novel female stimulus was introduced, while the 22-month-old rats failed to investigate the stimulus.[13]

Fuchs et al[14] reported that 19-month-old rats from the Emd:Wi-AF/Han strain showed an impairment in the acquisition of a one-way avoidance task, but acquired a two-way avoidance task (shuttle-box) as well as 3-month-old rats; 33-month-old rats showed a marked impairment in both tasks. Middle-aged (14-month-old) Long-Evans rats took significantly longer than young (3-month-old) animals to retrieve their rewards and made significantly more errors in an eight-arm radial maze paradigm.[15] In the Morris water maze, a progressive decline in spatial learning was demonstrated between groups of 3-, 12-, 18-, 24-, and 30-month-old female Sprague-Dawley rats. If time and distance covered to find the platform were compared, an impairment in acquisition appeared in the 24-month-old rats, and was markedly increased in the 30-month-old rats. However, if the rats were divided into cognitively impaired and not impaired groups, using the value of 2 standard deviations from the mean performance of the 3-month-old rats as the criterion, 8% of the rats were already cognitively impaired at 12 months and 45% at 18 months of age.[16] Similarly, 50% of female Dark Agouti rats already showed a learning deficit at 14 months of age, using a complex maze task, and 71% of the rats were impaired at 26 months of age.[17] However, dividing the rats into cognitively impaired and not impaired groups was too strict a criterion to identify MCI rats, which presumably fall into the not impaired group.

More subtle criteria are therefore needed. For instance, by testing Fischer-344 male rats in the Morris water maze, Lindner et al[18] were able to detect a difference in performance, evaluated as distance swam, between 2- and 16-month-old rats and observed that the aging rats had more impairment in the reference memory task, which was tested by keeping the target location in the same place, but using a longer intertrial interval than in the working memory task, in which the target location was changed, but the intertrial interval was short.

Increasing the retention time, ie, the interval between acquisition and testing, is a simple and effective procedure for unmasking memory deficits in aging rats. In a non-matching-to-sample task, 15- and 24-month-old Sprague-Dawley rats did not show any deficit if the delay between the sample and choice responses was 0 s, but an impairment was revealed when variable 0 to 24 s delay intervals were introduced.[19] By increasing the delay, memory impairment was detected in 17-month-old rats performing a delayed alternation task.[20]

Fourteen months is also the age at which a decline in memory ability, tested by an allocentric place determination task in a water maze,[21] was detected in Fisher-344 rats, individually followed throughout their life, as a decrease in accuracy. The decline progressed with age and could be reversed by inhibiting cholinesterase with physostigmine at the age of 22 to 23 months, but not at 26 to 27 months. An improvement in spatial learning was reported in navigation in a water maze, evaluated as time required to reach the platform, in 20-month-old Lister hooded rats receiving 40 to 80 mg/day aspirin in their drinking water.[22]

The above data, some of which are also presented in *Table II* for an easier comparison, make it possible to answer the question regarding the age at which cognitive impairment can be detected in the rat. It appears that the possibility of detecting an initial cognitive impairment in animals, mimicking MCI in humans, depends on many factors: the task that the animals are trained to acquire, the procedure used to train them to meet the criterion, the strain, and, most importantly, the interval between acquisition and recall. Environmental influences also play a role, as demonstrated by the finding that Long-Evans aging rats kept in an enriched environment made fewer errors in a maze than rats of the same age kept in an impoverished environment.[23]

With appropriate tests, a deficit can be detected early on, starting from 14 months of age. Fisher-344 rats show cognitive impairment slightly earlier than other strains, but by 16 to 18 months of age some subtle deficits can be observed in both sexes of most strains. The strain differences in water maze acquisition and recall were extensively investigated by Wyss et al[24] comparing Sprague-Dawley, Wistar-Kyoto, and spontaneously hypertensive rats (SHRs) in the Morris water maze paradigm. Sprague-Dawley rats showed that spatial learning began to decline between 12 and 18 months of age and fell off precipitously between 18 and 24 months of age. Both Wistar-Kyoto and SHR strains already showed impairment at 12 months of age.

In 14- to 19-month-old rats, many of the features that should characterize the MCI animal model, listed in *Table I*, are present: first, by definition, old age, then the subtle memory impairment and mild neuropathological changes. Among the latter, astrogliosis has been described in aging Wistar rats,[25] a loss of cholinergic neurons has already been observed in cognitively impaired rats at 14 months of age,[18] and a large decrease in acetylcholine (ACh) release from the cerebral cortex, hippocampus, and striatum has been reported in 19-month-old Wistar rats.[26] Moreover, in the age range of 14 to 19 months, the motor activity and feeding behavior of the rats are still similar to those of young adult rats. It should be mentioned that Gallangher[27] explicitly considers age-related cognitive decline in rats as a "naturally occurring animal model of MCI."

