The study of behavioral dysfunctions

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General introduction

Human aging, health, and quality of life

Probably everyone agrees with the statement ‘old age has its infirmities’, based on own experience or on our experience with aging relatives, friends, or neighbors. Everybody is familiar with the signs of growing old: the skin wrinkles and the hair turns gray. Functional and behavioral changes also occur. In the aged, sight and hearing slowly deteriorate. The ability of the eye to accommodate decreases, a process that starts at about 40 years of age, and the reaction to light and adaptation to dark decrease. Impairments of hearing, and even loss of perceptive hearing (presbycusis), occur. In particular, high-frequency tones are perceived less well with advancing age. The senses of smell and taste also deteriorate with age. Impairments in these senses, however, are less obvious and are in general not considered to be as disturbing as dysfunctions of sight or hearing. Similarly, the respiratory, digestive, and endocrine systems, the heart and the circulatory system, and the skeleton and muscles deteriorate with age (e.g. Butler, 1997; Lamberts, van den Beld & van der Lely, 1997).

The nervous system also ages. For example, in men the mean brain weight decreases non-linearly from 1391 grams in the third decade of life to 1161 grams in the ninth decade, and the decrease accelerates with higher age (Adams, Victor & Ropper, 1997, p. 612). Older studies reported a significant loss of cells in the brains of aged people, especially in structures such as the hippocampus and the cerebral cortex which are crucially involved in cognitive processes. In fact, this cell loss is considered to be the main reason why cognitive functions deteriorate in the aged. Recent stereological measurements, however, do not confirm these findings, making it less likely that neuronal cell death in the cerebral cortex and hippocampus contributes to the age-associated cognitive impairments of aging people. Instead, the cognitive deficits observed in the elderly might be due to more subtle changes at the neuronal level that compromise normal function (Morrison & Hof, 1997).

Perhaps the most notable symptom which provides an early indication of the aging of the brain is the deterioration of memory. There are also well recognizable neurological signs of aging of the nervous system, such as alterations in stance, posture, and gait. Although these alterations are a consequence of aging of the nervous system *per se* and are not caused by disease processes (Adams, Victor & Ropper, 1997, p. 1050), they do make the elderly more susceptible to diseases.

It is important to distinguish between the consequences of the normal aging process and of the first weak symptoms of disease. Early detection of age-related diseases might offer the opportunity to successfully intervene therapeutically, because intervention at a more advanced stage of a disease often drastically reduces the therapeutic efficacy (Molnar & Dalziel, 1997). Unfortunately, the distinction between disease and health is less clear in older people than it is in younger people. It is sometimes difficult to distinguish between the effects of aging *per se* and the effects of diseases which come with age. For example, symptoms of the early stages of Alzheimer’s disease, such as a deterioration of cognitive functions, might mistakenly be ascribed to the normal consequences of aging. Such false diagnoses are the main reason for missed opportunities to initiate treatment at a stage when disease progression might successfully be slowed down.

Although many people consider old age to be associated with disease and the need for help, 85% of all people aged 65 years and older are autonomous and are independent of the help of others. Normally, a healthily aging person is perfectly capable of coping adequately with these age-associated limitations, handicaps and burdens, and to lead a satisfying life despite these restrictions (Lehr, 1997). Most elderly people experience growing old as a normal phase in their development and accept the accompanying problems and limitations.

Many different theories of aging are currently being discussed and are under scientific investigation. These theories range from the somatic mutation theory, which assumes that the accumulation of spontaneous mutations impairs functions with increasing age, through the detrimental effects of free radicals, which attack and oxidize the molecular components of cells, to the supposition that wear and tear are the main factors responsible for the observed age-associated deterioration of functions (see Ricklefs & Finch, 1995, for a very illustrative introduction to this topic). However, none of these theories is able to explain all age-related impairments. A commonly accepted concept of aging is missing. It is, however, reasonable to suppose that the *maximum life span* is determined genetically. There are no reliable reports of people older than 120 years. The *individual life span* is modified to a considerable degree by environmental influences and by the personal life style (Finch & Tanzi, 1997). The maximum life span in humans appears to be unaffected by the factors which are responsible for the increasing mean life expectancy.

The prevalence of dementias and cerebrovascular diseases increases with age

Aging is associated with an increase in the prevalence of a number of diseases, such as dementias, cardiovascular and cerebrovascular diseases, and cancer. In fact, these diseases are usually the main cause of death in the aged.

Dementias

The number of elderly people with cognitive impairments is steadily increasing because the proportion of elderly people in the population is steadily increasing (Butler, 1997). When dysfunctions due to cognitive impairments become so severe that the elderly person can no longer successfully manage his or her normal daily activities and needs help, then he or she probably suffers from a dementia. This class of diseases is characterized by a (progressive) deterioration of intellect, memory, judgment, and abstract thinking (American Psychiatric Association, DSM IV, 1994). Many different types of dementias are recognized, which can be classified using different systems. One of these classifications distinguishes four main groups of dementias (Heinitz, 1997):

- *Primary degenerative dementias*, which predominantly affect the cerebral cortex, such as Alzheimer's disease, Pick's disease, and primary degenerative dementias of unspecified type, are the most frequent forms: 45 to 60% of people suffering from dementia are estimated to have a primary degenerative dementia.
- *Vascular dementias*, which are caused by cerebrovascular dysfunctions, are estimated to account for 15 to 25% of all cases.
- *Mixed forms of primary and vascular dementias* are estimated to account for 10 to 15% of all demented people.
- *Secondary dementias*, i.e. diseases which give the impression that the individual is demented are not primarily caused by pathological changes in the brain. Instead, diseases such as severe depression, serious infections such as acquired immune deficiency syndrome (AIDS), and side

effects of medications (note that the elderly often receive co-medication for a number of diseases) appear to have detrimental effects on cognitive functioning, which manifest themselves as attention deficits, lack of concentration, and apathy. The pattern of symptoms can be very similar to that seen in patients suffering from primary dementias (Heston & White, 1991). About 10 to 15% of all dementias are estimated to be secondary to other diseases.

