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Research Report

# Expression of $GABA_A$ receptor $\alpha_1$ subunit mRNA and protein in rat neocortex following photothrombotic infarction

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ABSTRACT

Photothrombotic infarcts of the neocortex result in structural and functional alterations of cortical networks, including decreased GABAergic inhibition, and can generate epileptic seizures within 1 month of lesioning. In our study, we assessed the involvement and potential changes of cortical $GABA_A$ receptor ($GABA_AR$) $\alpha_1$ subunits at 1, 3, 7, and 30 days after photothrombosis. Quantitative competitive reverse transcription-polymerase chain reaction (cRT-PCR) and semi-quantitative Western blot analysis were used to investigate $GABA_AR$ $\alpha_1$ subunit mRNA and protein levels in proximal and distal regions of perilesional cortex and in homotopic areas of young adult Sprague-Dawley rats. $GABA_AR$ $\alpha_1$ subunit mRNA levels were decreased ipsilateral and contralateral to the infarct at 7 days, but were increased bilaterally at 30 days. $GABA_AR$ $\alpha_1$ subunit protein levels revealed no significant change in neocortical areas of both hemispheres of lesioned animals compared with protein levels of sham-operated controls at 1, 3, 7, and 30 days. At 30 days, $GABA_AR$ $\alpha_1$ subunit protein expression was significantly increased in lesioned animals within proximal and distal regions of perilesional cortex compared with distal neocortical areas contralaterally (Student's *t*-test, $p<0.05$). Short- and long-term alterations of mRNA and protein levels of the $GABA_AR$ $\alpha_1$ subunit ipsilateral and contralateral to the lesion may influence alterations in cell surface receptor subtype expression and $GABA_AR$ function following ischemic infarction and may be associated with formative mechanisms of poststroke epileptogenesis.


## 1. Introduction

A balance of excitatory and inhibitory neurotransmission is required for normal functioning of the central nervous system. Inhibition is mediated by the principal neurotransmitter gamma-aminobutyric acid (GABA), which binds to ionotropic $GABA_A$ receptors ($GABA_ARs$) and metabotropic $GABA_B$ receptors. GABAergic neurons provide inhibitory control in the

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*Abbreviations:* GABA, gamma-aminobutyric acid; $GABA_ARs$, $GABA_A$ receptors; cRT-PCR, competitive reverse transcription-polymerase chain reaction; mRNA, messenger ribonucleic acid; DNA, deoxyribonucleic acid; dNTPs, deoxynucleoside triphosphates; cRNA, copy ribonucleic acid; cDNA, copy deoxyribonucleic acid, diethyl pyrocarbonate (DEP)-treated water; PVDF, polyvinylidene difluoride

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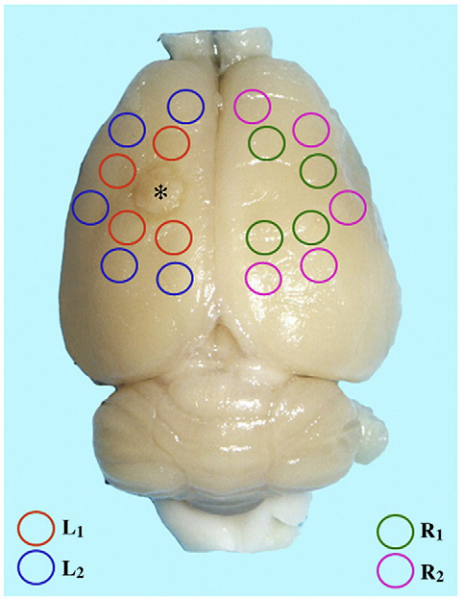


