Complex Biphasic Changes of Neuropeptide Concentrations in the Rat Limbic System During Pregnancy and Parturition

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Abstract: Sex hormones including estrogens affect brain areas involved in mood and cognition in addition to directly controlling reproduction and reproductive behavior. We studied the effect of pregnancy and puerperium on the concentrations of cholecystokinin (CCK), neuropeptide Y (NPY), substance P (SP) and galanin in tissue extracts from the rat striatum, frontal cortex and the hippocampal formation by means of radioimmunoassay. The most profound effects were found in the frontal cortex. Thus, cholecystokinin-like immunoreactivity (CCK-LI) was increased by 40 % during late pregnancy ($p<0.01$) compared to estrous whereas SP-LI and galanin-LI decreased 25 % and 10 %, respectively. Postpartum, CCK-LI decreased by 26% compared to pregnancy ($p<0.05$) whereas SP-LI and galanin-LI were increased to a level above estrous (SP, $P<0.01$; galanin, $P<0.05$). No significant effect was observed in NPY-LI in this area. In the striatum during late pregnancy the concentrations of cholecystokinin-LI increased by 29 % ($p<0.05$), NPY-LI by 22% ($p<0.05$) whereas SP-LI slightly increased (not significant). Postpartum, cholecystokinin-LI decreased by 25 % ($p<0.01$) compared to pregnancy and NPY by 16 % ($p<0.01$). SP continued to increase postpartum by 33 % ($p<0.05$) whereas no effect was observed on galanin-LI concentration. Surprisingly, we did not observe any changes in any peptide or groups measured in the hippocampal formation. The complex hormonal adjustments occurring during pregnancy and in the puerperium induce profound changes in the concentrations of several neuropeptides in regions of the rat brain involved in the control of mood and motor control.

Keywords: Rat, brain, neuropeptides, estradiol, postpartum depression.

INTRODUCTION

Gonadal steroids exert pronounced effects on brain areas involved in mood and cognition in addition to directly controlling reproductive behavior and control of reproductive functions [1-3]. During maternity, overall neuroendocrine activity is different from activity in females during other periods of the reproductive cycle. It has been shown that differences in sex steroid exposure are related to changes in neuropeptide levels in the extra-hypothalamic areas of the rat brain that are involved in the control of mood [4-9] . Neuropeptides co-exist with classical neurotransmitters [10] and are found to play important roles in modulating brain function [11].

Cholecystokinin, Galanin, Substance P and Neuropeptide Y

Cholecystokinin (CCK) [12-16] is widely distributed in the brain with the highest concentrations in cortical regions and one of several peptides implicated in behavioral and physiological function and also in mood disorders [17-19]. It has been suggested that there is a CCK projection from cortex to striatum [14] and that endogenous release of CCK influences motor behavior [18]. CCK has been found to be influenced by estradiol in both frontal cortex and hippocampal formation [4,6,20].

The neuropeptide Galanin is present in noradrenergic afferents from the locus coeruleus with widespread projections in the brain including the hippocampal formation and cortex [21] and is implicated in the regulation of mood [22]. Galanin is also a peptide influenced by both short- and long-term exposure to 17β-estradiol. Thus, administration of 17β-estradiol to ovary ectomized rats increases the concentration of Galanin-like immunoreactivity (LI) in the hippocampal formation and frontal cortex [8]. In addition, the tissue concentration of Galanin-LI during pro-estrous is higher in female cycling rats than during the diestrous and estrous phases [9].

Substance P (SP) [23] is another peptide suggested as playing a role in mediating behavioral and physiological function and in being involved in the regulation of mood in rodents. Thus, stressful stimuli and major depression have been found to induce increased levels/release of SP [23,24]. Indeed, SP antagonists have emerged as a novel type of drug with antidepressant efficacy that has also been shown to be promising in clinical trails [25].

The neuropeptideY (NPY) [26] is very abundant in the rodent brain [27-29] and induces potent anxiolytic effects in

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both rats [30] and mice [31] when centrally administered. This peptide has been shown to be implicated in depressive-like behavior - evidence that comes from - amongst others [32,33] - the findings of differential NPY expression in a genetic animal model of depression [34].

