Development/Plasticity/Repair

The Neonatal Ventromedial Hypothalamus Transcriptome Reveals Novel Markers with Spatially Distinct Patterning

Deborah M. Kurrasch,* Clement C. Cheung,* Florence Y. Lee, Phu V. Tran, Kenji Hata, and Holly A. Ingraham
Departments of Physiology and Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, California 94143

The ventromedial hypothalamus (VMH) is a distinct morphological nucleus involved in feeding, fear, thermoregulation, and sexual activity. It is essentially unknown how VMH circuits underlying these innate responses develop, in part because the VMH remains poorly defined at a cellular and molecular level. Specifically, there is a paucity of cell-type-specific genetic markers with which to identify neuronal subgroups and manipulate development and signaling *in vivo*. Using gene profiling, we now identify ~200 genes highly enriched in neonatal (postnatal day 0) mouse VMH tissue. Analyses of these VMH markers by real or virtual (Allen Brain Atlas; http://www.brain-map.org) experiments revealed distinct regional patterning within the newly formed VMH. Top neonatal markers include transcriptional regulators such as Vgll2, SF-1, Sox14, Satb2, Fezf1, Dax1, Nkx2-2, and COUP-TFII, but interestingly, the highest expressed VMH transcript, the transcriptional coregulator Vgll2, is completely absent in older animals. Collective results from zebrafish knockdown experiments and from cellular studies suggest that a subset of these VMH markers will be important for hypothalamic development and will be downstream of SF-1, a critical factor for normal VMH differentiation. We show that at least one VMH marker, the AT-rich binding protein Satb2, was responsive to the loss of leptin signaling ($Lep^{ob/ob}$) at postnatal day 0 but not in the adult, suggesting that some VMH transcriptional programs might be influenced by fetal or early postnatal environments. Our study describing this comprehensive "VMH transcriptome" provides a novel molecular toolkit to probe further the genetic basis of innate neuroendocrine behavioral responses.

Key words: VMH; SF-1; CNS development; gene profiling; hypothalamus; Vgll2; zebrafish

Introduction

The hypothalamus integrates various endocrine and autonomic physiologies and consists of anatomically distinct nuclei that together function to regulate sleep, circadian rhythm, energy homeostasis, sexual behaviors, and thermoregulation (Swanson, 1987). For most hypothalamic regions, the identity of neuronal subtypes within each nucleus remains poorly defined and has relied primarily on expression of specific neuropeptides or their receptors. In fact, even for well studied regions, such as the arcuate nucleus (ARC) and the paraventricular nucleus (PVN), the cellular complexity of these regions is essentially unknown. This lack of understanding of neuronal subtypes is especially true in the large ventromedial hypothalamus (VMH, VMN), which is located in the medial hypothalamus adjacent to the third ventricle. Based on rodent lesion analyses, this hypothalamic region appears critical for female reproductive behavior, initiating an innate fear response, maintaining daily rhythms of glucocorticoid secretion, and for normal feeding responses (Egawa et al., 1991; Cohen and Pfaff, 1992; Flier and Maratos-Flier, 1998). In rats, however, the most exaggerated response associated with VMH lesions is extreme hyperphagia, resulting in obesity (King, 2006b). Despite the intrinsic limitations of physical lesions studies, selective VMH genetic lesions of the leptin receptor (LEPR) or the glutamate synaptic vesicular transporter VGLUT2 also result in moderate disruption of normal energy homeostasis (Dhillon et al., 2006; Tong et al., 2007).

In rodent coronal brain sections, the VMH is easily recognized by the prominent bilateral Nissl-stained clusters located dorsal to the ARC and posteroventral to the PVN. These clusters are visible as early as embryonic day 15 (E15) (McClellan et al., 2006). In the adult, the VMH can be subdivided crudely into three anatomical regions: the dorsomedial (VMH_{DM}), central (VMH_{C}), and ventrolateral (VMH_{VL}). Functional studies suggest that the VMH_{DM} regulates energy homeostasis, whereas the VMH_{VL} controls female reproduction. To date, the VMH_{C} is not associated with any specific function, and there are no known molecular markers specific to this subregion.

Despite the implied role of the VMH in energy balance and other innate responses, identifying genes associated with or needed for the development of these circuits has not been forthcoming. Two obstacles prevent further dissection of the apparent cellular complexity of the VMH. First, there are no distinct layers

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*D.M.K. and C.C.C. contributed equally to this work.

Correspondence should be addressed to Holly A. Ingraham, Department of Physiology and Department of Cellular and Molecular Pharmacology, University of California, San Francisco, 1550 4th Street, San Francisco, CA 94143-2611. E-mail: holly.ingraham@ucsf.edu.

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or morphological characteristics in the VMH that would help classify neuronal subtypes as found for other brain regions. Second, only a few molecular markers have been described for the VMH as reviewed by McClellan et al. (2006). Indeed, a previous gene discovery approach in the adult hypothalamus identified only 10 VMH-enriched genes after comparing transcripts from laser-captured adult VMH, ARC, and dorsal medial hypothalamus (DMH) tissue, as summarized in supplemental Table S2 (available at www.jneurosci.org as supplemental material) (Segal et al., 2005). Their study identified the NR5A nuclear receptor SF-1 as the highest expressed VMH transcript consistent with the prominent expression of SF-1 in all three VMH subregions (Ikeda et al., 1995; Shinoda et al., 1995). The lack of molecular information for this large neuronal cluster prompted us to search for new VMH markers that would be expressed at a developmental stage [postnatal day 0 (P0)] when newly born neurons have already coalesced into a mature VMH cluster (Altman and Bayer, 1986; Pozzo Miller and Aoki, 1992) and when the neuroendocrine system must begin meeting the physiological demands of an external environment. This time point might not necessarily enrich for genes important for the birth or migration of VMH neurons from the ventricular zone that occurs between E10 and E17, as reviewed by McClellan et al. (2006), but would capture genes important for late developmental events involving VMH cell specification and function. Here, we report the first comprehensive neonatal VMH transcriptome, thereby providing a new molecular toolkit for understanding the development and function of this anatomically complex and physiologically diverse hypothalamic region.

Materials and Methods

Animals. Mice were kept on a 12 h light/dark cycle (lights off at 6:00 P.M.) and killed at 10:00 A.M. CD-1 wild-type mice were used for microarray ($n = 40$), *in situ* histochemistry ($n = 3$), and quantitative real-time PCR (qPCR) ($n = 4$) analyses. Heterozygous *sf-1*$^{+/-}$ and *Lep*$^{+/ob}$ mice were maintained on a C57BL/J6 background. Zebrafish were maintained at 28°C as described previously (Wehman et al., 2005). All research with animals was performed according to the guidelines approved by the Institutional Animal Care and Use Committee.

Tissue collection. Neonatal (P0) or adult mouse brains (CD-1) were embedded, sectioned (200 μm; VTI000s; Leica Microsystems, Nussloch, Germany), and the VMH was microdissected using visual outlines of the VMH capsule from six consecutive sections covering the region from 1.34 to 2.06 caudal to bregma, as defined by Paxinos and Franklin (2003). The remaining hypothalamus minus VMH tissue was dissected using the optic tract chiasm and the mammillary recess as the anterior and posterior limits, respectively. Total hypothalami were isolated from P0 or adult wild-type, *sf-1*$^{-/-}$ ($n = 4$), and *Lep*$^{ob/ob}$ ($n = 4$) using the same hypothalamic landmarks. Pooled tissues (microarray) or individual tissues (qPCR) were immediately placed in RNALater, and total RNAs were isolated using RNAqueous (Ambion, Austin, TX) and stored at −80°C.

Oligo microarray. MEEBO (Mouse Exonic Evidence Based Opensource) arrays were a generous gift from Dr. Joseph DeRisi (University of California, San Francisco, San Francisco, CA). VMH and hypothalamus minus VMH were obtained as described above. For each array, total RNAs (8 μg) of each sample were reverse transcribed and labeled with amino-allyl dUTP using reverse transcriptase and oligo-dT (Invitrogen, Carlsbad, CA). Labeled cDNAs were coupled to cyanine-3 or cyanine-5 dye (Monofunctional NHS-ester dye; GE Healthcare, Little Chalfont, UK), purified, combined, and hybridized to the microarray in a sealed chamber. Hybridization was performed at 65°C for 48 h. Slides were washed at 55°C in solution I (2× SSC, 0.03% SDS), solution II (1× SSC), and solution III (0.2× SSC) and quickly dried by centrifugation. Hybridized slides were scanned using an Axon Slide Scanner 4000B, and data were analyzed by Genepix software (Molecular Devices, Sunnyvale, CA).

In situ *hybridization.* Briefly, P0 brains were dissected, fixed immediately in 4% paraformaldehyde (PFA), and equilibrated in 30% sucrose/PBS. Brains were embedded and cryosectioned (20 μm each; 8–10 sections through the hypothalamus, total distance of ~2 mm) using the optic tract chiasm and the mammillary recess as the anterior and posterior limits, respectively (CM1900; Leica). digoxigenin (DIG)–cRNA probes were generated using a labeling kit (Roche, Indianapolis, IN) and hybridized to sections at 72°C overnight, washed in 0.2× SSC (65°C), and rinsed in TBS. Sections were incubated in a blocking solution and in diluted alkaline phosphatase (AP)-conjugated anti-DIG antibody (1:5000; Roche) overnight at 4°C, washed with TBS, equilibrated in developing buffer (in mM: 100 Tris, pH 9.5, 100 NaCl, and 50 $MgCl_2$), and developed in nitroblue-tetrazolium-chloride/5-bromo-4-chlor-indolyl-phosphate (NBT/BCIP) solution (Roche). Images were captured with an Olympus Optical (Tokyo, Japan) light microscope equipped with a CCD camera.

