

Estrogen timing and concentration affect the impact of age-related stress in a neuronal cell model

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Considering midlife, menopause and hormone therapy in Alzheimer's disease (AD) prevention

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Insights at a glance:

Neuroprotective actions of estrogen signalling are currently thought to be determined by time of initiation and relationship to existing damage.

We used a differentiated SH-SY5Y neuroblastoma model to assess the relationship between estrogen treatment timing and age-related stress at physiologically and clinically levels.

Late initiation could reduce neuroprotection even in the absence of stress, while concentration and timing effects vary by specific stress context.

Investigation of downstream effects on tau will support practical clinical integration, aiding inference of likely cognitive outcomes of treatment.

Current best hypotheses: Female sex, estrogen, ageing and AD

Women are at greater risk of developing AD in late-life, and while sex hormones likely play a role, there is a lack of consensus as to how, to what extent, and when increased risk manifests.

As the reduction in estrogen during the menopausal transition occurs concurrently with the earliest AD pathology, sex-specific endocrinological ageing should be considered during investigation of risk screening and intervention from midlife.

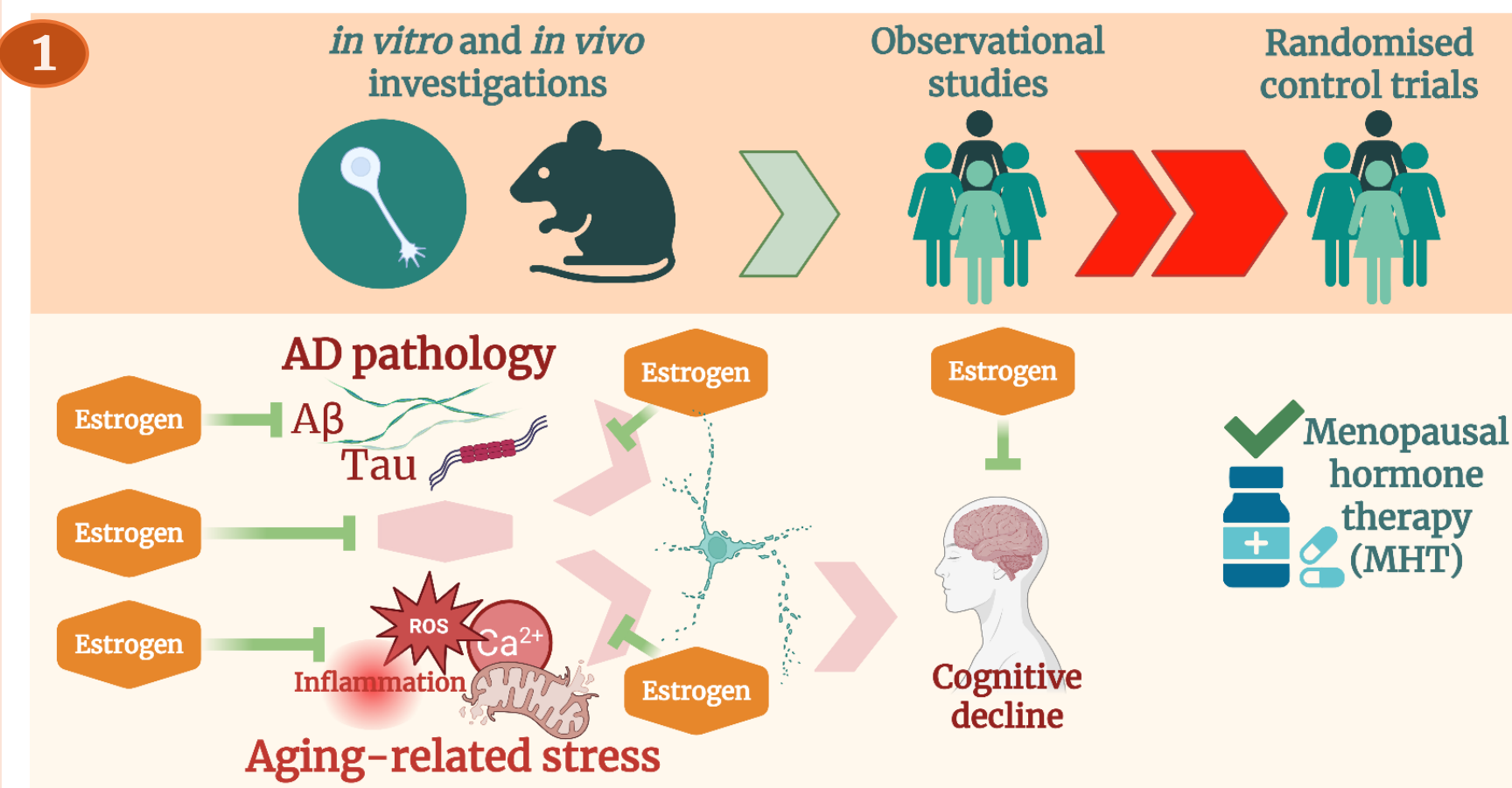


Figure 1. Preclinical and observational evidence of neuroprotection by estrogen motivated clinical investigation of MHT for protection against cognitive decline.

However, this has returned mixed results.

Figure 2. Two dominant hypotheses have emerged to reconcile this translation barrier from clinical and preclinical findings respectively:



Estrogen therapy could be beneficial if initiated close to the menopausal transition, and before a threshold of damage to brain cells has occurred.

But gaps in our basic understanding limit their application to clinical practice:

Figure 3. Physiological estrogen concentration in the brain is likely much lower than the bulk of prior *in vitro* studies have investigated.

