AN INTERNATIONAL ORGANIZATION DEDICATED TO PROTECTING THE RIGHTS OF ALL ANIMALS

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Kevin Shea
Administrator
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Axel Wolff, D.V.M., M.S.
Acting Director
Office of Laboratory Animal Welfare
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Via e-mail: kevin.a.shea@usda.gov; olaw@mail.nih.gov

Dear Mr. Shea and Dr. Wolff:

People for the Ethical Treatment of Animals (PETA) requests that the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) and the National Institutes of Health's (NIH) Office of Laboratory Animal Welfare (OLAW) investigate possible violations of the federal Animal Welfare Act (AWA) and noncompliance with the Public Health Service Policy on Care and Use of Laboratory Animals (PHS Policy) related to the use and treatment of common marmoset monkeys in neuroendocrine experiments. The procedures in question, led by NIH-funded principal investigator Agnès Lacreuse and conducted at the University of Massachusetts–Amherst (UMass; Certificate No. 14-R-0036), involve subjecting marmosets to multiple invasive procedures that appear to violate AWA regulations. The research objectives could alternatively be addressed ethically and non-invasively by using human volunteers and/or other human-relevant approaches.

In response to several Freedom of Information Act requests, PETA received more than 15 hours of video footage and hundreds of pages of documents related to experiments carried out by Lacreuse on common marmosets. A review of these documents, which include copies of grant applications for funded NIH projects R01CA246929 and R21AG074251 as well as the associated approved protocols from UMass (#70, #764, #2132, and #2376), reveal treatment of animals that appears to constitute violations of Animal Welfare Regulations (AWR) and noncompliance with PHS Policy.

These apparent violations include the following:

1. Failure on the part of the institutional animal care and use committee (IACUC) to ensure that the investigator provide a scientifically valid rationale for involving animals and for the appropriateness of the species and numbers of animals to be used [9 C.F.R. §2.31(e)(2)]

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2. Failure on the part of the IACUC to ensure that animals would not be used in more than one major operative surgery from which they were allowed to recover [9 C.F.R. §2.31(d)(1)(x)]
3. Failure to report the use of animals in the appropriate USDA category for pain and distress [9 C.F.R. §2.36]
4. Failure on the part of the IACUC to ensure that the principal investigator had considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals [9 C.F.R. §2.31(d)(1)(ii)]

PETA believes that the inadequate oversight by the UMass IACUC may have resulted in improper approval and ongoing use of live animals in these experiments.

1. Failure to ensure that a proposal to conduct an activity involving animals contains a scientifically valid rationale and purpose for procedures that impact the welfare of animals

C.F.R. Title 9, Section 2.31(e)(2) of the AWRs requires that a proposal to conduct an activity involving animals must contain a "rationale for involving animals, and for the appropriateness of the species and numbers of animals to be used." Similarly, the Guide for the Care and Use of Laboratory Animals (the *Guide*) requires that the IACUC review the proposed "rationale and purpose of the proposed use of animals" (p. 25). The *Guide* also requires that in its review of proposed animal use protocols, the IACUC consider, among other things, the "justification of the species and number of animals proposed" (p. 25).

The experiments Lacreuse is conducting involve subjecting marmoset monkeys to years of captivity, multiple invasive surgeries, hormone manipulation, frequent restraint, fluid restriction, fear- and stress-inducing behavioral tests, and frequent social separation. After eight to 10 years of experimentation, the animals are perfused and dissected.[1,2,3,4] The purported purpose of these experiments is to study the role of menopause and its associated disruptions in thermal regulation, sleep, and cognitive function in the increased risk for neurodegenerative diseases in women.

However, marmosets are an exceptionally inappropriate species to study for this purpose. Inherent differences in sex hormone function, aging, and gene expression between marmosets and humans; critical differences in endocrine and neurological processes between the two species; and the profound impact of captivity on marmosets' physiological systems render data from these experiments irrelevant to humans. Please consider the following information:

- Common marmosets do not naturally experience menopause. Their estrogen levels do not gradually decrease with age. They display no evidence of reproductive senescence or hormone-mediated osteoporosis—even when they are well into their more advanced ages of 14 and 16 years.[5] In fact, throughout their lifetimes, marmosets have elevated concentrations of plasma estradiol[6] and progesterone[7] compared to humans and Old World primates. This is true for marmosets who are intact and for those whose ovaries have been surgically removed.[8] Additionally, marmosets do not respond to circulating or extraneous hormones in the same way as humans and other primates. Compared to humans and other primates, marmosets display what is referred to as "generalized steroid hormone resistance,"[9] i.e., relatively high levels of steroid hormones in circulation and relatively low response to exogenous steroids, and they exhibit target-tissue resistance to gonadal steroid hormones.[10]

- In an attempt to induce menopause in marmosets, the Lacreuse laboratory surgically removes their ovaries. However, ovariectomized (OVX) marmosets do not exhibit the changes in metabolism, bodyweight, body composition, bone density, energy expenditure, physical activity, fasting glucose, or glucose tolerance or the measurable mood changes or cognitive impairments seen in peri- or post-menopausal women.[11,12] In fact, data obtained from OVX marmosets is often in direct contrast with data from human women, for whom surgically induced menopause is associated with more severe symptoms and health risks than those observed in spontaneous gradual menopause.[13] For example, women who have had surgically induced menopause are shown to have significantly greater cognitive impairments than those who undergo nonsurgical menopause.[14,15] The earlier the surgical induction of menopause, the more rapid the cognitive decline, the higher the risk of dementia, and the closer to Alzheimer's disease the pathology.[16,17] Induced menopause is associated with a higher risk for cardiovascular disease,[18,19] slower gait speed,[20] decreased bone mineral density, and increased fracture risk[21] compared to natural spontaneous menopause.

