Non human primate models for Alzheimers disease-related research and drug discovery

Using non-human primate models for Alzheimer's disease drug discovery

ABSTRACT

Introduction Pathophysiological mechanisms underlying Alzheimer's disease (AD) remain insufficiently documented for the identification of accurate diagnostic markers and purposeful target discovery and development. Nonhuman primates (NHPs) have important translational value given their close phylogenetic relationship to humans and similar developmental paths in (neuro)anatomy, physiology, genetics, and neural functions, as well as cognition, emotion, and social behavior.

Areas covered This review deals with the past and future role of NHP-based research in AD pathophysiology, diagnosis and drug discovery, and touches upon ethical and legal aspects.

Expert opinion Aging NHPs are not complete phenocopies of human AD. Conceivably, no other species or experimental model will ever develop the full spectrum of AD-typical alterations. Nevertheless, partial – and even negative – models can increase knowledge of disease mechanisms. Modeling complex brain disorders should not be based on a single model or species. Understanding brain diseases relies on knowledge of healthy brain functioning, and given their close phylogenetic relationship to humans, NHPs serve excellent tools in this respect. NHP-based studies remain essential in the development and validation of radiopharmaceuticals for early diagnostic imaging biomarkers, as well as in the efficacy and safety evaluation of new therapeutic approaches, with active immunization or vaccination approaches as front runners.

KEYWORDS

Alzheimer's disease; amyloid; ethics; immunization; neuroimaging; non-human primates; pathology; tau

ARTICLE HIGHLIGHTS

- NHPs have important translational value in neuroscience;
- The aging NHP brain undergoes structural and biochemical changes that parallel many of the changes occurring in the aging human brain;
- Aging NHPs are not complete phenocopies of human AD, but display AD-like pathological, neurochemical and functional alterations;
- NHP models will remain important in the development and validation of radiopharmaceutical;
- NHPs display immunosenescence and provide important tools for the safety and efficacy evaluation of immunization approaches.

1. INTRODUCTION

Animal-based research has brought about major advances in biomedical sciences based on a variety of different applications in basic, as well as applied research. Animal studies lead to advances in the scientific understanding of molecular, cellular, physiological and psychobiological processes. Valid animal models are indispensable to the drug discovery and development pipeline. In particular, animal models have a key role to play in target discovery and validation, which require proof that a molecular target is pivotal to a disease process, and that modulation of that target has potential therapeutic effects. In addition, laboratory animals are applied in the field of toxicology, for the production of therapeutic and diagnostic agents, as well as for educational purposes. Research with NHPs often serves as a critical link between basic science and human clinical application. As nicely summarized on websites accessible for the general public [1][2], or in scientific review papers, like recently by Phillips et al. [3], NHP-based research was at the basis of major scientific and medical advances. Historically, the polio vaccine, blood transfusions, organ transplantation among many other medical advances could not have been possible without NHP research. Also in the field of neuroscience, NHPs have indispensably advanced basic scientific and applied medical knowledge [1]-[3].

Alzheimer's disease (AD), the prototype of cortical dementias, presents with prominent cognitive deterioration in various domains, including episodic and semantic memory, executive and attentional processing, as well as visuospatial functioning [4]. AD and related dementias are equally associated with a high prevalence of behavioral and psychiatric disturbances, consisting of depression, apathy, agitation, aggression, sleep disorders, delusions and hallucinations (psychosis), activity disturbances, anxieties/phobias, irritability, disinhibition and euphoria/dysphoria [5]-[8]. The neuropathological hallmarks of AD include "positive" lesions such as amyloid plaques, cerebral amyloid angiopathy (CAA), neurofibrillary tangles, and neuroinflammatory responses, and "negative" lesions, such as neuronal and synaptic dysfunction and loss that have been associated with the cognitive and the behavioural and

neuropsychiatric symptoms [9]. In 2010 the number of dementia patients was estimated at 35.6 million patients worldwide. Due to the ever increasing life expectancy and the coinciding aging of the world population, this number is expected to double every 20 years exceeding 115 million by 2050 [10],[11].

Despite intensive research for many decades, underlying pathophysiological mechanisms remain insufficiently documented for purposeful target discovery and development, but also the identification of accurate diagnostic markers that can detect the earliest stages of the disease, prior to neurological damage, is crucial. The study of relevant animal species and animal models is essential in AD-related research as they enable the appraisal of early pathological processes – which are often not sufficiently accessible in patients. A multitude of species has been and continues to be applied in AD-related research, ranging from *Drosophila melanogaster*, *Caenorhabditis elegans*, *Danio rerio*, chick embryo's, various rodents and lagomorphs, dogs and cats, goats and sheep, pigs, bears, up to NHPs, and of course *Homo sapiens* [11],[12]-[15]. In general, animal models of human disease can be classified into spontaneous, induced, negative and orphan models, of which the latter two types do not apply to the field of Alzheimer modelling. Spontaneous models are presumed to develop their condition without experimental manipulation, but selective breeding is often compulsory to establish and maintain the desired line. Especially for psychiatric and neurological conditions, including AD, few spontaneous models exist and experimentally induced or amplified pathology is often considered necessary [16].

This paper will discuss the past and future role of NHP-based research in AD-related research and drug discovery, and will also touch upon ethical and legal aspects.

