Effects of garcinol administration on microtubule dynamics following cocaine-cue memory retrieval

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Introduction

Sustained abstinence from cocaine use is frequently compromised by exposure to stimuli that have previously been associated with drug taking. Such cues trigger drug-associated memories leading to craving and relapse. Our previous work has shown that altering cocaine-cue memories by interfering with the reconsolidation process is a potential therapeutic tool to prolong abstinence. We have previously shown that the histone acetyltransferase (HAT) inhibitor, garcinol, can impair the reconsolidation of cocaine-cue memories in a manner that is reactivation specific, temporally constrained, and long lasting (Monsey MS, Sanchez H & Taylor JR (2017), *Neuropsychopharm*, 42(3):587-597). Here, we examined the neuroproteomic profile in the lateral nucleus of the amygdala (LA) following cocaine-cue memory retrieval and systemic garcinol administration. We further examined the downstream effects of garcinol using primary neuronal cultures and cell lines.

Materials & Methods

Subjects. Adult male Sprague-Dawley rats, weighing 275-300 g, were housed individually on a 12 h light/dark cycle. Throughout the experiment food was restricted to maintain rats at 90-95% of their body weight following recovery from surgery. Water was provided *ad libitum.*

Surgical procedures. Rats were anesthetized with 75 mg/kg ketamine and 5 mg/kg Xylazine i.p. and implanted with indwelling catheters into the right jugular vein. They were allowed one week to recover from surgery with *ad libitum* access to food and water.

Behavioral procedures. Rats were trained on cocaine self-administration (SA) in operant conditioning chambers (Med Associates). Each operant box contained two retractable levers, a house light, a cue light, and a tone generator. Rats received 12 days of cocaine SA training. Each session lasted one hour, during which time an active lever presses resulted in a 1mg/kg I.V. infusion of cocaine while concurrently a cue light and tone (75 dB) were presented for 10 seconds. Inactive lever presses had no outcome. Self-administration was on a FR1 schedule where every active lever press resulted in 1 cocaine infusion and cue presentation. Rats then underwent lever extinction for 8 days, where lever pressing did not result in cocaine infusion or cue presentation. Rats were divided into 2 groups: to-be-vehicle or to-be-garcinol. The day after the last lever extinction session, rats underwent memory reactivation in a novel context where the cue was briefly presented. Non-reactivated controls were placed in the chamber, but received no cue presentation. Rats then received a systemic 10 mg/kg i.p. injection of garcinol or vehicle 30 minutes after reactivation. Rats were sacrificed by microwave irradiation at 90 minutes after reactivation.

Proteomic analysis. LA tissue was collected for proteomic analysis using a label free quantitative approach (LCMS/MS). Samples were desalted and reconstituted in running buffer (0.2% FA in water) with an equal amount of RT Cal mix standards spiked in at a ratio of 1:9 (standard: sample) to all samples. Processed samples were subjected to data dependent LCMS/MS analysis on a NanoACQUITY coupled in-line with a Q- Exactive Plus. Collected data was processed utilizing Progenesis QI, Mascot Search Engine, and Xcalibur software similarly described by Henderson et al., 2015. Data were analyzed with a significance cutoff of p<0.05 and False Discovery Rate of 1% or less.

Primary cell culture. Primary hippocampal, striatal, and cortical neurons were cultured and maintained for 14 days. They were plated at a density of 600,000 neurons per well and were grown in neurobasal medium with 1% penicillin/streptomycin, 1% Glutamax, and vitamin B27. On day 14 neurons were treated with either 5uM garcinol in DMSO or vehicle and collected in lysis buffer at 5, 15 or 30 minutes. Western blotting was performed probing for acetyl-alpha-tubulin (Lys-40), Alpha Tubulin, GAPDH, and FEZ1. Images were analyzed using a BIO-RAD ChemiDoc system.

Cell lines. Human Neuroblastoma SH-SY5Y Cells from ATCC were grown in Dulbecco's Modified Eagle Medium with 10% heat inactivated Fetal Bovine serum, 1% sodium pyruvate, 1% MEM Non-Essential Amino Acids Solution and 1% penicillin/streptomycin. Neurons were grown until 80% confluence and then treated with 2.5uM of garcinol or vehicle. Neurons were collected in lysis buffer at 5, 15, or 30 minutes. Samples were processed for Western blotting as above.

