8 Wybenga-Groot, L. *et al.* (2001) Structural basis for autoinhibition of the EphB2 receptor tyrosine kinase by the unphosphorylated juxtamembrane region. *Cell* 106, 745–757
9 Himanen, J.P. *et al.* (2001) Crystal structure of an Eph receptor-ephrin complex. *Nature* 414, 933–938
10 Lackmann, M. *et al.* (1997) Ligand for EPH-related kinase (LERK) 7 is the preferred high affinity ligand for the HEK receptor. *J. Biol. Chem.* 272, 16521–16530
11 Davis, S. *et al.* (1994) Ligands for EPH-related receptor tyrosine kinases that require membrane attachment or clustering for activity. *Science* 266, 816–819
12 Lo Conte, L. *et al.* (1999) The atomic structure of protein–protein recognition sites. *J. Mol. Biol.* 285, 2177–2198
13 Bruckner, K. *et al.* (1999) EphrinB ligands recruit GRIP family PDZ adaptor proteins into raft membrane microdomains. *Neuron* 22, 511–524
14 Stapleton, D. *et al.* (1999) The crystal structure of an Eph receptor SAM domain reveals a mechanism for modular dimerization. *Nat. Struct. Biol.* 6, 44–49
15 Thanos, C. *et al.* (1999) Oligomeric structure of the human EphB2 receptor SAM domain. *Science* 283, 833–836
16 Wang, H.U. *et al.* (1998) Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell* 93, 741–753
17 Gerety, S.S. *et al.* (1999) Symmetrical mutant phenotypes of the receptor EphB4 and its specific transmembrane ligand ephrin-B2 in cardiovascular development. *Mol. Cell* 4, 403–414

Tyler Cutforth
Center for Neurobiology and Behavior, Columbia University, 701 W. 168 St, New York, NY 10032, USA.

Celia J. Harrison*
Boston Biomedical Research Institute, 64 Grove St, Watertown, MA 02472, USA.
*e-mail: harrison@bbri.org

Meeting Report

Sex, cells and signals in the developing brain

Stacey A. Fannon, Regina M. Vidaver and Sherry A. Marts

Sex Begins in the Womb. Held at Crowne Plaza Cabana, Palo Alto, CA, USA, on 1 March 2002.

Hormones sculpt the neural architecture, creating a profound and permanent impact on physiology and behavior. Precisely how that sculpting occurs, and how hormones mediate signaling pathways in the brain, were discussed at 'Sex Begins in the Womb' – a multidisciplinary symposium convened by the Society for Women's Health Research. The meeting was part of a series of Society-sponsored conferences on the biology of sex differences and how those differences affect human health.

Sexually dimorphic circuitry

Recent research has established that the mammalian forebrain contains an entire circuitry that is sexually dimorphic. As Richard Simerly (Oregon Health Sciences University, Portland, OR, USA) explained, behavior depends in part on the number of cells in the various brain regions and the connections between them. In rodents, most sexually dimorphic regions contain more neurons in the male brain, but a few regions contain more neurons in females. Simerly focused his presentation on one such region, the anteroventral periventricular (AVPV) nucleus.

Located at the rostral end of the hypothalamus, the AVPV controls ovulation. Most of the neurons in the AVPV express genes encoding estrogen and progesterone receptors, making them responsive to circulating levels of these hormones. The AVPV connects to gonadotrophin-releasing hormone (GnRH)-producing neurons, and this output pathway is more robust in females than in males. By contrast, sensory input pathways to the AVPV tend to be more robust in males than in females.

As Simerly noted, hormones promote or diminish the survival or number of cells in brain nuclei in cell-type specific ways. This is exemplified in the AVPV, which has more proenkephalin-containing neurons in males than in females. However, if a female is treated with testosterone in the first few days of life, this neuron population shows a complete sex reversal, indicating that postnatal hormone exposure alone can direct development of this sex difference.

