Zárójelentés

Reprodukciót szabályozó hypothalamikus központok gén és fehérje expressziós mintázatának feltárása: az ösztradiol visszacsatolás mechanizmusai

OTKA nyilvántartási szám: ***K 100722***

Kutatóhely neve: Endokrin Neurobiológiai Kutatócsoport, MTA Kísérleti Orvostudományi Kutatóintézet

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I. Az eredeti munkaterv összefoglalója

Gonadotropin-releasing hormone (GnRH) egy fontos peptid, mely az agyalapi mirigy-ivarszerv endokrin rendszer működését szabályozza, fenntartva a szaporodást. Rágcsálók agyában mintegy 1500 GnRH idegsejt tölti be ezt a funkciót, melyek működését ideghálózatok és a vérben keringő hormonok szabályozzák. Funkcionális szempontból a női nemi hormon, az ösztradiol, kitüntetett szerepet játszik a reproduktív tengely hormon visszacsatolás általi szabályozásában. A pályázati munka fő célja feltárni az ösztradiol hormon hatását a GnRH idegsejtek és a velük kapcsolatban álló, a pozitív hormon visszacsatolást szabályozó struktúra, az elülső periventrikuláris idegmag (AVPV) génexpressziójára és fehérje termelésére. Molekuláris, strukturális és funkcionális módszertani megközelítéssel tervezzük:

1. Azonosítani GnRH idegsejtek gén expressziós profilját hím egerekben.

2. Felderíteni a GnRH idegsejtek nemhez kötött gén expressziós jegyeit.

3. Meghatározni az alacsony és magas ösztradiol szintek hatását a GnRH idegsejtek és az AVPV mag gén és fehérje kifejeződésére.

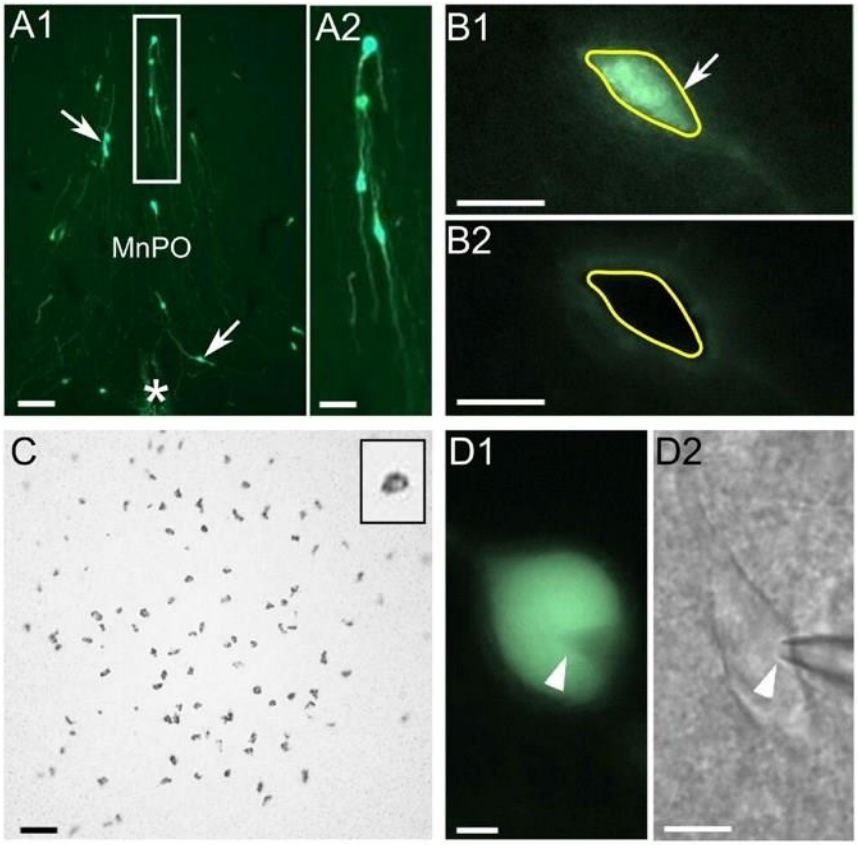
4. Adatbázist létrehozni a GnRH neuronokban működő, ösztrogén által szabályozott génekről, fehérjékről és mechanizmusokról.
5. Jellemezni az GnRH neuronok egy új típusú szabályzó rendszerét morfológiai, molekuláris biológiai és elektrofiziológiai módszerekkel. A vizsgálatok a reprodukciót központi szinten szabályozó új mechanizmusokat tárnak fel és hozzájárulhatnak a női szervezetre ható új gyógyszerek fejlesztéséhez.

II. Az elért eredmények összefoglalója

II/1. Elsődleges eredmények összegzése

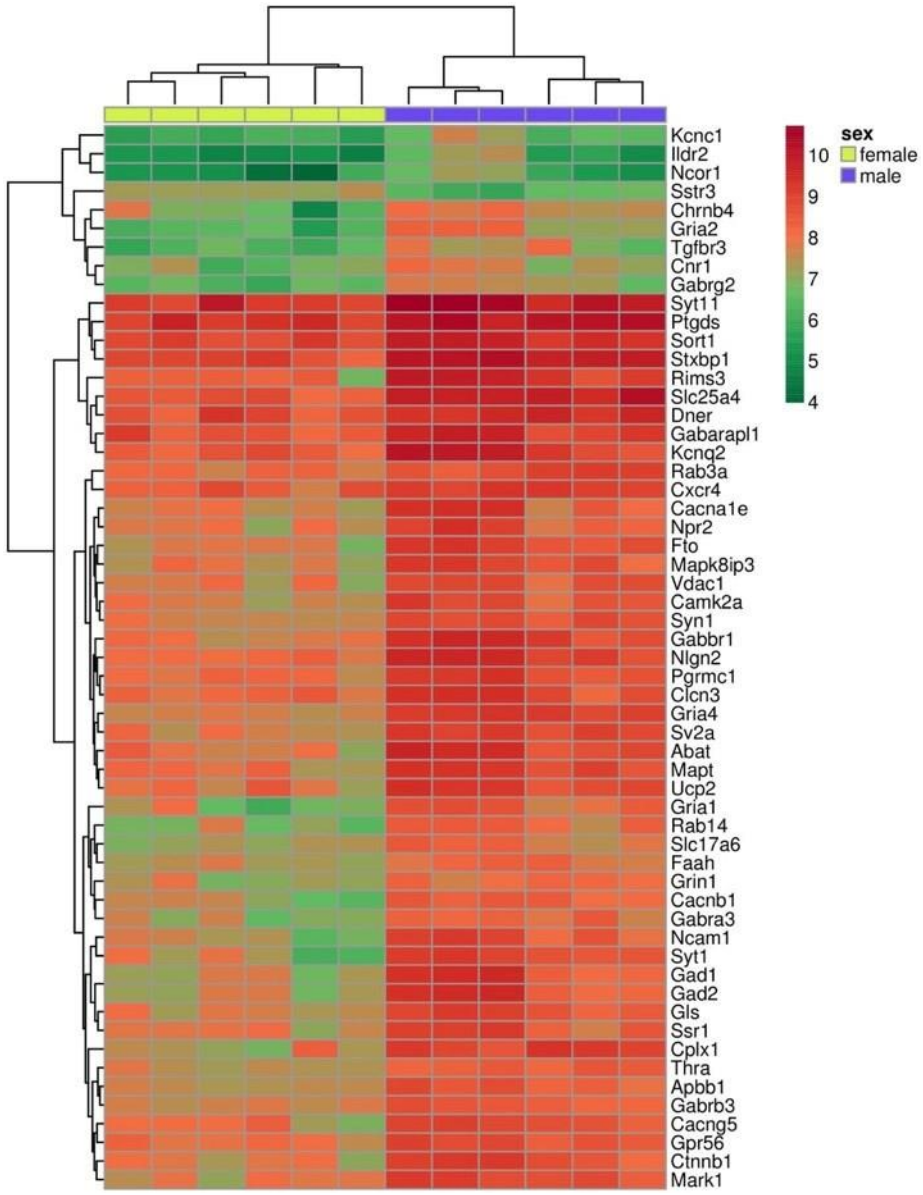
I. Módszert dolgoztunk ki a kisszámú és szórt agybéli elhelyezkedést mutató GnRH-GFP idegsejtek megbízható mintavételezésére lézer sugárral történő kimetszés (LCM) és patch-pipettával történő cytoplasma nyerés módszerek alkalmazásával. LCM módszerrel egyedi állatonként mintegy 250 GnRH-GFP idegsejtet tudtunk begyűjteni, reprezentálva a GnRH rendszer teljes rostrocaudális és medio-laterális kiterjedését. A fluoreszkáló idegsejt kimetszését nagy nagyítás mellett végeztünk, törekedve a kontaminációtól mentes mintavételre. A patch-pipettával végzett mintanyerést akut agyszeleteken végeztük, a GnRH-GFP idegsejtek citoplazmájának kiszívásával. A két módszerrel, független mintákon elvégzett kísérletek egybevágó eredményt adtak.

Az alábbi **1. ábra** demonstrálja a GnRH-GFP idegsejtek megjelenését egér előagyban (A1, A2), valamint azok citoplazma mintáinak kinyerésére alkalmazott LCM (B1, B2, C) és patch-pipetta (D1, D2) módszereket.



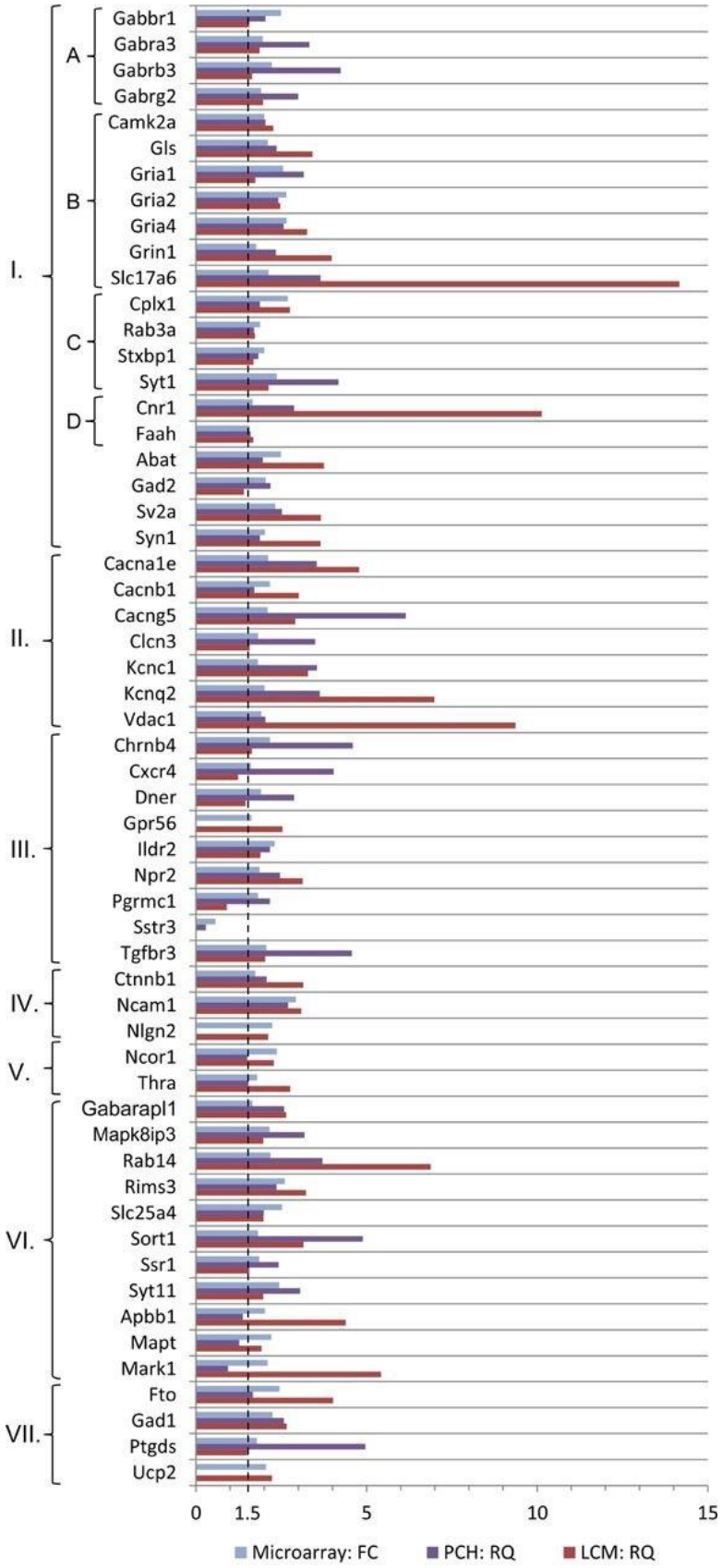
1. ábra. *GnRH-GFP idegsejtekből történő mintavétel LCM és patch-pipetta módszerek alkalmazásával*