On the basis of the assumption that gonadal steroid-sensitive neuropeptides might be involved in behavioral changes and mood-related behavior, the objective in the present work was to study the effects of pregnancy and parturition on the concentration of CCK, NPY, Galanin and SP-like immunoreactivity (LI). These four selected peptides were studied in the frontal cortex, hippocampal formation and striatum in near-term pregnant as well as puerperal rats and in a control group at estrous by means of radioimmunoassay (RIA).

MATERIALS AND METHODS

Animals and Test Procedure

The experiments were performed on 36 female Sprague Dawley rats (BK Universal, Uppsala, Sweden), all animals on the average 4 months of age at the beginning of the experiment. The experimental group with pregnant rats consisted of 22 rats, 8 days pregnant on arrival and the control group of 14 non-pregnant rats. They were all housed 2-4 in each cage at a constant room temperature (21 + 1 °C), with free access to water and standard rat chow, and with 12h light/dark cycles. The study was approved by the local animal research ethics committee and found to be in accordance with the guidelines issued by the Central Committee for Animal Research in Sweden.

The animals in group 1 (n = 10), (pregnant), were sacrificed on day 17 – 18 of pregnancy (verified by autopsy). The animals in group 2 (n = 12), (puerperal), were sacrificed 2 days after delivery. The control animals in group 3 (n = 14), (estrous), showed 4- or 5-day regular estrous cycles on the basis of examination of vaginal smear on a daily basis, on the same time schedule and checked for two cycles. The smear was examined by means of light-microscopy to establish the phase and the animals were sacrificed at estrous.

Blood-samples for the analysis of estradiol were taken from the femoral vein under general anesthesia (isoflurane) just before sacrifice. All rats were decapitated using a guillotine and the brains dissected [35,36] with particular reference to the frontal cortex, hippocampal formation and striatum (caudate and putamen). The tissues were removed, immediately weighed and frozen on dry ice.

Extraction of Tissue Samples

All tissues were cut into small pieces on ice, and 10 mL of 1 mol/L acetic acid (MERCK, Darmstadt, Germany) were added per gram tissue and boiled for 10 min. The tissues were homogenized with a polytron (CAT X520D, Scientific Industries, New York, NY, USA) and centrifuged at 1,500 x g in 4°C for 10 min. Immediately after collection, a second extraction was performed in 10 mL of distilled water per gram tissue. The supernatants from each sample were combined, lyophilized and stored at -70ºC. All samples were extracted and analyzed in randomized order.

Extraction of Plasma in Preparation for Analysis of 17β-Estradiol

Two mL diethyl ether was added to 100 µL plasma in glass tubes and vortex-mixed for 30 sec. The tubes were subsequently frozen in 95% ethanol containing dry ice, and after freezing of the aqueous fraction the supernatant were decanted into another glass tube. The ether was evaporated at 40°C. The extracted samples were dissolved in 0.05 mol/L phosphate buffer pH 7.4, containing 0.2% BSA and 0.1% Triton X-100 and kept at 40°C for 30 min before vortexing and cooling to room temperature.

Radioimmunoassay of SP - Galanin and NPY-LI and CCK

The RIA used in measuring SP- Galanin and NPY-LI has been shown by reverse-phase HPLC to overwhelmingly measure the intact peptides in extracts of rat brain [37,38]. The lyophilized samples were reconstituted in 1 mL of phosphate buffer (0.05 mol/L, pH 7.4), and 100 µL of each sample, antibody, and standard were mixed. The concentrations of SP- Galanin and NPY-LI were measured using, respectively; a rabbit anti-rat Galanin antiserum, RatGal4 which does not cross-react with neurokinin A, neuropeptide K, SP, neurokinin B, neuropeptide Y, gastrin, pancreatic polypeptide, glucagon or neurotensin [37]; a rabbit anti-porcine NPY antiserum which cross reacts 0.1% with avian pancreatic polypeptide, but not with other peptides [38]; an SP antiserum, SP2 reacting with SP and SP sulfoxide, but not with other tachykinins. CCK was analyzed using a commercial radioimmunoassay kit (Euria-CCK, RB 302, Euro-Diagnostica, Medeon, SE-20512, Malmö, Sweden) reacting 134% with CCK-33 but only 0.5% cross reactivity for gastrin. The detection limit of Galanin and NPY were 11 pmol/L, of SP 8 pmol/L and of CCK 0.3 pmol/L. Intra- and interassay coefficients of variation for Galanin and NPY were 7 and 12%, respectively, for SP 7 and 11% and for CCK 6% and 14%.

