performances. As in a jazz performance, where musicians are free to improvise provided that their improvisations fit within the structure of the tune, variations in animal cooperative performances also follow complex rules. Wrens produce variations in their duets but maintain the overall duet structure. How is the structure of the duet encoded in the song system, and how are variations generated from within this structure?

Over time, we hope not only to learn more about the specific mechanisms that plain-tailed wrens and other animals use to coordinate their behavior, but also to describe cooperative processes in mathematical terms. One application of these sorts of mathematical descriptions is to implement them in artificial systems, such as co-robots — robots that work with people. To safely cooperate with people, co-robots need to use algorithms that are well-tuned to the rules and mechanics of cooperation with people. These algorithms must be sufficiently predictable so that a person can work with the robot, but also sufficiently flexible to accommodate the natural range of variations that people make when they are cooperating.

Where can I find out more?

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Primer

Alzheimer's disease

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The German psychiatrist and neuropathologist Alois Alzheimer was fascinated by the symptoms of Auguste D., a 50-year-old woman admitted to the Frankfurt Psychiatric Hospital in 1901 who suffered from memory disturbances, paranoia and progressive confusion. After her death and autopsy, Alzheimer described histological alterations in her brain that later came to be known as amyloid plaques and neurofibrillary tangles (Figure 1). The case report was published in a psychiatric textbook some years later, and this peculiar and (at the time) seemingly rare illness was later named Alzheimer's disease.

Humans differ from other primates through their superior intellectual and mental abilities. When a gradual and chronic loss of these cognitive functions leads to a loss of independent living, the individual is described as being demented. Such declining cognitive functions encompass all mental processes involved in acquiring knowledge and practical skills, including memory, language, reasoning and attention. Today, Alzheimer's disease is the most prevalent neurodegenerative disorder, comprising approximately 60% of dementia cases. With steadily improving standards of living, people in developed regions of the world are living longer, and Alzheimer's disease is associated strongly with old age. The number of cases of Alzheimer's disease has been increasing steadily, and with today's aging population, the number of people with dementia worldwide is expected to quadruple by 2050 unless effective treatment or prevention becomes available. In this Primer, we consider the symptoms, biological basis and potential biomarkers of Alzheimer's disease.

Symptoms and risk factors
Separating changes associated with normal aging from the early symptoms of Alzheimer's disease is challenging because of the disease's gradual development and the subtlety of the initial changes in cognitive function. The first symptom is often a change in episodic memory, more specifically the amnestic syndrome, where free and cued recall abilities are impaired. Remembering new information becomes difficult, and as the disease progresses, executive dysfunction and problems in language and spatial orientation occur. Activities of daily living such as dressing and eating are usually not impaired until the disease progresses further. There are large variations in the course of the disease when it comes to types of symptom and rate of progression. Because there is no cure, most patients eventually reach the dementia phase of Alzheimer's disease, though as the disease usually starts in later life and can progress over many years, some patients may die prior to dementia.

Who will develop Alzheimer's disease, and is it possible to prevent it? The cause of Alzheimer's disease is probably multifactorial, consisting of a cocktail of environmental, lifestyle and genetic factors. Risk factors for Alzheimer's disease are classified as modifiable or non-modifiable, the latter being age and individual genetic profiles. There have been numerous studies attempting to identify modifiable risk factors. Those that have been reliably documented include cardiovascular risk factors such as hypertension, diabetes, obesity, physical inactivity and smoking. Other modifiable risk factors of importance are cognitive inactivity and a low level of education, perhaps due to what has been termed 'cognitive reserve', whereby individuals with higher education and mental stimulation are believed to have developed more synapses, allowing them to withstand neurodegeneration for a longer period of time.