2. THE VALUE OF NHP-BASED RESEARCH IN DRUG DISCOVERY FOR AD

2.1 NHP USE IN NEUROSCIENCE

It is relatively difficult to ascertain accurate figures of the global use of NHPs for research, as many countries do not disclose the number of NHPs used for this purpose. A survey targeting NHP-based

research published in 2001, estimated the annual number of NHPs used in research worldwide to be between 100,000 and 200,000 [17]. Overall, relatively few NHPs are used in biomedical research. The total number of animals used in 27 member states of the European Union in 2011 was just below 11.5 million. Since a long time, rodents and rabbits represent the majority (80% in 2011) of laboratory animals, followed by cold-blooded animals (12.5% in 2011) and birds (5.9% in 2011). NHPs accounted for 0.053% of the total number of animal used in 2011, of which 10.7% was applied to study human nervous system and mental disorders [18]. In the United States, on the other hand, NHPs accounted for 8.1% of the total number of animals used or housed for scientific purposes in 2015 [19]. It is important to note, however, that these statistics do not take into account rats, mice, birds or fish, as these animals are not covered by the Animal Welfare Act [20]. Approximately 15% of NHPs are applied in the field of neuroscience [21].

A 2001 survey indicated that approximately 65% of NHP-based studies involved Old World monkeys, versus 15.5% New World monkeys [17]. The Primate order has traditionally been divided into prosimians and simians. Prosimians (Suborder Strepsirrhini and Infraorder Tarsiiformes) resemble the earliest primates and include the lemurs of Madagascar, lorisiforms and Aye-aye. Simians entail the New World monkeys of South and Central America (Parvorder Platyrrhini, including e.g. marmosets, tamarins, capuchins and squirrel monkeys); the Old World Monkeys of Africa and Southeast Asia (Family Cercopithecidae, including e.g. baboons and macaques); and of course the Apes among which lesser apes (family Hylobatidae, including e.g. gibbons), and great apes (Family Hominidae, including e.g. gorillas, chimpanzees and humans) (Figure 1). The close phylogenetic relationship of NHPs to humans makes them excellent models for particular biological and medical phenomena. NHPs play a unique role in both fundamental biological research of the nervous system and special senses, as well as in applied neuroscience [3]. Much of our current understanding of nerve cell function is based on animal studies in, for example, cats, rats and even invertebrates where brain structure and circuitry is much less complex

as compared to humans [24]. However, the organization of nerve cells in a complex system such as the human brain is more likely understood by studying a similarly sized and complex primate brain. Important similarities include, among others, encephalization and sulcal characteristics, comparable numbers and densities of cortical neurons, a large prefrontal cortex which contains the areas responsible for working memory, executive function and aspects of decision making, similar nuclear organization, projection pathways and innervation pathways of the hippocampus, analogous blood-brain barrier structure and functioning, and the existence of mirror neurons [1],[24],[25]. However, it may be evident that brain structure and size differences exist across the primate phylogenetic tree, as illustrated in figure 1. Human, ape, and other anthropoid brains are not simply allometrically scaled versions of the same generalized design and exhibit qualitative and quantitative specializations, e.g. elaboration of the cerebellum in apes and of the frontal lobes in great apes, that form the source of cognitive and behavioral differences between NHP species [26].

2.2 NHP-BASED RESEARCH – DEFINING THE BOUNDARIES OF NORMAL AND PATHOLOGICAL AGING

Since the early 1980s lesion and single-cell recording studies in macaques formed the basis for the identification of brain regions critical for long-term memory and cognition, in particular, the medial temporal lobe memory system, which includes hippocampus and entorhinal, perirhinal, and parahippocampal cortices that have widespread and reciprocal connections with the neocortex, and the prefrontal cortex in particular [27]-[30]. Rhesus monkey hippocampus more closely resembles the human counterpart in terms of projection pathways, nuclear organization and innervation patters, in comparison to the rodent hippocampus [31]. Also with regard to other aspects of (explicit or implicit) learning and memory that not (only) involve the medial temporal lobe system, NHP-based studied were fundamental in gaining insight into related brain regions, networks and molecular mechanisms [32]. NHP and human brain are highly similar in cortical development and organization [33]. These fundamental

neuroscience insights aided in understanding the link between the neuropathological damage in particular brain regions in AD patients and the subsequent decline in various cognitive domains.

Not all NHP species are being studied equally intense in neuroscience, and in AD-related research in particular [34]-[38]. Indeed, the large bulk of NHP studies focusing on aging and AD-related neuropathology are performed in Old World monkeys, with rhesus monkeys (Macaca mulatta) and cynomologous monkeys (Macaca fascicularis) as front runners), compared to other macaque species. Squirrel monkeys (Saimiri sciureus) are the most intensely studied New World species in aging research, compared to for example, the marmoset (Callithrix jacchus), and the cotton-top tamarin (Saguinus oedipus). Among the prosimians, the grey mouse lemur (Microcebus murinus) is the most frequently studied. Studies in great apes are scarce, because of their long life span and limited availability in research settings. In the next sections, many relevant studies have been included to illustrate neuropathological alterations associated with aging and related to AD in NHP. However, we need to acknowledge that this is only a subset of studies and many more excellent NHP-based studies can be found in literature.

