Gene Expression Changes in the Septum: Possible Implications for MicroRNAs in Sculpting the Maternal Brain

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Abstract
The transition from the non-maternal to the maternal state is characterized by a variety of CNS alterations that support the care of offspring. The septum (including lateral and medial portions) is a brain region previously linked to various emotional and motivational processes, including maternal care. In this study, we used microarrays (PLIER algorithm) to examine gene expression changes in the septum of postpartum mice and employed gene set enrichment analysis (GSEA) to identify possible regulators of altered gene expression. Genes of interest identified as differentially regulated with microarray analysis were validated with quantitative real-time PCR. We found that fatty acid binding protein 7 (Fabp7) and galanin (Gal) were downregulated, whereas insulin-like growth factor binding protein 3 (Igfbp3) was upregulated in postpartum mice compared to virgin females. These genes were previously found to be differentially regulated in other brain regions during lactation. We also identified altered expression of novel genes not previously linked to maternal behavior, but that could play a role in postpartum processes, including glutamate-ammonia ligase (GluL) and somatostatin receptor 1 (Sst1r) (both upregulated in postpartum). Genes implicated in metabolism, cell differentiation, or proliferation also exhibited altered expression. Unexpectedly, enrichment analysis revealed a high number of microRNAs, transcription factors, or conserved binding sites (177 with corrected P-value < 0.05) that were significantly linked to maternal upregulated genes, while none were linked to downregulated genes. MicroRNAs have been linked to placenta and mammary gland development, but this is the first indication they may also play a key role in sculpting the maternal brain. Together, this study provides new insights into genes (along with possible mechanisms for their regulation) that are involved in septum-mediated adaptations during the postpartum period.

Introduction
A variety of behavioral, metabolic, structural, and neuroendocrine alterations occur during the transition from a virgin to a lactating state [1–4]. These physiological adaptations during lactation are believed to be critically important for the survival and development of the offspring. For example, in rodents, within hours of parturition, the mother retrieves the scattered pups, gathers them together in the nest, and adopts a nursing posture over the pups to permit suckling [5]. Adaptive changes in several neuroendocrine systems during lactation are also evident. For example, the release of both adrenocorticotropic hormone and corticosterone in response to various stressors is markedly suppressed [6,7]. A striking change in metabolic aspect during lactation is the negative energy balance resulting from the increased energy demand and decreased adaptive thermogenesis [8]. This energy-saving mechanism facilitates the availability of energy for milk production.

The mammalian septum is a heterogeneous forebrain structure that is divided into two main subdivisions, the medial septum (MS) and the lateral septum (LS), and has been linked to various aspects of maternal care, including offspring protection and pup retrieval [9–12]. Neuroanatomically, MS is densely interconnected with the hippocampus while LS has extensive reciprocal connections with numerous brain regions known to regulate affect and motivation, such as hypothalamus, amygdala, bed nucleus of the stria terminalis, periaqueductal gray, ventral tegmental area, and raphe nuclei [13–15]. Neurochemically, a high number of neurons in LS contain GABA and in subregions of LS, neurons also express other neurotransmitters, such as neurotensin, enkephalin, galanin, and somatostatin [13,14]. Neurons within MS are predominantly GABAergic and/or cholinergic [16]. LS has long been considered to play a critical role in regulating multiple affective, behavioral and cognitive processes, such as fear, anxiety, depression, aggression, maternal behavior, and social recognition [11,13,17–19]. MS functions mainly as a region that modulates processes related to attention and memory [20,21].

Alterations in neuronal activity and gene expression in several brain regions during the postpartum period have been reported. For example, during lactation, neuronal activity of neuropeptide Y (NPY) and agouti-related protein are increased in the arcuate nucleus of hypothalamus (Arc), a site known to be a core feeding
center, whereas proopiomelanocortin is reduced [22,23]. These changes in neuropeptide activity have been proposed to be important in integrating Arc-mediated food intake and energy balance. Expression changes of some genes in forebrain structures during lactation have been demonstrated to be involved in the control of maternal/parental and/or reproductive behaviors [24–26]. However, possible alterations in gene expression in the septum that may contribute to the septum-mediated adaptations during lactation remain largely unexplored. In this study, we employed microarray analysis to identify genes in the septum with altered expression during the postpartum period.

As part of this study, we were also interested in how identified gene expression differences may have occurred. Producing the maternal brain is a complex process and is associated with various contributing factors including hormonal changes and sensory input from different events, such as the mating, parturition, nursing, and additional interactions with offspring [27–29]. There are likely large numbers of transcription factors that contribute to the gene expression changes but which have not to date been evaluated. Newer data mining tools, such as gene set enrichment analysis (GSEA), provide a unique approach to gain insight into how altered gene expression occurs [30]. The databases include for each gene information on all known binding sites in that region, including those for a wide range of transcription factors and microRNAs (miRNAs), that may be involved in gene expression. miRNAs are of interest because they have been implicated in peripheral alterations, such as placenta and mammary gland development [31,32] in the maternal female, but to date no roles for miRNAs have been examined for the maternal brain. In this study, we used GSEA to gain insights into how large scale changes may have been occurring during pregnancy and early lactation with a subset of transcriptional regulators.

Results

Genes Identified as being Differentially Expressed in the Septum of Lactating Versus Virgin Mice

Using PLIER analysis with a P < 0.0025 cutoff value, we identified 116 genes that displayed significant changes in expression (Table 1). Two genes of interest, Gal (P = 0.018, fold change 1.83) and Sstrl (P = 0.004, fold change 1.22) were not included in Table 1 because their P-values were more than 0.0025. The full list of all 35,557 targets, their relative expression and P value ranking using PLIER algorithm is presented in Table S1. The genes identified as being differentially expressed in lactating versus virgin mice were distributed across a number of categories (Table 1). The function/category of each gene was sorted out individually based on the biological process using PubMed and GenBank databases.

Verification of Microarray Results by Quantitative Real-time PCR Analysis

Consistent with the findings from microarray analysis, mRNA levels of three representative genes, Ghrl (P = 0.023), Igfbp3 (P = 0.004) and Sstrl (P = 0.048) were identified as being significantly up-regulated in maternal mice as compared to virgin mice, whereas Fabp7 (P < 0.001) and Gal (P = 0.023) were significantly down-regulated (Fig. 1). While Npy1r mRNA levels were found to be significantly increased in lactating versus virgin mice in the microarray analysis, we did not confirm a difference between the two groups using highly sensitive real-time PCR technique (Fig. 1).

Gene Set Enrichment Analysis Results

When evaluating genes upregulated in postpartum mice, 177 unique regions involved in transcriptional regulation reached significance (corrected P-value < 0.05). The majority of these were miRNA binding sites (67%; 119 out of 177). For example, the site with the fifth highest p-value was a binding site for miRNA, Mir-183. For some binding sites, multiple miRNAs were listed. For example, for the 10th most significant region is the conserved binding site of AGCATTT, which can be bound by multiple miRNAs, including Mir-93 and Mir-302B. A partial (Table 2) and full list (Table S2) of the GSEA results are provided. The binding sites for 29 different transcription factors were identified as significantly upregulating gene expression in maternal mice (Table 3, Table S2). The database includes target data from multiple species and in some cases a given transcription factor (e.g., YY1) reached significance with multiple species. Here we only counted each transcription factor once and only listed those associated with mammals (e.g., mouse, human). Twenty seven additional conserved binding sites in close proximity to genes were also identified as being linked with upregulated expression in maternal mice (Table S2). For these regions, no known transcription factor or miRNA has been identified that target these regions.