Acute challenge assays. All studies involved male mice, housed five mice per cage, and maintained under a standard 12 h light/dark cycle (lights on from 6:00 A.M. to 6:00 P.M.) at 20°C. Mice were fed standard mouse chow containing 6% total energy as fat (Harlan Laboratories, Indianapolis, IN) *ad libitum*, except when noted. For the fasting studies, adult mice (10–14 weeks of age) were divided into two groups ($n = 5$) and placed into new cages at 10:00 A.M. (time 0). The *ad libitum* group was given unrestricted food access. Either 24 or 48 h later, the animals were killed, and tissues were collected as described above. For the restraint studies, adult male mice ($n = 5$) were secured in 50 ml conical tubes and placed inside their cage (one animal per cage); control mice were freely moving in another cage. After 30 min, the animals were killed, and tissues were collected. For the cold-stress studies, animals were transferred to individual cages without bedding and placed at 4°C or 25°C for 60 min. Animals were killed, and tissues were collected. Each assay was repeated at least two times.

Real-time qPCR. VMH was microdissected, and total RNAs were isolated as described above. Real-time PCR was performed as described previously (Kurrasch et al., 2004). All data are normalized to one tissue sample of hypothalamus minus VMH (taken to be 1), and the error bar represents the variation of the other hypothalamus minus VMH samples against this chosen normalized sample ($n = 4$). Before routine use, all primer sets (Primer Express version 2.0; Applied Biosystems, Foster City, CA) were validated to ensure amplification of a single product with appropriate efficiency. Data obtained from the PCR reaction were analyzed using the comparative cycle threshold C_T method (User Bulletin 2; PerkinElmer, Wellesley, MA). Primer sequences are listed in supplemental Table S3 (available at www.jneurosci.org as supplemental material).

Cellular luciferase reporter assays. Fragments (~200 bp) containing the SF-1 response element(s) were subcloned into a luciferase reporter construct as described (Nachtigal et al., 1998), and mutant promoter fragments were generated by site-directed mutagenesis altering the wild-type core SF-1 binding site of A**GG**TCA to A**t**aTCA. HepG2 cells were plated into a 24-wells plate at 10^5 cells per well and transfected using Fugene6 (Roche). Lysates from 48 h transfected cells were measured for luciferase activity using Veritus Microplate Luminometer (Turner BioSystems, Sunnyvale, CA) and performed three times.

Zebrafish morpholinos analysis. Morpholinos (MOs) were obtained from Open Biosystems (Hunstville, AL) or Gene Tools (Philomath, OR). Two morpholinos per gene were designed: one to bind sequences surrounding the initiating methionine and one to bind to 5′ untranslated region (UTR) or exon/intron regions. Each morpholino was tested against the genome for nondesired interactions. Sequences of MOs are listed in supplemental Table S3 (available at www.jneurosci.org as supplemental material). Morpholinos were injected according to previously published procedures (Summerton, 1999). MOs were resuspended in water to a working solution of 1 μM and injected into the yolk of one- to four-cell stage embryos ($n = 100$ embryos/MO; repeated three times). Effective doses were determined separately for each MO. Embryos were treated with 1-phenyl-2-thio-urea (200 μM) to block formation of melanocytes. Forty-eight hour postfertilization (hpf) embryos were collected, dechorinated, fixed overnight, and stored in 100% MeOH at 20°C until whole-mount *in situ* hybridizations performed.

Whole mount in situ *hybridization in zebrafish.* Embryos were rehy-

drated into PBS–0.1% Tween 20, treated with proteinase K (20 μg/ml; 8 min), refixed (4% PFA; 20 min), prehybridized (70°C; 1 h), and hybridized (70°C; overnight) with hypothalamic riboprobes. Embryos were washed (50% formamide/50% 2× SSC; 2× SSC; 0.2× SSC), blocked (5% sheep serum heat inactive), and incubated overnight at 4°C with α-DIG antibody followed by washing and a second incubation with NBT/BCIP in staining buffer (100 mM NaCl, 50 mM $MgCl_2$, 100 mM Tris-HCl, and 0.1% Tween 20). The color reaction was monitored for several hours and stopped by washing with PBS–0.1% Tween 20. The embryos were refixed and transferred to 90% glycerol for clearing. Embryos were captured using Spot camera.

Results

The neonatal mouse VMH transcriptome

To identify molecular markers enriched in the VMH at a late developmental stage, gene profiling was performed on microdissected tissue from newly born (P0) male mice with the VMH compared with the entire hypothalamus absent the VMH. We reasoned that this later time point would identify markers important for distinguishing distinct VMH cell types rather than identifying genes important for neuronal migration from the third ventricle. P0 also corresponds to a stage when the newborn animal requires a functional endocrine network for integrating sensory input and maintaining homeostasis in response to physiological demands. Moreover, significant overlap was found with our current gene list after profiling E18 VMH tissue with a less comprehensive microarray that only included RIKEN cDNAs (P. V. Tran, unpublished data). Using P0 VMH tissue, nearly 200 genes of 37,000 showed enrichment of ≥1.51-fold expression relative to the rest of the hypothalamus (supplemental Table S1, available at www.jneurosci.org as supplemental material). The top 50 neonatal VMH transcripts include a small subset of genes known previously to be expressed in the adult VMH (Table 1). Among these newly identified VMH markers are genes predicted to regulate transcription or mediate extracellular signaling, as well as several RIKEN and hypothetical cDNA transcripts that have yet to be annotated. Several transcription factors were identified, including *Vgll2*, *Sf-1*, *Sox14*, *Satb2*, *Fezf1*, *Dax1*, *COUP-TFII*, *Nkx2-2*, *Ldb2, Fbxw7*, *Lcorl*, *Nkx2.1*, *Grhl1*, *Neud4*, and *Isl1*. Of these transcription factors, known roles in hypothalamic development have been described only for SF-1 and Nkx2.1. Although SF-1 is not required for the birth of VMH neurons and is expressed only in postmitotic neurons (Tran et al., 2003), SF-1 is required for maintenance of normal VMH cytoarchitecture (Ikeda et al., 1995; Shinoda et al., 1995), for terminal differentiation (Tran et al., 2003), and proper condensation of the VMH nucleus (Davis et al., 2004). In the adult, these developmental deficits have been indirectly linked to energy dysregulation as evidenced by the fact that adrenal rescued *Sf-1* null adult mice display obesity (Majdic et al., 2002), and SF-1 heterozygous mice have decreased hypothalamic sympathetic output with a propensity for diet-induced obesity (Tran et al., 2006). In contrast to the restricted influence of SF-1 on VMH development, loss of nkx2.1 (Titf1) results in severe disruption of the entire hypothalamus except for the most ventral aspect as evidenced by an altered or absent expression pattern of SF-1, Sim-1, and Pax-6 (Marin et al., 2002). Other transcription factors on our list include ldb2, which has been linked to hypothalamic development (Caqueret et al., 2006) and Fezf1 (Hirata et al., 2006), COUP-TFII (Tripodi et al., 2004), and Satb2 (Britanova et al., 2005), which are all implicated broadly in neural development. Although the functional relevance of these other transcription factors in the VMH remains to be determined, it is likely that they will participate in specifying VMH patterning or cell fate.

In addition to transcription factors, we identified many membrane receptors/proteins, including G-protein-coupled receptors, *Gpr149*, *Nmbr*, *Cckbr*, *Htr1b*, *Cnr1*, *Htr2a*, and *Gpr176*, and ion channels, *Gabra5*, *Kcnj5*, *Kcnab1*, *Abcc4*, *Abcc8*, *Cacna2d1*, *Slc17a6*, *Clca1*, and *Fxyd7*. Of these, many have not been identified previously as being localized within the VMH. For example, the serotonin receptors Htr2a and Htr1b were found in our profiling, but Htr2c was not enriched (<0.5-fold) despite its known role in energy balance (Tecott et al., 1995). Another receptor involved in energy balance and found in the neonatal VMH is the cannabinoid receptor 1 (Cnr1). Indeed, $Cnr1^{-/-}$ mice are lean, hypophagic, and resistant to diet-induced obesity (Cota et al., 2003). Cnr1 may also specify VMH neurogenesis given that endocannabinoids have been suggested to regulate synaptogenesis (Berghuis et al., 2007) and attenuate BDNF signaling in the hippocampus and frontal cortex (Maj et al., 2007). Other membrane proteins implicated in the glucose-sensing properties of VMH neurons include VGLUT2 (Tong et al., 2007) and components of the ATP-dependent potassium channels, the potassium inwardly rectifying channel Kcnj11 (Kir6.2, 1.48-fold) (Miki et al., 2001), and its binding partner the sulfonylurea receptor Abcc8 (SUR1). Thus, the glucose-sensing properties of VMH neurons appear to be intact and well established at birth.

Although we identified many new VMH enriched transcripts, we failed to detect some well studied rat or mouse VMH adult markers, such as androgen receptor (AR) and estrogen receptor (Simerly et al., 1990; Ikeda et al., 2003), LEPR (Schwartz et al., 1996), and growth hormone secretagogue receptor (Zigman et al., 2006). We did not find a large number of new or previously identified neuropeptides, including NPY and galanin, which have been assigned as VMH peptides based on immunohistochemical analysis (McClellan et al., 2006). In fact, only four neuropeptides were enriched in the neonatal VMH, and these included *BDNF*, *pituitary adenylate cyclase-activating polypeptide* (*PACAP*), *prodynorphin*, and *Tac1* (*substance P*). Of these, functions in the VMH are best described for BDNF, in which it acts as a satiety factor downstream of melanocortin signaling (Xu et al., 2003). PACAP signaling is proposed to regulate the sympathoadrenal axis affecting the release of adrenal steroids (Hashimoto et al., 2006), and substance P release from ER-positive VMH neurons is proposed to affect sexual behavior (Daniels et al., 2003). Other known adult VMH peptides, such as *enkephalin*, *cholecystokinin* (*CCK*), and *somatostatin*, were not enriched in the neonatal VMH, but some were found at much higher levels in P21 hypothalami (F. Y. Lee and H. A. Ingraham, unpublished data). Collectively, these results suggest strongly that many adult neuropeptides and hormone receptors are not yet upregulated or enriched in the newborn VMH.