The aging NHP brain undergoes structural and biochemical changes that parallel many of the changes occurring in the aging human brain [34]-[42]. As far as sufficiently powered cognition-pathology correlation studies exist, there is somewhat contradictory evidence for the degree of neocortical or hippocampal neuronal loss, as well as its association with cognitive performance in aged NHPs; reports in aging NHPs range from significant cortical neuronal loss in one study of rhesus monkey brain [44], to maintained cortical neuronal counts in the majority of reports [34]-[36],[45]-[47]. On the other hand, more and consistent evidence was described for neuronal and synaptic loss and/or related alterations in various subcortical systems [36],[39]-[43], including the basal forebrain cholinergic system [48]-[52], and different monoaminergic systems, like the brainstem serotonergic system (e.g. in dorsal raphe nucleus or nucleus centralis superior) [53],[54], and the noradrenergic (e.g. alterations in locus coeruleus) [55],

as well as in peptidergic systems, for example the galaninergic system [56][57]. Important alterations in neuronal and synaptic processes in prefrontal cortex and hippocampus often precede other neuropathological alterations [36],[58]-[61]. Interestingly, a recent study applied structural and diffusion magnetic resonance imaging (MRI) to examine the brains of chimpanzees and rhesus monkeys across each species' adult lifespan, and compared these results with published findings in humans [62]. As in humans, gray matter volume decreased in aging chimpanzees and rhesus monkeys. Interestingly, chimpanzees showed a trend for decreased white matter volume with age, albeit proportionally later in life than in humans [62]. The longer lifespan of humans vs NHPs potentially provides time for greater deterioration of white matter, and hence, an increased prevalence of neurodegenerative diseases, including AD [62].

These neurobiological changes are likely at the origin of the multi-domain cognitive and behavioural deterioration in aging NHP, as is the case in humans [39]-[41],[63]. Another important translation advantage of NHPs is the shared complexity of higher order cognitive functions with humans [3],[64], and the possibility of applying operationally similar behavioral and neuropsychological paradigms in NHPs and humans [3],[65]. Studying NHP brain aging is, therefore, considered critical for our understanding of the neural basis of age-related cognitive impairment and to delineate the border (or continuum) between normal and pathological brain aging. From the drug development point of view, it is important to note that comprehensive studies comparing pharmacokinetics in humans, monkeys, dogs, and rats have shown that data in monkeys are the most predictive of human pharmacokinetic parameters [66].

Primates, are among the few animal species spontaneously developing age-dependent AD-resembling brain pathologies (Figure 2). With aging, all NHP species studied up to date show amyloid-related pathology, although important differences in age of onset, as well as the amount, distribution and appearance (including e.g. composition and biophysical characteristics) of amyloid pathology exist

[35],[37]. The genetic basis for these species differences, most probably, does not involve the Aβ coding sequence, since this is identical in NHPs and humans [34]. In brief, the majority of ageing NHP species display increasing amyloid-related pathology consisting of senile Aβ plaques containing both $A\beta_{1-40}$, and $A\beta_{1-42}$ [35]-[39]. In contrast to the majority of human plaques [70], $A\beta_{1-40}$ is often the more abundant peptide in NHP plaques [71]. Plaques can be accompanied by swollen neurites, glial activation (of astrocyte and/or microglial origin), and apolipoprotein E (ApoE) immunoreactivity, as described for example in aged rhesus monkeys [72]-[74]. It is well established that the ApoE epsilon4 allele is a risk factor for the development of AD, as it is associated with increased amyloid deposition. This increased plaque frequency in human AD brain has been largely attributed to an increase in $A\beta_{1-40}$-positive plaques [70],[75]. In contrast to humans, many animal species have only a single isoform of the ApoE protein [76]. Amino acid or nucleotide sequencing of in several NHP species has revealed similar characteristics as encoded by the human APOE epsilon4 allele [72],[77],[78]. These observations may form the basis for the predominance of $A\beta_{1-40}$-positive plaques in association with ApoE epsilon 4 in NHP brain. Interestingly, several studies have reported a similar neocortical-to-allocortical expansion of plaques as in human AD brain [79], although contrastingly, the hippocampus displays very limited, if any plaque pathology [72],[73],[80]-[82]. Ranging from prosimians to great apes, CAA is reported in the majority of NHP studies, and may even predominate parenchymal amyloidosis [74],[83]-[88]. Only a limited number of studies has scrutinized the link between amyloid burden and cognitive decline, but no correlation was observed [37],[89], similarly as in human AD [90]. The type of amyloid plaques observed in NHP brain also varies between species. Great apes in general present with diffuse plaques, whereas for example rhesus monkeys display neuritic plaques with aging [35]. In general and contrary to expectations, great apes studies often indicate only mild neurodegenerative alterations, whereas prosimians and monkeys show substantial brain amyloidosis and cytoskeletal abnormalities [34]. The limitedness of great ape studies might of course play a role in this, in addition to the lack of sufficiently aged animals bred and

studied in captivity. This hypothesis is indeed supported by very recent great ape studies reporting an age-related increase in Aβ-positive plaques and vasculature and indeed also the development of neuritic plaques in aged gorillas [89],[92].