**Fig. 1 – Position of tissue punches in the rat brain at 30 days following photothrombotic infarction. Tissue punches, 3 mm diameter, were made around the lesion in the left (L) ipsilateral hemisphere and homotopic areas of the right (R) contralateral hemisphere. $L_1$, $L_2$, $R_1$, and $R_2$ were designated to reflect the proximal and distal areas of the brain that were analyzed relative to the cortical lesion.**

brain and have an important role in selective neuronal degeneration following ischemia and epilepsy (Mileson et al., 1992; Sperk et al., 2004). Photothrombotic brain infarction results in morphological and physiological changes in multiple perilesional and remote areas of the brain (Neumann-Haefelin et al., 1998, 1999; Liu et al., 2002; Redecker et al., 2002; Frahm et al., 2004a,b, 2006) and an imbalance of excitatory and inhibitory neurotransmission (Schiene et al., 1996; Qu et al., 1998) that reflect various degrees of acute injury of cortex and its subsequent repair, recovery, and reorganization. In addition to augmentation of endogenous protective mechanisms following different pathophysiological conditions, alterations in the kinetics and pharmacology of $GABA_AR$s may be associated with the development of spontaneous seizure activity (Coulter, 2001; Treiman, 2001; Nishimura et al., 2005) and may contribute to the process of poststroke epileptogenesis (Kelly et al., 2001; Liu et al., 2002; Kharlamov et al., 2003, 2007; Karhunen et al., 2007); however, studies of the basic mechanisms of ischemia-induced epileptogenesis have had limited development (Kelly, 2002, 2007).

Because large photothrombotic infarcts of the neocortex, variably associated with the epileptic state, resulted in a significant increase of $\alpha_1$ subunit mRNA in the ipsilateral cortex 4 months after lesioning (Liu et al., 2002), we sought to explore these findings further by determining whether photothrombotic infarction triggered alterations of $GABA_AR$ $\alpha_1$ subunit mRNA and the corresponding polypeptide expression in different areas of neocortex at earlier time points after lesioning. The $\alpha_1$ subunit of $GABA_AR$s is the most common $\alpha$ isoform (McKernan and Whiting, 1996) and is the dominant subunit in importance for the assembly and functioning of

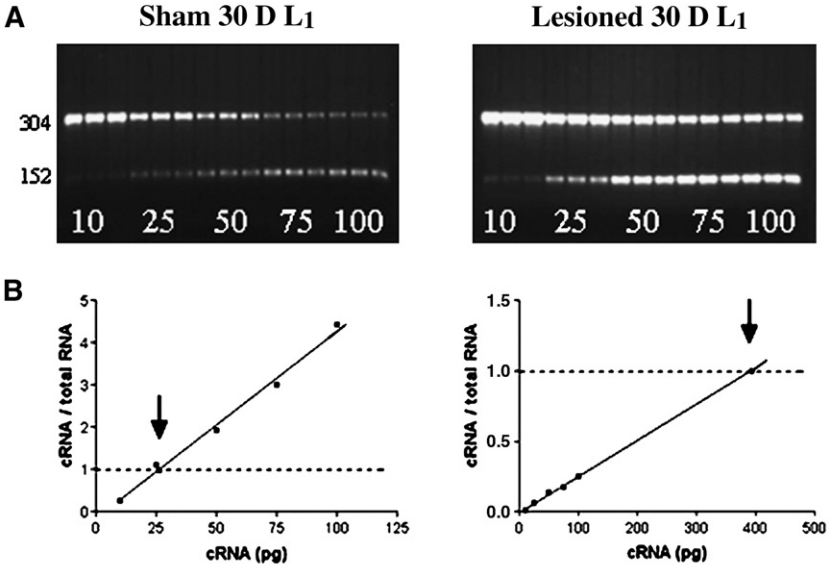


**Fig. 2 – Representative electrophoretic gels (A) and linear regression plots (B) of $\alpha_1$ subunit mRNAs for $GABA_AR$s from $L_1$-pooled samples of shams and lesioned animals generated by cRT-PCR at 30 days. (A) A series of concentrations of internal standard cRNAs (10, 25, 50, 75, and 100 pg) was added to each sample aliquot of total RNA (1.0 μg). On each gel, the cRT-PCR products are shown in triplicate; upper bands are products of target $\alpha_1$ subunit mRNA, whereas lower bands are *Bgl II*-digested internal standard PCR products. The increasing concentration of internal standards compete with $\alpha_1$ subunit mRNA for amplification. (B) Linear regression analysis of the ratios of cRNA/total RNA versus the amount of internal standard cRNA added to the reaction to generate the point of equivalency where the ratio is 1 (arrows), which represents the absolute concentration of target $GABA_AR$ $\alpha_1$ subunit mRNA.**