- In a recent review of the role of estrogen in cognitive aging, Russell *et al.* emphasized the exact problem with using surgically induced menopause to model perimenopausal cognitive impairments:

> [O]variectomy models the loss of E2 [estradiol], temporally this is markedly different from the changes that occur during natural menopause. In menopause, the less abrupt loss of E2 can produce cognitive deficits that continue to develop through later life. Furthermore, studies utilizing ovariectomy in young animals will produce a preclinical model that is inappropriate to interrogate the interaction between decreased estrogen, the aging brain, and cognitive dysfunction. The aging brain and associated cognitive dysfunction is developing concurrently with the menopausal transition in clinical populations. These age-related changes will not be occurring in the preclinical model.[22]

  In other words, in addition to the dramatically different effects experienced by marmosets whose ovaries have been removed and women with induced or spontaneous menopause, Lacreuse's experiments forgo the entire "transitional" stage of menopause, which is particularly problematic for her cognitive assessments.

- There are fundamental differences in gene expression and protein function in the brains of marmosets compared to humans.[23] There are differences in neurodevelopment[24,25] and neuroanatomy,[26,27] including in the timing, rate, and patterns of gray- and white-matter development across the animal's lifespan.[28,29,30,31,32,33] In marmosets, tau—a protein that makes up a major component of the neurofibrillary tangles in Alzheimer's disease—is actually much more similar to the protein found in rodents' brains than that found in humans.[34] Marmoset brains are also less sexually dimorphic than those of humans and other primates,[35] which will likely affect the applicability of any of Lacreuse's sex-related findings to humans. While marmosets exhibit some evidence of cognitive decline with age, they do not develop human-like Alzheimer's disease, a condition unique to humans that has never been successfully recapitulated in another species.[36,37,38,39,40]

- Marmosets' accelerated development makes them an inappropriate choice for studying the much more protracted and hormone-sensitive age-related changes in the human brain. In a

recent (2019) review, biological anthropologist, and experimenter at Yerkes National Primate Research Center (now Emory National Primate Research Center) Todd Preuss writes:

> The very small size of the marmoset brain makes it very likely that the functions of its cortical systems differ in important ways from those of larger-brained primates, if only because of the much more limited amount of neural machinery marmosets and other callitrichines have to work with. … Given the small size and rapid development of marmosets, it is tempting to view marmoset life history as a condensed version of that of longer-lived primates. Yet there is evidence primates vary in patterns of postnatal growth and development. Bogin (2007) indicates that cercopithecoid and hominoid development includes an extended period of slow growth, defining a juvenile stage that has no counterpart in marmosets. This difference, and the specializations of human development recognized by Bogin—namely, the addition of childhood and adolescent stages—imply differences in the hormonal control of development.[41]

Surgically induced, abrupt menopause in captive marmosets cannot mimic the complex genetic, environmental, or epigenetic factors known to influence the natural menopausal transition and its associated symptoms in humans and impacts the animals' well-being by requiring that they endure multiple invasive surgeries, hormone restraint, frequent restraint, fluid restriction, years of captivity, and eventually death. The IACUC appears to have failed to properly evaluate the proposed species to be used as well as the scientific rationale for the use of animals in the various components of these experiments relative to the impact that the tests have on the animals' welfare.

The lack of sound rationales for the use of animals in these experiments should have prevented them from being approved.

2. Failure to ensure that animals would not be used in more than one major operative survival surgery

Section 2143(a)(3)(D) of the AWA and Section 2.31(d)(1)(x) of the AWRs require that in its review of "proposed activities related to the care and use of animals," the IACUC must ensure that "no animal will be used in more than one major operative procedure from which [he or she] is allowed to recover" except in cases of "(i) scientific necessity; or (ii) other special circumstances as determined by the Secretary." Neither of these exceptions appear to be present or relevant here, as OVX marmosets do not exhibit a drop in estrogen levels similar to humans and the overnight recording equipment is surgically attached only for the convenience of the experimenters.

However, marmosets in Lacreuse's laboratory are subjected to multiple major surgeries. Female marmosets used in these experiments endure bilateral ovariohysterectomies, and males endure vasectomies and gonadectomies. Many male and female marmosets are then subjected to telemetry implantation surgeries so that experimenters can monitor brain activity, heart rate, and temperature during sleep and cognitive testing. To implant these devices, experimenters drill burr holes into the marmosets' skulls and screw electrodes directly into the bone.

The approved protocols for this procedure explicitly acknowledge the considerable harm to which marmosets are subjected, indicating that the animals may experience "pain … [and] irritation at

[the] surgery site." The protocol further states, "Monkey may stop eating, be lethargic, [and] scratch at the incision site."[42]

It appears that the IACUC improperly approved multiple major surgeries, including reproductive surgeries and drilling holes into their skulls, in direct contradiction of Section 2143(a)(3)(D) of the AWA and Section 2.31(d)(1)(x) of the AWRs.

3. Failure to report animal use in the appropriate USDA category for pain and distress

Section 2.36(a) and (b)(5–7) of the AWRs stipulates that research facilities must submit an annual report to the USDA, stating, "the common names and the numbers of animals upon which experiments, teaching, research, surgery, or tests were conducted," and classifying them under the appropriate USDA pain and distress category for the procedures in which the animals were used.