The prevalence of dementia doubles every 6 years between the ages of 60 and 85 years. However, it should be noted that a highly heterogeneous group of disorders which affect the aged in a variable way are subsumed under the diagnosis dementia. The prevalence of dementia of the Alzheimer type (DAT) doubles even more rapidly, namely every 4.2 years (Molnar & Dalziel, 1997).

Stroke

From the fifth decade onward, the risk of stroke generally doubles every 10 years. The incidence of cerebrovascular diseases is considerably higher (30%) in men than in women, but there are no gender differences regarding the distribution of the different types of cerebrovascular diseases (Gorelick, 1995). However, because women live longer than men, and because of the age-associated increase in the prevalence of cerebrovascular diseases, more women than men appear to be affected by these diseases (Schramm, 1997). Eighty to 90% of all cerebral ischemic strokes occur in people aged 65 years and older (Reuter, 1997), and 60% of primary cerebral hemorrhages occur in people aged 75 years and older (Schramm, 1997). Thus, ischemic cerebral stroke and hemorrhage are diseases which affect primarily old people. The accumulation of risk factors during life, such as exposure to harmful environmental influences (e.g. alcohol, tobacco, environmental toxins; Butler, 1997), or chronically impaired health (e.g. due to hypertension, diabetes mellitus, prior stroke), as well as genetic predisposition and their interaction with environmental influences might be responsible for the increase in cerebrovascular diseases with advancing age (Gorelick, 1995).

Quality of life

The incidence and prevalence data for dementias and cerebrovascular diseases show the enormous increase in the number of people affected with increasing age. However, the figures do not show the adverse consequences for each individual patient and his or her family, friends, the community and the health care system. Moreover, the caregivers are often of an advanced age and suffer from health problems themselves (Molnar & Dalziel, 1997). Thus, the costs to care for a patient might be high, and the quality of life of the caregiver might also be severely reduced. Caring for a dementing patient appears to have a stronger negative impact on the caregiver, i.e. spouse or other family members, than has care for patients suffering from other diseases (Walker, Salek, & Bayer, 1998). A number of instruments have been developed to assess the quality of life of patients and of his or her caregiver (reviewed in Walker, Salek & Bayer, 1998).

Quality of life depends on a variety of factors such as contact with the family, social interactions with friends and neighbors, a sound financial situation, good housing conditions, and on how the own biography is perceived (Lehr, 1997). A considerable portion of the experienced quality of life, however, depends on the functional state and on the health of the individual. They are good predictors of the perceived well-being and quality of life of the aged (Lehr, 1997). Improvement of the quality of life, especially in the elderly, is a major goal of medical care (Arnold, 1997; Dolan, 1998).

The average life span is increasing in industrialized and developing countries (Butler, 1997), mainly due to a decrease in disease-specific mortality. However, it seems inconsistent to increase the life span if this increase is not accompanied by a prolonged period of independence from the help of

others and a good quality of life. Figure 1 schematically shows that the number of years a person will experience chronic disabilities will also increase as the average life span increases (compare scenarios I and II in Fig. 1) if the onset of age-associated impairments is not postponed in parallel to an older age. Thus, both life span and health span should be increased (scenario III in Fig. 1). The main goal, however, should always be to ensure a good quality of life for as long as possible and to reduce the number of years with morbidity, i.e. years in which the quality of life is reduced due to chronic disabilities and dysfunctions.

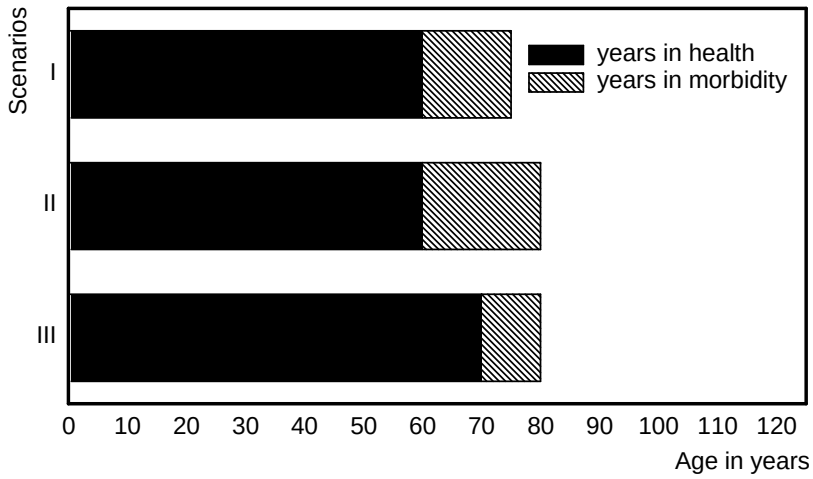

Figure 1. *Scenarios for prolongation of the average life span from 75 to 80 years. In scenario I, the mean life span is 75 years, where the last 15 years are characterized by an increase in age-related diseases and the accompanying chronic disabilities and dysfunctions. A 5-year increase in the average life span to 80 years, as a result of reduction in mortality, is shown in scenario II. Final aim, however, is a clear compression of the years with morbidity, as depicted in scenario III (adapted from Köhler, 1990).*

Unfortunately, we are far from being able to prevent or cure dementias and cerebrovascular diseases. To fight dementias and cerebrovascular diseases and to improve the quality of life in the elderly requires insight into the processes underlying the aging process and into the pathopysiological processes underlying stroke and dementias. This information is needed to identify and characterize putative therapeutics, i.e. to develop new therapies.