Modifiable risk factors have been estimated to represent 35% of the total risk of Alzheimer's disease, suggesting that increased focus on them could lead to prevention of up to one-third of all cases. Postponing symptom onset by only one year could potentially lower Alzheimer's disease prevalence by 11%, equivalent to more than 9 million cases worldwide
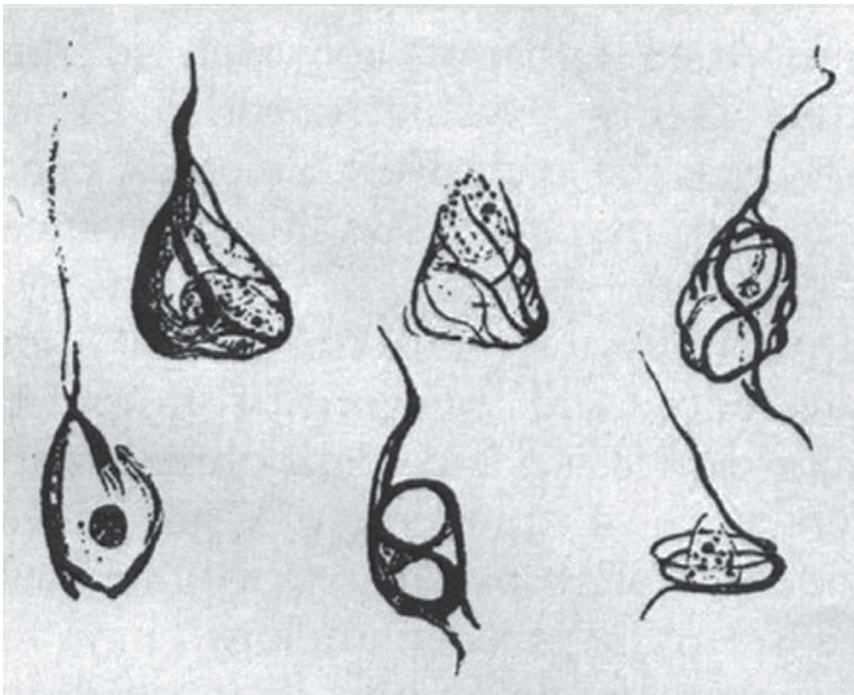
Figure 1. Alois Alzheimer`s original drawings of neurofibrillary tangles.
From Alzheimer (1911).

over the next 40 years. This would have a major impact on health economics.

The Alzheimer's disease continuum
The first diagnostic criteria for Alzheimer's disease from 1984 required the patient to be demented before the diagnosis could be made, and a definite diagnosis could only be made post mortem by neuropathological examination of the patient's brain. Autopsy studies, however, have shown that up to 30% of patients meeting the clinical criteria for Alzheimer's disease do not have typical brain pathology, suggesting other diseases may be present with similar symptoms to Alzheimer's disease. Furthermore, autopsy studies on elderly individuals with normal cognitive function have found typical Alzheimer's disease pathology in the brain, indicating that such alterations may be present without any symptoms of cognitive decline. Similar findings in young people with autosomal dominant Alzheimer's disease indicate that deposition of amyloid plaques may occur up to 20 years before onset of symptoms. These discoveries, together with the development of

biomarkers for Alzheimer's disease have led to a paradigm shift in the field of Alzheimer research, recognizing that symptoms and underlying brain pathology do not go hand in hand, but follow a non-linear dynamic continuum (Figure 2.)

During the past decade, new research and diagnostic criteria have been proposed, describing different phases of the Alzheimer's disease continuum. In the *presymptomatic* or *preclinical* phase, Alzheimer's disease pathology is already present in the brain even though the individual is cognitively intact. The *predementia* phase is characterized by mild cognitive impairment, in which patients have objective cognitive decline in one or more domains, but are still capable of independent living. The *dementia* phase is characterized by cognitive and functional decline to such an extent that daily life becomes impaired.

New diagnostic criteria that enable identification of people in the preclinical phase of Alzheimer's disease will be the key for early intervention, treatment and prognosis. As of today, we cannot cure affected neurons, and it is crucial that intervention is made before neuronal loss begins.

The amyloid hypothesis
A healthy brain contains about 86 billion neurons. One neuron may connect with up to 10,000 other neurons, passing signals via 1000 trillion synaptic connections. Alzheimer's disease leads to a gradual and irreversible loss of many of these neurons and synapses. The typical histopathological alterations thought to be the hallmark of the disease are the plaques and neurofibrillary tangles originally seen and drawn by Alzheimer. These are caused by an accumulation of amyloid-beta peptide in brain tissue, and hyperphosphorylation of microtubule-associated tau protein in neurons.