Contrary to cerebral amyloidosis, tauopathy on the other hand, is virtually lacking in aged NHP brain [35]-[39]. Both simians and prosimians show only couple examples of neurofibrillary tangles and dystrophic neurites, and very limited neuronal tau immunoreactivity, especially with regard to hyperphosphorylated tau [35]-[39]. A few studies indicated the presence of neurofibrillary tangles in aged mouse lemurs, although the hippocampus is relatively spared compared to the human situation [95]-[98]. For long, neurofibrillary degeneration was considered absent in aging great apes, though interestingly, one case report described AD-like tauopathy with paired helical filaments throughout the neocortex and in allocortical and subcortical structures in an aged chimpanzee [100]. This animal also showed a moderate degree of Aβ deposition in the cerebral vasculature and, although to a lesser extent, senile plaques [100]. Importantly, this chimpanzee displayed obesity and hypercholesterolemia [100], important risk factors for human AD known to accelerate neurodegeneration [101]. In aging baboons, increasing cytoskeletal changes associated with abnormally phosphorylated tau protein were demonstrated in neurons and glial cells [102]. Neurofibrillary tangles with paired helical filaments were apparently lacking, but the researchers warranted additional studies in baboons approximating the upper limit of longevity in this species [102]. Species differences in the propensity to develop neurofibrillary pathology may be attributable to variations in the amino acid sequence of tau(-related) proteins. Rhesus monkeys, for example, display a different transcriptional splicing pattern compared to humans with the inclusion of exon 8 in some messenger RNAs [103]. An alternative explanation has been sought in the evolutionary lifespan extension in the superfamily of apes (Hominoidea). Apes evolved to live considerably longer than monkeys (40 to 50 years versus 10 to 20 years, respectively), and may have developed enhanced neuroprotection against neurodegenerative mechanisms along the

way. Humans, on the other hand, have experienced a very rapid increase in longevity much more recently from an evolutionary point of view and neuroprotective mechanisms may not have evolved accordingly [34],[91],[99],[100].

Thus, despite their biological closeness to humans, none of the NHPs studied up to date has been shown to develop AD, which therefore remains a pure human condition. Nevertheless, NHPs can be considered a good model of brain aging and AD-related amyloidosis, and – as negative models for full-blown AD – may provide insight in the unique vulnerability and predisposition of us humans to develop AD [35],[105].

In this respect, NHP-based studies evaluating amyloid species-related neurotoxicity deserve to be mentioned [106]-[110]. Microinjection of plaque-equivalent concentrations of fibrillary Aβ in the aged rhesus monkey cerebral cortex resulted in profound neuronal loss, tau phosphorylation and microglial proliferation [106]. In another study [107], solubilized Aβ peptides were injected in the frontal cortex of aged NHP, thereby causing cortical amyloid lesions and cytoskeletal responses. In both studies, no neurotoxicity was observed in the young NHP brain, thereby indeed suggesting that Aβ neurotoxicity is a pathological response of the aging brain. Soluble Aβ oligomers are known to accumulate in the brains of AD patients and correlate with disease-associated cognitive dysfunction [110]. When injected into the lateral ventricle of macaques, Aβ oligomers diffused into the brain and accumulated in several regions associated with memory and cognitive functions, and were associated with synaptic loss, tau hyperphosphorylation, and glial activation [108]. Most importantly, oligomer injections induced AD-type thioflavin S- and Alz50-positive neurofibrillary tangle formation in the macaque (frontal) cortex, which was also confirmed by ultrastructural analysis with immunogold electron microscopy [108]. While surface and intracellular Aβ was detected in neurons, no extracellular aggregates developed throughout the Aβ oligomer-injected macaque brains [108]. NHP injection models also – at least partially – tackle an important limitation of NHP-based aging and AD-related research; it may take several years or even

decades for NHPs to spontaneously develop relevant pathology. In this regard, Li et al. [109] intracranially injected $A\beta_{1-42}$ peptides and thiorpan (inhibitor of neprylysin, responsible for Aβ clearance) in middle-aged rhesus monkeys, resulting after 7 weeks in significant intracellular neuronal Aβ accumulation accompanied by (cholinergic) neuronal atrophy and loss, as well as glial activation. These models clearly aid in determining the neurotoxic amyloid species that may form valuable targets in the drug discovery pipeline for AD, and provide an essential complement to the rodent (injection) models which only recapitulate neurofibrillary tangles when expressing mutant tau, as exemplified by the following seminal papers [111][112].

Additionally, NHP have also proven valuable animal models to scrutinize the possible toxic/neurodegenerative effects of selected substances or environmental conditions. Noteworthy are the following three research lines that will be mentioned here without further details; restricting of caloric intake decreasing amyloid accumulation [113],[114], exposure to certain environmental toxins (e.g. lead) during childhood may predispose individuals to develop AD in old age [115]-[117], and the consumption of certain metals (e.g. manganese and aluminum) may elicit AD-relevant neurodegenerative processes [118]-[120].

2.3 THE VALUE OF NHPS IN THE DEVELOPMENT OF IMAGING BIOMARKERS FOR AD

Historically, a definite AD diagnosis of AD could only be established postmortem after the attestation of the cardinal neuropathological features typical for AD brain. Solely based on clinical criteria, diagnostic accuracy is around 60-80% [121]-[124]. One approach to increase diagnostic accuracy was the development and application of various neuroimaging techniques, including computed tomography (CT), structural and functional MRI, positron emission tomography (PET), and single photon emission computed tomography (SPECT) [125]. Applications of AD-specific radiopharmaceuticals would allow the noninvasive appraisal of AD lesions and, hence, a definite premortem diagnosis. Especially the

identification of alterations in prodromal and even presymptomatic states is essential for disease staging, and, most importantly, the development of effective disease-modifying therapies, based on the true confirmation of the availability of the drug target, as well as the assessment and quantitative follow-up of new drugs intervening at a specific molecular pathophysiological process [126]. As recently nicely reviewed [125]-[127], various major molecular pathophysiological changes known to occur in AD form relevant targets to be visualized with specific ligands, including of course amyloid deposition and tauopathy (Figure 3), but also neuroinflammation (e.g. translocator protein), as well as the basal forebrain cholinergic system, given its role in learning and memory and involvement in AD, and other neurotransmitter system alterations. In this regard, gamma-aminobutyric acid (GABA)ergic deafferentation in AD brain has been associated with neuropsychiatric symptoms [130], and especially the $GABA_A$ receptor forms an important target for tracer development [127]. Given its role in motivation, cognition and learning, also the (mesolimbic and mesocortical) dopaminergic systems have been implicated in AD [131], and dopamine receptors are applied as targets for PET or SPECT tracer development [127]. Also the serotonergic (5-HT) system has been associated with AD. After all, it plays a complex role in the modulation of several psychological, emotional, and cognitive processes, and affects long-term and short-term memory and cognitive function through the regulating of other neurotransmitters systems, including the basal forebrain cholinergic system [132]. 5-HT_1-, 5-HT_4-, and 5-HT_6 receptors are of particular interest for tracer development, due to their important role in learning and memory processes [127].