In this series of experiments, only the surgical procedures and perfusion were classified as USDA pain and distress category D or as "involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used."[43] All other procedures approved by the IACUC, including hormone manipulations, restraint, social separation, and fluid restriction, were classified as category C, or as "involving no pain, distress, or use of pain-relieving drugs." However, the cumulative impact of the chronic captivity, multiple surgeries, hormone manipulation, and behavior testing do cause pain and distress to the animals. As shown in detail below, Lacreuse's experiments should be classified as category E, as the animals are being "subjected to painful or stressful procedures" that induce permanent physical and psychological harm and that are not being "alleviated through the use of anesthetics, analgesics, or tranquilizers."

- **Captivity:** The current standards for housing and enrichment for the marmosets in the Lacreuse laboratory do not meet the animals' needs to forage, climb, and engage in problem-solving and complex social interactions.[44,45] Marmosets in these experiments are kept caged, with no access to the outdoors, for up to 10 years. The lack of adequate mental and social stimulation in the laboratory, along with frequent subjection to common laboratory procedures, leads to chronic stress that negatively affects primates psychologically and physiologically. Primates held captive in laboratories and subjected to experimental procedures exhibit signs of extreme distress, including pacing, rocking, head-twisting, and eating their own feces. Highly traumatized primates will bite their own flesh, pull out their own hair, and engage in other forms of severe self-mutilation.[46,47,48,49] Primates in laboratories display aberrant immune-system functioning, including increased stress-related hormones, dysregulation of the hypothalamic-pituitary-adrenal axis, and depressed immune-system functioning.[50] Stress-induced immune dysregulation and systemic inflammation result in significant health consequences, including increased vulnerability to infection,[51] delayed wound healing and recovery from surgery,[52] and accelerated aging.[53] Captive marmosets are also prone to metabolic bone disease,[54] which results in bone lesions and fractures and may be the cause of the oral disease, including tooth decay, frequently affecting these animals. Experimenters currently believe this issue may be related to the higher vitamin D requirements of marmosets, differences in vitamin D metabolism in marmosets, or vitamin D deficiency caused by complete deprivation of sunlight in laboratory cages.[55] Marmosets in laboratories are also likely to suffer from secondary systemic amyloidosis[56] and insulin resistance.[57]

- **Marmoset wasting syndrome:** Marmosets in laboratories are at high risk of developing a condition referred to as "marmoset wasting syndrome" (also called "chronic lymphocytic enteritis"), a systemic inflammatory disorder that leads to weight loss, diarrhea, anemia, alopecia, weakness, intestinal inflammation, osteoporosis, paralysis, and death.[58,59] Efforts to curb the profound and deadly weight loss associated with improper nutrition have only caused additional health concerns. If they aren't wasting away, marmosets in laboratories are becoming obese and suffering from health complications associated with that condition, including altered glucose metabolism, reduced insulin sensitivity, increased risk of heart disease and diabetes, and various metabolic dysfunctions.[60]
- **Fluid restriction:** To ensure cooperation on the multitude of cognitive tests the laboratory subjects them to, the marmosets in the Lacreuse laboratory are deprived of one of their most basic needs—water—for hours on end. Water restriction can cause "decreased skin turgor, dry mucous membranes, increased plasma osmolality, and behavior suggestive of extreme thirst or hunger. Distressed primates might also show behavioral changes such as lethargy, agitation, or altered patterns of aggression,"[61] and smaller species of primates "may be especially susceptible to dehydration."
- **Restraint:** Marmosets endure frequent restraint for these experiments, including for blood draws and neuroimaging while conscious as well as thermal challenges. For awake neuroimaging conducted at UMass, marmosets are put into a restraint jacket and zip-tied to a head restraint device for up to five hours at a time. Marmosets are sensitive to physical restraint, and their response to restraint affects their heart and respiratory rates, temperature, and blood pressure.[62]
- **Letrozole administration:** In an attempt to induce human-like menopausal symptoms, the experimenters give the marmosets the drug letrozole to lower their estrogen levels even further than is caused by surgery. Letrozole is an aromatase inhibitor used to lower non–ovarian produced estrogen. Typically used to keep estrogen levels low in post-menopausal women with a risk or history of estrogen (E+) sensitive types of breast cancer, the side effects *in humans* include hot flashes, joint pain, dizziness, nausea, weight gain, edema, diarrhea, cognitive difficulties, and fatigue.

The extensive catalog of invasive, painful, and distressing procedures conducted on marmosets for these experiments and the irreversible harm they induce according to scientific literature clearly indicate that they should be classified as category E experiments, reflecting the unrelieved pain and distress that can be expected to be experienced by the monkeys over the course of these experiments.

4. Failure to consider alternatives to painful procedures

Section 2143(a)(3)(B) of the AWA and Section 2.31(d)(1)(ii) of the AWRs require that in its review of "proposed activities related to the care and use of animals," the IACUC ensure that the principal investigator has "considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals." The *Guide* also requires that the IACUC consider the "availability or appropriateness of the use of less invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation" (p. 12).

However, the animal study proposals for these experiments indicate that the experimenters failed to conduct an adequate search for alternative procedures. For the procedures the experimenters classified as USDA pain and distress category D, which were the major life surgeries and

perfusion, the search for alternatives to those procedures was limited to alternatives that would still involve performing surgical procedures on nonhuman primates. Non-animal alternatives were not considered.[63,64] The investigators did not search for alternative approaches to studying menopause, hormone levels, and brain structure and function non-invasively with humans or human-relevant methods.

