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HOT TOPICS

MDMA enhances pleasantness of affective touch

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A growing body of evidence indicates that ecstasy, or ±3, 4-methylenedioxymethamphetamine (MDMA) produces distinctive pro-social effects. MDMA is a popular recreational drug which purportedly produces feelings of empathy and interpersonal closeness. It has also been used therapeutically, where it enhances treatment for PTSD and social anxiety associated with autism when used in conjunction with psychotherapy [1, 2]. A number of controlled, double-blind studies have examined the behavioral effects of MDMA to identify the psychological and neural processes that underlie these effects, and distinguish it from other stimulant drugs [3–5]. For example, one consistent finding has been that MDMA decreases sensitivity to detecting negative emotions depicted in faces, while improving the ability to detect positive emotions. These two actions could facilitate both positive social interactions with strangers and interactions with a psychotherapist.

To fully understand the profile of social effects of a drug, it is of value to examine its effects on other basic modalities of social communication, aside from perceptions of visual stimuli. One modality that has received little attention is the sense of touch. Anecdotally, recreational MDMA users report that MDMA increases the pleasantness of social touch. In a recent study, we examined the acute effects of MDMA on responses to social, or affective, touch, in healthy young adults, under double blind conditions. Affective touch is touch that is experienced as hedonically pleasant, and has a known sensory pathway through activation of C-tactile afferents in the skin: slow stroking of the skin activates these afferents and is experienced as pleasurable, whereas faster stroking does not, and is not perceived as pleasant. Interestingly, the sensation of affective touch is thought to be mediated by both serotonin and oxytocin, which are also implicated in the behavioral actions of MDMA.

In our study [6], healthy volunteers ($N = 36$) participants received single doses of MDMA (0.75 or 1.5 mg/kg), 20 mg methamphetamine (MA) or placebo in a double-blind, within-subject design. MA was included as a prototypic stimulant drug, for comparison. At the time of peak drug effect, participants completed a touch task in which they rated the pleasantness (7 point Likert scale) of two different touch stimuli: slow stroking (3 cm/s) and faster stroking (10 cm/s) of the forearm. The touch was conducted with a soft brush rather than a hand to control for variation in skin temperature. Stroking at the slower frequency is known to preferentially activate CT-afferents, and thus is referred to as "affective touch." As expected, subjects rated the slow, affective, touch as more pleasant than fast touch. Further, and consistent with our expectations, MDMA significantly increased the perceived pleasantness of the slow touch, but not the fast touch. The prototypic stimulant MA produced its typical subjective effects but did not affect either pleasantness or intensity of the touch.

The results advance the field in two ways. First, they demonstrate the pro-social effect of MDMA in a novel modality, the sense of touch. The effect was dose-dependent, specific to MDMA (not MA) and limited to affective touch, compared to touch at other frequencies. Second, our use of the measure of affective touch opens a new door to psychopharmacological research investigating how drugs affect social behavior and perception.

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Intra-individual changes in methylome profiles: an epigenetic 'scar' of early-life adversity?

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Whereas genetics plays a critical role in vulnerability and resilience to mental illness, the contribution of early-life experiences to these outcomes has been strongly suggested in humans and supported by studies in animal models [1]. Early-life adversity (ELA), including poverty, chaotic environment, and abuse, predicts vulnerability to depression as well as lower cognitive function [1, 2]. Yet, at the individual level it is not possible to predict which child exposed to ELA will be vulnerable later in life. Lacking are predictive biomarkers that enable early diagnosis, prevention, and intervention [1–3].

There is evidence that the mechanisms by which early-life experiences influence the function of neurons (and neuronal networks) underlying vulnerability or resilience involve alterations of the repertoire and levels of gene expression via epigenetic processes. Among those, changes in DNA methylation at the individual gene and genomic scale may partially govern gene expression. It is not possible to examine DNA from brain samples in children, so that current approaches have largely employed peripheral cells including white blood cells or buccal swabs (mixed epithelial/white blood cells). Numerous studies have compared DNA methylation profiles from individuals experiencing adversity to those raised in 'typical' environments; yet cross-sectional studies are challenged by the large inter-individual variance of DNA methylation profiles.

We employed a novel intra-individual approach by testing buccal cell DNA methylation profiles of the same individual sampled twice: immediately before and after a defined period of ELA [3]. We imposed ELA of defined onset and termination during established sensitive developmental periods by raising rat pups for a week (postnatal days 2–10) in cages with limited bedding and nesting materials (simulated poverty). Prior work established that this experience provokes enduring anhedonia-like behaviors and significant deficits in hippocampus-dependent memory [4, 5]. Controls were sampled in parallel.

Traditional analyses of DNA methylation profiles across samples detected the effects of age, but did not distinguish pups exposed to ELA from controls. In contrast, DNA methylation changes between paired DNA samples from the same individual rat illustrated the impact of ELA. In ELA animals, methylation increased (predicting reduced expression) in genes coding for critical cellular/metabolic enzymes, ion channels, and receptors, whereas genes involved in pathways of death, inflammation, and cell-fate were less methylated, indicating their potential upregulation [3]. Thus ELA left an epigenetic 'signature': large-scale transcription-driven alterations of cellular fate, growth and function, consistent with –and potentially predicting–pathology.

This work, employing rodents, provides proof of principle for the potential power of intra-individual methylomics to identify epigenetic signatures of ELA which predict subsequent emotional and memory problems. The approach is translatable to the clinic [5, 6], as methylomic signatures in individual children might predict vulnerability or resilience to stress and/or mental illness. Current studies in infants are obtaining buccal-swab DNA twice, during the second week of life and at 1 year. ELA, derived from unpredictable environmental and maternal signals [5, 6] is assessed, and cognitive and emotional outcomes of individual infants are examined longitudinally.

In summary, intra-individual methylomics identifies epigenetic 'scars' and 'kisses' of early adversity, which may provide a predictive marker for vulnerability and resilience to certain mental illnesses.

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OPEN

The upside of stress: a mechanism for the positive motivational role of corticotropin releasing factor

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Intuitively, we know that salient environmental stimuli, even when stressful, can trigger an internal state of urgency that focuses our attention, motivates us to work harder, encourages us to explore new spaces or people, and helps us achieve specific goals. Despite its known benefits, the neural mechanisms that underlie these positive motivational qualities of acute stress remain poorly understood. In the past 10 years, evidence has emerged pointing to a new role for the neuropeptide corticotropin-releasing factor (CRF). CRF is a well-studied stress-associated neuropeptide. A rich literature exists demonstrating neuronal substrates of CRF-evoked energy mobilization, fear, and anxiety. However, when acting in the nucleus accumbens (NAc), CRF has been shown to promote exploratory behaviors and invigoration for reward [1, 2]. Yet, the cellular mechanism(s) mediating these positive motivational actions of CRF in the NAc were not known.

In a study published two months ago, we showed that CRF type 1 receptors (CRF-R1) are ubiquitously expressed on cholinergic interneurons within the NAc of adult male mice [3]. We found that CRF produces a robust increase in action potential firing in cholinergic interneurons via CRF-R1 activation and cAMP dependent mechanisms [3]. Cholinergic interneurons form dense axonal ramifications, and therefore, through acetylcholine modulation, can act as master regulators of accumbal output. Previous work had shown that selective ablation of cholinergic interneurons disrupts locomotion and enhanced dopamine transmission triggered by an acute stressor, indicating this cell population plays a critical role in adaptive stress processing [4]. Moreover, work from the Greengard laboratory and others has shown that suppression of cholinergic interneuron firing through a variety of cell-type specific transgenic or chemogenetic manipulations produces behaviors consistent with a depression-like state in mice [5] that is accompanied by a reduction in reward-evoked dopamine release [6]. Thus, we reasoned that the CRF mediated increase of cholinergic interneuron firing may facilitate pathways that drive the opposite behaviors: active coping and hedonic behaviors such as reward consumption. This notion is supported by our original findings that CRF both potentiates dopamine and facilitates appetitive behaviors when acting in the NAc. In this more recent report, we further show that this effect is, in part, due to activation of muscarinic type 5 receptors (M5) on dopamine neuron projections [3]. We propose that CRF's potentiation of cholinergic and dopaminergic transmission in the NAc is an underlying mechanism for the positive motivational qualities of acute stressors.

We further speculate that vulnerabilities to neuropsychiatric diseases such as anxiety, depression, and addiction may develop due to a diminution of the positive qualities of stress, not only an exacerbation of the negative qualities of stress. Studies in human subjects demonstrate disfunction in NAc activity in patients with depression compared to healthy controls in response to positive and negative stimuli using fMRI [7, 8]. Moreover, in mice, Gαs-DREADD activation in accumbal cholinergic interneurons was able to rescue depression-like phenotypes induced by chronic stress [5]. Thus, we remain hopeful that as we expand our understanding of the neuronal substrates that underlie the positive motivational qualities of stress and stress-associated neuropeptides like CRF, we may get closer to understanding the etiology of these diseases and improve treatment.

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Dopamine release drives motivation, independently from dopamine cell firing

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Despite decades of study, fundamental aspects of dopamine biology are still being revealed.