Given the (relative) similarity in brain structure , as well as important protein homology [133], NHPs play an important role in the preclinical evolution of radiopharmaceuticals with regard to safety, kinetics, selectivity and specificity. MicroPET imaging in human APP expressing transgenic mouse models, has not always proven to be a valid non-primate alternative [134]-[136]. These mice overexpress artificially high levels of mutated human APP, and produce human-type Aβ in the presence

of endogenous murine Aβ since they are heterozygous for the human mutated allele. Rodent peptides lack many of the posttranslational modifications that contribute to the insolubility of human plaques, and may also negatively influence the binding of radiopharmaceuticals based on subtle ultrastructural alterations in the combined rodent-human polymers [136],[137]. Also for other AD-relevant imaging targets, NHPs may prove more valuable than rodent models, as illustrated by an imaging study evaluating alpha7 nicotinic acetylcholine receptor agonist for their potential as PET tracers. Mouse brain lacked regional selectivity and selective receptor blockade, while the distribution volume was high in the hippocampus and thalamus but low in the cerebellum in the conscious monkey brain, indicating the potential value of the ligands for imaging these receptors in the human brain [138]).

2.4 ROLE OF NHP IN TREATMENT DEVELOPMENT AND EVALUATION

The compounds currently used for treatment of AD (donepezil, rivastigmine, galantamine and memantine) provide limited symptomatic relief [139]. Whether some of those agents are efficacious as disease-modifying treatments, remains a matter of debate and inconsistent finding may be partially attributed to the fact that clinical measures to evaluate the disease-modifying effect of an intervention are readily confounded by any symptomatic benefit of that intervention [140]. Ideally, intervention in the disease process will endorse restoration of neural integrity and function. As neurodegeneration advances in AD, and secondary or tertiary changes ensue, e.g. dendritic pruning, synapses loss and neurotransmitter deficits, such reversal becomes increasingly implausible. Tertiary prevention would then focus on managing manifest disease and its complications, and maximizing quality of life after AD diagnosis. Other approaches would provide secondary prevention by targeting the prodromal (i.e. predementia) stage of AD in an attempt to ward off further progression of mild symptoms, which of course relies on the identification of early disease signatures that are not as readily and reliably captured by the available neuropsychological metrics. Beyond question, the best approach is primary

prevention; neuroprotective agents that attenuate biological processes of AD pathogenesis in the latent clinical phase would delay or avoid the manifestation of symptoms altogether [141].

Most of currently internationally approved drugs for the treatment of AD, promote cholinergic function [139]. They provide relatively modest cognitive improvement, and are often (depending on the mode of administration) associated with important gastrointestinal side effects. Efficacious and well-tolerated symptomatic treatments, therefore, also form a significant unmet medical need in the AD field. Importantly, cholinergic dysfunction does not fully explain age-related cognitive deficits, and interactions between the basal forebrain cholinergic system and several other neurotransmitters and neuromodulators—including for example norepinephrine, dopamine, serotonin, GABA, opioid peptides, galanin, substance P, and angiotensin II—may be important in learning and memory [143] and form relevant AD drug development targets. Several NHP-based studied have contributed to these endeavors. The development of allosteric muscarinic M1 ligands to improve treatment efficacy while reducing side-effect-related attrition was to a large extent, based on target validation in NHP cognition studies [144]-[146]. Given the complex nature of AD pathogenesis, combination therapy is also considered an important strategy for effective treatment [147]. For example, the combined administration of the acetylcholinesterase inhibitor tacrine (Cognex) and the muscarinic agonist milameline improved scopolamine-induced cognitive impairment in rhesus monkeys at lower doses than required for the single-dosed cholinomimetic and without potentiating adverse side effects [148]. Similarly, the combination of the novel selective muscarinic 1 receptor positive allosteric modulator PQCA in with donepezil proved to be more efficacious in treating scopolamine-induced cognitive symptoms in rhesus macaques than both compounds separately, allowing lower dosages and significantly reducing cholinergic side effects [145],[146]. In this regard, concomitant cholinergic (physostigmine) and adrenergic (clonidine) stimulation proved more effective than either drug alone in enhancing memory performance in aged macaque monkeys. Part of the benefit may have been due to the ability of the

animals to tolerate significantly higher doses of physostigmine in the combination regimen [149]. Also non-cholinergic-based combination therapies have been investigated. As such, the application of WAY 100 635 (originally described as a 5-HT1A antagonist, but later on identified as a potent full agonist at the dopamine D4 receptor [150]), proved successful in alleviating cognitive impairments induced by the glutamatergic antagonist dizocilpine (MK-801) in common marmosets [151].

