SUPPLEMENTARY INFORMATION

Using Organoids to Model Sex Differences in the Human Brain

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Supplementary table 1: Table of steroid effects on synapses – selected studies

Hormone/Compound	Effects of treatment on synapse parameter (treatment timeframe)	Species and age (Model)	Reference
17β-estradiol	Increased excitatory postsynaptic amplitude and LTP amplitude (acute)	Rat pups* (hippocampal slices); adult and aged male rats (hippocampal slices)	(1, 2)
	Induced NMDA-independent LTP (acute)	Young male rats (hippocampal slices)	(3)
	Enhanced induction of LTP, increased the density of parallel fiber to Purkinje cell synapses (14 days)	Ovariectomized female and male adult mice (cerebellar slices)	(4)
	Increased field excitatory postsynaptic potentials (acute), increased LTP	Young male and female rats and mice (hippocampal slices)	(5)
	Enhanced LTP, increased field excitatory postsynaptic potentials (acute)	Young male rats (hippocampal slices)	(6)
	Enhanced synaptic response to stimulation of the vestibular nerve, but reduces spontaneous discharge in A and B neurons; enhanced excitatory post-synaptic potential and current	Male rats (medial vestibular nucleus brainstem slices)	(7)
	Induced LTP, increased density of small spines (acute)	Adult male rat (hippocampal slices)	(8)
	Decreased inhibitory postsynaptic currents and suppressed GABA release in females only	Adult ovariectomized female and male rats (hippocampal slices)	(9)
	Increased field excitatory postsynaptic potentials (acute)	adult male rats (hippocampal slices);	(10, 11)
	Increased fast glutamatergic transmission, increased number of actin spines, facilitated LTP	Young male rats and ovariectomized middle-aged female rats (hippocampal slices)	(12)
	Increased excitatory postsynaptic potentials (45 minutes)	Male and female mice (hippocampal slices)	(13)
	17β-estradiol treatment rescued aromatase knockout-associated deficits: increased LTP amplitude, increased synaptophysin and PSD95 protein levels (7 days)	Female mice	(14)
	Reduced glutamate decarboxylase in interneurons, reduced miniature inhibitory postsynaptic currents (24 hrs)	Embryonic rat* (primary culture containing hippocampal interneurons)	(15)

	Increased excitatory postsynaptic currents, increased miniature excitatory postsynaptic currents, enhanced oscillation of glutamate-induced calcium	Young male and female rats (hippocampal slices)	(16)
	Increased dendritic spine density and increased synaptic surface AMPAR content, increased GluN2A-NMDAR and decreasing GluN2B-NMDAR synaptic content (24 hrs)	Rat embryos* (primary neural culture), male mice	(17)
	Increased expression of PSD-95, increased spine density	Male and female rats (hippocampal slices and primary neural cultures)	(18)
	Increased spine density, formation of silent synapses (acute)	Embryonic rat* (cortical neuronal cultures)	(19)
	Increased synaptic excitability (48hrs), increased excitatory postsynaptic potentials and potentiation of responses to AMPA, kainate, and quisqualate, but not NMDA (acute)	Ovariectomized adult female rats (hippocampal slices)	(20)
	Increased field excitatory postsynaptic potentials, increased membrane levels of GluR1 (acute)	Embryonic rats* and mice* (cultured cortical and hippocampal neurons, acute hippocampal slices)	(21)
	Enhanced release of dopamine	Ovariectomized female rats (dorsolateral striatum)	(22)
Aromatase inhibitor (Letrozole)	Prevented LTP at fiber–Purkinje cell synapses (acute)	Adult male rats (cerebellar slices)	(23)
	Reduced basolateral amygdala spine synapse density in female mice only (7 days), prevented LTP induction in females only (60 minutes)	Male and female mice, male and female neonatal rats (corticoamygdalar slices)	(24)
Dihydrotestosterone	Increased spine synapse density (2 days)	ovariectomized adult female rats (hippocampus)	(25)
Estradiol benzoate	Increased field excitatory postsynaptic potentials (acute)	Adult ovariectomized female rats (hippocampal slices)	(26)
Flutamide (AR blocker)	Prevented LTD, No effect on LTP (acute)	Male rats (brainstem slices); Young and adult male rats (hippocampal slices)	(27, 28)
ICI 182,780 (ER blocker)	Prevented LTP, No effect on LTD	Male rats (brainstem slices); Young and adult	(27, 28)

		male rats (hippocampal slices)	
MPP (ERα Antagonist) and ERβ Antagonist (PHTPP)	Reduced LTP amplitude, in combination prevented LTP completely (acute)	Young and adult male rats (hippocampal slices)	(28)
PPT (ERα agonist)	Induced LTP, increased GluA1 surface staining (acute)	Cultured hippocampal neonatal male rat neurons	(3)

LTP - long-term potentiation

LTD - long-term depression

* sex not reported/not considered

acute treatment - <1 hour

effects on synapses – effects on electrophysiological parameters, synaptic proteins, synaptic puncta and spines

Supplementary table 2: Table of brain-region specific organoids

Organoid type	Advantages	Reference
Cortical spheroid	Dorsal or ventral forebrain spheroids that can be assembled *in vitro* to recapitulate fetal migration of interneurons and their functional integration with glutamatergic neurons. This model of cortical development follows a targeted approach that specifically studies interaction of excitatory and inhibitory neurons, and can provide a better understanding of E-I imbalance mechanisms.	(29)
Hippocampal organoid	Recapitulates hippocampus development, a key brain region influenced by sex steroids. Generates hippocampal granule-like, pyramidal-like neurons, and astrocyte-like cells.	(30)
Cerebellar organoid	Recapitulates early cerebellar development, the cerebellum being a non-cortical brain region showing sex-specific development. Generates precursors of cerebellar neurons (Purkinje-like, Golgi-like, DCN projection-like neurons, granule-like cells) with relevant morphology and electrophysiological properties.	(31)

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