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Modeling menopause: The utility of rodents in translational behavioral endocrinology research

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Abstract

The human menopause transition and aging are each associated with an increase in a variety of health risk factors including, but not limited to, cardiovascular disease, osteoporosis, cancer, diabetes, stroke, sexual dysfunction, affective disorders, sleep disturbances, and cognitive decline. It is often challenging to systematically evaluate the biological underpinnings associated with the menopause transition in the human population. For this reason, rodent models have been invaluable tools for studying the impact of gonadal hormone fluctuations and eventual decline on a variety of body systems. While it is essential to keep in mind that some of the mechanisms associated with aging and the transition into a reproductively senescent state can differ when translating from one species to another, animal models provide researchers with opportunities to gain a fundamental understanding of the key elements underlying reproduction and aging processes, paving the way to explore novel pathways for intervention associated with known health risks. Here, we discuss the utility of several rodent models used in the laboratory for translational menopause research, examining the benefits and drawbacks in helping us to better understand aging and the menopause transition in women. The rodent models discussed are ovary-intact, ovariectomy, and 4-vinylcyclohexene diepoxide for the menopause transition. We then describe how these models may be implemented in the laboratory, particularly in the context of cognition. Ultimately, we aim to use these animal models to elucidate novel perspectives and interventions for maintaining a high quality of life in women, and to potentially prevent or

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I, Heather A. Bimonte-Nelson, have no conflicts of interest to declare.

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Contributions

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postpone the onset of negative health consequences associated with these significant life changes during aging.

Keywords

Aging; Menopause; Rodent; Model; Hormones; Female

Introduction

Natural aging processes result in physiological changes in the brain and body of all organisms. In most mammalian species, females experience natural reproductive senescence in mid- to late- life. While the vast majority of species' lifespans do not far surpass their reproductive years, one clear exception to this common phenomenon is the human female, whose lifespan extends well beyond the reproductive life stage. For women, reproductive senescence typically occurs around the fifth decade of life, when their finite pool of immature ovarian follicles is depleted via a combination of ovulatory cycles and normal apoptosis (i.e., programmed cell death) called atresia. Menopause ensues when the menstrual cycle ceases due to anovulation, and is verified retrospectively after one year of amenorrhea [1–2]. Notably, menopause is not an abrupt event; indeed, the transition to menopause typically spans four to six years [3]. The onset of the menopause transition and subsequent post-reproductive life stage comes with a variety of physiological, behavioral, and brain changes that can impact quality of life [4–6]. Many health risk factors change with aging and after menopause, including, but not limited to, an increased risk for cardiovascular disease, osteoporosis, cancer, weight gain, diabetes, stroke, sexual dysfunction, affective disorders, sleep disturbances, and cognitive decline [1, 6–7].

Currently, the estimated average female lifespan at birth worldwide is 70 years [8], and life expectancy is increasing, especially for women [9]. Given these numbers, the post-reproductive life stage now encompasses about a third of a woman's lifespan on average. Presently, about 14.88% of United States (U.S.) residents are over the age of 65 (of which about 56% are female) [8]. By 2030, as the "baby boomer" generation ages and life expectancy continues to increase, approximately one out of five people will be aged 65 and older in the U.S. [10–11]. With over 6,000 women projected to reach menopause each day in the U.S. alone [1], it is imperative that we understand the impact of natural and surgical gonadal hormone loss as women reach the post-reproductive life stage in order to inform researchers and clinicians about options for women to maintain a high quality of life across the entire lifespan.

Remarkable work has been accomplished in the realm of clinical research on women's health. Yet, the biological underpinnings of the menopause transition can be challenging to systematically evaluate in humans due to heterogeneous variations in many life factors, including age, parity, diet, socioeconomic status, environmental exposures, and genetic characteristics. This rich variability in humans can make it difficult to dissociate genetic and environmental factors while evaluating specific variables associated with aging and menopause. As such, animal models have been instrumental in evaluating gonadal hormone

effects on various body systems, including developmental and cognitive processes, while also providing a more homogeneous population to study in the laboratory compared to humans [12]. There are several well-characterized laboratory animal models used to evaluate the effects of gonadal hormones and aging, including non-human primates and rodents. Non-human primate models are especially insightful for translational research because of their genetic, physiological, behavioral, and reproductive system similarities to humans [13–16]; however, they are costly and often require many years of study to elucidate the impact of aging and reproductive senescence due to their increased longevity relative to rodents. Rodent models, especially laboratory rats and mice, are particularly useful because of their well-defined aging trajectories and thoroughly studied brain and reproductive systems, as well as their approximate two to three year lifespan [17]. This review focuses on these rodent models, comparing and contrasting the benefits and drawbacks of rodent models of reproductive senescence, and discussing the utility of the rodent model for translational menopause and aging research.