Figure 3a. Limited clinical data for estimation of brain estrogen concentration.

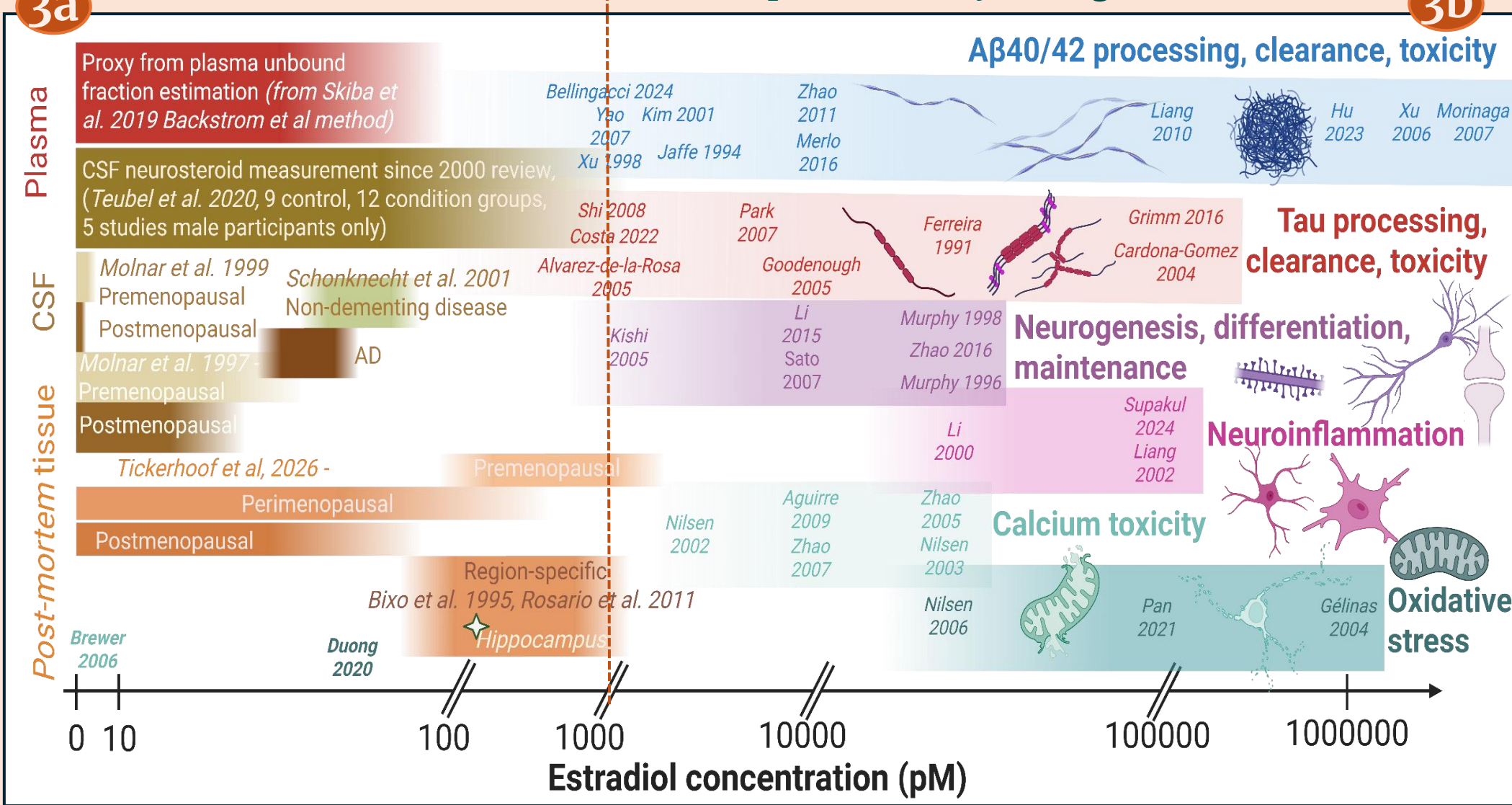
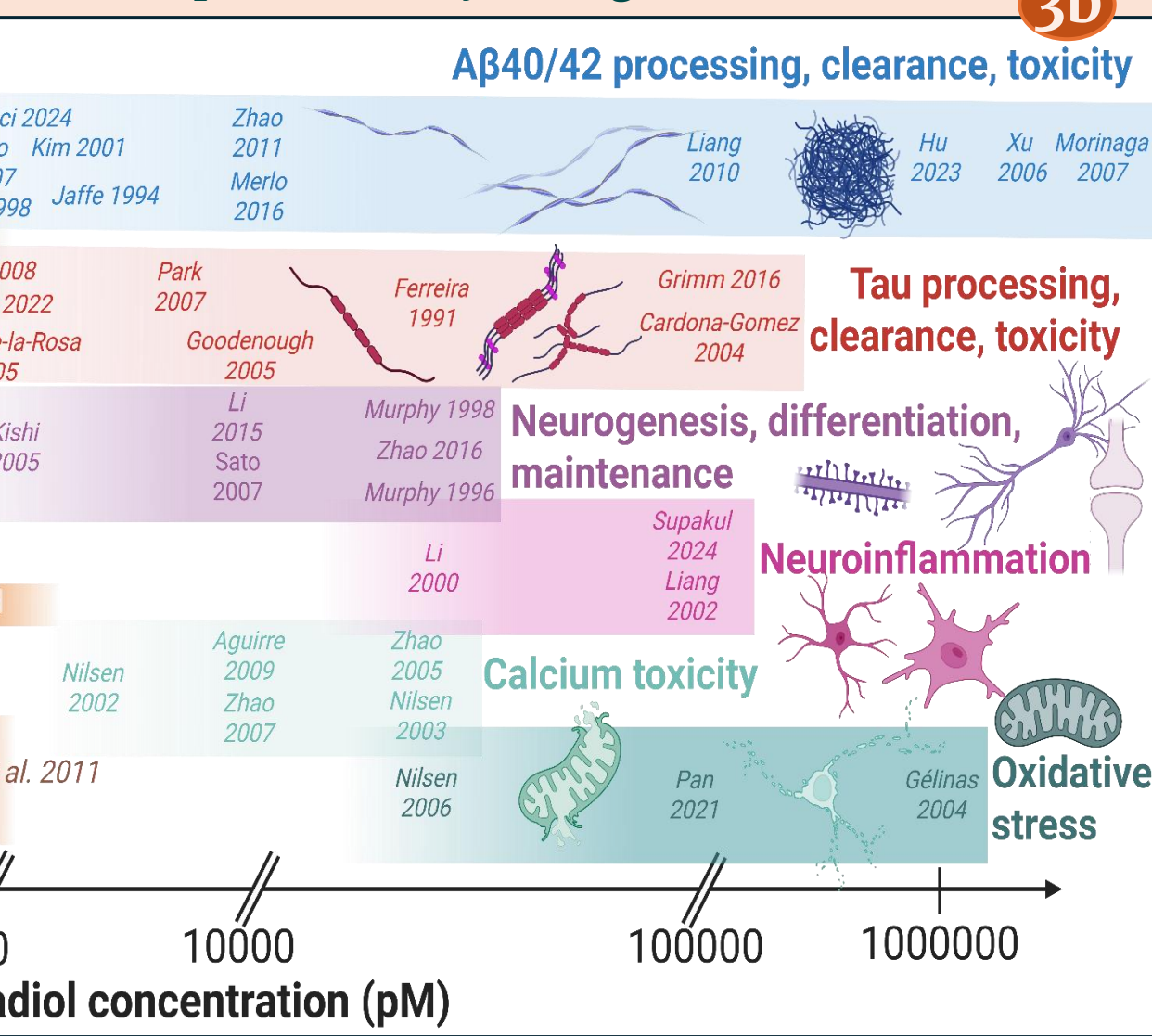


