SUPPLEMENTAL METHODS

Real-time PCR. Real-time PCR analysis was performed according to the comparative threshold cycle (C_{T} - cycle at which a significant increase in SYBR Green fluorescence is first detected) method (see SmartCycler manufacturer’s instructions). The calculation of expression ratio involves obtaining the difference (C_{T}) between the C_{T} of the target (eg. BDNF) and the normalizer (eg. HPRT):

\[ C_{T} = C_{T} \text{(target)} - C_{T} \text{(normalizer)} \]

This value is calculated for each experimental (eg. deprived visual cortex) and baseline (eg. visually-driven cortex) sample. The last step in the quantitation is to transform these values into expression ratios (in “visually-driven” cortex (ipsilateral to ME) compared to that in “deprived” visual cortex (contralateral to ME)) according to the formula:

\[ \text{Expression ratio} = 2^{-CT} \]

Since SYBR Green intercalates all double-stranded DNA (including primer-dimers), all real-time PCR runs included a melt curve for product identification and purity (see the SmartCycler Manual for details), and only reactions resulting in a single product were considered valid.

Microarray analysis. Each chip contains probe pair sets corresponding to ~12,000 genes and clustered ESTs. Each gene or EST interrogated by a GeneChip expression array is analyzed with 11-20 pairs of “specific” unique 25-mer Perfect Match (PM) and MisMatch (MM) oligonucleotide probes used for detection of expressed genes.

The output of the GeneChip software for each microarray yields “detection calls” indicating whether a transcript is reliably detected, and “signal” values calculated to assign a relative measure of abundance of a given transcript. The detection calls are based on statistical calculations of the difference in hybridization signals between PM and their control MM probe sets. The GeneChip software provides P-values for “detection calls” and defines a quantitative metric “signal” call (in arbitrary fluorescence units) for genes/ESTs being expressed as “present” (P) with P<0.04, “marginal” (M) with P value in the range 0.04-0.06, and “absent” (A) with P>0.06.

Affymetrix software uses two different and separate algorithms for evaluation of alteration in levels of gene expression between chips (ie. deprived vs. visually-driven visual cortex) to calculate significant changes and change quantity metrics for every probe set. A change algorithm generates “Change calls” (“Increase”, “Decrease”, “Marginal Increase/Decrease” and “No Change”) and “Fold change with P values”. A second algorithm produces a quantitative estimate of the magnitude and direction of change in gene expression in the form of a “Signal Log Ratio” (SLR) when two arrays are compared (See Affymetrix GeneChip Software for details). All gene regulation between deprived and visually-driven hemispheres is expressed in terms of expression ratio.