II. Sikerrel alkalmaztuk a közelmúltban kidolgozott pico profiling módszert a GnRH sejtminták transzkriptómjának microarray módszer általi karakterizálására. A vizsgálatokat együttműködésben végeztük az Institute for Research in Biomedicine (IRB), Functional Genomics Core, Barcelona, Spanyolország munkatársaival, a pico profiling módszer kidolgozóival. Az alkalmazott módszer jó minőségű mRNS-t eredményezett (RIN érték: 7) és microarray vizsgálatot követő heat-map analízisek homogén kísérleti csoportokról adtak számot. Az alábbi, **2. ábra** a hím és a metestrus ovarialis fázisban lévő nőstény patkányok GnRH idegsejtjeinek transzkriptóm jellemzését mutatja heat-map ábrázolás segítségével.



2. ábra. *Hím és nőstény (metestrus) állatokból származó GnRH idegsejtek transzkriptóm jellegzetességei heat-map elemzésben*

Az LCM mintákból nyert microarray eredményeket megbízhatóan megerősítettük független mintákban (patch pipetta mintavétel) végzett kvantitatív RT-PCR vizsgálattal. A két eltérő módszerrel és független mintákon nyert eredmények nagymérvű egyezőségét a **3. ábra** mutatja be, mintegy 57 gén vonatkozásában.



3. ***ábra.*** *GnRH idegsejtek transzkriptóm vizsgálati eredményeinek egybevágósága microarray és q-RT-PCR (PCH és LCM) elemzést követően* **III.** Feltártuk a hím és a metestrus ovarialis fázisban lévő nőstény egerek GnRH

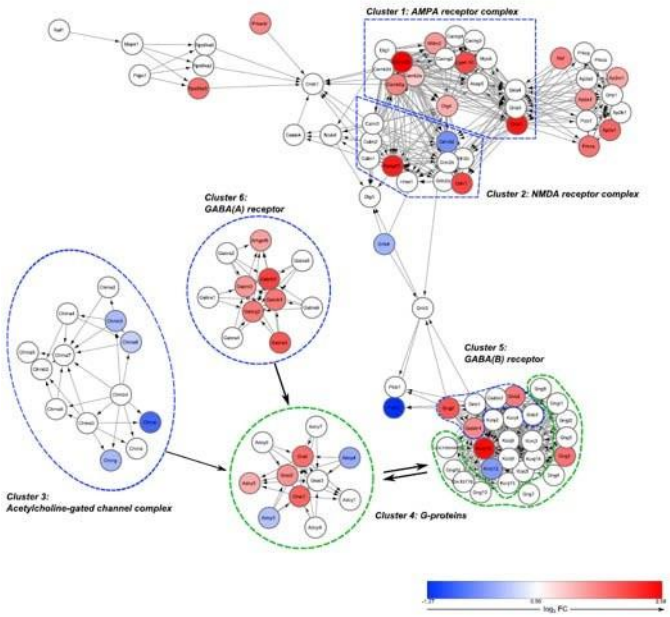
idegsejtjeinek transzkriptómját, valamint igazoltuk a génexpresszió ivarfüggő dimorfizmusát (**Közlemény 1)**. Megállapítottuk, hogy erős szelekciós feltétel mellett (FC >1.6), 543 gén mutatott differenciált expressziót, melyek közül 482 gén expressziója a hím állatokban mutatott dúsulást. A változást mutató gének ontológiai elemzése rámutatott, hogy a szexuális dimorfizmus alapvető GnRH sejtfolyamatot szabályozó mechanizmust érint, köztük a szinaptikus vesicula priming és docking folyamatát *(Syt1, Cplx1),* a GABA-erg *(Gabra3, Gabrb3, Gabrg2)* és a glutamáterg *(Gria1, Grin1, Slc17a6)* neurotranszmissziót, a peptiderg szignalizációt *(Sstr3, Npr2, Cxcr4)* és az intracelluláris ion homeosztázist *(Cacna1, Cacnb1, Cacng5, Kcnq2, Kcnc1)*.

IV. Meghatároztuk az alacsony ösztradiol szinttel jellemezhető metestrus ovariális ciklus fázisban, valamint a magas ösztradiol szinttel rendelkező proestrus fázisban lévő egerek GnRH idegsejtjeinek differenciált génexpressziós jegyeit, különös tekintettel az ovulációt szabályozó különféle neurotranszmitter rendszerek szerepére és azok funkcionális változására a pre-ovulatorikus GnRH surge előkészítésében (**Közlemény 3**). A proestrus és metestrus ciklusfázisban lévő nőstény egerekből származó GnRH idegsejtek összehasonlító transzkriptóm vizsgálata igazolta, hogy a két funkcionális állapot közti különbség jelentős és több mint 4000 gén differenciált expresszióját foglalja magába. Az alábbi gén ontológiai elemzés a GnRH idegsejteket beidegző neurotranszmitter rendszerek érintettségére utal:

Database	Pathway	pSize	NDE	pNDE	tA	pPERT	pG	pGFdr	pGFWER	Status
REACTOME	Neurotransmitter Receptor Binding And Downstream Transmission In The Postsynaptic Cell	109	41	9.11E-05	250.53	5.00E-06	1.03E-08	**3.37E-06**	3.37E-06	Activated
REACTOME	Activation of NMDA receptor upon glutamate binding and postsynaptic events	26	10	3.72E-02	50.73	5.00E-06	3.07E-06	**5.05E-04**	1.01E-03	Activated
KEGG	GABAergic synapse	64	25	1.08E-03	-4.55	2.20E-02	2.76E-04	**5.10E-03**	3.26E-02	Inhibited
KEGG	Dopaminergic synapse	116	40	8.73E-04	-0.18	9.48E-01	6.70E-03	**3.55E-02**	7.90E-01	Inhibited

A differenciált gén expresszió különféle transzmitter receptor alegységek és azok kapcsolt downstream szabályozó elemeinek, köztük a GABAerg (*Gabbr1*, *Gabra3*, *Gabrb3*, *Gabrb2*, *Gabrg2*), glutamáterg (*Gria1*, *Gria2*, *Grin1*, *Grin3a*, *Grm1*, *Slc17a6*), kolinerg (*Chrnb2, Chrm3, Chrm4*) és dopaminerg (*Drd3, Drd4*) szignál transzdukciós rendszerek érintettségében nyilvánult meg. Hasonló

szignifikáns eredményt kaptunk az adrenerg (*Adra1b*, *Adra2a*, *Adra2c*), adenosinerg (*Adora2a*, *Adora2b*), glicinerg (*Glra*), purinerg (*P2rx7*) és szerotoninerg (*Htr6*) receptor gének vonatkozásában. További expressziós változások egyes G-fehérjék (*Gnai1*, *Gnai2*, *Gnas*), adenilát cikláz (*Adcy3*, *Adcy5*), protein kináz (*Prkaca*, *Prkacb*, *Prkca*), valamint bizonyos transzporterek (*Slc1a4*, *Slc17a6*, *Slc6a17*) vonatkozásában voltak megfigyelhetők. Az eredményeket az **1. Táblázat** foglalja össze. Az eredmények megerősítik nevezett neurotranszmitter rendszerek részvételére vonatkozó megfigyeléseket a preovulatorikus GnRH-surge kialakításában. A serkentő rendszerek további aktivációja (**4. ábra**), míg a gátló rendszerek gátlása jellemzik a GnRH idegsejtek transzmitter receptorainak expressszióját végbemenő változásokat a proestrus napján.



4. ábra. *Differenciált expressziót mutató neurotranszmitter receptor gén csoportok GnRH idegsejtjeiben (poestrus)*

1. Táblázat

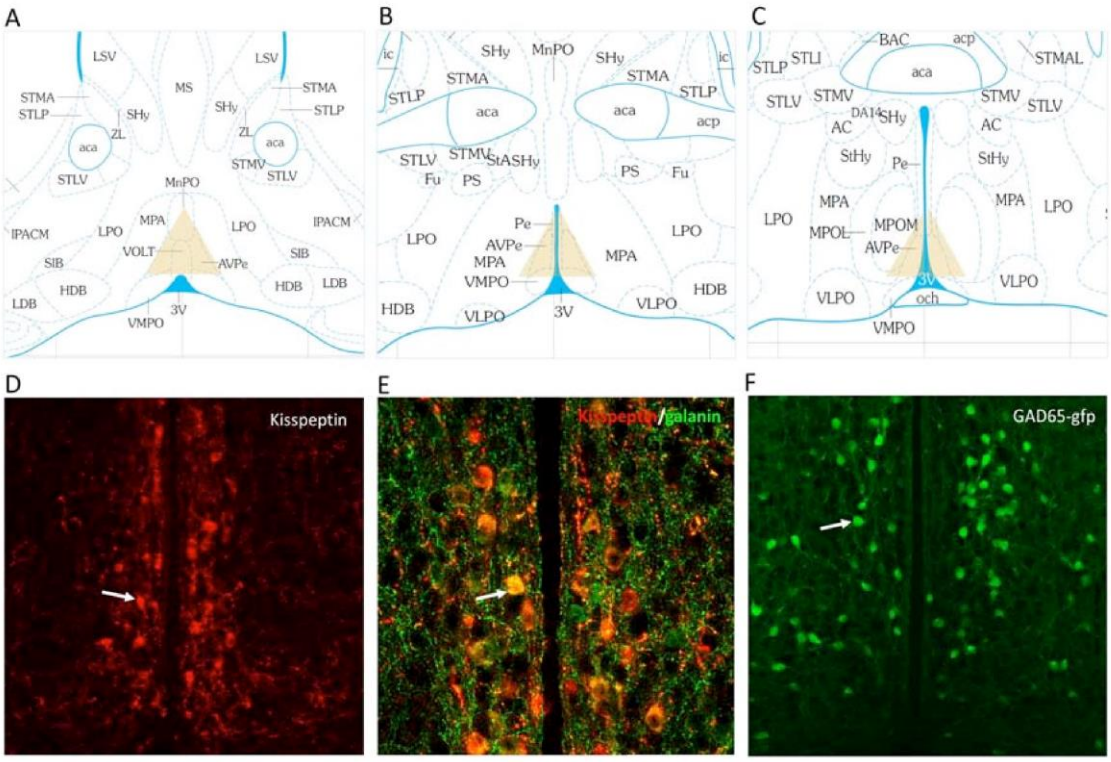
Signaling system	Symbol	Description	FC	FDR
Adenosin-ergic	Adora2a	adenosine A2a receptor	0,50	7,47E-04
	Adora2b	adenosine A2b receptor	0,59	7,64E-03
Adrenergic	Adra1b	adrenergic receptor, alpha 1b	0,49	5,18E-04