Sex steroid hormones such as estrogen influence brain development and plasticity through multiple signaling pathways [1], including indirect genomic actions of estrogen that are conveyed to the nucleus by a variety of second messengers and transcription factors. The best-characterized pathway for estrogen signaling is the direct genomic mechanism, whereby the steroid hormone enters the cell and binds to a nuclear estrogen receptor (ER). This receptor acts as a transcription factor to alter expression of certain genes that act on the cell in various ways. The developmental action of the ER was demonstrated by Simerly, who studied mice lacking ERα: in these mice, sexual differentiation of a population of dopamine-containing neurons was blocked. The same receptor appears to be crucial for formation of a sexually dimorphic neural pathway that delivers olfactory information to the AVPV: Simerly reported that, in a series of *in vitro* experiments, the development of this pathway depended on hormone exposure of the AVPV and also required ERα. By directly influencing the number of cells that mature in brain nuclei, and by specifying the formation of neural pathways between these cell groups, ERα plays a crucial role in determining the architecture of forebrain pathways that differ in males and females.

Key conference outcomes

- Behavior depends in part on the numbers of neurons in various parts of the brain, and the connections between them.
- The anteroventral periventricular (AVPV) nucleus in the female contains more neurons than does the male AVPV.
- Many of the effects of hormones on the brain are cell-type and temporally specific.
- Hormones promote or diminish the survival or the number of cells in brain nuclei in cell-type-specific ways.
- GABA is a predominant neurotransmitter with an excitatory function during development that changes to an inhibitory function in the adult brain.
- Maternal progesterone binds progesterone receptors (PR) in the fetal medial preoptic nucleus (MPN). PR might be involved in sexual differentiation of the MPN.

GABA signaling during sexual differentiation

Margaret McCarthy (University of Maryland, Baltimore, MD, USA) and her colleagues identified GABA signaling as a major divergence point in sexual

 0166-2236/02/$ – see front matter © 2002 Elsevier Science Ltd. All rights reserved. PII: S0166-2236(02)02175-6

differentiation of the brain. GABA is a predominant neurotransmitter, to which almost every cell in the brain is sensitive. In the adult brain, GABA generally mediates inhibition. In the developing brain, however, GABA is an excitatory transmitter.

Studies of hormonal action in the brain indicate that different brain regions control different functions. For example, the medial preoptic area (mPOA) is crucial for control and expression of male sexual behavior. By contrast, the ventral medial nucleus (VMN) is a major site controlling female sexual behavior, and the arcuate nucleus plays a modulatory role in the luteinizing hormone surge required for ovulation.

McCarthy presented data demonstrating that the developmental shift from excitatory to inhibitory GABA action is altered by the neonatal hormonal milieu and is, therefore, different in males and females. Excitatory GABA activates voltage-gated Ca^{2+} channels indirectly (by depolarizing the membrane), resulting in a transient increase in free cytosolic Ca^{2+} concentration. Neurons from the male brain that are exposed to estradiol exhibit Ca^{2+} transients that are twice the amplitude of those in untreated neurons. Estradiol-exposed neurons also have an increased probability of responding to GABA with membrane depolarization as development progresses [2]. Consequent to increased cytosolic Ca^{2+} concentration is activation of the transcription factor CREB (cAMP-response-element-binding protein). McCarthy and colleagues reported that the activation of CREB is dramatically increased in some regions of male brain following GABA-receptor activation, but is actually decreased in parts of the female brain [3]. Distinct patterns of activation and deactivation of CREB were seen in the mPOA, VMN and arcuate nucleus, underscoring the regional heterogeneity and divergence in excitatory versus inhibitory GABA activity that are postulated to direct development of a male versus a female brain.

