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Single-cell systems neuroscience: A growing frontier in mental illness

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Abstract: The development of effective treatments for psychiatric disease has been disappointing in recent decades given the advancements in neuroscience. Moreover, rising rates of mental illness such as addiction and depression compel scientists and physicians to discover novel and creative solutions. One such approach that has proven effective is systems neuroscience: A focus on networks as opposed to mechanism. Further, investigation at the single-cell and circuit level is likely to be fruitful in such endeavors as this resolution describes the functional psychopathology that allows for intervention.

Introduction

Rates and severity of mental illness in the United States have risen over the past decade (“Key Substance Use and Mental Health Indicators in the United States: Results from the 2019 National Survey on Drug Use and Health” 2020; Merikangas *et al.*, 2010; NIMH, 2020). Infection by the novel coronavirus and indirect effects of the pandemic on mental health suggest this increase is likely to continue and possibly accelerate (Vindegaard and Benros, 2020; Taquet *et al.*, 2020). In addition to individual suffering, the socioeconomic and politico-cultural consequences of this rise in mental illness compels investigators and clinicians alike towards realizing novel understandings and treatments as canonical psychiatric therapies are often burdened by low efficacy, high side-effect profiles, and ballooning costs (Holmes *et al.*, 2014; Holmes *et al.*, 2018; Ford and Young, 2021).

Neuropsychopathology studies, like virtually all biological investigation, are performed under the guise that the data from the experiments will eventually contribute to clinical treatment. Though neuroscientific understanding has developed rapidly in recent decades, novel psychiatric therapies remain elusive (Holmes *et al.*, 2014; Holmes *et al.*, 2018; Ford and Young, 2021). Even the recent psychedelic revolution is a rediscovery of medications from half a

century prior (Johnson *et al.*, 2019). The demand for efficacious clinical treatments with minimal side effects, however, remains high which galvanizes investigator creativity raising the attractiveness of newer approaches to neuropsychopathology such as systems biology.

Systems biology distinguishes itself from hypothesis-driven research in that understanding networks is the primary objective as opposed to elucidating mechanisms (Ideker *et al.*, 2001; Geschwind and Konopka, 2009). This difference shifts experimentation towards highthroughput measures at multiple levels of biological systems with an emphasis on physiological context and mathematical modeling. Experimental designs with such broad parameters that do not isolate individual variables had been previously untenable because of cost and reliability; However, advances in biotechnologies and computer science have diminished such barriers (Geschwind and Konopka, 2009). Moreover, this approach emphasizes network states and thus avoids the challenges of determining causality that are inherent in psychiatry (Stojanov *et al.*, 2011).

Indeed, the dam of systems neuroscience has burst, and datasets filled with pearls are flooding the field. At the level of organ systems, visceral feedback to central centers has been shown to have profound effects on mental health (Critchley and Harrison, 2013). The gut-brain connection has proven particularly powerful as intestinal microflora composition consistently demonstrate behavioral influence (Sylvia and Demas, 2018). Moreover, developments in imaging have allowed investigators to map neural modules

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and their relationships to psychopathology. This has led to the development of neuromodulatory interventions such as transcranial magnetic stimulation that is now Food and Drug Administration (FDA) approved for treatment-resistant depression and obsessive-compulsive disorder (Horvath *et al.*, 2010; Voelker, 2018). At the molecular level, genetic sequencing and transcriptome measures are yielding data-driven models of cellular signaling networks that challenge prior notions of neuronal phenotypes (Park *et al.*, 2014).

The emergence of single cell studies is particularly fruitful in this new frontier (Callaway, 2005). Nervous tissue contains many kinds of cells ranging from various types of neurons to astrocytes, oligodendrocytes, microglia, endothelial cells, and so on. The firing of any individual neuron—which, en masse, is still believed to be the modus for perception, thought, and behavior—is known to be influenced by the context in which that neuron finds itself (Pereira and Furlan, 2010; Callaway, 2005). Anatomic localization within a network, the neighboring neurons, synaptic and non-synaptic inputs, local paracrine signaling and glial influences in combination determine the role of that neuron within the network and its output. The complexity of this dance may never be fully understood even with quantum computers (Schiffer, 2019), but single-cell systems neuroscience is beginning to point us towards the prominent concepts at this level that elude mechanistic molecular studies and tissue-level or higher approaches. That is, single-cell studies are in a Goldilocks zone of biological levels for neuropsychopathology investigation—as signaling networks and circuits are deciphered at this level, knowledge of normal physiology and pathophysiology in mental illness is likely to translate to clinical treatments (Ford and Young, 2021).