Ordered according to feasibility, the major goals with respect to dementias are to preserve the ability of the patient to manage his or her daily activities of living for as long as possible, i.e. slow down the progression of the disease, to cure the disease, and finally, to prevent the onset of the disease.

For cerebrovascular diseases such as strokes and hemorrhages, ordered according to feasibility, the main goals are to preserve function after the infarct, to help the patient to regain as soon as possible as much of the lost or impaired functions as possible, to prevent new strokes, and to prevent strokes.

The degree of *behavioral impairment*, together with the degree of suffering and pain, is a substantial determinant of the perceived severity of age-associated and of disease-induced limitations to living a normal, independent life. In order to gain an understanding of the processes underlying these behavioral impairments, relevant animal models of aging, of brain infarction and of dementia are needed. Such models are invaluable tools to gain the scientific insight needed to reach the above-mentioned goals. These animal models should mimic the functional state, e.g. cognitive impairments and sensorimotor dysfunctions, associated with aging or disease. They are expected to provide insight into the processes underlying aging and disease, and to allow the assessment of effects of putative

therapeutics. An extremely important first step with respect to the use of animal models is a thorough evaluation of their reliability and validity.

Animal models for assessing brain-behavior relationships

In behavioral neurosciences such as neurobiology and comparative and physiological psychology, animal models provide a scientific approach to investigate brain-behavior relations. The final goal is to gain insight into human behavior and its underlying neuronal and neuroendocrinological processes.

The most relevant information, of course, can be derived from the study of humans themselves. However, this is not always possible. For example, behavioral dysfunctions and the underlying processes in the brain cannot be investigated in humans, except when they are assessed in a clinical setting with patients as subjects. Even then, it is difficult to evaluate the damage caused by accidents or by illness. The extent and location of the damage, and its 'history', are often unclear. Moreover, the neurobiological variables associated with behavioral dysfunctions cannot be controlled sufficiently in experimental and clinical studies with human patients. As a consequence, in order to reach meaningful and interpretable results, the high intrinsic variability in these studies must be compensated by large sample sizes (Dunnett & Barth, 1991).

A comparative approach that relies on animal models could be used to answer questions about behavioral dysfunctions and their underlying neural substrate. Animals with a known and reproducible dysfunction or damage may help us to understand brain (dys)functions and their effects on behavior. As Isaacson and colleagues (1971, p. 3) pointed out, the comparative approach aims at studying the effects of experimental manipulations of a brain structure in one or more species (including humans, if possible) in order to try to generalize about brain structures, functions, behavior, and how they are related.

What is a model?

In a broad sense, according to Kaplan (1973), *"(...) we may say that any system A is a model for the system B if the study of A is useful for the understanding of B without regard to any direct or indirect causal connection between A and B."* (p. 263).

In a more strict sense, *"(...) models are isomorphs of one another (...). Both systems have the same structure, in the sense that whenever a relation holds between two elements of one system a corresponding relation holds between the corresponding elements of the other system. The systems need not stand in any causal connection, for what is required is only that the relations correspond, and to satisfy this requirement it is enough that we can put them into correspondence, that is, think of them as corresponding."* (Kaplan, 1973, p. 263).

Consequently, in the behavioral neurosciences, *animal models are living experimental systems* (Tamura, Kawai & Takagi, 1997) *used to analyze brain-behavior relationships under controlled conditions* (Sanberg, 1986). McKinney (1984, p. 77) defined animal models as *"(...) experimental preparations developed in one species for the purpose of studying phenomena occurring in another species"*.

Normally a battery of psychological tests is used to assess aspects of behavior, where psychological test refers to careful observation in a standardized experimental setting (Bechtoldt, 1959). Testing then refers to the process by which these observations are collected (Bechtoldt, 1959).

Willner (1986, 1991) contrasts the animal model with two other, closely related, experimental methodologies. The first one is *drug screening*, and the second is *behavioral bioassay*. Drug screening tests are designed to distinguish between potentially effective and ineffective drugs, whereas behavioral bioassays are designed to assess the functional state of, for example, a specific brain system. Both approaches can be successful, *with no need to be isomorphs of a defined system that should be modeled*.

In drug screening, compounds are identified which are pharmacologically similar to a 'lead'-substance. Many substances are evaluated in a test which allows a high throughput (see also Stephens and Andrews, 1991). For example, in the 'four-plate-test' an observation period of only 1 minute is sufficient to assess the putative anxiolytic effect of a test compound. In this test, a mouse is put in a cage, the floor of which is subdivided into four equal segments made of metal. Each crossing to another segment is punished by a mild, electric shock (Stephens & Andrews, 1991).