Figure 3b. Inconsistent estrogen treatment concentration used across key *in vitro* investigations of neuroprotection by estrogen.



A new approach: Clinically relevant *in vitro* investigation

Developing a physiologically and clinically relevant *in vitro* model for integrated investigation of the 'Window of Opportunity' and 'Healthy Cell Bias' hypotheses:

Figure 4. A fully-differentiated SH-SY5Y neuroblastoma cell model for assessment of interactions between estrogen concentration, timing, and age-related stress.

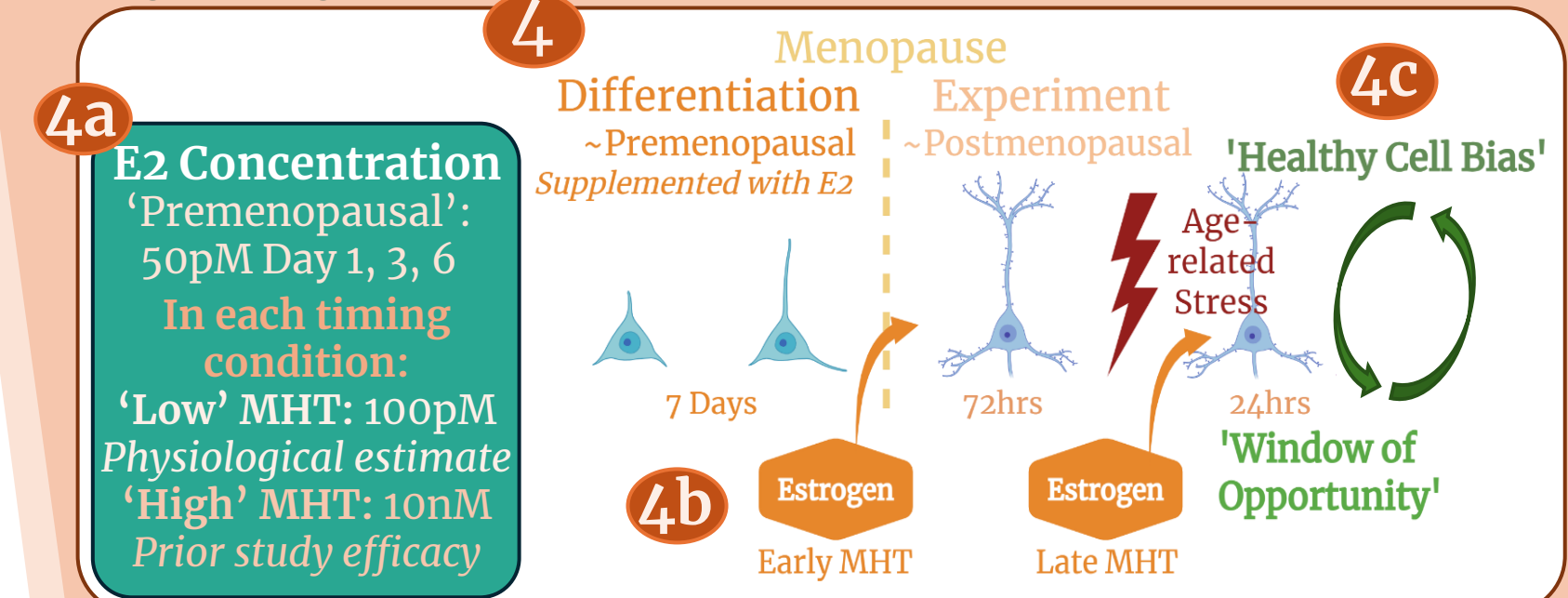


Figure 4a. Concentrations of dominant human estrogen (17β-estradiol (E2)) treatment used. 4b. Following differentiation, estrogen treatment is removed (96hrs), continued (96hrs, media replaced 72hrs) or reinitiated following a delay (removed 72hrs, reinitiated for 24hrs). This offers clinical proxies of menopause and subsequent MHT at various times of initiation. 4c. Various age-related stressors are initiated at 72hrs (prior to estrogen replacement/reinitiation) to assess context-specific relationships with estrogen timescale.

Age-related stress: Inflammation LPS 100µg/ml⁻¹ 1hr+24hr w/estrogen
Oxidative stress H2O2 100µM 1hr, wash, 24hr estrogen
Hyperexcitotoxicity Glutamate 20mM

Screening the impact of estrogen and stress interactions on neurotoxicity – Probe conditions for investigation of subtle changes

ReadyProbes™ viability imaging kit (Thermo Scientific) staining
Live/dead counting using ImageXpress™ automated Pico reader

Data collection ongoing – data presented: Points represent individual well images (1007 over 31 biological repeats, 5 treatment conditions and 4 stress conditions – 'None' 461; 'Glutamate' 279; 'Oxidative' 181, 'LPS' 86.). Median total cell number in each well image: 5019.

Generalised linear mixed modelling (GLMM): Interactive effects of concentration, timing, stress:

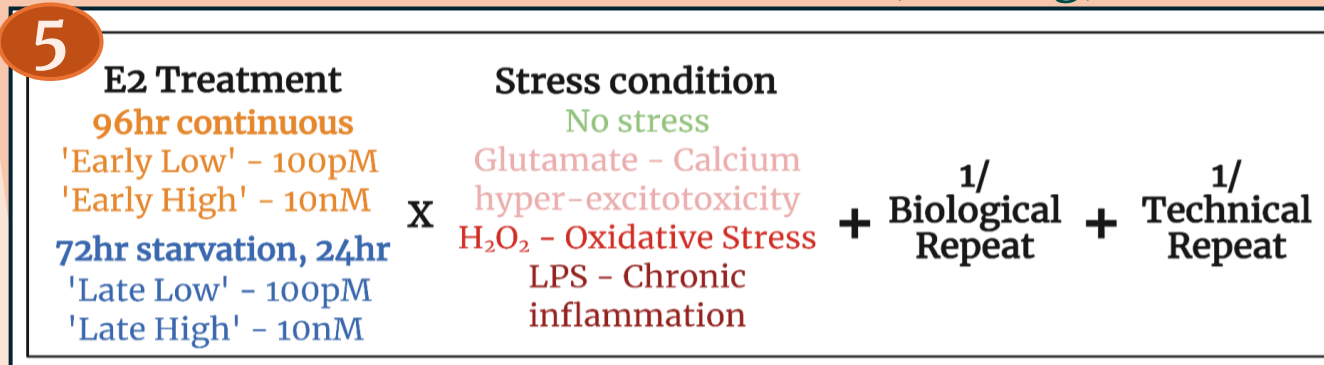


Figure 5. GLMM structure to investigate interactions between concentration, timing and stress.

Model development ongoing – estimates presented:

Bars represent model estimates for *Palive* (probability a cell is alive) using a logit link function. Error bars represent model estimated 95% confidence intervals. Uncorrected p-values for planned contrasts comparing each treatment against control within each stress condition.

Estrogen, stress and neurotoxicity: Concentration, timing and context

Data collection and model development are ongoing. Please refer to 'A new approach:' section (left) for description of data presentation across all graphs.

The 'Window of Opportunity':

What is the effect of late initiation in the absence of stress?

