Nucleus- and cell-specific gene expression in monkey thalamus

Karl D. Murray, Prabhakara V. Choudary, and Edward G. Jones*

Center for Neuroscience and Department of Psychiatry and Behavioral Sciences, University of California, Davis, CA 95616

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Nuclei of the mammalian thalamus are aggregations of neurons with unique architectures and input–output connections, yet the molecular determinants of their organizational specificity remain unknown. By comparing expression profiles of thalamus and cerebral cortex in adult rhesus monkeys, we identified transcripts that are unique to dorsal thalamus or to individual nuclei within it. Real-time quantitative PCR and *in situ* hybridization analyses confirmed the findings. Expression profiling of individual nuclei microdissected from the dorsal thalamus revealed additional subsets of nucleus-specific genes. Functional annotation using Gene Ontology (GO) vocabulary and Ingenuity Pathways Analysis revealed overrepresentation of GO categories related to development, morphogenesis, cell–cell interactions, and extracellular matrix within the thalamus- and nucleus-specific genes, many involved in the Wnt signaling pathway. Examples included the transcription factor *TCF7L2*, localized exclusively to excitatory neurons; a calmodulin-binding protein *PCP4*; the bone extracellular matrix molecules *SPP1* and *SPARC*; and other genes involved in axon outgrowth and cell matrix interactions. Other nucleus-specific genes such as *CBLN1* are involved in synaptogenesis. The genes identified likely underlie nuclear specification, cell phenotype, and connectivity during development and their maintenance in the adult thalamus.

development | excitatory neurons | inhibitory neurons | thalamocortical | Wnt signaling pathway

The mammalian thalamus is made up of groupings of neurons that reflect its evolutionary and developmental history, its function as a sensory relay, and its involvement in forebrain activities that underlie states of consciousness (1–4). The three major subdivisions of thalamus (epithalamus, dorsal thalamus, and ventral thalamus) emerge during embryogenesis from the wall of the diencephalic alar plate (5–7). Aggregation of fate-determined postmitotic neurons leads to the formation of multiple subnuclei within these divisions characterized by different chemo-, cyto-, and myeloarchitectures and differing patterns of connections.

The establishment of architecture and connections in all brain regions is modulated by molecular cues that govern cell aggregation, neurotransmitter phenotype, axon guidance, and synaptogenesis (8, 9). The unique architecture, connectivity, and transmitter/receptor characteristics of each thalamic nucleus are unlikely to be established or maintained in the absence of molecular genetic guidance. Clues to the nature of underlying mechanisms can be found by identifying genes that give a molecular identity to thalamic nuclei. Sets of regulatory genes distinguish the three major thalamic subdivisions in the developing and adult rodent and primate (10, 11), but expression occurs across multiple nuclei, in regional rather than nucleus-specific patterns. Some examples of nucleus-specific expression, however, do occur, e.g., *Id-2* in the primate centre médian nucleus (CM; ref. 10). Expression of neurotransmitter- or receptor-related genes in the thalamus also tends to be regional rather than nucleus-specific, but there are exceptions to this rule as well (12, 13).

To determine the extent to which subnuclei of the major thalamic divisions are molecularly distinct, we used high-density oligonucleotide arrays to identify thalamus-specific genes in

adult monkeys. By comparing expression profiles of thalamus and cerebral cortex, we identified genes not hitherto known to be expressed in thalamus. Further profiling of microdissected nuclei identified more comprehensive sets of genes with nucleus-specific expression. Confirmation of gene expression by RT-PCR, *in situ* hybridization histochemistry, and/or immunohistochemistry revealed remarkable cell- and nucleus-specific patterns of expression. Functionally, the genes are related to development and cell–cell interactions, implying their involvement in the establishment and maintenance of thalamic nuclear and cellular specificity.

Results

Genes specific to thalamus were identified by comparing expression patterns in posterior thalamus (PT) with those in visual cortex (VC) and frontal cortex (FC). The vast majority of genes called “present” were equally distributed between cortex and thalamus. Of 1,978 genes called “present” in PT, VC, and FC, 89 (4.4%) were enriched in PT by ≥ 1.2 -fold. A more stringent cutoff of ≥ 3 -fold reduced this number to 31 (1.5%) thalamus-specific genes [supporting information (SI) Table 3].