HPLC–purified, 125I-labeled rat SP - Galanin and porcine NPY (4000 cpm) using chloramine-T were prepared in our laboratory and subsequently used as radioligands. Rat Galanin(1-29) and rat NPY(1-36) were used as calibrators (all from Neosystem, Strasbourg, France). All samples were extracted and analyzed in random order.

Samples and calibrators were measured on a Gamma-Master 1277 (LKB Wallac, Turku, Finland).

RIA of 17β-Estradiol

7β-estradiol was analyzed using a commercially available radioimmunoassay kit (Estradiol Double Antibody kit KE2D, Diagnostic Products Co, Los Angeles, CA, USA) and measured on a GammaMaster 1277 (LKB Wallac). The intra- and inter-assay coefficients of variation were 6% and 8%, respectively.

STATISTICS

For neuropeptides and estradiol, medians and quartiles were used to display the central tendency and variation respectively. Multivariate analysis of variance (SYSTAT version 10, SPSS, Inc., 2000) was used for the initial test of

effects (estrous, pregnant and postpartum states) in the experiment. In case of significance ($p<0.05$) in the multivariate ANOVA, Kruskal-Wallis one-way nonparametric "ANOVA" with multiple comparisons was used for significance testing due to the non-normality of the results in several of the groups.

RESULTS

Frontal Cortex

In the frontal cortex, the concentration of CCK-LI increased by 40% during pregnancy ($p<0.05$) followed by a decrease of 26% ($p<0.05$) from pregnancy to postpartum Fig. (**1**). In contrast, SP-LI was also found to be significantly decreased by 21% in this area during pregnancy ($p<0.05$) but increased by 48% postpartum ($p<0.05$). This increase was not an increase of SP back to the levels found during estrous. Thus, there was a significant difference between estrous and postpartum ($p<0.01$). The tissue concentrations of Galanin-LI also decreased in this area by 10% during pregnancy ($p<0.05$) and increased by 39% ($p<0.05$) postpartum. NPY-LI concentration was, however, not altered in this brain region.

Striatum

During late pregnancy the tissue concentration of CCK-LI in the striatum was 29% higher during pregnancy ($p<0.05$) and then decreased by 25% postpartum ($p<0.01$). Similarly, the tissue concentrations of NPY-LI were increased by 22% ($p<0.05$) in the striatum (caudate and putamen) during pregnancy compared to estrous and then decreased by 16% ($p<0.01$) two days after delivery Fig. (**2**). SP-LI, showed in contrast a slightly increased pattern in the striatum during pregnancy (not significant) and a continuing increase of 33% postpartum compared to pregnancy ($p<0.05$). The SP-LI levels increased in post partum group versus those of estrous by 24% and this increase was significant ($p<0.01$).

Hippocampal Formation

In the hippocampal formation no effect on NPY, CCK, Galanin or SP-LI concentrations was observed during late pregnancy or postpartum on Fig. (**3**).

Plasma 17β-Estradiol

The concentrations of 17β-estradiol in plasma were 41% higher during pregnancy ($p<0.001$) and subsequently decreased by 35% from pregnancy to postpartum ($p<0.001$) Fig. (**4**). No significant differences were observed in 17β-estradiol concentrations between estrous and postpartum.

DISCUSSION

The present study provides evidence that pregnancy and parturition differentially influence the levels of CCK, SP, NPY and Galanin in rat brain areas implicated in the regulation of behavioral function, stress and mood. The greatest change of neuropeptide concentrations two days after delivery and during sex-hormone withdrawal was observed in the frontal cortex and striatum, respectively. Surprisingly, no effects on the concentration of any of these four peptides were observed in the hippocampal formation – an area known to be sensitive to sex-hormones – especially estrogen