1.1 The Human Menopause Transition

It is a well-accepted tenet that women are born with a finite pool of immature ovarian follicles. Histological follicle counts and mathematical models of ovarian follicle reserves estimate an average of 295,000 follicles per ovary at birth to 180,000 follicles per ovary at puberty; as the menopause transition approaches, estimates are reduced to between 100–1000 immature follicles per ovary [18–19]. At puberty, the ovulatory cycle begins as a result of the maturation of the hypothalamic-pituitary-gonadal (HPG) feedback loop. Women experience regular ovulation, and therefore a consistent menstrual cycle, for about 40 years. Approximately 400 eggs are ovulated across a woman's reproductive life stage; the remainder — and vast majority — of ovarian follicles undergo atresia [20–21].

The female reproductive cycle involves an intricate feedback system between the brain, pituitary gland, and reproductive tract. The hypothalamus synthesizes and releases gonadotropin releasing hormone (GnRH) from GnRH neurons. GnRH signals the anterior pituitary gland to synthesize and secrete the gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH) into the general bloodstream. Immature ovarian follicles undergo various developmental stages over the course of several months and are classified by size and the different cell types that comprise the growing follicle (Figure 1) [18, 22]. Once the follicle pool naturally depletes over time, menopause ensues. What initiates the transition to menopause, whether it is the declining follicle pool and/or brain changes resulting in dysregulation in the HPG axis, is a topic of debate. Phyllis Wise's elegant work in rodents and non-human primates suggests that the central nervous system is of chief importance for the onset of reproductive senescence and that, ultimately, a breakdown in communication between the brain and ovaries results in anovulatory cycles and eventual cessation of the menstrual cycle. This landmark research, discussed in section 3.1.1, suggests that a complex set of changes in neuroendocrine and neurotransmitter signaling involving hypothalamic GnRH neurons and alterations in glutamatergic, GABAergic, and monoaminergic signaling, likely play roles in early stages of the transition to a reproductively senescent state for rodents, non-human primates, and women alike [23–28].

irregular estrous cycles, aptly termed estropause. A constant/persistent estrus state is common, wherein the estrus phase is prolonged and anovulatory cycles may occur (Figure 3A). Animals may then transition into an anestrus state, where ovulatory cycles halt and low levels of gonadal steroids are present [53–55]. Rats, more often than mice, will also pass through a phase of repetitive pseudopregnancy prior to transitioning into an anestrus state, or they may stay in a pseudopregnant state for their remaining lifespan, wherein they ovulate irregularly (and sometimes supraovulate), resulting in corpora lutea that are maintained for an extended period of time producing high progesterone levels [50, 55]. Rodents also experience dysregulated HPG axis activity in estropause, including decreased LH release responsiveness to estrogen signaling in middle-aged rats [56–57]. A chief difference between humans and rodents is the presence of potentially mature ovulatory follicles in the aged rodent. Rodents experience some natural ovarian follicular depletion and are also suspected to have a finite follicle pool [58]; however, some preliminary evidence for adult neo-oogenesis in rodents has challenged this idea [59–60]. Because a hallmark of human menopause is complete ovarian failure, it is important to keep these differences in mind when choosing an appropriate animal model of menopause. Several rodent models of reproductive aging are implemented in the laboratory to aid in understanding of brain and body aging and the menopause transition, considered in detail below.

3.1 Rodent Models of Menopause

3.1.1 Ovary-intact: A model of the aging hypothalamic-pituitary-gonadal axis

Similarly to women, rodents experience regular reproductive cycles in adulthood, as well as age-related dysregulation of this cycle and the HPG axis, ovarian changes, and gonadal hormone fluctuations. Many researchers have utilized the ovary-intact female rat as a model of human menopause to evaluate the changing brain, pituitary, and ovary interactions with age [23–24, 61]. Phyllis Wise's research, beginning in the 1970's, demonstrated that as aging occurs in ovary-intact rats, changes occur in the rhythmicity of hypothalamic GnRH release that initiates the LH surge. These alterations, originating in the brain, precede observable changes in normal estrous cyclicity in aging rodents [62–63]. These alterations are particularly evident during the proestrus phase, wherein a LH and FSH surge precedes ovulation. Wise's laboratory found that middle-aged animals had lower pre-ovulatory circulating estradiol levels, and that before the LH surge, there was a temporal delay in rising GnRH concentrations in the median eminence, which connects the hypothalamus to the pituitary gland and facilitates gonadotropin release via the hypophyseal portal blood stream; decreased serum LH, estradiol, and progesterone levels following the LH surge in middle-aged animals point to an age-related disruption in circadian rhythm function that consequently alters normal estrous cycle patterns [62]. Wise and others have also found that the amplitude of the LH, FSH, and prolactin surges was reduced in middle-aged, ovary-intact animals [63–64]. These landmark studies gave rise to the theory of aging of neural pacemakers, which suggests both chronological and endocrine aging are crucial factors for understanding the transition to reproductive senescence, and that the brain plays a significant role in these processes. Using the ovary-intact rat model, the Gore laboratory has evaluated aging and its relationship to neuroendocrine control and feedback mechanisms. This team found increased GnRH mRNA levels in middle-aged and aged rats compared to young

