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Review

Neuronal Organoid Engineering and Disease-Focused High-Throughput Neuropharmacology: Advances, Limitations, and Translational Strategies

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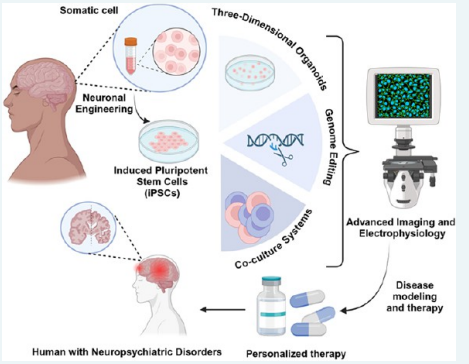
ABSTRACT: Although animal models offer the physiology of the entire organism, various cell populations, and circuit-level behaviors, their predictive ability for polygenic neuropsychiatric disorders may be limited by species-specific neurodevelopment and genetics. Consequently, despite decades of neuropharmacological research, many CNS-targeted drug candidates still fail in late-stage clinical trials. This review summarizes how neuronal-engineering platforms, especially patient-derived induced pluripotent stem-cell (iPSC) organoids and neuron-glia cocultures, enable high-throughput screening (HTS) pipelines with greater clinical fidelity. This review focuses explicitly on neuropsychiatric disorders such as major depressive disorder, schizophrenia, bipolar disorder, and anxiety, and emphasizes human cell-derived organoid and neuron-glia coculture models tailored to their circuit-level pathophysiology. Organoid-enabled HTS couples human genetics with automated phenotyping, accelerating identification of circuit-level drug effects while reducing animal use. The remaining issues are integrating multiomics data, vascularization, and batch variability. These gaps will be filled, and precision psychiatry will become attainable with the continued advancements in biomaterials, single-cell analytics, and machine learning, by highlighting how human iPSC-derived organoids and advanced neuronal engineering recapitulate pathology and enable scalable drug screening. This review addresses a critical bottleneck in psychiatric drug development and outlines how these innovations can help close the bench-to-bedside gap in neuropsychiatric drug discovery.



KEYWORDS: *neuronal engineering, iPSC-derived brain organoids, high-throughput neuropharmacology, precision neuropsychiatry, neuron−glia cocultures, neuropsychiatric drug discovery*

Neuropsychiatric disorders, including schizophrenia, bipolar disorder, major depressive disorder, and autism spectrum disorder, place a significant strain on individuals, families, and societies at large.[1] According to the Global Burden of Disease 2019 Mental Disorders Collaborators, mental disorders accounted for 125.3 million years lived with disability, or 14.6% of all global YLDs in 2019.[2] The World Health Organization projects that the annual economic cost of mental disorders will rise from about $2.5 trillion in 2010 to roughly $6 trillion by 2030.[3] Yet fewer than one in ten CNS drug candidates that enter Phase I ultimately reach approval, 5.9% in neurology and 7.3% in psychiatry, versus 7.9% across all therapeutic areas between 2011 and 2020.[4] Attrition is often linked to gaps in translational fidelity. Conventional two-dimensional cell cultures and animal models remain indispensable for mechanism and safety, yet they may not fully reproduce human-specific circuit maturation, synaptic pruning, or the highly polygenic architecture of psychiatric disease.[5,6] Indeed, only approximately 13% of differentially expressed genes in a popular rodent schizophrenia model overlapped with human post-mortem brain data. Neuronal engineering now offers a powerful bridge between human genetics and pharmacology. For example, patient somatic cells can be reprogrammed into iPSCs with ever-improving efficiencies (1−2% for integration-free methods), then guided into neurons, astrocytes, and oligodendrocytes. CRISPR/Cas9 genome editing allows isogenic controls differing by a single variant, isolating genotype-phenotype effects.[7−9]

Three-dimensional (3D) brain organoids extend these capabilities by permitting multiple neural cell types to self-organize into layered architectures that exhibit spontaneous oscillatory activity. Automated liquid-handling and imaging platforms apply more than 2000 compounds daily to 96-well midbrain organoids with >90% interbatch reproducibility.[10,11]

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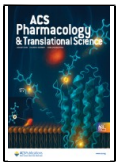

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In one such study, organoids derived from 17 schizophrenia patients exhibited a 40% reduction in basal oxygen consumption rate and differential expression of approximately 2500 genes; treatment with 15 μM clozapine partially rescued oxygen consumption by 15% and normalized MAP2 expression.[10]

To keep our scope operational, we adopt a concise framework for modeling neuropsychiatric disease in human-derived systems: Cell composition, excitatory glutamatergic neurons with inhibitory interneurons at 3−5:1 E:I; astrocytes 10−30% to support synaptogenesis and network stability; microglia 5−10% when inflammatory tone or pruning is assayed; add oligodendrocyte-lineage cells when myelination readouts are intended. Second, Functional hallmarks include spontaneous/evoked network activity (MEA mean firing rate, burst frequency/duration, synchrony), calcium-imaging kinetics, synaptic-plasticity surrogates (mEPSC/mIPSC; dendritic spine and PSD-95/synaptophysin/vGLUT1/vGAT puncta), and mitochondrial/omic state. Finally, HTS-ready readouts, automated spine/synapse counts, MEA features, FLIPR-style Ca^{2+} kinetics, cytokine panels (e.g., IL-6 under defined immune challenges), and OCR/ECAR. (iv) Minimum reporting, donor numbers/provenance and isogenic controls, days-in vitro, exact cell-type ratios and densities, acquisition parameters, and QC (Z'-factor, %CV).

In this review, neuropsychiatric disorders refer to conditions primarily characterized by disturbances in mood, cognition, or behavior that arise from circuit-level dysfunction, most prominently major depressive disorder, schizophrenia, bipolar disorder, and anxiety disorders. Neurodegenerative diseases differ from these entities (e.g., Parkinson's or Alzheimer's) in that network-level dysconnectivity, polygenic risk, neuromodulatory imbalance, and synaptic plasticity defects are more common than overt cell loss as the defining early feature. Accordingly, we prioritize human-derived organoids and neuron-glia cocultures that capture these circuit-relevant phenotypes for target discovery and high-throughput screening (HTS).