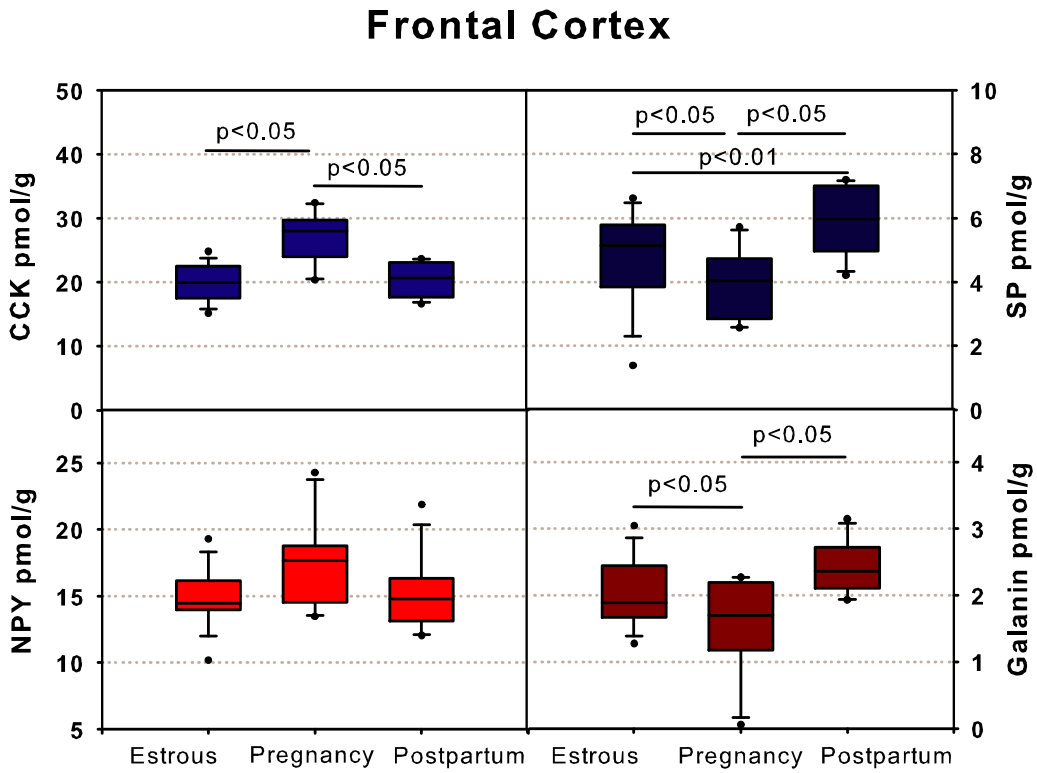

Fig. (1). Box plots displaying the concentrations of neuropeptide Y (NPY), cholecystokinin (CCK), galanin and substance P (SP) – like immunoreactivity-(LI) in extracts from rat frontal cortex during estrous, the 17 – 18th day of pregnancy and two days postpartum.

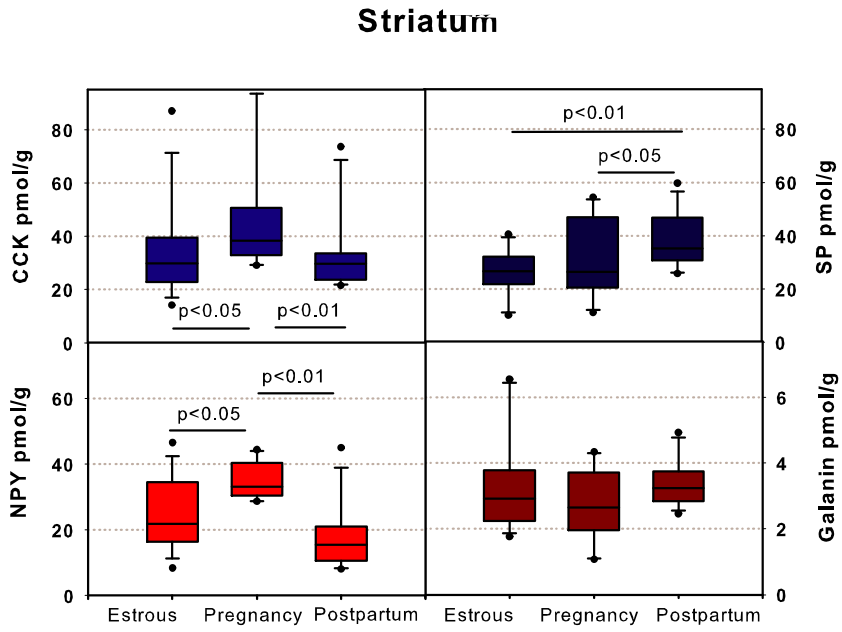

Fig. (2). Box plots displaying the concentrations of neuropeptide Y (NPY), cholecystokinin (CCK), galanin and substance P (SP) – like immunoreactivity-(LI) in extracts from striatum during estrous, the 17 – 18th day of pregnancy and two days post- partum.