Aging rats have been widely used for testing drugs potentially useful for treating memory deficits and senile dementia. A few examples of drug-induced memory improvement, taken from the author's experience, will be mentioned. A recovery of age-associated impairment in the acquisition of a passive avoidance response was observed in 18-month-old rats treated with phosphatidylserine intraperitoneally (IP) for 7 days.[28] Nerve growth factor (NGF), administered intracerebroventricularly (ICV) for 14 days, restored age-impaired object recognition in 20-month-old rats.[29] A single administration of aniracetam was able to restore object recognition in 22-month-old rats.[12] Drugs aimed to facilitate learning may have no effect in young rats, but improve the performance in aging rats and the size of the improvement is, to some extent, proportional to the severity of cognitive impairment. For instance, the effect of the ampakine BDP-12[15] in restoring retention to the level of young rats was larger in middle-aged rats with minimal experience in the maze than in those with extensive maze experience. The α_2-adrenoceptor agonist medetomidine, tested on a delayed alternation task, exerted no effect in young rats, a small effect at 7 to 11 months, and significant improvement in performance at 17 to 18 months of age.[20] On the other hand, according to Takefumi et al,[21] physostigmine ameliorated the performance of a place navigation task in 22- to 23-month-old rats, but lost its effect in 26- to 27-month-old rats.

To conclude this section, the "middle-aged rat" appears to be a useful and convenient model for MCI, but with the caveat that the therapeutic efficacy of very few of the many candidate drugs tested on this model was later confirmed beyond doubt in clinical trials. Therefore, the model may generate "false-positive" drugs, ie, drugs very active in the animal tests, but with limited or no clinical efficacy.

Rats with cerebrovascular pathology

The correlation between hypertension and memory impairment is well known[30] and has been repeatedly confirmed.[31,32] Moreover, MCI may be present in the initial stages of cerebrovascular diseases.[1,33]

SHRs are considered a model of human hypertension and cardiovascular disease. In these animals, a learning impairment, expressed as more days needed to reach criterion and more errors made, can be observed in a radial maze test at 12 months of age, earlier than in normotensive rats of the same strain[34,35] and other strains at the same age.[24] Less efficient learning, demonstrated by longer latencies in finding the hidden platform, with normal swim speed, was observed by comparing SHRs with normotensive Wistar-Kyoto rats.[36] The longer time needed for learning[34] and remembering[36] observed in the

Strain	Age (months)	Behavioral test	Reference
Wistar	16	Passive avoidance	7
Emd:Wi-AF/Han	19	One-way active avoidance	11
Wistar	16-18	Object recognition	12
Fischer-344	15	Social memory/recognition	13
Long-Evans	24	Morris water maze	14
Long-Evans	14	Eight-arm radial maze	15
Fischer-344	16	Morris water maze	16
Sprague-Dawley	15	Delayed non-matching-to-sample task	19
Fisher-344	14	Water maze–allocentric place determination	21
Wistar	17	Delayed alternation	20

Table II. Age at which a cognitive impairment can be detected in the rat according to the different behavioral tests used.

SHR model is reminiscent of the slowing in cognitive performance, accompanied by relatively mild impairments of memory, that characterize vascular cognitive impairment in humans.[37]

SHRs show hypertensive brain damage including astrogliosis, cytoskeletal breakdown, and hippocampal atrophy at an early age,[38] and subtle cholinergic deficits.[35,36] Long-term treatment with angiotensin-converting enzyme (ACE) inhibitors lowers blood pressure and prevents the cognitive impairment.[39] However, the cognitive impairment in SHRs can also be improved by cognition enhancer agents, such as oxiracetam.[40]

In conclusion, SHRs show mild cognitive deficits and limited neuropathological lesions, including some damage to the cholinergic system. Therefore, they mimic the initial phases of the vascular cognitive impairment, which may stabilize or progress toward vascular dementia with much severer cognitive impairments. Models of this progression to vascular dementia, could include transient cerebral ischemia,[41] bilateral middle artery occlusion,[42] and global cerebral ischemia,[43] which all induce extensive neuropathological changes associated with severe cognitive impairment.

Aging and transgenic mice

For a long time, mice have been less popular than rats for studying brain aging, mainly because much less neuroanatomical and neurochemical information was available on mice than on rats. In recent years, the generation of many types of transgenic mice, including those overexpressing amyloid precursor protein (APP), have brought mice to the forefront of aging research since they present extensive Aβ deposition and can be considered an animal model of AD.