Dopamine release in the forebrain has at least two distinct functions: it invigorates current behavior (motivation) [1] and influences future behavior (learning) [2]. The learning role seems, at least in part, to involve brief bursts of dopamine cell firing signaling reward prediction errors [3]. This "phasic" dopamine signal helps adjust future reward expectations, through the modification of synaptic strengths in forebrain targets [4]. This is a compelling account of reinforcement learning mechanisms, but does not describe how dopamine achieves more immediate motivational functions.

It has been argued that motivation is mediated by distinct, slower changes in "tonic" dopamine cell firing. This now appears not to be the case [5]. In rats working for sugar rewards, we directly compared the firing of optogenetically identified midbrain dopamine cells (in the ventral tegmental area) with forebrain dopamine release (measured using microdialysis, voltammetry, and optical sensors). We found that dopamine release increases with reward expectation—and thereby enhances the animals' willingness to expend effort [1, 5]. Crucially, however, we found no corresponding change in dopamine cell firing.

Instead, this motivational aspect of dopamine release seems to be locally controlled within forebrain subregions. In both striatum and cortex we found specific "hotspots" (nucleus accumbens core and ventral prelimbic cortex) where dopamine release covaried with reward expectation [5]. These spatial foci stand in contrast to the canonical concept of dopaminergic reward prediction errors being "broadcast" throughout the forebrain.

There are many mechanisms that can achieve local control of dopamine release [6], most obviously the nicotinic acetylcholine receptors on dopamine terminals. The axons of striatal cholinergic interneurons form a very dense network of release sites closely intermingled with dopamine varicosities. Artificial stimulation of striatal cholinergic neurons very rapidly evokes dopamine release.

Although the local control of dopamine release has long been studied, its functional and computational significance is only now coming into focus. A better understanding of how dopamine release is regulated in behaving animals may provide a critical foundation for our understanding of neurological and psychiatric disorders, and the development of novel pharmacological therapies.

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Physiological roles for neuromodulation via $G_{i/o}$ GPCRs working through Gβγ–SNARE interaction

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Activation of presynaptic $G_{i/o}$-coupled receptors by hormones, neurotransmitters (NT) and neuromodulators leads to decreased neurotransmission. This decreased release provides an important control mechanism for autoreceptors to guard against over-activation, and an important homeostatic mechanism. For heteroreceptors, it is a critical component of synaptic integration mediating circuitry-level effects. Fast membrane-delimited inhibition of secretion may occur via Gβγ regulation of voltage-dependent Ca^{2+}channels (VDCCs). However, a direct interaction between Gβγ and soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins also leads to inhibition of exocytosis downstream of Ca^{2+} entry [1]. This mechanism is not only more acute and direct in controlling evoked release, leaving secondary effects of presynaptic Ca^{2+} unaffected, but is also able to modify components of exocytosis not available to mechanisms that control release probability. These include modifying the concentration of neurotransmitter released [2] by interacting with a region of the SNARE complex that controls fusion rate, but also modifying spontaneous release, which has important roles in its own right. The same synapses can have different $G_{i/o}$-GPCR-triggered modulation of neurotransmitter release by different mechanisms. For example, in hippocampal neurons, $GABA_B$ receptors cause decreased Ca^{2+} entry and $5HT_{1b}$ receptors inhibit exocytosis by directly acting on SNAREs at the same synapse: this allows for presynaptic neural integration [3]. What could be the mechanistic basis of this specificity? There is considerable evidence that unique Gβγ isoforms play specific roles in mediating interactions with both receptors and effectors. Our recent *in vivo* proteomic studies of Gβγ specificity suggest that it might come from receptor selection of particular Gβγ subunits [4], and the affinity of those Gβγ's for the SNARE complex (unpublished).

Understanding of the physiological role of Gβγ-SNARE interaction has lagged because of a lack of tools. But recent progress in understanding the molecular basis of this interaction, in particular a target for Gβγ at the C-terminal of SNAP25 [5] has yielded a transgenic SNAP25Δ3 mouse with a selectively disturbed Gβγ–SNARE interaction. This mouse has normal evoked exocytosis and normal GABAergic inhibition of VDCC, but disturbed inhibition of exocytosis through Gβγ–SNARE interaction. The SNAP25Δ3 mouse provides clear evidence that the Gβγ–SNARE locus is physiologically important for regulation, because it has a number of interesting phenotypes both central and peripheral, including elevated stress-induced hyperthermia, impaired supraspinal nociception, defective spatial learning, impaired gait, and depressive-like behavior [6].

Most interestingly, the two Gβγ-mediated inhibitory mechanisms, co-occurring at the same synapse, are synergistic with each other: a completely unexpected result. This observation suggests that combinations of neurotransmitters may shape neuromodulation, potentially giving rise to novel effects on circuits. Thus, synaptic integration can occur as much presynaptically as postsynaptically. The specificity of the two mechanisms raises the possibility that targeting the Gβγ-SNARE interaction may be a therapeutic strategy, and, further, that therapeutic pairing of drugs that affect each mechanism may themselves work synergistically, an exciting possibility.

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The striatum specifies the statistics of behavior

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Many neurodegenerative disorders with motor phenotypes (e.g. Parkinson's and Huntington's disease) target an evolutionarily conserved, subcortical group of nuclei called the basal ganglia (BG). The input nucleus to this circuit is the largest structure in the BG, the striatum. Over 95% of the cells in the striatum are a unique type of GABAergic principal cell, the spiny projection neuron (SPN), which samples inputs from cortex and relays these signals to the rest of the BG. Because of the intimate relationship between BG and behavior, understanding the physiological function of the BG—and how those functions are altered during disease—requires the ability to carefully characterize behavior. Prior work on BG function in both humans and animals has relied on manual scoring of video recordings. The use of these methods has led to a model in which the BG initiates or suppresses broad classes of movement [1]. Specifically, SPNs expressing the DRD1 receptor (direct SPNs, or dSPNs), which contain a direct projection to the output of the BG, are thought to promote movements, while SPNs expressing the DRD2 receptor are thought to suppress them (indirect SPNs, or iSPNs).

Figure 1 Recently, our group has developed a machine learning-based approach called Motion Sequencing to better define the structure of ongoing behavior. In the context of the BG, these (and related) experiments have revealed that SPNs do not simply initiate or suppress broad classes of movements; instead, SPNs both reflect the content of individual movements (i.e., behavioral "syllables") and directly control how movements are sequentially organized (i.e., behavioral "grammar") [2, 3].

Activity measurements from single dSPNs and iSPNs in freely moving mice demonstrated that both SPN types contain a "map" of syllables [4]. Similar populations of SPNs were active during similar syllables, and different neurons were active for dissimilar syllables. In our work, activity of dSPNs and iSPNs was monitored simultaneously, while also measuring exploratory mouse movements in 3D. We found that dSPNs and iSPNs encode action through a complementary code: more information about the animal's ongoing behavior is contained in both dSPN and iSPN activity compared with either in isolation [2] (see also [3]). Similar to [4], we found that the ensembles of dSPNs and iSPNs associated with any given behavior overlapped with those recruited during similar behaviors. Neurons active during a rear up in the center of the arena were likelier to be active during other behaviors with similar pose dynamics, for example rears along the wall or curved rears leading into a turn. Thus, the activity of SPNs could be directly mapped onto the landscape of pose dynamics between different behaviors. Furthermore, chemical lesion of the dorsolateral portion of the striatum led to aberrant sequencing of both spontaneous movements and sensory-guided movements. However, the execution of individual syllables was spared, similar to observations made in the context of grooming sequences in the rat [5].

Taken together, new recording technologies and automated methods for annotating behavior allowed our group, and others, to show that individual SPNs contain a relational map of action, dSPNs and iSPNs encode action complementarily, and SPNs control how movements are organized sequentially over time. This work reveals a more general role for both dSPNs and iSPNs in the process of selecting which action to perform among dozens of possible actions on a moment-by-moment basis. Thus, clinically, pathologies of the BG may be manifest both in the form and kinematics of movements and in the statistics of how movements are deployed over time.

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CODE AVAILABILITY

Details on how to obtain open-source code for the automated analysis of behavior are available at http://datta.hms.harvard.edu/research/behavioral-analysis/.