The behavioral bioassay is used to test, for example, the effects of compounds on specific neurotransmitter receptors. For example, unilateral, neurotoxic lesioning of the dorsal raphe nucleus by 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a serotonergic agonist, induces unilateral circling in rats. Serotonergic receptor antagonists are able to inhibit this circling behavior, without inducing the behavior by themselves (De Vry et al., 1991).

Drug screening and behavioral bioassay are two experimental methodologies, distinct from animal models, but they are not mutually exclusive. There is a fluent transition from drug screening and behavioral bioassay to animal models: the more precise the assumptions (or the knowledge) about underlying relations and processes, the more the criteria for an animal model will be fulfilled.

Model building as an iterative process

Model building can be considered as an iterative process (Britt, 1997). This process is depicted in Fig. 2 as a flow-diagramm. It starts with a *definition (or selection) stage* in which the central question is to select which aspects of human normal or abnormal behavior, i.e. which 'phenotype' should be modeled (Gershenfeld & Paul, 1998). Models of human behavior or of behavioral dysfunctions are dealing with extremely complex phenotypes which cannot be measured directly (Smoller & Tsuang, 1998). In the *consensus stage*, consensus must be reached about the criteria, definitions and assumptions about what are expected to be valid representations of the phenotype(s) to be modeled. They must be broken down to testable components, i.e. into elemental phenotypes (Smoller & Tsuang, 1998) which should preferentially be testable in both humans and animals (Robbins, 1998). These testables need to be defined operationally; simplifications are unavoidable at this *deduction stage*.

The next stage consists of constructing or refining the model. Then, the model is tested, using experimental approaches during the *model testing stage*. The results of testing are critically discussed and evaluted in the *model evaluation stage*. If the model is considered as acceptable, then the knowledge gained from the model approach can be used to refine or correct the concepts of whatever phenotype is being modeled in the induction stage. If the model proves to be unacceptable or

inadequate, it must be questioned whether the criteria used to build the model must be reconsidered, or whether the model needs reconstruction or refinement, based on the criteria agreed upon previously. There are no generally accepted criteria to terminate this process. However, a model will be considered as adequate if it reliably and validly (see below) represents the phenotype it is intended to model. Consequently, the process of model building will be finished as soon as the model is considered as adequate, or when it is judged as inadequate, a scientific *cul-de-sac*, with no realistic perspecitve for further improvement (see, for example Eijkenboom and van der Staay, 1999, who concluded that vincristine-induced hippocampal lesions in rats do not establish a suitable animal model of learning and memory deficits). It is not very likely that an animal model will ever reach one of these two states.

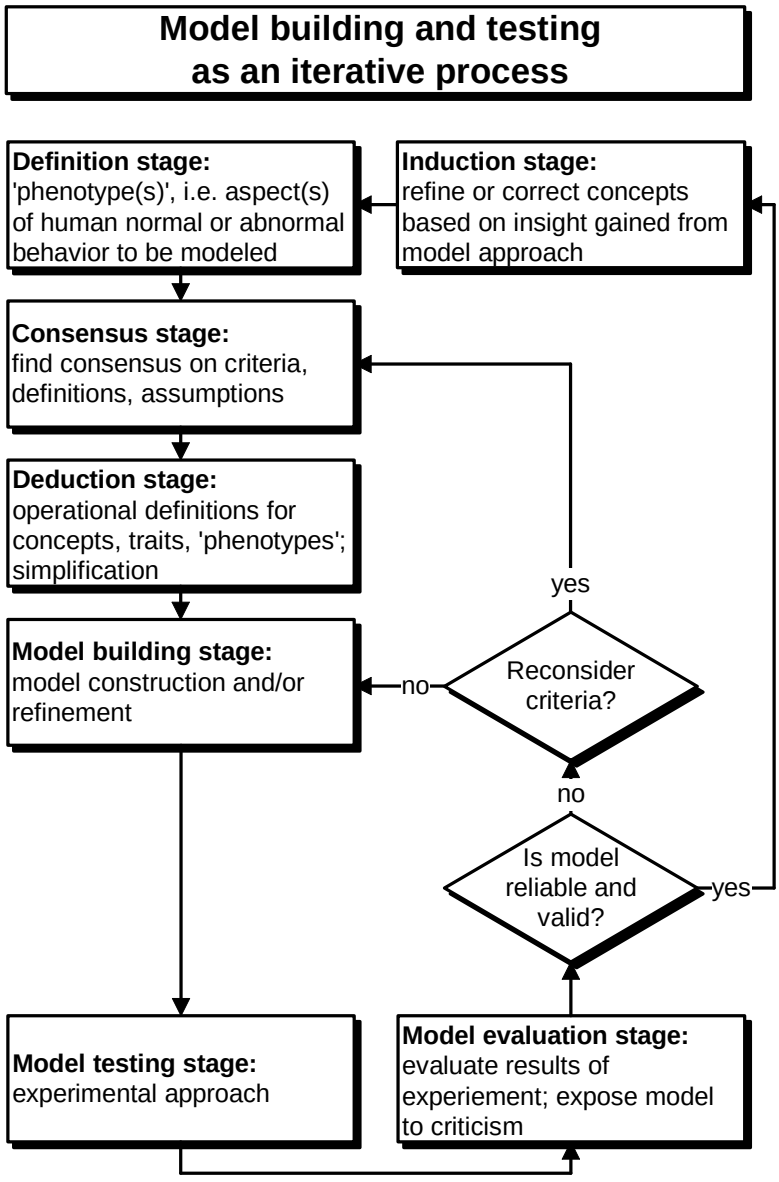

Figure 2. *Flow diagram representing model building as an iterative process (see text for further explanations).*

The concept(s) of validity

With respect to animal models of behavioral dysfunctions, such as psychiatric disorders, additional criteria have been proposed which are related to their validity, i.e. whether a particular, operationally defined measure or variable actually measures the trait it is supposed to measure. According to Willner (1986, 1991) animal models should possess *face validity*, *predictive validity*, and *construct validity*. This distinction between different forms of validity helps to identify the weaknesses or limitations of a particular model and provides a framework for comparing models (Willner, 1991).