An example of this approach is our recent work measuring the effects of opioid withdrawal on gene expression in single brain cells in the central nucleus of the amygdala (CeA) of rats (O'Sullivan *et al.*, 2019). We combined single-cell laser capture microdissection with microfluidic RT-qPCR to measure a selected transcriptome of neurons, microglia, and astrocytes with anatomic specificity (O'Sullivan *et al.*, 2020). The pre-experimental hypothesis, based on our prior hypothesis-generating work (Freeman *et al.*, 2012a; Freeman *et al.*, 2013; Freeman *et al.*, 2012b), was that neuroinflammation, likely originating from microglia, would be present 24 hours following acute naltrexone-precipitated opioid withdrawal. Rather, we found a striking transcriptional response in astrocytes. A review of the literature contextualized this finding and led us to a profound conjecture—that neuroinflammation mediated by astrocytes in the CeA contributes to the negative emotion characteristic of opioid withdrawal and that this neuroinflammation increases the negative reinforcement of withdrawal (O'Sullivan and Schwaber, 2021). Moreover, this work translates to other levels of biological systems including molecular signaling, organ systems, and behavior, and has implications for the mechanisms of action of new therapies.

Astrocytes had the most perturbed transcriptome of the assayed cell types, but neurons and microglia also demonstrated a significant increase in the expression of tumor necrosis factor alpha (TNF- α) (Fig. 1). This finding was validated with Western blot and immunofluorescence and suggests that this cytokine is a primary molecular signal in the negative emotional sequelae of opioid withdrawal. TNF- α has been shown to lower neuronal excitation threshold resulting in an increased probability of actional potential firing (Schäfers and Sorkin, 2008; Vezzani and Viviani, 2015). Taken together, these findings suggest TNF- α