Amyloid beta peptides are composed of a various number of amino acids, and generated through proteolytic cleavage of amyloid precursor protein (APP) by several enzyme complexes known as secretases. Cleavage by α-secretase and subsequently by the γ-secretase complex forms non-amyloidogenic products of APP. An alternative amyloidogenic pathway, with cleavage of APP first by the β-secretase BACE1, and subsequently by the γ-secretase complex, leads to an accumulation of insoluble amyloid beta proteins in the brain.

The accumulation of amyloid beta is thought to trigger a neurotoxic cascade, leading to plaque formation, phosphorylation of tau protein, formation of neurofibrillary tangles, inflammation, synaptic loss and eventually neuronal death. The pathology usually starts in the brain regions involved in learning and memory, more specifically the hippocampus and entorhinal cortex in the temporal lobe, eventually spreading to the entire cortex.

There has been a substantial amount of skepticism towards the amyloid hypothesis ever since its first description in the 1980s. Critics often claim APP has neurotropic effects, sharing similar structural features with the precursor of epidermal growth factor, suggesting that increased expression of APP and subsequent increases in amyloid deposition are a *response* to neuronal injury rather than a pathological driver of the disease. The large amount of failed therapeutic trials targeting amyloid deposition is often used as confirmation of this theory. However, the strong correlation between mutations in the

PSEN1, *PSEN2* and *APP* genes and trisomy 21, which all lead to increased accumulation of amyloid plaques and the development of early-onset familial Alzheimer's disease, strongly supports the amyloid hypothesis. PSEN1 and PSEN2 are important components of the γ-secretase complexes responsible for the cleavage and release of Aβ. The *APP* gene is located in chromosome 21 and mutations in the gene itself or trisomy 21 result in increased levels of amyloid beta. Also Aβ42 peptides isolated from late-onset Alzheimer's disease human brains cause memory deficits, long-term synaptic depression and decreased synapse density when injected to rodent brains, thereby providing further support for the amyloid hypothesis.

Genetics

The gene for apolipoprotein E *(APOE)* was the first identified susceptibility gene for late-onset Alzheimer's disease, which is by far the most common form. ApoE is a protein mainly involved in cholesterol metabolism and transport, and it transports cholesterol to neurons via ApoE receptors. The *APOE* gene comes in three main isoforms; ε2, ε3 and ε4, which have a worldwide frequency of 1–5%, 50–90%, and 5–35%, respectively. The isoforms have different impacts on accumulation and clearance of amyloid beta in the brain, and the reduced ability of the ApoEε4 isoform of the protein to remove deposited amyloid may be the reason that *APOEε4* is the most important genetic risk factor for late-onset Alzheimer's disease. Having one copy of *APOEε4* increases the risk for late-onset Alzheimer's disease threefold, while individuals with two copies have an eight- to twelve-fold increased risk of developing the disease.

Recent studies also show that the overall heritable risk of sporadic late-onset Alzheimer's disease is complex, with a large polygenic contribution. In addition to the ApoE polymorphism, genome-wide association studies have identified several other genetic loci to be associated with the disease, suggesting that there are many other susceptibility genes contributing to its intricate biological pathway. Polygenic risk scores have been developed based on these loci, enabling calculation of an individual genetic risk profile. In the
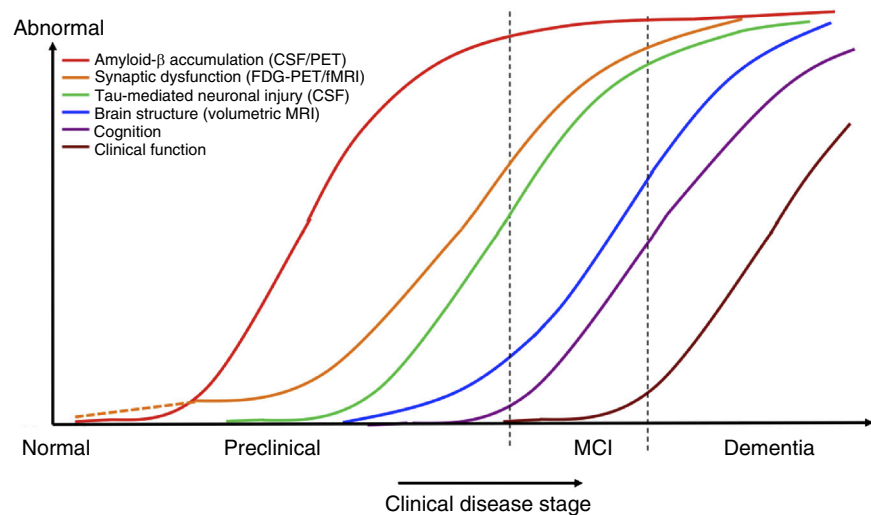
Figure 2. Model based on research data indicating sequential changes in dynamic biomarkers of Alzheimer's disease over time.
Reproduced with permission from Jack *et al*. (2010).