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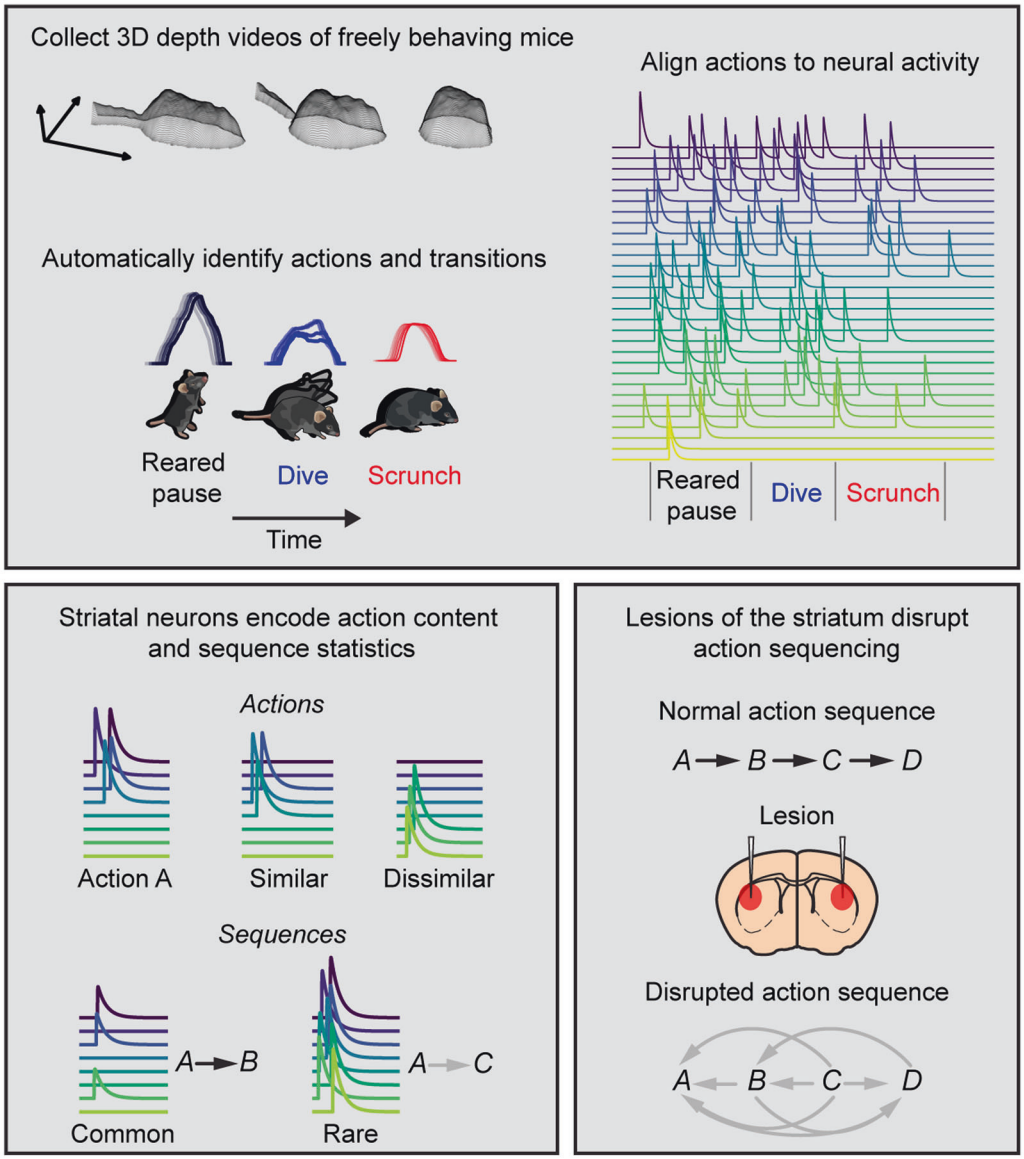

Fig. 1 Top, Schematic of the experimental workflow used in [2]. First, depth videos are collected from freely behaving mice from while simultaneously recording neural activity from SPNs. Then, behaviors and transitions are automatically identified using machine learning methods previously developed by our group [6]. Bottom left, Simultaneous measurement of 3D behavior and SPN activity revealed that SPNs encode the content and sequence structure of actions. Bottom right, Lesion experiments demonstrated that the striatum specifies how behaviors are sequenced

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Contributions of nonneuronal brain cells in substance use disorders

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INTRODUCTION

Investigations into the neurobiology of substance use disorders (SUD) have historically focused on neurons. However, recent years have brought a notable shift toward recognition of contributions of nonneuronal cells to SUDs. Here, we provide a brief overview of this emerging topic and suggest future research directions.

DRUG EFFECTS ON GLIAL CELLS

Accumulating evidence indicates that drug exposure can have dynamic and long-lasting effects on glial cells in the brain, including astrocytes, microglia, and oligodendrocytes. Among the first reported effects of rodent drug self-administration on astrocytes was decreased expression of mediators of glutamate homeostasis in the nucleus accumbens following self-administration of cocaine, heroin, nicotine, and ethanol. Using the membrane-associated Lck-GFP fluorescent reporter, more recent studies indicate that extinction from cocaine self-administration is associated with reduction of structural properties and synaptic co-localization of astrocytes in the nucleus accumbens [1], further suggesting adverse effects of contingent cocaine exposure on neuron–astrocyte communication.

Inflammatory glial responses are also observed following drug use, particularly following chronic opiate treatments associated with tolerance and hyperalgesia. For example, upregulation of proinflammatory cytokines and toll-like receptor signaling on microglia and astrocytes is associated with these processes. However, engagement of these pathways following self-administration has remained less clear. Notably, recent reports demonstrate that self-administration of cocaine also leads to activation of microglia within the reward circuitry [2, 3]. Likewise, consequences of drug self-administration on oligodendrocytes are largely unknown. While there have been reports on effects of drug use on myelination and white matter integrity, detailed consequences to structure and activity of oligodendrocytes remain obscure. Intriguingly, a recent transcriptome analysis within the prefrontal cortex of male rats revealed upregulation of markers of oligodendrocyte maturation and differentiation following heroin self-administration [4].

ROLES OF GLIAL CELLS IN BEHAVIORS ASSOCIATED WITH SUD

Beyond the effects of drugs on properties of nonneuronal cells, a growing number of investigations have also assessed the functional contributions of glial cells to behaviors associated with SUDs. For example, ligand stimulation of astrocyte-specific Gq DREADD receptors in the nucleus accumbens leads to reduced reinstatement to cocaine seeking [5]. Further, VTA astrocytes drive avoidance behaviors via stimulation of GABAergic neurons, and stimulation of VTA astrocytes can block cocaine conditioned place preference [6]. Selective overexpression of the oligodendrocyte precursor-specific protein, Sox10, in the prefrontal cortex decreases motivation to self-administer heroin [4]. Future studies should evaluate whether and how oligodendrocyte activity elsewhere in the brain might influence drug taking or relapse. These results collectively suggest that manipulation of glial cells can alter drug-seeking behaviors, and in particular that stimulation of glial cells within the reward circuitry may in some cases oppose drug seeking.

In summary, accumulating evidence suggests that glial cells are critical players in the complex mechanisms of drug seeking, and may represent viable candidates for SUD treatment strategies. In order to identify new glia-based therapeutic targets, continued studies will be required to elucidate more complete glia-specific adaptations associated with SUD, and how amelioration of these adaptations can stem vulnerability to relapse.

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TRPV1 and MOR working in tandem: implications for pain and opioids use

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Opioids are widely used medications for the relief of moderate to severe pain. While they remain one of the strongest analgesics for pain associated with cancer, trauma, or surgery, prolonged treatment often comes with undesirable side effects, including analgesic tolerance that causes dose escalation and addiction.

Three opioid receptors: mu-, delta-, and kappa-opioid receptors (MOR, DOR, and KOR, respectively) are found in the afferent pain pathway and participate in opioid-induced analgesia [1]. These receptors respond to exogenous (i.e morphine) and endogenous opioids (β-endorphin, enkephalins, and the dynorphins) that exert efficient inhibitory control of pain at sites of inflammation and in the central nervous system. The endogenous opioid system represents an evolutionarily important pain-coping strategy during tissue healing and several studies have shown that opioid receptors on afferent nociceptors respond to peripherally acting endogenous opioids released by immune cells, including $CD4^{+}$T cells that are recruited in the later phase of inflammation [2]. Overall, pain sensitization and opioid signaling appear intertwined in the establishment of chronic inflammatory pain. Activation of the mu-opioid receptor (MOR) by opioids results in the binding of β-arrestin2 to the receptor. This interaction prevents receptor signaling, and elicits desensitization, which in turn reduces the pain-relieving effect and requires increased opioid administration, enhancing the unwanted side effects of MOR activation [3]. Previous work reported an improvement of the opioid therapeutic window in acute inflammation, suggesting a mechanism by which inflammation renders opioid receptors to be more responsive [4]. We recently identified the transient receptor potential vanilloid type 1 (TRPV1), a main target of inflammatory mediators, as a central hub protein that primes pain-relieving effects of opioids. MOR is predominantly expressed in $TRPV1^{+}$ nociceptors and chemical stimulation of the TRPV1 channel was found to prevent G protein-coupled receptor kinase 5-dependent phosphorylation of agonist-bound MOR [5]. We found that activation of TRPV1 diverts the MOR effector β-arrestin2 to the nucleus, and this process coincides with enhanced mitogen-activated protein kinases (MAPK) signaling. With β-arrestin2 removed from the membrane-anchored MOR, the receptor is free of β-arrestin2 and hence unable to desensitize and internalize. We next used the complete Freund's adjuvant (CFA) model of chronic inflammatory pain to show that TRPV1 knockout mice do not exhibit endogenous opioid analgesia during resolution of inflammation. Finally, using morphine-treated animals, we found that absence of TRPV1 expression promotes peripheral opioid receptor desensitization. Altogether, our findings suggest that agonists of TRPV1 prevent β arrestin2-biased signaling of MOR, which enhances analgesia by maintaining peripheral opioid receptor function (Fig. 1) [6]. As chronic inflammatory conditions like arthritis or inflammatory bowel diseases are often associated with persistent pain, further studies will reveal whether the dysregulated interplay between TRPV1 and β-arrestin2 contributes to the transition from acute to chronic pain.

