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# Glutamatergic or GABAergic neuron-specific, long-term expression in neocortical neurons from helper virus-free HSV-1 vectors containing the phosphate-activated glutaminase, vesicular glutamate transporter-1, or glutamic acid decarboxylase promoter

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ABSTRACT

Many potential uses of direct gene transfer into neurons require restricting expression to one of the two major types of forebrain neurons, glutamatergic or GABAergic neurons. Thus, it is desirable to develop virus vectors that contain either a glutamatergic or GABAergic neuron-specific promoter. The brain/kidney phosphate-activated glutaminase (PAG), the product of the GLS1 gene, produces the majority of the glutamate for release as neurotransmitter, and is a marker for glutamatergic neurons. A PAG promoter was partially characterized using a cultured kidney cell line. The three vesicular glutamate transporters (VGLUTs) are expressed in distinct populations of neurons, and VGLUT1 is the predominant VGLUT in the neocortex, hippocampus, and cerebellar cortex. Glutamic acid decarboxylase (GAD) produces GABA; the two molecular forms of the enzyme, GAD65 and GAD67, are expressed in distinct, but largely overlapping, groups of neurons, and GAD67 is the predominant form in the neocortex. In transgenic mice, an ~9 kb fragment of the GAD67 promoter supports expression in most classes of GABAergic neurons. Here, we constructed plasmid (amplicon) Herpes Simplex Virus (HSV-1) vectors that placed the *Lac Z* gene under the regulation of putative PAG, VGLUT1, or GAD67 promoters. Helper virus-free vector stocks were delivered into postrhinal cortex, and the rats were sacrificed 4 days or 2 months later. The PAG or VGLUT1 promoters supported ~90% glutamatergic neuron-specific expression. The GAD67 promoter supported ~90% GABAergic neuron-specific expression. Long-term expression was observed using each promoter. Principles for obtaining long-term expression from HSV-1 vectors, based on these and other results, are discussed. Long-term glutamatergic or GABAergic neuron-specific expression may benefit specific experiments on learning or specific gene therapy

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approaches. Of note, promoter analyses might identify regulatory elements that determine a glutamatergic or GABAergic neuron.



## 1. Introduction

Due to the heterogeneous cellular composition of specific brain areas, neuronal subtype-specific expression is required for many potential uses of direct neural gene transfer. The two predominant types of forebrain neurons are glutamatergic or GABAergic neurons, although the classification of classes of neurons within each type remains controversial (Mott and Dingledine, 2003; Nelson et al., 2006; Sugino et al., 2006). Thus, it is desirable to develop vectors that support either glutamatergic or GABAergic neuron-specific expression. One approach is to exploit promoters that are specific for either glutamatergic or GABAergic neurons. Vectors containing such promoters could restrict recombinant expression to either type of neuron, and, conversely, analyses of these promoters might identify the critical regulatory elements that determine a glutamatergic or GABAergic neuron.