Ellenbroek and Cools (1990) consider predictive validity, face validity, and construct validity, in that order, as a hierarchy of categories of validity, where construct validity is the highest category. Willner (1986), by contrast, sees these categories as relatively independent. Whereas predictive and face validity primarily address the empirical state of a model, the construct validity addresses its theoretical status and is therefore considered as a more basic concept (Willner, 1986).

Although students of animal behavior are aware of the importance of considering the validity of the models they use, papers dealing explicitly with this topic are scarce. Most papers are restricted to the concept of construct validity (e.g. Royce, 1977; Markou & Koob, 1991), perhaps because it has received a lot of attention in psychological testing theories, but there are some papers dealing with other aspects of validity (Willner, 1986; Ellenbroek & Cools, 1990; Moser, 1990).

Predictive validity

The outcome of a test is frequently used to predict, for example, future behavior. A test with high predictive validity makes it possible to venture a sound prognosis (Lienert, 1969). Ingram and Reynolds (1986) assessed the predictive validity of scores in a battery of sensorimotor tests carried out at a particular age in mice with respect to the lifespan of these animals. They found that a better performance at 24 months of age predicted a longer lifespan. An animal model possesses predictive validity if it predicts behavior in the situation it is supposed to model, i.e. if it allows extrapolation of the effect of a particular experimental manipulation from one species to other species, including humans. For example, a drug characterized as a cognition enhancer in animal models of cognitive impairment or dementia should also act as a cognition enhancer in humans.

Face validity (or phenomenological validity)

With respect to models of behavioral dysfunctions, face validity is usually restricted to the similarity of symptomatology (Willner, 1986). For example, according to McKinney and Bunney (1969), an animal model should at least meet the requirement that it *resembles* the condition to be modeled with respect to its etiology, its symptomatology, its underlying processes, and its treatment. In most cases, however, these requirements will not be met. In fact, the etiology and the underlying (pathological) processes of many neuropathological diseases such as dementias (e.g. of the Alzheimer type; Roses, 1996; Nitsch, 1996) and stroke (Adams, Victor & Ropper, 1997), and even normal aging, which also might lead to behavioral dysfunctions (Evans et al., 1984; Flicker et al., 1985; Era, Jokela & Heikkinen, 1986; Masoro, 1991), are still only poorly understood.

Construct validity

Construct validity refers to the theoretical clarification of what a test measures (Lienert, 1961). Animal models possess construct validity if their procedures are theoretically sound. Implicitly, a construct is defined by a network of associations (Cronbach & Meehl, 1955; Runkel & McGrath, 1972, pp. 162-163). The construct validity is not established by determining the relation between a test and an

accepted criterion. Instead, it aims to establish relationships which are based on the definition of a *trait*. This network of associations has been elaborated by Campbell and Fiske (1955). Their multitrait-multimethod matrix approach allows assessment of the reliability and the validity of measures which are believed to be related to a specific trait. Unfortunately, this approach has not yet been adopted by students of animal behavior. A first, incomplete, attempt to use the multitrait-multimethod approach in the validation of measures for the trait anxiety has demonstrated that this approach is suited to animal research (van der Staay, Kerbusch & Raaijmakers, 1990; van der Staay, 1992).

In addition to these different concepts of validity which are of special relevance to animal models (Willner, 1986; Ellenbroek & Cools, 1990), other concepts of validity have been proposed (see, for example Lienert, 1969; Runkel & McGrath, 1972, pp. 158-172; Fischer, 1974).

Table 1. *Schematic overview of animal models for the study of behavioral dysfunctions. The scheme has been modified from Gamzu (1985). Examples from the categories of animal models printed in italics are described and discussed in this book. The scheme focuses on the type of subject (independent variable) and is not concerned with the type of dependent variable measured.*

Normals	**Deficits**	
	Naturally occurring	Experimentally induced
Normal subjects, i.e. animals without any observable behavioral deficit	Old animals	Transgenic and knockout animals
	Genetic lines	*Animals with CNS-specific lesions or with cerebral ischemic damage*
	Selected extremes from a particular animal population, e.g. good vs. poor learners	Animals with disruptions induced electrically, pharmacologically, or by hypoxia, anoxia

Types of animal models to study behavioral dysfunctions

The animal models which have been proposed for the study of behavioral dysfunctions can be classified into two main groups: those using normal subjects and those using subjects with behavioral deficits. The second group can further be subdivided into models which are based on naturally occurring deficits or dysfunctions and models in which deficits or dysfunctions are induced experimentally (see Table 1).

Animal models of behavioral dysfunction serve two main aims:

- first, to enhance our understanding of the underlying substrates and mechanisms, i.e. the brain-behavior relation. This is done experimentally by, for example, inducing dissociations between processes, subprocesses and modulating influences, either pharmacologically or through the destruction of neural tissue (D'Mello & Steckler, 1996).
- second, to assess the effects of putative neuroprotective, anti-degenerative, revalidation-supporting, and/or cognition-enhancing compounds or treatments (Allain et al., 1998).