control subjects; yet, gene transcription for GnRH decreased with age, pointing to a potential posttranscriptional mechanism for the age-related increase in GnRH mRNA [65]. GnRH and LH release are, in part, mediated by excitatory glutamate signaling via input from N-methyl-D-aspartate (NMDA) receptors; alterations in these inputs likely disrupt positive and negative feedback mechanisms of the HPG axis regulation [65]. Although the number of GnRH neurons does not appear to change with age, there are age- and reproductive senescence- related changes in the gene expression of the subunits that constitute the NMDA receptor [65–66]. Gore and others have suggested that during aging, there is attenuated NMDA receptor-mediated glutamatergic activation of GnRH-producing neurons in brain regions important for HPG feedback [65, 67–68]. This disrupted balance between excitatory and inhibitory neurotransmission is related to the transition to reproductive senescence [61]. These alterations in neuroendocrine function may occur prior to animals displaying outward behavioral or estrous cycle abnormalities, and would likely be difficult to target studying human clinical populations. Therefore, utilizing the ovary-intact rat model to evaluate the natural age-related cellular and molecular changes in brain regions involved in normal reproductive functioning and feedback is crucial to understanding neurological changes that occur in women, and to the development of novel therapeutic targets to mitigate negative symptoms associated with age and reproductive senescence.

A drawback to modeling menopause in the ovary-intact rodent is the difference in the ovarian follicle reserve and steroid hormone changes as aging ensues in the rat compared to women. Because rodents do not typically exhibit follicle-deplete ovaries by the onset of reproductive senescence like women do, circulating gonadal hormone levels in an aged ovary-intact rodent do not closely resemble those of menopausal women. Women in the post-menopausal state have very low circulating levels of 17 β -estradiol and progesterone and significantly elevated FSH and LH levels (Figure 3B) [52, 69]. Rodents, on the other hand, experience several reproductive-aging states and typically maintain moderate circulating 17 β -estradiol levels, despite HPG axis dysregulation [55] (discussed in 2.1). Variations in the onset and expression of HPG axis changes in the ovary-intact female rat could add undesirable variability to this menopause model; however, as we gain understanding of these changes, and if we acknowledge these variations when interpreting findings, we can use this natural variation to our experimental advantage. Vaginal cytology monitoring is a common, minimally invasive way to monitor estrous cycle activity (Figure 4). Yet, vaginal cytology can become erratic in middle age due to dysregulated HPG axis activity. If a researcher intends to use a particular hormone profile of a middle-aged rodent, such as constant estrous, for an experiment, it is likely that only a subset of animals in a colony will fall under this profile at the appropriate age and timing for the experiment. While studying the natural diversity that occurs in the ovary-intact model and taking these estrous cycle variations into account benefits correlation data evaluations, it is limiting in causal-type inferences. It is preferable to methodically control reproductive status and age at evaluation in experimental animals in order to dissociate the specific effects of reproductive senescence from advanced aging, since confounding factors due to age alone may muddle results.

Some stocks or strains of laboratory rodents are considered better models than others for aging research. One example is the National Institute on Aging's (NIA) recommendation to use the Fischer-344 (F344) inbred strain for aging research due to their longevity and well-

defined aging patterns. Sprague Dawley and Long Evans are also commonly utilized rat strains in aging research. Most laboratory rodent colonies do not have aging animals in stock; those that are available are often limited to retired breeders. In menopause research, reproductive history is a variable of chief importance – indeed, several laboratories have demonstrated that sexual experience and parity influence cognitive and brain outcomes [70–74]. For example, these studies suggest a history of pregnancy is related to enhanced spatial memory performance compared to nulliparous animals, and brain responsiveness to estrogen treatment may be influenced by reproductive history. Thus, when employing the ovary-intact rodent model for aging and menopause work, scientists must carefully consider the stock or strain of rodent as well as their age and life history to obtain an appropriate aging model for their research questions regarding the transition to reproductive senescence.