[2]. We have previously shown in several studies that estradiol does induce effects on concentrations of Galanin, CCK (unpublished data) and NPY in the hippocampal formation of both female rats and mice [4,5,8,9].

As mentioned above, we found profound changes between pregnancy and parturition in neuropeptide concentration in the frontal cortex, especially of SP, CCK and Galanin - LI. The prefrontal cortex is involved in the control of higher brain functions such as control of shifting behavioral demands, and is highly connected with amygdala [39]. In addition, prefrontal cortex projects to the hypothalamus and the brain stem nuclei mediating neuroendocrine and autonomomic responses to stress and has reciprocal connections to the hippocampus and the ventral and dorsal striatum as well as the dorsal raphe serotonergic neurons [40].

In the present study, SP-LI increased 48% postpartum as compared with pregnancy. Through its receptor NK1 located on noradrenergic cells in the LC, SP has been found to control the release of noradrenalin in the medial prefrontal cortex [41]. Galanin-LI, in line with the effect on SP-LI, was decreased during pregnancy and then increased by 39% postpartum. This peptide, present in both dorsal raphe and locus coeruleus projecting to the frontal cortex and hippocampus has been implicated in both stress-related behavior as well as anxiety [22] e.g. from studies of the bed nucleus of stria terminalis located immediate adjacent to the central nucleus of amygdala [42]. On the contrary, we found that CCK-LI concentration was reduced in the frontal cortex postpartum by 26%. CCK is a peptide that has been implicated in mood disorders based on studies showing anxiety symptoms and the properties of CCK receptor 2 agonist to provoke panic attacks [19,43]. We found, however, no effect on NPY-LI concentration in the control group (estrous), during pregnancy or postpartum in this area.

In striatum (caudate/putamen), SP-LI increased during pregnancy and continued to increase postpartum by 33%. SP-LI in striatum has been found to be markedly changed in an animal model of depression [32]. In contrast, we found a significant decrease of NPY-LI in striatum, 16% lower postpartum than during pregnancy. Previous studies on correlations between anxiety and changed levels of NPY-LI concentration in caudate-putamen have found NPY-LI levels to be associated with changed levels of NPY [31,33,34,44]. CCK-LI concentration was also decreased in this area postpartum by 25%.

Withdrawal of estrogen has been extensively investigated in rodents and found to produce depressive-like symptoms [45-47]. For example, depression-like behavior is decreased in the third trimester when plasma levels of estradiol are high [48] and increased postpartum [46]. Since several neuropeptides are sensitive to estrogen there is a possibility that neuropeptides are involved in the regulation of brain function and affecting mood through their modulation of classical neurotransmitters. However, it is important to note that there are other hormones whose concentrations are dramatically changed during this period, for example the pituitary hormone prolactin [49].

CONCLUDING REMARKS

In summary, the present results support our hypothesis that the neuropeptidergic system is influenced during pregnancy and postpartum, which perhaps contributes to the etiology of the change in mood-related behavior in rodents after withdrawal of ovarian hormones. However, these mechanisms are as yet unrevealed and likely to be very complex and will have to be investigated in combinations with behavioral analysis. Nonetheless, taking all our findings together, this study show that pregnancy and puerperium are temporally linked to changes in SP, CCK, NPY and Galanin con-