Results

Figure 1: Differential protein expression in the LA following cocaine-cue memory reactivation and garcinol administration

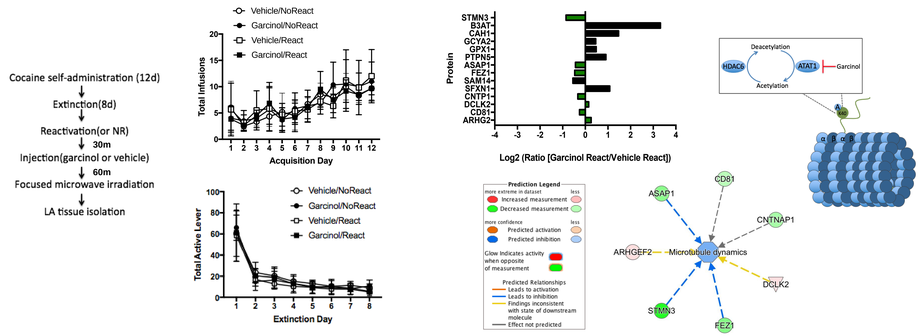


Figure 2: Garcinol decreases alpha-tubulin acetylation in primary hippocampal, striatal, and cortical neurons

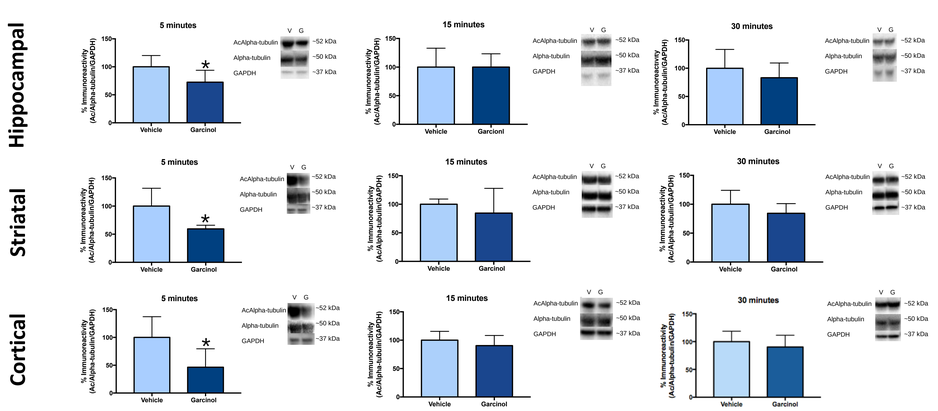


Figure 3: Garcinol decreases alpha-tubulin acetylation in human neuroblastoma SH-SY5Y neurons

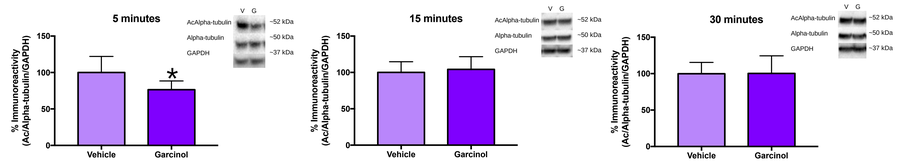
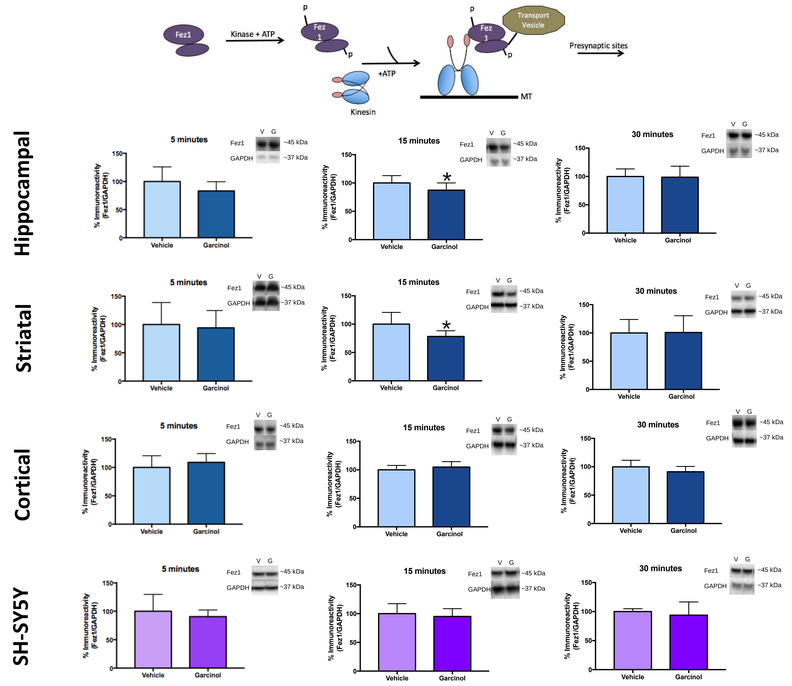


Figure 4: Garcinol decreases Fez1 protein expression in hippocampal and striatal primary neurons



Conclusions

- Garcinol administration following cocaine-cue memory retrieval drives the differential expression of 14 proteins in the LA, half of which are associated with changes in microtubule dynamics.
- In primary hippocampal, striatal, and cortical neurons, as well as in human neuroblastoma SH-SY5Y neurons, garcinol decreases levels of alpha-tubulin acetylation at 5 minutes, but not at 15 or 30 minutes after administration.
- Garcinol additionally decreases levels of the kinesin adaptor protein, Fez1, protein expression in hippocampal and striatal primary neurons 15 minutes after administration.

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