3.1.2 Ovariectomy: A model of surgical menopause

The gold standard in the preclinical field for evaluating gonadal hormone effects in female animal models is the ovariectomy, or surgical removal of the ovaries, often abbreviated Ovx. Rodent anatomy differs from women in that rodents have a bifurcated uterus, called the uterine horn, which accommodates large litters (Figure 5). The standard Ovx procedure is to bilaterally excise the ovaries, oviducts (i.e., the fallopian tubes), and tips of the uterine horn from the peritoneal cavity, leaving the ligated uterine horns intact [75]. Full recovery from Ovx occurs within one week. Ovx most accurately models surgical menopause in women. Ovx is an ideal model to evaluate specific effects of gonadal hormone deprivation and subsequent exogenous hormone treatments on the brain and periphery because it creates a “blank gonadal hormone slate.” As an example, estrogens may be administered after Ovx, alone or in combination with other ovarian hormones, with variations in the type of estrogen administered, dose, route of administration, and treatment duration while controlling for interactions with endogenous levels of sex steroid hormones. Combined exogenous therapies, such as 17 β -estradiol plus progesterone, may be used to evaluate their interactions on a particular system, more closely modeling HT regimens that many menopausal women take. Our laboratory and many others have utilized the Ovx model to evaluate the potential for neuroprotective effects of exogenous ovarian hormone administration on the brain. For example, administering HT to Ovx females has been shown to enhance performance on mazes evaluating spatial memory compared to Ovx females that were not receiving estrogen treatment (discussed in section 4.1). Higher circulating 17 β -estradiol levels have been associated with better spatial memory performance for both young and middle-aged Ovx rats [47], and chronic, long-term exogenous estrogen plus progesterone treatment enhances radial-arm maze performance in middle age [45]. Importantly, the Ovx model has also shown limitations of efficacy in gonadal hormones, including some estrogens. For example, when estrone, a weaker metabolite of 17 β -estradiol and the main component in Premarin, was given at a tonic dose to middle-aged Ovx rats, it had a negative impact on spatial memory at a high dose [76].

A drawback to the Ovx model in the context of translational research is that the majority of women retain their whole reproductive tract throughout the menopause transition. While removing the ovaries prior to the onset of reproductive senescence allows researchers the control to evaluate the impact of particular hormones without aging as a confounding factor,

the age at which Ovx occurs in rodents may also result in divergent effects [77–78], particularly for cognition [44]. Additionally, the sudden loss of ovarian steroids is not characteristic of the majority of transitionally menopausal women; compounded with this, the post-menopausal ovary continues to release androgens and low levels of other steroids [79–80]. Consequently, the Ovx model results in a considerably different hormone profile, as well as an altered HPG axis, compared to animals with an intact reproductive tract. Nonetheless, the Ovx model is a classic technique that merits much praise for its utility in evaluating novel phenomena in the context of aging and menopause research. While it is imperative to understand the impact of specific hormone and drug effects on the brain and body systems with a “blank gonadal hormone slate,” in the context of the menopause transition, these factors associated with reproductive senescence do not operate in a vacuum, and understanding how exogenous hormone formulations interact with intact systems (i.e., a more representative model of human menopause) is translationally important.

3.1.3 4-vinylcyclohexene diepoxide: A model of transitional menopause

The drug 4-vinylcyclohexene diepoxide, or VCD, a metabolite of 4-vinylcyclohexene, is produced when the industrial chemical butadiene is utilized to manufacture various plastic-based goods [81–82]. VCD is used commercially to reduce fecundity and species proliferation of rodent pests without the use of hazardous poisons that endanger the environment [83]. In the early 2000's, VCD was introduced to biobehavioral animal research as a rodent model of transitional menopause because it was found to selectively target and deplete the non-growing ovarian follicle pool in rodents via atresia, resulting in accelerated follicular depletion and eventual ovarian failure in rodents [79–81, 84–93]. VCD is thought to function through accelerating atresia in primordial and primary ovarian cells — but not disrupting growing follicles — by altering the expression and distribution of the Bcl-2 family of proteins that regulate apoptosis, including Bcl-xL, Bax, and Bak proteins within ovarian follicles, thus initiating cytochrome c release into the cell cytosol, triggering downstream caspase signaling activity, all of which are related to accelerated follicle atresia [49, 84–85, 91–93].