OVERVIEW OF TRADITIONAL DRUG SCREENING AND DISCOVERY METHODS

Animal Models: Ubiquitous Use, Poor Translation. Despite regulatory requirements that virtually every IND-enabling safety package includes a rodent species, rats were used in 96% of short-term and 93% of long-term general-toxicology studies submitted between 2010 and 2019. The translational yield for CNS programs remains poor.[12] Across 2011−2020, only 5.9% of neurology and 7.3% of psychiatry candidates that entered Phase I reached approval, versus 7.9% for all therapeutic areas.[4] Nonrodent, large-animal models add little fidelity relative to cost: comparative in vitro data show minipigs replicate 70% and dogs 80% of aggregate human CYP450 activity, leaving sizable metabolic gaps.[13] Meanwhile, the price of a single purpose-bred cynomolgus macaque now exceeds $25,000−30,000, and housing alone approaches $14,600 per year, pushing first-year outlays well above $40,000 per animal.[14] Even when budgets allow groups of six nonhuman primates (NHPs) per arm, a common practice, statistical power to detect a 30% treatment effect seldom exceeds 45%, mirroring the broader 'power failure' problem in preclinical neuroscience.[15]

Two-Dimensional Cellular Systems—High-Content but Limited Depth. 2D monolayer assays, such as cell painting, deliver unparalleled information density and speed. By staining six cellular compartments and analyzing the images with CellProfiler, they routinely capture more than 1700 morphological features from every single cell.[16] Modern high-content imagers scan an entire ANSI/SLAS-compatible 96-well plate in 3.5 min. At the typical seeding density of 3000 cells $well^{-1}$, which equates to about 1.2×10^3 cells s^{-1}, a 10,000-compound screen (105 plates) can be imaged in one workday and analyzed within a 10-day campaign.[17]

The Cell-Painting approach now scales public resources such as the JUMP consortium. The benchmark CPJUMP1 data set alone provides 3 million five-channel images and single-cell profiles for 75 million cells, whereas the consortium's full cpg0016-JUMP release covers 136,000 matched chemical and genetic perturbations (more than 116,000 compounds and over 20,000 genetic perturbations).[18,19] When 75 million single-cell signatures from the CPJUMP1 pilot are embedded in low-dimensional space, chemically and genetically matched perturbations cluster together—a visual proof-of-concept for mechanism-of-action discovery (Figure 1).[17]

However, this breadth of readouts comes at the expense of biological depth. Flat cultures lack the extracellular-matrix gradients, mechanical forces, and multicellular architecture that govern drug penetration and tissue metabolism in vivo, leading to systematic mis-estimation of compound potency.[20] Because most monolayer screens rely on decades-old immortalized

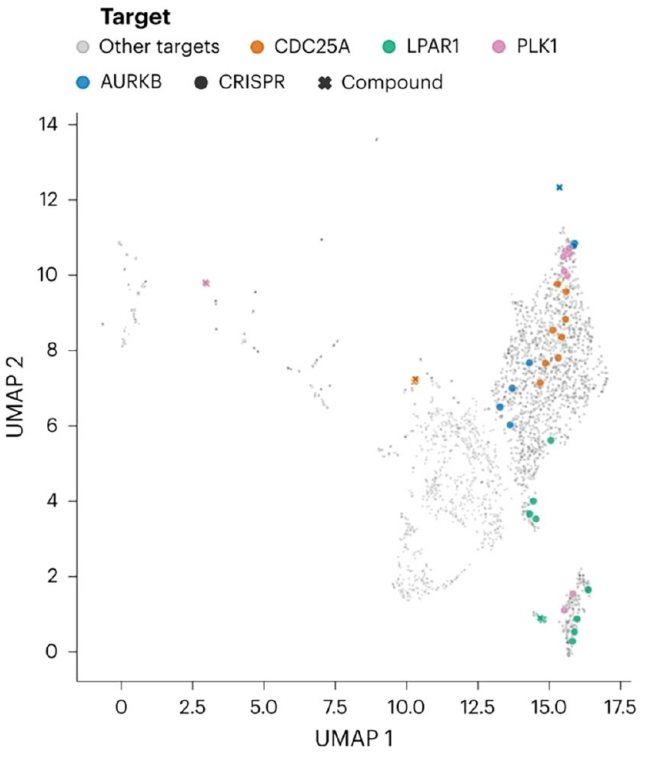

Figure 1. Low-dimensional organization of the CPJUMP1 morphology atlas. UMAP embedding of image-based profiles from A549 cells treated with 303 small-molecule compounds (orange) or paired CRISPR knockouts (purple) at the long time point. Highlighted pairs illustrate how sister compounds and genetic perturbations targeting the same protein (PLK1 and AURKB) converge in morphological space, demonstrating the data set's utility for linking chemistry to gene function. Reproduced with permission from ref 18. Copyright 2024 Nature.