A significant overrepresentation of thalamus-enriched probe sets was found for Gene Ontology (GO) terms related to development, morphogenesis, and cell signaling. The terms structural molecule activity (six genes, $P < 0.001$), cell differentiation (six genes, $P < 0.001$), morphogenesis (13 genes, $P < 0.01$), and extracellular matrix (ECM) (three genes, $P < 0.01$) were all overrepresented. Of the 17 most overrepresented genes (SI Table 4), 12 were associated with cellular growth and development (*FGFR2*, *HSPA2*, *ILGFBP7*, *MOG*, *NDRG1*, *NHLH2*, *NTNG1*, *PCP4*, *PRDX1*, *SPARC*, *SPP1*, and *WWP1*). Four genes associated with cell–cell interactions (*NTNG1*, *RLN*, *SPARC*, and *SPP1*) were localized to the ECM.

Validation of the microarray results by RT-PCR was limited to the 31 genes enriched in PT by ≥ 3 -fold. Twenty-five (81%) were successfully amplified (Figs. 1 and 2, Table 1). Twenty (65%) displayed elevated expression in PT vs. VC.

Transcriptional profiling of samples microdissected from anterior nuclei of dorsal thalamus, CM, mediodorsal nucleus, pulvinar, and ventral posterior nuclei confirmed and extended the thalamus-specific results. Unsupervised hierarchical clustering broadly parsed thalamic nuclei into groups displaying similar

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Abbreviations: ECM, extracellular matrix; CM, centre médian nucleus; FC, frontal cortex; GO, Gene Ontology; PT, posterior thalamus; Pf, parafascicular nucleus; VC, visual cortex; dLGN, dorsal lateral geniculate nucleus.

Data deposition: The sequences reported in this paper have been deposited in the GEO database (accession no. GSE6708).

*To whom correspondence should be addressed. E-mail: ejones@ucdavis.edu.

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Fig. 1. RT-PCR measures of regional transcript expression levels confirm microarray results. Bar graph indicates relative expression between PT and VC for transcripts overrepresented in thalamus by ≥ 3 -fold in microarray analysis. Dotted line indicates a ratio of 1, denoting no change in expression. Error bars indicate standard deviation of the mean. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and β -actin genes were included as internal controls. Asterisks indicate probe sets analyzed by *in situ* hybridization histochemistry.

gene expression profiles (Fig. 3). When further analyzed by the Drawn Gene function in GeneSpring software (Agilent Technologies, Foster City, CA) (Fig. 3), >550 genes expressed in a nucleus-specific manner were identified within the dorsal thalamus (SI Dataset 1).

Ingenuity Pathways Analysis of the nucleus-enriched genes also showed significant overrepresentation of functions related to development and cell signaling (Fig. 4). Analysis of candidate genes for potential interactions revealed putative protein networks involving development and cell signaling as top functional categories (Table 2). Other top network functions included cell cycle, cancer, and gene expression. Strikingly, members of the canonical Wnt/ β -catenin signaling cascade featured prominently in four of the five top networks (Table 2).

The distribution of genes validated by RT-PCR and of certain of those defined by nucleus-specific assay was examined by *in situ* hybridization. Four patterns of expression were observed (Table 1): (i) restricted to thalamus, (ii) enriched in thalamus, (iii) equal in thalamus and cortex, and (iv) associated with fiber tracts. For example, expression of *SPP1*, *TCF7L2*, and *CBLN1* was restricted to thalamus. Expression of *SPARC*, *PCP4*, and *SEPT4* was enriched in thalamic nuclei. Expression of *CALR*, *FGFR2*, and *ST18* was equal in cortex and thalamus. Expression of genes associated with myelin production *MAG*, *MOG*, and *MOBP* was restricted to fiber tracts.