Indeed, a review of the literature indicates that this entire battery of tests could have been conducted ethically with human volunteers or other human-relevant methods. Please consider the following examples:

- *In vivo* imaging at various stages of the menopausal transition of women who are at risk of developing or living with various neurological disorders, [65,66] postmortem analysis of brain tissues from patients,[67] and large-scale epidemiological studies[68,69] are helping researchers understand the role of estrogen in various human diseases and types of behavior.[70]
- Cutting-edge technology, including pluripotent stem cell models,[71,72] three-dimensional cell-culture models,[73,74] and organ-on-a-chip technology,[75,76] are being used not only to serve as more accurate and detailed models of human neurodegenerative disease but also to test the effects of estrogen at the cellular level.[77,78] The effects of estrogen on cognition,[79,80,81] brain structure and function,[82,83,84,85] mood,[86,87,88] hot flash frequency and severity,[89,90] sleep disturbances,[91,92] and risk for neurodegenerative diseases have all been successfully studied in human volunteers.
- Researchers studying women have investigated whether hormone replacement therapy is associated with a lower risk for the amyloid β-deposits associated with Alzheimer's disease[93] and whether resveratrol, a phytoestrogen available in many foods, can ameliorate symptoms associated with menopause.[94,95] A recent study of more than 2,500 post-menopausal women indicated that the degree of cognitive decline experienced was associated with corresponding depressive symptoms.[96]

Clearly, a multitude of non-animal methods are available for studying the role of menopause and hormone levels on aging and neurodegenerative risk. The IACUC apparently failed to ensure that the investigator considered available alternatives to procedures that will cause more than momentary or slight pain and distress to animals.

5. PHS Policy

The issues described above also appear to violate the PHS Policy's U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training.[97]

In particular, Principle II of the PHS Policy states, "Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society." Principle III maintains that "[t]he animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should be considered." And Principle IV states, "Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals" (p. 4). Accordingly, we request that OLAW evaluate these concerns and, if

corrective measures are not taken, "restrict or withdraw approval of [Animal Welfare] Assurances" (p. 24).

The IACUC that approved these procedures apparently failed to consider the lack of relevance of these experiments to humans, the inappropriateness of the species, the impact of the procedures on the animals, and the availability of non-animal methods when reviewing the proposed animal use protocols associated with Lacreuse's experiments.

Conclusion

As detailed above, apparent violations of the AWRs and noncompliance with the PHS Policy by the UMass IACUC include the following:

1. Approval for Lacreuse to experiment on marmosets, even though marmosets are not the appropriate species for the research project, discomfort and pain were avoidable, and the rationale for the use of animals was poorly evaluated
2. Approval for Lacreuse to perform multiple major operative procedures on marmosets
3. Failure to report the appropriate USDA pain and distress categories
4. Failure to consider alternatives to procedures that cause more than momentary or slight pain or distress to animals

Accordingly, PETA requests that APHIS and OLAW investigate this matter and order corrective action and appropriate penalties.

Thank you for your attention to this important matter.

Sincerely,

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[1]Edwards M, Lam S, Ranjan R, Pereira M, Babbitt C, Lacreuse A. Letrozole treatment alters hippocampal gene expression in common marmosets (*Callithrix jacchus*). *Horm Behav*. 2023;147:105281. doi:10.1016/j.yhbeh.2022.105281

[2]Rothwell ES, Workman KP, Wang D, Lacreuse A. Sex differences in cognitive aging: a 4-year longitudinal study in marmosets. *Neurobiol Aging*. 2022;109:88-99. doi:10.1016/j.neurobiolaging.2021.09.015

[3]Nephew BC, Febo M, Cali R, et al. Robustness of sex-differences in functional connectivity over time in middle-aged marmosets. *Sci Rep*. 2020;10(1):16647. doi:10.1038/s41598-020-73811-9

[4]Gervais NJ, Viechweg SS, Mong JA, Lacreuse A. The middle-aged ovariectomized marmoset (*Callithrix jacchus*) as a model of menopausal symptoms: Preliminary evidence. *Neuroscience*. 2016;337:1-8.