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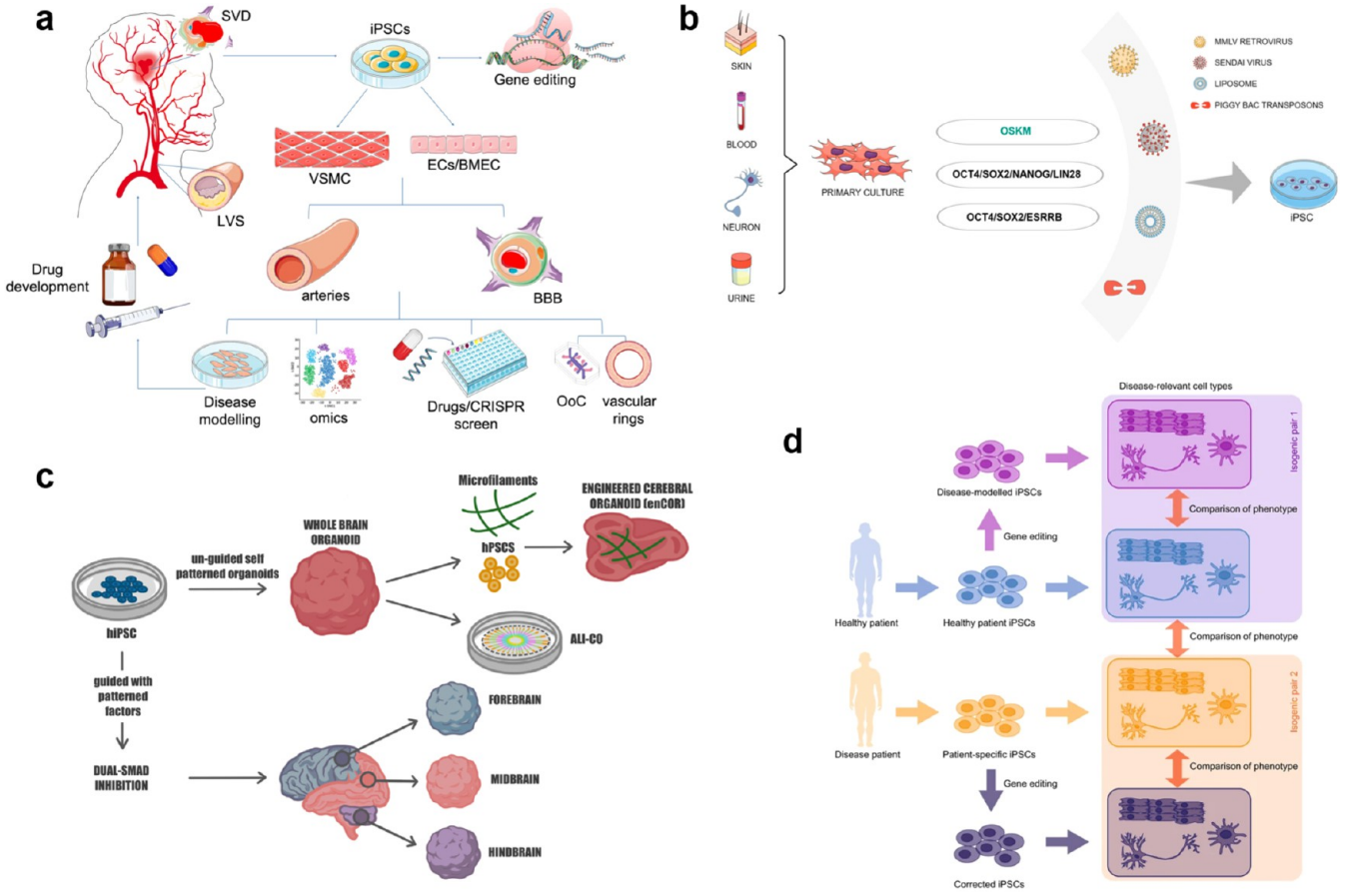

Figure 2. iPSC-based platforms for vascular and neural disease modeling and genome editing. (a) Application of human induced pluripotent stem cell (iPSC) technology to develop vascular models comprising vascular smooth muscle cells, endothelial cells, and brain microvascular endothelial cells for in vitro modeling of large- and small-vessel stroke, aiming to identify novel therapeutic targets. Reproduced with permission from ref 37. Copyright 2023 μ_BC. (b) Primary cultures can be established from skin, blood, neural, and urinary tissues; reprogramming to pluripotency is achieved using transcription factors (OSKM) delivered via retroviral, Sendai viral, liposomal, or transposon-based vectors. Reproduced with permission from ref 38. Copyright 2024 Elsevier. (c) Human iPSCs can be differentiated into neural organoids via unguided (self-organized, heterogeneous whole-brain organoids) or guided protocols (region-specific organoids), further refined by microfilament engineering and air−liquid interface cultures to enhance quality and reproducibility. Reproduced with permission from ref 39. Copyright 2017 MDPI. (d) Genome editing enables the creation of isogenic iPSC lines by introducing or correcting disease-causing variants, allowing precise comparison of molecular and cellular phenotypes to dissect genotype−phenotype relationships. Reproduced with permission from ref 40. Copyright 2017 Springer Nature.

lines, they also miss patient-specific genetic backgrounds and accumulate genetic drift over time.[21] For neuroscience, the limitations are acute: induced-neuronal monolayers rarely develop the nested oscillatory network activity that emerges spontaneously in cerebral organoids, a hallmark of functional circuit maturation.[22] Perhaps unsurprisingly, programs that originate from 2D phenotypic screens in neurology advance from Phase I to approval only 5.9% of the time, well below the industry average.[23] In contrast, patient-derived organoid drug-sensitivity testing in metastatic gastrointestinal cancer has been shown to predict clinical response with 88% positive and 100% negative predictive values, a roughly 5-fold improvement in translational fidelity.[24] Collectively, these observations establish cell painting and related 2D assays as indispensable first-pass filters for chemical space, while underscoring the need to advance confirmed hits into human-derived 3D systems that more faithfully replicate in vivo biology.

In Vivo Models beyond Rodents. Nonhuman primates (NHPs), such as macaques, remain the only laboratory species that faithfully reproduce the human brain's laminar architecture and long-range cortico-cortical wiring. Recent diffusion- and resting-state MRI studies have mapped one-to-one homologues for at least nine visual and association areas, showing that mesoscale structural and functional connectivity is essentially conserved between the two species.[25] The translational fidelity of NHPs is now at a steep and rapidly increasing cost. Because such costs (and parallel ethical constraints) limit cohort sizes, most contemporary NHP neuroscience papers still study only four to six animals. Formal power analyses confirm that, with a two-tailed $\alpha = 0.05$, even an optimiztic 30% effect (Cohen's $d = 1.5$) is detected with barely 41% power at $n = 4$ and 52% at $n = 6$, far short of the 80% benchmark for reproducibility.[26] At the opposite end of the spectrum, invertebrate and lower-vertebrate models provide the throughput required for large-scale screens. Still, they share less than half of the common-variant risk loci implicated in complex psychiatric disorders, limiting their face validity for polygenic disease.[27] These twin pressures, high costs and low N in primates, and limited genetic homology in simpler organisms, have catalyzed rapid migration toward patient-derived induced pluripotent stem-cell (iPSC) platforms. HiPSC-based models retain each donor's full polygenic