Of the animal models summarized in Table 1, three main categories, namely *aging*, *CNS-specific lesions,* inducing cholinergic system (dys)functions, and *cerebral ischemia*, induced experimentally by

occlusion of brain arteries, will be considered more closely. In the experiments described in this book we used the three categories of models, either alone or in combination. *Genetic lines* are an additional aspect of some of the experiments reported. With respect to behavior, emphasis is mainly on learning, especially (spatial) discrimination learning, and on sensorimotor (dys)functions.

Genetic strains

One approach is to characterize and select *specific genetic strains or genotypes* which show a strong expression of a particular trait or characteristic of interest, for example increased emotional reactivity (e.g. van der Staay, Kerbusch & Raaijmakers, 1990; Fernández-Teruel et al., 1994; Fujita, Annen & Kitaoka, 1994) or a high voluntary alcohol intake (e.g. Deitrich, 1993). Thus, various behavioral, anatomical and neurochemical characteristics are associated with genetic variation. For example, there are strain differences in cognitive functioning, i.e. in tests designed to measure learning and memory, in rats (e.g. van der Staay & Blokland, 1996a), and mice (e.g. Klapdor & van der Staay, 1996).

Cholinergic neurotransmission

Central cholinergic neurotransmission, which appears to undergo massive changes in patients suffering from dementia of the Alzheimer type, has been found to show clear genetic variability in rodents. Overstreet and co-workers (1984; overview: Overstreet, 1992) genetically selected a line of rats with increased sensitivity to the acetlycholinesterase (AChE) inhibitor, diisopropyl fluorophosphate. Roderick (1960) showed that cortical cholinesterase activity responded to bi-directional selection in two genetically heterogeneous populations of different origin. Genetic variability of AChE in the cortex was also found by Kerbusch and coworkers (Kerbusch, van der Staay & Hendriks, 1981) in a classical Mendelian cross-breeding study with rats, and by Kerbusch (1974) and Raaijmakers (1978) in diallel cross studies with mice.

The aging rodent

The survival characteristics of populations are also under genetic control, although, according to Finch and Tanzi (1997), the heritability of lifespan appears to be relatively small. Takeda and co-workers (Takeda et al., 1981; Takeda, 1999) have selected two sublines of mice, each consisting of a number of independent breeding series, one of which shows biological characteristics of accelerated aging (SAM-P: senescent-accelerated prone mouse), whereas the other shows normal aging (SAM-R: senescent-accelerated resistant mouse). The SAM-P mouse shows an earlier onset of age-related deterioration in learning and memory and the deterioration is correlated with the accelerated aging (Miyamoto et al. 1986).

These examples demonstrate that parameters of the cholinergic system and survival characteristics respond to genetic selection. Further evidence for genetic factors in aging is provided by the fact that different inbred strains of mice (Russell, 1972; Ordy, 1975) and rats (Burek, 1978; Masoro, 1980; Gleiser & Shain, 1986) show considerable differences in the mean and distribution of their lifespan.

Old animals have been suggested to be good animal models for human aging (e.g. Schuurman et al., 1986; Gallagher & Pelleymounter, 1988; Barnes, 1990). Certainly, small rodents possess a number of clear advantages for aging research: they have a relatively short lifespan (2 to 3 years), their environment can be strictly controlled, and they show age-related behavioral impairments (e.g. Elias & Elias, 1976). The age-associated impairments of cognitive functioning (e.g. Campbell, Krauter & Wallace, 1980; van der Staay, van Nies & Raaijmakers, 1990; van der Staay, Krechting, Blokland & Raaijmakers, 1990; van der Staay & de Jonge, 1993) and of sensorimotor performance (e.g.

Campbell, Krauter & Wallace, 1980; Ingram & Reynolds, 1986; Markowska et al., 1990) of rodents are well documented.

Pathological conditions, for example Alzheimer's disease.

Old animals have also been proposed as animal models for senile dementia (e.g. Schuurman et al., 1986). It is, however, a matter of debate whether the old animal can be considered as a model for gerontopathological states seen in humans. A general limitation of this model is that it lacks any true analogy to the human disease state it is supposed to model (Gamzu, 1985). As Mervis (1981) pointed out, animal models of the aging brain suffer from the major limitation that animals do not show the pathologies that characterize the age-related neuropathologies seen in human brains, for example which characterize those associated with Alzheimer-type dementia. Senile neuritic plaques and neurofibrillary tangles, both key morphological changes of this disease, have not been detected in the aging rodent brain.

CNS-specific lesions: lesioning of the cholinergic projections

In the central nervous system of patients suffering from Alzheimer's dementia, the activity of cortical choline acetyltransferase (ChAT), the enzyme that synthesizes acetylcholine (ACh), is reduced and there are fewer markers of other neurotransmitter systems, such as serotonergic, glutamatergic, and peptidergic systems (McGeer & McGeer, 1975, 1978; Gottfries et al., 1983; Winblad et al. 1985; Lieberman & Abou-Nader, 1986; Farooqui, Liss & Horrocks, 1988; Procter, 1996). The most pronounced decline, however, is in the activity of ChAT (McGeer & McGeer, 1978; Perry, 1980; Collerton, 1986). A severe functional deterioration of the central cholinergic system (Coyle, Price & DeLong, 1983; Procter, 1996) is one of the most important and consistent symptoms of Alzheimer-type dementia (Collerton, 1986).