doi:10.1016/j.neuroscience.2016.08.056
[5]Abbott DH, Barnett DK, Colman RJ, Yamamoto ME, Schultz-Darken NJ. Aspects of common marmoset basic biology and life history important for biomedical research. *Comp Med*. 2003;53(4):339-350.
[6]Kraynak M, Flowers MT, Shapiro RA, Kapoor A, Levine JE, Abbott DH. Extraovarian gonadotropin negative feedback revealed by aromatase inhibition in female marmoset monkeys. *Am J Physiol Endocrinol Metab*. 2017;313(5):E507-E514. doi:10.1152/ajpendo.00058.2017
[7]Chen H, Hu B, Huang GH, Trainor AG, Abbott DH, Adams JS. Purification and characterization of a novel intracellular 17 beta-estradiol binding protein in estrogen-resistant New World primate cells. *J Clin Endocrinol Metab*. 2003;88(1):501-504. doi:10.1210/jc.2002-021488
[8]Kraynak M, Flowers MT, Shapiro RA, Kapoor A, Levine JE, Abbott DH. Extraovarian gonadotropin negative feedback revealed by aromatase inhibition in female marmoset monkeys. *Am J Physiol Endocrinol Metab*. 2017;313(5):E507-E514. doi:10.1152/ajpendo.00058.2017
[9]Li LH, Donald JM, Golub MS. Review on testicular development, structure, function, and regulation in common marmoset. *Birth Defects Res B Dev Reprod Toxicol*. 2005;74(5):450-469. doi:10.1002/bdrb.20057
[10]Chen H, Hu B, Huang GH, Trainor AG, Abbott DH, Adams JS. Purification and characterization of a novel intracellular 17 beta-estradiol binding protein in estrogen-resistant New World primate cells. *J Clin Endocrinol Metab*. 2003;88(1):501-504. doi:10.1210/jc.2002-021488
[11]Saltzman W, Abbott DH, Binkley N, Colman RJ. Maintenance of bone mass despite estrogen depletion in female common marmoset monkeys (*Callithrix jacchus*). *Am J Primatol*. 2019;81(2):e22905. doi:10.1002/ajp.22905
[12]Kraynak M, Colman RJ, Flowers MT, Abbott DH, Levine JE. Ovarian estradiol supports sexual behavior but not energy homeostasis in female marmoset monkeys. *Int J Obes (Lond)*. 2019;43(5):1034-1045. doi:10.1038/s41366-018-0156-4
[13]Rodriguez M, Shoupe D. Surgical Menopause. *Endocrinol Metab Clin North Am*. 2015;44(3):531-542. doi:10.1016/j.ecl.2015.05.003
[14]Nappi RE, Sinforiani E, Mauri M, Bono G, Polatti F, Nappi G. Memory functioning at menopause: impact of age in ovariectomized women. *Gynecol Obstet Invest*. 1999;47(1):29-36. doi:10.1159/000010058
[15]Ryan J, Scali J, Carrière I, et al. Impact of a premature menopause on cognitive function in later life. *BJOG*. 2014;121(13):1729-1739. doi:10.1111/1471-0528.12828
[16]Bove R, Secor E, Chibnik LB, et al. Age at surgical menopause influences cognitive decline and Alzheimer pathology in older women. *Neurology*. 2014;82(3):222-229. doi:10.1212/WNL.0000000000000033
[17]Georgakis MK, Beskou-Kontou T, Theodoridis I, Skalkidou A, Petridou ET. Surgical menopause in association with cognitive function and risk of dementia: a systematic review and meta-analysis. *Psychoneuroendocrinology*. 2019;106:9-19. doi:10.1016/j.psyneuen.2019.03.013
[18]Price MA, Alvarado BE, Rosendaal NTA, Câmara SMA, Pirkle CM, Velez MP. Early and surgical menopause associated with higher Framingham Risk Scores for cardiovascular disease in the Canadian Longitudinal Study on Aging. *Menopause*. 2021;28(5):484-490. doi:10.1097/GME.0000000000001729
[19]Zhu D, Chung HF, Dobson AJ, et al. Type of menopause, age of menopause and variations in the risk of incident cardiovascular disease: pooled analysis of individual data from 10 international studies. *Hum Reprod*. 2020;35(8):1933-1943. doi:10.1093/humrep/deaa124
[20]Velez MP, Alvarado BE, Rosendaal N, et al. Age at natural menopause and physical functioning in postmenopausal women: the Canadian Longitudinal Study on Aging. *Menopause*. 2019;26(9):958-965. doi:10.1097/GME.0000000000001362
[21]Sullivan SD, Lehman A, Nathan NK, Thomson CA, Howard BV. Age of menopause and fracture risk in postmenopausal women randomized to calcium + vitamin D, hormone therapy, or the combination: results from the Women's Health Initiative Clinical Trials. *Menopause*. 2017;24(4):371-378. doi:10.1097/GME.0000000000000775
[22]Russell JK, Jones CK, Newhouse PA. The role of estrogen in brain and cognitive aging. *Neurotherapeutics*. 2019;16(3):649-665. doi:10.1007/s13311-019-00766-9
[23]Bailey J. Monkey-based research on human disease: the implications of genetic differences. *Altern Lab Anim*. 2014;42(5):287-317. doi:10.1177/026119291404200504
[24]Charvet CJ, Finlay BL. Comparing adult hippocampal neurogenesis across species: translating time to predict the tempo in humans. *Front Neurosci*. 2018;12:706. doi:10.3389/fnins.2018.00706
[25]Sakai T, Komaki Y, Hata J, et al. Elucidation of developmental patterns of marmoset corpus callosum through a comparative MRI in marmosets, chimpanzees, and humans. *Neurosci Res*. 2017;122:25-34. doi:10.1016/j.neures.2017.04.001
[26]Fukushima M, Ichinohe N, Okano H. Neuroanatomy of the marmoset. In: Marini RP, Wachtman LM, Tardif SD, Mansfield K, Fox JG, eds. *The Common Marmoset in Captivity and Biomedical Research*. Academic Press; 2019:43-62. doi:10.1016/b978-0-12-811829-0.00003-0
[27]Charvet CJ, Palani A, Kabaria P, Takahashi E. Evolution of brain connections: integrating diffusion MR