Discussion

This study used Affymetrix microarray analysis and real-time PCR techniques to examine CNS gene expression changes in the septum during lactation. We identified 116 genes (P < 0.0025 as analyzed by PLIER) and 2844 genes (using a lower cutoff of P < 0.05) which were distributed across a number of categories in biological process, as being differentially expressed in lactating versus virgin mice. Some of the genes identified are consistent with previous studies testing gene expression changes during lactation. Our study also found a list of novel genes that may play a role in the transition from a non-parental to a parental state. Further, using data mining approaches and GSEA we identified a number of miRNAs and transcription factors that may play a role in producing the maternal brain.

Identification of Expression Changes During Lactation Consistent with Previous Studies

In the septum, mRNA of Igfbp3 increased about 30% during lactation (Table 1), and was confirmed by qPCR (Fig. 1). This result is consistent with previous findings that Igfbp3 was increased in the arcuate nucleus of hypothalamus [33], and in large hypothalamic CNS regions in previous maternal array studies [34]. Igfbp3 is a member of the superfamily of insulin-like growth factor (IGF) binding proteins and is expressed in the brain. In vivo studies show that Igfbp3 inhibits IGF action by modulating the bioavailability and distribution of IGFs [35]. One possibility is that increased Igfbp3 in the septum during lactation would result in reduced IGF action, but this would need to be determined. Given that IGF negatively regulates food intake and body weight [36], the increased expression of Igfbp3 would be expected to increase food intake through an IGF-mediated mechanism, consistent with the low levels of IGF in association with the metabolic adaptations that occur during lactation [37]. Based on its role in neurogenesis [38], Igfbp3 has been linked to the involvement in certain disease processes, such as ischemia, hypoxia and autism [39,40]. There are multiple pathways by which Igfbp3 could possibly interact with maternal circuitry.
Table 1. List of genes showing highest significant differences in gene expression between lactating and virgin mice when analyzed with the probe logarithmic intensity error statistics (PLIER) ($P<0.0025$).

| Accession# | Gene | Gene title | Fold change |
|------------|------|------------|-------------|
| NM_019735  | Apip | APAF1 interacting protein | 1.11 |
| NM_026121  | Bag4 | BCL2-associated athanogene 4 | 1.17 |
| NM_001033136 | Fam82a2 | family with sequence similarity 82, member A2 | 1.07 |
| NM_010477  | Hspd1 | heat shock protein 1 | 1.10 |
| NM_026160  | Map1lc3b | microtubule-associated protein 1 light chain 3 beta | 1.07 |
| NM_013490  | Chka | choline kinase alpha | 1.14 |
| NM_010027  | Ddt | D-dopachrome tautomerase | 1.16 |
| NM_001033300 | Gmps | guanine monophosphate synthetase | 1.05 |
| NM_134017  | Mat2b | methionine adenosyltransferase II, beta | 1.08 |
| NM_007591  | Calr | calreticulin | 1.10 |
| NM_017367  | Ccni | cyclin I | 1.07 |
| NM_009865  | Cdhl10 | cadherin 10 | 1.09 |
| NM_009699  | Csf2 | colony stimulating factor 2 (granulocyte-macrophage) | 0.87 |
| NM_019561  | Ensa | endosulfine alpha | 1.07 |
| NM_023794  | Etv5 | ets variant gene 5 | 1.19 |
| NM_021272  | Fabp7* | fatty acid binding protein 7 | 0.69 |
| NM_146601  | Hhip | huntingtin interacting protein 1 | 1.09 |
| NM_008343  | Igfbp3* | insulin-like growth factor binding protein 3 | 1.30 |
| NM_005664  | Mcm2 | minichromosome maintenance deficient 2 mitotin (S. cerevisiae) | 0.91 |
| NM_022889  | Pes1 | pescadiollo homolog 1 | 1.11 |
| NM_033573  | Prcc | papillary renal cell carcinoma (translocation-associated) | 1.06 |
| NM_009009  | Rad21 | RAD21 homolog (S. pombe) | 1.08 |
| NM_009082  | Rpl29 | ribosomal protein L29 | 0.95 |
| NM_018754  | Sfn | stratifin | 0.89 |
| NM_026232  | Sgol1 | shugoshin-like 1 (S. pombe) | 0.88 |
| NM_016737  | Stip1 | stress-induced phosphoprotein 1 | 1.11 |
| NM_177033  | Vwc2 | von Willebrand factor C domain containing 2 | 1.08 |
| NM_016845  | Acrbp | proacrosin binding protein | 1.10 |
| NM_023598  | And5b | AT rich interactive domain 5B (MRF1-like) | 1.14 |
| NM_008010  | Fgfr3 | fibroblast growth factor receptor 3 | 1.15 |
| NM_008494  | Lfng | LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase | 1.13 |
| NM_023248  | Sbds | Shwachman-Bodian-Diamond syndrome homolog (human) | 1.08 |
| NM_025590  | Acot11 | acyl-CoA thioesterase 11 | 1.13 |
| NM_035115  | AcoX2 | acyl-Coenzyme A oxidase 2, branched chain | 0.87 |
| NM_021299  | Ak3 | adenylylate kinase 3 | 1.11 |
| NM_025275  | Am22 | archaealysin family metallopeptidase 2 | 1.10 |
| NM_001038699 | Fn3k | fructosamine 3 kinase | 1.09 |
| NM_008131  | Glut* | glutamate-ammonia ligase (glutamine synthetase) | 1.06 |
| NM_028894  | Lonrf3 | LON peptidase N-terminal domain and ring finger 3 | 1.24 |
| NR_028335  | Ntsc2 | 5’-nucleotidase, cytosolic II | 1.07 |
| NM_026794  | Nudt9 | nudix (nucleoside diphosphate linked moiety X)-type motif 9 | 1.09 |
| NM_009787  | Pdia4 | protein disulfide isomerase associated 4 | 1.15 |
| NM_027959  | Pdia6 | protein disulfide isomerase associated 6 | 1.20 |
## Table 1. Cont.

| Accession# | Gene      | Gene title                                      | Fold change |
|------------|-----------|------------------------------------------------|-------------|
| NM_008832  | Phka1     | phosphorylase kinase alpha 1                    | 1.17        |
| NM_001083110 | Pja1  | praja1, RING-H2 motif containing                | 1.08        |
| NM_025882  | Pole4     | polymerase (DNA-directed), epsilon 4 (p12 subunit) | 0.94        |
| NM_011275  | Rnaseh1   | ribonuclease H1                                  | 1.07        |
| NM_025683  | Rpe       | ribulose-5-phosphate 3-epimerase                | 1.08        |
| NM_001163571 | Semp3  | SUMO/sentrin specific peptidase 3               | 1.10        |
| NM_001177833 | Smox  | spermine oxidase                                | 1.14        |

### Phosphorylation, dephosphorylation

| Accession# | Gene      | Gene title                                      | Fold change |
|------------|-----------|------------------------------------------------|-------------|
| NM_013557  | Eif2ak1   | eukaryotic translation initiation factor 2 alpha kinase 1 | 1.07        |
| NM_001004144 | Git1   | G protein-coupled receptor kinase-interactor 1  | 1.06        |
| NM_144843  | Mtrm6     | myotubularin related protein 6                  | 1.07        |
| NM_001110218 | Ppm1h  | protein phosphatase 1H (PP2C domain containing)  | 1.15        |
| NM_027673  | Tssk4     | testis-specific serine kinase 4                 | 0.91        |

