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Research report

Effects of HIV/TAT protein expression and chronic selegiline treatment on spatial memory, reversal learning and neurotransmitter levels in mice

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HIGHLIGHTS

- TAT protein expression in the brain facilitates reversal learning in mice.
- TAT expression tended to increase the conversion of glutamate to glutamine.
- MAO inhibitor selegiline decreased the metabolism of dopamine and serotonin.
- Chronic selegiline treatment increased glutamate levels in the caudate putamen.
- Chronic selegiline treatment does not alter spatial memory or reversal learning.

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ABSTRACT

Neurotoxic viral protein TAT may contribute to deficits in dopaminergic and cognitive function in individuals infected with human immunodeficiency virus. Transgenic mice with brain-specific doxycycline-induced TAT expression (TAT+, TAT- control) show impaired cognition. However, previously reported TAT-induced deficits in reversal learning may be compromised by initial learning deficits. We investigated the effects of TAT expression on memory retention/recall and reversal learning, and neurotransmitter function. We also investigated if TAT-induced effects can be reversed by improving dopamine function with selegiline, a monoamine oxidase inhibitor. Mice were tested in the Barnes maze and TAT expression was induced after the task acquisition. Selegiline treatment continued throughout behavioral testing. Dopamine, serotonin and glutamate tissue levels in the prefrontal/orbitofrontal cortex, hippocampus and caudate putamen were measured using high performance liquid chromatography. Neither TAT expression nor selegiline altered memory retention. On day 2 of reversal learning testing, TAT+ mice made fewer errors and used more efficient search strategies than TAT- mice. TAT expression decreased dopamine turnover in the caudate putamen, increased serotonin turnover in the hippocampus and tended to increase the conversion of glutamate to glutamine in all regions. Selegiline decreased dopamine and serotonin metabolism in all regions and increased glutamate levels in the caudate putamen. In the absence of impaired learning, TAT expression does not impair spatial memory retention/recall, and actually facilitates reversal learning. Selegiline-induced increases in dopamine metabolism did not affect cognitive function. These findings suggest that TAT-induced alterations in glutamate signaling, but not alterations in monoamine metabolism, may underlie the facilitation of reversal learning.

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Abbreviations: 3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxy-indoleacetic acid; 5-HT, serotonin; AIDS, acquired immunodeficiency syndrome; ANOVA, analysis of variance; CPu, caudate putamen; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; GABA, γ-aminobutyric acid; GFAP, glial fibrillary acidic protein; Gln, glutamine; GLU, glutamate; HAD, HIV associated dementia; HIV, human immunodeficiency virus; HPLC, high performance liquid chromatography; HVA, homovanillic acid; LSD, least significant difference; MAO, monoamine oxidase; ORB, orbitofrontal cortex; PFC, prefrontal cortex; Ret, retention; Rv, reversal learning; Sel, selegiline; SEM, standard error of the mean; SIV, simian immunodeficiency virus; SME, significant main effects; WM, working memory.

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1. Introduction

Mild neuropsychological impairments associated with Human Immunodeficiency Virus (HIV) infection are relatively common, occurring in approximately 50% of people with AIDS [1] receiving combination antiretroviral therapy [2]. HIV-related brain dysfunction is associated with frontal-subcortical mediated patterns of cognitive deficits, characterized by impairments in working memory, processing speed, executive function, learning and motor skills [1,3,4]. This collection of symptoms suggest the brain regions most commonly damaged in HAD are the basal ganglia, hippocampus, and cerebral cortex [5]. Due to the persistence of cognitive deficits in HIV patients, identifying neurobiological mechanisms and subsequently, therapeutics for HIV-related cognitive deficits is a growing area of interest in the field of HIV research [3].

HIV-induced neurodegeneration involves, in part, HIV viral products including the non-structural protein TAT which plays a central role in the pathogenesis of the HIV infection (for review, [6]) and may contribute to cognitive deficits in treated patients. For example, the TAT protein has been found in the *post-mortem* brain tissue of patients with HIV [7,8]. Transgenic mice that express the viral TAT protein under the glial fibrillary acidic protein (GFAP) promoter provide a useful in vivo model to study the impact of TAT protein in cognitive function. TAT-induced mice show neuropathology similar to that observed in HIV-infected humans including apoptosis, astrocytosis, neurodegeneration of the cortex, degeneration of dendrites, inflammation and premature death [9]. TAT protein also induces dysfunction of dopaminergic neurotransmission in corticolimbic brain circuits [10–13] that are involved in memory and executive function [14–16]. TAT expression in mice leads to impaired learning, memory and cognitive flexibility (reversal learning) [17,18]. However, an important caveat to these studies is that TAT expression was induced prior to the task acquisition. Therefore, deficits in learning may compromise the subsequent testing of memory retrieval and reversal learning. Thus, it is important to design experiments that can discretely assess memory and reversal learning *independent* of concomitant impairments in learning after TAT expression.