	Adra2a	adrenergic receptor, alpha 2a	0,37	5,07E-04
	Adra2c	adrenergic receptor, alpha 2c	0,56	1,22E-03
Cholinergic	Chrm3	cholinergic receptor, muscarinic 3, cardiac	0,63	7,01E-03
	Chrm4	cholinergic receptor, muscarinic 4	0,58	1,20E-02
	Chrnb2	cholinergic receptor, nicotinic, beta polypeptide 2 (neuronal)	**2,65**	2,17E-02
	Chrne	cholinergic receptor, nicotinic, epsilon polypeptide	0,52	4,98E-03
	Chrng	cholinergic receptor, nicotinic, gamma polypeptide	0,64	8,04E-03
Dopamin-ergic	Drd3	dopamine receptor D3	0,60	4,99E-02
	Drd4	dopamine receptor D4	0,45	8,28E-05
GABAergic	Gabrp	gamma-aminobutyric acid (GABA) A receptor, pi	0,65	1,46E-02
	Gabarapl1	gamma-aminobutyric acid (GABA) A receptor-associated protein-like 1	**1,60**	2,69E-02
	Gabbr1	gamma-aminobutyric acid (GABA) B receptor, 1	**2,80**	5,95E-04
	Gabra3	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 3	**1,90**	1,77E-02
	Gabrb2	gamma-aminobutyric acid (GABA) A receptor, subunit beta 2	**2,12**	5,95E-04
	Gabrb3	gamma-aminobutyric acid (GABA) A receptor, subunit beta 3	**3,05**	1,53E-02
	Gabrd	gamma-aminobutyric acid (GABA) A receptor, subunit delta	0,58	4,98E-03
	Gabrg2	gamma-aminobutyric acid (GABA) A receptor, subunit gamma 2	**2,20**	2,96E-02
	Gabarap	gamma-aminobutyric acid receptor associated protein	**1,82**	4,36E-03
G-proteins and downstream effectors	**Gnai1**	guanine nucleotide binding protein (G protein), alpha inhibiting 1	**2,59**	1,49E-03
	Gnai2	guanine nucleotide binding protein (G protein), alpha inhibiting 2	**1,88**	4,71E-02
	Gnas	GNAS (guanine nucleotide binding protein, alpha stimulating) complex locus	**1,73**	8,27E-04
	Adcy3	adenylate cyclase 3	0,50	9,04E-03
	Adcy5	adenylate cyclase 5	**2,70**	1,84E-02
	Prkaca	protein kinase, cAMP dependent, catalytic, alpha	**2,06**	5,66E-04
	Prkacb	protein kinase, cAMP dependent, catalytic, beta	**1,86**	1,22E-03
	Prkca	protein kinase C, alpha	**1,78**	7,51E-03
	Plcb2	phospholipase C, beta 2	0,42	3,99E-04
Glutamatergic	**Gria2**	glutamate receptor, ionotropic, AMPA2 (alpha 2)	**3,13**	1,35E-03

	Gria1	glutamate receptor, ionotropic, AMPA1 (alpha 1)	**2,36**	2,84E-02
	Grin1	glutamate receptor, ionotropic, NMDA1 (zeta 1)	**2,24**	2,73E-02
	Grin3a	glutamate receptor ionotropic, NMDA3A	**1,80**	2,11E-02
	Gls	glutaminase	0,56	4,53E-03
	Grm1	glutamate receptor, metabotropic 1	0,53	5,78E-03
	Grin2d	glutamate receptor, ionotropic, NMDA2D (epsilon 4)	0,37	1,49E-04
Serotonergic	Htr1b	5-hydroxytryptamine (serotonin) receptor 1B	0,58	1,14E-02
	Htr6	5-hydroxytryptamine (serotonin) receptor 6	0,63	9,10E-03
Purinergic	P2rx7	purinergic receptor P2X, ligand-gated ion channel, 7	0,59	2,20E-03
Solute carrier family	**Slc17a6**	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6	**1,77**	1,46E-02
	Slc1a4	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6	**2,24**	1,46E-02
	Slc6a17	solute carrier family 6 (neurotransmitter transporter), member 17	**3,55**	1,30E-03
Others	Glra1	glycine receptor, alpha 1 subunit	0,49	2,76E-03

VI. Kimutattuk, hogy az elülső periventrikuláris idegmag (AVPV) területén elhelyezkedő idegelemek proestrus délután időszakában jelentős transzkripcionális átalakuláson mennek keresztül, elsősorban a peptid és neurotranszmitter szabályozó rendszerek vonatkozásában, mely folyamatok szerepet játszanak a pozitív ösztradiol visszacsatolás közvetítésében és az ovuláció indukciójában.

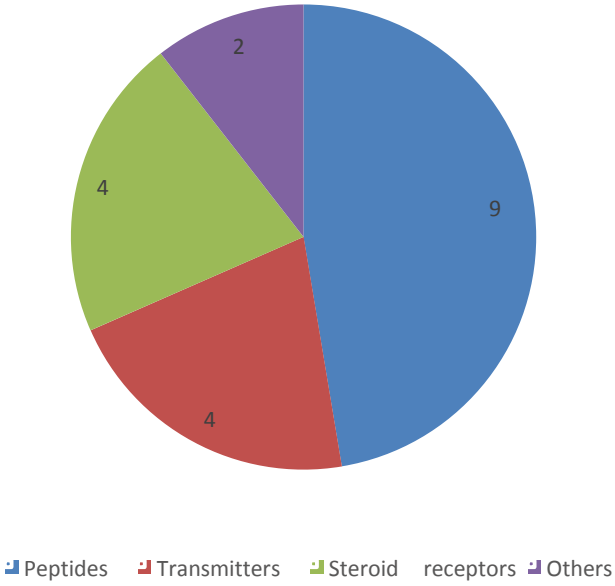
Vizsgálatainkban 6 proestrus és 6 metesrus ovariális cikulus fázisban lévő egér AVPV területét LCM segítségével kimetszettük, majd a mintákban a gén expressziót kvantitatív PCR vizsgálattal tanulmányoztuk. A kimetszett agyterületet, valamint annak néhány jellegzetes peptid és transzmitter sejtcsoportját az **5. ábrán** foglaltuk össze.



5. ábra. Az AVPV elhelyezkedése a bazális előagy területén (A-C). Kisspeptin (D, E), galanin (E) és GAD65-gfp (F) expressziója az AVPV-ben

Az AVPV régió transzkriptóm analízise 93 génre terjedt ki, melyeket irodalmi adatok alapján, továbbá saját tudományos érdeklődésünk mentén választottunk ki. Szignifikáns ($p<0.05$) génexpressziós változásokat észleltünk 19 gén esetében, melyek transzmitter, peptid és szteroid hormon szignalizációs folyamatokat érintenek **6. ábra**.

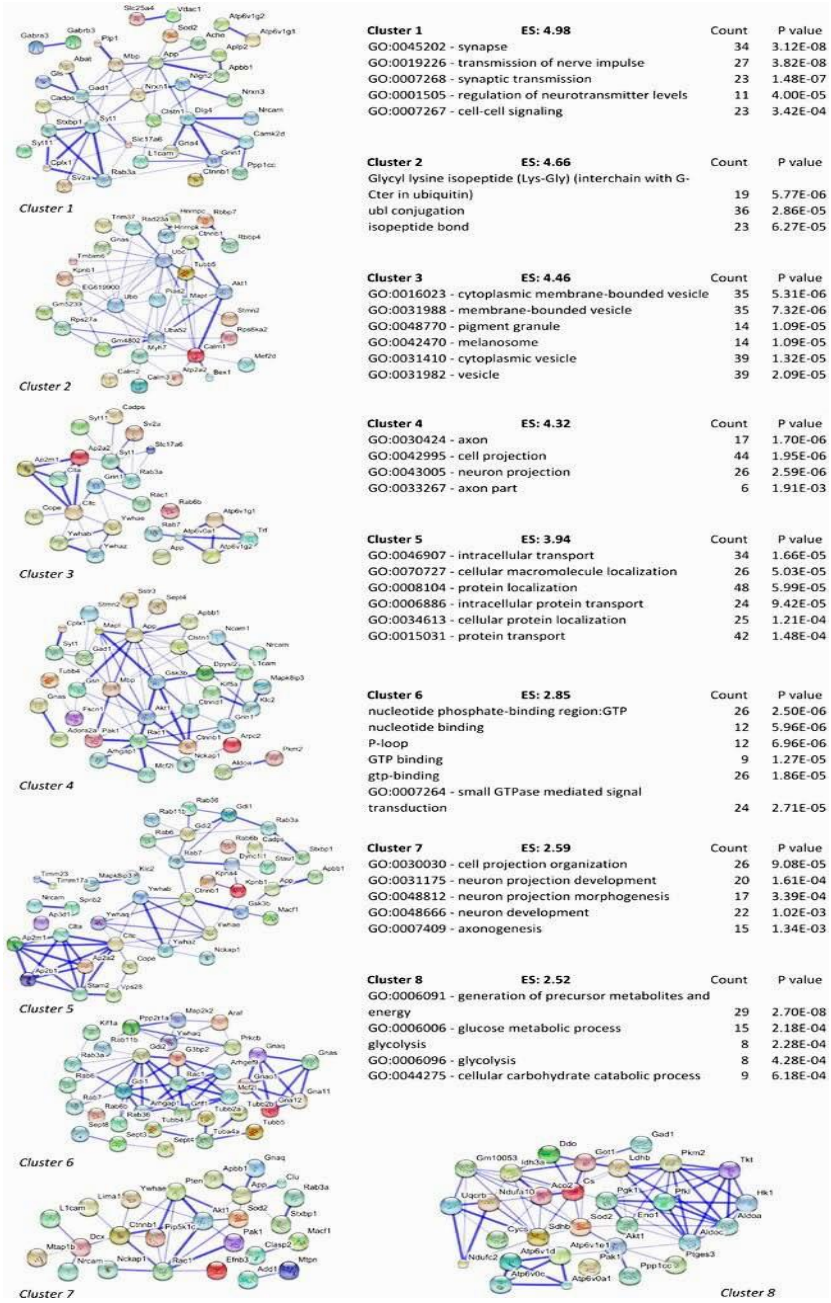
6. ábra. Proestrus által regulált funkcionális géncsoportok az AVPV területén



Reprezentatív példaként kisspeptin (31.37), tirozin hidroxiláz (3.12) és ösztrogén receptor alfa (3.27) mRNS-ek relatív mennyiségének (RQ) változást említjük.