tractography with gene expression highlights increased corticocortical projections in primates. *Cereb Cortex*. 2019;29(12):5150-5165. doi:10.1093/cercor/bhz054
[28]Seki F, Hikishima K, Komaki Y, et al. Developmental trajectories of macroanatomical structures in common marmoset brain. *Neuroscience*. 2017;364:143-156. doi:10.1016/j.neuroscience.2017.09.021
[29]Walker LC, Jucker M. The exceptional vulnerability of humans to Alzheimer's disease. *Trends Mol Med*. 2017;23(6):534-545. doi:10.1016/j.molmed.2017.04.001
[30]Drummond E, Wisniewski T. Alzheimer's disease: experimental models and reality. *Acta Neuropathol*. 2017;133(2):155-175. doi:10.1007/s00401-016-1662-x
[31]Duyckaerts C, Potier MC, Delatour B. Alzheimer disease models and human neuropathology: similarities and differences. *Acta Neuropathol*. 2008;115(1):5-38. doi:10.1007/s00401-007-0312-8
[32]Neha, Sodhi RK, Jaggi AS, Singh N. Animal models of dementia and cognitive dysfunction. *Life Sci*. 2014;109(2):73-86. doi:10.1016/j.lfs.2014.05.017
[33]Heuer E, Rosen RF, Cintron A, Walker LC. Nonhuman primate models of Alzheimer-like cerebral proteopathy. *Curr Pharm Des*. 2012;18(8):1159-1169. doi:10.2174/138161212799315885
[34]Sharma G, Huo A, Kimura T, et al. Tau isoform expression and phosphorylation in marmoset brains. *J Biol Chem*. 2019;294(30):11433-11444. doi:10.1074/jbc.RA119.008415
[35]Reinius B, Saetre P, Leonard JA, et al. An evolutionarily conserved sexual signature in the primate brain. *PLoS Genet*. 2008;4(6):e1000100. doi:10.1371/journal.pgen.1000100
[36]Walker LC, Jucker M. The exceptional vulnerability of humans to Alzheimer's disease. *Trends Mol Med*. 2017;23(6):534-545. doi:10.1016/j.molmed.2017.04.001
[37]Drummond E, Wisniewski T. Alzheimer's disease: experimental models and reality. *Acta Neuropathol*. 2017;133(2):155-175. doi:10.1007/s00401-016-1662-x
[38]Duyckaerts C, Potier MC, Delatour B. Alzheimer disease models and human neuropathology: similarities and differences. *Acta Neuropathol*. 2008;115(1):5-38. doi:10.1007/s00401-007-0312-8
[39]Neha, Sodhi RK, Jaggi AS, Singh N. Animal models of dementia and cognitive dysfunction. *Life Sci*. 2014;109(2):73-86. doi:10.1016/j.lfs.2014.05.017
[40]Heuer E, Rosen RF, Cintron A, Walker LC. Nonhuman primate models of Alzheimer-like cerebral proteopathy. *Curr Pharm Des*. 2012;18(8):1159-1169. doi:10.2174/138161212799315885
[41]Preuss TM. Critique of pure marmoset. *Brain Behav Evol*. 2019;93(2-3):92-107. doi:10.1159/000500500
[42]University of Massachusetts–Amherst. Lacreuse_2016-0065_Sleep, hot flashes and cognition: a nonhuman primate model for menopausal symptoms. (Exhibit 1: Protocol information received by PETA via Freedom of Information Act request.) June 22, 2021:57.
[43]APHIS. Animal Care Tech Note. Accessed November 16, 2023. https://www.aphis.usda.gov/publications/animal_welfare/fs-pain-distress-categories.pdf
[44]Duarte MHL, Goulart VDLR, Young RJ. Designing laboratory marmoset housing: what can we learn from urban marmosets? *Appl Anim Behav Sci*. 2012;137(3-4):127-136. doi:10.1016/j.applanim.2011.11.013
[45]Bakker J, Ouwerling B, Heidt PJ, Kondova I, Langermans JAM. Advantages and risks of husbandry and housing changes to improve animal wellbeing in a breeding colony of common marmosets (*Callithrix jacchus*). *J Am Assoc Lab Anim Sci*. 2015;54(3):273-279.
[46]Novak MA. Self-injurious behavior in rhesus monkeys: new insights into its etiology, physiology, and treatment. *Am J Primatol*. 2003;59(1):3-19. doi:10.1002/ajp.10063
[47]Lutz C, Well A, Novak M. Stereotypic and self-injurious behavior in rhesus macaques: a survey and retrospective analysis of environment and early experience. *Am J Primatol*. 2003;60(1):1-15. doi:10.1002/ajp.10075
[48]Gottlieb DH, Capitanio JP, McCowan B. Risk factors for stereotypic behavior and self-biting in rhesus macaques (*Macaca mulatta*): animal's history, current environment, and personality. *Am J Primatol*. 2013;75(10):995-1008. doi:10.1002/ajp.22161
[49]Lutz CK, Coleman K, Worlein J, Novak MA. Hair loss and hair-pulling in rhesus macaques (*Macaca mulatta*). *J Am Assoc Lab Anim Sci*. 2013;52(4):454-457.
[50]Novak MA, Hamel AF, Kelly BJ, Dettmer AM, Meyer JS. Stress, the HPA axis, and nonhuman primate well-being: a review. *Appl Anim Behav Sci*. 2013;143(2-4):135-149. doi:10.1016/j.applanim.2012.10.012
[51]Avitsur R, Levy S, Goren N, Grinshpahet R. Early adversity, immunity and infectious disease. *Stress*. 2015;18(3):289-296. doi:10.3109/10253890.2015.1017464
[52]Godbout JP, Glaser R. Stress-induced immune dysregulation: implications for wound healing, infectious disease and cancer. *J Neuroimmune Pharmacol*. 2006;1(4):421-427. doi:10.1007/s11481-006-9036-0
[53]Flynn MG, Markofski MM, Carrillo AE. Elevated inflammatory status and increased risk of chronic disease in chronological aging: inflamm-aging or *inflamm-inactivity*? *Aging Dis*. 2019;10(1):147-156. doi:10.14336/AD.2018.0326
[54]Olson EJ, Shaw GC, Hutchinson EK, et al. Bone disease in the common marmoset: radiographic and histological