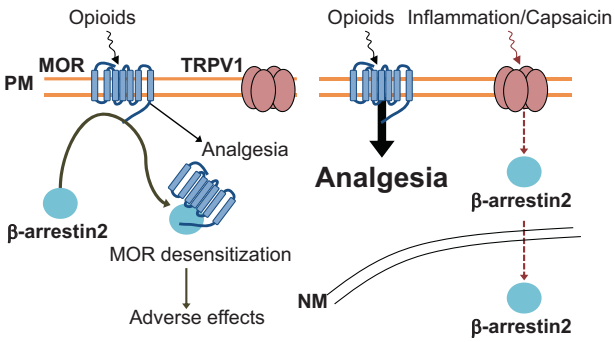

Fig. 1 Illustration of TRPV1–MOR interplay with (right) and without (left) inflammation. MOR desensitization, mediated by β-arrestin2 recruitment to the receptor, promotes analgesic tolerance. Activation of TRPV1 with capsaicin, or during inflammation, prevents β arrestin2-biased signaling of MOR, and thus enhances analgesia

With current research efforts focused on optimizing new types of opioids that circumvent the adverse side effects, these findings could lead to new combination therapies using TRPV1 agonists like vanilloids and cannabinoids as effective analgesics that may also be useful to prevent opioid tolerance.

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Towards objective definition of psychopathology in post-traumatic stress disorder

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Owing to their reliance on imprecise clinical phenotypic definitions, current psychiatric diagnoses capture a broad range of neurobiological alterations across patients. The difficulties that arise from these definitions are particularly striking for post-traumatic stress disorder (PTSD). For example, the revision of its diagnostic criteria from DSM-IV to DSM5 resulted in only a ~ 50% overlap in case definition [1]. One way to overcome challenges inherent to these clinical definitions is to anchor patient definitions in objectively quantifiable measures [2].

To identify biologically and clinically meaningful PTSD subtypes, we began with the perspective that cognitive task behavior may be a particularly useful way to anchor patient phenotypes so that they are both objective and face-valid [3]. Within cognition, verbal memory is the domain most impaired in PTSD patients on average [4]. We therefore treated verbal memory in a normative perspective, dividing patients based on whether they performed within or outside the healthy norm, and examined resting-state functional magnetic resonance imaging (fMRI) network connectivity to understand mechanisms involved with differences in memory [3]. This is akin to a typical medical test, which are often framed within a normative perspective. We found, and then replicated (total $N = 357$), that after correction for multiple comparisons, connectivity in one brain system (the ventral attention network; VAN) was reduced only in PTSD patients with impaired verbal memory, relative to either controls or patients with intact memory.

Critically, moreover, memory and VAN connectivity predicted treatment outcome, thus demonstrating clinical relevance, despite the discovery of the memory–VAN connection coming out of a mechanistic characterization rather than one primarily targeting treatment prediction [3]. Patients in one of the samples went on to receive either prolonged exposure psychotherapy, a gold-standard treatment for PTSD, or a wait list intervention control. Those patients with impaired memory and VAN connectivity failed to respond to prolonged exposure (and did not differ in the wait list arm), whereas those without both abnormalities responded well. Finally, to understand how these insights may be useful in driving new therapeutics, we used simultaneous non-invasive transcranial magnetic stimulation and electroencephalography (TMS/EEG) to map the brain's response to single TMS pulses at various locations and implicated a region in the right prefrontal cortex [3].

These results suggest that by anchoring on an objective measure (i.e., verbal memory), clinically and mechanistically meaningful biological differences can be observed and replicated. The TMS findings further suggest an avenue for developing novel interventions for memory–VAN impaired patients, by targeting the right prefrontal cortex.

More broadly, these findings open up a path for transcending traditional clinical phenomenology and grounding clinically meaningful case definition in observable biomarkers. To ultimately impact clinical care, we anticipate that tools such as EEG (a cheaper and more clinic-ready tool than fMRI) and machine learning (to make relevant brain signatures more robust) will be required. Nonetheless, our results suggest that it is more a question of how, rather than whether, these types of biomarkers could transform diagnosis and treatment in psychiatry.

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Linking actions with their consequences within the ventrolateral orbital cortex

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Humans and rodents can associate actions with their outcomes and modify expectations when associations change. These cognitive adaptions accommodate change and presumably optimize decision-making. For example, we might modify our driving route when construction blocks our path or abstain from alcohol when we need to drive, expecting that both actions will deliver us safely home. Across species, medial prefrontal cortical regions are involved in linking actions with valued outcomes, but contributions of the orbitofrontal cortex remain contentious. One issue is that the orbitofrontal cortex occupies a large territory, yet is sometimes treated as a homologous structure. A related concern is the assumption that ventromedial subregions, like lateral regions, specialize in stimulus−outcome associations (linking cues, rather than actions, with likely outcomes) agnostic to action−outcome associations. Nevertheless, poor or aberrant decision-making is commonplace in neuropsychiatric illnesses, necessitating a full dissection of how action-outcome associations form, update, and solidify.

Parkes et al. [1] made important in-roads in resolving controversies. Rats were trained to associate two actions with a single food reward; then the actions were paired with different, unique rewards in the days preceding a devaluation test. Inactivation of the ventrolateral orbitofrontal cortex (VLO) either during the final training days or during the subsequent probe test blocked the ability of rats to choose actions based on the value of respective rewards. Meanwhile, VLO inactivation had no effect when action−outcome contingencies had not changed during training. In another investigation, inactivation of the VLO immediately following the violation of a familiar action−outcome association in mice occluded optimal responding in a later test, even when the VLO was back "on-line" [2]. Thus, the VLO appears necessary for stabilizing newly formed or updated action−outcome associations, which then guide future behavior.

Notably, linking actions with their outcomes involves dendritic spine plasticity on excitatory neurons in the VLO [2]. Specifically, updating action−outcome expectations reduces thin-type dendritic spines, considered immature, on layer V neurons. Meanwhile, the proportion of mushroom-shaped spines, considered mature, increases, potentially solidifying newly modified action−outcome associations to optimize future decision-making. Remarkably, inactivating VLO neurons upon the violation of familiar action−outcome associations not only blocks response updating, but also inhibits dendritic spine plasticity in an activity-dependent manner [2]. These patterns strongly suggest that dendritic spine plasticity on excitatory VLO neurons is *necessary* for forming action−outcome associations, consistent with evidence that orbitofrontal neurons are capable of forming and maintaining long-term reward-related memory to support behavioral adaptations [3].

Orbitofrontal neurons display a rich diversity of functionally distinct populations based on input/output patterns, many of which make unique contributions to flexible decision-making [3, 4]. A key question is thus: What inputs to the VLO help to form/update action−outcome associations? Are these inputs distinct from those supporting other associations, e.g., stimulus−outcome associations? Basolateral amygdala (BLA) projections are one candidate. BLA lesions alter the reward-related coding properties of orbitofrontal neurons [5], and BLA → orbitofrontal cortical connections appear necessary for certain forms of reinforcement learning [4], including in primates ([6] and references therein). Whether and how these "bottom-up" connections are involved in forming action−outcome associations should be resolved.

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Regulation of fear extinction and relapse by hippocampal engrams

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Maladaptive fears are often treated using therapies based on extinction—re-exposure to a feared stimulus in a safe environment. Although these treatments can be effective, relapse is common [1]. Since the time of Pavlov, relapse has been thought to indicate that extinction is new learning, not unlearning [2]. From this perspective, relapse is a mnemonic phenomenon in which two opposing memory traces vie for expression. Recent work suggests that the hippocampus is an arena in which this competition plays out.

Experiments in mice demonstrate that the hippocampal dentate gyrus (DG) generates a contextual fear "engram." Immediate-early gene-based tagging of neurons active during acquisition of contextual fear—fear of the chamber in which a footshock was given—shows that these neurons are reactivated during recall of contextual fear [3]. Furthermore, optogenetic experiments demonstrate that this reactivation is necessary and sufficient for expression of contextual fear [3, 4]. Two recent studies investigated what happens to hippocampal fear ensembles when contextual fear is extinguished.

The first of these studies, by Lacagnina et al. [5], used a transgenic mouse line to tag neurons active during acquisition or extinction of contextual fear. During a test session shortly after extinction training, fear acquisition neurons were suppressed and extinction neurons were reactivated. A month after extinction training mice displayed spontaneous recovery (relapse) of fear, and the pattern reversed: fear acquisition neurons were reactivated while extinction neurons were suppressed. The results suggest that the DG generates distinct fear acquisition and extinction representations, and competition between these representations determines whether fear is suppressed or recovers after extinction. Consistent with this interpretation, optogenetic manipulations demonstrated that reactivation of extinction neurons is necessary for suppression of fear after extinction, whereas reactivation of fear acquisition neurons is necessary for spontaneous recovery [5]

The other recent study, by Khalaf et al. [6], highlights that fear acquisition neurons also play an important role in extinction learning. In this study, neurons active during recall of a remote fear memory (acquired a month before extinction training) were tagged. Reactivation of these neurons was necessary for effective extinction, and artificial stimulation of these neurons improved extinction. For extinction to be effective, the fear acquisition memory must be reactivated during extinction training. Whether these findings apply to recent fear memories, like those studied in the Lacagnina et al. [5] experiments, is not yet known.