VII. Adatbázist hoztunk létre a hím és nőstény egerek GnRH idegsejtjeinek transzkriptóm jegyeiről, melyek jelentős részét a kutató közösség számára nyilvánossá tettünk. Egyrészt a tudományos eredményeket közlő folyóirat honlapján supplementum anyagként (https://www.karger.com/ProdukteDB/miscArchiv/000/430/818/000430818_sm.html), másrészt az NIH vonatkozó gén adatbázisában, a Gene Expression Omnibus rendszerében (http://www.ncbi.nlm.nih.gov/geo/). Azonosító: GSE66806. Adatok nyilvánosságra kerülésének védelmi dátuma: 2018. 12. 21. Az ivari nem és ösztradiol szint specifikus génexpressziós adatokból prediktív fehérje-fehérje interakcióra utaló elemzéseket végeztünk a String 9.0 program segítségével, melyek alapján a GnRH neuronokban végbemenő szabályozó folyamatok molekuláris mechanizmusaira tudtunk következtetni (**Közlemény 1**).

Az egyes ontológiai funkcionális géncsoportokat és a prediktív fehérje-fehérje interakciókat az alábbi **7. ábra** szemlélteti.



Cluster 1	ES: 4.98	Count	P value
GO:0045202 - synapse		34	3.12E-08
GO:0019226 - transmission of nerve impulse		27	3.82E-08
GO:0007268 - synaptic transmission		23	1.48E-07
GO:0001505 - regulation of neurotransmitter levels		11	4.00E-05
GO:0007267 - cell-cell signaling		23	3.42E-04

Cluster 2	ES: 4.66	Count	P value
Glycyl lysine isopeptide (Lys-Gly) (interchain with G-Cter in ubiquitin)		19	5.77E-06
ubl conjugation		36	2.86E-05
isopeptide bond		23	6.27E-05

Cluster 3	ES: 4.46	Count	P value
GO:0016023 - cytoplasmic membrane-bounded vesicle		35	5.31E-06
GO:0031988 - membrane-bounded vesicle		35	7.32E-06
GO:0048770 - pigment granule		14	1.09E-05
GO:0042470 - melanosome		14	1.09E-05
GO:0031410 - cytoplasmic vesicle		39	1.32E-05
GO:0031982 - vesicle		39	2.09E-05

Cluster 4	ES: 4.32	Count	P value
GO:0030424 - axon		17	1.70E-06
GO:0042995 - cell projection		44	1.95E-06
GO:0043005 - neuron projection		26	2.59E-06
GO:0033267 - axon part		6	1.91E-03

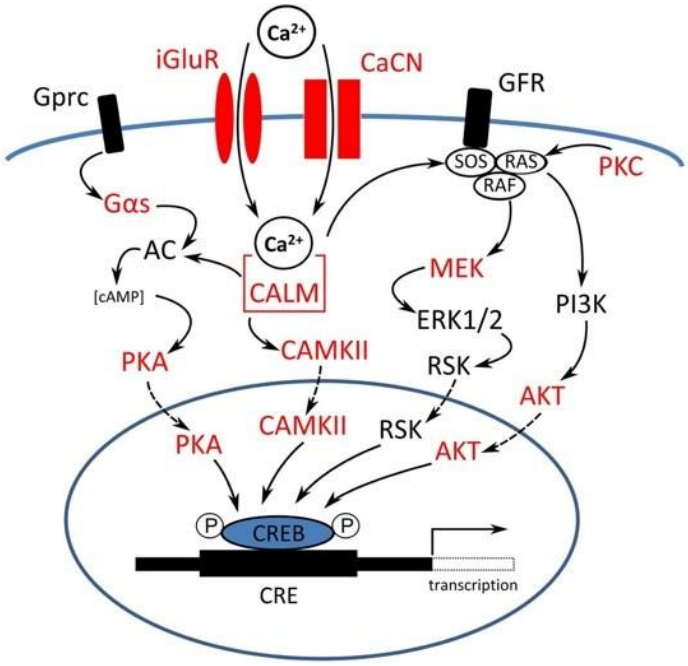
Cluster 5	ES: 3.94	Count	P value
GO:0046907 - intracellular transport		34	1.66E-05
GO:0070727 - cellular macromolecule localization		26	5.03E-05
GO:0008104 - protein localization		48	5.99E-05
GO:0006886 - intracellular protein transport		24	9.42E-05
GO:0034613 - cellular protein localization		25	1.21E-04
GO:0015031 - protein transport		42	1.48E-04

Cluster 6	ES: 2.85	Count	P value
nucleotide phosphate-binding region:GTP		26	2.50E-06
nucleotide binding		12	5.96E-06
P-loop		12	6.96E-06
GTP binding		9	1.27E-05
gtp-binding		26	1.86E-05
GO:0007264 - small GTPase mediated signal transduction		24	2.71E-05

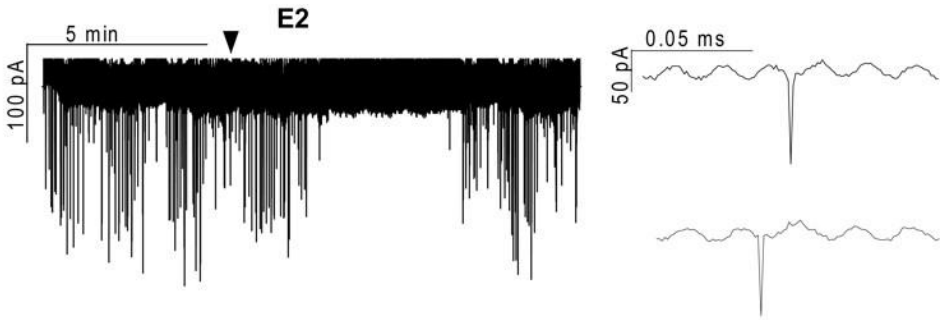
Cluster 7	ES: 2.59	Count	P value
GO:0030030 - cell projection organization		26	9.08E-05
GO:0031175 - neuron projection development		20	1.61E-04
GO:0048812 - neuron projection morphogenesis		17	3.39E-04
GO:0048666 - neuron development		22	1.02E-03
GO:0007409 - axonogenesis		15	1.34E-03

Cluster 8	ES: 2.52	Count	P value
GO:0006091 - generation of precursor metabolites and energy		29	2.70E-08
GO:0006006 - glucose metabolic process		15	2.18E-04
glycolysis		8	2.28E-04
GO:0006096 - glycolysis		8	4.28E-04
GO:0044275 - cellular carbohydrate catabolic process		9	6.18E-04

A szexuális dimorfizmus több szignál átviteli útvonal érintettségét is magába foglalja, amint azt az alábbi **8. ábra** összegezi:

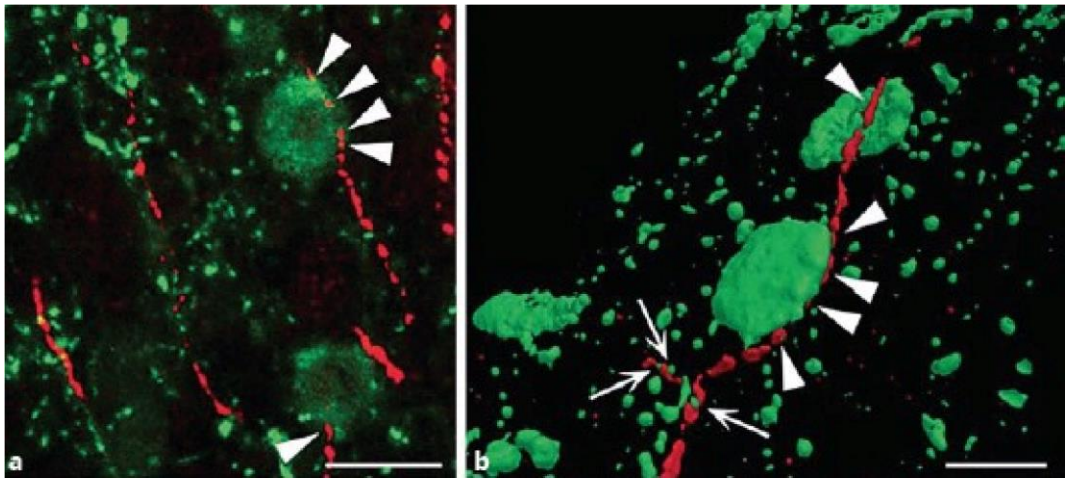


VIII. Bizonyítottuk, hogy metestrus ciklus fázisban az alacsony ösztradiol szint mellett a GnRH idegsejtek tüzelési aktivitása és poszt-szinaptikus áramai gátlódnak (**9. ábra**). Igazoltuk, hogy a szabályozásban a munkacsoportunk által a GnRH idegsejtekben korábban feltárt béta típusú ösztrogén receptor és a GABAerg idegvégződésekre ható retrográd endogén kannabinoid szignalizáció meghatározó szerepet töltenek be (**Közlemény 4**).



9. ábra. *Ösztradiol (10 pM) gátló hatása a GnRH idegsejtek tüzelésére metestrusban*

IX. Tanulmányoztuk a GnRH idegsejtek kisspeptin (KP)-erg beidegzését egér agyban. Igazoltuk a szabályozás szinaptikus voltát és azonosítottuk a KPidegrostok eredő sejtjeit a hypothalamusban (**Közlemény 5**). Bizonyítottuk a neuronális kapcsolat reciprok jellegét (**10. ábra**). (**Közlemény 6**).



10. ábra. *GnRH immunreaktív idegrostok (piros) kapcsolata kisspeptint (zöld) szintetizáló idegsejtekkel az AVPV területén*

II/2. További, kiegészítő eredmények összegzése

A GnRH idegsejtek nem és ösztradiol hormonszint specifikus expressziós eredményeinek birtokában a vizsgálatokat kiterjesztettük agykérgi (frontális kéreg, hippocampus) és agytörzsi (ventrális tegmentális area) régiók vizsgálatára is rágcsálókban, valamint a humán GnRH neuronrendszer vonatkozó szabályozó rendszereinek feltárására.

X. Kimutattuk, hogy ösztrogén hormon szintek és specifikus ösztrogén receptor agonisták hatékonyan szabályozzák a frontális kéreg és a hippocampus transzkriptómját, befolyásolva a neuronális plaszticitás (**2. Táblázat**), neurogenezis és immunmoduláció különféle mechanizmusait és celluláris/molekuláris folyamatait (**Közlemények 2, 7, 8, 9**).