findings. *Vet Pathol*. 2015;52(5):883-893. doi:10.1177/0300985815589354
[55]Goodroe A, Wachtman L, Benedict W, et al. Current practices in nutrition management and disease incidence of common marmosets (*Callithrix jacchus*). *J Med Primatol*. 2021;50(3):164-175. doi:10.1111/jmp.12525
[56]Ludlage E, Murphy CL, Davern SM, et al. Systemic AA amyloidosis in the common marmoset. *Vet Pathol*. 2005;42(2):117-124. doi:10.1354/vp.42-2-117
[57] Perez-Cruz C, Rodriguez-Callejas JD. The common marmoset as a model of neurodegeneration. *Trends Neurosci*. 2023;46(5):394-409.
[58]Otovic P, Smith S, Hutchinson E. The use of glucocorticoids in marmoset wasting syndrome. *J Med Primatol*. 2015;44(2):53-59. doi:10.1111/jmp.12159
[59]Cabana F, Maguire R, Hsu CD, Plowman A. Identification of possible nutritional and stress risk factors in the development of marmoset wasting syndrome. *Zoo Biol*. 2018;37(2):98-106. doi:10.1002/zoo.21398
[60]Ross CN, Colman R, Power M, Tardif S. Marmoset metabolism, nutrition, and obesity. *ILAR J*. 2021;ilab014. doi:10.1093/ilar/ilab014
[61]Willems RA. Regulatory issues regarding the use of food and water restriction in laboratory animals. *Lab Anim (NY)*. 2009;38(10):325-328. doi:10.1038/laban1009-325
[62]Ludlage E, Mansfield K. Clinical care and diseases of the common marmoset (*Callithrix jacchus*). *Comp Med*. 2003;53(4):369-382
[63]University of Massachusetts–Amherst. Lacreuse_2019-0069_DHED rescue of Letrozole-induced deficits. (Exhibit 2: Protocol information received by PETA via Freedom of Information Act request.) June 22, 2021:60.
[64]University of Massachusetts–Amherst. Sex differences in cognitive and brain aging: a primate model. (Exhibit 3: Protocol information received by PETA via Freedom of Information Act request.) June 22, 2021:60.
[65]Coupé P, Manjón JV, Lanuza E, Catheline G. Lifespan changes of the human brain in Alzheimer's disease. *Sci Rep*. 2019;9(1):3998. doi:10.1038/s41598-019-39809-8
[66]Couto PJ, Millis RM. PET imaging of epigenetic influences on Alzheimer's disease. *Int J Alzheimers Dis*. 2015;2015:575078. doi:10.1155/2015/575078
[67]Barroeta-Espar I, Weinstock LD, Perez-Nievas BG. Distinct cytokine profiles in human brains resilient to Alzheimer's pathology. *Neurobiol Dis*. 2019;121:327-337. doi:10.1016/j.nbd.2018.10.009
[68]Guthrie KA, Larson JC, Ensrud KE, et al. Effects of pharmacologic and nonpharmacologic interventions on insomnia symptoms and self-reported sleep quality in women with hot flashes: a pooled analysis of individual participant data from four MsFLASH trials. *Sleep*. 2018;41(1):zsx190. doi:10.1093/sleep/zsx190
[69]Spencer BE, Jennings RG, Brewer JB, Alzheimer's Disease Neuroimaging Initiative. Combined biomarker prognosis of mild cognitive impairment: an 11-year follow-up study in the Alzheimer's Disease Neuroimaging Initiative. *J Alzheimers Dis*. 2019;68(4):1549-1559. doi:10.3233/JAD-181243
[70]Raz L, Hunter LV, Dowling NM, et al. Differential effects of hormone therapy on serotonin, vascular function and mood in the KEEPS. *Climacteric*. 2016;19(1):49-59. doi:10.3109/13697137.2015.1116504
[71]Adhya D, Annuario E, Lancaster MA, Price J, Baron-Cohen S, Srivastava DP. Understanding the role of steroids in typical and atypical brain development: advantages of using a "brain in a dish" approach. *J Neuroendocrinol*. 2018;30(2):e12547. doi:10.1111/jne.12547
[72]Choi SA, An JH, Lee SH, et al. Comparative evaluation of hormones and hormone-like molecule in lineage specification of human induced pluripotent stem cells. *Int J Stem Cells*. 2019;12(2):240-250. doi:10.15283/ijsc18137
[73]Amin ND, Paşca SP. Building models of brain disorders with three-dimensional organoids. *Neuron*. 2018;100(2):389-405. doi:10.1016/j.neuron.2018.10.007
[74]Jorfi M, D'Avanzo C, Kim DY, Irimia D. Three-dimensional models of the human brain development and diseases. *Adv Healthc Mater*. 2018;7(1):10.1002/adhm.201700723. doi:10.1002/adhm.201700723
[75]Jahromi MAM, Abdoli A, Rahmanian M, et al. Microfluidic brain-on-a-chip: perspectives for mimicking neural system disorders. *Mol Neurobiol*. 2019;56(12):8489-8512. doi:10.1007/s12035-019-01653-2
[76]Isoherranen N, Madabushi R, Huang SM. Emerging role of organ-on-a-chip technologies in quantitative clinical pharmacology evaluation. *Clin Transl Sci*. 2019;12(2):113-121. doi:10.1111/cts.12627
[77]Cairns J, Ingle JN, Dudenkov TM, et al. Pharmacogenomics of aromatase inhibitors in postmenopausal breast cancer and additional mechanisms of anastrozole action. *JCI Insight*. 2020;5(16):e137571. doi:10.1172/jci.insight.137571
[78]Elliot SJ, Catanuto P, Pereira-Simon S, et al. Catalase, a therapeutic target in the reversal of estrogen-mediated aging. *Mol Ther*. 2021;S1525-0016(21)00326-9. doi:10.1016/j.ymthe.2021.06.020
[79]Wroolie TE, Kenna HA, Williams KE, et al. Differences in verbal memory performance in postmenopausal women receiving hormone therapy: 17β-estradiol versus conjugated equine estrogens. *Am J Geriatr Psychiatry*. 2011;19(9):792-802. doi:10.1097/JGP.0b013e3181ff678a
[80]Yoon BK, Chin J, Kim JW, et al. Menopausal hormone therapy and mild cognitive impairment: a randomized, placebo-controlled trial. *Menopause*. 2018;25(8):870-876. doi:10.1097/GME.0000000000001140
[81]Conley AC, Albert KM, Boyd BD, et al. Cognitive complaints are associated with smaller right medial temporal