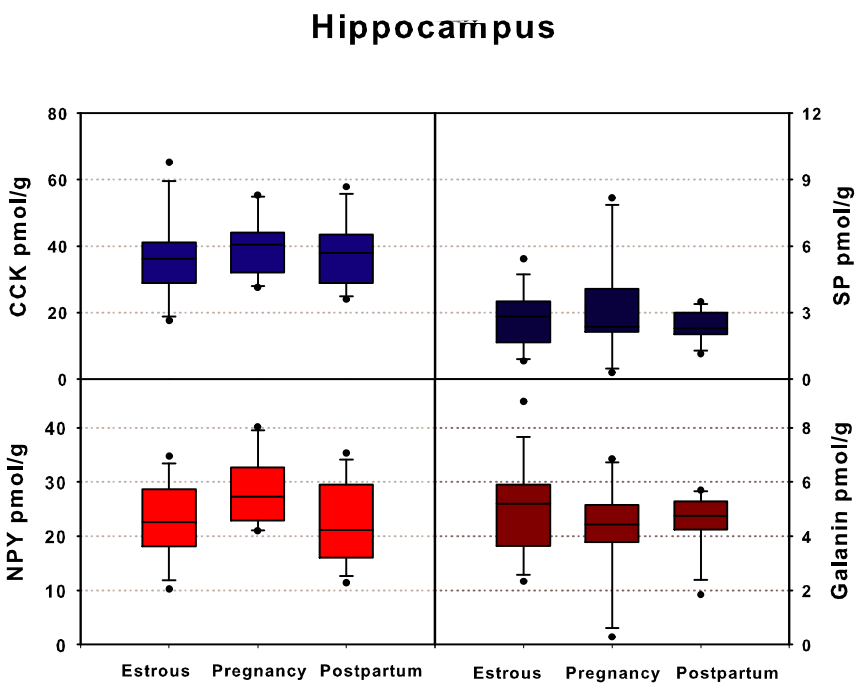

Fig. (3). Box plots displaying the concentrations of neuropeptide Y (NPY), cholecystokinin (CCK), galanin and substance P (SP) in extracts from hippocampus during estrous, the 17 – 18th day of pregnancy and two days post- partum.

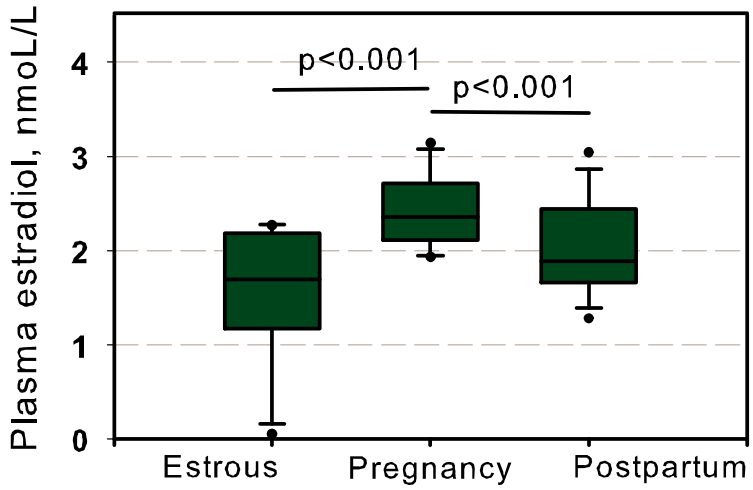

Fig. (4). Box plots displaying the concentration of 17β-estradiol in plasma during estrous, the 17-18th day of pregnancy and two days post-partum.

centrations in rat brain areas highly connected to the regulation of mood-related behavior.

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ABBREVIATIONS

CCK = Cholecystokinin

LI = Like immunoreactivity

SP = Substance P

RIA = Radioimmunoassay

REFERENCES

[1] Epperson CN. Postpartum major depression: detection and treatment. Am Fam Physician 1999; 59: 2247-54, 2259-60.

[2] McEwen B. Estrogen actions throughout the brain. Recent Prog Horm Res 2002; 57: 357-84.

[3] Shively CA, Bethea CL. Cognition, mood disorders, and sex hormones. ILAR J 2004; 45: 189-99.

[4] Hilke S, Hökfelt T, Darwish M, Theodorsson E. Cholecystokinin levels in the rat brain during the estrous cycle. Brain Res 2007; 1144: 70-3.

[5] Hilke S, Holm L, Man K, Hökfelt T, Theodorsson E. Rapid change of neuropeptide Y levels and gene-expression in the brain of ovariectomized mice after administration of 17 beta-estradiol. Neuropeptides 2009; 43: 327-32.

[6] Holland KL, Norby LA, Micevych PE. Peripubertal ontogeny and estrogen stimulation of cholecystokinin and preproenkephalin mRNA in the rat hypothalamus and limbic system. J Comp Neurol 1998; 392: 48-57.

[7] Österlund MK, Hurd YL. Estrogen receptors in the human forebrain and the relation to neuropsychiatric disorders. Prog Neurobiol 2001; 64: 251-67.

[8] Rugarn O, Hammar M, Theodorsson A, Theodorsson E, Stenfors C. Sex differences in neuropeptide distribution in the rat brain. Peptides 1999; 20: 81-6.

