Centering the Needs of Transgender, Non-Binary, and Gender-Diverse Populations in Neuroendocrine Models of Gender-Affirming Hormone Therapy

Supplemental Information

Definitions and Terminology Recommendations

In this piece, TNG will include all individuals whose gender does not align with their sex-assigned-at-birth, including specific identities such as transgender, nonbinary, genderqueer, transfeminine, transmasculine, agender, bigender, autigender, genderfluid, and genderflux. Some Two-Spirit individuals also identify within the TNG umbrella, though not all, as gender as a category does not easily superimpose on historical contexts. We use cisgender (gender congruent) to refer to individuals whose gender does align with their sex-assigned-at-birth. This review is written from the Anglo-centric concept of gender that is dominant within the current Western biomedical establishment. Regarding referenced animal studies, we will defer to reported sex of animals (male/female) when discussing the mechanisms of gonadal hormones. For recommendations on terminology usage in primary research articles and experimental design, see (1).

Puberty and Adolescent GAHT

A particular area of research important to TNG youth is the effect of puberty blockers on mood, social behavior, cognition, and memory. TNG people, healthcare providers, and numerous scientific and professional societies approve of puberty blockers, such as the GnRH agonist leuprolide, as the standard of care for TNG youth (2–8). While much is known about pubertal changes to behavior and neuroanatomy, how hormones regulate pubertal gene expression changes remains largely unexplored. Adolescence is associated with significant neural circuit modifications, including changes in excitatory-inhibitory balance and synaptic weights (9,10). For example, hippocampal long-term potentiation is enhanced in prepubertal female mice. This enhancement is lost in adulthood due to increasing synaptic inhibition but observed only in intact female mice. It remains unknown if pubertal increases in E2 or ERα are responsible for the developmental increase in inhibition (9). Another study found pubertal increases in ovarian hormones in mice mediate an increase in inhibition in the prefrontal cortex that accompanies a decrease in behavioral flexibility. However, prepubertal ovariectomy does not alter behavioral performance (10). These complexities reinforce the need for well-designed experiments with multiple readouts when assessing the mechanisms of pubertal hormonal action on neurons as there may be important developmental compensations relevant for GAHT. Additionally, during puberty the brain is uniquely susceptible to stress (11), and gonadal hormone receptors are highly expressed across brain regions involved in the hypothalamic-pituitary-adrenal (HPA) axis (12) (**Figure 2**). As TNG populations experience extreme amounts of chronic stressors, animal studies of GAHT mechanisms need to address the paucity of research on overlapping stress and gonadal hormone signaling, particularly during adolescence.

Currently, there is limited research with inconsistent results on the impact of GnRH agonists on cognition and memory in animal models or in human studies (6,13,14). A recent study examined the effects of leuprolide on a variety of behavioral tasks to assess learning and memory as well as social and affective behavior in mice: whereas leuprolide reduces observed sex differences in some tasks, it had no effect on learning and memory (14). However, leuprolide was administered in sexually mature, young adult mice (P42-84 days). Current standards of care recommend puberty suppression for transgender adolescents during early puberty (2,3). Future studies should be conducted as close to the onset of puberty in the

studied organism (~P24-42 days in laboratory mice; **Figure 1C**) as possible to best model how GnRH agonists impact relevant neurophysiology and behavior. In a case study of one trans girl treated with puberty blockers, operational memory (executive function), using the Wechsler Intelligence Scale, decreased slightly, with speed processing and memory lower after 28 months of treatment compared to before puberty blockers. However, this may be due to baseline cognitive performance and not treatment (15). A few studies have examined the cognitive effects of blocking steroid signaling in pre- or peri-pubertal humans and none in the context of gender-affirming healthcare. In peripubertal cisgender boys, cognition and memory were not significantly altered by treatment with aromatase inhibitor, letrozole, which reduces estrogen production (16). A consensus study suggested that for human studies involving puberty blockers and assessment of neurological outcomes, a minimum of three time points should be used – before, during, and after puberty blockers (5). Additionally, the report suggests statistical modeling of sex and heterogeneity and the use of multiple comparison groups (untreated TNG youth matched on pubertal stage, cisgender youth matched on pubertal stage, and an independent sample from a large-scale youth development database). For GAHT models, we suggest that investigators examine puberty blockers at multiple doses, during relevant developmental time periods (post-weaning to young adult), and examine behavioral outcomes before, during, and after the cessation of puberty blockers (**Figure 1**).

Gonadal Hormones and Social Behavior in Adults

Gonadal hormones act on the SBN to influence adult social behavior during both the detection and action phases, as can be seen in rodent female-typical sexual behavior. During proestrus, E2 acts at ERα in neurotensin^{+} medial preoptic area (MPOA$^{Nts/Esr1}$) neurons to increase the gain of their responses to male sensory cues (17). MPOA$^{Nts/Esr1}$ neurons project to the ventral tegmental area and their optogenetic activation is reinforcing to mice (17). Optogenetic inactivation of the MPOA$^{Nts/Esr1}$ population reduced approach behavior to males and male urine (17), demonstrating a role for these neurons in the initial detection of social sensory cues. Additionally, proestrus E2 acts at ERα in PR^{+} neurons in the ventrolateral division of the ventromedial hypothalamus (VMHvl) to promote axonal outgrowth to the anteroventral periventricular hypothalamic nucleus (AVPV) (18). This novel axonal outgrowth strengthens the VMHvl^{PR+}→AVPV synaptic connection during estrus and is required for the display of lordosis (18). E2's control of rodent lordosis is not limited to intact females: both ovariectomized rodents treated with acute E2 and orchiectomized rodents treated with longitudinal E2 display lordosis in response to a sexually motivated male (18–20). Similar effects have been seen for other social behaviors influenced by sex hormones (14,21–24). These data demonstrate that exogenous longitudinal hormone treatments can promote species-specific social behaviors consistent with those displayed by conspecifics with similar endogenous hormone state regardless of the sex assigned to the animal. This suggests that gonadal hormones can restructure adult hormone-sensitive neural circuits, biasing individuals towards a suite of behaviors congruent with their hormone state.

Supplemental References

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