### Protein folding

| Accession# | Gene      | Gene title                                      | Fold change |
|------------|-----------|------------------------------------------------|-------------|
| NM_009838  | Cct6a     | chaperonin containing Tcp1, subunit 6a (zeta)   | 1.08        |
| NM_021422  | Dnaja4    | Dnaj (Hsp40) homolog, subfamily A, member 4     | 1.17        |
| NM_198899  | Ugg1t1    | UDP-glucose glycoprotein glucosyltransferase 1   | 1.09        |

### Protein ubiquitination, deubiquitination

| Accession# | Gene      | Gene title                                      | Fold change |
|------------|-----------|------------------------------------------------|-------------|
| NM_019927  | Anh1      | ariadne ubiquitin-conjugating enzyme E2 binding protein homolog 1 (Drosophila) | 1.05        |
| NM_133247  | Usp33     | ubiquitin specific peptidase 33                 | 1.08        |

### Regulation of transcription

| Accession# | Gene      | Gene title                                      | Fold change |
|------------|-----------|------------------------------------------------|-------------|
| NM_017406  | Atf6b     | activating transcription factor 6 beta          | 1.08        |
| NM_011714  | Baz1b     | bromodomain adjacent to zinc finger domain, 1B  | 1.08        |
| NM_019682  | Dynll1    | dynein light chain LC8-type 1                   | 1.09        |
| NM_008065  | Gabpa     | GA repeat binding protein, alpha                | 1.12        |
| NM_001080817 | Prdm10 | PR domain containing 10                        | 1.06        |
| NM_027748  | Taf3      | TAF3 RNA polymerase II, TATA box binding protein (TBP)-associated factor | 1.08        |
| NM_001077364 | Tsc22d3 | TSC22 domain family, member 3                  | 1.34        |

### RNA-related function

| Accession# | Gene      | Gene title                                      | Fold change |
|------------|-----------|------------------------------------------------|-------------|
| NM_026623  | Nudt21    | nudix (nucleoside diphosphate linked moiety X)-type motif 21 | 1.13        |
| NM_016813  | Nxf1      | nuclear RNA export factor 1 homolog (S. cerevisiae) | 1.09        |
| NM_026045  | Prpf18    | PRP18 pre-mRNA processing factor 18 homolog     | 1.08        |
| NM_027911  | Raver1    | ribonucleoprotein, PTB-binding 1                | 0.93        |
| NM_026886  | Srm4      | serine/arginine repetitive matrix 4             | 1.12        |
| NM_198102  | Tra2a     | transformer 2 alpha homolog (Drosophila)        | 1.21        |

### Signal transduction

| Accession# | Gene      | Gene title                                      | Fold change |
|------------|-----------|------------------------------------------------|-------------|
| NM_001128084 | Arhgap21 | Rho GTPase activating protein 21                | 1.04        |
| NM_011332  | Ccl17     | chemokine (C-C motif) ligand 17                 | 0.83        |
| NM_009895  | Cish      | cytokine inducible SH2-containing protein       | 1.13        |
| NM_010172  | F7        | coagulation factor VII                          | 0.89        |
| NM_181748  | Gpr120    | G protein-coupled receptor 120                  | 0.89        |
| NM_008630  | Mt2       | metallothionein 2                               | 0.93        |
| NM_010934  | Npy1r *   | neuropeptide Y receptor Y1                      | 1.23        |
| NM_027571  | P2ry12    | purinergic receptor P2Y, G-protein coupled 12   | 0.92        |
| NM_008882  | Plxna2    | plexin A2                                      | 1.05        |
| NM_009067  | Ralbp1    | ralA binding protein 1                          | 1.08        |
| NM_001039089 | Sel1l  | sel-1 suppressor of lin-12-like (C. elegans)   | 1.07        |
| NM_008507  | Sh2b3     | SH2B adaptor protein 3                          | 1.05        |

### Translation

| Accession# | Gene      | Gene title                                      | Fold change |
|------------|-----------|------------------------------------------------|-------------|
Fabp7 transcript was reduced about 30% during lactation in this study and was also found to be significantly decreased in maternal rodents in Arc [33] and hypothalamic CNS regions [34] in previous array studies. The mammalian Fabp7 is expressed in radial glial cells, astrocytes, and neuronal cell precursors, functioning as a modulator of cell differentiation, proliferation, and neurogenesis [41]. Interestingly, Fabp7 was preferentially expressed in numerous astrocytes in the amygdala and septum, regions known to be critically involved in the regulation of fear and anxiety [42]. In addition, previous studies show that this gene modulates the processes of sleep, memory, and anxiety [42,43]. Several lines of evidence associate Fabp7 with neuropsychiatric diseases such as schizophrenia [44]. Fabp7-deficient mice displayed enhanced fear memory and anxiety in adulthood [42].

Together with the functional significance of the septum in fear, anxiety and memory [15,20,21], these findings suggest the possibility that Fabp7 plays an important role in emotional changes that occur during the postpartum period.

We found a decrease of galanin in the gene array, which was confirmed by qPCR. Significant reduction of galanin during lactation has been observed in previous array studies [33,34]. Galanin is a 29 amino-acid (30 in humans) neuropeptide and is richly expressed in the CNS. Although galanin was not listed in the top identified genes in terms of significance ($P = 0.018$), it was selected as a gene of interest since this peptide has been known to regulate a variety of behavioral processes, including food intake, sexual behavior, learning, memory, reward, cognition, sleep, seizure, as well as emotion/mood-related behaviors, such as stress,

Table 1. Cont.

| Accession#   | Gene       | Gene title                                                                 | Fold change |
|--------------|------------|----------------------------------------------------------------------------|-------------|
| NM_013854    | Abcf1      | ATP-binding cassette, sub-family F (GCN20), member 1                       | 1.07        |
| NM_029735    | Eprs       | glutamyl-prolyl-tRNA synthetase                                             | 1.06        |
| NM_030722    | Pum1       | pumilio 1 (Drosophila)                                                     | 1.07        |
| Transport    | NM_001122820 | adaptor-related protein complex 3, mu 2 subunit                          | 1.06        |
| NM_025828    | Lman2      | lectin, mannoside-binding 2                                                | 1.10        |
| NM_001013374 | Lman2l     | lectin, mannoside-binding 2-like                                            | 1.09        |
| NM_019983    | Rabg5f1    | RAB guanine nucleotide exchange factor (GEF) 1                             | 1.08        |
| NM_009199    | Slc1a1     | solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1 | 1.12        |
| NM_011394    | Slc20a2    | solute carrier family 20, member 2                                         | 0.92        |
| NM_014000    | Slc2a1     | solute carrier family 2 (facilitated glucose transporter), member 1       | 1.13        |
| NM_028064    | Slc39a4    | solute carrier family 39 (zinc transporter), member 4                     | 0.89        |
| NM_172269    | Vps18      | vacuolar protein sorting 18 (yeast)                                        | 1.06        |
| Others       | NM_027907  | Agxt2b1 | alanine-glyoxylate aminotransferase 2-like 1                              | 1.64        |
| NM_025675    | Atpdb4     | ATP binding domain 4                                                       | 0.92        |
| NM_177759    | Ccc60      | coiled-coil domain containing 60                                           | 0.84        |
| NM_001081345 | Chd2       | chromodomain helicase DNA binding protein 2                              | 1.08        |
| NM_030137    | Cstad      | CSA-conditional, T cell activation-dependent protein                       | 0.79        |
| NM_145570    | Fam176a    | family with sequence similarity 176, member A                             | 1.19        |
| NM_001033440 | Gm1587     | predicted gene 1587                                                       | 0.89        |
| NM_001024720 | Hmcn1      | hemicentin 1                                                               | 0.85        |
| NM_033222    | Lzlf1      | leucine zipper transcription factor-like 1                                | 1.09        |
| NM_001102613 | Phld3      | pleckstrin homology-like domain, family B, member 3                        | 0.86        |
| NM_027241    | Pol3gl     | polymerase (RNA) III (DNA directed) polypeptide G like                     | 1.07        |
| NM_175388    | Rnf169     | ring finger protein 169                                                    | 1.07        |
| NM_022324    | Sdf2l1     | stromal cell-derived factor 2-like 1                                       | 1.24        |
| NM_183136    | Spink8     | serine peptidase inhibitor, Kazal type 8                                  | 1.22        |
| NM_175106    | Tmem177    | transmembrane protein 177                                                 | 1.12        |
| NM_029074    | Tmem188    | transmembrane protein 188                                                 | 1.09        |
| NM_153525    | Tmem41b    | transmembrane protein 41B                                                 | 1.15        |
| NM_025749    | Zfp474     | zinc finger protein 474                                                   | 0.90        |
| NM_146203    | Zfp764     | zinc finger protein 764                                                   | 0.90        |