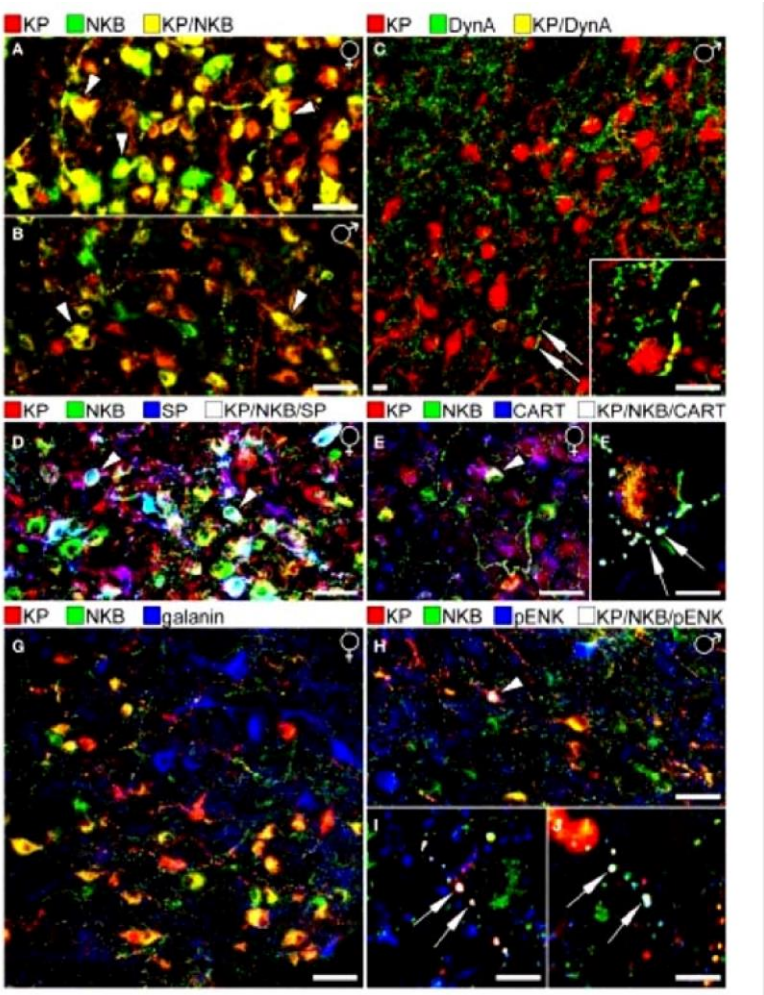
GO ID	GO terms and corresponding genes	Gene #	*P*	*P* _fdr
4724	**Glutamatergic synapse** Gng12, Dlgap1, Cacna1a, Shank2, Gls, Gnb5, Kcnj3, Grm1, Grm8	9	4,68E-04	1,33E-01
4725	**Cholinergic synapse** Chrm4, Gng12, Pik3ca, Fos, Cacna1a, Chrm1, Gnb5, Kcnj3	8	1,51E-03	1,52E-01
3013	**RNA transport** Tpr, Srrm1, Eif3d, Eif4g1, Thoc2, Eif3e, Upf3a, Nupl1, Pabpc1, Ranbp2	10	1,86E-03	1,52E-01

4144	**Endocytosis** Asap1, Epn2, Arfgap1, Folr1, Vps45, Cxcr4, Nedd4, RT1-A1, Sh3gl2, Erbb4, Grk6, Vps4a	12	2,11E-03	1,52E-01
4919	**Thyroid hormone signaling pathway** Hif1a, Pik3ca, Med1, Ncor1, Slc9a1, Crebbp, Pfkfb2, Atp2a2	8	2,87E-03	1,63E-01
3018	**RNA degradation** Dhx36, Cnot6l, Edc4, Pabpc1, Ddx6, Lsm7	6	4,68E-03	1,92E-01
4723	**Retrograde endocannabinoid signaling** Gng12, Cacna1a, Mgll, Mapk10, Gnb5, Kcnj3, Grm1	7	4,69E-03	1,92E-01
3015	**mRNA surveillance pathway** Papolg, Pabpn1, Srrm1, Upf3a, Cpsf3,Pabpc1	6	1,07E-02	3,84E-01

2. Táblázat. *Krónikus ösztrogén receptor béta agonista (DPN) kezelés hatása a hippocampus gén expressziójára és egyes szabályozó útvonalára középkorú, petefészek irtott nőstény patkányban*

XI. Funkcionális fMRI vizsgálatokkal igazoltuk, hogy a ventrális tegmentális területről felszálló és az agykéreg működését szabályozó, priméren dopamin (DA)-erg neuronrendszer működését ösztrogén receptor alfa és béta típusú agonistákkal hatékonyan lehet módosítani (**Közlemény 10**). Igazoltuk továbbá a VTA DA idegsejtjeinek oldalsó hypothalamus területéről érkező orexin tartalmú beidegzését (**Közlemény 11**), valamint feltártuk a VTA hypothalamikus eredetű afferens rendszereit, különös tekintettel a GABA- és glutamáterg fenotípusú idegsejt csoportokra (**Közlemény 12**).

XII. Feltérképeztük a humán GnRH idegsejtek neuronális afferens viszonyait és a sejtekben GnRH-val együtt termelődő hormonok jellegét, valamint a komplex fenotípus nemhez és életkorhoz kötött sajátosságait. Bizonyítottuk a humán GnRH neuronok GABA és glutamáterg rendszerek általi beidegzését (**Közlemény 13**). Feltérképeztük a GnRH neuronrendszer egyik hatékony szabályozó rendszerének a kisspeptint termelő idegsejteknek kémiai arculatát (**Közlemény 14**), kimutatva több hormon ko-szintézisét a sejtekben (**11. ábra**), (**Közlemények 15, 16, 17**). Nemek közötti dimorfizmust írtunk le a basalis hypothalamus területén a KP és neurokinin B immunreaktív rendszerek vonatkozásában (**Közlemény 18)**.



11. ábra. *Humán GnRH idegsejtek komplex kémiai fenotípusának szemléltetése*

III. Az elért *főbb* eredmények (2012-2016) scientometriai összegzése

Közlemények száma	**18**
Összesített impakt érték	**66,287**
Összes idézet száma	**134**
Független idézetek száma	**96**

IV. Az eredményeket megjelenítő közlemények jegyzéke és azok kivonatai

1.Vastagh Cs; Rodolosse A; Solymosi N; Farkas I; Auer H; Sárvári M; Liposits Zs
Differential Gene Expression in Gonadotropin-Releasing Hormone Neurons of Male and Metestrous Female Mice
NEUROENDOCRINOLOGY 102:(1-2) pp. 44-59., 2015
IF: 4.373

Abstract
Background: Gonadotropin-releasing hormone (GnRH) neurons play a pivotal role in the regulation of the hypothalamic-pituitary gonadal axis in a sex-specific manner. We hypothesized that the differences seen in reproductive functions of males and females are associated with a sexually dimorphic gene expression profile of GnRH neurons. Methods and Results: We compared the transcriptome of GnRH neurons obtained from intact metestrous female and male GnRH-green fluorescent protein transgenic mice. About 1,500 individual GnRH neurons from each sex were sampled with laser capture microdissection followed by whole-transcriptome amplification for gene expression profiling. Under stringent selection criteria (fold change >1.6, adjusted p value 0.01), Affymetrix Mouse Genome 430 PM array analysis identified 543 differentially expressed genes. Sexual dimorphism was most apparent in gene clusters associated with synaptic communication, signal transduction, cell adhesion, vesicular transport and cell metabolism. To validate microar- ray results, 57 genes were selected, and 91% of their differential expression was confirmed by real-time PCR. Similarly, 88% of microarray results were confirmed with PCR from independent samples obtained by patch pipette harvesting and pooling of 30 GnRH neurons from each sex. We found significant differences in the expression of genes involved in vesicle priming and docking (Syt1, Cplx1) , GABAergic (Gabra3, Gabrb3, Gabrg2) and glutamatergic (Gria1, Grin1, Slc17a6) neurotransmission, peptide signaling (Sstr3, Npr2, Cxcr4) and the regulation of intracellular ion homeostasis (Cacna1, Cacnb1, Cacng5, Kcnq2, Kcnc1) . Conclusion: The striking sexual dimorphism of the GnRH neuron transcriptome we report here contributes to a better understanding of the differences in cellular mechanisms of GnRH neurons in the two sexes.

2.Sárvári M, Kalló I, Hrabovszky E, Solymosi N, Rodolosse A, Vastagh C, Auer H, Liposits Z
Hippocampal Gene Expression Is Highly Responsive to Estradiol Replacement in Middle-Aged Female Rats.
ENDOCRINOLOGY;156(7):2632-45. 2015
IF: 4.503

Abstract
In the hippocampus, estrogens are powerful modulators of neurotransmission, synaptic plasticity and neurogenesis. In women, menopause is associated with increased risk of memory disturbances, which can be attenuated by timely estrogen therapy. In animal models of menopause, 17β-estradiol (E2) replacement improves hippocampus-dependent spatial memory. Here, we explored the effect of E2 replacement on hippocampal gene expression in a rat menopause model. Middle-aged ovariectomized female rats were treated continuously for 29 days with E2, and then, the hippocampal transcriptome was investigated with Affymetrix expression arrays. Microarray data were analyzed by Bioconductor packages and web-based softwares, and verified with quantitative PCR. At standard fold change selection criterion, 156 genes responded to E2. All alterations but 4 were transcriptional activation. Robust activation (fold change > 10) occurred in the case of transthyretin, klotho, claudin 2, prolactin receptor, ectodin, coagulation factor V, Igf2, Igfbp2, and sodium/sulfate symporter. Classification of the 156 genes revealed major groups, including signaling (35 genes), metabolism (31 genes), extracellular matrix (17 genes), and transcription (16 genes). We selected 33 genes for further studies, and all changes were confirmed by real-time PCR. The results suggest that E2 promotes retinoid, growth factor, homeoprotein, neurohormone, and neurotransmitter signaling, changes metabolism, extracellular matrix composition, and transcription, and induces protective mechanisms via genomic

effects. We propose that these mechanisms contribute to effects of E2 on neurogenesis, neural plasticity, and memory functions. Our findings provide further support for the rationale to develop safe estrogen receptor ligands for the maintenance of cognitive performance in postmenopausal women.

3.Csaba Vastagh, Annie Rodolosse, Norbert Solymosi and Zsolt Liposits,
Altered expression of genes encoding neurotransmitter receptors in GnRH neurons of 1 proestrous mice
NEUROENDOCRINOLOGY, 2016 (submitted)

Abstract
Gonadotropin-releasing hormone (GnRH) neurons play a key role in the central regulation of reproduction. In proestrous female mice, estradiol triggers the pre-ovulatory GnRH surge, however, its impact on the expression of neurotransmitter receptor genes in GnRH neurons has not been explored yet. We hypothesized that proestrus is accompanied by substantial changes in the expression profile of genes coding for neurotransmitter receptors in GnRH neurons. Therefore, we compared the transcriptome of GnRH neurons obtained from intact, proestrous and metestrous female GnRH-GFP transgenic mice, respectively. About 1500 individual GnRH neurons were sampled from both groups and their transcriptome was analyzed using microarray and RT-PCR. Differential gene expression was most apparent in receptor subunits and downstream elements ofthe GABA-ergic (*Gabbr1*, *Gabra3, Gabrb3, Gabrb2, Gabrg2*), glutamatergic (*Gria1, Gria2, Grin1*, *Grin3a*, *Grm1*, *Slc17a6*), cholinergic (*Chrnb2, Chrm3, Chrm4*) and dopaminergic (*Drd3, Drd4*) signaling pathways. The study uncovered significant differences in gene expression of adrenergic (*Adra1b, Adra2a*, *Adra2c*), adenosinergic (*Adora2a*, *Adora2b*), glycinergic (*Glra*), purinergic (*P2rx7*) and serotonergic (*Htr6*) receptors. In concert with these events, expression of G-proteins (*Gnai1*, *Gnai2*, *Gnas*), adenylate-cyclases (*Adcy3, Adcy5*), protein kinase A (*Prkaca, Prkacb*) and protein kinase C (*Prkca*) and certain transporters (*Slc1a4, Slc17a6, Slc6a17*) were also changed. The marked differences found in the expression of genes involved in neurotransmitter signaling of GnRH neurons at pro- and metestrous stages of the ovarian cycle indicate the differential contribution of these neurotransmitter systems to the induction of the preovulatory GnRH surge, the known prerequisite of the subsequent hormonal cascade inducing ovulation.