Transcripts at the bottom of our list that are predicted to be low in the VMH (<0.4-fold) but are high in other hypothalamic regions include several known hypothalamic markers, including *hypocretin*, *arginine vasopressin*, *corticotrophin-releasing hormone* (*CRH*), *oxytocin*, *thyrotropin-releasing hormone receptor*, *LRHR* (*luteinizing hormone releasing hormone*), *Brn2*, *Sim1*, and *carboxypeptidase*. These results confirm that our microdissection technique and gene profiling method are valid.

VMH markers exhibit regional and temporal expression patterns

We next examined the expression patterns of several top VMH markers in neonatal brains to confirm that they were VMH-enriched markers. For all candidates assessed, a prominent, tightly restricted expression pattern was observed in the ventral

Table 1. Top 50 VMH-enriched genes

Rank	Symbol	Gene name	Accession #	Fold
1	Vgll2	Vestigial-like 2 homolog	NM_153786	8.42
2[a]	Nr5a1	Nuclear receptor subfamily 5, group A, member 1 (SF1, Ad4BP)	NM_139051	7.75
3[a]	Sox14	SRY-box containing gene 14	XM_284529	7.35
4	Nptx2	Neuronal pentraxin 2	NM_016789	6.85
5	RIKF11	RIKEN cDNA 9130024F11	AK020268	6.13
6[a]	Bdnf	Brain-derived neurotrophic factor	NM_007540	5.37
7	Satb2	Special AT-rich sequence binding protein 2	AK035129	4.68
8	Gpr149	G-protein-coupled receptor 149	NM_177346	4.43
9	Fezf1	Fez family zinc finger 1	XM_133029	4.38
10	Ddn	Dendrin	AK122363	4.29
11[a]	Cbln1	Cerebellin 1 precursor protein	NM_019626	4.28
12	RIKG08	RIKEN cDNA C130093G08	AK082001	4.27
13	Mst1r	Macrophage stimulating 1 receptor	NM_009074	3.83
14	Tnfrsf8	Tumor necrosis factor receptor superfamily, member 8	NM_009401	3.79
15	Nmbr	Neuromedin B receptor	NM_008703	3.30
16[a]	Adcyap1	Adenylate cyclase activating polypeptide 1 (PACAP)	NM_009625	3.28
17[a]	Nr0b1	Nuclear receptor subfamily 0, group B, member 1 (Dax1)	NM_007430	3.18
18	Pdyn	Prodynorphin	NM_018863	3.16
19	Amigo2	Adhesion molecule with Ig-like domain 2	NM_178114	3.13
20	RIKK11	RIKEN cDNA 1700024K11	CN839877	3.12
21	Plp1	Proteolipid protein (myelin) 1	AK077564	3.05
22	Nkx2-2	NK2 transcription factor related, locus 2	NM_010919	2.94
23[a]	Gabra5	GABA receptor, subunit α 5	NM_176942	2.89
24[a]	Gda	Guanine deaminase	AK080065	2.81
25	RIKC22	RIKEN cDNA C230097C22	BB396351	2.74
26[a]	Nr2f2	Nuclear receptor subfamily 2, group F, member 2 (COUP-TFII)	NM_009697	2.73
27	RIKB13	RIKEN cDNA E030019B13	BC064451	2.73
28	Camkv	CaM kinase-like vesicle-associated	NM_145621	2.70
29	Ldb2	LIM domain binding 2	NM_010698	2.65
30	Fbxw7	F-box and WD-40 domain protein 7, archipelago homolog	NM_080428	2.62
31[a]	Cckbr	Cholecystokinin B receptor	NM_007627	2.60
32	Cdh4	Cadherin 4	NM_009867	2.58
33	Col6a3	Procollagen, type VI, alpha 3	XM_484897	2.53
34	Sema3a	Sema domain, Ig, short basic domain, secreted, 3A	NM_009152	2.52
35	RIKP06	RIKEN cDNA 1700007P06	AK005744	2.48
36	Rerg	RAS-like, estrogen-regulated, growth inhibitor	NM_181988	2.46
37	Acvr1c	Activin A receptor, type IC	XM_194020	2.44
38	A2bp1	Ataxin 2 binding protein 1, transcript variant 2 (Fox-1)	NM_021477	2.40
39[a]	Tac1	Tachykinin 1 (Substance P)	NM_009311	2.36
40	Lcorl	Ligand-dependent nuclear receptor corepressor-like, transcript v0.2	NM_172153	2.36
41	Card14	Caspase recruitment domain family, member 14	NM_130886	2.32
42		cDNA sequence BC034076	NM_177649	2.27
43[a]	Titf1	Thyroid transcription factor 1 (Nkx2.1)	NM_009385	2.27
44	B3gnt3	UDP-GlcNAc:βGal β-1,3-*N*-acetylglucosaminyltransferase 3	NM_028189	2.25
45	Tcfcp2l2	Transcription factor CP2-like 2 (Grhl1)	NM_145890	2.24
46	Kcnj5	Potassium inwardly rectifying channel, subfamily J, member 5	NM_010605	2.22
47	Ntn3	Netrin 3	NM_010947	2.21
48	Calb1	Calbindin-28K	AK038856	2.21
49	Tmem35	Transmembrane protein 35	NM_026239	2.20
50	RIKI05	RIKEN cDNA C030019I05	NM_177075	2.20

VMH candidates are ranked by fold expression relative to hypothalamus minus VMH. Results from P0 male mice on a MEEBO microarray (~37,000 genes).

[a]Previously known genes.

medial region of neonatal hypothalami similar to that observed for SF-1 (Fig. 1*A*) (supplemental Fig. S1, available at www.jneurosci.org as supplemental material). Regardless of their rank order, all transcripts were expressed in the VMH, with some transcripts showing low expression in the ARC or DMH (Amigo2, Sox14, and Nptx2) (Fig. 1*A*). Using qPCR, we also established that these top transcripts were also enriched in the adult VMH (Fig. 1*B*). Indeed, virtual expression analyses using Allen Brain Atlas (http://www.brain-map.org) (Lein et al., 2007) showed that several of these top transcripts and even those lower on our list (~1.5-fold) displayed prominent expression in the adult VMH (supplemental Table S1, Fig. S2, available at www.jneurosci.org as supplemental material). Thus, the nearly 200 genes identified here provide a comprehensive list of known and novel VMH-enriched markers.

Currently, SF-1 is the only definitive factor known to regulate VMH differentiation and development. We assumed that other regulators important for early and late stages of VMH development will be enriched or restricted to this nucleus. Similar to SF-1, factors involved in the earliest stages of VMH development would be expressed concomitantly with the migration and formation of this nucleus or between E11 and E17. Expression of our

top marker Vgll2 (VITO-1) exhibits this very pattern. Indeed, the ontogeny of Vgll2 expression shows that this transcriptional cofactor is present in the developing embryo, with expression peaking in the newly born animal (P0). However, by P7, expression was diminished, and, by weaning at P21, this VMH marker is completely absent (Fig. 2). Additionally, although Vgll2 appears to overlap with SF-1 at P0 and P7, it exhibits a much more restricted pattern and is confined to the dorsal medial region. Similarly, at the earliest stage examined, E12, both markers exhibit overlapping but distinct paraventricular expression patterns (Fig. 2, E12). Based on data in the Allen Brain Atlas and differential profiling at P0 versus P21 (Lee and Ingraham, unpublished data), we predict that a small but sizable number of VMH genes (10%) will exhibit patterns similar to Vgll2 and be significantly downregulated or silenced in the adult VMH. The dynamic expression pattern exhibited by Vgll2 suggests strongly that this transcriptional cofactor will play an important role in the earliest stages of VMH neural development.

Closer examination of patterns displayed by the top VMH transcripts revealed overlapping but remarkably distinct regional patterning in the VMH when assessed in serial adjacent neonatal brain sections (Fig. 3*A*). As mentioned above, Vgll2 was restricted to the dorsomedial region throughout the entire VMH (Fig. 3*A*) (supplemental Figs. S3, S5, available at www.jneurosci.org as supplemental material). In this particular plane of sectioning, other markers such as Amigo2 and Sox14 were expressed in the VMH_{DM} and VMH_C but appeared to be weakly expressed in the VMH_{VL}. However, Satb2 and Nptx2 lacked strong expression in the VMH_{VL} and instead showed enriched expression in the VMH_C. Furthermore, virtual analyses of other VMH markers also showed distinct spatial patterning in the adult brain, albeit with the inherent limitations of virtually matched sections (supplemental Fig. S4*A*, available at www.jneurosci.org as supplemental material). Collectively, our findings imply that the VMH consists of multiple neuronal subtypes, which can now be defined more fully by future detailed colocalization studies.

Another provocative observation was the prominent and selective expression of some VMH markers in the medial amygdala (Fig. 3*B*) (supplemental Fig. S4*B*, available at www.jneurosci.org as supplemental material). Although this brain region remains poorly understood at a molecular level, the amygdala receives input from and projects to the VMH via the stria terminalis; it has also been implicated in anxiety and fear responses (King, 2006a). Among these new VMH transcripts, the zinc-finger transcriptional repressor Fezf1 was highly expressed in the neonatal amygdala. Fezf1 is also expressed in the olfactory epithelium and is required for olfactory bulb development (Hirata et al., 2006). Thus, Fezf1 appears to mark the entire olfactory–amygdala–VMH circuitry. Based on this expression pattern, it will be of interest to determine whether Fezf1 coordinately regulates development of this reproductive and feeding circuit.