future, polygenic risk scores could be valuable in selecting young individuals at risk for Alzheimer's disease, allowing for early intervention with respect to modifiable risk scores.

Biomarkers

An ideal biomarker for Alzheimer's disease should have high specificity and sensitivity, clearly separating the disease from other types of dementia as well as from healthy individuals. Biomarkers used for the detection of Alzheimer's disease can be divided into two main groups; measurements of specific proteins in cerebrospinal fluid (CSF), and neuroimaging techniques. Validated biomarkers in CSF include reduction in the level of β-amyloid (Aβ42), increased total tau and phosphorylated tau-181. A combination of these three biomarkers increases the diagnostic validity for sporadic Alzheimer's disease compared to healthy controls with a combined sensitivity of >95% and specificity of >85%.

Magnetic resonance imaging (MRI) and positron emission tomography (PET) can be used to measure amyloid and tau deposition, brain metabolism and brain volume. Amyloid PET uses a labeled amyloid tracer, and the cortico/cerebellar standardized uptake value/ratio is calculated as an index for Aβ-deposits. Certain regions of interest in the brain are determined, and the uptake is compared to a cerebellar

reference. This allows objective measurement, with higher sensitivity and specificity than visual inspections of scans. Studies have confirmed its ability to separate Alzheimer's disease patients from control subjects, and Aβ42 in CSF and amyloid plaque deposition in brain are inversely correlated. Low β-amyloid (1–42) and high total tau and phosphorylated tau-181 in CSF and/or high retention of amyloid tracer measured with amyloid PET in connection with Alzheimer's disease are required as pathophysiological markers according to the latest research criteria.

A decline in Aβ42 in CSF can be detected up to 20 years before onset of symptoms, whereas amyloid PET can detect pathological deposition of amyloid plaques up to 15–20 years before the first symptom occurs. Considering the high costs of Alzheimer's disease dementia, it has been suggested that in the future, middle-aged people should be screened through sampling of CSF, selecting those with low Aβ42 for further investigation with PET. If pathological amyloid deposition is confirmed, these individuals could be selected for early, presymptomatic therapeutic interventions when available.

But although CSF sampling is relatively inexpensive, it is an invasive procedure, and therefore has a certain risk of complications. On the other hand, amyloid PET is a highly expensive
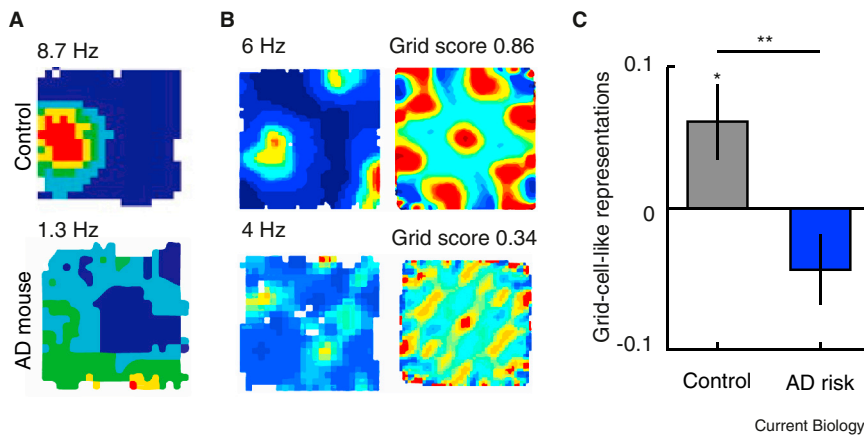
Figure 3. Spatial representations are impaired in mouse models of AD and young human adults at genetic risk for AD.