Given the progressive nature of AD, neuroprotective and disease-modifying strategies are highly desired. Considerable research efforts – including preclinical evaluation in NHPs – have focused on muscarinic M1 agonists that would not only be able to provide symptomatic relief [144]-[147], but also to modify the disease process by attenuating amyloid and tau-related pathology [144],[152],[153].

Nerve growth factor (NGF), the prototype of neurotrophic factors, has received important focus as a promising candidate to halt AD progression [154]. Since NGF would require chronic, targeted intracerebral release, gene transfer has emerged as an alternative delivery method. NHP-based research was the platform to assess safety, toxic effects, and effectiveness of the invasive intracerebral treatment before its transfer to the clinic [155],[156]. Given its central role in AD pathophysiology, amyloid has of course received much interest as a potential neuroprotection target. Secretase-based approaches, as well as drugs aiming to prevent the formation of Aβ aggregates, to dissolve existing aggregates, or to enhance Aβ clearance from the brain, have been successfully studied in NHPs [157],[158]. In addition, immunotherapeutic strategies have received much interest. Amyloid-targeting immunotherapy is either based on active immunization with Aβ peptides or on passive transfer of Aβ-specific antibodies. The clinical advancement of immunization approaches in AD was significantly hampered by the occurrence of aseptic meningoencephalitis in a subset (6%) of patients actively immunized with human $A\beta_{1-42}$ (AN1792) in a 2002 phase IIA clinical trial [159]. The immunization approach had previously proven therapeutically efficacious in transgenic human APP mouse models, with reduction of established plaque burden and/or prevention of plaque deposition [160]-[163], as well as improvement or

prevention of cognitive deficits [164],[165]. Unfortunately, the encephalitis was not predicted in these models. Interestingly, postmortem neuropathological evaluation in two of the encephalitis patients, unexpectedly revealed low plaque burden in extensive neocortical areas [166]. No regression of CAA, nor of tau pathology in neurofibrillary tangles and neuropil threads was noted. Amyloid plaques appeared to have cleared by Aβ-immunoreactive macrophages. The development of encephalitis was linked to T cell lymphocytosis and CAA-associated microhemorrhages [166],[167]. No improvement in memory or other cognitive functions was apparent in aseptic meningoencephalitis-free patients that likewise discontinued immunization [168]. Despite these initial complications, researchers continue their endeavors to optimize safe immunization approaches for AD.

NHPs have played an important role in further deciphering the conditions that promote the development of encephalitis. Potential sources of immunization-related meningoencephalitis may be connected with the specific Aβ epitope that is targeted, the type of adjuvant, and the magnitude of amyloid burden, especially CAA [169]. Because of highly analogous immune systems, as well as important brain and AD-related pathology similarities, specific NHP species (in particular rhesus monkeys) provide highly relevant animal models with an important translational value in immunization development and testing for AD, as exemplified by several amyloid-focusing immunization studies [170]-[175].

2.5 THE USE OF NHPS FROM AN ETHICAL AND LEGAL POINT OF VIEW

The very similarities between humans and NHPs at the level of physiology, cognition, behavior, psychology, reproduction, social complexity, in fact NHPs being sentient beings, make them interesting and vital animal models in neuroscience, but also form the basis for important ethical considerations and restraints with regard to their scientific application [176]. The ethical dilemma of NHP-based research is the same that applies for all animal-based research: do we, humans, have the moral right to

cause suffering, distress, pain, or lasting harm in scientific endeavors to alleviate or prevent human disease or suffering? Three main theoretical approaches can be applied: contractarianism, utilitarianism, and the animal rights view [177],[178]. Contractarianism states that animals lack moral status and only human long-term interests matter. Contractarianism may apply a graded moral status along the phylogenetic ladder [179], with NHP-based research being more objectionable compared to, for example, rodent studies. According to utilitarism, animals are morally relevant because they can suffer, and the related cost-benefit of harm-benefit assessment has been implicated in many current ethical matrices to evaluate animal experimentation. The limited number of NHPs used in scientific research, is hence justified by the potential huge benefit for the aging population struck by the increasing incidence of neurodegenerative disorders. Finally, according to the animal rights view we should distinguish between interests and rights. Rights must be respected and interests should not overrule them. Advocates of animal rights expand this approach and apply it to all sentient animals, which would totally ban the study of NHPs given their high level of sentience [177][178]. The moral acceptability of animal research, and especially NHP-based studies, is less questionable when the 3Rs principle is applied, requiring researchers, where possible, to *replace* in vivo experiments with alternatives, *reduce* the number of animals used, *refine* methods to minimize animal discomfort or suffering [180]. In addition, animal research is conducted under very strict government and institution oversight and regulations by scientists specially trained and qualified for the task.

The EU Directive 2010/63/EU aimed at strengthening legislation, improve laboratory animal welfare, as well as to firmly anchor the 3Rs principle [181]. The 2010 EU Directive endorses the viewpoint that the use of NHPs in biomedical research is still necessary, however, their use in translational or applied research or toxicity testing is restricted to procedures which are “undertaken with a view to the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening clinical conditions in human beings” [182]. The use of great apes is generally not permitted, unless it is believed

that the actions are essential to preserve the species or in relation to an unexpected outbreak of a life-threatening or debilitating clinical condition in human beings. Enacted in 1966, the Animal Welfare Act (AWA; 7 U.S.C. § 2131) is the only Unites States federal law that regulates the commercial breeding, the public exhibition and commercial transport of various species (including NHP—such as chimpanzees and monkeys), but also the treatment of animals in research [20]. A great ape research ban, or at least severe restrictions on the use of great apes in research, is currently in place in various countries, thereby ruling that chimpanzees, gorillas, and orangutans are cognitively so similar to humans that using them as test subjects is unethical. For a long time, the United States was the only developed country that continued large-scale confinement and use of chimpanzees for research purposes. The National Institute of Health (NIH) has steadily backed away from the use of great apes, in particular chimpanzees, since 2010 under The Chimpanzee Health Improvement, Maintenance and Protection Act [20]. After retiring about 310 chimpanzees in 2013, NIH announced to be ending its support for invasive research on chimpanzees and the remaining 50 NIH-owned animals that still remained available for research to sanctuaries in November 2015 [184][185].