Fold change greater than 1.0 represents increases, while less than 1.0 indicates decreases in lactating versus virgin mice.

*Indicates gene expression result obtained from microarray analysis was further verified using qPCR.

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fear, anxiety and depression [45–47]. Given that LS has many roles in common with galanin in the processes of fear, anxiety and depression, galanin may be an interesting candidate gene for regulation of LS-mediated affective processes.

Identification of Novel Genes During Lactation

The upregulation of Glul mRNA in the septum of maternal mice was noteworthy for a few reasons. The astrocytic Glul or enzyme glutamine synthetase (GS), is a key enzyme involved in the glutamate-glutamine cycle. Glutamate is metabolized mainly to glutamine by the catalyzation of Glul. Further, glutamine may also be utilized for de novo synthesis of GABA, a major inhibitory neurotransmitter. Thus, Glul modulates levels of glutamate, glutamine, and GABA in the brain [48], and influences GABAergic neurotransmission [49]. As GABA signaling regulates maternal defense [11], it may be that Glul plays a role in the emergence of offspring protection during the postpartum period.

Research on the role of excitatory neurotransmitter glutamate in maternal behavior has so far received little attention, but future

![Figure 1. Quantitative real-time PCR analysis of Fabp7, Gal, Glul, Igfbp3, Npy1r and Sstr1 expression in the septum.](image)

Relative expression distribution of mRNA (y-axis) represented as a ratio of maternal versus virgin mice, was calculated using Ywhaz and CycA as reference genes, and shown by box-and-whisker plot as medians (dashed lines), interquartile range (boxes) and ranges (whiskers). Ratios over one indicate genes with higher expression in maternal relative to virgin mice, and ratios of less than one indicate genes with lower expression in maternal as opposed to virgin mice. Confirming array results, postpartum mice exhibited increased Glul, Igfbp3, Sstr1 and decreased Fabp7 and Gal mRNA levels relative to virgin control mice. Npy1r was not confirmed. *P<0.05 maternal mice versus virgin control.

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Table 2. List of all miRNAs found to be significantly linked with upregulated gene expression in postpartum mice using GSEA.

| MIR-1   | MIR-30E-5P | MIR-138 | MIR-195 | MIR-301 | MIR-381 | MIR-501 |
|---------|------------|---------|---------|---------|---------|---------|
| MIR-9   | MIR-31     | MIR-139 | MIR-197 | MIR-302A-D | MIR-382 | MIR-505 |
| MIR-15A-B | MIR-32   | MIR-141 | MIR-199A-B | MIR-320 | MIR-409-3P | MIR-512-3P |
| MIR-16   | MIR-33     | MIR-142-3P | MIR-200A-C | MIR-323 | MIR-409-5P | MIR-512-5P |
| MIR-17-3P | MIR-34A-C | MIR-142-5P | MIR-202 | MIR-324-3P | MIR-410 | MIR-515-3P |
| MIR-17-5P | MIR-92    | MIR-143 | MIR-203 | MIR-326 | MIR-422A-B | MIR-516-5P |
| MIR-18A-B | MIR-93   | MIR-144 | MIR-204 | MIR-329 | MIR-424 | MIR-518A-2 |
| MIR-19A-B | MIR-96   | MIR-145 | MIR-205 | MIR-335 | MIR-429 | MIR-518C |
| MIR-20A-B | MIR-101  | MIR-148A-B | MIR-206 | MIR-337 | MIR-432 | MIR-519A-D |
| MIR-21   | MIR-103   | MIR-149 | MIR-211 | MIR-346 | MIR-448 | MIR-520A-H |
| MIR-22   | MIR-105   | MIR-150 | MIR-212 | MIR-361 | MIR-449 | MIR-524 |
| MIR-24   | MIR-106A-B | MIR-152 | MIR-214 | MIR-363 | MIR-452 | MIR-526B |
| MIR-25   | MIR-107   | MIR-153 | MIR-216 | MIR-365 | MIR-485-3P | MIR-527 |
| MIR-26A-B | MIR-122A | MIR-154 | MIR-218 | MIR-367 | MIR-485-5P |
| MIR-27A-B | MIR-124A | MIR-155 | MIR-219 | MIR-369-3P | MIR-487 |
| MIR-28   | MIR-125A-B | MIR-181A-D | MIR-221 | MIR-372 | MIR-493 |
| MIR-30A-3P | MIR-128A-B | MIR-182 | MIR-222 | MIR-373 | MIR-494 |
| MIR-30A-3P | MIR-129   | MIR-183 | MIR-223 | MIR-374 | MIR-495 |
| MIR-30B-D | MIR-130A-B | MIR-186 | MIR-224 | MIR-377 | MIR-496 |
| MIR-30E-3P | MIR-132   | MIR-194 | MIR-299-5P | MIR-378 | MIR-497 |

For a given binding site near an upregulated gene, in some cases only one miRNA is identified, but for other sites multiple miRNAs are identified. In the list, if a dash separates two letters, that indicates all miRNAs within those letters were also significant (e.g., MIR-181A-D indicates miRNAs 181A, 181B, 181C, and 181D). Table S2 provides additional information on the binding sites for the miRNAs and p-values.

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work evaluating glutamate and maternal care appears to be merited.

We identified increased Sstr1 transcript in the septum during lactation. The neuropeptide, somatostatin, has been linked to hormone release, cell proliferation, and many pathophysiological processes of brain disorders [50]. Somatostatin exerts its actions by interacting with specific G-protein coupled receptors. Sstr1 acts as an inhibitory autoreceptor located on somatostatin neurons and is involved in the modulation of the ultradian release of growth hormone from pituitary [51]. As somatostatin has been shown to regulate GABA receptor functions [52], and GABA signaling is involved in maternal defense, it will be interesting to know whether and how Sstr1 regulates this aspect of maternal care.