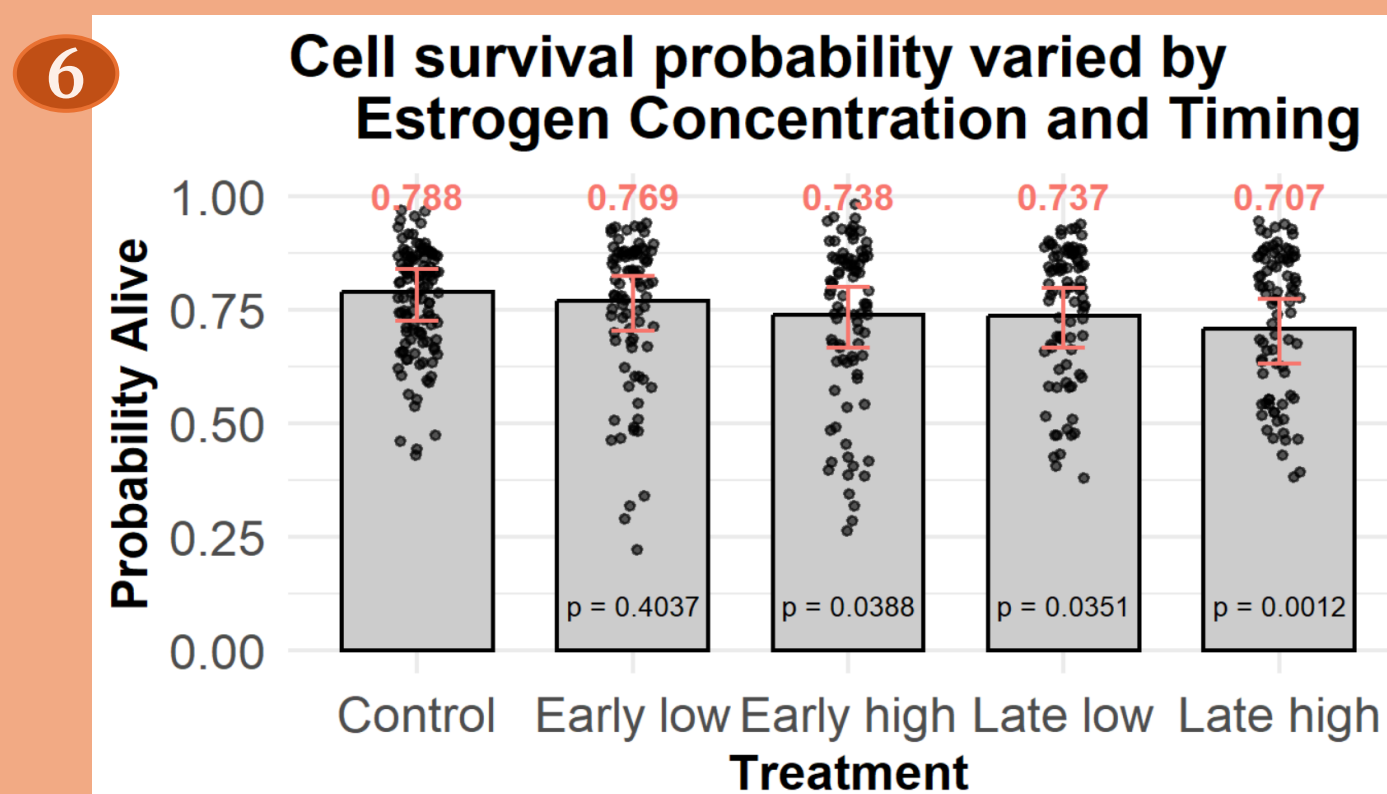


Figure 6. In the absence of stress, higher concentration and later initiation significantly reduce cell survival versus control, although effect size is small.

The 'Healthy Cell Bias' in the context of the 'Window of Opportunity'

Does age-related stress pronounce treatment differences?

Figure 7. Late and low concentration estrogen treatment significantly exacerbates oxidative stress-induced reductions in cell survival.