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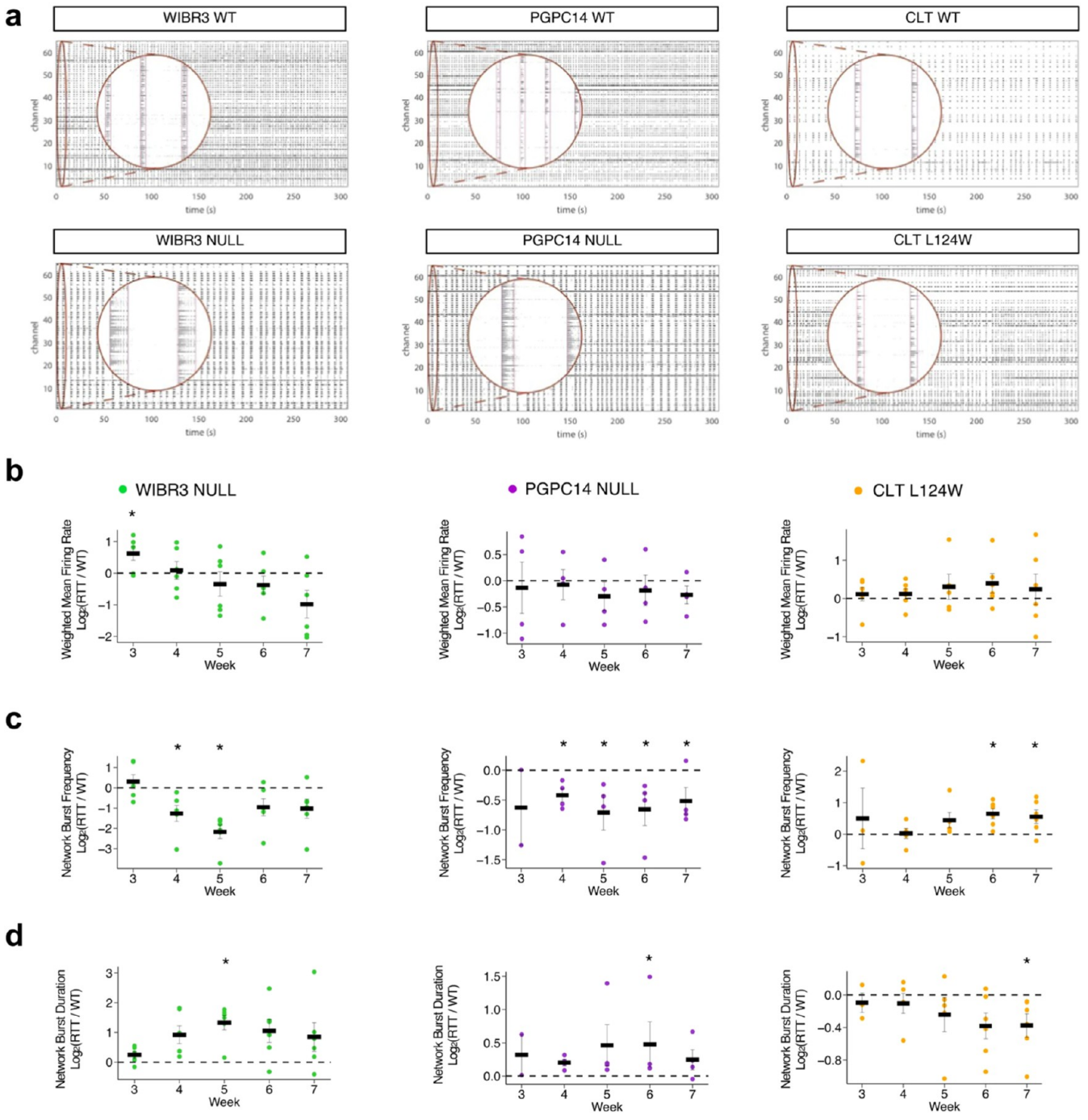

Figure 3. MECP2 mutations induce bidirectional changes in neuronal network bursting. (a) 300 s raster plots of spontaneous activity in iPSC-derived networks; each row corresponds to one MEA electrode, and black ticks represent action potentials. (b–d) Weighted mean firing rate, burst frequency, and burst duration (log_2 fold change vs isogenic WT) for each cell line. MECP2-null lines (WIBR3 and PGPC14) exhibited significantly reduced burst frequency and transiently increased burst duration, whereas the hypomorphic L124W variant (CLT) caused elevated burst frequency and shorter bursts at later stages. Data represent $n = 6$ replicate plates for WIBR3, $n = 4$ for PGPC14, and $n = 6$ for CLT; mean ± SEM; $^*P < 0.05$. Reproduced with permission from ref 45. Copyright 2022 Nature.

background, permit isogenic CRISPR editing, and can be differentiated into 2D neuronal cultures, 3D cerebral organoids, or multilineage assemblies. Panels exceeding 100 lines capture common alleles down to 1% minor-allele frequency, enabling genome-wide polygenic risk stratification and high-throughput pharmacology at a scale infeasible in NHPs.[27] Consequently, current discovery pipelines increasingly reserve primate studies for late-stage, circuit-level validation of therapeutic leads that first emerge from iPSC screens rather than for primary hypothesis generation.[28]

System-Wide Limitations and Economic Pressure for Change. Traditional paradigms often inaccurately predict efficacy and safety, failing to replicate the complexities of human neuropsychiatry, despite these contributions. A significant drawback is the reliance on simplified cellular systems and animal models, which may not accurately represent the complexity of neuropsychiatric disorders in

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humans.[29] The lack of human genetic diversity and the challenge of replicating intricate cellular interactions and brain circuitry present significant hurdles in understanding disease pathophysiology and finding practical therapeutic approaches. Moreover, the elevated failure rates in clinical trials underscore the need for enhanced preclinical models to more accurately predict clinical outcomes.[30]

Inadequate translational power remains the main obstacle in conventional paradigms. Notably, 56% of neuropsychiatric Phase II failures (2010−2020) were due to lack of efficacy despite promising preclinical results, and species-specific toxicities still accounted for 17% of late-stage failures.[31,32] Safety translation is scarcely better since species-specific toxicities still account for 17% of late-stage failures, often after tens of millions of dollars have been invested.[31] The financial burden comprises these biological shortcomings. When animal housing, behavioral phenotyping, histopathology, and data analysis are included, rodent disease-model screens cost roughly $55,000−75,000 per compound, whereas a fully automated midbrain-organoid high-throughput screen delivers equivalent phenotypic resolution for about $450 per compound-over 2 orders of magnitude cheaper for comparable phenotypic readouts.[33]

Ultimately, ethical concerns, limited resources, and scalability challenges associated with animal models and in vivo methods underscore the need for alternative strategies. These alternatives should provide more efficient, cost-effective, and ethically viable methodologies for drug discovery.[34]