The introduction of VCD as a model of transitional menopause has been an integral contribution to science. This innovative model more closely mimics the human menopause transition compared to the ovary-intact or Ovx models by effectively depleting the resting follicle pool but allowing for the retention of follicle-deplete ovarian tissue, resulting in an ovarian and hormone profile more similar to a vast majority of women undergoing the natural menopause transition who retain their reproductive organs in the post-reproductive life stage. In rodents, VCD is typically administered via a series of intraperitoneal injections ranging from 80 mg/kg-160 mg/kg [79–81, 94–96]. The Jackson Laboratory offers a VCD-treated mouse model available for purchase (<https://www.jax.org/strain/100010>), or researchers can administer the drug to rodents in the laboratory. Recently, an oral bait has been developed for rodents that contains a combination of VCD and triptolide, a Chinese herb thought to accelerate the follicular depletion of later-stage ovarian follicles (e.g., pre-ovulatory antral follicles) [83], which could be used both in the laboratory to decrease the number of necessary VCD injections and to target later-stage follicle development, as well as commercially to reduce wild rodent pest populations by further decreasing fertility. This

model is still undergoing development but is an excellent addition to the VCD-related models [83]. The chief drawback of the VCD model is that it is toxic in high doses [82]. However, the use of VCD in the laboratory has been refined as a model of transitional menopause and no significant effects have been indicated on other organs under appropriate administration regimens [81, 94–95], although it may be an acute stressor to the animal during drug administration. Animals may decrease in body weight during injections, but generally return to baseline weights after drug administration.

Resulting serum hormone and gonadotropin profiles after VCD-induced follicular depletion have been shown to be similar to the hormone profiles observed in ovary-intact menopausal women [69, 79–80, 97–98]; it is noteworthy that gonadotropin and sex steroid hormone profiles from VCD-induced follicle depletion may vary based on species, dose, age, duration of administration, and length of time since VCD administration [95]. In addition to the circulating hormone profile produced by VCD, this is an excellent model of follicle atresia in women. Ovarian follicle counts are considered a reliable marker of reproductive capacity. In clinical research, antral follicle count (AFC) is often used as a biomarker for reproductive capacity in the context of fertility [99], and more recently, in the transition to menopause [100–102]. AFC may be a more accurate marker than circulating serum gonadal hormone and gonadotrophin levels, as these levels fluctuate erratically in the human menopause transition [101]. Because the VCD model allows for the retention of post-menopausal ovarian tissue, researchers can now evaluate how ovarian follicle count relates to reproductive senescence in rodents. This model can also inform our understanding of the impact of intact post-menopausal ovarian tissue with its continued release of androgens, particularly androstenedione (discussed in 4.1.3), [80] and the relationship to other low levels of circulating steroid hormones from extra-ovarian sources, such as the adrenals, breast, and adipose tissues in the post-menopausal life stage. Overall, the VCD menopause model has a substantial advantage over the ovary-intact and Ovx models in that it results in an ovarian follicle and circulating steroid hormone profile more similar to the majority of women transitioning into the post-menopausal life stage.

The VCD model is growing in popularity in the field of menopause and aging research. Our laboratory has implemented the VCD model in the context of spatial memory performance, and showed that transitional menopause differentially impacts spatial memory as well as the efficacy of the estrogenic HT, conjugated equine estrogens (CEE; tradename: Premarin) compared to Ovx animals [97–98]. Others have shown VCD-induced reproductive senescence is associated with insulin resistance, bone loss, atherosclerotic lesion development, and changes in anxiety-like behaviors [90, 103–105], all of which can be negative health risks related to aging and the menopause transition in women. These studies point to the benefits and utility of the VCD model, and suggest that it should be further tapped in the context of menopause research in order to elucidate novel therapies in an intact system.

4.1 Evaluating hormones, behavior, and brains in rodent menopause models

4.1.1 Standing on the shoulder of giants: building on classic frameworks of behavioral endocrinology research

Systematic investigations into hormones, brain, and behavior have a long history. Beginning in the early 20th century, gonadal hormones were found to be crucial not only for normal reproductive tract and mammary gland development, estrous cycles, and pregnancy, but also for the phenotypic display of sex-specific adult mating behaviors [106–110]. Soon after, Phoenix, Goy, Gerall, and Young presented the theory of organizational/activational effects of gonadal hormones on the body and brain in 1959, which suggested that critical periods exist for hormone exposures. Organizational effects were thought to be permanent and occur early in development to set up the brain to respond in a particular way later in life, while activational effects were considered to occur later in life and only when a particular hormone is present. In other words, later behaviors are activated based upon early organization of the brain and body systems [111–112]. Since these initial reports, we have learned that ovarian hormones have both organizational and activational effects on the female brain, and that related factors likely play into sensitivity and efficacy of HT during aging and reproductive senescence.