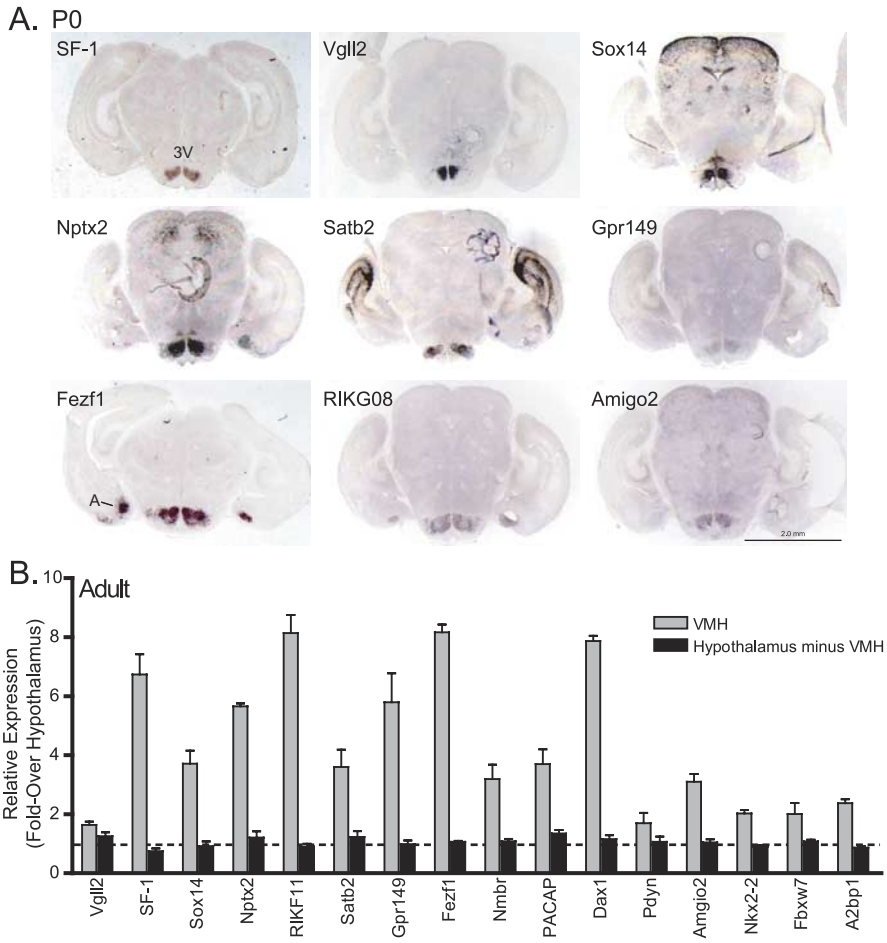

Figure 1. Expression patterns and levels of top VMH markers. *In situ* hybridization of top VMH markers in P0 embryos and qPCR in adult. ***A***, Expression of novel VMH markers in P0 mouse pups is shown for coronal brain sections (20 μm thick). The pattern for the current definitive VMH marker, SF-1, is shown in the top left panel. Anatomical landmarks include the third ventricle (3V) and the amygdala (A). Scale bar, 2 mm. ***B***, Relative transcript levels are shown for several VMH markers in adult male mice (>8 weeks). Relative levels of each transcript were assayed in the VMH (gray bars) and in the hypothalamus minus the VMH (black bars). Fold induction is relative to the hypothalamus minus the VMH and taken to be 1 (horizontal dashed line), with one of four control samples randomly set to equal 1. All other samples, including additional control and experimental samples, are normalized to this one value (or 1 tissue). The variation among each group of tissues is shown by error bars.

Functional characterization of VMH genes in the zebrafish hypothalamus

To explore functionally the potential role of these new VMH-enriched genes in hypothalamic development, we turned to zebrafish because one can quickly assess the functional role of genes in development using morpholino knockdowns. Additionally, there is remarkable conservation in genetic programs governing vertebrate organogenesis and CNS development. Indeed, analysis of sensory neurosecretory cell types in zebrafish and a distantly related annelid species revealed expression of regulatory factors, such as Nkx2.1 and otp, that are required for proper hypothalamic development in mice (Tessmar-Raible et al., 2007). Furthermore, peptides and pathways involved in the hypothalamic control of energy homeostasis are also conserved in zebrafish, including the mammalian feeding-related peptides CCK, NPY, melanin-concentrating hormone (MCH), pro-opiomelanocortin (POMC), and ghrelin, (for review, see Volkoff et al., 2005). In addition, hypocretin (orexin) appears to be functionally con-

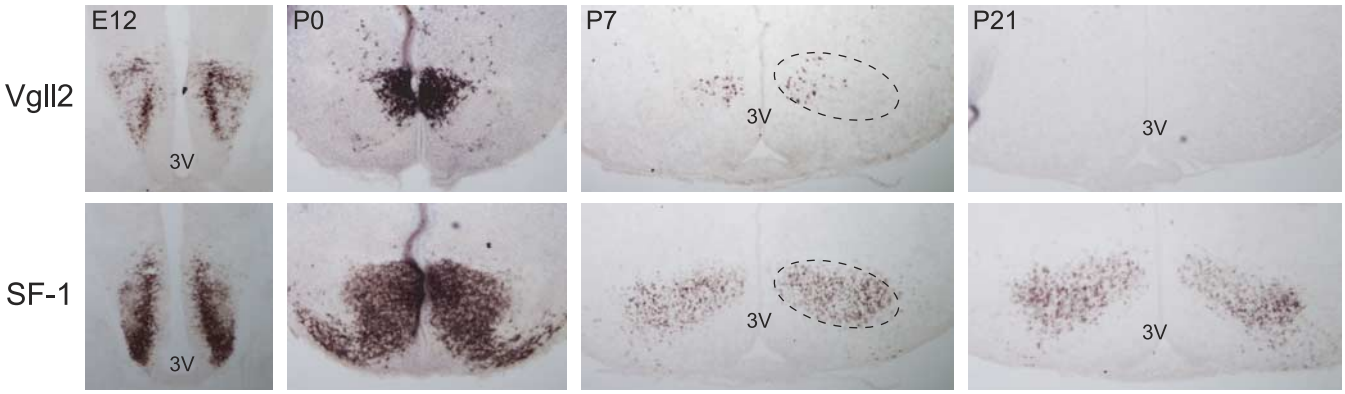

Figure 2. Expression of Vgll2 is present in the embryo and postnatal VMH but is absent in the early adult. *In situ* hybridization of Vgll2 and SF-1 in E12, P0, P7, and P21 brains. Vgll2 (top) and SF-1 (bottom) expression is shown for coronal brain sections (20 μm). The VMH is marked with a dashed oval, and the third ventricle (3V) is labeled for orientation.

served in lower vertebrates as evidenced by an insomnia-like phenotype in zebrafish after overexpression (Prober et al., 2006). Although the exact anatomy of hypothalamic nuclei remains to be fully defined in zebrafish, the collective functional data coupled with the distinct spatial patterning of neuroendocrine markers (D. M. Kurrasch and H. A. Ingraham, unpublished data) and projections (Forlano and Cone, 2007) strongly suggest that the neuroendocrine system is highly conserved between mammals and zebrafish.

Before any functional analyses, we determined whether the zebrafish orthologs of neonatal VMH markers, nr5a subfamily members (duplicated SF-1 orthologs), fezf1, and nkx2.1a would display the same restricted expression pattern in a subpopulation of hypothalamic neurons in zebrafish as in rodents. Indeed, a prominent expression profile is observed in the medial hypothalamus for nr5a1a, nr5a2, and fezf1 (Fig. 4*A*). Expression of these VMH neonatal markers does not overlap with hypocretin or gnrh (Fig. 4*A*,*B*) (data not shown), demonstrating that regional specificity exists in both mice and teleosts. At this developmental time point in fish (48 hpf), nkx2.1a is expressed broadly in the medial and lateral hypothalamus.

To ascertain whether some of these newly revealed VMH genes are involved in hypothalamic development, we used morpholino antisense technology to block or knockdown translation of targeted mRNAs in the developing zebrafish embryo. Targeted genes were limited to those present as a single copy in the genome; duplicated genes were not chosen for this analysis to avoid compensatory effects. Morpholinos were designed and injected at the one-cell stage targeted against nmbr, a2bp1, fezf1, sox14, satb2, and crip2. Injected embryos were analyzed for disruptions in hypothalamic patterning of VMH genes just after completion of early organogenesis at 24 hpf (data not shown), mid-development at 48 hpf (Fig. 4*B*), and during the embryo-to-larvae transition at 96 hpf, which is approximately equivalent to P0 in mammals (data not shown). Using nr5a1a and nr5a2 as presumptive VMH markers in zebrafish, we observed significant disruption in their bilateral hypothalamic expression after knocking down sox14, a2bp1, fezf1, and satb2 at all time points (Fig. 4*B* and data not shown). Expression patterns remained unchanged with nmbr morpholinos (Fig. 4*B*) or vehicle control (data not shown). The extent of disruption included loss of an organized condensed symmetrical pattern to a disorganized round pattern that is not longer bilateral; these patterns are displayed left to right, with the strongest phenotype shown on the far right (sox14-MO). This disruption of zebrafish patterning was specific for the medial hypothalamus as evidenced by normal expression patterns for the lateral hypothalamic marker hypocretin (Fig. 4*B*) or arcuate/pituitary marker (POMC; data not shown). These data suggest that regulatory factors found in the VMH transcriptome will likely be required for normal vertebrate hypothalamic development. Our findings also serve to underscore how parallel studies in mice and zebrafish provide a strategy for quickly surveying functional roles of newly identified genes in vertebrate organogenesis.