Expression of *TCF7L2*, *PCP4*, *CBLN1*, and *SPP1* was delimited by the borders of thalamic nuclei. *TCF7L2* expression was

restricted to nuclei of the dorsal thalamus (Fig. 2), except for some aspects of the ventral thalamus (the deep lamina of the pregeniculate nucleus, the posterior division of the zona incerta, and among scattered cells of the field of Forel), as well as in a small focus in the medial habenular nucleus of the epithalamus (Fig. 2). *PCP4* was expressed throughout dorsal thalamus, with the notable exception of the CM and parafascicular nucleus (Pf); *CBLN1*, by contrast, was expressed only in CM and Pf (Fig. 4). *PCP4* was also expressed in the striatum (caudate nucleus and putamen) and in layer V of the cerebral cortex. *SPP1* and *PCP4* were expressed only in the parvo- and magnocellular layers of the dorsal lateral geniculate nucleus; *TCF7L2* was expressed only in the parvocellular, s layers, and interlaminar layers (Fig. 2).

Immunocytochemistry for *TCF7L2* protein showed enrichment in the dorsal thalamus (SI Fig. 5). In double-labeling experiments with markers of excitatory and inhibitory neurons, *TCF7L2* immunostaining was absent from GABA neurons (SI Fig. 5) in dorsal thalamus, reticular nucleus, zona incerta, or pregeniculate nucleus. *TCF7L2* was coexpressed in dorsal thalamic neurons immunostained for α type II calcium/calmodulin-dependent protein kinase, a marker for excitatory neurons (14–16) (SI Fig. 5). *SPP1* and *PCP4* immunoreactive cells could be costained for neuronal markers, whereas *SPARC* immunoreactive cells could not be costained for neuronal, astrocytic, or oligodendrocytic markers, implying they may be microglial cells (data not shown).

Discussion

Thalamic nuclei, classically defined by cyto-, myelo-, and chemoarchitecture and by connectional and physiological characteristics, are molecularly distinct. Expression profiling revealed a subset of genes that were enriched or exclusively expressed in nuclei of adult thalamus. Most prominent were genes associated with development, cell signaling, morphogenesis, the ECM, and the Wnt signaling pathway. Cell signaling molecules encoded by several candidate genes are localized to ECM, suggesting that molecules within the extracellular environs are important determinants of how thalamic cells aggregate into nuclei.

The results demonstrate that human oligonucleotide arrays can be successfully used for large-scale transcriptional profiling of other Old World primate brains, as demonstrated by the delineation of novel thalamic markers, e.g., *TCF7L2*, *PCP4*, and *CBLN1*, whose expression is not only limited to specific thalamic nuclei, but in the case of *TCF7L2*, restricted to excitatory neurons. Two members of the bone matricellular family, *SPP1* (Osteopontin) and *SPARC* (Osteonectin), with cell type- and nucleus-specific patterns of expression, were also detected. Although from the same protein family, *SPP1* and *SPARC* are expressed in neurons and nonneuronal cells, respectively, suggesting they may contribute to nuclear and cellular identities in unique ways.

The thalamus-specific genes identified, whether highly restricted (e.g., *SPP1* and *CBLN1*) or broad (e.g., *TCF7L2* and *PCP4*) in their expression, were invariably delimited by classical nuclear boundaries, and none redefined thalamic boundaries. Similar adherence of expression patterns to cytoarchitecture occurs in mouse hippocampus (17, 18) and amygdala (19), often providing less equivocal delineations of borders than cytoarchitecture alone (20). Establishment of nuclear identity from homogenous masses of cells in the developing thalamus is associated with determination of cell size, aggregation, packing density, and establishment of connections; the GO associations of the majority of the genes identified implicate them in these activities.

Expression of two genes, *PCP4* and *SPP1*, was restricted to cells of dorsal thalamus, but none to ventral thalamus, although *TCF7L2* marked subpopulations of non-GABA cells in the pregeniculate nucleus, zona incerta, and field of Forel. In ferrets, Kawasaki *et al.* (21) demonstrated molecular distinctions be-