These studies reveal new ensemble mechanisms of fear extinction and relapse and raise some interesting questions. For instance, what aspects of extinction training stimulate creation of hippocampal extinction representations, and how do these representations suppress fear? Do they do so by activating extra-hippocampal fear-suppressive networks? In the Lacagnina study, why did fear acquisition neurons become more active over time and extinction neurons less active? Is it because of intrinsic differences between the two populations, changes in upstream input pathways, or different plasticity mechanisms involved in fear acquisition and extinction? Addressing these and other questions raised by the Lacagnina and Khalaf papers may provide keys to making extinction more resistant to relapse.

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The complexity of pharmacology of cannabidiol (CBD) and its implications in the treatment of brain disorders

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Cannabidiol (CBD) is one of the major cannabinoid constituents of the C*annabis* plant. Recently, CBD has sparked the interest of medical researchers because of its more than 65 identified molecular targets. Of those, mostly studied in brain disorders are cannabinoid, $5HT_{1A}$ receptors, G-protein receptor protein 55 (GPR55), transient receptor potential (TRP) channels, and cytochrome P450s [1]. Here we discuss possible mechanisms of actions of CBD in several brain disorders.

The evidence suggests that the antiepileptic potential of CBD may be via its modulation of TRP (vanilloid 1 and TRPA), potassium channels, NMDA receptors, and more recently by the interaction with GPR55 to reduce neuronal excitability [1, 2]. Although in the US, CBD is currently prescribed as an adjuvant treatment for seizures in Lennox-Gastaut and Dravet syndromes, as well as tuberous sclerosis complex, it is still unknown if CBD's antiepileptic properties are due to its direct interaction with the molecular targets, or possibly through potentiating effects of antiepileptic treatments by modulation of cytochrome P450s [1, 3].

CBD exhibited anxiolytic properties by acting on the $5HT_{1A}$ receptors in animal models [1]. Most recently, an in vitro study showed that CBD might also elicit anxiolytic effects by allosterically modulating $GABA_A$ receptors [4]. Human studies using CBD were limited to assessing the short-term effects of CBD on social anxiety disorder (SAD) [1].

By the mechanism of action on the CB1 receptor, CBD attenuated behavioral responses to different forms of aversive memories in rodent PTSD models [5]. Although in human studies, CBD was associated with reduced PTSD symptomatology, the evidence is only limited to case studies, while possibly being confounded by the co-administration of other psychiatric treatments [6].

The antidepressant properties of CBD by activation of $5HT_{1A}$ receptors were revealed in animal models of depression [1]. However, to date, CBD's effects on clinical depression have not been studied.

CBD has been proposed to have anti-psychotic effects by modulating dopamine D2, cannabinoid receptors, and TRPV1 channels; however, these mechanisms are somewhat speculative, given the lack of reproducibility of findings. In human studies, CBD produced conflicting evidence to either augment or improve the symptoms of schizophrenia [1].

The anti-addictive potential of CBD was demonstrated in animal models of cannabis, opioid, alcohol, methamphetamine, and cocaine use disorders. Although CBD's molecular pathways are still poorly understood, they may include neuronal excitability, $5HT_{1A}$ receptors and possibly cannabinoid and opioid systems. In small-scale clinical trials, CBD reduced cigarette consumption and heroin cue-induced craving. The anecdotal evidence also shows the positive effects of CBD on reducing symptoms of cannabis and alcohol use disorders, yet these effects need further investigation in larger trials [1].

In summary, the complexity of CBD pharmacology is due to CBD's ability to interact with several molecular targets, making it a good candidate for further therapeutic investigation. Currently, in the US, CBD is only prescribed for treatment of childhood epilepsies, while other indications are still under exploration. To fully elucidate its true therapeutic potential in other brain disorders, CBD needs to be tested in larger-scale randomized, placebo-controlled trials.

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OPEN

Is depression a disorder of electrical brain networks?

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Major depressive disorder (MDD) is one of the most prevalent and disabling neuropsychological disorders in the world, with 15% of adults expected to experience depression sometime in their lives. Current treatment options are largely ineffective, as only 50–70% of patients experience remission after multiple rounds of treatment [1]. Thus, there is a clear and immediate need for the development of novel therapeutics that prevent MDD. Nevertheless, this endeavor has been hampered by limited knowledge of the biology underlying the disorder.

A well-validated murine model of depression, chronic social defeat stress (CSDS) [2], can differentiate between mice that exhibit MDD-like behavior following stress exposure, termed "susceptible", and those that do not, termed "resilient". Our lab's prior work exploring network dynamics linked to CSDS susceptibility found that susceptible mice exhibited greater prefrontal cortex (PFC)-dependent limbic synchrony [3]. Since susceptible and resilient mice experienced identical stress exposure but exhibited different network dynamics after CSDS, we hypothesized that differences in network dynamics exist prior to stress exposure and could serve as a biomarker for the vulnerable population of test mice (i.e., mice that *will* exhibit MDD-like behavior *following* future exposure to CSDS).

To test this hypothesis, multicircuit recordings during acute threat were collected from test mice before and after exposure to CSDS, and processed using discriminative cross-spectral factor analysis (dCSFA), a model of machine learning [4]. The dCSFA method was chosen for its interpretability (i.e., relatability to specific neural phenomena) and prediction (i.e., discrimination of behavioral variables). This approach identified four electrical network features, termed "electome factors". These networks were validated using techniques previously demonstrated to increase vulnerability (e.g., early life stress, inflammation, and overexpression of the gene *Sdk1* in the ventral hippocampus). Only one of these electome factors, *Electome Factor 1 (EF1)*, was responsive to vulnerability manipulations and, consequently, validated as a network underlying vulnerability. Furthermore, techniques for treating susceptible mice after CSDS (e.g., ketamine administration and suppression of activity in PFC) did not have any significant effect on *EF1*, though these treatments suppressed other electomes associated with susceptibility. Activity in this network originates in the PFC and ventral striatum, relays through the amygdala and ventral tegmental area, and converges in the ventral hippocampus. Together, these results indicate that *EF1* is a biomarker of vulnerability and is distinct from MDD-like susceptibility.

Alternative techniques have identified networks indicative of individual vulnerability to social stress in rats [5]. Though vulnerability identification has not progressed to humans yet, recent functional magnetic resonance imaging studies in depressed patients have revealed distinct functional networks [6]. Furthermore, differences in functional connectivity successfully predict different subtypes of depression as well as responsiveness to treatment, suggesting that network-level analyses may provide an avenue for developing more successful treatments for depression.

Our findings demonstrate that network-level spatiotemporal dynamics can indicate previously obscured vulnerable individuals within heterogeneous populations. These results could support the development of novel therapeutic mechanisms targeted at preventing the emergence of MDD or encouraging resilience in vulnerable populations. Furthermore, they encourage exploration of electome networks that may signal other emotional states in health and disease.

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MDMA-assisted psychotherapy for posttraumatic stress disorder: A promising novel approach to treatment

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Posttraumatic stress disorder (PTSD) treatment guidelines have unequivocally designated psychotherapy as a first-line treatment, despite well-documented neurobiological alterations in this disorder [1]. Even with psychotherapy, PTSD often remains chronic and severe. There is urgency to discover novel compounds and new treatment strategies for PTSD.

One approach is the use of medication to leverage the effects of psychotherapy. A promising example is the use of 3,4-methylenedioxymethamphetamine (MDMA). MDMA was synthesized in 1912 by Merck and discovered in the early 1970s to enhance effects of psychotherapy. The subsequent classification of MDMA as a Schedule 1 controlled substance made its use in therapy illegal and created obstacles to clinical research, including assessment of safety.

Nonetheless, new research has emerged demonstrating the efficacy of MDMA as an enhancer of psychotherapy for PTSD. Recently, a pooled analysis was published from six small randomized, double-blind, controlled clinical trials of MDMA [2]. Patients were enrolled to manualized psychotherapy sessions in two or three 8 h sessions, spaced a month apart. They were given either active doses of MDMA ranging from 75 to 125 mg ($n = 72$) or placebo/control doses 0–40 mg ($n = 31$). Non-drug sessions lasting 90 min preceded the first MDMA exposure and three to four weekly sessions following the drug-facilitated session. Two clinicians facilitated an introspective process in which the patient revisited past experiences while under a mental state produced by MDMA that presumptively minimized fear, arousal, and avoidance of painful material. Under MDMA, patients experienced significantly greater reductions in PTSD symptom scores than under placebo, with a treatment effect of 0.8. After two experimental sessions, double the participants in the active group (54.2%) did not meet PTSD diagnostic criteria than in the control group (22.6%). Based on these pooled results Food and Drug Administration (FDA) granted MDMA a breakthrough therapy designation for the treatment of PTSD. Yet, it will be important to continue to assess safety of MDMA, particularly when higher or multiple doses are used [3].