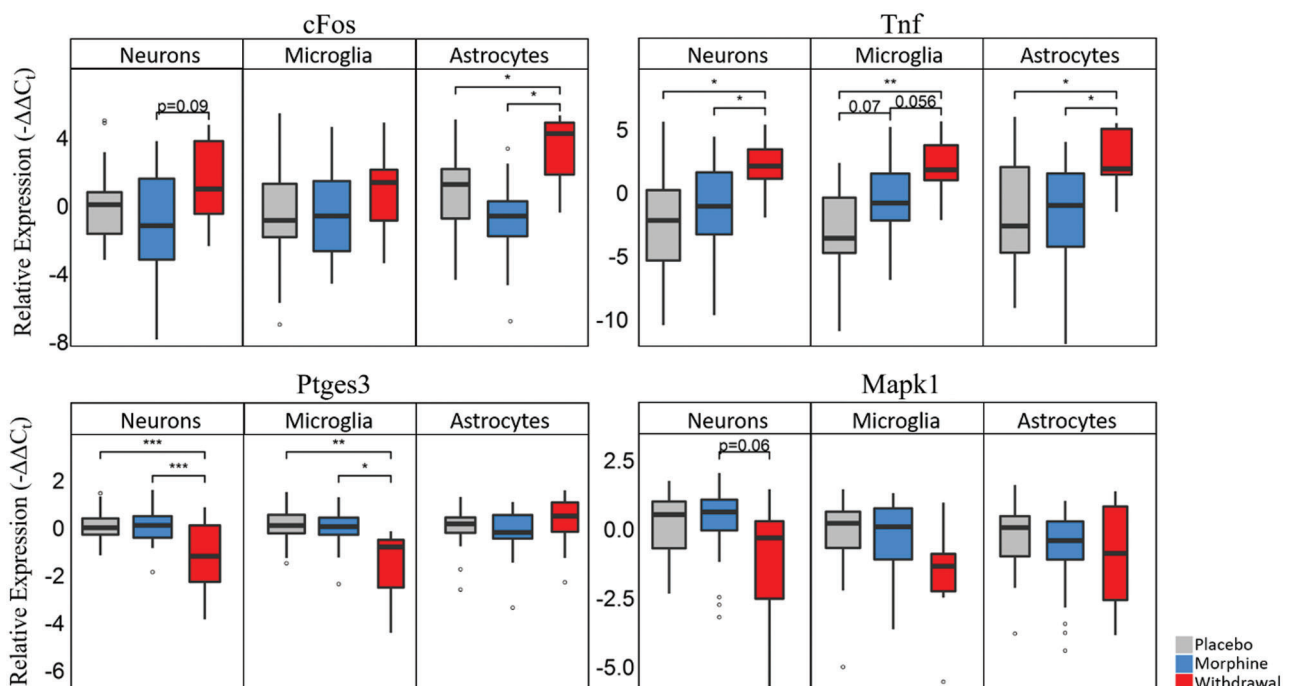


FIGURE 1. Boxplots of select genes demonstrating significant differential gene expression.

Statistics were calculated using nested ANOVA (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$ $n = 4$ animals for all treatments). Originally published in O'Sullivan *et al.* (2019).

paracrine signaling, likely mediated by astrocytes, in the CeA during opioid withdrawal may lead to hyperactivity that contributes to negative emotion and reinforcement of drug taking.

At the systems level, the CeA has strong bidirectional connections to the nucleus tractus solitarius (NTS)—the primary viscerosensory nucleus in the central nervous system (Schwaber *et al.*, 1982). This neuroanatomy suggests that the emotions experienced secondary to limbic activity are substantially influenced by peripheral feedback; an insight that has been shown convincingly (Maniscalco and Rinaman, 2018). Additionally, we found that gut microflora demonstrated remarkable perturbation in opioid withdrawal (Fig. 2) (O’Sullivan *et al.*, 2019). This finding was correlated with astrocyte activation in the amygdala suggesting these two observations may be linked via interoceptive vagal afferents and the NTS.

These single-cell findings translate to the behavioral level as well. We conjecture that these connections form a visceral-emotional neuraxis in which peripheral perturbation sensed by vagal afferents is transmitted to the amygdala forming an

interoceptive antireward pathway (O’Sullivan and Schwaber, 2021). This antireward circuit is inhibited by drug use and stimulated by substance withdrawal. We hypothesize that the mesolimbic dopamine reward pathway functions in parallel to this circuit with inverse activity consistent with the opponent-process model (Pierce and Kumaresan, 2006; Wise, 2008; Solomon and Corbit, 1974). These reward and antireward pathways combine to provide positive and negative motivators that reinforce behavior. Compulsive drug-seeking and taking, then, is an imbalance in this behavioral paradigm that is influenced at the molecular level.

This model informs future studies and the mechanism of action of recent therapies. Astrocyte-mediated inflammation in CeA has been demonstrated to provoke anxiety-like behavior, (Yang *et al.*, 2016) but the temporal dynamics of this process in opioid withdrawal and the contribution of visceral feedback remain unknown. Subdiaphragmatic vagotomy studies in rat methamphetamine self-administration suggest this circuit has an important influence on addiction (Everett *et al.*, 2021), but further investigation is warranted.