gray-matter volume in younger postmenopausal women. *Menopause*. 2020;27(11):1220-1227. doi:10.1097/GME.0000000000001613
[82]Kantarci K, Tosakulwong N, Lesnick TG, et al. Effects of hormone therapy on brain structure: a randomized controlled trial. *Neurology*. 2016;87(9):887-896. doi:10.1212/WNL.0000000000002970
[83]Kantarci K, Tosakulwong N, Lesnick TG, et al. Brain structure and cognition 3 years after the end of an early menopausal hormone therapy trial. *Neurology*. 2018;90(16):e1404-e1412. doi:10.1212/WNL.0000000000005325
[84]Sommer T, Richter K, Singer F, et al. Effects of the experimental administration of oral estrogen on prefrontal functions in healthy young women. *Psychopharmacology (Berl)*. 2018;235(12):3465-3477. doi:10.1007/s00213-018-5061-y
[85]Bayer J, Rusch T, Zhang L, Gläscher J, Sommer T. Dose-dependent effects of estrogen on prediction error related neural activity in the nucleus accumbens of healthy young women. *Psychopharmacology (Berl)*. 2020;237(3):745-755. doi:10.1007/s00213-019-05409-7
[86]Gleason CE, Dowling NM, Wharton W, et al. Effects of hormone therapy on cognition and mood in recently postmenopausal women: findings from the randomized, controlled KEEPS-Cognitive and Affective Study. *PLoS Med*. 2015;12(6):e1001833. doi:10.1371/journal.pmed.1001833
[87]Henningsson S, Madsen KH, Pinborg A, et al. Role of emotional processing in depressive responses to sex-hormone manipulation: a pharmacological fMRI study. *Transl Psychiatry*. 2015;5(12):e688. doi:10.1038/tp.2015.184
[88]Lascurain MB, Camuñas-Palacín A, Thomas N, et al. Improvement in depression with oestrogen treatment in women with schizophrenia. *Arch Womens Ment Health*. 2020;23(2):149-154. doi:10.1007/s00737-019-00959-3
[89]Tsiligiannis S, Wick-Urban BC, van der Stam J, Stevenson JC. Efficacy and safety of a low-dose continuous combined hormone replacement therapy with 0.5 mg 17β-estradiol and 2.5 mg dydrogesterone in subgroups of postmenopausal women with vasomotor symptoms. *Maturitas*. 2020;139:20-26. doi:10.1016/j.maturitas.2020.05.002
[90]Constantine GD, Simon JA, Kaunitz AM, et al. TX-001HR is associated with a clinically meaningful effect on severity of moderate to severe vasomotor symptoms in the REPLENISH trial. *Menopause*. 2020;27(11):1236-1241. doi:10.1097/GME.0000000000001602
[91]Geiger PJ, Eisenlohr-Moul T, Gordon JL, Rubinow DR, Girdler SS. Effects of perimenopausal transdermal estradiol on self-reported sleep, independent of its effect on vasomotor symptom bother and depressive symptoms. *Menopause*. 2019;26(11):1318-1323. doi:10.1097/GME.0000000000001398
[92]Pauwaert K, Goessaert AS, Ghijselings L, et al. Hormone therapy as a possible solution for postmenopausal women with nocturia: results of a pilot trial. *Menopause*. 2021;28(5):502-510. doi:10.1097/GME.0000000000001741
[93]Kantarci K, Lowe VJ, Lesnick TG, et al. Early postmenopausal transdermal 17β-estradiol therapy and amyloid-β deposition. *J Alzheimers Dis*. 2016;53(2):547-556. doi:10.3233/JAD-160258
[94]Thaung Zaw JJ, Howe PRC, Wong RHX. Sustained cerebrovascular and cognitive benefits of resveratrol in postmenopausal women. *Nutrients*. 2020;12(3):828. doi:10.3390/nu12030828
[95]Evans HM, Howe PRC, Wong RHX. Clinical evaluation of effects of chronic resveratrol supplementation on cerebrovascular function, cognition, mood, physical function and general well-being in postmenopausal women—rationale and study design. *Nutrients*. 2016;8(3):150. doi:10.3390/nu8030150
[96]Gong B, Wu C. The mediating and moderating effects of depression on the relationship between cognitive function and difficulty in activities of daily living among postmenopausal women. *Menopause*. 2021;28(6):667-677. doi:10.1097/GME.0000000000001773
[97]Office of Laboratory Animal Welfare. *Public Health Service Policy on Humane Care and Use of Laboratory Animals*. National Institutes of Health. Updated 2015. Accessed August 21, 2023. https://olaw.nih.gov/sites/default/files/PHSPolicyLabAnimals.pdf.