MDMA-assisted psychotherapy provides a novel approach for examining how the use of a medication that dramatically alters a cognitive state can facilitate a deeper psychotherapeutic process [4]. It offers a contrast to the current use of medications and psychotherapy, which are often not well integrated and/or provided under the auspices of a single clinician. The fewer in number, but lengthier sessions in the presence of MDMA also redefine concepts regarding the appropriate approach and length of a therapy session involving engagement with traumatic material [5].

Working with FDA in America and the European Medicines Agency in Europe, the pooled data formed the basis for expansion into multi-site Phase 3 trials of MDMA therapy for PTSD. These are sponsored by a non-profit organization that has raised funds through philanthropy, which offers yet another model of drug and therapeutic development. Study centers in the United States are now underway. The European sites—in the Netherlands, United Kingdom, Germany, Finland, Portugal and the Czech Republic—are in the process of seeking approvals and are projected to start later in 2019, putting MDMA on course to becoming a licensed treatment in 2021 [6].

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Spatial transcriptomics: putting genome-wide expression on the map

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Extensive efforts are underway to comprehensively characterize changes in the human brain transcriptome in neurodevelopmental and neuropsychiatric disorders. Although bulk RNA-sequencing (RNA-seq) in postmortem human brain has identified many molecular associations with psychiatric disease, analysis of homogenate brain tissue can mask the heterogeneity of associations within and across specific cell types. Although computational approaches exist to deconvolute cell-type-specific effects from bulk data, these approaches largely control for cellular heterogeneity across samples [1] and do not pinpoint candidate cell types harboring transcriptional differences [2]. However, rapid progress towards characterizing the cell types that make up the brain has been achieved by major advances in single-cell (sc) and single-nuclei (sn) approaches, which are revealing molecular profiles of distinct cell populations. However, these methods still require tissue dissociation, which removes molecularly defined cell types from their spatial environment. This is problematic, because the ability to assess gene expression as a function of neuroanatomy and cytoarchitectural organization will be critical for interpreting molecular and genetic associations.

Methods such as laser-capture microdissection allow for transcriptome-wide profiling in a defined spatial area, but tissue is removed from the surrounding spatial context, making it difficult to analyze gradients of gene expression. The rapidly accelerating field of spatial transcriptomics utilizes techniques that examine the location of hundreds of gene targets in intact tissue slices. In situ sequencing and fluorescent in situ hybridization-based technologies have achieved high levels of multiplexing in single cells of mouse brain using padlock probes or barcoding strategies in combination with sequential rounds of probing, imaging, and stripping (reviewed in refs. [3, 4]). However, even for methodologies that can accommodate hundreds of transcripts simultaneously, molecular crowding within cells leads to fluorescence overlap posing significant microscopy and computational challenges [3, 5].

Although not yet sc in resolution, additional platforms such as Slide-seq [6] and SPATiAL transcriptomics [7] have emerged to provide positional context of genome-wide expression in intact tissue sections. SPATiAL generates transcriptome-wide RNA-seq data through capture of polyadenylated RNA on arrays containing positional molecular barcodes that are introduced during cDNA synthesis, which occurs on the surface of the tissue section. Barcoded cDNA is then cleaved, prepared into libraries, and sequenced on a standard Illumina platform. Each RNA is then mapped back to its spatial location within 1 of ~1000 different molecular positions in a 6 mm^2 array. Although these approaches have not yet been widely applied to postmortem human brain, SPATiAL has been used to identify perturbations in transcriptional pathways for several normal and pathological human tissues, including the spinal cord [8].

Ultimately, understanding the molecular basis of psychiatric disease will require linking molecularly defined cell types to measures of function, including correlates of morphology, physiology, and connectivity. Analyzing gene expression within the existing tissue architecture in the human brain is the next step. These rapidly evolving spatial transcriptomics techniques open possibilities for combining spatial expression maps with sc/snRNA-seq data using spatial registration approaches to add anatomical dimensions to existing datasets and further refine cell-type classifications in the human brain. Importantly, these spatial transcriptomic approaches capture gene expression in the cytoplasm and neurites (Fig. 1), which has been largely missed by snRNA-seq approaches in human brain tissue. This is important, because synaptically localized mRNAs may be especially relevant for understanding genetic risk for psychiatric disorders as

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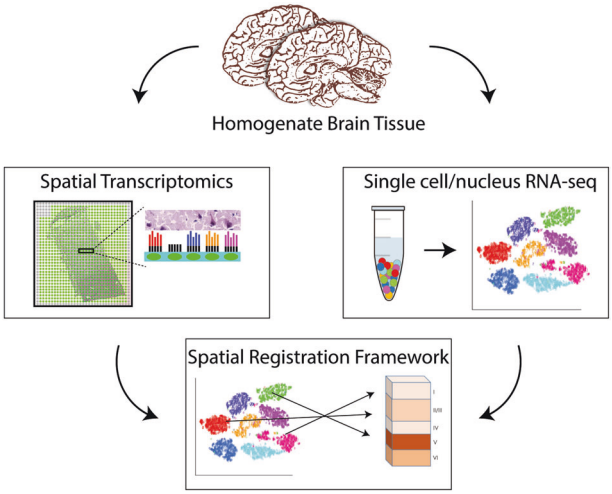

Fig. 1 Spatial resolution of cell-type-specific gene expression in the human brain. Bulk homogenate tissue contains heterogeneous cell types with distinct spatial orientations. Spatial transcriptomic approaches, such as SPATiAL, use spotted arrays with positional molecular barcodes that tag cDNA synthesized in intact tissue sections with a spatial location allowing gene expression to be mapped back to a histological image (i.e., specific cortical layers). Parallel sc/sn RNA-seq approaches define gene expression in individual cells dissociated from homogenate tissue. Integrating spatial transcriptomic data with sc/snRNA-seq data opens possibilities for adding anatomical dimensions to existing datasets, to better understand cell-type-specific molecular profiles in the human brain during development and psychiatric disease

genome-wide association study signals are preferentially enriched for synaptic neuropil transcripts [9].

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Role for kappa-opioid system in stress-induced cocaine use uncovered with PET

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Cocaine use disorder (CUD) continues to affect nearly a million American adults. While significant efforts have been made to find an effective pharmacotherapeutic, primarily targeting the brain's reward system, there remain few treatment options. In the past 30 years, animal models and post-mortem human studies have elucidated the role of the dynorphin–kappa opioid receptor (KOR) system in the maintenance of cocaine use, particularly during the negative affect state, also termed the "dark side" of addiction [1]. Animal models have shown that blockade or genetic deletion of the KOR attenuates stress-induced cocaine-seeking behavior [2]. Importantly, this effect is selective for stress: blocking the KOR does not change cocaine-primed reinstatement [2]. Post-mortem human studies have shown increases in KOR in limbic areas in fatal overdose victims [3]. However, there has been little advancement to evaluate the effects of KOR antagonists in individuals with CUD, despite the lack of currently available treatment options.

Our recent study sought to evaluate the association between the dynorphin–KOR system and a laboratory model of stress-induced relapse and binge cocaine use using positron emission tomography (PET) [4]. Imaging with the KOR selective agonist [^{11}C]GR103545 in volunteers with CUD showed a significant association between KOR binding and cocaine self-administration following stress induced by a cold-pressor test. This result suggests that CUD individuals with higher levels of KOR binding are more prone to relapse to cocaine use under stressful conditions compared to those with lower levels of KOR availability. Additionally, after a 3-day cocaine binge in the

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laboratory, a subsequent PET scan showed reduced [^{11}C] GR103545 binding compared to CUD subjects' baseline scans. This reduction in radioligand binding suggests that increased endogenous dynorphin is competing for KOR-binding sites and supports the prior animal literature showing that binge cocaine use modifies this neurotransmitter system. Together, these findings support the negative affect state hypothesis of addiction and implicate that the dynorphin–KOR system is a central part of this state in humans with CUD.

Prior to our combined human laboratory and PET imaging study, there was a limited understanding of the potential clinical role for KOR antagonists in CUD. A multi-site placebo-controlled trial of combined buprenorphine (a potent kappa antagonist and partial mu agonist) and extended-release naltrexone (a non-specific opioid receptor antagonist) showed mixed results [5], although this may have been affected by the mu receptor activity of naltrexone. More recently, a selective KOR antagonist LY2456302 was shown to be well-tolerated in CUD patients, showing feasibility for future clinical trials [6].

The confluence of data in animal models, post-mortem human studies, and now human PET imaging robustly supports the need for clinical studies of KOR antagonists that focus on stress-induced relapse. Future success with KOR antagonists will depend on targeting the "dark side" of addiction, by selectively recruiting participants who relapse in the setting of stress and dysphoria.