Important ethical and financial restraints with regards to NHP research, as well as the development of other animal models, form the driving force in phasing out NHP application in biomedical research. Nevertheless, NHP-based research often remains to serve as a pivotal translational link between basic science and human clinical application, which is especially true in neuroscience. The comparison of cognitive and brain aging in apes and humans can elucidate unique characteristics of the human aging phenotype, and provide insight into the origins and treatment of human-specific neurodegenerative diseases such as AD.

3. EXPERT OPINION

An important phase of AD research has involved the investigation of genetic factors and concomitant molecular mechanisms via in vitro techniques and transgenic rodent models [13],[185]-[188]. Because of the transgene overexpression and interactions with endogenous proteins and peptides, the translational value of these models can be debated. For example, important AD phenotypes like macroscopic atrophy or neuronal loss are very modest or nonexistent in Aβ-driven transgenic rodent models. Models poorly replicate the biochemical complexity (truncations and post-translational modifications of Aβ or Tau) and often do not reflect the biochemical resilience of human pathology. Therapeutic response in models sometimes poorly translates into clinical trials, due to inadequately performed preclinical studies or misinterpretations of the models by largely ignoring the partial character of these models (e.g. amyloidosis versus AD models). Mice may be bred on different genetic backgrounds, often heterogeneous, and transgenes can be unstable across generations, leading to limited reproducibility [189]. NHPs are the closest species to humans in terms of biological make-up, so they are thought to have a high degree of sentience. Consequently, they are not the first in line choice as model organism in medical research. In several fields of research, nevertheless species from a lower neurophysiological level, like the more commonly applied mice and rats, may not suffice. Especially with regard to the assessment of innovative treatment strategies, NHP research has the potential to satisfy the need to assess functional, cognitive and behavioural outcomes, in addition to the physiological effects in species with similar brain and immune system structure and functionality. However, this may be hampered by a virtual lack of longitudinal follow-up studies to evaluate aging effects on cognition and behavior in sufficiently large NHP cohorts.

Inbred mouse species of mice and even transgenic models cannot predict accurately for how long a drug, biological, or vaccine will work or possibly cause adverse effects in an outbred population. An outbred population with specific characteristics, e. g. aged NHP, which better resemble the human population, is often the most relevant model. In any case, rodent (or other small animal models) and

primate experimental models need to be used in parallel in order to obtain robust and complementary information. Alongside other models, NHPs should have a unique place in the overall aging and neurodegenerative research strategy.

To our opinion, NHP-based studies in the field of neurodegeneration and the drug discovery pipeline in particular, will remain essential in the development and validation of radiopharmaceuticals for the identification and follow-up of early diagnostic imaging biomarkers, as well as in the preclinical efficacy and safety evaluation of new therapeutic approaches, with active immunization or vaccination approaches as front runners. Important in this matter may be the fact that aged NHPs also display immunosenescence [190], as needs to be taken into account with the development of immunization methods in an aged human population [191].

The quest for models with even further improved translational validity needs of course to be continued. Manipulating genes in NHPS is far more difficult than in mice. For instance, it took almost three decades of research effort to create transgenic NHPs after the first transgenic mouse was established. Then it took almost another decade to generate the first transgenic NHP neurodegenerative disease models [193]. The development of efficient and reliable ways to make precise, targeted changes to the genome of living cells is a long-standing goal for biomedical researchers. Precise gene editing systems, including zinc finger nucleases (ZFNs), transcription activator-like endonucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)-associated Cas9 endonucleases, which are programmable site-specific nucleases, have recently been designed to target genes of interest and represent powerful research tools [192]. The bacterial CRISPR/Cas9 system allows sequence-specific gene editing in many cells and organisms and holds promise as a tool to generate innovating models of human diseases [194]. The development of engineered mice only takes months, at a fraction of the costs compared to the classic transgenesis tools, allowing the development of complete new generations of inbred and outbred mice for more precise identification of genes and variants that

impact disease phenotypes [195]. In the near future, these innovative gene-editing tools might also be applied in NHP compared to generate highly translation NHP models for human diseases, including AD.

In conclusion, aging NHP are not complete phenocopies of human AD. It may well be the case that no other species or experimental model will ever develop the full spectrum of neuropathological, neurochemical, cognitive, behavioural and functional alterations typical for AD. Nevertheless, also partial – and even negative models – are valuable and increase knowledge and understanding of disease mechanisms. Key is to broaden focus and acknowledge the values and, first and foremost, also the shortcomings of particular models and species. Animal modeling of complex brain disorders like AD, should and cannot be based on one particular models or animal species. Only by comparing animal models based on various species, ranging from Drosophila, Caenorhabditis elegans over Zebrafish, towards rodents and NHPs, but of course also Homo sapiens, we will be able to gain maximal insight in AD pathogenesis and therapeutic options. As described in this review, and several other excellent reviews [35]-[39], NHPs have important translational value given their close phylogenetic relationship to humans and quite similar brain structure and functioning. Understanding all brain diseases relies on us understanding how the healthy brain works during normal functioning, and NHP serve excellent tools in this respect. In our opinion, NHP-based studies in the field of neurodegeneration and the drug discovery pipeline in particular, will remain essential in the development and validation of radiopharmaceuticals for the identification and follow-up of early diagnostic imaging biomarkers, as well as in the preclinical efficacy and safety evaluation of new therapeutic approaches, with active immunization or vaccination approaches as front runners.

