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Module 1, Video 4: Sex differences during normal and pathological aging

Cognition naturally declines with age. Of the many factors that contribute to the risk of suffering from neurodegenerative diseases, aging predominates. Further, more than half of all individuals over 65 are women and women have a longer lifespan than men. Thus, their prevalence of neurodegenerative diseases is much higher, with only Parkinson's disease exhibiting an increased risk in men [1], demonstrating a need to understand sex differences in normal and pathological brain aging.

However, a recent analysis of over 15,000 studies published between 1994 and 2014 showed that over 40% of preclinical studies did not report the animal's age, and 20% failed to report both sex and age [2, 3], highlighting an important research gap. In this video, we will cover sex differences during normal AND pathological aging, using Alzheimer's disease as an example.

In women, menopause, and the resultant decline in sex hormones, is associated with a number of physiological changes. These changes coincide with cognitive declines and the increased risk of various neurodegenerative diseases [4, 5][6][7, 8][9-11]. Similarly, aging rodents also undergo many changes as they enter reproductive senescence [12][13, 14] [15][16-18], including effects on cognition, making them an attractive model for studying the effects of estrogen loss on memory.

Overall, reproductive senescence in rodents is similar to menopause in humans, but some distinct differences should be noted. Notably, approximately half of rodents enter a persistent estrous state and have continuously high hormone levels and some remaining follicles for the remainder of their life. We will discuss these differences between rodents and humans further in Video 10.

The timing of rodent ovarian cycle cessation also makes them an ideal model for studying the effects of estrogen loss on memory. By 12 months, approximately 10% of female rats are acyclic. This number increases to 40% by 18 months and 75% by 24 months [19]. Thus, rodents are typically considered "aged" at approximately 2 years old and "middle aged" from 16-18 months. But most memory and cognition studies are conducted in rodents between 12 and 16 months, limiting the translational relevance of these memory studies conducted in "young" animals.

Age-related changes in the brain are also noted in multiple species. Effects on the hippocampus are widely studied in aging due to its role in learning and memory and documented association with circulating estrogen levels [16]. Deficits in hippocampal-dependent spatial cognition are

associated with the normal loss of estrous cycling in both rats and mice [20, 21]. Although there are many tests to assess age-related cognitive decline [22][23], much of this work has focused on the Morris water maze for several reasons. First, while the Morris water maze in itself is stressful, its results are not confounded by food or water deprivation to promote motivation during the test. Second, cognition can be assessed rather quickly. This task has also recently been adapted for use in Alzheimer's patients, potentially improving its translational relevance for assessing cognitive decline [24].

Similar to humans, spatial memory impairments emerge gradually in rodents with age. Few studies have assessed sex differences in cognition over time. But females show significant deficits in Morris water maze spatial memory at an earlier age than males: for females, about 12 months in rats and 17 months in mice, whereas for males, about 18 and 25 months, respectively [25-28]. While the exact mechanism of sex differences in age-related spatial cognitive decline is unknown [29, 30], it is clear that these changes coincide with the cessation of circulating ovarian hormones [31]. Thus, sex differences in normal lifespan memory trajectories are important to consider.

One way to determine how changes in ovarian hormones contribute to memory decline in females is with hormone replacement therapy. In women, hormone replacement therapy can prevent cognitive decline [32]. However, the timing is important, with cognitive benefits only observed if replacement is initiated close to the onset of menopause. These findings are echoed in rodents. In rats, hormone replacement improves spatial memory performance only when initiated within 3 months of ovariectomy, not at 5 or 10 months after ovariectomy [33-35].

Men also show age-associated declines in cognition. These declines are associated with lower testosterone levels and a higher risk of Alzheimer's disease [36]. Administering testosterone, which is aromatized to estrogen, to aged male rats improves working memory, whereas dihydrotestosterone, which is not aromatized to estrogen, has no effect [37]. Thus, hormone therapy in aged males AND females may have beneficial effects on some aspects of cognition.

Alzheimer's disease shows sex differences in its prevalence, clinical manifestations, disease course, and prognosis—particularly across the life span [38].

Although these differences could be related to the increased life expectancy of women, longevity alone may not fully explain why 2/3 of Alzheimer's disease patients are women. The incidence of Alzheimer's also diverges later in life, with females slightly higher than males. Alzheimer's disease is marked by the accumulation of amyloid-beta plaques and neurofibrillary tangles, which contribute to neuronal loss and cognitive and physical disability [40]. These

hallmarks are recapitulated in animal models. While much of this work has been done in male rodents, there are known sex differences in the potential pathways that contribute to the accumulation of amyloid-beta and neurofibrillary tangles. For example, inflammation increases in both sexes with age, but microglia, which are capable of clearing aggregates in the brain, are more inflammatory in females than in males and show different inflammatory molecule profiles, factors that may be directly attributed to differences in estrogen receptors [15].

Women with the genetic risk factor APOE4 also have a greater risk of developing Alzheimer's, show accelerated progression of the disease, and have more severe memory and cognitive decline than men with this allele. Similar findings have been observed in APOE4 mouse models [41-43].

The decline in sex hormone levels in women also coincides with their increased risk for Alzheimer's disease. Some of these findings are recapitulated in mice where ovariectomy increases soluble amyloid-beta levels in mice and worsens behavioral performance; these changes are attenuated by estradiol hormone replacement [44][41]. Therefore, sex differences should be a priority in the development of Alzheimer's disease therapeutics from preclinical to clinical studies [45].

In this video, we covered how sex differences in aging may contribute to normal and pathological cognitive changes. Some of these cognitive effects can be attenuated by hormone replacement therapy in females when administered at the optimal timing. Sex disparities in neurodegenerative diseases such as Alzheimer's disease highlight the importance of including both sexes in aging research.

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