[9] Hilke S, Theodorsson A, Fetissov S, *et al*. Estrogen induces a rapid increase in galanin levels in female rat hippocampal formation--possibly a nongenomic/indirect effect. Eur J Neurosci 2005; 21: 2089-99.

[10] Hökfelt T, Johansson O, Ljungdahl A, Lundberg JM, Schultzberg M. Peptidergic neurones. Nature 1980; 284: 515-21.

[11] Hökfelt T, Bartfai T, Bloom F. Neuropeptides. opportunities for drug discovery. Lancet Neurol 2003; 2: 463-72.

[12] Beinfeld MC, Palkovits M. Distribution of cholecystokinin (CCK) in the rat lower brain stem nuclei. Brain Res 1982; 238: 260-5.

[13] Dockray GJ. Immunochemical evidence of cholecystokinin-like peptides in brain. Nature 1976; 264: 568-70.

[14] Hökfelt T, Blacker D, Broberger C, *et al*. Some aspects on the anatomy and function of central cholecystokinin systems. Pharmacol Toxicol 2002; 91: 382-6.

[15] Rehfeld JF. Immunochemical studies on cholecystokinin. II. Distribution and molecular heterogeneity in the central nervous system and small intestine of man and hog. J Biol Chem 1978; 253: 4022-30.

[16] Rehfeld JF. Immunochemical studies on cholecystokinin. I: Development of sequence-specific radioimmunoassays for porcine triacontatriapeptide cholecystokinin. J Biol Chem 1978; 253: 4016-21.

[17] Bradwejn J, Koszycki D, Couetoux du Tertre A, *et al*. The panicogenic effects of cholecystokinin-tetrapeptide are antagonized by L-365,260, a central cholecystokinin receptor antagonist, in patients with panic disorder. Arch Gen Psychiatry 1994; 51: 486-93.

[18] Ladurelle N, Keller G, Roques BP, Dauge V. Effects of CCK8 and of the CCKB-selective agonist BC264 on extracellular dopamine content in the anterior and posterior nucleus accumbens. a microdialysis study in freely moving rats. Brain Res 1993; 628: 254-62.

[19] Rehfeld JF. Cholecystokinin and panic disorder--three unsettled questions. Regul Pept 2000; 93: 79-83.

[20] Micevych PE, Eckersell CB, Brecha N, Holland KL. Estrogen modulation of opioid and cholecystokinin systems in the limbic-hypothalamic circuit. Brain Res Bull 1997; 44: 335-43.

[21] Melander T, Hökfelt T, Rökaeus A. Distribution of galaninlike immunoreactivity in the rat central nervous system. J Comp Neurol 1986; 248: 475-517.

[22] Kuteeva E, Wardi T, Lundström L, *et al*. Differential role of galanin receptors in the regulation of depression-like behavior and monoamine/stress-related genes at the cell body level. Neuropsychopharmacology 2008; 33: 73-2585.

[23] Hökfelt T, Kuteeva E. Substance P. a neuropeptide. Am J Psychiatry 2006; 163: 578.

[24] Sergeyev V, Fetissov S, Mathe AA, *et al*. Neuropeptide expression in rats exposed to chronic mild stresses. Psychopharmacology 2005; 178: 115-24.

[25] Ebner K, Muigg P, Singewald G, Singewald N. Substance P in stress and anxiety. NK-1 receptor antagonism interacts with key brain areas of the stress circuitry. Ann N Y Acad Sci 2008; 1144: 61-73.

[26] Tatemoto K, Carlquist M, Mutt V. Neuropeptide Y--a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 1982; 296: 659-60.

[27] Chronwall BM, DiMaggio DA, Massari VJ, Pickel VM, Ruggiero DA, O'Donohue TL. The anatomy of neuropeptide-Y-containing neurons in rat brain. Neuroscience 1985; 15: 1159-81.

[28] de Quidt ME, Emson PC. Distribution of neuropeptide Y-like immunoreactivity in the rat central nervous system—II: Immunohistochemical analysis. Neuroscience 1986; 18: 545-618.

[29] de Quidt ME, Emson PC. Distribution of neuropeptide Y-like immunoreactivity in the rat central nervous system—I: Radioimmunoassay and chromatographic characterisation. Neuroscience 1986; 18: 527-43.

[30] Holmes A, Heilig M, Rupniak NM, Steckler T, Griebel G. Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. Trends Pharmacol Sci 2003; 24: 580-8.