4.Flóra Bálint; Zsolt Liposits; Imre Farkas
Estrogen Receptor Beta and 2-arachidonoylglycerol Mediate the Suppressive Effects of Estradiol on Frequency of Postsynaptic Currents in Gonadotropin-Releasing Hormone Neurons of Metestrous Mice: An Acute Slice Electrophysiological Study
FRONTIERS IN CELLULAR NEUROSCIENCE, 2016
IF: 4.289

Abstract
Gonadotropin-releasing hormone (GnRH) neurons are controlled by 17b-estradiol (E2) contributing to the steroid feedback regulation of the reproductive axis. In rodents, E2 exerts a negative feedback effect upon GnRH neurons throughout the estrus-diestrus phase of the ovarian cycle. The present study was undertaken to reveal the role of estrogen receptor subtypes in the mediation of the E2 signal and elucidate the downstream molecular machinery of suppression. The effect of E2 administration at low physiological concentration (10 pM) on GnRH neurons in acute brain slices obtained from metestrous GnRH-green fluorescent protein (GFP) mice was studied under paradigms of blocking or activating estrogen receptor subtypes and interfering with retrograde 2-arachidonoylglycerol (2-AG) signaling. Whole-cell patch clamp recordings revealed that E2 significantly diminished the frequency of spontaneous postsynaptic currents (sPSCs) in GnRH neurons (49.62 _ 7.6%) which effect was abolished by application of the estrogen receptor (ER) a/b blocker Faslodex (1 mM). Pretreatment of the brain slices with cannabinoid receptor type 1 (CB1) inverse agonist AM251 (1 mM) and intracellularly applied

endocannabinoid synthesis blocker THL (10 mM) significantly attenuated the effect of E2 on the sPSCs. E2 remained effective in the presence of tetrodotoxin (TTX) indicating a direct action of E2 on GnRH cells. The ERb specific agonist DPN (10 pM) also significantly decreased the frequency of miniature postsynaptic currents (mPSCs) in GnRH neurons. In addition, the suppressive effect of E2 was completely blocked by the selective ERb antagonist PHTPP (1 mM) indicating that ERb is required for the observed rapid effect of the E2. In contrast, the ERa agonist PPT (10 pM) or the membraneassociated G protein-coupled estrogen receptor (GPR30) agonist G1 (10 pM) had no significant effect on the frequency of mPSCs in these neurons. AM251 and tetrahydrolipstatin (THL) significantly abolished the effect of E2 whereas AM251 eliminated the action of DPN on the mPSCs. These data suggest the involvement of the retrograde endocannabinoid mechanism in the rapid direct effect of E2. These results collectively indicate that estrogen receptor beta and 2-AG/CB1 signaling mechanisms are coupled and play an important role in the mediation of the negative estradiol feedback on GnRH neurons in acute slice preparation obtained from intact, metestrous mice.

5.Kalló I, Vida B, Deli L, Molnar CS, Hrabovszky E, Caraty A, Ciofi P, Coen CW, Liposits Z:
Co-localisation of kisspeptin with galanin or neurokinin B in afferents to mouse GnRH neurones, J
NEUROENDOCRINOLOGY 24: (3) 464-476, 2012
IF:4.934

Abstract
The gonadotrophin-releasing hormone (GnRH) secreting neurones, which form the final common pathway for the central regulation of reproduction, are directly targeted by kisspeptin (KP) via the G protein-coupled receptor, GPR54. In these multiple labelling studies, we used ovariectomised mice treated with 17b-oestradiol (OVX + E2) or vehicle (OVX + oil) to determine: (i) the ultrastructural characteristics of KP-immunoreactive (IR) afferents to GnRH neurones; (ii) their galanin or neurokinin B (NKB) content; and (iii) the co-expression of galanin or NKB with KP in the two major subpopulations of KP neurones located in the rostral periventricular area of the third ventricle (RP3V) and the arcuate nucleus (Arc). Electron microscopic investigation of the neuronal juxtapositions revealed axosomatic and axodendritic synapses; these showed symmetrical or asymmetrical characteristics, suggesting a phenotypic diversity of KP afferents. Heterogeneity of afferents was also demonstrated by differential co-expression of neuropeptides; in OVX + E2 mice, KP afferents to GnRH neurones showed galaninimmunoreactivity with an incidence of 22.50 _ 2.41% and NKB-immunoreactivity with an incidence of 5.61 _ 2.57%. In OVX + oil animals, galanin-immunoreactivity in the KP afferents showed a major reduction, appearing in only 5.78 _ 1.57%. Analysis for co-localisation of galanin or NKB with KP was extended to the perikaryal level in animal models, which showed the highest KP incidence; these were OVX + E2 females for the RP3V and OVX + oil females for the ARC. In the RP3V of colchicine- treated OVX + E2 animals, 87.84 _ 2.65% of KP-IR neurones were galanin positive. In the Arc of the colchicine-treated OVX + oil animals, galanin immunoreactivity was detected in only 12.50 _ 1.92% of the KP expressing neurones. By contrast, the incidence of co-localisation with NKB in the Arc of those animals was 98.09 _ 1.30%. In situ hybridisation histochemistry of sections from OVX + E2 animals identified galanin message in more than a third of the KP neurones in the RP3V (38.67 _ 11.57%) and in the Arc (42.50 _ 12.52%). These data suggest that GnRH neurones are innervated by chemically heterogeneous KP cell populations, with a small proportion deriving from the Arc group. The presence of galanin within KP axons innervating GnRH neurones and the oestrogen-dependent regulation of that presence add a new dimension to the roles played by galanin in the central regulation of reproduction. Key words: confocal microscopy, pre-embedding electron microscopy, immunohistochemistry, in situ hybridisation, double- and triple-labelling.

6.Kallo I, Vida B, Bardoczi Z, Szilvasy-Szabo A, Rabi F, Molnar T, Farkas I, Caraty A, Mikkelsen J, Coen CW, Hrabovszky E, Liposits Z
Gonadotropin-Releasing Hormone Neurones Innervate Kisspeptin Neurones in the Female Mouse Brain
NEUROENDOCRINOLOGY 98: (4) 281-289, 2013

IF: 3.537

Abstract
Kisspeptin (KP) neurones in the rostral periventricular area of the third ventricle (RP3V) and arcuate nucleus (Arc) are important elements in the neuronal circuitry regulating gonadotropin- releasing hormone (GnRH) secretion. KP and cosynthesised neuropeptides/neurotransmitters act directly on GnRH perikarya and processes. GnRH neurones not only form the final output pathway regulating the reproductive functions of the anterior pituitary gland, but also provide neuronal input to sites within the hypothalamus. The current double-label immunohistochemical studies investigated whether GnRH-immunoreactive (IR) projections to the RP3V and/or Arc establish morphological connections with KP-IR neurones at these sites. To optimise visualisation of KP immunoreactivity in, respectively, the RP3V and Arc, ovariectomised (OVX) oestrogen-treated and OVX oil-treated female mice were studied. Confocal laser microscopic analysis of im- munofluorescent specimens revealed GnRH-IR axon varicosities in apposition to approximately 25% of the KP-IR neurones in the RP3V and 50% of the KP-IR neurones in the Arc. At the ultrastructural level, GnRH-IR neurones were seen to establish asymmetric synaptic contacts, which usually reflect excitatory neurotransmission, with KP-IR neurones in both the RP3V and Arc. Together with previous data, these findings indicate reciprocal connectivity between both of the KP cell populations and the GnRH neuronal system. The functional significance of the GnRH-IR input to the two separate KP cell populations requires electrophysiological investigation.

7.Sarvari M, Hrabovszky E, Kallo I, Solymosi N, Liko I, Berchtold N, Cotman C, Liposits Z
Menopause leads to elevated expression of macrophage-associated genes in the aging frontal cortex: rat and human studies identify strikingly similar changes
J NEUROINFLAMM 9: (1) 264, 2012
IF: 4.351

Abstract
Background: The intricate interactions between the immune, endocrine and central nervous systems shape the innate immune response of the brain. We have previously shown that estradiol suppresses expression of immune genes in the frontal cortex of middle-aged ovariectomized rats, but not in young ones reflecting elevated expression of these genes in middle-aged, ovarian hormone deficient animals. Here, we explored the impact of menopause on the microglia phenotype capitalizing on the differential expression of macrophage-associated genes in quiescent and activated microglia. Methods: We selected twenty-three genes encoding phagocytic and recognition receptors expressed primarily in microglia, and eleven proinflammatory genes and followed their expression in the rat frontal cortex by real-time PCR. We used young, middle-aged and middle-aged ovariectomized rats to reveal age- and ovariectomyrelated alterations. We analyzed the expression of the same set of genes in the postcentral and superior frontal gyrus of pre- and postmenopausal women using raw microarray data from our previous study. Results: Ovariectomy caused up-regulation of four classic microglia reactivity marker genes including Cd11b, Cd18, Cd45 and Cd86. The change was reversible since estradiol attenuated transcriptional activation of the four marker genes. Expression of genes encoding phagocytic and toll-like receptors such as Cd11b, Cd18, C3, Cd32, Msr2 and Tlr4 increased, whereas scavenger receptor Cd36 decreased following ovariectomy. Ovarian hormone deprivation altered the expression of major components of estrogen and neuronal inhibitory signaling which are involved in the control of microglia reactivity. Strikingly similar changes took place in the postcentral and superior frontal gyrus of postmenopausal women. Conclusions: Based on the overlapping results of rat and human studies we propose that the microglia phenotype shifts from the resting toward the reactive state which can be characterized by upregulation of CD11b, CD14, CD18, CD45, CD74, CD86, TLR4, down-regulation of CD36 and unchanged CD40 expression. As a result of this shift, microglial cells have lower threshold for subsequent activation in the forebrain of postmenopausal women.