Dean et al[44] studied age-related behavioral differences in the C57BL mouse and observed that, in the passive avoidance test, the number of mice failing to avoid the dark chamber in which the mice had previously been shocked is higher at 9 than at 3 months of age. Similarly, 10-month-old mice need a higher number of trials to criterion in a T-maze task than the 3-month-old mice. The cognitive impairment revealed by these tests becomes progressively more severe at 23 and 31 months of age. Impairment in the acquisition and retention of the water maze task was detected in 18- to 19-month-old C57BL mice and was associated with a decrease in the volume of the septal cholinergic neurons.[45]

It has already been mentioned that MCI can be considered to be a transitional state between normal aging and dementia. Since transgenic mice presenting Aβ deposits are considered to be a model of AD, it should be possible to detect a prodromal phase of the disease with the features of MCI. The learning deficit related to age and Aβ plaques was investigated by Chen et al[46] in PDAPP transgenic mice and nontransgenic mice of different ages. Age did not affect the object recognition test in transgenic or wild-type mice. Conversely, in the Morris maze test, an age-related impairment of spatial memory was evident in both groups of mice, but was clearly more profound in the transgenic mice, and a relationship was found between the Aβ plaque burden and learning impairment. However, only in the group of young (9 months) transgenic mice was it possible to detect an impairment in learning the first platform location that was associated with a minimal plaque burden and could reasonably be considered as MCI. Retardation in initial learning and in learning a new escape location had also been previously observed[47] in transgenic mice for APP695 in which no amyloid deposition was detected. In contrast to these results, Westerman et al[48] demonstrated that, in Tg 2576 mice overexpressing human APP695 with the "Swedish" mutation, spatial memory impairment, evaluated in the Morris water maze, could be detected beginning from the age of 6 to 11 months and coincided with the appearance of the insoluble form of Aβ. In testing a similar strain in a passive avoidance task, it was demonstrated[49] that, in the transgenic mice, memory impairment appeared at about 8 months of age and progressed with aging. At 8 months, there were few senile plaques and an initial decrease in ACh content in several brain regions including the cortex and the hippocampus. The present analysis was confined to the relatively few papers in which memory and learning were investigated in transgenic mice in the early stages of the disease development, omitting the large number of papers in which old mice with a marked cognitive impairment and extensive neuropathology were used. We believe that transgenic mice overexpressing mutated human APP, in an early stage, around 8 to 10 months of age, and showing an initial cognitive impairment, few Aβ deposits, and some cholinergic deficit could be considered a model of MCI. These mice have been used for testing treatments, such as vaccination, aiming to prevent Aβ deposition and subsequent memory loss.[50,51] A 7-month treatment with cholinesterase inhibitors reduced neither Aβ deposition nor memory

impairment.[52] Conversely, in 6-month-old Tg 2576 mice, a 6-month treatment with *Ginkgo biloba* did not prevent the plaque deposition and protein oxidation, but reduced the spatial memory impairment.[53] These papers demonstrate that transgenic mice overexpressing Aβ may be useful models for testing drugs potentially active in preventing or delaying the conversion from MCI to AD.

Aging monkeys

Monkeys of different species have been widely used for studying the effect of age on memory, beginning from the classic papers by Bartus et al.[54,55] A review comparing the memory changes occurring in normal aging in humans, nonhuman primates, and rats was published in 2003 by Erickson and Barnes.[56] Given the similarities in cognitive aging between human and nonhuman primates, MCI should also occur in the latter. However, in most papers in which the age-associated changes in cognitive processes have been investigated in monkeys, only two groups of monkeys, young and old, were compared. In rhesus monkeys, the species that is most commonly used, the age of "young" animals ranges between 3 and 10 years and that of the "old" between 23 and 30 years. Within the latter age range, memory impaired and unimpaired monkeys can be recognized,[57] but impairment also depends on the task that the animals are required to perform. For instance,[58] aged monkeys were impaired in a delayed response test of visuospatial memory when the retention interval of the task was increased from 0 to 10 s. When trained in a delayed non-matching-to-sample test of visual object recognition memory, the aged animals took longer to learn the task, but were only minimally impaired if recognition memory was tested at retention intervals ranging from 10 s to 22 h. In contrast to their relatively intact performance in the object recognition task, the same monkeys were dramatically impaired in a second version of the test that required subjects to remember the temporal order in which objects were presented. In a comparison of four groups of monkeys aged 3 to 6, 14 to 17, 20 to 24, and 26 to 30 years,[59] the only behavioral deficit in the 14- to 17-year-old group was detected using a difficult visuospatial orientation task. Impairment in the delayed response task with long delay, assessing spatial memory, appeared in the 20- to 24-year-old monkeys, while object recognition was still preserved and was only impaired in the oldest group.

