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Module 2, Video 10: Sex differences in maturation and aging

Most research is done with young adult animals. However, experiments using animals outside of young adulthood or spanning multiple ages is also important, but requires special considerations. This is particularly important when both males and females are used in the same experiment. Dramatic changes in gonadal hormones across the lifespan can affect experimental outcomes [1]. These changes occur on different timelines for males and females across species. In this video, we will cover logistical design considerations for using males and females across the lifespan.

Puberty occurs later in males than in females. This is problematic if you want to study this time period. So, how can you best address timing differences in puberty while still keeping your experimental variables controlled? In other words, should you age-match your animals even though one sex will not be undergoing puberty or should you assess a span of time that covers puberty in both sexes? The answer depends upon your experimental questions and desired outcomes. Puberty is accompanied by hormonal changes, switches in how the brain reacts to certain proteins, and changes in brain structure that are both dependent and independent of hormones [2-5]. Thus, WHEN animals are tested can have profound effects on behavioral and other outcomes. It is important to research the specific timing for the species and strain you plan to use.

It's also important to consider how exposure to stress or endocrine-disrupting chemicals during critical windows of development may contribute to subsequent alterations in puberty onset. For example, in females, early-life stress has been shown to accelerate sexual maturation in both humans [6, 7] and rodents [8-10]. In males, however, early-life stress has either no effect [11] or delays puberty onset [9, 12], although inconsistencies could be due to difficulties in measuring puberty onset in male rodents [13].

Another important example is the timing of shipping animals. Shipping animals of both sexes during puberty or pregnancy can have short-term and long-lasting consequences on behavioral outcomes and hormone responses [14-20], likely due to the stress of the shipping process.

These two examples highlight the importance of how shifts in pubertal timing are important to consider when planning experiments.

Studying aging in rodents also requires special considerations. Unlike humans, rats and mice begin to experience irregular estrous cycles at 9-12 months of age. Menopause also manifests differently in rodents than in humans [21]. By definition, menopause in humans occurs when menstruation has ceased, ovarian follicular activity is lost, and hormone levels fall [22-24]. In rodents, however, following the onset of irregular estrous, some rodents will transition directly

to an anestrous state. This state is similar to menopause in humans, including no ovulation and low levels of gonadal steroids, but mature ovulatory follicles can still exist [21]. Before anestrous, rats more often than mice, can enter a pseudopregnancy phase that can continue for the rest of their lives, meaning that these animals never mimic the physiological conditions necessary to study menopause in humans [25]. There are also surgical and non-surgical options as well as some newly developed genetic models to model menopause in rodents. The right model will depend on your experimental question and outcomes [26, 27].

It should be noted that male rodents also have varying levels of testosterone across their lifespan, with the highest concentrations in adulthood/middle age [48]. Gonadectomy in male animals has good translatability to andropause syndrome in men.

The age of the animal can also matter for specific types of methodologies that you might consider employing. One example is hippocampal long-term potentiation (or LTP). LTP is an extensively studied phenomena in neuroscience and shows significant changes across the lifespan [49-52]. Many of these changes are directly related to gonadal hormone fluctuations across the lifespan, which leads to different levels of hippocampal neuron excitability. Cyclic changes in hippocampal LTP across the estrous cycle have also been observed.

However, a 2009 review study found that 59% of hippocampal LTP studies were performed in animals that had not yet reached adulthood [53]. Further, 30% of these studies also pooled together animals whose ages varied by 3-4 weeks. Thus, the effects of the experimental manipulation cannot be distinguished from the effects of hormonal variations when the results of animals spanning critical periods of development are analyzed together [53]. Therefore, it is critical to design experiments that carefully consider the factors that can change with both age and sex.

Tamoxifen has become an important tool for investigating gene function in mice. It allows researchers to temporally control gene deletion using the Cre/loxP system to determine whether a gene is required in an adult animal. A point mutation is introduced to the ligand-binding domain of estrogen receptor alpha, resulting in a receptor that only binds the synthetic selective estrogen receptor modulator tamoxifen, and not endogenous estrogens.

Tamoxifen shows mixed agonist/antagonist activity for ERα, depending on the tissue and cell type. Tamoxifen has also been used to delay precocious puberty [54], and thus will delay puberty onset when used to delete a gene before puberty. Even its use in adult animals can confound some experimental results because, similar to estrogen, tamoxifen treatment causes an acute drop in food intake and body weight [55, 56]. Transient treatment with tamoxifen also has short-term effects on glucose tolerance and insulin secretion [57], and strikingly persistent effects on lipid metabolism and fat mass [58]. These factors highlight the importance of

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including tamoxifen-only controls in all behavioral analyses when it is used at any age to induce gene deletion.

As demonstrated in this video, age and sex are both important experimental factors that must be considered when designing your study. Critical changes occur during puberty on different timelines for males and females and hormone fluctuations continue across the rodent lifespan, all of which can affect behavioral outcomes. Experimentally controlling for hormone variations induced by age can mitigate these effects when males and females are used in the same experiment.

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