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Targeting neuroinflammation with brain penetrant P2X7 antagonists as novel therapeutics for neuropsychiatric disorders

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Several lines of scientific data suggest a correlation between neuroinflammation and CNS disorders such as multiple sclerosis, Alzheimer's disease, Parkinson's disease, epilepsy, chronic pain and neuropsychiatric disorders (depression, bipolar disorder and schizophrenia). Many of these CNS diseases are comorbid with classical peripheral inflammatory disorders such as rheumatoid arthritis, inflammatory bowel disease, psoriasis, to name a few. The scientific underpinning of this comorbidity in patients is perhaps driven by a compromised immune surveillance system wherein the immune cells and cytokines/chemokines in the periphery cross-talk to the CNS either directly (BBB integrity compromise) or indirectly via modulating meningeal immunity. In addition, there is an inherent '*central*' component of neuroinflammation that is driven by microglial cells within the CNS. As such, neuroinflammatory drug targets on microglia cells within the CNS have been an emerging area of research within the neuroscience community, both in academia, industry and in public-private consortia.

P2X7 is an ATP activated ion channel, that is expressed abundantly on microglia (and peripheral immune cells) and is a critical mediator of neuroinflammation via release of NLRP3 dependent IL-1β and IL-18 [1]. In several preclinical models of stress in rodents, independent research groups have demonstrated an activated NLRP3-IL1β pathway; as such intervention of this pro-inflammatory pathway by targeting the P2X7 ion channel with CNS penetrant antagonists have demonstrated efficacy in models of psychiatric diseases [2] and provides optimism for novel therapeutic options for patients with mood disorders. When ATP is present in high concentrations in extracellular space as a danger signal, the P2X7-NLRP3- IL1β signaling is activated initiating the microglial neuroinflammatory cascade. The functional expression of this pathway may or may not be associated with enhanced expression of P2X7 at the protein level

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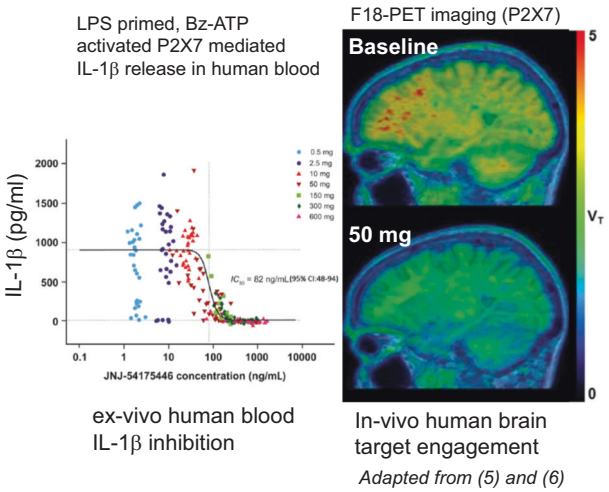

Fig. 1 Incremental human plasma exposure of the P2X7 antagonist JNJ-54175446 caused a dose dependent attenuation of P2X7 dependent ex-vivo IL-1β release (ex-vivo LPS primed, ex-vivo Bz-ATP as 2nd stimulus) from human subjects (left). The dose of 50 mg JNJ-54175446 produced central target engagement as assessed by displacing the P2X7 PET ligand, [^{18}F]JNJ-64413739 (right)

and an absence of P2X7 upregulation should not be taken as proof of absence of P2X7 in the disease process. Patients with inflammatory burden and activated immune cells with enhanced IL-1β and IL-18 signaling, ought to be tested in controlled clinical studies with doses of P2X7 antagonists that satisfy target engagement of brain P2X7 channels in humans.

We have recently disclosed two brain penetrant P2X7 antagonists in JNJ-54175446 [3] and JNJ-55308942 [4]. In a phase I single ascending dose study in human subjects, JNJ-54175446 demonstrated dose dependent increases in plasma exposure, CSF exposure and ex-vivo inhibition of IL-1β from human blood (Fig. 1) [5]; more importantly, the P2X7 antagonist demonstrated robust occupancy at the human brain P2X7 as was assessed by a PET study (Fig. 1) [6]. Preliminary data from two Phase 1 human experimental medicine studies indicates a central effect of JNJ-54175446 in sleep deprived and amphetamine challenged human subjects (ACNP 2018) setting the stage for proof of concept testing in treatment resistant depression (http://www.isrctn.com/ISRCTN44411633). We remain optimistic based on the preclinical body of evidence that P2X7 antagonism will bring in novel therapeutic options for patients and their physicians, either as mono/adjunctive therapies to address the unmet need in neuropsychiatry.

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Biological fingerprints for psychosis

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It is generally acknowledged that once the nosology of psychiatric disorders finds a basis in neurobiology, the field will be more successful at specifying, finding treatments, and predicting outcomes for psychiatric conditions.

THE NEED FOR BIOLOGY-BASED PARSING IN PSYCHOSIS

Leaders in psychiatry have called for biologic characteristics to be the basis for designating psychiatric diagnostic groups. Most recent efforts have focused on genetics for biological classification, despite the weak genetic effects for the psychoses [1]. Geneticists themselves have concluded that their results "strongly suggest that our diagnostic categories do not define pathophysiological entities" [2]. Therefore, seeking clues to biology, we have turned to a comprehensive characterization of quantitative behavioral, cognitive, electro-physiologic, and imaging traits (each with some but not complete biological understanding) in a cross-diagnostic psychosis population.

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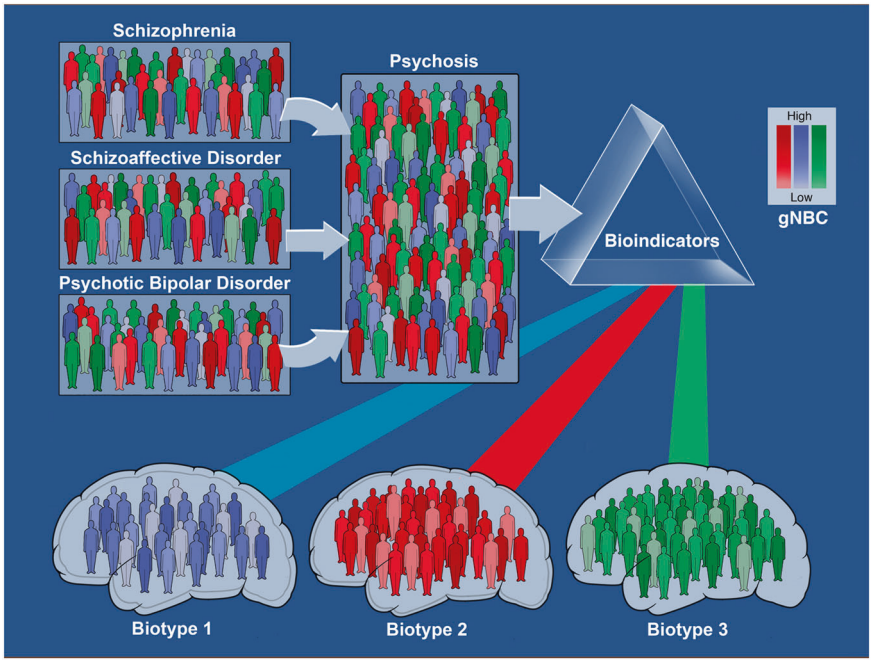

Fig. 1 Psychosis is a defining characteristic of schizophrenia, schizoaffective disorder, and psychotic bipolar disorder, all mechanistically complex syndromes. These clinical syndromes were not distinguishable by the B-SNIP biomarker panel, with >70 individual biomarkers. Psychosis subjects, therefore, were combined into a single group, independent of conventional diagnosis. Then, biomarker variables were used to define subgroups with shared neurobiological variance. Two biomarker dimensions, "cognitive control" and "sensorimotor reactivity," provided a means for creating biomarker-defined subgroups (called psychosis Biotypes). Biotype-1, -2, and -3 had distinctive neurobiological characteristics, including on variables *not* used in their definition (external validators). Within each Biotype, the level of cognition (see color intensity) can vary from low to high. Neural characteristics as they segregate in these groups could be the basis for distinct molecular and therapeutic targets

B-SNIP EXPERIENCE AND REPLICATION

The Bipolar-Schizophrenia Network for Intermediate Phenotypes (B-SNIP) began by seeking quantitative markers for DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition)-defined psychoses in 933 probands, and 1059 relatives with 459 healthy controls (HCs) [3]. Finding no markers strong enough for diagnostic distinctions [4], we proceeded to use computational approaches to develop biologically based clusters of psychosis probands from these deep phenotyping data. Using principal component analysis and *k*-means clustering, three distinct groups emerged, named Biotype-1, -2, and -3 (B-1; B-2; B-3). The groups show distinct biological characteristics: B-1 has *low* electroencephalography (EEG) power, profound cognitive dysfunction, gray matter reductions throughout neocortex, and reduced abstract thinking. B-2 has *high* EEG power, moderate cognitive dysfunction, a reduction in fronto-temporal gray matter volume, and increased thought disorder. B-3 is mostly normal on these features, with EEG power and cognition similar to the HCs and a distribution of gray matter reduction limited to limbic cortex, albeit with mild psychosis [5]. Four years later in an entirely new, well-phenotyped sample with 919 probands, remarkably high Biotype replication was found with the original sample with intraclass correlation coefficients of 0.85–0.90 [6].