Fig. 2. Autoradiograms and immunocytochemical preparations from monkey thalamus and cortex. Images from film autoradiograms show abundance of transcripts of *TCF7L2* (A, E, I, and M), *PCP4* (B, F, and J), *SPP1* (C, G, and K), and *SPARC* (D, H, and L) in thalamus (A–D and I–M) relative to cortex (E–H). Expression of *TCF7L2* and *SPP1* is restricted to thalamus, whereas *PCP4* and *SPARC* are expressed in other subcortical regions as well. Higher-magnification images of the dorsal lateral geniculate nucleus (dLGN) illustrate the differential expression of *TCF7L2*, *PCP4*, and *SPP1* in dLGN (I–L). Dashed line indicates separation between magnocellular (1 and 2) and parvocellular (3–6) layers of the dLGN, as marked in adjacent Nissl section (L). *In situ* hybridization for *TCF7L2* mRNA (M) and immunoreactivity for TCF7L2 (N and O) reveal expression in posterior division of the zona incerta (M), the deep lamina of the pregeniculate nucleus (N), and the field of Forel (O). (Scale bars: A–H, 3 mm; M, 1 mm; I–L, N, and O, 500 μ m.) CN, caudate nucleus; FF, field of Forel; LP, lateral posterior nucleus; MB, midbrain; MD, mediodorsal nucleus; OT, optic tract; P, putamen; Prg, pregeniculate nucleus; R, reticular nucleus; SPf, sub-Pf; VL, ventral lateral nucleus; VMb, basal ventral medial nucleus; VP, ventral posterior inferior nucleus; VPL, ventral posterior lateral nucleus; VPM, ventral posterior medial nucleus; ZI, zona incerta.

tween the dLGN, a component of dorsal thalamus and the perigeniculate nucleus, a part of the reticular nucleus. In their study, *PCP4* was more heavily expressed in larger putative Y cells

of the dLGN than in smaller putative X cells. We observed *PCP4* expression in both magno- and parvocellular laminae of monkey dLGN and in most other dorsal thalamic nuclei. However, *PCP4* expression was absent from the CM and Pf nuclei that form the caudal group of intralaminar nuclei. CM, absent in rodents and many other mammals, is particularly elaborated in primates and displays a unique expression profile compared with other thalamic nuclei (Fig. 3 and SI Dataset 1). CM and Pf also exclusively express *CBLN1* and the transcription factor *Id-2* (10) and are characterized by patterns of GABA and glutamate receptor expression that are very different from those in other dorsal thalamic nuclei (12, 13). *CBLN1*, a glycoprotein that is structurally related to the C1q and tumor necrosis factor families of proteins, in the cerebellum is associated with synaptogenesis in Purkinje cells (22).

The transcription factor, *TCF7L2*, was highly expressed and restricted to excitatory neurons in dorsal thalamus and to non-GABA neurons in the ventral thalamus. *TCF7L2* is specific for thalamus, unlike other markers of excitatory thalamic neurons, such as α type II calcium/calmodulin-dependent protein kinase, which are expressed in other forebrain regions as well (13, 14). *TCF7L2* is a member of the high mobility group box

Table 1. Candidate genes validated by *in situ* hybridization

Expression pattern	Gene symbol
Restricted to thalamus	<i>SPP1</i> <i>TCF7L2</i>
Enriched in thalamus	<i>SPARC</i> <i>SEPT4</i> <i>PCP4</i>
Equal in cortex and thalamus	<i>CALR</i> <i>FGFR2</i> <i>ST18</i>
Associated with fiber tracts	<i>MAG</i> <i>MOBP</i> <i>MOG</i>
Cortex specific or enriched	<i>NONE</i>
None detected	<i>GPR37</i> <i>C11ORF9</i>

Fig. 3. GeneSpring analysis of five thalamic nuclei. Nuclei of the dorsal thalamus display unique gene expression profiles. (A) Genes with significant expression ($P < 0.05$) in at least one of the five regions examined were hierarchically clustered by similarity in expression profile. The resulting heat map of the dendrogram tree reveals groups of genes with high (red) expression levels in one thalamic nucleus and moderate (black) or low (green) levels in the other four nuclei. (B) Genes with thalamic nucleus-specific expression profiles were identified by using the Drawn Gene function in the GeneSpring analysis program. For each region examined, sets of genes whose expression profiles significantly correlated ($R^2 > 0.95$) to a template pattern (red) were identified.

proteins of the T cell factor/lymphoid enhancer factor subfamily that mediate Wnt protein signaling through the canonical β -catenin pathway (23–25). Wnts direct the emergence of functionally distinct regions along the developing neuraxis and have consequently been implicated in the formation of neuronal connectivity (26–30). The Wnt pathway also influences axonal remodeling through interactions with the cytoskeleton (31, 32), and interfering with Wnt signaling disrupts synapse formation (31, 33). The Wnt/ β -catenin signaling cascade featured prominently in the top functional networks (Table 2). Hence, Wnt/ β -catenin signaling is a candidate for involvement not only in