It should be noted that many genes implicated in metabolism, cell differentiation, or proliferation exhibited altered expression pattern during lactation, suggesting that septum may be an important neural site for lactation-induced metabolic and structural alterations. As can be seen in Table 1, 18 out of 116 genes were linked to cell division, death, or proliferation, while about 16% were metabolism-related genes. However, the mechanism by which these genes regulate metabolic and structural adaptations during lactation remains to be evaluated. This study provides potential candidate genes for future research to answer these questions.

| Table 3. List of transcription factors (and corrected p-values) that are significantly linked to upregulation of subsets of genes in maternal mice using GSEA. |
|-----------------|----------------|----------------|----------------|----------------|
| ELK1            | 0.00000        | RFX1           | 0.00000        | TFDP2 0.0210   |
| YY1             | 0.00000        | GABPB2         | 0.0010         | MYC 0.0080     |
| NRF2            | 0.00000        | E2F            | 0.0010         | SOX9 0.0080    |
| ATF2            | 0.00000        | HSFI1          | 0.0020         | HLF 0.0080     |
| E4F1            | 0.00000        | HIFA1          | 0.0020         | ZF5 0.0090     |
| E4BP4           | 0.00000        | ATF4           | 0.0040         | HSFI2 0.0130   |
| NFMyel1         | 0.00000        | E2F1           | 0.0060         | NFMyel1 0.0130 |
| NRF1            | 0.00000        | SREBF1         | 0.0070         | TFDP1 0.0190   |

GSEA website (http://www.broadinstitute.org/gsea/index.jsp) provides information on the conserved binding motif along with all gene targets. Table 3 provides information on p-values and names used by GSEA. Abbreviations: ATF, activating transcription factor; E2F, E2F transcription factor; E4F, E4F transcription factor; ELK1, ELK1, member of ETS oncogene family; ERR1, oestrogen receptor related 1; GABPB2, GA repeat binding protein, beta 2; HIF1, hypoxia inducible factor 1; HLF, hepatic leukemia factor; HSFI1, heat shock factor; MAX, max protein; MYC, myelocytomatosis oncogene; NMYC, neuroblastoma myc-related oncogene; NRF1, nuclear respiratory factor 1; SOX9, paired box gene 3; RFX1, regulatory factor X, 1; Sox1, splicing factor 1; SOX9, SRY-box containing gene 9; SREBF1, sterol regulatory element binding transcription factor 1; TFDP, transcription factor Sp; YY1, YY1 transcription factor; ZF5, zinc finger protein 5.

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Genes Previously Shown to be Differentially Expressed During Lactation Not Identified in the Present Study

Expression of neuropeptide Y, preproenkephalin, and oxytocin has been reported to be increased during lactation [22,24,33,34], whereas proopiomelanocortin was down-regulated [53]. Although these changes were not observed in this study, one possible explanation for negative results is a region-specific effect as these neuropeptides were examined in other brain regions such as hypothalamus and Arc, while the septum was tested in this study. Alternatively, as discussed below, using the whole septum as a target, rather than subregions of septum, may contribute to the lack of difference. In addition to the above two possible factors, time lapse between the parturition and the gene analysis may be another contributing factor. Previous studies have shown that gene expression in specific brain regions undergoes a time-course change from parturition through lactation [24,34,35]. For example, preproenkephalin mRNA in the anterior arcuate nucleus of lactating versus virgin females significantly increased on postpartum day 10, but did not differ on postpartum day 3 [24], suggesting that gene expression is dynamic and alters during the postpartum period. Future study will be directed towards understanding the time-course effect on gene expression during the postpartum period.

Gene Set Enrichment Analysis

One surprising result from this study was the finding of a large number of miRNAs and transcription factors (N = 177) that were significantly linked to the upregulation of genes in maternal mice, whereas no significant links were found with downregulated maternal expression. Why so many regulators for upregulation, but not one for downregulation was found is not clear, but the finding may reflect the underlying processes of developing the maternal brain. It was also surprising to find a high number of miRNAs that were linked to upregulated gene expression. The identification of 29 transcription factors, including Elk-1, YY1, and HSFI1, that were linked to upregulated genes provides possible new insight into how the maternal brain may be produced. Although key roles for estrogen and progesterone receptors have been found in producing the maternal brain [27,28], steroid receptors were not identified here, with the exception of estrogen related receptor 1 (Err1). One explanation is that steroid receptors alter expression of other transcription factors and the ones identified here may be key agents for some of the final changes in maternal gene expression in septum. Some of the transcription factors identified by GSEA were also found to have altered expression from the PLIER array analysis (P<0.05), such as HSFI1. It is not necessarily surprising that transcription factors or miRNAs linked by GSEA to altered expression are themselves not found to be differentially expressed because their action could have occurred prior to the day of tissue collection (e.g., during pregnancy or early postpartum).

It was also noteworthy that some conserved binding sites were found that were linked to maternally upregulated genes, although there are currently no known factors that bind to these sites. Future studies that determine whether or how these sites are acted upon will be critical for developing an understanding of the relevance of these binding sites.

Methodological Considerations and Limitations

A number of factors contribute to the formation of the maternal brain, including the experience of mating, pregnancy, parturition, lactation, and the sensory input from the pups. All these factors orchestrate many of changes in gene expression that occur during
the postpartum period. This study seeks to explore the results of this constellation of experiences by examining the differences in gene expression between virgin mice, who have never been exposed to any of these experiences, and lactating mice, who have been exposed to all of them. Thus, the observed changes in gene expression in postpartum female mice do not simply reflect maternal care or lactation, but rather the integration of multiple factors. In this study, we did not discriminate between virgins at different stages of the estrus cycle although estrous stage can influence gene expression [57]. Virgin mice were either in estrus, diestrus I and diestrus II (periods of relatively consistent estradiol and progesterone levels), but not proestrus. In this way, only differences large enough to rise above any noise created by varying estrus states appear in the results. While this noise may obscure differences in gene expression, the present approach serves to highlight general differences in virgin and postpartum mice. However, subtler differences between the virgin and lactating mice may be missed. It should be noted that virgin female mice were fed a breeder diet to provide identical food to both groups. This feeding paradigm may have had a somewhat different effect on
Table 4. Primers of interest and reference genes used for quantitative real-time PCR assay.

| Gene       | Full name                                                                 | NCBI accession number | Primer sequence forward | Primer sequence reverse |
|------------|---------------------------------------------------------------------------|-----------------------|-------------------------|-------------------------|
| CycA       | Cyclophilin A                                                              | NM_008907.1           | 5'-TGCTGGACAAACACAACACC-3' | 5'-GCTCTTTCACCTTCCAAA-3' |
| Fabp7      | Fatty acid binding protein 7                                                | NM_021272.3           | 5'-TAAGCTGTGTTGTCGTTGG-3' | 5'-CCCCAAGGGATAGTCAGCAG-3' |
| Gal        | Galanin                                                                   | NM_010253.3           | 5'-GAGACCAATCCATGCCCAC-3' | 5'-ATGGTCTCAGGACCTTCTAGG-3' |
| Glu        | Glutamate-ammonia ligase                                                   | NM_008131.3           | 5'-TGAGAGAACCATCCTATTCATG-3' | 5'-TAAGCGTAATGAACTGGAGACC-3' |
| Igfbp3     | Insulin-like growth factor binding protein 3                               | NM_008343.2           | 5'-GGAAACCCATTAAAGGATAG-3' | 5'-GCCACAGTCCCAAGTAGATC-3' |
| Npy1r      | Neuropeptide Y receptor 1                                                  | NM_010934.4           | 5'-GCCACTCAGACATTTCTAAC-3' | 5'-CTCCACAGTCCCAAGTAGATC-3' |
| Sstr1      | Somatostatin receptor 1                                                    | NM_009216.3           | 5'-GCTGTCCAATTTGAGTATGGTC-3' | 5'-CAGGTCTAGTGGAATTCCGCG-3' |
| Ywhaz      | Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide | NM_011740.3 | 5'-TCCTTATCCCCTTCTGGCGAC-3' | 5'-ATAATCCATACATTACCGGTT-3' |

*CycA primer sequences were described in published literature [74].