INNOVATIVE APPROACHES IN NEURONAL ENGINEERING

Induced Pluripotent Stem Cells: Patient-Specific Models. Neuronal engineering has the potential to revolutionize this field by combining stem cell technology, genome editing, and tissue engineering. This will enable the development of in vitro models that replicate disease pathophysiology and identify novel targets and therapies. Reprogramming patient fibroblasts or peripheral blood cells with second-generation, nonintegrating Sendai vectors now yields induced pluripotent stem cells (iPSCs) at efficiencies approaching one colony per 8000 input cells (1.2%) within 17 days. In contrast, NKX3−1-based protocols can increase reprogramming efficiency to 1.5% and shorten the timeline to 12 days.[9] Guided differentiation converts these iPSCs into region-specific neurons, astrocytes, and oligodendrocytes in 30 days, producing electrophysiologically mature cortical pyramidal neurons with mean action-potential half-widths of 1.4 ms and spontaneous firing rates of 3−5 Hz by day 45 in vitro.[35] CRISPR-Cas9 base editing achieves single-nucleotide precision with <0.1% off-target rate, enabling the construction of isogenic control pairs that differ solely at a candidate variant, thereby isolating genotype-phenotype relationships.[36] IPSC technology has been applied to develop vascular models that incorporate vascular smooth muscle cells, endothelial cells, and brain microvascular endothelial cells, thereby facilitating the in vitro modeling of both large- and small-vessel strokes (Figure 2). Following the steps, researchers can create patient-specific neural models ranging from vascularized 2D cultures to complex 3D organoids.

Genome Editing: Unraveling Disease Mechanisms. Isogenic cell pairs, in which disease-relevant genetic modifications are either introduced or deleted, can be generated using genome editing techniques. This approach presents a promising chance to learn how specific mutations affect disease processes and what their functional consequences are.[37] Additionally, these technologies facilitate the exploration of interactions between genes and the environment, enabling the identification of potential therapeutic targets.

Large-scale CRISPR interference (CRISPRi) screens have now been conducted directly in human iPSC-derived cortical neurons using a pooled library that targets 2,893 neurodevelopment-associated genes with six sgRNAs per gene. The screens are run at a ≥ 500-fold representation of sgRNA to maintain uniform library complexity. Analyses of these data identify chromatin-remodeling factors as key convergent nodes. For instance, heterozygous loss of CHD8 results in a 33−54% reduction in synaptophysin and PSD-95 puncta, and an 80% decrease in spontaneous firing, providing a direct link between CHD8 dosage and synaptic output.[41−43] Using CRISPR/Cas9 or base-editing tools, researchers can introduce or correct disease-relevant mutations to create isogenic cell line pairs differing by only that variant. One can directly assess a mutation's impact on disease processes by comparing their phenotypes. This approach revealed how specific mutations contribute to neuronal dysfunction (Figure 3). Also, it facilitates gene-environment interaction studies by, for instance, introducing mutations in cells exposed to stressors.[44−46]

Three-Dimensional Organoids: Mimicking Brain Complexity. Organoids have been used to model neuropsychiatric disorders. For example, patient-derived cerebral organoids can recapitulate schizophrenia-associated phenotypes. Brain organoids are created by guiding induced pluripotent stem cells (iPSCs) through specific differentiation protocols to mimic the development of various brain regions. These 3D structures replicate the cellular heterogeneity and organization of the human brain, including cortical and hippocampal areas, making them excellent tools for studying schizophrenia and depression.[47] Notaras et al. generated 3D cerebral organoids from iPSC lines of 15 schizophrenia (Scz) patients and 5 matched controls to probe early developmental pathology. Histology revealed a marked increase in apoptosis among SOX2-positive ventricular progenitors in Scz organoids, accompanied by a concomitant depletion of MAP2-positive cortical neurons, establishing progenitor loss as an intrinsic disease phenotype. A 24 h BrdU pulse/7-day chase showed that 48.6% of labeled progenitors in controls matured into CTIP2+ early born neurons, whereas only 16.1% did so in the Scz cultures, demonstrating a profound block in neurogenesis. Tandem-mass-tag LC-MS quantified the organoid proteome and identified 222 peptides, approximately 5.9% of all detected proteins, as differentially expressed, underscoring subtle yet specific molecular shifts. Among these were four schizophrenia GWAS candidates (PTN, COMT, PLCL1, PODXL) and peptide fragments mapping to the neuronal transcription factor BRN2, indicating convergence on known risk pathways. Single-cell RNA-seq of 26,335 transcriptomes confirmed selective depletion of SOX2+/PAX6+ progenitors and MAP2+/DCX+ neurons in patient organoids. Canonical neural and glial identities comprised 93% of control cells but only 75% of Scz cells, the remainder being diverted toward neuro-endothelial and choroid-plexus-like lineages. Mechanistic rescue experiments pinpointed two levers of pathology. Lentiviral re-expression of BRN2 restored MAP2+ neuron numbers without affecting progenitor death, while supplementation with 25 ng mL^{-1} pleiotrophin (PTN) normalized both progenitor survival

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and neuronal differentiation, returning MAP2+ BrdU+ neuron counts to control levels. Together, the data position patient-derived organoids as faithful models of cell-specific Scz vulnerability, capturing coordinated defects in progenitor viability, neurogenic programming, and growth-factor support that precede overt brain circuitry changes.[47]

In one study, brain organoids elicited from iPSCs were employed to model schizophrenia to reveal dysfunction in neuron connectivity and pathological synaptic switch. A total of 2500 genes were deemed significant by FDR correction; of these, 23% of novel schizophrenia GWAS genes showed differences in schizophrenia organoids (10 upregulated, 15 downregulated). These were mainly related to synaptic biology and neurodevelopmental pathways. Mitochondrial function was impaired, with basal oxygen consumption rates reduced by 40% compared to control organoids.[48]

Cerebral organoids formed by embedding 9000 iPSCs in Matrigel microdroplets self-organize into laminated neuroepithelia within 10 days and express layer-V marker CTIP2 by day 40, paralleling midgestation cortex.[49] Multielectrode recordings detect coherent γ oscillations (30−80 Hz) from week 6 onward, and calcium-imaging quantifies network burst rates of 0.18 ± 0.05 Hz values that match fetal EEG signatures at gestational week 25.[22]