From the above experiments, it appears that MCI can be detected in middle-aged monkeys, presumably about 15 years old, if difficult visuospatial tasks are presented to them. Conversely, since in old monkeys the cognitive processes are usually well preserved, we can induce a MCI by a moderate increase in delay in the delayed non-matching-to-sample task or the delayed response spatial memory task. However, paradoxically, it could be claimed that since old monkeys never show the catastrophic loss of memory, massive degeneration of the cholinergic system, and extensive Aβ plaque deposition occurring in AD, the memory impairment in the aging monkey could be considered a model of MCI.

The cognitive deficit in the old monkeys can be improved, with individual marked differences, by drug treatments. Among the drugs tested are cholinesterase inhibitors,[60,61] arginine vasopressin,[62] dopamine D_1 agonists,[63] adrenergic agonists,[64] and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor allosteric modulators.[65] However, like in the rat, few drugs shown to improve cognition in aging monkeys have become useful therapeutic agents in man.

Conclusions

This survey of the literature on aging in laboratory animals reveals that there are several possible models of MCI. If they are listed according to cost and availability, their order of preference would be middle-aged rats and mice, transgenic mice overexpressing Aβ, at an early phase before extensive deposit formation, and aging monkeys. SHRs are a model of MCI that occurrs in hypertensive patients. Strain differences are not relevant while the selection of the behavioral task is very important for detecting the cognitive deficit at an early age. Although the animal models described in this review have frequently been used to test potentially useful drugs for the treatment of the memory deficits, which is the main symptom of MCI, a validation of the models through comparable therapeutic results in both animal and man has been rarely obtained. ❑

Deterioro cognitivo leve: modelos animales

El deterioro cognitivo leve (DCL) es parte del envejecimiento cognitivo y se considera un estado de transición entre el envejecimiento normal y la demencia, en la cual se puede convertir. Para una comprensión de los mecanismos patogénicos del DCL y para el desarrollo de fármacos para su tratamiento se requieren modelos animales apropiados. En esta revisión se identifican las características que debe tener un modelo animal de DCL, como edad avanzada, deterioro discreto de la memoria, cambios neuropatológicos leves y cambios en el sistema colinérgico, y también la edad en la cual estas características se pueden detectar en los animales de laboratorio. Estas características deben presentarse en animales viejos con actividad motora y conducta alimentaria normales. Los modelos animales pueden ser ratas y ratones de edad media, ratas con isquemia cerebral, ratones transgénicos con sobreexpresión de proteína precursora de amiloide y presenilina 1 (evaluadas en una etapa precoz) o monos viejos. Los déficits de memoria pueden ser detectados seleccionando pruebas conductuales adecuadamente difíciles, y los déficits se pueden asociar con alteraciones neuropatológicas. La literatura revisada demuestra que bajo ciertas condiciones estas especies animales pueden ser consideradas modelos de DCL, y el deterioro cognitivo en estos modelos responde al tratamiento con fármacos.

Déficit cognitif léger : modèles animaux

*Le déficit cognitif léger (*Mild Cognitive Impairment, *MCI) est un aspect du vieillissement cognitif considéré comme un état de transition entre le vieillissement normal et la démence vers laquelle il peut évoluer. Des modèles animaux appropriés sont nécessaires afin de comprendre les mécanismes pathogènes du MCI et de développer les médicaments qui le traiteront. Dans cet article, nous identifions les critères qui devraient caractériser un modèle animal de MCI, à savoir un âge avancé, un déficit mnésique discret, des modifications neuropathologiques légères et des modifications du système cholinergique, et l'âge auquel il est possible de détecter ces caractéristiques chez les animaux de laboratoire. Elles devraient apparaître chez des animaux vieillissants avec une activité motrice et un comportement alimentaire normaux. Les modèles animaux peuvent être des rats et des souris d'âge moyen, des rats avec une ischémie cérébrale, des souris transgéniques dont la préséniline 1 (testée à un stade précoce) et la protéine précurseur amyloïde sont surexprimées, ou des singes vieillissants. Il est possible de détecter les déficits mnésiques en sélectionnant judicieusement des tâches comportementales difficiles et de retrouver une association avec des altérations neuropathologiques. Une revue de la littérature montre que, sous certaines conditions, ces espèces animales peuvent être considérées comme des modèles de MCI et que le déficit cognitif répond au traitement médicamenteux dans ces modèles.*

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