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Research Report

# A novel analytical brain block tool to enable functional annotation of discriminatory transcript biomarkers among discrete regions of the fronto-limbic circuit in primate brain

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ARTICLE INFO

*Article history:*
Accepted 11 December 2014
Available online 18 December 2014

*Keywords:*
Systems
RNA-seq
Area 25
Hippocampus
Anterior cingulate cortex
Amygdala

ABSTRACT

Fronto-limbic circuits in the primate brain are responsible for executive function, learning and memory, and emotions, including fear. Consequently, changes in gene expression in cortical and subcortical brain regions housing these circuits are associated with many important psychiatric and neurological disorders. While high quality gene expression profiles can be identified in brains from model organisms, primate brains have unique features such as Brodmann Area 25, which is absent in rodents, yet profoundly important in primates, including humans. The potential insights to be gained from studying the human brain are complicated by the fact that the post-mortem interval (PMI) is variable, and most repositories keep solid tissue in the deep frozen state. Consequently, sampling the important medial and internal regions of these brains is difficult. Here we describe a novel method for obtaining discrete regions from the fronto-limbic circuits of a 4 year old and a 5 year old, male, intact, frozen non-human primate (NHP) brain, for which the PMI is exactly known. The method also preserves high quality RNA, from which we use transcriptional profiling and a new algorithm to identify region-exclusive RNA signatures

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http://dx.doi.org/10.1016/j.brainres.2014.12.031

for Area 25 (NFκB and dopamine receptor signaling), the anterior cingulate cortex (LXR/RXR signaling), the amygdala (semaphorin signaling), and the hippocampus ($Ca^{++}$ and retinoic acid signaling). The RNA signatures not only reflect function of the different regions, but also include highly expressed RNAs for which function is either poorly understood, or which generate proteins presently lacking annotated functions. We suggest that this new approach will provide a useful strategy for identifying changes in fronto-limbic system biology underlying normal development, aging and disease in the human brain.



## 1. Introduction

In human and non-human primate (NHP) brains, messenger RNA and microRNA expression patterns change profoundly and coincidently during post-natal development, maturation and aging (Dannemann et al., 2012; Hu et al., 2011; Somel et al., 2010, 2011). Consequently, it has been widely anticipated that analysis of changes in gene expression in cortical and subcortical brain regions of NHP, particularly those associated with executive function and emotional control in the fronto-limbic circuit, might yield mechanistic insights into many important human disorders. These disorders could include entities as diverse as posttraumatic stress disorder (PTSD) (Admon et al., 2013; Choi et al., 2011; Myers et al., 2013; Taghva et al., 2013), major depressive disorder (Badawy et al., 2013; Engel et al., 2013; Godsil et al., 2013; Lozano et al., 2008; Seminowicz et al., 2004; Sibille et al., 2004), autism spectrum disorders (Gotts et al., 2012), schizophrenia (Guillozet-Bongaarts et al., 2014; Lee, 2013; Penzes et al., 2013), and dementias associated with Alzheimer Disease (Grieve et al., 2005; Kensinger et al., 2002), Parkinson's Disease (Bonelli and Cummings, 2007; Ibarretxe-Bilbao et al., 2008), and cognitive decline (Arnsten et al., 1995). However, very little is known regarding the genomic and epigenomic signatures characterizing individual components of the healthy fronto-limbic circuit, and how they might change as a function of age. We have therefore reasoned that there might be an operational advantage to focusing experimental attention on the NHP brain. Importantly, the time between death and availability for preservation can be minimized, and precisely controlled. By contrast, this is seldom true for the human brain, whether diseased or healthy, and circadian considerations also impact profoundly on the human brain transcription profile (Li et al., 2013). However, since human and NHP brain genomes in the superior frontal gyrus precisely parallel each other over the individual NHP and human lifespans (Somel et al., 2010), we have reasoned that there would be a great advantage to developing methods to isolate discrete frontal and limbic NHP brain regions, while at the same time preserving high quality RNA for genomic and epigenomic analysis.

The transcription profile changes as a function of age in a region-specific manner in both rodent cortex (Inukai et al., 2012; Lee et al., 2000; Loerch et al., 2008; Zahn et al., 2007), and human cortex (Erraji-Benchekroun et al., 2005; Hu et al., 2011; Lu et al., 2004). However, few parallels have been identified when directly comparing gene expression changes in brains from human and other species. In the only study of this kind of which we are aware, Somel et al. (2010)) were able to map parallel developmental and aging changes in microRNAs (miRs) and messenger RNAs (mRNAs) in gross samples of the surface-accessible superior frontal gyrus from frozen postmortem brains of healthy *Rhesus macaque,* and in the same region which had been dissected from frozen healthy human brains obtained from an NICHD/NIH-sponsored repository of frozen human brains. Relevantly, the major changes in gene expression in NHP and human superior frontal gyrus coordinately change with age when differences in lifespan are taken into account (Somel et al., 2010). However, it is not known to what extent other parts of the brain parallel these findings, including components of the fronto-limbic circuit, Furthermore, inasmuch as most repositories of diseased and control human brains are often available only in an intact, frozen state, it is a challenge to access and isolate centrally located brain regions. In addition, the time of death for humans, especially "controls", is seldom known with certainty. For example, deaths from heart attack or stroke in otherwise psychologically healthy humans often occurs after midnight. By contrast, suicides usually occur during the day, when timing is more certain (Li et al., 2013). It is therefore the purpose of this paper to describe a workflow for the frozen NHP brain that not only solves the microdissection problem for deeper brain structures, but also yields high quality RNA that can be used to identify quantitative, region-specific gene expression from within the fronto-limbic circuit.

Our strategy has been to build on our personal experiences with atlas-assisted isolation of discrete regions of fresh rodent brains (Jacobowitz, 1974; Palkovits, 1973), and fresh or fixed human brain (Jacobowitz et al., 1994). However, as described above, substantial changes in approach needed to be developed for microdissecting large frozen intact brains. In the case of brains from small rodents, we previously developed the Jacobowitz Brain Block ™ (Jacobowitz, 1974). Here, we describe a new brain block, specifically designed for the NHP brain, that can be deployed for physically slicing of either fresh or frozen intact brains into a series of rostral-to-caudal 3 mm slices. Specific regions of frozen brain can then be further microdissected, and micropunched, to isolate specific cortical and subcortical regions of the brain, while also preserving high quality RNA. Having verified that the method generates intact, high quality RNA, we addressed the following: (1) whether fronto-temporal regions from different NHPs yield quantitatively similar levels of RNAs: we find *r*-values of 0.9 or better for two NHP brains of similar age; (2)