Strong et al. used FLIPR calcium imaging on 16 neural spheroid formulations (PFC-like vs VTA-like neurons, with astrocytes), extracting 10 robust kinetic features. Principal component analysis separated spheroids by cell-type composition, illustrating that functional phenotypes cluster by dominant cell type. This demonstrates that engineered spheroids can produce quantifiable, cell-type-specific activity profiles, supporting their use in HTS.[50]

Proteomic profiling of organoids from 25 individuals (17 with schizophrenia and 8 controls) showed that only 2.62% of the proteomic landscape was differentially expressed, but key proteins for neuronal differentiation (e.g., MAP2, GAP43, TUBB3) were downregulated in schizophrenia organoids.[51] Studies on organoids from monozygotic twins discordant for schizophrenia have shown that excitatory-to-inhibitory neuronal imbalances are driven by increased GABAergic specification and reduced excitatory neuron proliferation. The Wnt pathway, critical for cortical development, was reduced by 30−40% in affected organoids compared to controls.[52] For authoritative overviews that consolidate state-of-the-art brain organoid and assembloid methods, comparative analyses of iPSC- versus ASC/PDO organoids, and strategies to enhance physiological maturity.[53,54]

Coculture Systems: Modeling Cellular Interactions. Neural engineering techniques enable the development of coculture systems that integrate multiple cell types, including endothelial cells, microglia, and astrocytes.

By simulating the complex cellular interplay of the brain, coculture systems (e.g., neurons with astrocytes, microglia, or endothelial cells) more accurately model neuroinflammation and neurodevelopmental interactions.[55] For example, adding astrocytes to neuron cultures increases mEPSC frequency and synaptic puncta density, whereas the addition of microglia at 5% of the total cells elicits a 4-fold surge in IL-6 release within 24 h under lipopolysaccharide challenge, modeling neuroinflammatory escalation, which mirrors the neuroinflammatory escalation seen in vivo.[56] These coculture systems serve as valuable platforms for investigating the dynamic interplay among different cell populations and their potential contributions to neuropsychiatric disorders. As a common feature of neuropsychiatric disorders, synaptic dysfunction and neuroinflammatory processes are associated with dysregulation of glial cells, particularly microglia and astrocytes.[57] In line with our coculture/interaction framing, comprehensive reviews encompass neural and multilineage assembloids, head-to-head iPSC- vs ASC/PDO organoid paradigms, methodological advances (including neuroimmunology applications), and maturation-enhancement strategies.[58−60] A cross-platform summary of model categories, assay families, and validated readouts relevant to neuropsychiatric screening is provided in Supporting Table S1.

APPLICATIONS OF NEURONAL ENGINEERING IN DISCOVERY

iPSC-Derived Models. Human iPSC models enable patient-specific phenotyping and isogenic control. With optimized patterning protocols, >95 % $OLIG2^+$ motor neuron progenitors can be generated in under 12 days using CHIR99021, SB431542, and DMH-1, and expanded 10^4-fold across five passages without fate drift.[61]

In patient-derived cortical organoids and excitatory neuron cultures from schizophrenia cohorts, disease-relevant phenotypes such as reduced dendritic complexity, down-scaled synaptic protein abundance, altered burst synchrony, and impaired mitochondrial respiration have been reproducibly quantified; pharmacological challenge with antipsychotics partially normalizes electrophysiological readouts and synaptic markers, providing HTS-amenable end points. In major depressive disorder, stress-hormone or inflammation-sensitized neuron-glia cocultures reveal synaptogenesis deficits and activity-dependent plasticity changes that respond to fast-acting antidepressant mechanisms, enabling responder-stratification assays. Bipolar disorder iPSC-derived neurons exhibit state-dependent hyperexcitability and circadian rhythm perturbations, while anxiety-relevant inhibitory interneuron models capture GABAergic imbalance. Together, these disease-focused preparations provide quantitative, circuit-level readouts for scalable screens and mechanism-based validation. These neuropsychiatric-specific models collectively illustrate how human genetics and circuit phenotyping can be integrated into HTS workflows for target discovery, lead optimization, and pharmacodynamic profiling.

In addition to genetic modeling, iPSCs enable the examination of intricate connections between genes and environmental influences, providing a solid foundation for exploring disease causes and evaluating therapeutics. While 2D systems enable uniform exposure to nutrients and simpler imaging, 3D systems better mimic in vivo environments, including extracellular matrix architecture, cell−cell interactions, and diffusion gradients. Each 3D design presents trade-offs among complexity, scalability, and physiological relevance.[62] Representative neuropsychiatry-focused screens, quantitative end points, and biomarkers used in drug discovery workflows are collated in Supporting Table S2.

Organoid Technologies. 3D brain organoids generated from induced pluripotent stem cells (iPSCs) offer a unique opportunity to replicate the human brain's cellular structure and functional dynamics. These systems facilitate the examination of intercellular communication, brain circuit development, and the function of glial cells in disease pathology. Brain organoids have effectively replicated key pathologies of AD, such as amyloid-β plaques and tau

