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BRAIN RESEARCH

Research Report

Gene profiling of pooled single neuronal cell bodies from laser capture microdissected vervet monkey lateral geniculate nucleus hybridized to the Rhesus Macaque Genome Array

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ABSTRACT

This report is based on an ongoing study to examine gene expression differences in monkey lateral geniculate nucleus (LGN). Here, samples from an Old World species, the vervet monkey (*Cercopithecus aethiops*), were cross-hybridized to the Rhesus Macaque Genome Array (Affymetrix). Microarray analysis was performed using laser capture microdissected populations of individual neuronal cell bodies isolated from the LGN compared to heterogeneous samples from whole lamina. Our results indicated that cross-species hybridization of microdissected brain tissue samples from vervet monkeys to the Rhesus array produced reliable and biologically relevant data sets. We present the first list of genes enriched in the large neuronal cell bodies of the LGN. We found that these cell bodies are concentrated with genes involved in metabolic processes and protein synthesis, whereas signaling molecules including chemokines and integrins were expressed at higher levels within heterogeneous samples. Our data set also provides support for a contribution of Wnt signaling in adult monkey LGN.

1. Introduction

Old World primates offer unique advantages for gene profiling studies of brain areas (Karssen et al., 2006). They are particularly indispensable when studying anatomical or functional aspects of the brain where common laboratory models such as rodents appreciably diverge from humans. Some notable examples include parallel visual pathways (Callaway, 2005; Lee et al., 1996; Wassle, 2004), neural correlates of social behavior (Karssen et al., 2006), and aging (Fraser et al., 2005). There are practical difficulties in handling human brain tissue for gene profiling studies because it is not easily available, and then only so in uncontrolled post mortem form and usually preserved with formalin fixatives. Brain tissue from Old World non-human primates can be obtained from laboratory populations at precise developmental stages in frozen form, which is more amenable for experimental design of molecular studies, such as RNA isolation and microarray analysis. However, these advantages are offset by cost and ethical concerns that make monkey tissue samples precious commodities. It is therefore essential to maximize the quality of data obtained from gene profiling studies while using the smallest possible amounts of tissue. To this end, laser capture microdissection (LCM) technology has now become widely used to precisely delineate homogenous

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Abbreviations: LCM, laser capture microdissection; LGN, lateral geniculate nucleus; FDR, false discovery rate; qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction; N, neuronal cell body samples; L, heterogeneous layer samples

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brain cell populations for use in microarray studies. Linked with RNA amplification protocols, LCM allows for gene profiling using starting sample sizes as small as single cells (Luzzi et al., 2003).

High-throughput monkey brain profiling studies with commercial oligonucleotide microarrays have to date been performed almost exclusively by cross-species hybridization to human arrays (Ace and Okulicz, 2004; Caceres et al., 2003; Chismar et al., 2002; Dillman and Phillips, 2005; Lachance and Chaudhuri, 2004; Lemos et al., 2006; Marvanova et al., 2003; Murray et al., 2007; Redmond et al., 2003; Spindel et al., 2005). The sequence similarity between Old World monkeys and humans is about 85–92% (King et al., 1988; Sibley et al., 1990). In practice, this causes reduced overall signal intensity and a significant increase in the noise level of the data, resulting in high false-discovery rates and false-negative misses (Marvanova et al., 2003; Walker et al., 2006). Studies utilizing cross-species hybridization of monkey samples to human arrays have nevertheless produced reliable identification of independently validated candidate genes. These may have however come at the expense of substantial data loss (Chismar et al., 2002; Karssen et al., 2006). Cross-species hybridizations become further problematic when examining more closely related brain areas, where fewer differentially expressed genes are estimated to exist and are therefore likely to be masked by data noise (Evans et al., 2003).

Although longer oligonucleotide arrays can be used (Walker et al., 2006), and methods have been developed to circumvent the performance issues of monkey samples cross-hybridized to human arrays (Wang et al., 2004), sequence divergence continues to remain as a major concern with differences as low as 1% capable of impacting hybridization performance (Bar-Or et al., 2007; Gilad et al., 2005). For vervet monkey (*Cercopithecus aethiops*), the Old World species used as a model in our laboratory, percent identity with Rhesus of the 3′ regions of selected genes is estimated at around 98% whereas it is roughly 94% with human sequences (Spindel et al., 2005). A potential improvement would thus be to use a microarray based on Rhesus sequences as a means to reduce the evolutionary divergence at the nucleotide level. The commercial introduction of the Affymetrix Rhesus Macaque Genome Array is a welcome development in this regard. A recent evaluation of macaque tissue samples hybridized to this array has confirmed its dependability and validity as a research tool (Duan et al., 2007).