SF-1 is required for full expression of selective VMH genes

Given that SF-1 is needed to differentiate and/or maintain the population of VMH neurons after birth, loss of SF-1 is expected to affect expression of some neonatal VMH markers. SF-1 is also predicted to regulate directly some of these newly identified VMH markers. Expression levels and patterns of some VMH markers were determined in SF-1 wild-type and null mice at P0. At this stage, one observes a distinct cell cluster expressing SF-1 transcripts, even after the loss of a functional SF-1 protein (Fig. 5*A*) (Tran et al., 2003). At later stages, the morphology and structure of the VMH is more severely compromised (Majdic et al., 2002). As expected, VMH markers were greatly diminished in *sf-1*$^{-/-}$ mice (Fig. 5*A*). However, loss of SF-1 did not affect expression of these genes in other brain regions; for instance, Fezf1 expression was maintained and possibly upregulated in the medial amygdala, and expression of Nkx2-2 and A2bp1 persisted in the zona limitas and dorsal thalamus, respectively (Fig. 5*A*). Additional qPCR analyses on neonatal hypothalami showed that levels of many, but not all, VMH transcripts were significantly lower in the SF-1 null background (Fig. 5*B*). Thus, while expression of many VMH markers diminishes with loss of SF-1 protein, our qPCR results suggest that additional analysis of these markers is needed to determine whether SF-1 is expressed in all subtypes of VMH neurons.

Given that SF-1 coordinately regulates multiple genes during endocrine organogenesis and in the adult endocrine system, we wanted to assess whether SF-1 might also directly regulate some of these newly identified VMH markers. After scanning upstream promoter regions of several genes (−3 kb), we identified one or more potential SF-1 binding sites (C/ACAGGTCA) in *Nptx2*, *Fezf1*, *A2bp1*, *Nkx2-2*, *Fbxw7*, *Ddn*, *Slitrk1*, *Slitrk5*, and *Crip2*. Cellular reporter assays in HepG2 cells showed that wild-type, but not mutated, promoter regions of these candidate genes are activated directly by SF-1 (Fig. 5*C*). We noted that, for some promoters containing multiple SF-1 response elements, only se-

lective elements were activated (data not shown). Together, these data imply that SF-1 will regulate CNS targets that are potentially important for VMH neuronal differentiation and adult physiology.

Loss of leptin signaling affects a VMH transcript in the developing but not adult hypothalamus

To begin to determine whether these new VMH transcripts are differentially regulated in other genetic backgrounds, we surveyed expression of some top VMH transcripts in P0 and adult *Lep*$^{ob/ob}$ mice. No changes in the relative transcript levels were detected for these VMH markers in adult *Lep*$^{ob/ob}$ mice. As expected, NPY and POMC were upregulated and downregulated, respectively, in adult *Lep*$^{ob/ob}$ mice (Fig. 6*B*), confirming previous results and reflecting the normal hypothalamic response after loss of leptin signaling (Bates et al., 2003). No changes in expression of these VMH markers were detected after acute challenges in adult wild-type mice (24 and 48 h fasting, physical restraint, and cold stress; data not shown). However, in the newborn animal, the AT-rich binding, cut-domain homeobox Satb2 was downregulated, whereas NPY and POMC remained unchanged (Fig. 6*A*). These data suggest that the transcriptional responses to hormone signaling by Satb2, and presumably other newly identified VMH factors, will differ in the newborn versus adult.

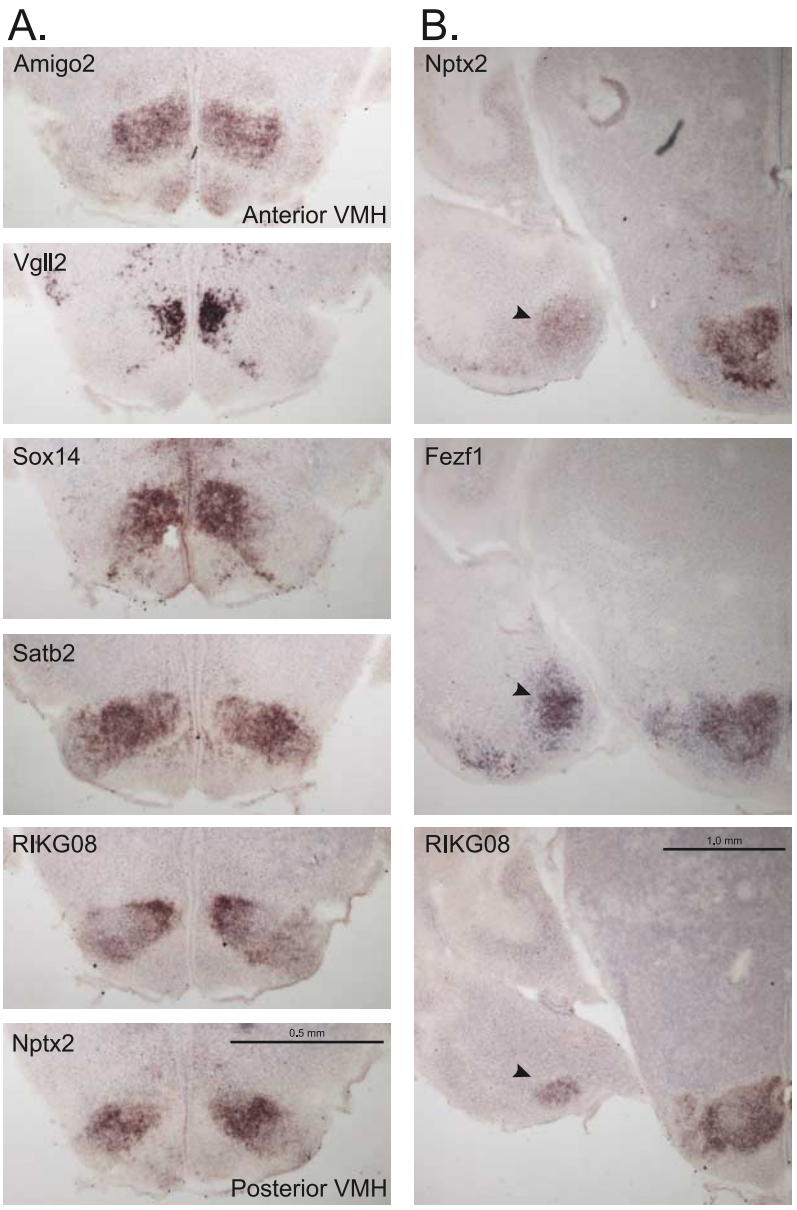

Figure 3. Expression patterns of new markers reveal spatially distinct expression patterns in the VMH, with some expressed in the medial amygdala. ***A***, Expression patterns are shown for six different VMH markers in serial coronal brain sections obtained from P0 male mice (20 μm) beginning with the most anterior section (Amigo2, top) and extending posterior (Nptx2, bottom). Scale bar, 0.5 mm. ***B***, Expression of Nptx2, Fezf1, and RIKG08 in the medial amygdala (arrowhead) is shown relative to expression in the VMH (for other genes found to be expressed in the VMH and in the amygdala, see supplemental Fig. S2, available at www.jneurosci.org as supplemental material). Scale bar, 1.0 mm.

Discussion

Here we report a comprehensive list of molecular markers that are highly specific or enriched in the neonatal and adult VMH. Although our list includes many of the known VMH markers, the majority of genes reported here are either new or not previously appreciated as being expressed in the VMH. This VMH transcriptome represents a functionally diverse set of molecular probes to define further VMH neuronal subtypes. Among the genes on this list, we found many transcription factors as top markers. Our limited functional analysis in zebrafish predicts that these factors will be important for specifying VMH cell fate and patterning. Moreover, the remarkably distinct expression patterns exhibited by these markers offer a preliminary glimpse into the cellular complexity of the VMH that likely underlies the wide range of innate behavioral responses associated with this hypothalamic region.

Our strategy to profile the neonatal VMH nucleus resulted in a list of markers that greatly increases the number of available transcripts for probing VMH function. In comparing our list with VMH-enriched adult transcripts identified by Segal et al. (2005), we found SF-1 as the second highest expressed gene in the VMH. Our top VMH marker Vgll2 would not have been detected by their study because it is downregulated in the adult. Among the other nine genes on their list, six are found in our top ~100 VMH-enriched genes, one showed no enrichment in neonatal VMH, and two genes were not represented on our chip. Thus, despite the differences in experimental design, microdissection versus laser capture and neonate versus adult, a very small but overlapping subset of markers was identified in both studies, albeit with many more transcripts identified in this study. Indeed, our VMH transcriptome identifies scores of new transcripts not previously noted to be expressed in the VMH; the majority of these genes are also expressed at some level in the adult VMH (Allen Brain Atlas). Our preliminary results suggest that ~10% of