[31] Karlsson RM, Holmes A, Heilig M, Crawley JN. Anxiolytic-like actions of centrally-administered neuropeptide Y, but not galanin, in C57BL/6J mice. Pharmacol Biochem Behav 2005; 80: 427-36.

[32] Husum H, Vasquez PA, Mathe AA. Changed concentrations of tachykinins and neuropeptide Y in brain of a rat model of depression. lithium treatment normalizes tachykinins. Neuropsychopharmacology 2001; 24: 183-91.

[33] Redrobe JP, Dumont Y, Quirion R. Neuropeptide Y (NPY) and depression. from animal studies to the human condition. Life Sci 2002; 71: 2921-37.

[34] Caberlotto L, Hurd YL. Reduced neuropeptide Y mRNA expression in the prefrontal cortex of subjects with bipolar disorder. Neuroreport 1999; 10: 1747-50.

[35] Glowinski J, Iversen LL. Regional studies of catecholamines in the rat brain. I: The disposition of [3H]norepinephrine, [3H]dopamine and [3H]dopa in various regions of the brain. J Neurochem 1966; 13: 655-69.

[36] Paxinos GW. The rat brain in stereotaxic coordinates. San Diego, USA Academic Press 1998.

[37] Theodorsson E, Rugarn O. Radioimmunoassay for rat galanin. immunochemical and chromatographic characterization of immunoreactivity in tissue extracts. Scand J Clin Lab Invest 2000; 60: 411-8.

[38] Theodorsson-Norheim E, Hemsen A, Lundberg JM. Radioimmunoassay for neuropeptide Y (NPY): chromatographic characterization of immunoreactivity in plasma and tissue extracts. Scand J Clin Lab Invest 1985; 45: 355-65.

[39] Holmes A, Wellman CL. Stress-induced prefrontal reorganization and executive dysfunction in rodents. Neurosci Biobehav Rev 2009; 33: 773-83.

[40] Jankowski MP, Sesack SR. Prefrontal cortical projections to the rat dorsal raphe nucleus. ultrastructural features and associations with serotonin and gamma-aminobutyric acid neurons. J Comp Neurol 2004; 468: 518-29.

[41] Hahn MK, Bannon MJ. Stress-induced C-fos expression in the rat locus coeruleus is dependent on neurokinin 1 receptor activation. Neuroscience 1999; 94: 1183-8.

[42] Barrera G, Hernandez A, Poulin JF, Laforest S, Drolet G, Morilak DA. Galanin-mediated anxiolytic effect in rat central amygdala is not a result of corelease from noradrenergic terminals. Synapse 2006; 59: 27-40.

[43] Wang H, Wong PT, Spiess J, Zhu YZ. Cholecystokinin-2 (CCK2) receptor-mediated anxiety-like behaviors in rats. Neurosci Biobehav Rev 2005; 29: 1361-73.

[44] Heilig M, Zachrisson O, Thorsell A, *et al*. Decreased cerebrospinal fluid neuropeptide Y (NPY) in patients with treatment refractory unipolar major depression: preliminary evidence for association with preproNPY gene polymorphism. J Psychiatr Res 2004; 38: 113-21.

[45] Galea LA, Wide JK, Paine TA, Holmes MM, Ormerod BK, Floresco SB. High levels of estradiol disrupt conditioned place preference learning, stimulus response learning and reference memory but have limited effects on working memory. Behav Brain Res 2001; 126: 115-26.

[46] Suda S, Segi-Nishida E, Newton SS, Duman RS. A postpartum model in rat: behavioral and gene expression changes induced by ovarian steroid deprivation. Biol Psychiatry 2008; 64: 311-9.

[47] Walf AA, Frye CA. Antianxiety and antidepressive behavior produced by physiological estradiol regimen may be modulated by hypothalamic-pituitary-adrenal axis activity. Neuropsychopharmacology 2005; 30: 1288-1301.

[48] Frye CA, Walf AA. Hippocampal 3alpha, 5alpha-THP may alter depressive behavior of pregnant and lactating rats. Pharmacol Biochem Behav 2004; 78: 531-40.

[49] Törner L, Toschi N, Pohlinger A, Landgraf R, Neumann ID. Anxiolytic and anti-stress effects of brain prolactin. improved efficacy of antisense targeting of the prolactin receptor by molecular modeling. J Neurosci 2001; 21: 3207-14.

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