With this framework in mind, the field has continued to study hormone-brain-behavior interactions using rodent models in many dimensions and domains in the context of aging and menopause, including depression, anxiety, and cognition. We and many others have investigated the effects of dose, task-specific effects, type of hormone, menopause type, as well as route, timing, and duration of treatment administration on both brain and behavior in female rodents [43–45, 113–121].

4.1.2 Mazes and memory

Mazes are a classic and effective way to study memory in the rodent and are sensitive to the effects of gonadal hormone changes associated with aging, Ovx, and follicular depletion. Multiple memory types can be assessed using maze paradigms, including *reference memory*, a form of long-term memory used to remember information that remains constant, and *working memory*, a type of short-term memory used to update information. Rodents have a general preference to navigate using spatial cues, so many maze tasks are geared toward allowing rodents to use their tendency to orient and remember in space by providing distal spatial cues around the room. For example, the standard Morris water maze (MM) tests spatial reference memory by requiring a rodent to learn to navigate to a hidden platform in a large tub of water by using spatial cues around the room. MM can also be used to evaluate overnight forgetting, a measure of memory retention, where performance on the last trial of the previous day is compared to performance on the first trial of the following day. The radial-arm maze and its water variant (water radial-arm maze, WRAM) assess spatial working and reference memory such that animals are tasked with finding several food rewards or hidden platforms that are not replaced within a day (Figure 6). This results in an increasing working memory load as trials progress; animals must remember where not to return after locating the reward within a day [113]. This measure of increasingly taxed

working memory is especially key for translational menopause research. Often, verbal memory is impacted by the menopause transition and gonadal hormone loss, and in particular, the presence or absence of estrogens [122–123]. A challenge with animal models is that researchers cannot implement verbal memory tasks; however, a key part of these tests is remembering a particular number of items, so the memory load associated with the radial-arm maze paradigms is a similar measure that we can quantify using an animal model.

4.1.3 Understanding hormone therapy effects on spatial memory and the brain using preclinical models

Our laboratory uses Ovx and VCD rat models to evaluate cognitive and brain changes associated with aging and the menopause transition, as well as how common estrogenic HT regimens, including 17 β -estradiol, CEE, and progestogen components, such as progesterone and medroxyprogesterone acetate (MPA, a synthetic progestin that was combined with CEE in the WHI clinical trials, and is the main component in the birth control Depo Provera), impact spatial working and reference memory in water maze tasks. For example, we found that in middle-aged Ovx rats, MPA and progesterone impaired spatial working and reference memory on the WRAM, and that MPA exposure at any time point — whether early in adulthood, middle-age, or both stages — impaired memory, even when MPA was no longer detectable in circulation. This effect occurred specifically at the highest working memory load, where animals have the most items of information to remember; Ovx animals treated with MPA in middle-age also had impaired overnight forgetting on the MM, a measure of memory retention [124–125]. Related research utilizing the Ovx model showed that, depending on dose, CEE benefited spatial working and reference memory after a delay in the WRAM and a delayed match-to-sample task (DMS), another common water escape task used to evaluate spatial working and recent memory [126].

These behavioral effects were associated with observable brain changes. MPA decreased glutamic acid decarboxylase (GAD), the rate-limiting enzyme for the inhibitory neurotransmitter GABA, in the hippocampus and increased GAD in the entorhinal cortex, two regions important for learning and memory retention [124]. CEE increased the number of choline acetyltransferase (ChAT; the synthesizing enzyme for acetylcholine) – positive cells in the basal forebrain, and increased the neurotrophins brain derived neurotrophic factor and nerve growth factor in the cingulate gyrus and perirhinal cortex [97, 126]. How then, can we reconcile some of the WHIMS findings? We showed that that beneficial effects of CEE are dependent upon menopause etiology. That is, Ovx animals administered CEE had enhanced spatial working and reference memory performance compared to control subjects on the WRAM and after a delay on the DMS, while VCD-treated animals given CEE had impaired performance compared to controls on the WRAM [98]. Importantly, more WRAM errors were positively correlated with higher serum androstenedione levels [98], an androgen and the primary hormone released by the post-menopausal ovary. Androstenedione can be aromatized to estrone, which is the primary estrogen component in CEE that was previously shown to impair memory at high doses [76]. These collective findings corroborated the WHIMS reports concerning the potential increased risk for memory impairment in the CEE +MPA groups, as women given that HT combination had a uterus, and likely experienced transitional menopause [34–35].