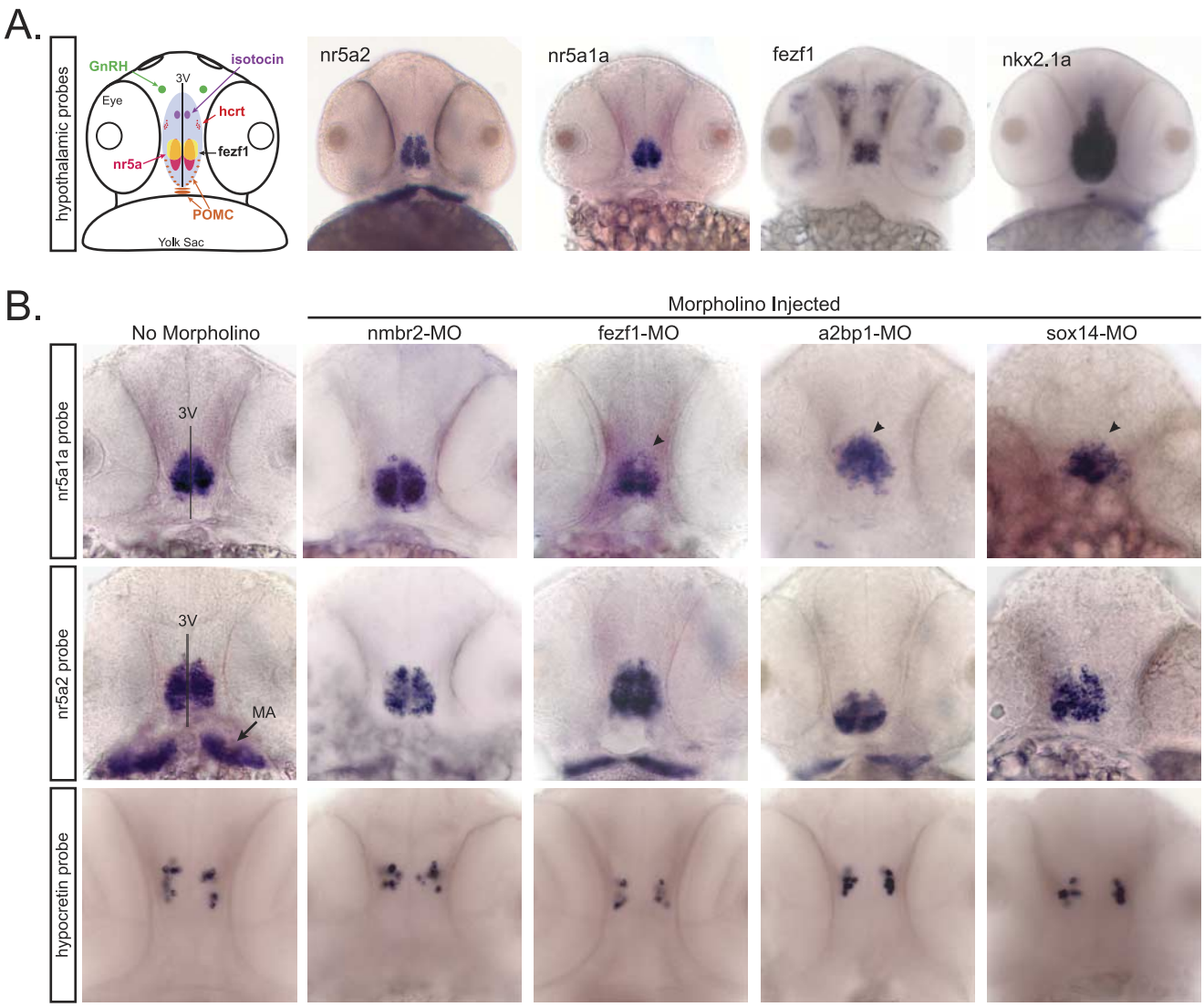

Figure 4. Knockdown of VMH markers disrupts medial hypothalamic patterning in developing zebrafish. ***A***, Diagram depicting the spatial patterning of known hypothalamic markers and VMH-specific makers in the 48 hpf zebrafish brain. Whole-mount *in situ* hybridization expression profiles are shown for orthologs of mouse neonatal VMH-enriched markers. Other anatomical landmarks include the developing eye and yolk sac. Images represent the most ventral side of the embryo with the dorsal side down. ***B***, Whole-mount expression patterns for wild-type and morpholino-injected 48 hpf zebrafish are shown for nr5a1a (top 5 panels), nr5a2 (middle 5 panels), or hypocretin (bottom 5 panels). Sequences for all antisense morpholinos, including nmbr-MO, sox14-MO, a2bp1-MO, and fezf1-MO, are provided in supplemental Table S3 (available at www.jneurosci.org as supplemental material). Disruption of the nr5a1a and nr5a2 expression are noted by the lack of a discernable third ventricle boundary (arrowhead). Images are displayed from no phenotype (nmbr-MO), to weakly disrupted (fezf1-MO), and to severely disrupted (sox14-MO). MA, Mandibular arch. For each condition, two distinct morpholinos directed against the initiator methionine or splice junction/5′ UTR were used independently and together. Data shown are representative of two separate experiments using at least 80 embryos.

these P0 genes will be substantially downregulated in the adult (Lee and Ingraham, unpublished results). Together, searching for VMH enriched-genes at a late developmental time point (P0) rather than at earlier (E10–E17) or much later stages (P21 to adult) yielded a remarkably comprehensive set of new molecular VMH markers that are likely to be important for both embryonic and adult functions. A similar strategy could prove useful for other brain regions in which markers and cell types remain poorly defined.

It is surprising that only a few neuropeptides are expressed in the neonatal VMH. Further inspection of our gene profiling data find that the hormone receptors (CRH receptor 2), AR, and oxytocin receptor are all elevated in the P0 VMH but fell short of the threshold level we set as significant (≥1.51-fold). Other listed VMH peptides including CCK and enkephalin are expressed at values well below onefold at P0. As such, these peptides must be upregulated sometime after birth, suggesting that the physiological demand for peptide signaling is lower in the newborn animal. This notion is consistent with the fact that mutations in endocrine pathways do not lead to overt phenotypes until after birth when homeostasis in response to the external environment is required. It will be of interest to determine when expression of these peptides becomes enriched in the VMH. This raises another obvious question: what is the precise function of the few VMH peptides that were found to be expressed early in the VMH? Although established roles for PACAP, substance P (Tac1), and prodynorphin in VMH development are still unclear, the marked expression of BDNF observed in the P0 VMH suggests that this neurotrophic factor is important for some aspects of VMH development in addition to its purported role in mediating melanocortin signaling for satiety.

Our top candidate Vgll2 exhibits an extremely robust and

selective expression pattern in the VMH_{DM} and supersedes SF-1 as the top developmental marker for the VMH. Based on known functions of the *Drosophila* ortholog *vestigial* in organogenesis, Vgll2 is hypothesized to promote skeletal muscle differentiation by functioning as a cell-specific transcriptional cofactor (Chen et al., 2004). Vgll2 has not been found in the CNS until now and instead was purported to be in Rathke's pouch or the pituitary anlagen (Mielcarek et al., 2002). We find that Vgll2 is not enriched in the adult VMH or expressed in a prepubescent mouse (P21), suggesting that this gene has a role specific to VMH development. Given that SF-1 and Vgll2 have overlapping but distinct expression patterns at E12, it is possible that each of these factors marks a slightly different progenitor population. Our results show that Vgll2 is one of the most restricted markers of the purported "metabolic center" of the VMH, namely the VMH_{DM}. Vgll2 is excluded from the proposed "reproductive center" of the VMH (VMH_{VL}) that contains the estrogen and progesterone receptors (Flanagan-Cato, 2000). Other adult VMH_{DM} markers include the leptin and ghrelin receptors (Zigman et al., 2006). Although the *in vivo* function of Vgll2 remains to be established, it is possible that this transcriptional cofactor plays an important role in establishing, but not necessarily maintaining, expression of metabolic VMH_{DM}-specific markers. It should be noted that other factors affecting energy balance such as BDNF, tubby, and VGLUT2 are actually enriched in the VMH_{VL} or are found throughout the entire VMH (Coleman and Eicher, 1990; Kernie et al., 2000; Rios et al., 2001; Majdic et al., 2002; Collin et al., 2003; Xu et al., 2003; Tran et al., 2006; Tong et al., 2007). This fact underscores the need for molecular definition of neuronal subtypes within the VMH rather than relying solely on the current "two-center" organizational scheme of the VMH. Clearly, having highly expressed and regionally restricted VMH markers such as Vgll2 will make it possible to begin unraveling the cellular and functional complexity of this neuroendocrine center.

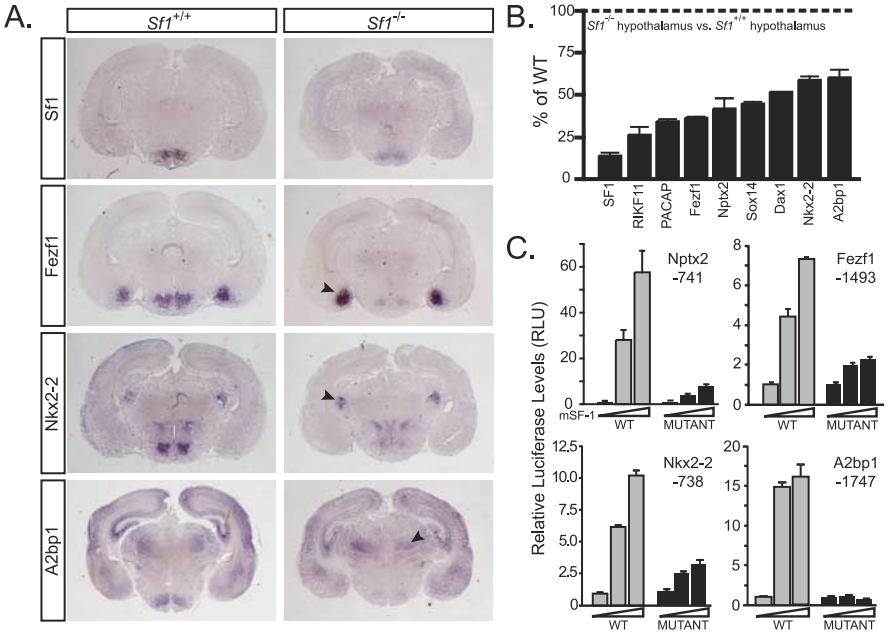

Figure 5. Expression of VMH-enriched transcripts requires SF-1. ***A***, Expression patterns for Fezf1, Nkx2-2, and A2bp1 are shown in *sf-1*$^{-/-}$ P0 male mice. For comparison, SF-1 is shown (top). Expression of these markers is maintained in other brain regions (arrowheads). ***B***, Relative transcript levels of VMH-enriched genes are shown for *sf-1*$^{-/-}$ mice (whole hypothalamus); expression in wild-type (WT) mice for each transcript is set at 100% (data not shown). ***C***, Relative luciferase activity (RLU) is shown after transfection of wild-type or mutant promoter plasmids (100 ng; Materials and Methods) and increasing amounts of pCMV–SF-1 (0, 100, and 200 ng).

