Are Astrocytes the Missing Hidden Layer? Harnessing the Tripartite Synapse Architecture for Cognitive Control of Behavior

Gregory J. Simandl (gregory.simandl@marquette.edu)
College of Biomedical Sciences; Marquette University, WI, USA

Robert C. Twining, PhD (robert.twining@marquette.edu)
College of Biomedical Sciences; Marquette University, WI, USA

Sidney Peck (sidney.peck@marquette.edu)
College of Biomedical Sciences; Marquette University, WI, USA

David A. Baker, PhD (david.baker@marquette.edu)
College of Biomedical Sciences; Marquette University, WI, USA

Abstract:

Brain function is often attributed to the activity of neuronal circuits, yet some are beginning to speculate that phylogenetic gains in intelligence or other complex forms of cognition required increases in information processing that exceed the capacity of networks comprised solely of neurons. Conceptually, astrocytes represent a novel biological source for increasing the computational capacity of the brain. However, evidence supporting this important idea is largely lacking. An opportunity to use a biological system to test this intriguing idea is to manipulate system xc- (Sxc) to disrupt astrocyte to neuron communication (SXC:A-N). Sxc is a glutamatergic release mechanism exclusively expressed *in vivo* on astrocytes, thereby positioning this mechanism to relay integrated astrocytic information to neuronal networks. Here, we created and behaviorally characterized a line of genetically-modified rats lacking Sxc-mediated signaling between astrocytes and neurons (MSXC:A-N). MSXC:A-N did not alter simple forms of brain function, but significantly impaired rat performance on tasks requiring executive function (i.e., decision making). We propose that biological networks are capable of dynamically increasing computational processing power by engaging astrocytes, and by extension, SXC:A-N.

Keywords: astrocyte; cognition; evolution; transformers

Introduction

Evolutionary gains in behavioral control likely required increased biological computational processing capacity to enable more complex evaluation of behavioral options. An open question is whether the capacity to recruit the unique processing features of astrocytes contributed to evolutionary gains.

Astrocytes, a type of glial cell, are ubiquitous in the central nervous system. A single astrocyte contacts up to 100,000 synapses in the rodent brain, and over a million synapses in the human brain (Bushong et al., 2002; Saint-Martin and Goda., 2023). Previously thought of as a support cell, astrocytes have recently been shown to play a role in behavioral deficits seen in numerous psychiatric disorders (Hess et al., 2023; Soto et al., 2023). The reason for this may be that astrocytes are able to: 1) integrate information across numerous synapses; 2) slowly process information to keep prior states relevant for longer; 3) provide additional signaling nodes to regulate neuronal activity.

To investigate this, we disrupted astrocyte to neuron communication in the rat brain by blocking activity from an astrocyte-specific signaling protein, system x_c^- (SXC:A-N), using genetic-engineering (MSXC:A-N) and pharmacology (SSZ). MSXC:A-N rats displayed enhanced cocaine drug seeking behavior, but there are numerous behavioral elements that contribute to this phenotype. Therefore, we wanted to determine if disrupting SXC:A-N impacted either simple (reward/impulse processing), complex cognitive processing (decision making), or both. MSXC:A-N rats performed similarly to WT counterparts in simple tasks (i.e., hedonic-based feeding and anxiety-based urges) but showed a deficit in tasks that require complex cognition (i.e., decision making). Additionally, SSZ rats display similar deficits in decision making as MSXC:A-N rats, providing support for the idea that astrocytes are necessary for cognitive tasks that require additional signaling complexity.

Methods

Self-Administration and Reinstatement

Adult WT and MSXC:A-N rats were trained to self-administer cocaine during once-daily 2-hour sessions, followed by extinction training. Once rats met criterion, reinstatement was conducted with a low dose of cocaine (3mg/kg, i.p) prior to testing to assess drug-seeking behavior (Madayag et al., 2010).

Simple Behavioral Measures

2-Meal Paradigm Adult WT and MSXC:A-N rats were trained to consume their daily standard chow (homeostatic; HS) in a 2hr period after the onset of dark phase (Meal 1). Upon stabilization of feeding patterns and weight gain, rats were offered a 15 min meal (Meal 2) of either HS or high calorie western diet (hedonic; HD) 30 minutes after M1 (Hurley et al., 2016). Calorie consumption during each meal was recorded for 7 days and consumption on the final day was analyzed.

Marble Bury Adult WT and MSXC:A-N rats were placed in an enclosed chamber filled with 5cm of bedding and 20 glass marbles arranged in a 4x5 array on top. After 15 minutes, rats were removed and burying behavior was graded. Marbles submerged >66% were considered buried (Reimer et al., 2015)

Complex Behavioral Measures

Rat Gambling Task Adult WT, MSXC:A-N, and SSZ rats were trained to choose between four reward/punishment schedules associated with location-specific light stimuli. Schedules differed in reward probability, reward magnitude, and punishment duration. Rats must develop a strategy to obtain the maximum pellet count by the end of the session. If only one stimulus was chosen throughout the 30-minutes session, rats would obtain the follow pellet counts: P1, 295; P2, 411; P3, 135; and P4, 99 (Kim et al., 2017). Therefore, P2 preference is the optimal stimulus choice because it results in the highest cumulative reward density. Pharmacological treatment (Sulfasalazine, SSZ; 16mg/kg, i.p) was administered 2h prior to daily test sessions.