8.Sarvari M, Kallo I, Hrabovszky E, Solymosi N, Liposits
Ovariectomy and subsequent treatment with estrogen receptor agonists tune the innate immune system of the hippocampus in middle-aged female rats

PLOS ONE 9: (2) e 88540, 2014
IF: 3.53

Abstract
The innate immune system including microglia has a major contribution to maintenance of the physiological functions of the hippocampus by permanent monitoring of the neural milieu and elimination of tissue-damaging threats. The hippocampus is vulnerable to age-related changes ranging from gene expression to network connectivity. The risk of hippocampal deterioration increases with the decline of gonadal hormone supply. To explore the impact of hormone milieu on the function of the innate immune system in middle-aged female rats, we compared mRNA expression in the hippocampus after gonadal hormone withdrawal, with or without subsequent estrogen replacement using estradiol and isotype-selective estrogen receptor (ER) agonists. Targeted profiling assessed the status of the innate immune system (macrophage-associated receptors, complement, inhibitory neuronal ligands), local estradiol synthesis (P450 aromatase) and estrogen reception (ER). Results established upregulation of macrophage-associated (Cd45, Iba1, Cd68, Cd11b, Cd18, Fcgr1a, Fcgr2b) and complement (C3, factor B, properdin) genes in response to ovariectomy. Ovariectomy upregulated Cd22 and downregulated semaphorin3A (Sema3a) expression, indicating altered neuronal regulation of microglia. Ovariectomy also led to downregulation of aromatase and upregulation of ERa gene. Of note, analogous changes were observed in the hippocampus of postmenopausal women. In ovariectomized rats, estradiol replacement attenuated Iba1, Cd11b, Fcgr1a, C3, increased mannose receptor Mrc1, Cd163 and reversed Sema3a expression. In contrast, reduced expression of aromatase was not reversed by estradiol. While the effects of ERa agonist closely resembled those of estradiol, ERb agonist was also capable of attenuating the expression of several macrophage-associated and complement genes. These data together indicate that the innate immune system of the aging hippocampus is highly responsive to the gonadal hormone milieu. In ovariectomized female rats, estradiol replacement exerts potent immunomodulatory effects including attenuation of microglia sensitization, initiation of M2-like activation and modulation of complement expression by targeting hippocampal neurons and glial cells through ERa and ERb.

9.Miklós Sárvári, Imre Kalló, Erik Hrabovszky, Norbert Solymosi, Annie Rodolosse and Zsolt Liposits
Long-Term Estrogen Receptor Beta Agonist Treatment Modifies the Hippocampal Transcriptome in Middle-Aged Ovariectomized Rats
FRONTIERS IN CELLULAR NEUROSCIENCE, 2016 (submitted)

Abstract
Estradiol (E2) robustly activates transcription of a broad array of genes in the hippocampal formation of middle-aged ovariectomized rats via estrogen receptors (ERα, ERβ and GPER). Selective ERβ agonists also influence hippocampal functions, although their downstream molecular targets and mechanisms are not known. In this study, we explored the effects of long- term treatment with ERβ agonist diarylpropionitrile (DPN, 0.05 mg/kg/day, sc.) on the hippocampal transcriptome in ovariectomized, middle-aged (13 month) rats. Isolated hippocampal formations were analyzed by Affymetrix oligonucleotide microarray and quantitative real-time PCR. Four hundred ninety-seven genes fulfilled the absolute fold change higher than 2 (FC>2) selection criterion. Among them 370 genes were activated. Pathway analysis identified terms including glutamatergic and cholinergic synapse, RNA transport, endocytosis, thyroid hormone signaling, RNA degradation, retrograde endocannabinoidsignaling and mRNA surveillance. PCR studies showed transcriptional regulation of 58 genes encoding growth factors (Igf2, Igfb2, Igf1r, Fgf1, Mdk, Ntf3, Bdnf), transcription factors (Otx2, Msx1), potassium channels (Kcne2), neuropeptides (Cck, Pdyn), peptide receptors (Crhr2, Oprm1, Gnrhr, Galr2, Sstr1, Sstr3), neurotransmitter receptors (Htr1a, Htr2c, Htr2a, Gria2, Gria 3, Grm5, Gabra1, Chrm5, Adrb1) and vesicular neurotransmitter transporters (Slc32a1, Slc17a7). Proteinprotein interaction analysis revealed networking of clusters associated with the regulation of growth/troph factor signaling, transcription, translation, neurotransmitter and neurohormone signaling mechanisms and potassium channels. Collectively, the results reveal the contribution of ERβ-mediated processes to the regulation of transcription, translation, neurogenesis, neuromodulation and

neuroprotection in the hippocampal formation of ovariectomized, middle-aged rats and elucidate regulatory channels responsible for DPN- altered functional patterns. These findings support the notion that selective activation of ERβ may be a viable approach for treating the neural symptoms of E2 deficiency in menopause.

10.Sárvári Miklós, Deli Levente, Kocsis Pál, Márk László, Maász Gábor, Hrabovszky Erik, Kalló Imre, Gajári Dávid, Vastagh Csaba, Sümegi Balázs, Tihanyi Károly, Liposits Zsolt
Estradiol and isotype-selective estrogen receptor agonists modulate the mesocortical dopaminergic system in gonadectomized female rats
BRAIN RESEARCH 1583: 1-11, 2014
IF: 2.843

Abstract
The mesocortical dopaminergic pathway projecting from the ventral tegmental area (VTA) to the prefrontal cortex (PFC) contributes to the processing of reward signals. This pathway is regulated by gonadal steroids including estradiol. To address the putative role of estradiol and isotype-selective estrogen receptor (ER) agonists in the regulation of the rodent mesocortical system, we combined fMRI, HPLC–MS and qRT-PCR techniques. In fMRI experiments adult, chronically ovariectomized rats, treated with either vehicle, estradiol, ERα agonist 16α-lactone-estradiol (LE2) or ERβ agonist diarylpropionitrile (DPN), received a single dose of D-amphetamine-sulphate (10 mg/kg, i.p.) and BOLD responses were monitored in the VTA and the PFC. Ovariectomized rats showed no significant response to amphetamine. In contrast, the VTA of ER agonist-substituted ovariectomized rats showed robust amphetamine-evoked BOLD increases. The PFC of estradiol-replaced animals was also responsive to amphetamine. Mass spectroscopic analysis of dopamine and its metabolites revealed a two-fold increase in both dopamine and 3,4-dihydroxyphe- nylacetic acid content of the PFC in estradiol-replaced animals compared to ovariecto- mized controls. qRT-PCR studies revealed upregulation of dopamine transporter and dopamine receptor in the VTA and PFC, respectively, of ER agonist-treated ovariectomized animals. Collectively, the results indicate that E2 and isotype-selective ER agonists can powerfully modulate the responsiveness of the mesocortical dopaminergic system, increase the expression of key genes related to dopaminergic neurotransmission and augment the dopamine content of the PFC. In a broader sense, the findings support the concept that the manifestation of reward signals in the PFC is dependent on the actual estrogen milieu of the brain.

11.Hrabovszky E, Molnar CS, Borsay BA, Gergely P, Herczeg L, Liposits Z
Orexinergic input to dopaminergic neurons of the human ventral tegmental area
PLOS ONE 8: (12) , 2013
IF: 3.53

Abstract
The mesolimbic reward pathway arising from dopaminergic (DA) neurons of the ventral tegmental area (VTA) has been strongly implicated in reward processing and drug abuse. In rodents, behaviors associated with this projection are profoundly influenced by an orexinergic input from the lateral hypothalamus to the VTA. Because the existence and significance of an analogous orexigenic regulatory mechanism acting in the human VTA have been elusive, here we addressed the possibility that orexinergic neurons provide direct input to DA neurons of the human VTA. Dual-label immunohistochemistry was used and orexinergic projections to the VTA and to DA neurons of the neighboring substantia nigra (SN) were analyzed comparatively in adult male humans and rats. Orexin B-immunoreactive (IR) axons apposed to tyrosine hydroxylase (TH)-IR DA and to non-DA neurons were scarce in the VTA and SN of both species. In the VTA, 15.062.8% of TH-IR perikarya in humans and 3.260.3% in rats received orexin B-IR afferent contacts. On average, 0.2460.05 and 0.0560.005 orexinergic appositions per TH-IR perikaryon were detected in humans and rats, respectively. The majority (86–88%) of randomly encountered orexinergic contacts targeted the dendritic compartment of DA neurons. Finally, DA neurons of the SN also received orexinergic innervation in both species. Based

on the observation of five times heavier orexinergic input to TH-IR neurons of the human, compared with the rat, VTA, we propose that orexinergic mechanism acting in the VTA may play just as important roles in reward processing and drug abuse in humans, as already established well in rodents.

12.Imre Kalló, Csilla S. Molnár, Sarolta Szöke, Csaba Fekete, Erik Hrabovszky and Zsolt Liposits
Area-specific analysis of the distribution of hypothalamic neurons projecting to the rat ventral tegmental area, with special reference to the GABAergic and glutamatergic efferents. IF: 3,544

Abstract
The ventral tegmental area (VTA) is a main regulator of reward and integrates a wide scale of hormonal and neuronal information. Feeding-, energy expenditure-, stress, adaptation- and reproduction-related hypothalamic signals are processed in the VTA and influence the reward processes. However, the neuroanatomical origin and chemical phenotype of neurons mediating these signals to the VTA have not been fully characterized. In this study we have systematically mapped hypothalamic neurons that project to the VTA using the retrograde tracer Choleratoxin B subunit (CTB) and analyzed their putative gamma-aminobutyric acid (GABA) and/or glutamate character with in situ hybridization in male rats. 23.93 ± 3.91% of hypothalamic neurons projecting to the VTA was found in preoptic and 76.27 ± 4.88% in anterior, tuberal and mammillary hypothalamic regions. Nearly half of the retrogradely-labeled neurons in the preoptic, and more than one third in the anterior, tuberal and mammillary hypothalamus appeared in medially located regions. The analyses of vesicular glutamate transporter 2 (VGLUT2) and glutamate decarboxylase 65 (GAD65) mRNA expression revealed both amino acid markers in different subsets of retrogradely-labeled hypothalamic neurons, typically with the predominance of the glutamatergic marker VGLUT2. About one tenth of CTB-IR neurons were GAD65-positive even in hypothalamic nuclei expressing primarily VGLUT2. Some regions were populated mostly by GAD65 mRNA-containing retrogradely-labeled neurons. These included the perifornical part of the lateral hypothalamus where 58.63 ± 19.04% of CTB-IR neurons were GABAergic. These results indicate that both the medial and lateral nuclear compartments of the hypothalamus provide substantial input to the VTA. Furthermore, colocalization studies revealed that these projections not only use glutamate but also GABA for neurotransmission. These GABAergic afferents may underlie important inhibitory mechanism to fine-tune the reward value of specific signals in the VTA.