Despite the fact that SF-1 is known to coordinately regulate genetic programs in peripheral endocrine tissues, SF-1 target genes in the VMH have remained elusive. Here we demonstrate that some of these new VMH markers are direct targets of SF-1. Knowing the network of SF-1-regulated genes, especially during brain development, will be instructive for understanding why loss of SF-1 results in a failure of VMH neurons to differentiate and to project to other brain regions (Tran et al., 2003; Davis et al., 2004). It is likely that some of the several cell adhesion molecules identified here, such as *Amigo2*, *Cdh4*, *Sema3a*, *Slit3*, and *Netrin3*, are directly regulated by SF-1 and affect axonal guidance. Another unanticipated gene that is regulated directly by SF-1 and is at the top of our list is the secreted glycoprotein Nptx2 (Narp). Nptx2 and other neuronal pentraxins are proposed to regulate activity-dependent synaptic plasticity via AMPA receptor clustering in hippocampal spines based primarily on *in vitro* studies; these functions suggest that neuronal pentraxins participate in learning and memory (O'Brien et al., 1999). However, deleting all three members of this family in mice resulted in a mild impairment of retinal activity, whereas hippocampal long-term potentiation and long-term depression appeared normal (Bjartmar et al., 2006). In the hypothalamus, others have suggested that lateral hypothalamic neuropeptides, hypocretin and MCH, use neuronal pentraxins to influence synapse formation of target neurons (Reti et al., 2002). In light of these findings, additional studies are needed to define the precise function of Nptx2 in the VMH and may thereby uncover novel roles for this interesting but still poorly understood protein family.

We speculate that several genes on our list will participate in genetic programs that establish VMH patterning, migration, and the development of neural circuits. Our limited zebrafish knockdown experiments show that the transcription factors Fezf1 and Sox14 are required for early patterning of the medial hypothalamus. Although these results need to be confirmed in mice, mutations in these developmental factors might underlie adult metabolic disease, as shown previously for the basic helix–loop–helix/PAS transcription factor Sim1 (Michaud et al., 2001). Aside from regulating overall hypothalamic patterning, these new VMH factors are likely to be important for establishing proper hypothalamic brain circuitry during critical developmental stages, including postnatal periods. In this regard, Satb2 represents a likely candidate for this role. Indeed, this AT-rich binding protein, cut homeodomain transcription factor, is proposed to affect neuronal cell fate based on expression patterns in the embryonic brain (Britanova et al., 2005). We find that Satb2 transcript levels are responsive to leptin signaling in the neonatal but not in the adult. Interestingly, Satb2 also regulates osteocalcin, a potential endocrine hormone that is made in the bone and is needed for normal glucose homeostasis (Lee et al., 2007). Our findings are intriguing given that Bouret and Simerly (2006) showed that neonatal leptin plays a neurotrophic role during a critical development window and is needed for establishing nor-

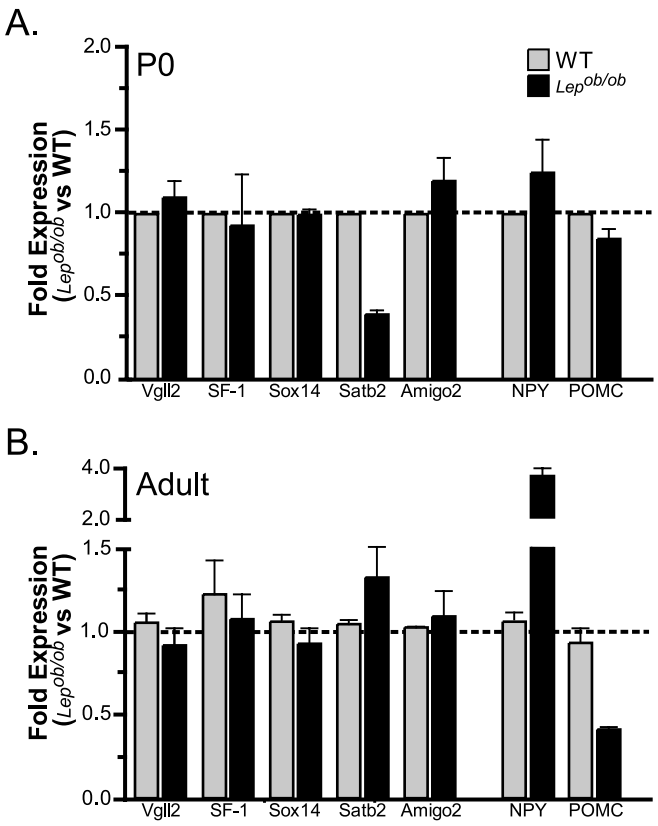

Figure 6. Reduction of Satb2 in the neonatal but not adult VMH. Relative levels of VMH transcripts as well as ARC transcripts, NPY, and POMC, as indicated on the *x*-axis are shown from isolated total hypothalami obtained from neonatal (P0; ***A***) and adult (***B***) mice. Levels for wild-type (gray bars) and for $Lep^{ob/ob}$ (black bars) are shown in both panels. Fold induction is relative to the hypothalamus minus the VMH with the wild-type (WT) control sample assigned a value of 1 (horizontal dashed line).

mal ARC projections. Determining the downstream targets of Satb2 in the VMH is needed to more fully define the role of this transcription factor in the hypothalamus.

In summary, this neonatal VMH transcriptome now allows for the novel molecular classification of this functionally complex neuroendocrine center. Identifying the developmental regulatory networks for the VMH and other hypothalamic regions should shed insight into the fetal origins of adult endocrine diseases (Barker et al., 1989), as well as the mechanistic underpinnings of innate homeostatic responses.

References

Altman J, Bayer SA (1986) The development of the rat hypothalamus. Adv Anat Embryol Cell Biol 100:1–178.

Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ (1989) Weight in infancy and death from ischaemic heart disease. Lancet 2:577–580.

Bates SH, Stearns WH, Dundon TA, Schubert M, Tso AW, Wang Y, Banks AS, Lavery HJ, Haq AK, Maratos-Flier E, Neel BG, Schwartz MW, Myers Jr MG (2003) STAT3 signalling is required for leptin regulation of energy balance but not reproduction. Nature 421:856–859.

Berghuis P, Rajnicek AM, Morozov YM, Ross RA, Mulder J, Urban GM, Monory K, Marsicano G, Matteoli M, Canty A, Irving AJ, Katona I, Yanagawa Y, Rakic P, Lutz B, Mackie K, Harkany T (2007) Hardwiring the brain: endocannabinoids shape neuronal connectivity. Science 316:1212–1216.

Bjartmar L, Huberman AD, Ullian EM, Renteria RC, Liu X, Xu W, Prezioso J, Susman MW, Stellwagen D, Stokes CC, Cho R, Worley P, Malenka RC, Ball S, Peachey NS, Copenhagen D, Chapman B, Nakamoto M, Barres BA, Perin MS (2006) Neuronal pentraxins mediate synaptic refinement in the developing visual system. J Neurosci 26:6269–6281.

Bouret SG, Simerly RB (2006) Developmental programming of hypothalamic feeding circuits. Clin Genet 70:295–301.

Britanova O, Akopov S, Lukyanov S, Gruss P, Tarabykin V (2005) Novel transcription factor Satb2 interacts with matrix attachment region DNA elements in a tissue-specific manner and demonstrates cell-type-dependent expression in the developing mouse CNS. Eur J Neurosci 21:658–668.

Caqueret A, Boucher F, Michaud JL (2006) Laminar organization of the early developing anterior hypothalamus. Dev Biol 298:95–106.

Chen HH, Maeda T, Mullett SJ, Stewart AF (2004) Transcription cofactor Vgl-2 is required for skeletal muscle differentiation. Genesis 39:273–279.

Cohen RS, Pfaff DW (1992) Ventromedial hypothalamic neurons in the mediation of long-lasting effects of estrogen on lordosis behavior. Prog Neurobiol 38:423–453.

Coleman DL, Eicher EM (1990) Fat (fat) and tubby (tub): two autosomal recessive mutations causing obesity syndromes in the mouse. J Hered 81:424–427.

Collin M, Backberg M, Ovesjo ML, Fisone G, Edwards RH, Fujiyama F, Meister B (2003) Plasma membrane and vesicular glutamate transporter mRNAs/proteins in hypothalamic neurons that regulate body weight. Eur J Neurosci 18:1265–1278.

Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thone-Reineke C, Ortmann S, Tomassoni F, Cervino C, Nisoli E, Linthorst AC, Pasquali R, Lutz B, Stalla GK, Pagotto U (2003) The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest 112:423–431.

Daniels D, Miselis RR, Flanagan-Cato LM (2003) Hypothalamic co-localization of substance P receptor and transneuronal tracer from the lordosis-relevant lumbar epaxial muscles in the female rat. Neurosci Lett 338:111–114.

Davis AM, Seney ML, Stallings NR, Zhao LP, Parker KL, Tobet SA (2004) Loss of steroidogenic factor 1 alters cellular topography in the mouse ventromedial nucleus of the hypothalamus. J Neurobiol 60:424–436.