Gene expression changes relative to regular rodent chow as breeder diet differentially modulates expression of genes implicated in food intake and energy balance due to its high fat content [50,59]. One limitation of using whole septum is that we are unable to determine whether changes occur throughout septum or are specific to subregions. Future studies examining subregions can address this issue. Also, if changes in gene expression occur in opposite directions within these subregions, then the difference may be missed as false negatives. A related general issue is that microarray analysis can produce false negatives. The chips target a subset of regions along a given mRNA transcript and it is possible with different targeting that significant differences may have been found. Although different algorithms produce different false negative rates [60,61], the approach used here, PLIER, is the current recommended algorithm as it introduces a higher sensitivity to changes in abundance for targets near background [60]. One example of a possible false negative is that we recently glutamic acid decarboxylase (GAD67) mRNA in the septum to be upregulated in lactating versus virgin mice (unpublished observation), while no difference was observed in this study. Microarray technology has become a useful tool for analyzing gene expression profiling and in identifying new genes or molecular pathways involved in multiple processes [62–64]. Five out of six genes identified as being differentially expressed (except Npy1r) in this study were confirmed by real-time PCR, validating our microarray analysis as reliable means to evaluate gene expression.

Conclusions

Using high density oligonucleotide microarrays, we observed a distinct gene expression profile in the septum of maternal mice. Given that the identified genes may play a role in diverse biological processes, this study provides new insights into the genes linked to the septum and brain changes that promote the emergence of maternal care during the postpartum period. Our findings suggest a potential key role for miRNAs in creating the maternal brain and could open an important new area of research. Future studies could involve directly evaluating and manipulating miRNAs to determine the relative importance of miRNAs in producing a maternal brain.

Materials and Methods

Animals

Untested nulliparous female mice from a line of mice previously selected for high maternal defense (original stock was outbred hsd:ICR mice) [Mus domesticus] (Harlan, Madison, WI) were used. Selected mice were used because they exhibit the reliable emergence of a number of maternal characteristics, including nursing and offspring protection, and thus provide a solid platform for comparing maternal and non-maternal brains. As for any work that uses one strain, there is a limitation in knowing whether results apply to other strains. Only with comparisons across strains and species can reliable alterations with the postpartum period be identified, but here we provide a start for examining septal changes in gene expression with maternal behavior in mice. All animals were age matched (~70 days old at time of dissection). For mating, females were housed with a breeder male (hsd:ICR strain) for 2 weeks. At the same time, virgin females were co-housed to provide similar social stimuli. When breeder males were removed, all females (pregnant and virgin) were housed individually and provided precut nesting material until dissections. Thus, virgin and postpartum females experienced similar levels of co-housing and single housing. The timing of cohousing and isolation was performed to minimize the effects of isolation-induced stress. Polypropylene cages were changed once weekly, but when pups were born (postpartum Day 0), cages were not changed for the postpartum female or the age matched virgin control for the remainder of the experiment. All animals were housed in the same room and cages of virgin and lactating females were alternated.
with one another on the same shelves. A 14:10 light/dark cycle with lights on at 06:00 h CST was used. Female mice were given ad lib access to breeder chow (Harlan) and tap water. All procedures were carried out in strict accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and studies were approved by the University of Wisconsin Animal Care and Use Committee.

**Tissue Collection and RNA Extraction**

On postpartum Days 6–8, brains were removed from lactating females between 10:00 and 12:00 h. Brains from age-matched, virgin females were also removed on the same day and dissections were alternated between the two groups so that an equal number of dissections from each group were obtained. The animals were lightly anesthetized with isoflurane, killed by cervical dislocation and then decapitated. Following decapitation, virgin females were examined for stage of estrous cycle using a vaginal lavage [65,66]. Female mice in vaginal proestrus were not used because this stage is associated with mating in mice. For the microarray study, the stages of the six virgin mice were estrous, diestrus I, and diestrus II (N = 2 each). Although estrous stage can influence gene expression [57], the use of mice from different estrous states (excluding proestrus) provides a generic view of the virgin female and should provide insights into reliable differences between maternal and virgin mice. The whole brain was removed, snap frozen in isopentane on dry ice, and then stored at −80°C until sliced. Brain sections were sliced in a cryostat (Leica, CM1850, Bannockburn, IL, USA) with the first two sections at 300 microns and the last section at 200 microns and then mounted on glass slides. Target tissue was captured using a micropunch technique [67]. Microdissection of frozen brain sections was made with Brain Punch Set (Stoelting, Wood Dale, IL, USA) under a dissecting microscope. The whole septum including lateral and medial parts (Fig. 2) was collected bilaterally from Bregma 1.10 to 0.14 and pooled, so that each mouse provided one sample of the septum. Microdissections from ten animals in each group were flash frozen on dry ice and stored at −80°C until being processed for either gene array analysis or real-time PCR. Total RNA was extracted and prepared in matched pairs (virgin versus lactating) using an Aurum Total RNA Fatty and Fibrous Tissue Kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer’s instructions. Following isolation, the integrity of RNA was assessed using Agilent RNA 6000 Nano Chips with Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA). The purity of RNA was tested, and the yield of RNA was determined using NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Purified total RNA was stored at −80°C until processed.

**High-Density Oligonucleotide Array Hybridization**

Six out of ten samples in each group (n = 6 per group) were randomly chosen for use in the microarray experiment. Microarray analysis was performed with the GeneChip Mouse Gene 1.0 ST array (Affymetrix, Santa Clara, CA, USA) using targets derived from total RNA isolated from septum as described above. cDNA for array hybridization was synthesized from 200 ng of total RNA using an Ambion GeneChip WT Expression Kit (Ambion, Austin, TX, USA) according to the manufacturer’s specifications. Briefly, total RNA was used to synthesize double-stranded cDNA, which was then used as a template for single-stranded cRNA synthesis. This cRNA was in turn used as a template for a second round of single-stranded cDNA synthesis, and the resultant DNA-RNA hybrids were then degraded using RNase H. Amplified cDNA was then fragmented and biotinylated using an Affymetrix WT Terminal Labeling Kit (Affymetrix, Santa Clara, CA, USA) according to the manufacturer’s specifications. Fragmented and labeled cDNA samples were hybridized with the arrays at 45°C for 16 hours. The hybridized arrays were then washed and stained according to manufacturer specifications, and arrays were scanned at 570 nm on an Affymetrix GC3000 G7 Scanner. Data were extracted and processed from scans using Affymetrix Command Console v. 3.1.1.1229. cDNA synthesis, fragmentation, labeling, array hybridization, staining, and scanning were performed by the Gene Expression Center at the University of Wisconsin-Madison.

**Probeset Level Summarization and Microarray Statistical Analysis**

Probeset level summarization and data normalization were performed with the PLIER algorithm with GC bin background correction using Affymetrix Power Tools v. 1.12.0. The microarray data discussed in this publication, both raw and summarized, have been deposited in NCBI’s Gene Expression Omnibus [68], and are accessible through GEO Series accession number GSE30836 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE30836). GEO reporting fulfills the requirements of the MIAME. Inferential statistics for differential expression between maternal and virgin samples were calculated using the array-specific empirical Bayesian implementation of ANOVA in the BioConductor package limma v.3.6.9 [69]. Both nominal and false discovery rate corrected p-values were calculated, and fold-change differences for each gene were calculated in Excel by dividing the limma-calculated average maternal expression by the limma-calculated average virgin expression.