There is a profound degeneration of ACh-releasing cells in the nucleus basalis of Meynert (nbM), which is localized in the basal forebrain, in Alzheimer patients (Coyle, Price & DeLong, 1983; Davison, 1987). As this nucleus provides the major cholinergic input to the neocortex, an experimental approach to mimic this sign of Alzheimer's disease consists of lesioning the animal homologue of the nbM, the nucleus basalis magnocellularis (nbm), in rodents.

Ischemia induced by occlusion of the middle cerebral artery

Occlusions of the middle cerebral artery (MCA) in rats or mice provide an animal model to investigate the pathophysiology of permanent focal cerebral ischemia (Welsh et al., 1987), to screen potentially neuroprotective substances (e.g. Obana, Pitts & Nishimura, 1988; Gotti et al., 1990; Hara et al., 1991; Yamamoto et al., 1991; Park & Hall, 1994; Hunter, Green & Cross, 1995; Sauter & Rudin, 1995), or to assess ischemia-induced behavioral and neurological disturbances (e.g. Tamura et al., 1985; Bederson et al., 1986; Yamamoto et al., 1988, Markgraf et al., 1992; van der Staay, Augstein & Horváth, 1996a,b).

Tests to assess behavioral deficits

This schematic overview of animal models (see Table 1) focused on the type of subject, i.e. the independent variable, and was not concerned with the question how the dependent variables are measured. Two different categories of dependent variables must be considered in animal models of

behavioral dysfunction. First, the pathologic features, such as the degree of neuronal damage or the extent of the damage to circuit and systems (e.g. changes in neurotransmitter activity, cell loss, extent and location of lesions or infarcts), and second, the behavioral features (dysfunctions induced). We used spatial discrimination tasks to assess behavioral dysfunction at the cognitive level in the experiments described in this book.

Spatial discrimination learning

Aging in humans is generally accompanied by a decline in memory performance. One of the types of memory that shows an age-related decline is the memory for spatial information (Evans et al., 1984; Light & Zielinski, 1983; Moore, Richards & Hood, 1984; Perlmutter et al., 1981; Sharps & Gollin, 1987). Similarly, visuospatial discrimination is disturbed in patients suffering form Alzheimer's disease (Adams, Victor & Ropper, 1997). Age-related deficits in spatial memory are not exclusively restricted to humans: aged rats often show an impaired performance in spatial learning tests (Barnes, 1988a; Gallagher & Pelleymounter, 1988) and pathological changes are detected in a number of selected neural regions involved in spatial memory performance (Flood & Coleman, 1988). As Barnes states, *"(...) in both primates and rodents tasks that have a strong spatial component tend to give old animals particular difficulties. This offers an interesting point of convergence between the human and the animal literature, as aged humans also have difficulty with certain spatial problems."* (1990, p. 187). Thus, aged rats might serve as a useful model of age-related memory dysfunction and spatial discrimination tasks might be useful to assess cognitive impairments.

Types of spatial discrimination tasks

A broad range of mazes has been established to assess spatial discrimination performance in rodents (e.g. Hodges, 1996). These mazes can be broadly classified as 'sequential choice' or 'alley' mazes and 'free choice' mazes (Crannell, 1942; Lachman & Brown, 1957). Sequential mazes consist of a fixed starting position and one correct route to the goal, which, for example, might either provide a food reward, or the opportunity to escape form an aversive testing environment (see also Chapter 3.1). In 'free choice' spatial discrimination tasks, food reward or an opportunity to escape can be found in all or only a subset of alternative locations, e.g. at the ends of the arms of a radial maze (e.g. Olton, Becker & Handelmann, 1979; Levin, Kaplan & Boardman, 1997) or at the entrance to an escape tunnel (Barnes, 1979). The animal is free to visit the alternative locations in whichever order and along whichever route it wants.

The distinction between 'sequential choice' and 'free choice mazes might be somewhat artificial, because the circular maze (Barnes, 1979; Bardgett, Newcomer & Taylor, 1996) and the Morris water escape task (Morris, 1984), for example, share some characteristics of both types of tasks: there are no constraints in the order to negotiate the maze or in the route(s) to the goal, except the boundaries of the testing apparatus itself. However, only one correct goal (the escape platform in the Morris water escape task, and the escape tunnel in the circular maze) is provided.

In spatial orientation tasks it is important to distinguish between tasks measuring working memory (WM) and those measuring reference memory (RM) (Honig, 1978; Olton, Becker & Handelmann, 1979). The rat must remember a list of places already visited in order to avoid revisits. This list of locations already visited in a trial is held in the WM (Olton & Samuelson, 1976), and the information it contains is relevant only within a specific trial. The RM holds trial-independent information about, for example, the locations where the food reward or the escape opportunity can be found. We used three different spatial discrimination tasks in the experiments described in this book:

- The seven-choice task in a radial maze was used as representative of ‘alley mazes’. This task is believed to measure spatial RM.
- The holeboard was selected as a representative of appetitively motivated ‘free-choice’ mazes. The holeboard measures both spatial WM and RM simultaneously, as only a subset of all holes is baited with a food reward (van der Staay, van Nies & Raaijmakers, 1990).
- The Morris water escape task was used as another representative of ‘free-choice’-mazes. Learning in this task is aversively motivated. The standard Morris task measures predominantly RM (Mundy, Barone and Tilson, 1990). WM versions of the Morris water escape task have also been developed (e.g. Whishaw, 1987, 1995; van der Staay & de Jonge, 1993; Petrie, 1995). Both versions of the Morris water escape task have been used.