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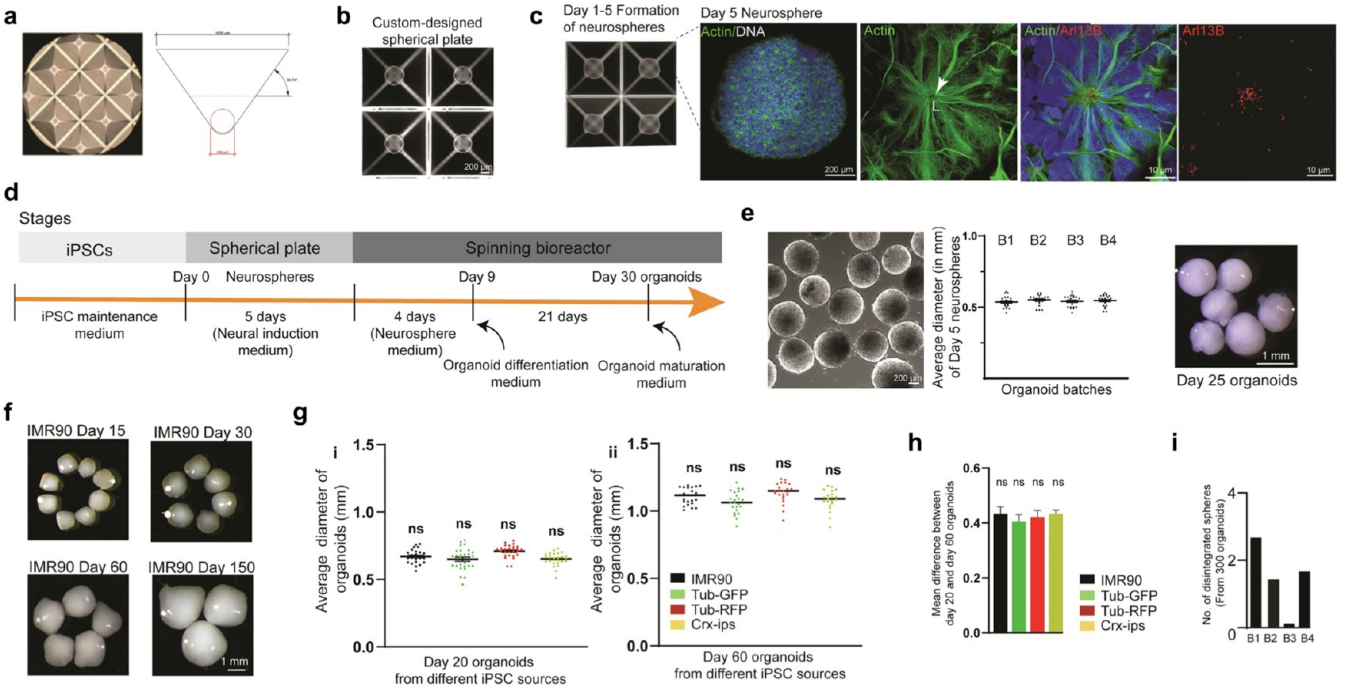

Figure 4. High-quantity (Hi-Q) cerebral organoid workflow. (a) Custom 24-well spherical microplate containing 185 microwells per well; (b) transfer of uniform neurospheres to spinner-flask bioreactors; (c–i) growth kinetics and batch reproducibility of 15,000 organoids across 39 batches. Reproduced with permission from ref 65. Copyright 2024 Nature.

hyperphosphorylation, offering a physiologically appropriate model for investigating disease progression and evaluating therapies. Notably, 3D organoid models of Alzheimer's disease recapitulate classic lesions. A human neural organoid model exhibited diffuse Aβ aggregation and tau hyperphosphorylation, which could be mitigated by β- and γ-secretase inhibitors.[63]

Organoids recapitulate complex tissue architectures, allowing multicell-type codevelopment, cortical layer formation, and oscillatory activity within 35–50 days.[64] These models are scalable, reproducible, and directly compatible with high-throughput imaging pipelines. Ramani et al. developed an organoid system using custom microplates and spinner-flask bioreactors to address the challenge of batch-to-batch variability and enable the scalability required for high-throughput screening. This platform enabled the production of up to 15,000 homogeneous cerebral organoids across 39 batches with high reproducibility and size uniformity (Figure 4).[65]

Enhancing Clinical Translation. Recent innovations are dramatically improving translation. For instance, Lampart et al. developed the "JeWells" platform, enabling live imaging of >1000 cerebral organoids per chip at subcellular resolution within 30 min, vastly increasing screening throughput and consistency.[66] In parallel, computational models (integrating pharmacokinetic/pharmacodynamic data) can optimize dosing predictions, reducing preclinical-to-clinical discrepancies.[31] Moreover, *in vivo* digital phenotyping (e.g., via wearable sensors) is emerging to capture patient biomarkers in real time; these data streams can be used to tune organoid assays and validate that organoid responses reflect clinical symptom dynamics. Such multimodal platforms essentially "close the loop" between bench and bedside, enabling human-relevant mechanistic validation at scale. These contrasts between 2D monolayers and 3D organoids are illustrated in Figure 5.

Disease-Focused Use Cases for Neuropsychiatric Drug Discovery. Patient-derived human models capture cell-type-specific synaptic deficits with actionable readouts. In iPSC-derived cortical neurons from 7 SCZ donors compared to 7 control donors, layer III neurons exhibited a significant reduction in dendritic spine density, which was rescued by overexpressing the NRXN3–204 isoform. Treatment with 10 μM clozapine for 24 h increased spine and synaptic puncta density, partially normalizing the phenotype.[69] In cerebral organoids from 25 donors (17 SCZ, 8 controls), 2.62% of the quantified proteome was differentially expressed, with down-regulation of neuronal/synaptic markers (e.g., MAP2, GAP43, TUBB3) and enrichment for risk-gene products (PTN, COMT, PLCL1, PODXL). These quantitative proteomic and cellular phenotypes define HTS-amenable end points (spine/synapse counts, proteomic panels) for antipsychotic MoA profiling.[70]

Disease-relevant plasticity can be quantified in human cells. In human iPSC-derived NPCs, 1 μM ketamine increased proliferation within 24 h with significant up-regulation of IGF2 and p11; IGF2 knockdown reduced baseline proliferation by 18% and blunted ketamine's effect by 17%, establishing an IGF2-dependent mechanism. Such NPC proliferation and IGF2-pathway transcriptomics are scalable HTS end points for rapid-acting antidepressant discovery.[71]

IPSC-derived dentate-gyrus-like neurons from BD patients exhibit mitochondrial abnormalities and neuronal hyperexcitability; notably, lithium normalized hyperexcitability only in neurons from clinical lithium responders, providing a cellular correlate for pharmacological stratification (Nature, 2015). MEA and patch-clamp metrics (mean firing rate, burst structure) provide quantitative, responder-stratified end points for screening mood stabilizers.[72]

HTS-compatible GABAergic assays align with anxiolytic mechanisms. Human iPSC-derived GABA neurons show