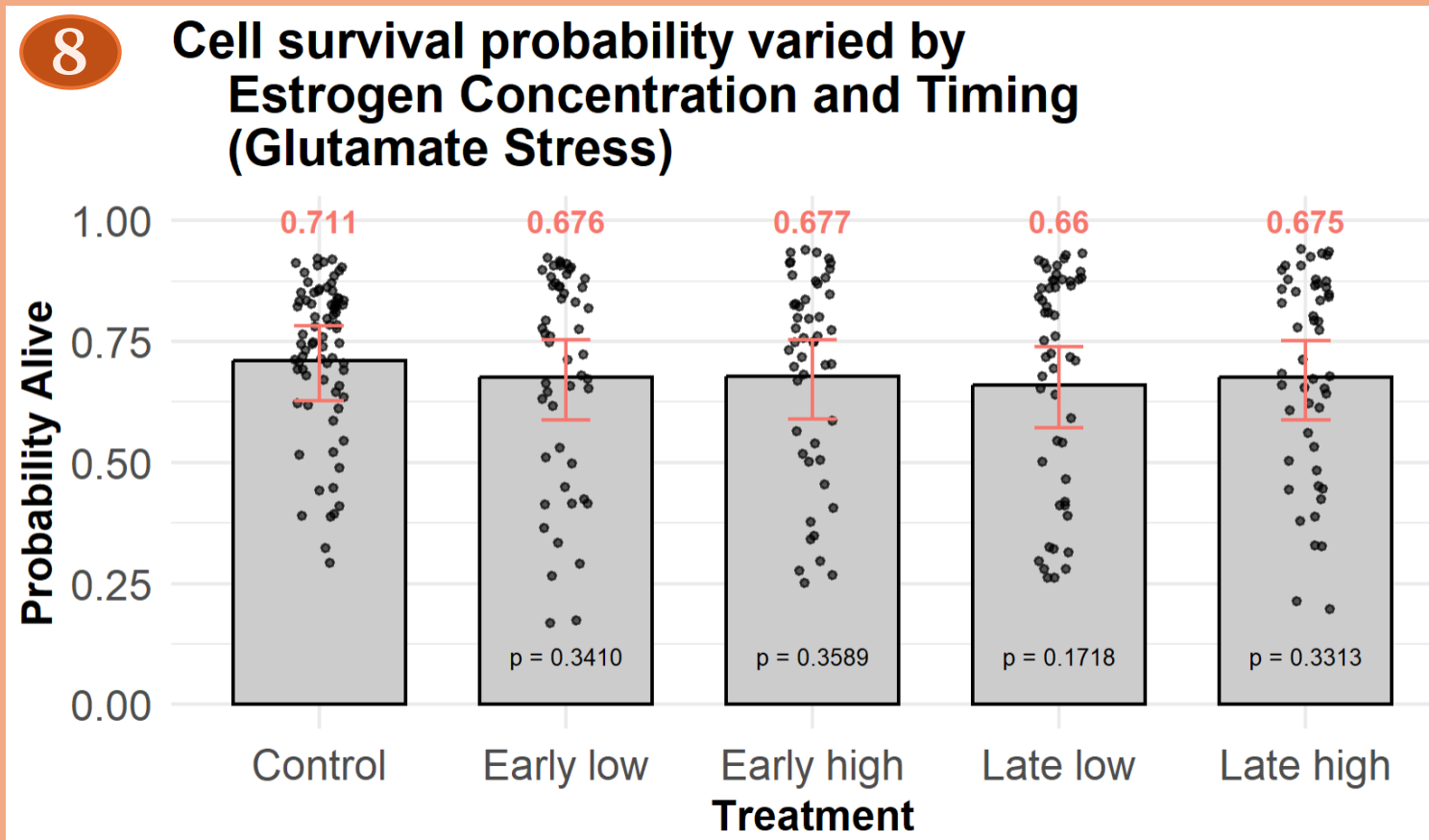
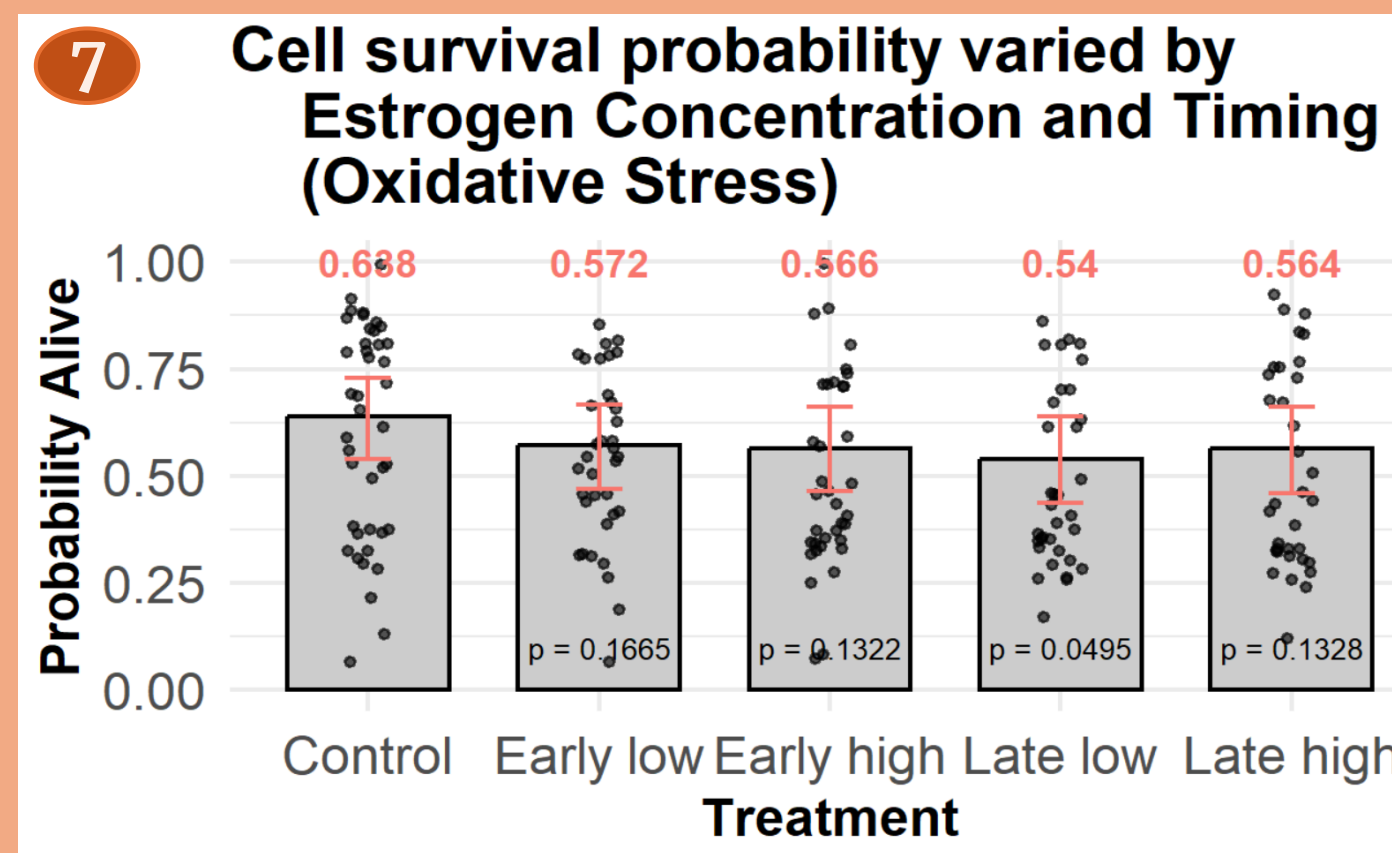


Figure 8. No significant effects of estrogen treatment on glutamate-induced reductions in cell survival are observed.