PHENOMENOLOGICAL AND BIOLOGICAL GROUPS ARE ORTHOGONAL

Each psychosis Biotype includes individuals with representative DSM-5 psychosis diagnoses, with biomarker characteristics falling orthogonal to conventional diagnoses, with each of the Biotypes containing all conventional diagnoses. Biological variability is still not low within Biotypes and we predict more subgroups within each Biotype. We describe, as well, a common complex cognition measure that includes cognition determinants, cognition measures, and biological correlates of cognition, potentially creating distinctive sub-classes within Biotypes (Fig. 1).

IT TAKES A BIOMARKER BATTERY FOR COMPREHENSIVE COVERAGE

To develop these large cross-cutting psychosis Biotypes took a *biomarker battery*, not just single biomarkers, a battery broad enough to capture neural complexity in the psychosis phenotype. Many different disorders likely reside within each Biotype, suggesting that these three organizing Biotypes are analogous to finding the overarching categories of *Dropsey*, with "cardiac," "pulmonary," and "renal" determinants.

MOVING FORWARD WITH BIOLOGICALLY BASED HYPOTHESES

The usefulness of these Biotypes for clinical care and disease understanding needs to be demonstrated, to establish that biomarker clusters predict treatments, correlate with disease, or define a genetic fingerprint. Moreover, we predict that these Biotypes will guide our search for pathophysiology, even though biomarker biology is not completely worked out. We see all of these applications as the goal of this work and anticipate breakthroughs based on biomarker-guided studies.

FUNDING AND DISCLOSURE

B-SNIP collaborators, in addition to the authors, include Drs. Elliot Gershon (Chicago), Matcheri S. Keshavan (Boston), Godfrey D. Pearlson (Hartford), and John A. Sweeney (Cincinnati). All PIs do equal work. The research reported here was funded by NIMH: MH077851, MH077862, MH078113, MH077945, and MH077852. The authors declare no competing interests.

ADDITIONAL INFORMATION

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Selection criteria for neurophysiologic biomarkers to accelerate the pace of CNS therapeutic development

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In recent years, the field of psychiatric neuroscience has generated numerous biological markers that have contributed to our understanding of central nervous system disorders. Despite the abundance of available "biomarkers" [1] and improved understanding of pathophysiology, drugs for the treatment of Alzheimer's Disease, schizophrenia, and other CNS disorders continue to fail at high rates and at substantial cost (e.g., aducanab and encenicline). In the preclinical stages of development, many assays, including those that purport to measure the same underlying cognitive constructs across species, have limited evidence for predicting responses in humans. Promising drugs graduate from preclinical to later clinical stages of development and are tested on broad diagnosis-based cohorts, without consideration of individual variations in the brain functions that govern treatment sensitivity. This "one-size-fits-all" approach limits the ability to differentiate treatment-sensitive individuals who may be hidden among non-responders in conventional group level analyses [2].

To address these and other limitations, NIH and/or FDA have established future research frameworks (e.g., Research Domain Criteria) and called for translational biomarkers that can rapidly detect treatment sensitivity and/or early response to interventions and thereby accelerate the development of novel therapeutics [1]. Based on prior review processes for selecting cognitive tests for clinical trials [3], we propose an expanded set of criteria (Table 1) for neurophysiologic measures that can be broadly used across multiple categories of biomarkers and surrogate endpoints including pharmacodynamic/response, predictive, prognostic, monitoring, and susceptibility/risk [1].

Proposed criteria for candidate translational neurophysiologic biomarkers have admittedly high standards for established psychometric properties and functional characteristics, applicable to both infra-human and human versions of the measures. Early "target engagement" identified in single-dose or limited-dose experimental medicine designs is both feasible [2, 4, 5] and particularly valuable but may not generalize across settings. Since the type and calibration of equipment, as well as data processing and analysis methods all can have a substantial impact on psychometric properties, criteria established on one testing platform may not be applicable to another. For example, results obtained from high-density EEG recordings with advanced signal processing algorithms that leverage spatiotemporal relationships for sophisticated artifact reduction and analysis may not generalize to lower-fidelity recording systems with much more limited signal processing options.

While preclinical assays are often used in specialized laboratories, validation of human response homology tested in less controlled, real-world clinical trial environments is an important next-step for validation. Some translational neurophysiologic measures already fulfill all or many of the Table 1 criteria, including mismatch negativity (MMN), P3a, auditory steady state response (ASSR) and prepulse inhibition of startle (PPI), and have been used effectively in experimental medicine designs [2, 4, 5] and multi-site consortia [6]. Notably, even high-density EEG assessments can be feasibly scaled up for valid use in multi-center trials with proper training and centralized data processing and management.

We propose an initial set of criteria to guide development of neurophysiologic biomarkers for predicting psychotherapeutic sensitivity. These criteria offer the potential to advance treatments for major brain disorders beyond the "one-size-fits-all" approach based on fuzzy diagnostic categories, towards a more precise, personalized, and biologically informed strategy for matching CNS interventions with sensitive patient subgroups.

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Table 1. Proposed criteria for neurophysiologic biomarkers

Psychometric properties of translational biomarkers
• Established substantial test-retest reliability (intraclass correlations > 0.8)
• Suitable for use as a repeated measure (i.e., no practice, maturation, instrumentation, testing or statistical regression effects)
Functional characteristics
• Early sensitivity to single- or limited "doses" of pharmacologic agents, cognitive training or other CNS interventions
• Consistent relationships to important domains of clinical, cognitive and/or psychosocial functioning in humans
Scalable for use in real-world multi-site global clinical trial settings
• Equipment should be low cost with identical interchangeable calibrated systems and components
• Measures are robust to variations in testers and testing environments
• Tests can be administered by non-specialists with appropriate training, certification, and centralized quality assurance and oversight
• Does not require special testing environments, suitable for valid use in varied multi-center settings
• Objective automated analysis methods that are amenable to centralized data processing blinded to group and conditions

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Sleep and EEG biomarkers as avenues toward new treatment approaches in Angelman syndrome

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Angelman syndrome (AS) is characterized by severe intellectual, speech and motor deficits [1]. The cause of AS is either disruption of the maternal ubiquitin-protein ligase E3A gene (*UBE3A*) (30%) or deletion of chromosome 15 at 15q11-q13 (70%). The deleted region includes *UBE3A* and *GABRB3*, *GABRA5*, and *GABRG3*, genes that encode the gamma-aminobutyric acid (GABA) type A receptor subunits β3, α5, and γ3. Several medical co-morbidities are associated with AS.

Sleep disturbance is a common medical co-morbidity, occurring in up to 80% of individuals with AS. Difficulty falling and staying asleep and reduced total sleep time are most common. The etiology of sleep disturbances in AS is multifactorial, involving genetic, co-morbid medical, and behavioral factors. Deletion of the *GABRB3-GABRA5-GABRG3* gene grouping, which occurs in most cases of AS, probably contributes to the high prevalence of sleep problems and co-morbid epilepsy [2]. Epilepsy occurs in 80–90% of individuals with AS and can contribute to sleep problems. In a study of sleep disturbances and epilepsy in 290 subjects with AS, disturbed sleep was described by caregivers of 58% of the sample [3]. Among these subjects, 79% had epilepsy, and 69% of those with both sleep problems and epilepsy had multiple seizure types. Subjects with epilepsy non-responsive to more than two antiepileptic drugs (AEDs) had more significant sleep disturbances than those successfully treated with up to two AEDs. From a behavioral standpoint, urinary

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incontinence overnight may cause mid-nocturnal awakenings, and agitation in response to separation from preferred caregivers may play a role in the disturbed sleep.

Recent studies are attempting to identify biomarkers of the pathophysiology of sleep disturbances in AS using EEG. In a retrospective study, den Bakker and colleagues [4] identified two quantitative readouts of dysregulated sleep composition in children with AS: increased gamma coherence and fewer sleep spindles. Increased long-range gamma-band coherence during sleep and wakefulness suggests that, despite reduced structural connectivity, there may be fewer inhibitory constraints on efferent projections in the brain of individuals with AS. Sleep spindles, which are reduced in several neurodevelopmental disorders, are important for memory consolidation and learning. Another group tested the hypothesis that genes other than *UBE3A* located on 15q11-q13 cause differences in pathophysiology between AS genotypes [5]. In children and adolescents with AS, they found that an abnormality in the delta-band EEG indexes *UBE3A*-related pathophysiology, while theta- and beta-band EEG abnormalities index contributions from other genes, most likely the *GABRB3-GABRA5-GABRG3* gene cluster.

Building upon the identification of biomarkers for disturbed sleep in AS, a Phase 2, 12-week randomized double-blind, placebo-controlled trial of gaboxadol (OV101), an extracellular delta-selective $GABA_A$ receptor agonist, was completed in 78 adults and adolescents [6]. At week 12, OV101 resulted in global improvement in significantly more subjects than did placebo. Sleep onset latency, overall sleep, and motor function were found to contribute to this global response.

Safe and potentially efficacious targeted therapies are being developed for interfering symptoms in AS. With continued progress, it is hoped that interventions will one day alter the course of this severe developmental disorder.

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