4.1.4 Optimizing parameters for hormone therapy: dose, timing, and healthy cognitive aging

Dose is key to elucidating estrogens' effects; in Ovx rats, low physiological levels of estradiol benzoate (EB; similar to diestrus levels) benefitted performance on a land radial-arm maze, while higher doses of EB that resulted in high physiological levels (similar to proestrus levels) impaired performance [127–128]. Optimal dosing may also depend on age — Foster and colleagues reported that older animals required higher EB doses to achieve similar memory performance to younger animals receiving lower doses on a MM memory retention probe trial [44]. Importantly, rodent research supports the tenet that the aging brain can still respond to 17β -estradiol or EB treatment even after a period of deprivation, although sensitivity to hormone treatment may be reduced after a long withdrawal period [43, 129–132], pointing to a critical window of opportunity for HT early in the menopause transition. Although the presence of estrogen is thought to be necessary to benefit memory in middle age, the Daniel laboratory demonstrated that transient exposure to 17β -estradiol benefitted spatial memory performance on the land radial-arm maze in middle-aged Ovx rats, even several months after hormone treatment ended [133]. Of note, estrogen-containing HT is not the only way to maintain or enhance cognitive performance during aging. For example, cognitive practice can mitigate age-related decline for a familiar task in males and females; in addition, females that have cognitive practice can transfer this enhanced performance to a novel task [134–135].

Rodent models of menopause have yielded invaluable insight into how aging and reproductive senescence impact cognition, allowing us to peer into critical windows of sensitivity to potentially maximize HT efficacy. Using these translational models, we can methodically test the effects of lifelong hormone exposures related to reproductive history, including endogenous hormone fluctuations (e.g., pregnancy, lactation) and exogenous hormone exposures (e.g., hormonal contraceptives, HT), to obtain a global view and better understanding of how these factors may interact and result in long-lasting effects on the trajectory of brain aging and reproductive senescence.

5.1 Conclusions

Since the early twentieth century, rodent models have been instrumental to our current understanding of the reproductive system, HPG feedback loops, and steroid hormone effects —particularly estrogens— on a wide range of systems, including cardiovascular disease, bone health, stroke, cancers, affective disorders, and cognitive processes, including spatial learning, memory, and attentional processes. In addition, we have the advantageous capacity to evaluate behavioral and brain changes at the cellular and molecular level much more quickly than would be possible using human subjects. This utility affords basic scientists the opportunity to explore novel brain targets and drug delivery methods [136] for favorable cognitive outcomes and healthy brain aging profiles that have the potential to translate to the clinical research setting.

These three core rodent models — ovary-intact, Ovx, and VCD — form the basis of our abilities as preclinical scientists to aid understanding of the female reproductive system, as well as brain and behavioral changes that occur with perturbations and alterations to this

system, such as removal of steroid hormones, depletion of ovarian follicles, or exogenous HT treatment (Figure 7). Indeed, these models allow basic scientists to systematically and methodically evaluate longitudinal impacts of hormones and aging, as well as directly assess brain, ovarian, uterine, and other body tissues from which we can glean insight into the physiological correlates associated with the aging female and the menopause transition. All of these elements showcase the utility of rodent models as translational models of human menopause and aid in our understanding of risk factors associated with aging and menopause, providing direction and potential solutions for individualized treatments for women in mid-life to lower risk factors for disease and to maintain a high quality of life.

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Abbreviations

HT	hormone therapy
VCD	4-vinylcyclohexene diepoxide
WRAM	water radial-arm maze
DMS	delayed match-to-sample task

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Highlights

- Frameworks and mechanisms of rodent and human reproductive senescence
- Strengths and weaknesses of the most commonly used rodent menopause models
- Testing cognition and the brain in rodent menopause models using a systems approach
- Optimizing preclinical models for translation to promote healthy aging in women

Figure 1.

Stages of Ovarian Follicle Development Stages of ovarian follicle development, beginning at the primordial resting stage and growing in size and shape through the antral (pre-ovulatory) mature follicle. Once the ovum is ovulated, the remaining follicle forms the corpus luteum, a temporary endocrine structure that produces high amounts of progesterone and some estradiol. The corpus luteum degenerates into the corpus albicans if no egg fertilization occurs.

Figure 2.