Glutamatergic or GABAergic neuron-specific promoters might be obtained from specific genes for neurotransmitter biosynthetic enzymes or vesicular transporters. The brain/kidney phosphate-activated glutaminase (PAG (Banner et al., 1988)), the product of the GLS1 gene, produces the majority of the glutamate for release as neurotransmitter (Hertz, 2004), and PAG knockout mice show a reduction in depolarization-evoked glutamate release (Masson et al., 2006). PAG has been used as an immunohistochemical marker for glutamatergic neurons (Kaneko and Mizuno, 1988; Kaneko et al., 1992; Sakata et al., 2002; Van der Gucht et al., 2003). The rat PAG promoter was cloned and partially characterized by DNA transfection studies in a cultured kidney cell line (Taylor et al., 2001), but no analyses in neuronal cells have been reported to date. The three vesicular glutamate transporters (VGLUT1, VGLUT2, VGLUT3) are expressed in distinct populations of neurons (review (Fremeau et al., 2004)). VGLUT1 is the predominant VGLUT in the neocortex, hippocampus, cerebellar cortex, and basolateral nuclei of the amygdala; VGLUT2 is found in the thalamus, deep cerebellar nuclei, hypothalamus, brainstem, and in some neurons in layer 4 of neocortex; and VGLUT3 is found in neurons traditionally viewed as non-glutamatergic (Bellocchio et al., 2000; Fremeau et al., 2001, 2004; Herzog et al., 2001; Takamori et al., 2000, 2001; Varoqui et al., 2002). VGLUT1 knockout mice show large reductions in glutamatergic neurotransmission and quantal size (Wojcik et al., 2004). There are no published studies on the VGLUT1 promoter. Glutamic acid decarboxylase (GAD) produces GABA, and the two GAD isoforms are encoded by two genes, GAD65 and GAD67 (Erlander et al., 1991). GAD65 and GAD67 are expressed in distinct, but largely overlapping, types of neurons, and GAD67 is the predominant form in the neocortex (Erlander et al., 1991; Esclapez et al., 1994; Feldblum et al., 1993). Both the GAD65 (Bali et al., 2005; Lopez-Bendito et al., 2004; Makinae et al., 2000) and GAD67 (Chattopadhyaya et al., 2004; Di Cristo et al., 2004; Heinke et al., 2004; Kobayashi et al., 2003; Ma et al., 2006; Oliva et al., 2000, 2002; Tamamaki et al., 2003) promoters have been analyzed in transgenic mice, and an ~9 kb fragment of the GAD67 promoter is sufficient to support expression in most types of GABAergic neurons.

Helper virus-free Herpes Simplex Virus (HSV-1) plasmid vectors (Fraefel et al., 1996) (amplicons) are attractive for gene transfer into neurons because they efficiently transduce neurons, have a large capacity (51 kb or 149 kb HSV-1 vectors have been reported (Wade-Martins et al., 2003; Wang et al., 2000)), and can support long-term neuronal-specific, or neuronal subtype-specific, expression from specific cellular promoters. Specifically, the preproenkephalin (preproENK) promoter supported long-term (2 months) expression in specific brain areas containing enkephalinergic neurons (ventromedial hypothalamus or amygdala, helper virus system (Kaplitt et al., 1994)). Large fragments of the tyrosine hydroxylase (TH; 6.8 kb or 9 kb) promoter supported long-term (2 months) expression in midbrain dopaminergic neurons (helper virus system (Jin et al., 1996; Song et al., 1997); helper virus-free system (Wang et al., 1999)). Of note, vectors containing the TH promoter supported 40% to 60% nigrostriatal neuron-specific expression (Song et al., 1997; Wang et al., 1999), compared to only 5% using an vector containing the HSV-1 immediate early 4/5 promoter (Song et al., 1997). A vector containing a neurofilament heavy gene (NF-H) promoter supported neuronal-specific expression, but expression was only short-term (Wang et al., 1999). To obtain long-term, neuronal-specific expression, we previously constructed chimeric promoters that fused an upstream enhancer from the TH promoter to the NF-H promoter (TH-NFH promoter) or placed a β-globin insulator (INS) upstream of the TH-NFH promoter (INS-TH-NFH promoter) (Zhang et al., 2000). The TH-NFH promoter supported long-term expression in two different neocortical areas (1 month), hippocampus (2 months), or striatum (6 months; helper virus-free system) (Zhang et al., 2000). At 6 months after gene transfer, vectors containing the INS-TH-NFH promoter supported expression in ~11,400 striatal neurons (using 3 sites for gene transfer), and expression was maintained for 14 months (Sun et al., 2004). The TH-NFH or INS-TH-NFH promoters supported ~90% neuronal-specific expression in the striatum, hippocampus, or neocortex (Sun et al., 2004; Zhang et al., 2000, 2005). However, glutamatergic or GABAergic neuron-specific expression has not been reported using a virus vector system.

In this study, we inserted the PAG, VGLUT1, or GAD67 promoters into HSV-1 vectors, and delivered these vectors into rat postrhinal (POR) cortex. The PAG or VGLUT1 promoters supported ~90% glutamatergic neuron-specific expression, and the GAD67 promoter supported ~90% GABAergic neuron-specific expression. These promoters supported expression for 2 months after gene transfer.