Fig. 4. Microarray results confirmed by *in situ* hybridization histochemistry (A) and functional analysis of thalamus-enriched genes (B). *In situ* hybridization and functional annotation validate thalamic nucleus-specific gene expression. (A) Images from film autoradiograms show differential expression of *CBLN1* (Upper) and *PCP4* (Lower) in monkey dorsal thalamus, corroborating the microarray results (Right). (B) The Ingenuity Pathways Analysis program enabled biological/functional annotations of candidate genes identified by the Drawn Gene Function in GeneSpring analysis. The top five functions are compared across all regions. Abbreviations are as in Fig. 2.

formation and maintenance of dorsal thalamic connectivity but also in the plasticity of connections in the adult. This notion is supported by the observation that mice null for the low-density lipoprotein receptor-related protein 6, a required signaling coreceptor for the Wnt/ β -catenin pathway, exhibit profound disruptions in the development of dorsal thalamus and epithalamus (34).

The present observations illustrate that nuclei of the dorsal thalamus are comprised of groups of cells sharing common molecular phenotypes. The variety of cellular and molecular mechanisms likely to be required for establishing and maintaining the specific architectural, connectional, and functional signatures of each thalamic nucleus is reflected in the range of functions associated with the thalamus-enriched genes.

Materials and Methods

Animals. Brains from eight adult male rhesus monkeys (*Macaca mulatta*) were used. All procedures were carried out by using

Region	Score	Focus genes	Wnt/ β -catenin canonical pathway genes	Top biological functions
Ven DT	24	18	0	Cell-to-cell signaling and interaction, cellular assembly and organization, cellular function and maintenance
CM	23	15	3	Skeletal and muscular system development and function, cancer, cell cycle
Med DT	33	21	2	Embryonic development, cellular assembly and organization, cellular development
Pulv	18	10	3	Cell cycle, carbohydrate metabolism, small molecule biochemistry
An DT	33	16	3	Gene expression, cancer, cellular growth and proliferation

Chemicon, Temecula, CA), prospero-related homeobox 1 (Prox1; Covance, Berkeley, CA), Tcf7l2 (Upstate Biotechnology, Lake Placid, NY, and Zymed, San Francisco, CA), SPP1 (Osteopontin; Developmental Studies Hybridoma Bank, Iowa City, IA), and SPARC (Osteonectin; US Biologicals, Swampscott, MA).

Thalamic nuclei were identified by cytoarchitectural features observed in Nissl-stained sections (ref. 7; www.brainmaps.org). Autoradiographic images were captured by using a Nikon D1X camera (Nikon, Melville, NY). Brightfield photomicrographs were obtained by using a Nikon Eclipse 1000 microscope equipped with a Quantix CCD camera (Photometrics, Tuscon, AZ). Fluorescent images were obtained with a Zeiss

(Oberkochen, Germany) LSM 510 META laser-scanning confocal microscope. Z-stacked images were collected, collapsed, saved as TIFF files, imported into Adobe Photoshop, and labeled in Adobe Illustrator (Adobe Systems, San Jose, CA).