The Ovarian Hormone Cycle During the Reproductive Stage A) The rodent estrous cycle is characterized by four phases. Proestrus is the shortest phase, lasting less than a day, wherein a critical level of increasing 17β -estradiol is thought to trigger the LH surge, inducing ovulation. FSH and progesterone also peak during proestrus. The proestrus phase is followed by the estrus phase. In estrus, LH, FSH, and progesterone decline to baseline levels, while 17β -estradiol levels are moderately low. In metestrus, 17β -estradiol is low, and a transient increase in FSH occurs. Some 17β -estradiol and high amounts of progesterone are released from the corpora lutea following ovulation, increasing circulating progesterone levels between the metestrus and diestrus phases. Gonadal hormones return to baseline as the estrous cycle begins again. B) The human menstrual cycle has three distinct phases. The follicular phase is characterized by steadily increasing 17β -estradiol levels. At a critical level of 17β -estradiol, LH surges, with the trigger for ovulation occurring in the perioviulatory phase. Ovulation is followed by the luteal phase, wherein progesterone levels increase and 17β -estradiol is present at a moderate level.

Figure 3.

Ovarian Hormone Levels in Reproductive Senescence A) Rodents commonly enter a persistent estrus state during reproductive senescence (estropause), characterized by moderate to high 17β-estradiol levels and moderate progesterone, LH, and FSH levels as a result of disrupted feedback between the ovaries and hypothalamus/pituitary. b) Human reproductive senescence (menopause) is characterized by low, sometimes undetectable, levels of 17β-estradiol and progesterone, but increased levels of the gonadotropins, FSH and LH.

Figure 4.

The Estrous Cycle Representative images of the rat estrous cycle with vaginal cytology.

Animals in estrus exhibit cornified cells in their vaginal smears. Metestrous smears contain a combination of cornified cells, leukocytes, needle-like, and round epithelial cells. Diestrus smears are characterized by a large number of leukocytes, and proestrus smears contain round epithelial cells and some cornified cells, sometimes presenting in clustered groups.

Figure 5.
Human and Rodent Reproductive Tracts A comparison of the human and rodent reproductive tracts, with analogous structures labeled.

Commonly utilized mazes to study menopause and hormone therapy effects on learning and memory in the rat and mouse model		
MAZE:	<div>Radial-arm maze</div> <div> </div>	<div>Morris water maze</div> <div> </div>
COMMON PROTOCOL:	Water-escape maze or land maze with food reward are both commonly used. Platform is removed or food is eaten, once located. Animals must remember & avoid the previously located spatial locations (working memory). If a subset of the arms are platformed or have food, the arms that continuously need to be avoided require reference memory.	Water-escape maze where animals search for a hidden platform. The platform stays in the same spatial location across all test days & trials to test spatial reference memory, or the platform gets moved across days & remains in the same place within a day to measure spatial working & recent memory. On the last day, a probe trial is completed whereby the platform is removed & platform localization is assessed.
EVALUATES:	Spatial working memory alone (if all arms but the start arm are rewarded) or working & reference memory simultaneously (if a subset of the arms are rewarded), working memory as load increases across trials, delayed retention when an extended time delay is given between trials (usually 30 minutes for mice, or 4 to 8 hours for rats).	Spatial reference memory & overnight forgetting (if platform is in the same location on all days and trials) or spatial working & recent memory (if platform is moved to a new location on each day).
REPRESENTATIVE FINDINGS & PUBLICATIONS FOR THE RESPECTIVE COGNITIVE MAZE TASK:	<ul style="list-style-type: none">•CEE dose-dependently benefited memory in Ovx rats [126]•CEE impaired memory in VCD transitionally menopause rats, but improved memory in Ovx surgically menopausal rats [98]•Low physiological estradiol levels benefited, but high levels impaired, memory in Ovx rats [128]•Transient estradiol exposure enhanced performance in Ovx rats [133]•Estradiol treatment immediately following Ovx, but not after a deprivation period, enhanced memory in rats [43]•Androstenedione impaired memory at the highest memory load; blocking the aromatization of androstenedione to estrone with anastrozole prevented these impairments in young Ovx rats [137]	<ul style="list-style-type: none">•Estradiol facilitated learning in Ovx young and middle-aged rats, and higher estradiol levels were correlated with less swim distance and better spatial reference memory on the probe trial [47]•MPA impaired overnight forgetting in aged rats [124]•Higher estrogen doses were needed in aged rats to exhibit performance comparable to younger animals given lower doses [44]

Figure 6. Commonly utilized mazes to study menopause and hormone therapy effects on learning and memory in the rat and mouse model A comparison of common maze paradigms utilized with rodent models of menopause, and examples of representative results using the mazes.

Figure 7.
Rodent Models of Menopause An illustration and comparison of three validated rodent models of menopause.