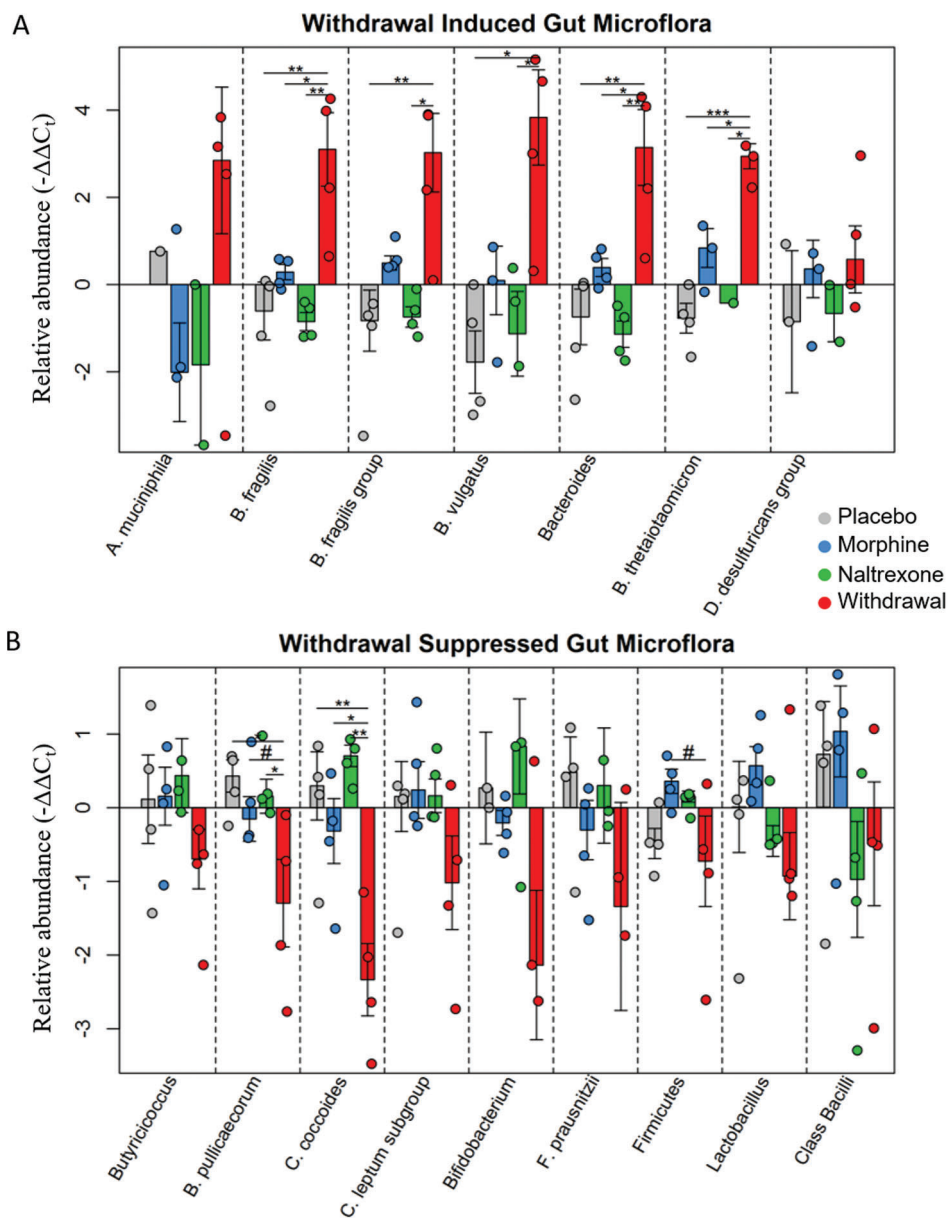


FIGURE 2. Relative abundance of gut microflora from rat cohort. Barplots display relative abundance of bacterial species ($-\Delta\Delta C_t$ values). # $p < 0.1$, * $p < 0.05$, ** $p < 0.008$, *** $p = 0.0009$; two-way ANOVA $n = 4$ animals for each treatment. Originally published in O’Sullivan *et al.* (2019).

Moreover, anti-neuroinflammatory interventions such as ibudilast have shown promise in treating addiction to multiple substances in clinical trials (Cooper *et al.*, 2016; Heinzerling *et al.*, 2020; Worley *et al.*, 2016; Metz *et al.*, 2017; Comer and Johnson, 2013). It reduced the physical and emotional symptoms of opioid withdrawal syndrome. Furthermore, there is evidence that ibudilast's mechanism of action may be a peripheral decrease in inflammation consistent with our model (Li *et al.*, 2020). Indeed, peripheral stimulation for opioid withdrawal syndrome has recently been approved by the FDA (Miranda and Taca, 2018). The BRIDGE device provides peripheral stimulation to auricular cranial nerves during opioid withdrawal and investigators have shown that this input decreases neuronal firing in the amygdala *via* the NTS. This device likely treats opioid withdrawal symptoms by decreasing CeA neuronal activity *via* peripheral stimulation consistent with our findings and model (O'Sullivan *et al.*, 2019; O'Sullivan and Schwaber, 2021; O'Sullivan *et al.*, 2021).

In conclusion, single-cell studies are situated at a unique level of biological analysis that is particularly fruitful for developing much needed psychiatric treatments. Single-cell experiments with a network focus not only inform our understanding of neural circuits, but also paracrine signaling by a spectrum of cell types and provide context for organ systems and behavioral understandings. The models generated from such studies require validation by more mechanistic approaches, but these models and the hypotheses they suggest can provide insight into the mechanisms of action of novel therapies.

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