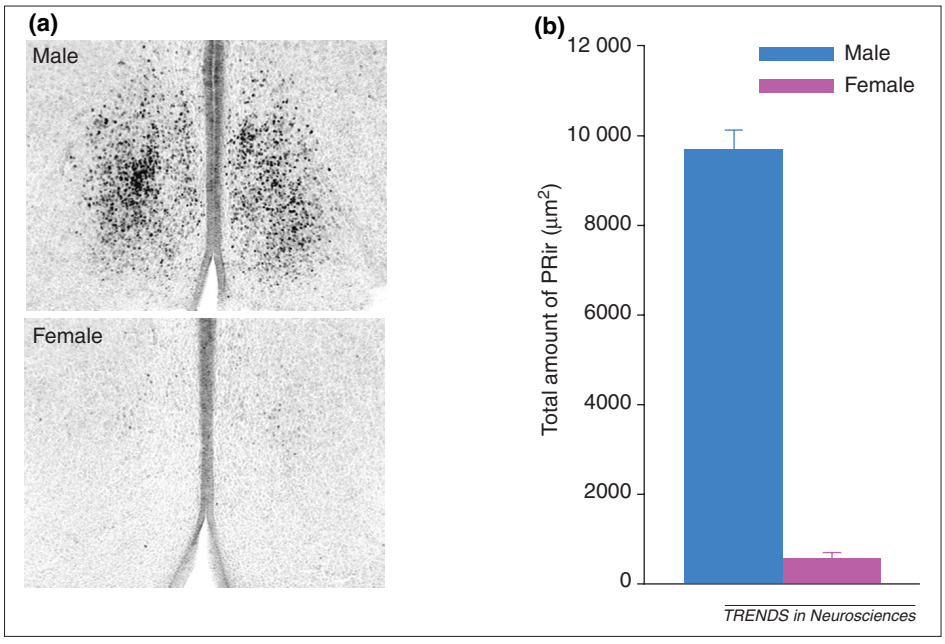

Fig. 1. (a) Progesterone receptor immunoreactivity (PRir) in the medial preoptic nucleus (MPN) of male and female neonatal rats on the day of birth. (b) Total amount of PRir (μm^2) within the MPN on the day of birth. There is a significant difference between the areas of PRir in males and females. Error bars, ± SE. Reproduced, with permission, from Ref. [4] (© The Endocrine Society, 1998).

Maternal–fetal hormone interaction

Maternal hormones might also influence the developing brain. In particular, progesterone circulates at high levels during pregnancy and lactation. Christine Wagner (State University of New York at Albany, NY, USA) presented her findings on effects of progesterone on the brains of pregnant rats and their fetuses. Progesterone levels in the pregnant rat peak between gestational days 15 and 19, then drop precipitously just before birth (approximately gestational day 23). These levels correlate closely with fetal levels, suggesting that the mother is the source of progesterone in fetal circulation. Immunocytochemical evaluation of progesterone receptor (PR) immunoreactivity in the medial preoptic nucleus (MPN) showed that male rats have high levels of PR, whereas levels are low in females [4] (Fig. 1). These findings also suggest that the PR could be involved in the sexual differentiation of this region.

Wagner noted that fetal testosterone is aromatized to estradiol, and that both hormones exert effects on the developing brain. In the absence of testosterone, a female brain develops. Wagner reported that neonatal treatment of females and castrated males with testosterone and the PR antagonist, RU-486, elicited sexually different responses. In females, RU-486 attenuated the masculinizing effects of testosterone on the volume of the central subdivision of the MPN. But in the males, RU-486 augmented the effects of testosterone. These opposite effects suggest that males and females are differentiated at birth in a way that postnatal testosterone cannot change [5].

Other presentations examined neurobehavioral effects of exogenous hormones, maternal stress and other aspects of the intrauterine environment. A summary of the meeting is available from the conference organizers at http://www.womens-health.org

Acknowledgements

We would like to acknowledge the assistance of Nancy Evans in the preparation of this meeting report.

References

1 McEwen, B.S. (2001) Invited review: Estrogens' effects on the brain: multiple sites and molecular mechanisms. *J. Appl. Physiol.* 91, 2785–2801
2 Perrot-Sinal, T.S. *et al.* (2001) Estradiol enhances increases in cytosolic calcium induced by excitatory GABA in neonatal hypothalamic neurons. *Endocrinology* 142, 2238–2243
3 Auger, A.P. *et al.* (2001) Excitatory versus inhibitory GABA as a divergence point in steroid-mediated sexual differentiation of the brain. *Proc. Natl. Acad. Sci. U. S. A.* 98, 8059–8064
4 Wagner, C.K. *et al.* (1998) Potential role of maternal progesterone in the sexual differentiation of the brain. *Endocrinology* 139, 3658–3661
5 Quadros, P.S. *et al.* (2002) Progesterone receptors and the sexual differentiation of the medial preoptic nucleus. *J. Neurobiol.* 51, 24–32

Stacey A. Fannon
Regina M. Vidaver*
Sherry A. Marts
Society for Women's Health Research, 1828 L Street, NW Suite 625 Washington, DC 20036, USA.
*Regina@womens-health.org