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1. Jones EG (1998) *Adv Neurol* 77:49–71.
2. Llinás R, Ribary U, Contreras D, Pedroarena C (1998) *Philos Trans R Soc London B* 353:1841–1849.
3. Llinás R, Ribary U (2001) *Ann NY Acad Sci* 929:166–175.
4. Llinás RR, Paré, D (1991) *Neuroscience* 44:521–535.
5. Puelles L, Rubenstein JL (1993) *Trends Neurosci* 16:472–479.
6. Puelles L, Rubenstein JL (2003) *Trends Neurosci* 26:469–476.
7. Jones EG (2006) *The Thalamus* (Cambridge Univ Press, Cambridge, UK), 2nd Ed.
8. Jan YN, Jan LY (2003) *Neuron* 40:229–242.
9. Tessier-Lavigne M (2002) *Harvey Lect* 98:103–143.
10. Jones EG, Rubenstein JL (2004) *J Comp Neurol* 477:55–80.
11. Nakagawa Y, O'Leary DD (2001) *J Neurosci* 21:2711–2725.
12. Huntsman MM, Leggio MG, Jones EG (1996) *J Neurosci* 16:3571–3589.
13. Jones EG, Tighilet B, Tran BV, Huntsman MM (1998) *J Comp Neurol* 397:371–393.
14. Benson DL, Isackson PJ, Hendry SH, Jones EG (1991) *J Neurosci* 11:1540–1564.
15. Benson DL, Isackson PJ, Gall CM, Jones EG (1992) *Neuroscience* 46:825–849.
16. Liu XB, Jones EG (1996) *Proc Natl Acad Sci USA* 93:7332–7336.
17. Lein ES, Zhao X, Gage FH (2004) *J Neurosci* 24:3879–3889.
18. Zhao X, Lein ES, He A, Smith SC, Aston C, Gage FH (2001) *J Comp Neurol* 441:187–196.
19. Zirlinger M, Kreiman G, Anderson DJ (2001) *Proc Natl Acad Sci USA* 98:5270–5275.
20. Lein ES, Callaway EM, Albright TD, Gage FH (2005) *J Comp Neurol* 485:1–10.
21. Kawasaki H, Crowley JC, Livesey FJ, Katz LC (2004) *J Neurosci* 24:9962–9970.
22. Hirai H, Pang Z, Bao D, Miyazaki T, Li L, Miura E, Parris J, Rong, Y, Watanabe M, Yuzaki M, Morgan JL (2005) *Nat Neurosci* 8:1534–1541.
23. Cho EA, Dressler GR (1998) *Mech Dev* 77:9–18.
24. Korinek V, Barker N, Willert K, Molenaar M, Roose J, Wagenaar G, Markman M, Lamers W, Destree O, Clevers H (1998) *Mol Cell Biol* 18:1248–1256.
25. Lee YJ, Swencki B, Shoichet S, Shivdasani RA (1999) *J Biol Chem* 274:1566–1572.
26. Charron F, Tessier-Lavigne M (2005) *Development (Cambridge, UK)* 132:2251–2262.
27. Christiansen JH, Coles EG, Wilkinson DG (2000) *Curr Opin Cell Biol* 12:719–724.
28. Patapoutian A, Reichardt LF (2000) *Curr Opin Neurobiol* 10:392–399.
29. Ciani L, Salinas PC (2005) *Nat Rev Neurosci* 6:351–362.
30. Pleasure SJ (2001) *Trends Neurosci* 24:69–71.
31. Hall AC, Lucas FR, Salinas PC (2000) *Cell* 100:525–535.
32. Krylova O, Messenger MJ, Salinas PC (2000) *J Cell Biol* 151:83–94.
33. Luo ZG, Wang Q, Zhou JZ, Wang J, Luo Z, Liu M, He X, Wynshaw-Boris A, Xiong WC, Lu B, et al. (2002) *Neuron* 35:489–505.
34. Zhou CJ, Pinson KI, Pleasure SJ (2004) *J Neurosci* 24:7632–7639.
35. Jones EG, Hendry SHC, Liu XB, Hodgins S, Potkin SG, Tourtellotte WW (1992) *J Neurosci Methods* 44:133–144.
36. Bunney WE, Bunney BG, Vawter MP, Tomita H, Li J, Evans SJ, Choudary PV, Myers RM, Jones EG, Watson SJ, et al. (2003) *Am J Psychiatry* 160:657–666.
37. Choudary PV, Molnar M, Evans SJ, Tomita H, Li JZ, Vawter MP, Myers RM, Bunney WE, Jr, Akil H, Watson SJ, et al. (2005) *Proc Natl Acad Sci USA* 102:15653–15658.
38. Anderle P, Duval M, Draghici S, Kuklin A, Littlejohn TG, Medrano JF, Vilanova D, Roberts MA (2003) *BioTechniques* Suppl:36–44.
39. Draghici S, Khatri P, Bhavsar P, Shah A, Krawetz SA, Tainsky MA (2003) *Nucleic Acids Res* 31:3775–3781.
40. Khatri P, Draghici S, Ostermeier GC, Krawetz SA (2002) *Genomics* 79:266–270.
41. Murray KD, Isackson PJ, Jones EG (2003) *Neuroscience* 122:407–420.