Dhillon H, Zigman JM, Ye CP, Lee CE, McGovern RA, Tang VS, Kenny CD, Christiansen LM, White RD, Edelstein EA, Coppari R, Balthasar N, Cowley MA, Chua S Jr, Elmquist JK, Lowell BB (2006) Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. Neuron 49:191–203.

Egawa M, Inoue S, Sato S, Takamura Y, Murakami N, Takahashi K (1991) Restoration of circadian corticosterone rhythm in ventromedial hypothalamic lesioned rats. Neuroendocrinology 53:543–548.

Flanagan-Cato LM (2000) Estrogen-induced remodeling of hypothalamic neural circuitry. Front Neuroendocrinol 21:309–329.

Flier JS, Maratos-Flier E (1998) Obesity and the hypothalamus: novel peptides for new pathways. Cell 92:437–440.

Forlano PM, Cone RD (2007) Conserved neurochemical pathways involved in hypothalamic control of energy homeostasis. J Comp Neurol 505:235–248.

Hashimoto H, Shintani N, Baba A (2006) New insights into the central PACAPergic system from the phenotypes in PACAP- and PACAP receptor-knockout mice. Ann NY Acad Sci 1070:75–89.

Hirata T, Nakazawa M, Yoshihara S, Miyachi H, Kitamura K, Yoshihara Y, Hibi M (2006) Zinc-finger gene Fez in the olfactory sensory neurons regulates development of the olfactory bulb non-cell-autonomously. Development 133:1433–1443.

Ikeda Y, Luo X, Abbud R, Nilson JH, Parker KL (1995) The nuclear receptor steroidogenic factor 1 is essential for the formation of the ventromedial hypothalamic nucleus. Mol Endocrinol 9:478–486.

Ikeda Y, Nagai A, Ikeda MA, Hayashi S (2003) Sexually dimorphic and estrogen-dependent expression of estrogen receptor beta in the ventromedial hypothalamus during rat postnatal development. Endocrinology 144:5098–5104.

Kernie SG, Liebl DJ, Parada LF (2000) BDNF regulates eating behavior and locomotor activity in mice. EMBO J 19:1290–1300.

King BM (2006a) Amygdaloid lesion-induced obesity: relation to sexual behavior, olfaction, and the ventromedial hypothalamus. Am J Physiol Regul Integr Comp Physiol 291:R1201–R1214.

King BM (2006b) The rise, fall, and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. Physiol Behav 87:221–244.

Kurrasch DM, Huang J, Wilkie TM, Repa JJ (2004) Quantitative real-time

polymerase chain reaction measurement of regulators of G-protein signaling mRNA levels in mouse tissues. Methods Enzymol 389:3–15.

Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G (2007) Endocrine regulation of energy metabolism by the skeleton. Cell 130:456–469.

Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, Boe AF, Boguski MS, Brockway KS, Byrnes EJ, Chen L, Chen L, Chen TM, Chin MC, Chong J, Crook BE, Czaplinska A, Dang CN, Datta S, Dee NR (2007) Genome-wide atlas of gene expression in the adult mouse brain. Nature 445:168–176.

Maj PF, Collu M, Fadda P, Cattaneo A, Racagni G, Riva MA (2007) Long-term reduction of brain-derived neurotrophic factor levels and signaling impairment following prenatal treatment with the cannabinoid receptor 1 receptor agonist (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone. Eur J Neurosci 25:3305–3311.

Majdic G, Young M, Gomez-Sanchez E, Anderson P, Szczepaniak LS, Dobbins RL, McGarry JD, Parker KL (2002) Knockout mice lacking steroidogenic factor 1 are a novel genetic model of hypothalamic obesity. Endocrinology 143:607–614.

Marin O, Baker J, Puelles L, Rubenstein JL (2002) Patterning of the basal telencephalon and hypothalamus is essential for guidance of cortical projections. Development 129:761–773.

McClellan KM, Parker KL, Tobet S (2006) Development of the ventromedial nucleus of the hypothalamus. Front Neuroendocrinol 27:193–209.

Michaud JL, Boucher F, Melnyk A, Gauthier F, Goshu E, Levy E, Mitchell GA, Himms-Hagen J, Fan CM (2001) Sim1 haploinsufficiency causes hyperphagia, obesity and reduction of the paraventricular nucleus of the hypothalamus. Hum Mol Genet 10:1465–1473.

Mielcarek M, Gunther S, Kruger M, Braun T (2002) VITO-1, a novel vestigial related protein is predominantly expressed in the skeletal muscle lineage. Gene Expr Patterns 2:305–310.

Miki T, Liss B, Minami K, Shiuchi T, Saraya A, Kashima Y, Horiuchi M, Ashcroft F, Minokoshi Y, Roeper J, Seino S (2001) ATP-sensitive K^+ channels in the hypothalamus are essential for the maintenance of glucose homeostasis. Nat Neurosci 4:507–512.

Nachtigal MW, Hirokawa Y, Enyeart-VanHouten DL, Flanagan JN, Hammer GD, Ingraham HA (1998) Wilms' Tumor 1 and Dax-1 modulate the orphan nuclear receptor SF-1 in sex-specific gene expression. Cell 93:445–454.

O'Brien RJ, Xu D, Petralia RS, Steward O, Huganir RL, Worley P (1999) Synaptic clustering of AMPA receptors by the extracellular immediate-early gene product Narp. Neuron 23:309–323.

Paxinos G, Franklin KBJ (2003) The mouse brain in stereotaxic coordinates: compact Ed 2. San Diego: Academic.

Pozzo Miller LD, Aoki A (1992) Postnatal development of the hypothalamic ventromedial nucleus: neurons and synapses. Cell Mol Neurobiol 12:121–129.

Prober DA, Rihel J, Onah AA, Sung RJ, Schier AF (2006) Hypocretin/orexin overexpression induces an insomnia-like phenotype in zebrafish. J Neurosci 26:13400–13410.

Reti IM, Reddy R, Worley PF, Baraban JM (2002) Prominent Narp expression in projection pathways and terminal fields. J Neurochem 82:935–944.

Rios M, Fan G, Fekete C, Kelly J, Bates B, Kuehn R, Lechan RM, Jaenisch R (2001) Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Mol Endocrinol 15:1748–1757.

Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG (1996) Identification of targets of leptin action in rat hypothalamus. J Clin Invest 98:1101–1106.

Segal JP, Stallings NR, Lee CE, Zhao LP, Socci N, Viale A, Harris TM, Soares MB, Childs G, Elmquist JK, Parker KL, Friedman JM (2005) Use of laser-capture microdissection for the identification of marker genes for the ventromedial hypothalamic nucleus. J Neurosci 25:4181–4188.

Shinoda K, Lei H, Yoshii H, Nomura M, Nagano M, Shiba H, Sasaki H, Osawa Y, Ninomiya Y, Niwa O, Morohashi KI, Li E (1995) Developmental defects of the ventromedial hypothalamic nucleus and pituitary gonadotroph in the Ftz-F1 disrupted mice. Dev Dyn 204:22–29.

Simerly RB, Chang C, Muramatsu M, Swanson LW (1990) Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J Comp Neurol 294:76–95.

Summerton J (1999) Morpholino antisense oligomers: the case for an RNase H-independent structural type. Biochim Biophys Acta 1489:141–158.

Swanson LW (1987) The hypothalamus. Amsterdam: Elsevier Science.

Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, Julius D (1995) Eating disorder and epilepsy in mice lacking 5-HT2c serotonin receptors. Nature 374:542–546.

Tessmar-Raible K, Raible F, Christodoulou F, Guy K, Rembold M, Hausen H, Arendt D (2007) Conserved sensory-neurosecretory cell types in annelid and fish forebrain: insights into hypothalamus evolution. Cell 129:1389–1400.

Tong Q, Ye C, McCrimmon RJ, Dhillon H, Choi B, Kramer MD, Yu J, Yang Z, Christiansen LM, Lee CE, Choi CS, Zigman JM, Shulman GI, Sherwin RS, Elmquist JK, Lowell BB (2007) Synaptic glutamate release by ventromedial hypothalamic neurons is part of the neurocircuitry that prevents hypoglycemia. Cell Metab 5:383–393.

Tran PV, Lee MB, Marin O, Xu B, Jones KR, Reichardt LF, Rubenstein JR, Ingraham HA (2003) Requirement of the orphan nuclear receptor SF-1 in terminal differentiation of ventromedial hypothalamic neurons. Mol Cell Neurosci 22:441–453.

Tran PV, Akana SF, Malkovska I, Dallman MF, Parada LF, Ingraham HA (2006) Diminished hypothalamic bdnf expression and impaired VMH function are associated with reduced SF-1 gene dosage. J Comp Neurol 498:637–648.

Tripodi M, Filosa A, Armentano M, Studer M (2004) The COUP-TF nuclear receptors regulate cell migration in the mammalian basal forebrain. Development 131:6119–6129.

Volkoff H, Canosa LF, Unniappan S, Cerda-Reverter JM, Bernier NJ, Kelly SP, Peter RE (2005) Neuropeptides and the control of food intake in fish. Gen Comp Endocrinol 142:3–19.

Wehman AM, Staub W, Meyers JR, Raymond PA, Baier H (2005) Genetic dissection of the zebrafish retinal stem-cell compartment. Dev Biol 281:53–65.

Xu B, Goulding EH, Zang K, Cepoi D, Cone RD, Jones KR, Tecott LH, Reichardt LF (2003) Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci 6:736–742.

Zigman JM, Jones JE, Lee CE, Saper CB, Elmquist JK (2006) Expression of ghrelin receptor mRNA in the rat and the mouse brain. J Comp Neurol 494:528–548.