**Verification of Microarray Results with Quantitative Real-time PCR (qPCR)**

To confirm gene expression results obtained from microarray analysis, six genes: fatty acid binding protein 7 (Fabp7), galanin (Gal), glutamate-ammonia ligase (GluL/glutamine synthetase (GS), insulin-like growth factor binding protein 3 (Igfbp3), neuropeptide Y receptor 1 (Npy1r), and somatostatin receptor 1 (Sstr1) were evaluated using qPCR. These genes were chosen because of their possible involvement in multiple lactation-induced processes and the function of the genes is described in the Discussion. A SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA) was used to reverse transcribe 100 ng of RNA to cDNA in an Eppendorf MasterCycler Personal PCR machine (Eppendorf, Hamburg, Germany) using poly-T 20mer primers. The cDNA was then amplified using a SsoFast EvaGreen Supermix kit (Bio-Rad, Hercules, CA, USA) in a StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA, USA). The amplification mixtures (20 μL) contained 1x SsoFast EvaGreen Supermix, 160 ng template cDNA, and 500 nM forward and reverse primers. Each sample was run in triplicate and standard amplification procedures were used. The cycling profile is as follows: an initial melting step at 95°C for 30 sec followed by 40 cycles of a 95°C melting step for 5 sec, a 58°C annealing step except Fabp7 (at 60°C) for 20 sec, and a 72°C elongation step for 20 sec. Primers for genes of interest and reference genes (Table 4) were designed and screened for specificity using NCBI Primer-BLAST. Ywhaz and Ccna were used as reference genes, as they are found to be among the most stable genes in rodent brain [70,71] and found not to be different between lactating and virgin females in our arrays results. Following amplification, a standard curve was generated to assess the empirical PCR reaction efficiency, and a dissociation curve analysis was performed to insure specificity of PCR products.
values were calculated using the StepOnePlus software. The expression ratio of mRNA of genes in lactating relative to virgin (normalized to the reference genes Ywhaz and CycA) was calculated using a relative expression software tool REST 2009, which corrects for empirical PCR efficiency, allows for the use of multiple reference genes, and utilizes a randomization test of significance [72]. The N’s used for qPCR were as follows: virgin, N = 10; maternal, N = 10. Four females were added to each group from the array mice and the virgin states were estrous (N = 2), diestrous I (N = 4), and diestrous II (N = 4).

Gene Set Enrichment Analysis (GSEA)

GSEA is a technique that calculates up or down regulation of precompiled lists of genes and was performed using GSEA software v. 2.07 [30]. The specific gene sets described here came from the MSigDB C3 computationally-curated gene set of miRNA and transcription factor binding sites. For transcription factor binding sites, data from multiple species were used, including mice, humans, and rats. For miRNA, only human data sets were available, but binding sites for given miRNAs in mice and humans are highly conserved [73]. The analysis used z-transformed values for PLIER-normalized array intensities, t-tests to calculate individual gene inferential statistics, gene set permutation, and a weighted test statistic. The GSEA output provides both nominal and corrected p-values for each target.

Supporting Information

Table S1 Full list of all targets, their relative expression and P value ranking using PLIER algorithm. (XLS)

Table S2 List of target sites linked significantly with upregulation of subsets of genes in postpartum mice by GSEA. (DOC)

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Author Contributions

Conceived and designed the experiments: CZ SG. Performed the experiments: CZ MS TD. Analyzed the data: CZ MS SG. Contributed reagents/materials/analysis tools: SG. Wrote the paper: CZ SG.