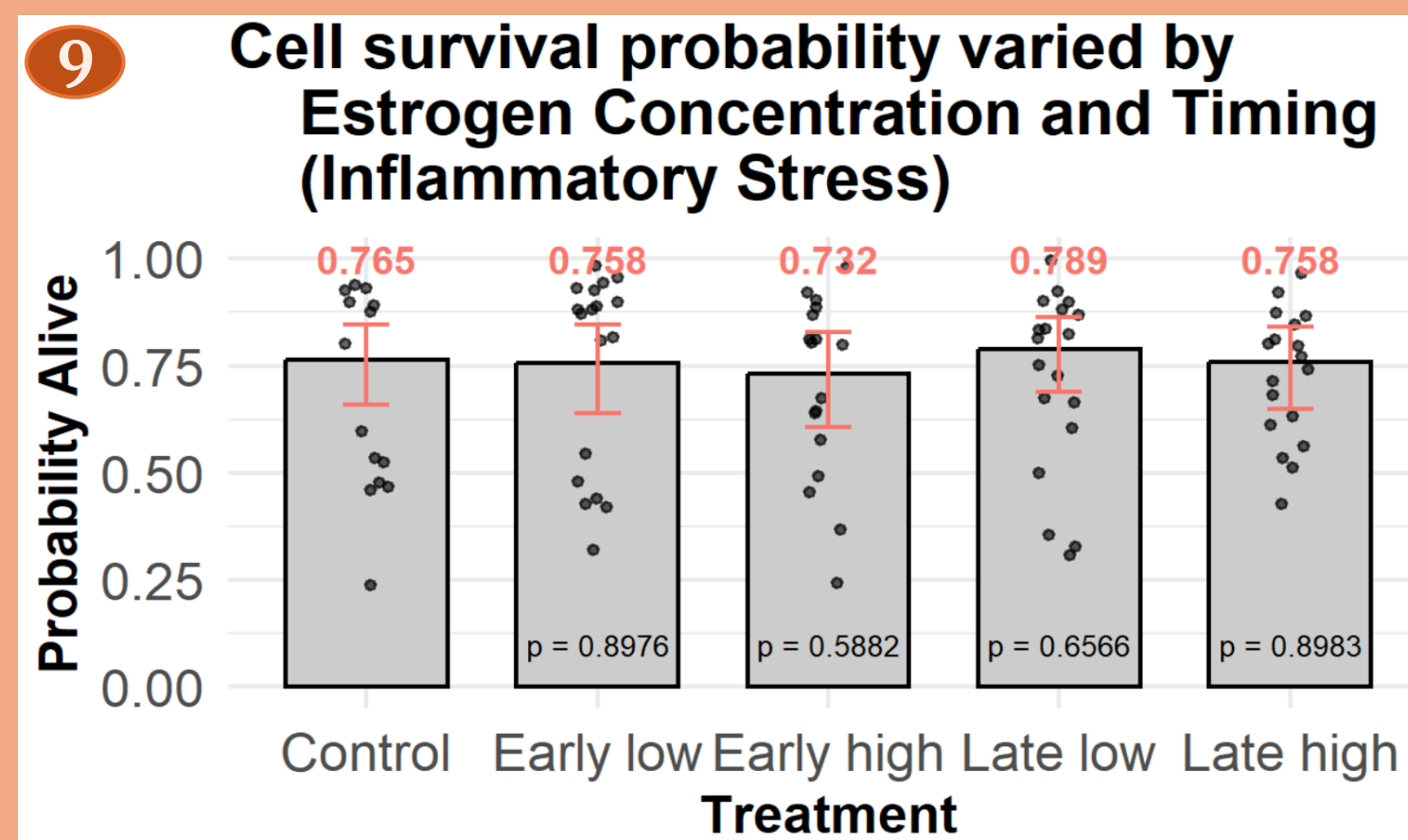
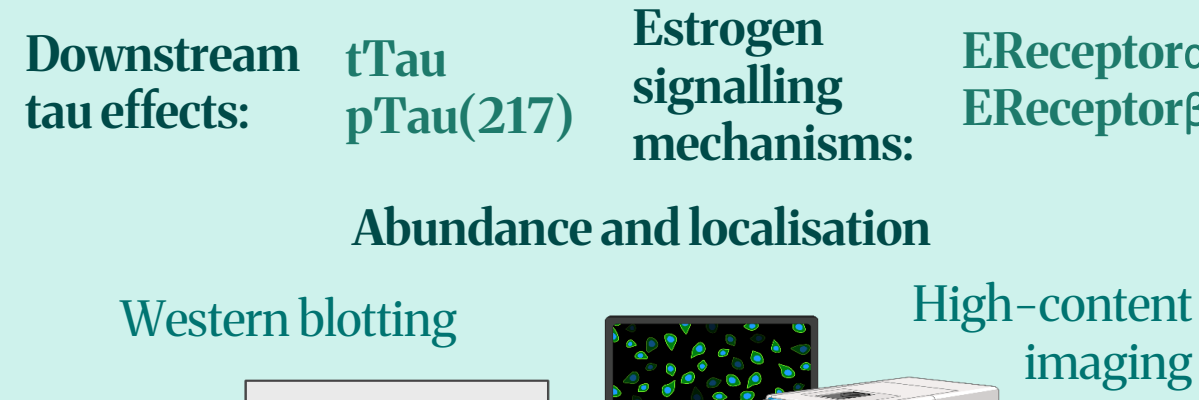


Figure 9. No significant effects of estrogen treatment on glutamate-induced reductions in cell survival are observed. Limited data so far for this condition.

Integration in clinical research: Assessing *in vitro* insights from midlife

Can we answer clinically relevant questions – How could a clinician tell who might experience neuroprotective benefits from MHT? From what dose? When? How?

Future sensitive measures may capture sub-neurotoxic effects:



How can preclinical findings be implemented clinically?