This report is drawn from our ongoing efforts at examining gene expression differences in visual areas of the vervet monkey. We are particularly interested in gene expression differences in

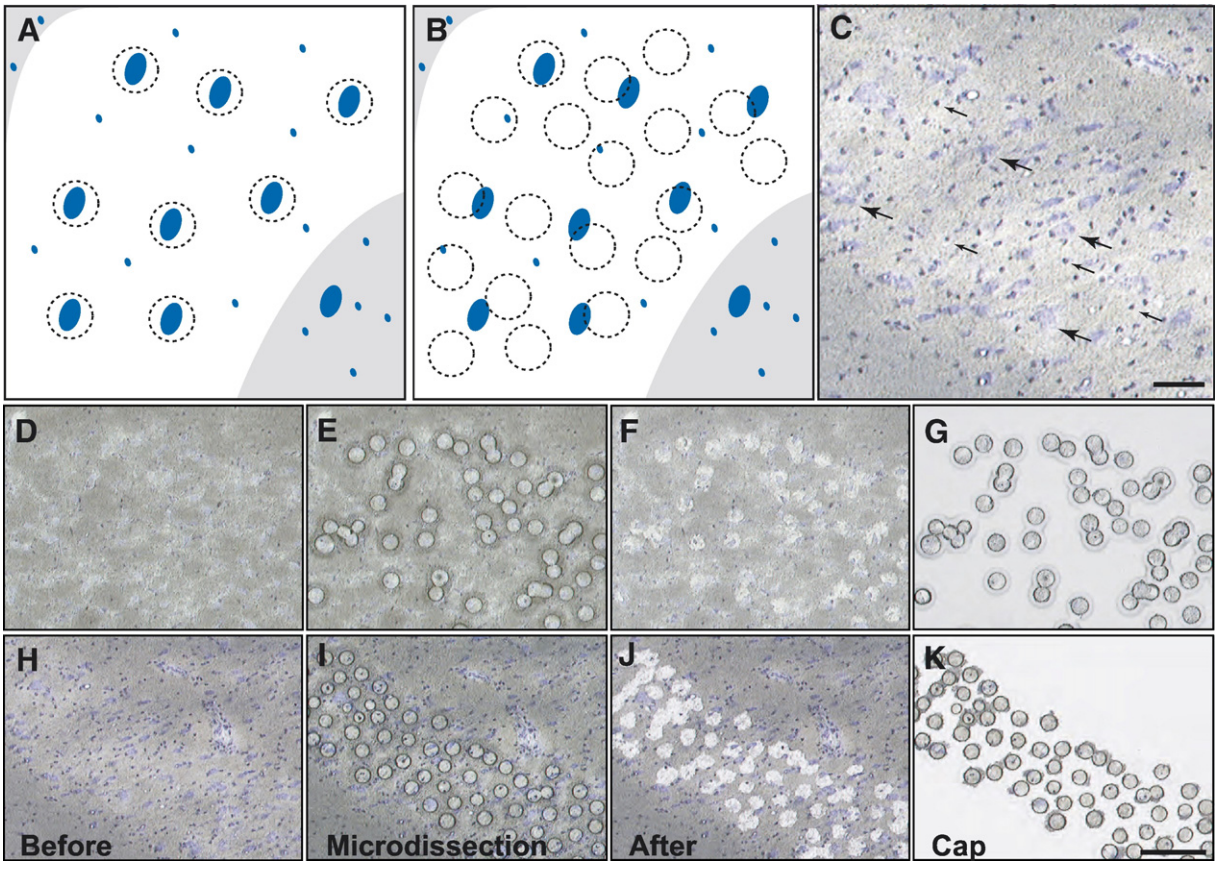

Fig. 1 – Laser capture microdissection strategy. Schematic representation of microdissection of neuronal cell body (A) and heterogeneous layer (B) samples. Dashed circles, LCM pulses; large blue ovals, nucleus of cresyl violet stained neuron; small blue ovals, nucleus of cresyl violet stained glial cell; white, lamina of LGN; gray, intercalated layers of LGN (koniocellular layers). Typical appearance of LGN cells stained with cresyl violet and dehydrated through ethanol (C) showing the distinction between larger neuronal cell bodies (large arrowheads) and smaller glia (small arrowheads). Photomicrographs from typical LCM of neuronal cell body (D–G) and heterogeneous layer (H–K) samples before microdissection (D, H), showing LCM pulses during microdissection (E, I), remaining tissue after microdissection (F, J), and final captured tissue on the LCM cap (G, K). Scale bars = 100 μm.