HIV infection has been shown to preferentially target the basal ganglia, leading to decreased caudate/basal ganglia volume [19,20]. Both caudate atrophy and decreased dopamine levels have been associated with impaired cognitive performance in HIV-infected humans [20–22]. TAT infusions into the striatum resulted in decreased levels of potassium-evoked dopamine release 24 and 48 h later [10] and TAT has been shown, *in vitro*, to induce rapid and reversible effects on dopamine uptake and storage [12,13]. Dopamine function in the prefrontal cortex (PFC) and caudate putamen (CPu) of rodents has been associated with memory retrieval and reversal learning [23,24]. Furthermore, interactions with dopamine systems via other brain regions such as the orbitofrontal cortex (ORB) [25] and hippocampus [26] are also important for both memory and adaptive responses. Selegiline, a monoamine oxidase (MAO) inhibitor, decreases dopamine and serotonin (5-HT) metabolism [27], in addition to having antioxidant and neuroprotective functions [28]. Selegiline treatment improved age-related memory deficits in rodents [29] as well as memory deficits induced by a variety of insults in rodent models [30–32]. Moreover, selegiline treatment in monkeys with SIV has improved dopamine-related function in the brain [33]. Based on these findings, we hypothesized that selegiline may be promising treatment for TAT-induced memory impairments by improving brain dopamine function with possible downstream effects on glutamate and γ-aminobutyric acid (GABA) systems.

The goal of the present study was to determine the impact of TAT expression and chronic selegiline treatment on memory retention, reversal learning and neurotransmitter function. To discretely assess memory and reversal learning without confounding alterations in learning, mice were trained to learn the location of the escape tunnel in the Barnes maze test *prior* to TAT expression or selegiline treatment. Subsequently, the effects of TAT expression, with and without selegiline, on memory retention/recall and reversal learning were assessed. Dopamine, 5-HT, glutamate and GABA function in regions associated with memory and reversal learning including the PFC, ORB, CPu and hippocampus was determined using high performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Animals

A total of 60 male mice all containing the GFAP-null alleles but only half containing the TAT protein transgene were used in this study. Inducible TAT transgenic mouse colonies with a C57BL/6J background were obtained by generation of two separate transgenic lines Teton-GFAP mice and TRE-Tat86 mice, and then cross-breeding of these two lines of transgenic mice as previously described in Ref. [9]. The mice were housed in groups of 2–4 in a humidity- and temperature-controlled animal facility on a 12 h/12 h reverse light/dark cycle (lights off at 7:00 AM) with *ad libitum* access to food and water. Behavioral testing was conducted during the dark phase of the light/dark cycle. All of the experiments were conducted in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and National Research Council's Guide for the Care and Use of Laboratory Animals and approved by the University of California San Diego Institutional Animal Care and Use Committee.

2.2. Experimental design

A graphical representation of the experimental design is presented in Fig. 1. TAT- (n = 30) and TAT+ (n = 30) mice were trained to learn the spatial location of the escape tunnel during the acquisition trials followed by the probe test in the Barnes maze. Subsequently, TAT+ and TAT- mice were divided into two equally performing groups based on latency, strategy and reference errors during the final three days of acquisition trials. Then all mice were treated with a doxycycline hyclate regimen (100 mg/kg, Sigma, St. Louis, MO, USA) consisting of intraperitoneal injections once a day for 7 days at 08:00 and starting two days after the probe test. This doxycycline regimen is based on the previously demonstrated efficacy of TAT induction at this dose of doxycycline [9,17]. Selegiline hydrochloride (Sel+; Sigma), 2 mg/kg subcutaneously once per day at 17:00 [29], or saline (Sel-) treatment began two days after the first doxycycline administration and continued throughout behavioral testing. The final number of mice included in each test group were as follows: TAT-/Sel- n = 16, TAT+/Sel- n = 16, TAT-/Sel+ n = 13 and TAT+/Sel+ n = 15. Effects of TAT and selegiline on memory retention were assessed 15-days after the completion of acquisition trials and followed immediately with reversal trials. Brain samples were taken the day following the final reversal trials.

2.3. Barnes maze test

The Barnes maze testing was conducted similar to that described previously [34,35]. The maze consisted of a white, acrylic, circular disc (90 cm diameter) that was elevated 90 cm above the floor, with 20 equally spaced holes (San Diego Instruments, San Diego, CA) with a black acrylic escape box (20 × 5 × 6 cm) placed under one of the holes. The maze was surrounded by four spatial cues at the height of the maze. Illumination in the center of the maze was