Mouse models of Alzheimer's disease show a degradation of the spatial activity of both place cells (A) in the hippocampus and grid cells in the entorhinal cortex. (B) Top: control mice. Bottom: mice with AD pathology. In (A) and the left panel of (B) are shown firing rate maps (adapted from Cacucci *et al.* (2008), copyright (2008) National Academy of Sciences, USA, and Fu *et al*. (2017), respectively). Red colours indicate higher firing frequency of a cell at a given location in the environment. The right panel in (B) shows spatial autocorrelograms of grid cell activity. Grid scores, a measure of how regular the hexagonal grid cell pattern is, are reduced in mice with Alzheimer's disease pathology. (C) Grid-cell-like representations in the entorhinal cortex of young adults are reduced in the *APOEε*4 group compared to the control group (adapted from Kunz *et al*. (2015)).

imaging technique, exposes the patient to (short-term) radiation, and has limited availability even in wealthy countries. There has been a considerable amount of research on various blood-based biomarkers in Alzheimer's disease, identifying the intricacy of the pathobiology of the disease, but none yet fulfil the diagnostic accuracy of amyloid PET/CSF core biomarkers. A recently published paper has shown promising results for a blood-based (plasma) amyloid β-biomarker using immunoprecipitation coupled with mass spectrometry. The ideal screening method, however, would be a blood-based biomarker that separates Alzheimer's disease from other dementias, and also monitors disease progression and effect of therapy. No such biomarker exists today.

There are also several ethical issues to screening. Should the clinician communicate to cognitively normal middle-aged individuals that they are in a preclinical stage of Alzheimer's disease, and if so how?

A potential new biomarker for early detection of Alzheimer's disease

Through decades of research, the hippocampal formation has been demonstrated to contribute substantially to memory formation and retrieval, as well as to navigation and the representation of space. Studies in freely moving rodents have revealed place-modulated neuronal activity in two key structures of the hippocampal formation; place cells in the hippocampus and grid cells in the entorhinal cortex. Reports of similar neuronal substrates in monkeys, bats and humans showed that neuronal coding in these areas is highly conserved across species, and suggests an importance for many fundamental processes in the brain.

While hippocampal place cells fire whenever an animal enters a certain place in its environment (the neuron's place field), entorhinal grid cells fire periodically at multiple locations and form a six-fold rotationally symmetrical grid-like firing pattern across space (the neuron's grid field). The neuronal population code emerging from these two spatially tuned cell types is assumed to provide an internal, cognitive map. Importantly, recent evidence suggests that the functional role of grid cells and place cells extends well beyond navigation through space. For example, if rats have to recognize a certain pitch of frequency in a tone, grid cells show representation of discrete 'frequency locations' on the continuous dimension of pitch.

The grid-cell system also seems to map visual space even without actual navigation. In both monkeys and humans, studies found the six-fold rotationally symmetrical grid-cell-like activity typical of grid cells that depended on where subjects were looking on a computer screen. Lastly, the grid cell system has been implicated in the organization of abstract conceptual spaces in humans.

Human grid-cell representations can be investigated with functional magnetic resonance imaging (fMRI), where changes in blood oxygenation levels correlate with neuronal activity. When analyzing blood oxygenation level dependent (BOLD) responses as a function of the participant's movement direction in virtual reality, a six-fold rotational dependency of entorhinal activity (hexadirectional signal) was found that correlated with how well participants remembered locations. This fMRI-based method provides a promising, non-invasive window into the population activity of grid-like coding in humans.

Importantly, the entorhinal cortex is among the first regions affected by Alzheimer's disease-related pathology. Entorhinal grid cell-like representations are already altered in young adults at genetic risk for Alzheimer's disease (*APOEe4* heterozygotes), decades before potential onset of cognitive decline or general deficits in spatial memory. APOEe4 carriers also showed subtle changes in navigational strategy, such as moving close to the boundary in a virtual arena environment. These behavioral differences, as well as increased activity in parts of the hippocampus, could reflect compensatory mechanisms for impaired entorhinal processing. Compromised grid-cell-like representations have subsequently been associated with age-related navigational deficits and studies in matured mice at genetic risk for Alzheimer's disease-related pathology showed impairments in both grid cell activity and spatial memory (Figure 3).