13.Hrabovszky E, Molnar CS, Nagy R, Vida B, Borsay BA, Racz K, Herczeg L, Watanabe M, Kallo I, Liposits Z
Glutamatergic and GABAergic Innervation of Human Gonadotropin-Releasing Hormone-I Neurons
ENDOCRINOLOGY 153: (6) 2766-2776, 2012
IF: 4.717

Abstract
Amino acid (aa) neurotransmitters in synaptic afferents to hypothalamic GnRH-I neurons are criticallyinvolved in the neuroendocrine control of reproduction. Although in rodents the major a neurotransmitter in these afferents is _-aminobutyric acid (GABA), glutamatergic axons also innervate GnRH neurons directly. Our aim with the present study was to address the relative contribution of GABAergic and glutamatergic axons to the afferent control of human GnRH neurons. Formalin-fixed hypothalamic samples were obtained from adult male individuals (n _ 8) at autopsies, and their coronal sections processed for dual-label immunohistochemical studies. GABAergic axons were labeled with vesicular inhibitory aa transporter antibodies, whereas glutamatergic axons were detected with antisera against the major vesicular glutamate transporter (VGLUT) isoforms, VGLUT1 and VGLUT2. The relative incidences of GABAergic and glutamatergic axonal appositions to GnRH-immunoreactive neurons were compared quantitatively in two regions, the infundibular and paraventricular nuclei. Results showed that GABAergic axons established the most frequently encountered type of axo-somatic apposition. Glutamatergic contacts occurred in significantly lower numbers, with similar contributions by their VGLUT1 and VGLUT2 subclasses. The innervation pattern was different on GnRH dendrites where the combined incidence of glutamatergic (VGLUT1 _ VGLUT2) contacts slightly exceeded that

of the GABAergic appositions. We conclude that GABA represents the major aa neurotransmitter in axo-somatic afferents to human GnRH neurons, whereas glutamatergic inputs occur somewhat more frequently than GABAergic inputs on GnRH dendrites. Unlike in rats, the GnRH system of the human receives innervation from the VGLUT1, in addition to the VGLUT2, subclass of glutamatergic neurons.

14.Hrabovszky E, Sipos MT, Molnar CS, Ciofi P, Borsay BA, Gergely P, Herczeg L, Bloom SR, Ghatei MA, Dhillo WS, Liposits Z
Low Degree of Overlap Between Kisspeptin, Neurokinin B, and Dynorphin Immunoreactivities in the Infundibular Nucleus of Young Male Human Subjects Challenges the KNDy Neuron Concept
ENDOCRINOLOGY 153: (10) 4978-4989, 2012
IF: 4.717

Abstract
Previous immunohistochemical and *in situ* hybridization studies of sheep, goats, and rodents indicated that kisspeptin (KP), neurokinin B (NKB), and dynorphin A (DYN) are extensively colocalized in the hypothalamic arcuate nucleus, thus providing a basis for the KP/NKB/DYN (KNDy) neuron concept; in both sexes, KNDy neuropeptides have been implicated in the generation of GnRH neurosecretory pulses and in the negative feedback effects of sexual steroids to the reproductive axis. To test the validity and limitations of the KNDy neuron concept in the human, we carried out the comparative immunohistochemical analysis of the three neuropeptides in the infundibular nucleus (Inf; also known as arcuate nucleus) and stalk of young male human individuals (_37 yr). Results of quantitative immunohistochemical experiments established that the regional densities of NKB immunoreactive (IR) perikarya and fibers, and the incidence of afferent contacts they formed onto GnRH neurons, were about 5 times as high as those of the KP-IR elements. Dual-immunofluorescent studies confirmed that considerable subsets of the NKB-IR and KP-IR cell bodies and fibers are separate, and only about33%of NKB-IR perikarya and75%of KP-IR perikarya were dual labeled. Furthermore, very few DYN-IR cell bodies could be visualized in the Inf. DYN-IR fibers were also rare and, with few exceptions, distinct from the KP-IR fibers. The abundance and colocalization patterns of the three immunoreactivities showed similar trends in the infundibular stalk around portal blood vessels. Together these results indicate that most NKB neurons in the Inf do not synthesize detectable amounts of KP and DYN in young male human individuals. These data call for a critical use of the KNDy neuron terminology when referring to the putative pulse generator system of the mediobasal hypothalamus. We conclude that the functional importance of these three neuropeptides in reproductive regulation considerably varies among species, between sexes, and at different ages.

15.Hrabovszky E, Borsay BA, Racz K, Herczeg L, Ciofi P, Bloom SR, Ghatei MA, Dhillo WS, Liposits Z
Substance p immunoreactivity exhibits frequent colocalization with kisspeptin and neurokinin B in the human infundibular region PLOS ONE 8: (8) e72369, 2013
IF:4.934

Abstract
Neurons synthesizing neurokinin B (NKB) and kisspeptin (KP) in the hypothalamic arcuate nucleus represent important upstream regulators of pulsatile gonadotropin-releasing hormone (GnRH) neurosecretion. In search of neuropeptides co-expressed in analogous neurons of the human infundibular nucleus (Inf), we have carried out immunohistochemical studies of the tachykinin peptide Substance P (SP) in autopsy samples from men (21-78 years) and postmenopausal (53-83 years) women. Significantly higher numbers of SP-immunoreactive (IR) neurons and darker labeling were observed in the Inf of postmenopausal women than in age-matched men. Tripleimmunofluorescent studies localized SP immunoreactivity to considerable subsets of KP-IR and NKB-IR axons and perikarya in the infundibular region. In postmenopausal women, 25.1% of NKB-IR and 30.6% of KP-IR perikarya contained SP and 16.5% of all immunolabeled cell bodies were triple-labeled. Triple-, double- and single-labeled SPIR axons innervated densely the portal capillaries of the infundibular stalk. In

quadruple-labeled sections, these axons formed occasional contacts with GnRH-IR axons. Presence of SP in NKB and KP neurons increases the functional complexity of the putative pulse generator network. First, it is possible that SP modulates the effects of KP and NKB in axo-somatic and axo-dendritic afferents to GnRH neurons. Intrinsic SP may also affect the activity and/or neuropeptide release of NKB and KP neurons via autocrine/paracrine actions. In the infundibular stalk, SP may influence the KP and NKB secretory output via additional autocrine/paracrine mechanisms or regulate GnRH neurosecretion directly. Finally, possible co-release of SP with KP and NKB into the portal circulation could underlie further actions on adenohypophysial gonadotrophs.

16.Skrapits K, Borsay BA, Herczeg L, Ciofi P, Bloom SR, Ghatei MA, Dhillo WS, Liposits Z, Hrabovszky E
Colocalization of cocaine- and amphetamine-regulated transcript with kisspeptin and neurokinin B in the human infundibular region PLOS ONE 9: (8) e103977, 2014
IF: 3.234

Abstract
Kisspeptin (KP)- and neurokinin B (NKB)- synthesizing neurons of the hypothalamic arcuate nucleus play a pivotal role in the regulation of pulsatile gonadotropin-releasing hormone (GnRH) secretion. Unlike in rodents and sheep, the homologous KP and NKB neurons in the human infundibular region rarely express dynorphin- but often exhibit Substance P (SP) immunoreactivity, indicating remarkable species differences in the neurochemical phenotype of these neurons. In search for additional neuropeptides in human KP and NKB neurons, we carried out immunofluorescent studies on hypothalamic sections obtained from five postmenopausal women. Colocalization experiments provided evidence for the presence of cocaine- and amphetamine-regulated transcript (CART) in 47.966 % of KP-immunoreactive (IR) and 30.064.9% of NKB-IR perikarya and in 17.062.3% of KP-IR and 6.262.0% of NKB-IR axon varicosities. All three neuropeptides were present in 33.364.9% of KP-IR and 28.264.6% of NKB-IR somata, respectively, whereas triple-labeling showed lower incidences in KP-IR (14.361.8%) and NKB-IR (5.962.0%) axon varicosities. CART-IR KP and NKB neurons established contacts with other peptidergic cells, including GnRH-IR neurons and also sent projections to the infundibular stalk. KP and NKB fibers with CART often contained SP as well, while being distinct from CART fibers co-containing the orexigenic peptide agouti-related protein. Presence of CART in human, but not rodent, KP and NKB neurons represents a new example of species differences in the neuropeptide repertoire of mediobasal hypothalamic KP and NKB neurons. Target cells, receptor sites and physiological significance of CART in the efferent communication of KP and NKB neurons in primates require clarification.

17. Skrapits K, Borsay BA, Herczeg L, Ciofi P, Liposits Z, Hrabovszky E
Neuropeptide co-expression in hypothalamic kisspeptin neurons of laboratory animals and the human
FRONTIERS IN NEUROSCIENCE 9: 29, 2015
IF: 3.656

Abstract
Hypothalamic peptidergic neurons using kisspeptin (KP) and its co-transmitters for communication are critically involved in the regulation of mammalian reproduction and puberty. This article provides an overview of neuropeptides present in KP neurons, with a focus on the human species. Immunohistochemical studies reveal that large subsets of human KP neurons synthesize neurokinin B, as also shown in laboratory animals. In contrast, dynorphin described in KP neurons of rodents and sheep is found rarely in KP cells of human males and postmenopausal females. Similarly, galanin is detectable in mouse, but not human, KP cells, whereas substance P, cocaine- and amphetamineregulated transcript and proenkephalin-derived opioids are expressed in varying subsets of KP neurons in humans, but not reported in ARC of other species. Human KP neurons do not contain neurotensin, cholecystokinin, proopiomelanocortin-derivatives, agouti-related protein, neuropeptide Y, somatostatin or tyrosine hydroxylase (dopamine). These data identify the possible co-transmitters of human KP cells.

Neurochemical properties distinct from those of laboratory species indicate that humans use considerably different neurotransmitter mechanisms to regulate fertility.

18.Molnar CS, Vida B, Sipos MT, Ciofi P, Borsay BA, Racz K, Herczeg L, Bloom SR, Ghatei MA, Dhillo WS, Liposits Z, Hrabovszky E
Morphological Evidence for Enhanced Kisspeptin and Neurokinin B Signaling in the Infundibular Nucleus of the Aging Man
ENDOCRINOLOGY 153: (11) 5428-5439, 2012
IF:4.717

Abstract
Peptidergic neurons synthesizing kisspeptin (KP) and neurokinin B (NKB) in the hypothalamic infundibular nucleus have been implicated in negative sex steroid feedback to GnRH neurons. In laboratory rodents, testosterone decreases KP and NKB expression in this region. In the present study, we addressed the hypothesis that the weakening of this inhibitory testosterone feedback in elderly men coincides with enhanced KP and NKB signaling in the infundibular nucleus. This central hypothesis was tested in a series of immunohistochemical studies on hypothalamic sections of male human individuals that were divided into arbitrary "young" (21–49 yr, n_11) and "aged" (50–67 yr, n _ 9) groups. Quantitative immunohistochemical experiments established that the regional densities of NKBimmunoreactive (IR) perikarya and fibers, and the incidence of afferent contacts they formed onto GnRH neurons, exceeded several times those of the KP-IR elements. Robust aging-dependent enhancements were identified in the regional densities of KP-IR perikarya and fibers and the incidence of afferent contacts they established onto GnRH neurons. The abundance of NKB-IR perikarya, fibers, and axonal appositions to GnRH neurons also increased with age, albeit to lower extents. In dualimmunofluorescent studies, the incidence of KP-IR NKB perikarya increased from 36% in young to 68% in aged men. Collectively, these immunohistochemical data suggest an aging-related robust enhancement in central KP signaling and a moderate enhancement in central NKB signaling. These changes are compatible with a reduced testosterone negative feedback to KP and NKB neurons. The heavier KP and NKB inputs to GnRH neurons in aged, compared with young, men may play a role in the enhanced central stimulation of the reproductive axis. It requires clarification to what extent the enhanced KP and NKB signaling upstream from GnRH neurons is an adaptive response to hypogonadism or, alternatively, a consequence of a decline.