Parallel investigation: midlife females experiencing menopausal symptoms

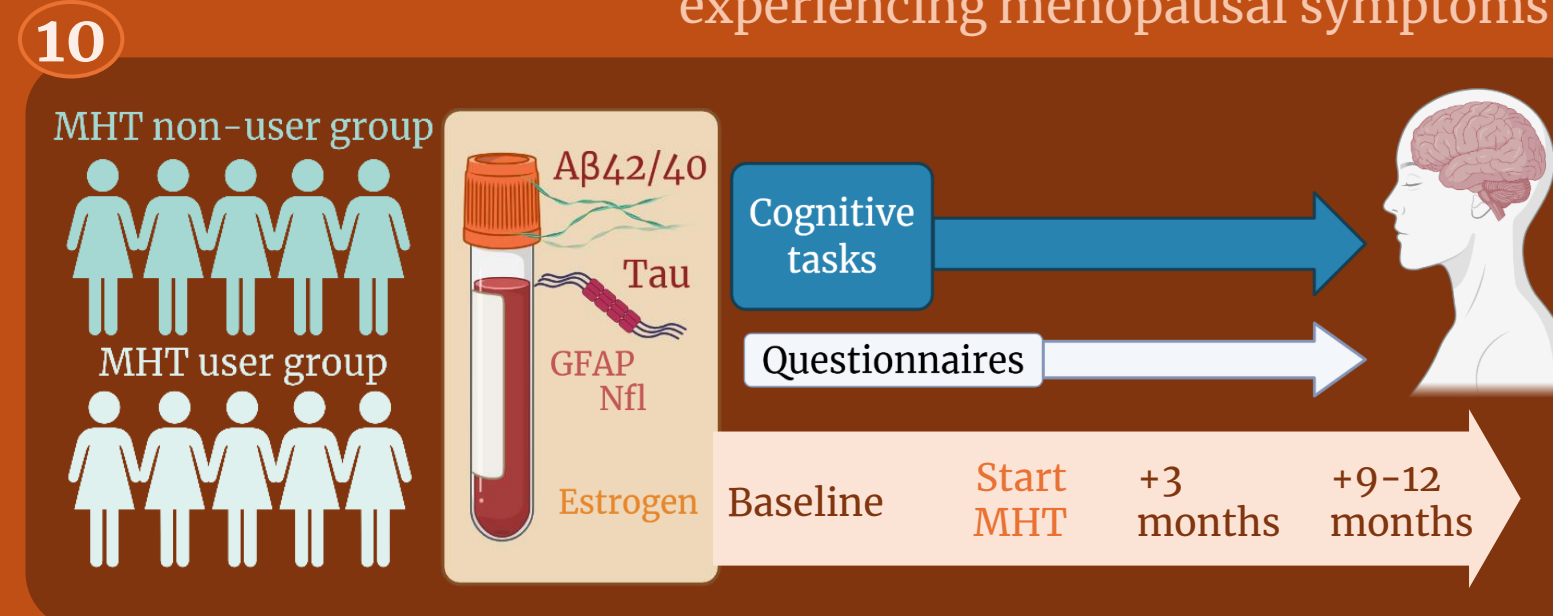


Figure 10. Parallel *in vivo* clinical investigation with hormone therapy users and non-users aged 45–65. Cognitive tasks focussed to load on the medial temporal lobe as site of earliest tau deposition. Baseline plasma biomarkers will be probed for association with cognitive change.

Assessing baseline characteristics and likely outcomes:

Can blood markers help us test the 'Healthy Cell Bias' in humans?

Tracking cognitive effects from point of MHT initiation:

Does cognitive testing reveal domain-specific sensitivity to hormone therapy and early AD biomarkers?

Using biomarkers and cognitive tracking, could we develop screening methods to help infer cognitive outcomes following MHT initiation?

Key References:

Mielke MM. Sex and Gender Differences in Alzheimer's Disease Dementia. *Psychiatr Times*. 2018; Liu C, Li Y, Nwosu A, Ang TFA, Liu Y, Devine S, et al. Sex-specific biomarkers in Alzheimer's disease progression: Framingham Heart Study. *Alzheimers Dement Amst Neth*. 2022; Snyder HM, Asthana S, Bain L, Brinton R, Craft S, Dubal DB, et al. Sex biology contributions to vulnerability to Alzheimer's disease: A think tank convened by the Women's Alzheimer's Research Initiative. *Alzheimers Dement J Alzheimers Assoc*. 2016; Theriault J, Pascoal TA, Lussier FZ, Tissot G, Chamoun M, Bezgin G, et al. Biomarker modeling of Alzheimer's disease using PET-based Braak staging. *Nat Aging*. 2022; Calvo N, Einstein G. Steroid hormones: risk and resilience in women's Alzheimer disease. *Front Aging Neurosci*. 2023; Blair IA. Analysis of estrogens in serum and plasma from postmenopausal women: past, present, and future. *Steroids*. 2010; Henderson VW, Brinton RD. Menopause and mitochondria: windows into estrogen effects on Alzheimer's disease risk and therapy. *Prog Brain Res*. 2010; Pozzi S, Benedusi V, Maggi A, Vegeto E. Estrogen action in neuroprotection and brain inflammation. *Ann NY Acad Sci*. 2006; Davey DA. Alzheimer's Disease, Dementia, Mild Cognitive Impairment and the Menopause: A 'Window of Opportunity'? *Women's Health*. 2013; Backstrom T, Carstensen H, Sodergard R. Concentration of estradiol, testosterone and progesterone in cerebrospinal fluid compared to plasma unbound and total concentrations. *J Steroid Biochem*. 1976; Greenstein BD, Puig-Duran E, Franklin M. Accurate, rapid measurement of the fraction of unbound estradiol and progesterone in small volumes of undiluted serum at 37°C by miniature steady-state gel filtration. *Steroids*. 1977

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