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FIGURE LEGENDS

Figure 1

Phylogenetic tree of primate evolution with mean brain weight (g) and homocentric encephalization quotients (EQ), as described in [22]. EQ is a measure of relative brain size defined as the ratio between actual brain mass and predicted brain mass for an animal of a given size, which is hypothesized to be a rough estimate of the intelligence or cognition of the animal, although this is a matter of debate. Since *Homo sapiens* has the highest EQ, a homocentric EQ, calculated as brain weight/body weight$^{0.64906}$, in which *Homo sapiens* has the highest value of 1.0 or 100%, can also be applied. This equation is derived by drawing a line through the average log (base 10) values of modern *Homo* to the origin of zero brain and body weights. The advantage of the homocentric EQ is that all other animal (or primate, in this case) species are expressed as a direct percentage of the human value. Values from the following species were included as representatives from their evolutionary clade; for Lemurs, lorises, pottos: ring-tailed lemur (*Lemur catta*); for Tarsiers: the spectral tarsier (*Tarsius spectrum* also called *Tarsius tarsier*); for New World monkey: Common Squirrel Monkey (*Saimiri sciureus*); for Old World monkeys: Rhesus monkey (*Macaca mulatta*); for Lesser apes: white-handed gibbon (*Hylobates lar*); for Orangutans: Bornean orangutan (Pongo pygmeus); for Chimpanzees and Bonobos: common chimpanzee (*Pan troglodytes*); for Gorillas: western gorilla (Gorilla gorilla). Images of brains were obtained from the University of Wisconsin and Michigan State Comparative Mammalian Brain Collections where the preparation of all these images and specimens were funded by the National Science Foundation, as well as by the National Institutes of Health (Retrieved from [23]).

Figure 2

Comparison of Alzheimer's disease (AD) pathological hallmarks in human AD patient (left panels) and aged cynomolgus monkey (Macaca fascicularis) brain (right panels) [67]. Neuropathological features include (A+D) amyloid plaques present in brain parenchyma visualized with anti-A$\beta_{1\text{-}42}$

immunohistochemical staining with, respectively, 4G8 and AB5078P antibodies, (B+E) cerebral amyloid angiopathy (CAA) visualized with anti-$A\beta_{1-42}$ immunohistochemical staining using, respectively, 4G8 and AB5078P antibodies, (C+F) neurofibrillary tangles visualized with, respectively, NFT200 and anti-human p-tau threonine 231 (p-tau T231, MAB3420SP) antibodies for immunohistochemical staining. Compact plaques were observed in frontal lobes (arrows in B), whereas diffused shapes were noted in parietal, temporal lobes and hippocampus in aged cynomolgus brain. CAA was found is small veins and capillaries of aging cynomolgus brain (arrows in D), while in human AD, CAA is most prominent in arterioles and less frequent in veins and capillaries [68]. Some evidence of early tauopathy was present in aging cynomolgus brain (arrows in F); p-tau T231-positive structures appeared only in the cytoplasm of the neurons, analogous to the early stage of tangle formation in human AD brain [69]. These observations add to the utility of aged cynomolgus monkeys as a spontaneous model of AD. Images from human AD brains were obtained with permission from the Institute Born-Bunge biobank database. Images from cynomolgus monkey brain reprinted with permission from [67].

Figure 3

Abnormally aggregated tau is the hallmark pathology of tauopathy neurodegenerative disorders and is a target for development of both diagnostic tools and therapeutic strategies across the tauopathy disease spectrum, which includes of course Alzheimer's disease, but also some forms of frontotemporal dementia, as well as progressive supranuclear palsy and corticobasal degeneration. Development of carbon-11- or fluorine-18-labeled radiotracers with appropriate affinity and specificity for tau would allow noninvasive quantification of tau burden using positron emission tomography (PET) imaging. Nonhuman primates have a high translational value in the development and preclinical evaluation of lead radioligands prior to human PET imaging trials, as exemplified here with representative sagittal microPET images of rhesus monkeys imaged with three different new tracers; [^{11}C] N-methyl lansoprazole (A, summed images 0−60 min post i.v. injection of the radiotracer), [^{18}F] N-methyl

lansoprazole (B, summed images 0−90 min post i.v. injection of the radiotracer) and [^{18}F] lansoprazole (C, summed images 0−60 min post i.v. injection of the radiotracer). Panel D shows a representative sagittal view of a rat imaged with [^{11}C] N-methyl lansoprazole (summed images 0−60 min post i.v. injection of the radiotracer). Lansoprazole is a proton-pump inhibitor that has nanomolar affinity for certain forms of tau and selectivity for tau over amyloid [128]. Panels A and B clearly show brain penetrance of the tracers, while no brain penetrance is observed for the third tracer in rhesus monkey brain. Panel D shows no discernible brain uptake of [^{11}C] N-methyl lansoprazole radiotracer in rats that did show brain penetrance in rhesus monkeys (A). Reprinted with permission from [129]. Copyright 2014 American Chemical Society.