In summary, the entorhinal cortex and hippocampus play central roles in spatial navigation and memory and form a system that organizes memory-guided behaviors. Unsurprisingly, the degradation of this system in Alzheimer's disease is associated with severe impairments

in memory, navigation and cognition in general. Estimates of grid cell-like-representations based on fMRI data therefore hold promise as a potential early prognostic marker for the development of cognitive and memory impairment in Alzheimer's disease.

Treatment

In spite of comprehensive research and an immense number of therapeutic trials, there remains no curative treatment for Alzheimer's disease. Disease modifying treatments available today are designed to delay symptoms and cognitive decline in symptomatic patients. Acetylcholinesterase inhibitors for mild to moderate Alzheimer's disease, and memantine, an N-methyl-D-aspartate receptor antagonist for moderate and severe Alzheimer's disease, aim at slowing progression and controlling symptoms by replacing neurotransmitters known to be altered in Alzheimer's disease. Meta analyses show a short-term effect on cognitive function, but no effect on progression of disease.

Based on the amyloid hypothesis, several therapeutic trials have sought to decrease and remove amyloid beta depositions in the brain. Despite some success in plaque removal, none have succeeded in phase III trials because no marked improvement in cognitive function has been demonstrated. One explanation for this may be that the intervention came too late in the disease continuum, often in the early dementia phase, when brain pathology has evolved too far and the neuronal damage is too large. Patient selection has also often been based on a clinical diagnosis alone, so it has been unclear whether the participants have amyloid pathology or not. Optimizing selection of participants and better understanding the pathophysiological processes of Alzheimer's disease are essential to develop novel disease-modifying drugs.

Future considerations

Alzheimer's disease research is still at an early stage compared to many other medical research fields. The establishment and understanding of the nonlinear dynamic Alzheimer's disease continuum has led to an important paradigm shift in the field, but many aspects remain to be clarified. Further studies on the complex biological pathways are essential.

The entorhinal cortex and hippocampus are usually the first regions affected by Alzheimer's disease pathology. They play a key role in spatial navigation and memory, and form a system that appears to organize memory-guided behaviors based on continuous sensory or conceptual dimensions. The degradation of this system in Alzheimer's disease is therefore associated with severe impairments in memory and navigation. fMRI-based estimates of grid cell-like-representations in the entorhinal cortex hold promise as a potential early prognostic marker for the development of cognitive and memory impairment in Alzheimer's disease.

Existing Alzheimer's disease biomarkers support accurate and early diagnosis, and also aid selection of suitable individuals for intervention, thus increasing optimism for upcoming therapeutic trials. Nevertheless, the existing biomarkers have limited relevance and multiple biomarkers will need to be developed; for understanding pathophysiological changes associated with the Alzheimer's disease continuum, identifying asymptomatic individuals at risk of disease with high sensitivity and specificity, distinguishing Alzheimer's disease from other forms of dementia, measuring the effect of therapy, and excluding individuals at risk of developing dangerous side effects of specific treatments. So the hunt for new biomarkers will continue. In this regard, the development of blood-based biomarkers would be advantageous as blood sampling is not considered particularly invasive, samples are quickly and easily obtained, the risk is minimal, and in a medico-social context they would be by far the most cost-effective.

Although effective therapy to stop or delay Alzheimer's disease progression has long been, and remains, a major goal, this does not have to be drug-based. One crucial aspect that has only recently received marked attention regards the clear possibilities for disease prevention, or at least delay of disease onset, that can often be achieved through simple changes in lifestyle. Campaigns through the media regarding modifiable risk factors could be easily arranged, and would have the added advantage of beneficial effects on other major aspects of public health, like obesity, vascular disease and diabetes. It would also be extremely cost-effective compared to the development of drug-based therapies. Indeed, intervention studies focusing on vascular risk factors, physical activity and cognitive training have shown promising results in delaying cognitive decline, and as of today are a better therapy against Alzheimer's disease than any current drug-based treatment.

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