References

1. Montagnese CM, Poulain DA, Vincent JD, Theodosiou DT (1987) Structural plasticity in the rat suprachiasmatic nucleus during gestation, post-partum lactation, and suckling-induced pseudogestation and lactation. J Endocrinol 115: 97–105.
2. Russell JA, Douglas AJ, Ingram CD (2001) Brain preparations for maternity–adaptive changes in behavioral and neuroendocrine systems during pregnancy and lactation. An overview. Prog Brain Res 133: 1–38.
3. Neumann ID (2003) Alterations in behavioral and neuroendocrine stress coping strategies in pregnant, parturient and lactating rats. Prog Brain Res 133: 143–152.
4. Levy F, Glaser G, Keller M (2011) Plasticity of the parental brain: a case for neurogenesis. J Neuroendocrinol 23: 981–993.
5. Rosenblatt JS, Lehrman DS (1963) Maternal behavior in the laboratory rat. In: Rheingold HL, ed. Maternal behavior in mammals. New York: John Wiley and Sons. pp 8–57.
6. Lightman SL (1992) Alterations in hypothalamic-pituitary responsiveness during lactation. Ann N Y Acad Sci 632: 340–346.
7. Windle RJ, Wood S, Shanks N, Perks P, Colde GL, et al. (1997) Endocrine and behavioural responses to noise stress: comparison of virgin and lactating female rats during non-disrupted maternal activity. J Neuroendocrinol 9: 407–414.
8. Smith MS, Grove KL (2002) Integration of the regulation of reproductive function and energy balance: lactation as a model. Front Neuroendocrinol 23: 225–256.
9. Zhao C, Li M (2010) c-Fos identification of neuroanatomical sites associated with maternal behavior in the rat. Neuroscience 165: 1043–1053.
10. Fleischer S, Stotnick BM (1978) Disruption of maternal behavior in rats with lesions of the septal area. Physiol Behav 21: 189–200.
11. Lee G, Gammie SC (2009) GABA/A receptor signaling in the lateral septum regulates maternal aggression in mice. Behav Neurosci 123: 1169–1177.
12. D’Anna KL, Gammie SC (2009) Activation of corticotropin-releasing factor receptor 2 in lateral septum negatively regulates maternal defense. Behav Neurosci 123: 356–368.
13. Risold PY, Swanson LW (1997a) Connections of the rat lateral septal complex. Brain Res Brain Res Rev 24: 115–195.
14. Risold PY, Swanson LW (1997b) Chemoarchitecture of the rat lateral septal nucleus. Brain Res Brain Res Rev 24: 91–113.
15. Sheehan TP, Chambers RA, Russell DS (2004) Regulation of affect by the lateral septum: implications for neuropsychiatry. Brain Res Brain Res Rev 46: 21–117.
16. Jakab RL, Lezath C (1995) Septum. In: Paxinos G, ed. The Rat Nervous System. San Diego: Academic Press. pp 405–442.
17. Everts HG, Koellhaas JM (1999) Differential modulation of lateral septal vasopressin receptor blockade in spatial learning, social recognition, and anxiety-related behaviors in rats. Behav Brain Res 99: 7–16.
18. Lukas M, Bredeow R, Landgraf R, Neumann ID, Veenuena AH (2011) Early life stress impairs social recognition due to a blunted response of vasopressin release within the septum of adult male rats. Psychoneuroendocrinology 36: 843–853.
19. Veenuena AH, Beiderbeck DJ, Lukas M, Neumann ID (2010) Distinct correlations of vasopressin release within the lateral septum and the bed nucleus of the stria terminalis with the display of intimate aggression. Horm Behav 58: 271–287.
20. Klein RL, Hirko AC, Meyers CA, Grimes JR, Muzycka N, et al. (2000) NGF gene transfer to intrinsic basal forebrain neurons increases cholinergic cell size and protects from age-related, spatial memory deficits in middle-aged rats. Brain Res 873: 144–151.
21. Rudlick CN, Gibbs RB, Woolley GS (2000) A role for the basal forebrain cholinergic system in estrogen-induced inhibition of hippocampal pyramidal cells. J Neurosci 20: 4479–4490.
22. Smith MS (1995) Lactation alters neuropeptide-Y and proopiomelanocortin gene expression in the arcuate nucleus of the rat. Endocrinology 133: 1258–1265.
23. Chen P, Li C, Haskell-Laenvano C, Cone RD, Smith MS (1999) Altered expression of agouti-related protein and its colocalization with neuropeptide Y in the arcuate nucleus of the hypothalamus during lactation. Endocrinology 140: 2645–2650.
24. Ottinger MA, Rosewell KL, Weiland NG, Margaretten KT, Wise PM (1995) Effect of lactation on hypothalamic preproenkephalin gene expression. J Neuroendocrinol 7: 341–346.
25. Broad KD, Kendrick KM, Sirinathsinghi DJ, Keverne EB (1993) Changes in oxytocin immunoreactivity and mRNA expression in the sheep brain during pregnancy, parturition and lactation and in response to oestrogen and progesterone. J Neuroendocrinol 5: 435–444.
26. Kurenda KO, Tachikawa K, YodaIha S, Tsumooka Y, Numan M (2011) Neuronomolecular basis of parental behavior in laboratory mice and rats: with special emphasis on technical issues of using mouse genetics. Prog Neuro-psychopharmacol Biol Psychiatry 35: 1205–1231.
27. Giordano AL, Siegel HJ, Rosenblatt JS (1989) Nuclear estrogen receptor binding in the preoptic area and hypothalamus of pregnancy-terminated rats: correlation with the onset of maternal behavior. Neuroendocrinology 50: 240–258.
28. Numan M, Roach JK, del Cerro MC, Guillamón A, Segovia S, et al. (1999) Expression of intracellular progesterone receptors in rat brain during different reproductive states, and involvement in maternal behavior. Brain Res 830: 350–371.
29. Kinsley CH, Amory-Meyer E (2011) Why the maternal brain? J Neuroendocrinol 23: 971–983.
30. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, et al. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102: 15545–15550.
31. Haggstrom OM, Markert UR (2011) MicroRNAs in pregnancy. J Reprod Immunol 88: 106–111.
32. Silveri L, Tilly G, Vilotte JL, Le Provost F (2006) MicroRNA involvement in estrous cycle and ovarian cycle. Hum Reprod 21: 954–961.
33. Xiao XQ, Grove KL, Lau SY, Mccweeney S, Smith MS (2005) Desoxyribonucleic acid microarray analysis of gene expression pattern in the arcuate nucleus/ventromedial nucleus of hypothalamus during lactation. Endocrinology 146: 4931–4938.
34. Gammie SC, Hasen NS, Awad TA, Auger AP, Jessen HM, et al. (2005) Gene array profiling of large hypothalamic CNS regions in lactating and randomly cycling virgin mice. Brain Res Mol Brain Res 139: 201–211.
35. Clemmons DR, Underwood LE (1991) Nutritional regulation of IGF-I and IGF binding proteins. Annu Rev Nutr 11: 393–412.
36. Lautero TJ, Marsen L, Daughaday WH, Baile CA (1987) Evidence for the role of insulin-like growth factor II (IGF-II) in the control of food intake. Physiol Behav 40: 755–758.
37. Butler ST, Marc AL, Pelton SH, Radcliffe RP, Lucy MC, et al. (2003) Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-I and GH receptor 1A. J Endocrinol 176: 205–217.
38. Kalluri HS, Dempsey RJ (2011) IGF-1R3 inhibits the proliferation of neural progenitor cells. Neurochem Res 36: 406–411.
39. Gluckman P, Klement N, Guan J, Mallard C, Sirimanne E, et al. (1992) A role for IGF-I in the rescue of CNS neurons following hypoxic-ischemic injury. Biochem Biophys Res Commun 182: 593–599.
40. Mills JL, Hediger ML, Molloy CA, Chrousos GP, Manning-Courtney P, et al. (2007) Elevated levels of growth-related hormones in autism and autism spectrum disorder. Clin Endocrinol (Oxf) 67: 230–237.
41. Arai Y, Funatsu N, Numayama-Tsuruta K, Nomura T, Nakamura S, et al. (2003) Role of Fabp7, a downstream gene of P6x, in the maintenance of neuroepithelial cells during early embryonic development of the rat cortex. J Neurosci 23: 9752–9761.
42. Ovada Y, Abdelwahab SA, Kitakata N, Sakagami H, Takane H, et al. (2006) Altered emotional behavioral responses in mice lacking brain-type fatty acid-binding protein gene. Eur J Neurosci 24: 175–187.
43. Gerstner JR, Vanderheyden WM, Shaw PJ, Landry CF, Yin JC (2011) Fatty-acid binding proteins modulate sleep and enhance long-term memory consolidation in Drosophila. PLoS One 6: e15890.
44. Gross L (2007) A candidate gene for a biological marker of schizophrenia in mice. PLoS Biol 5: e320.
45. Karlsson RM, Holmes A (2006) Galanin as a modulator of anxiety and depression and a therapeutic target for affective disease. Amino Acids 31: 231–239.
46. Hokfelt T, Sjoerdsma A, Bjoerklund A, Holmberg G, Stenevi U, et al. (1976) Gene Expression Changes in the Septum
47. Hokfelt T, Broberger C, Dietz M, Xo ZQ, Shi T, et al. (2005) Galanin and NPY, galanin in cognition and affect. Prog Neuropsychopharmacol Biol Psychiatry 25: 343–362.
48. Fonnum F, Paulsen RE (1990) Comparison of transmitter amino acid levels in the rat brain. Epilepsia 51: 1446–1455.
49. Cremer CM, Bidmon HJ, Gorg B, Palomero-Gallagher N, Escobar JL, et al. (1999) Galanin and NPY, galanin in cognition and affect. Prog Neuropsychopharmacol Biol Psychiatry 25: 343–362.
50. Olias G, Viollet C, Kusserow H, Epelbaum J, Meyerhof W (2004) Regulation of galanin gene expression in the ventromedial hypothalamus of the rat: temporal qualities and synergy with progesterone. Brain Res Mol Brain Res 5: 51–58.
51. Stroh T, van Schouwenburg MR, Beaudet A, Tannenbaum GS (2009) IGFBP-3 inhibits the proliferation of neural progenitor cells. Neurochem Res 36: 406–411.
52. Schaffhauser AO, Madiehe AM, Braymer HD, Bray GA, York DA (2002) Effects of a high-fat diet and strain on hypothalamic gene expression in rats. Obes Res 10: 1188–1196.
53. Romano GJ, Mobbs CV, Howells RD, Pfaff DW (1989) Estradiol treatment results in decreased expression of galanin mRNA in the hypothalamus. Brain Res 46: 9–16.
54. da Costa AP, Wood S, Ingram CD, Lightman SL (1996) Region-specific reduction in stress-induced c-fos mRNA expression during pregnancy and lactation. Brain Res 742: 177–194.
55. Shenouda SK, Alahari SK (2009) MicroRNA function in cancer: oncogene or a tumor suppressor? Cancer Metastasis Rev 28: 369–378.
56. Schrauwers AO, Madiehe AM, Braymer HD, Bray GA, York DA (2002) Effects of a high-fat diet and strain on hypothalamic gene expression in rats. Obes Res 10: 1188–1196.
57. Rosati B, Grau F, Kuehler A, Rodriguez S, McKinnon D (2004) Comparison of different probe-level analysis techniques for oligonucleotide microarrays. Biotechniques 35: 316–322.
58. Stroh T, van Schouwenburg MR, Beaudet A, Tannenbaum GS (2009) IGFBP-3 inhibits the proliferation of neural progenitor cells. Neurochem Res 36: 406–411.