Sensorimotor tests

The majority of patients with infarcts caused by occlusion of the MCA do not suffer from spatial orientation problems but often experience sensorimotor dysfunctions (Adams, Victor & Ropper, 1997). For this reason we used not only the seven-choice task and the Morris water escape task in its RM version and its WM version, but also sensorimotor tests to assess the effects of occlusion of the MCA. The tests were selected from the literature and have previously proven to be sensitive to the effects of aging, or to the effects of experimentally induced damage to the brain.

Short description of the experiments

We have examined various animal models of behavioral dysfunctions. This section provides an overview of the experiments performed. The reasons why we chose a particular experimental approach are discussed in the introductions to the separate chapters. Three types of animal models are considered:

- old animals, as a model of normal human aging,
- animals with lesions of the nucleus basalis magnocellularis as an example for CNS-specific lesions, and more specific, as a model for Alzheimer’s disease, and
- animals with permanent occlusion of the MCA as an example of experimentally induced brain ischemia, and more specific, as a model for ischemic stroke.

In the majority of experiments, we assessed spatial discrimination performance in either the holeboard, seven-choice task, or in different versions of the Morris water escape task. In some experiments we also evaluated sensorimotor functions. Alternative approaches, and suggestions of how to improve animal models and how to evaluate behavioral, especially cognitive, (dys)functions, are discussed.

In **Chapter 2**, we evaluated the effects of age on various aspects of spatial discrimination learning of rats in Morris water escape tasks.

In a series of three experiments, we compared the acquisition-curves of aged rats of three different strains with those of their younger conspecifics in Chapter 2.1. In a second series of experiments, we investigated the effects of age on performance of rats in the standard Morris water escape task and in a repeated acquisition version of this task, which measures spatial WM (Chapter 2.2). Because longitudinal studies are quite time-consuming, even in rodents with their relatively short life-span, the

most frequently applied experimental design in aging research consists of cross-sectional comparisons between age groups. In the third series of experiments, we evaluated whether the Morris water escape task is appropriate for a longitudinal study with aged rats. The results are described in Chapter 2.3. Finally, we assessed the replicability of the standard Morris water escape experiments in 24-month-old Wistar rats, by comparing the performance of aged rats across thirty-six experiments (Chapter 2.4).

In **Chapter 3**, we evaluated lesioning of the nbm by the neurotoxin ibotenic acid as an animal model of dementia of the Alzheimer type. We assessed the effects of bilateral ibotenic acid lesions of the nbm on the acquisition of spatial discrimination of adult rats in a complex spatial holeboard discrimination task in Chapter 3.1.

We studied the effects of bilateral nbm lesions on the performance of rats in a seven-choice problem in a radial alley maze in a series of experiments. In the first experiment of Chapter 3.2, we investigated the effects of nbm lesions on the acquisition of this task. In the second experiment, we assessed the effects of the lesions on the retention of the seven-choice task, on the acquisition of a new problem, and on the re-acquisition of the originally acquired problem. In addition, we also investigated whether the lesion affected the number of proactive and retroactive errors in the second experiment. The results of this analysis are reported in Appendix 6.3. In the third experiment of Chapter 3.2, we investigated the effects of different lesion coordinates on the acquisition of the seven-choice task. Finally, in Chapter 3.3, we tested the effects of nbm lesions on spatial learning and on a battery of neurological tests, using young and aged Wistar rats.

In **Chapter 4**, we assessed the effects of unilateral MCA occlusion (MCA-O) on the behavior of mice and rats. In Chapter 4.1, we investigated the recovery of sensorimotor functions in Wistar Kyoto rats with cerebral infarction, induced by unilateral MCA-O. In Chapter 4.2, we present the results of three experiments which addressed strain differences and effects of the occlusion site on sensorimotor impairments in rats with cerebral infarction. Using CFW1 mice, we studied the effects of unilateral occlusion of the MCA on the acquisition of the Morris water escape task in the first experiment of Chapter 4.3, and the effects of the MCA-O on the retention of the standard version of the Morris water escape task, which had been acquired before MCA-O, in the second experiment. Finally, we investigated the effects of occluding the MCA in C57BL mice which had been trained in the repeated acquisition version of the Morris task (Chapter 4.4) which measures predominantly spatial WM.

The **Appendices** provide additional information which we consider to be relevant to an understanding of the experiments we performed. Appendix 6.1 gives an example of the calculation of the measure ‘choice correspondence of reinforced visits’. We used this measure in Chapter 3.1 to determine whether rats develop a food search strategy in a holeboard discrimination task. We present further evidence of the sensitivity of the seven-choice task in a radial alley maze, which was used in Chapters 3.2 and 3.3, for studying the effects of age on learning in Appendix 6.2. We addressed the question whether nbm lesions affect the number of proactive and retroactive errors in Appendix 6.3, based on data from the second experiment reported in Chapter 3.2. Finally, the question whether the deficits in cortical ChAT activity induced by lesioning of the nbm recover over time, and whether the process of recovery is different for young and aged rats was raised in Chapter 3.3. We addressed this question by using the results from the experiment reported in Chapter 3.3 and additional data from an unpublished pilot study. The results of this exploratory analysis are reported in Appendix 6.4.