Results

MSXC:A-N Increases Drug-Induced Reinstatement of Drug Seeking

Drug seeking behavior, characterized by lever pressing in preclinical models, is a measure of relapse potential. Both WT and MSXC:A-N rats consumed similar amounts of

cocaine during acquisition and maintenance phases. After extinction training, when exposed to a sub-threshold dose of cocaine, MSXC:A-N rats exhibited significantly more lever presses than WT rats ($t_{(28)}$=2.723, p<0.05; **Fig 1A**). Additionally, MSXC:A-N rats displayed elevated drug seeking compared to their last day of extinction ($t_{(13)}$=-5.805, p<0.05; **Fig 1A**).

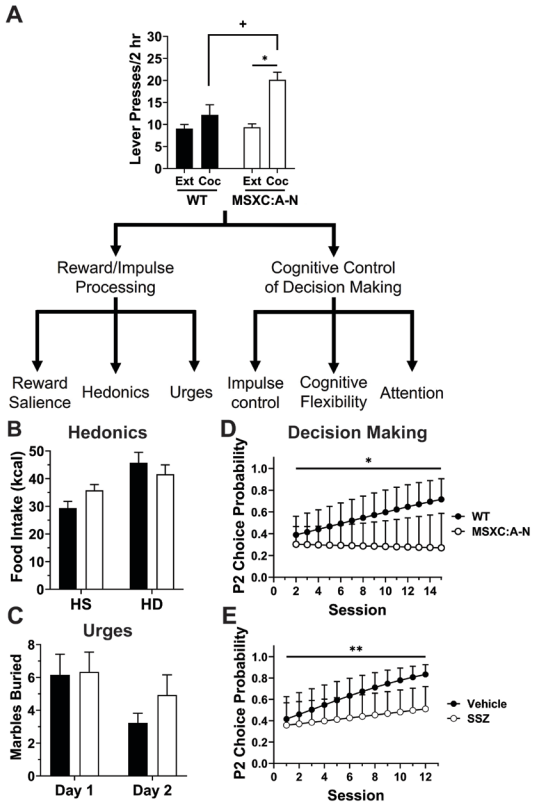

Figure 1. A) Drug seeking behavior requires information integration from multiple circuits, therefore, it is difficult to discern the cause of reinstatement. We utilized various behavioral paradigms to dissect behavior into simple (reward/impulse processing) and complex (decision making) processing to more precisely identify circuits that rely on SXC:A-N and contribute to drug-seeking. **B-C)** Simple processing measures of hedonic-based feeding (2-meal) and anxiety-driven urges (marble bury). **D-E)** Complex processing measures of decision-making (rat gambling) using genetic engineering and pharmacology to disrupt SXC:A-N.

MSXC:A-N Does Not Impact Simple Processing

Drug seeking behavior can be influenced by how appetitive the reward is (hedonics) and internal states, like anxiety (urges). We found that both WT and MSXC:A-N rats consumed similar kCal for both standard chow (HS) and a more appetitive western diet (HD) (**Fig 1B**). Additionally, we found that both WT and MSXC:A-N rats displayed similar anxiety-driven burying behavior (**Fig 1C**). These results indicate that SXC:A-N may not be necessary in simple cognitive processes.

MSXC:A-N Impairs Complex Processing

Decision making in drug seeking behavior appears binary - seek out the drug or abstain – but there are multiple dimensions to drug seeking. We wanted to assess whether disrupting SXC:A-N produced deficits in decision making, which may contribute to drug seeking. Results of a Generalized Linear Mixed Effects Model (GLMM) revealed that WT rats display a significantly higher optimal choice probability (P2) across sessions compared to MSXC:A-N rats, illustrating that WT rats develop a preference for the optimal strategy ($X^2_{(1,\ N=20)}$=4.171, p<0.05; **Fig 1D**). Additionally, WT rats display a similar choice preference compared to SSZ rats ($X^2_{(1,\ N=40)}$=8.562, p<0.05; **Fig 1E**), providing support that disrupting SXC:A-N impairs decision making.

Astrocyte Transformer: Self-Attention Theory of Information Integration

It is well established that astrocytes and neurons form a communication network, although efforts to translate this biological framework to AI are ongoing. While it is known that astrocytes can regulate hundreds of thousands of synapses across space and time due to their chemical signaling properties (Ca^{2+} transients), exactly how they accomplish this is still unknown. It was recently suggested that neuron-astrocyte interactions have similar properties as the AI transformer architecture (Kozachkov et al., 2023). Astrocytes microdomains process neuronal input from individual synapses (X_i; **Fig 2A**) and translate this information into Ca^{2+} waves, which can be spatially and temporally averaged and held as a reference for later signaling events. We believe that astrocytes utilize self-attention mechanisms not just as a form of plasticity to optimize neuronal transmission, but as a way for astrocytes to relay processed information back to neurons via signaling proteins such as SXC:A-N (**Fig 2A-B**). Our data illustrate the potential for investigating astrocyte to neuron communication as a hidden layer in AI transformer architecture.

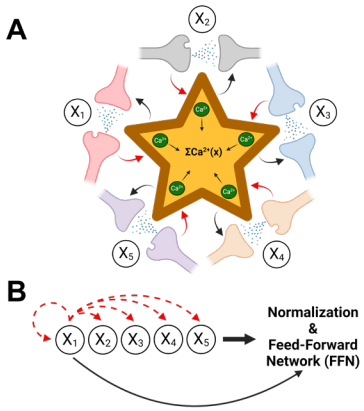

Figure 2. A) Tripartite synaptic architecture of a single astrocyte processing neuronal signals from multiple synapses. **B)** Conceptual hypothesis of astrocyte self-attention.

Acknowledgments

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