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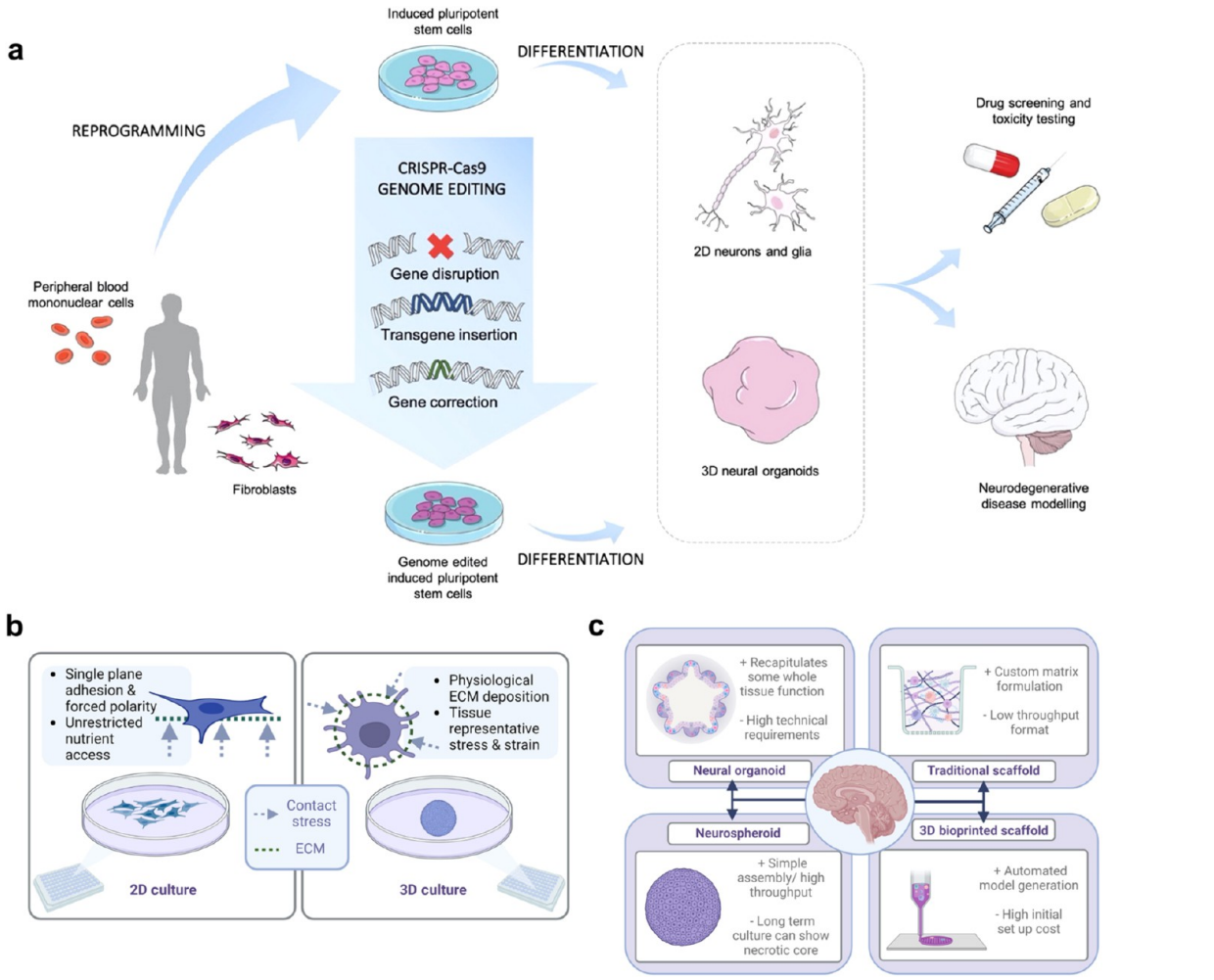

Figure 5. 2D and 3D models of human iPSCs for neurodegeneration research. (a) Human iPSCs as versatile tools for modeling neurodegenerative diseases and drug screening. Reproduced with permission from ref 67. Copyright 2020 American Chemical Society. (b) Comparison of traditional 2D vs 3D culture: single-plane adhesion in 2D induces polarity and limits cell−cell/ECM interactions, whereas 3D culture provides physiological ECM deposition and tissue-relevant mechanical cues. Reproduced with permission from ref 68. Copyright 2023 Elsevier. (c) Four major neural 3D model categories and their trade-offs: neural organoids, traditional scaffold-based models, neurospheroids, and 3D-bioprinted scaffolds (schematic; not to scale). Reproduced with permission from ref 68. Copyright 2023 Elsevier.

concentration-dependent GABA_A currents that are potentiated by benzodiazepines (e.g., diazepam, chlordiazepoxide) and neurosteroids, and blocked by bicuculline/picrotoxin, enabling pharmacologically validated quantitative current-amplitude and MEA network readouts for anxiolytic discovery. These assays can be embedded in cocultures and spheroids to capture E/I-balance features relevant to anxiety pathophysiology and HTS.[73]

Challenges and Future Directions. Recent advancements in microfluidic technology now enable truly high-capacity neuronal assays compatible with screening-level throughput. Additionally, integrating cutting-edge technologies such as CRISPR-based lineage tracing, 3D bioprinting, and scaffold-guided morphogenesis is crucial for creating disease-specific microenvironments that accurately replicate in vivo pathology. This convergence is pivotal for accelerating progress in regenerative neurotherapies and precision medicine. Figure 6 summarizes four emerging biochip-based drug screening platforms ranging from patient-derived neuron reprogramming to organ-on-chip systems. These technologies collectively provide scalable, genetically tunable platforms for modeling neurodegenerative diseases and discovering therapeutics. In parallel, "digital organoid" frameworks integrate longitudinal imaging, single-cell omics, and mechanistic PK/PD modeling into computational twins that predict drug responses and optimize dosing in silico. Such platforms provide a data backbone to rank organoid phenotypes by expected clinical benefit and to refine screening conditions iteratively.[74] Figure 6 summarizes four emerging biochip-based drug screening platforms.

APPLICATIONS IN DRUG DISCOVERY AND DISEASE MODELING

Human stem cell-derived models are now being deployed at multiple stages of drug discovery, from high-throughput screens to personalized medicine. Below, several applications with case studies are highlighted. In a recent high-throughput CRISPR-Cas9 screen combined with single-cell RNA-seq ("CHOOSE" protocol) targeting 36 autism risk genes in cerebral organoids, Li et al. observed a 25% shift in progenitor-to-neuron ratios and identified key chromatin regulators, such as ARID1B, that altered lineage commitment.[79] Therefore, these technologies provide a mechanistic bridge between

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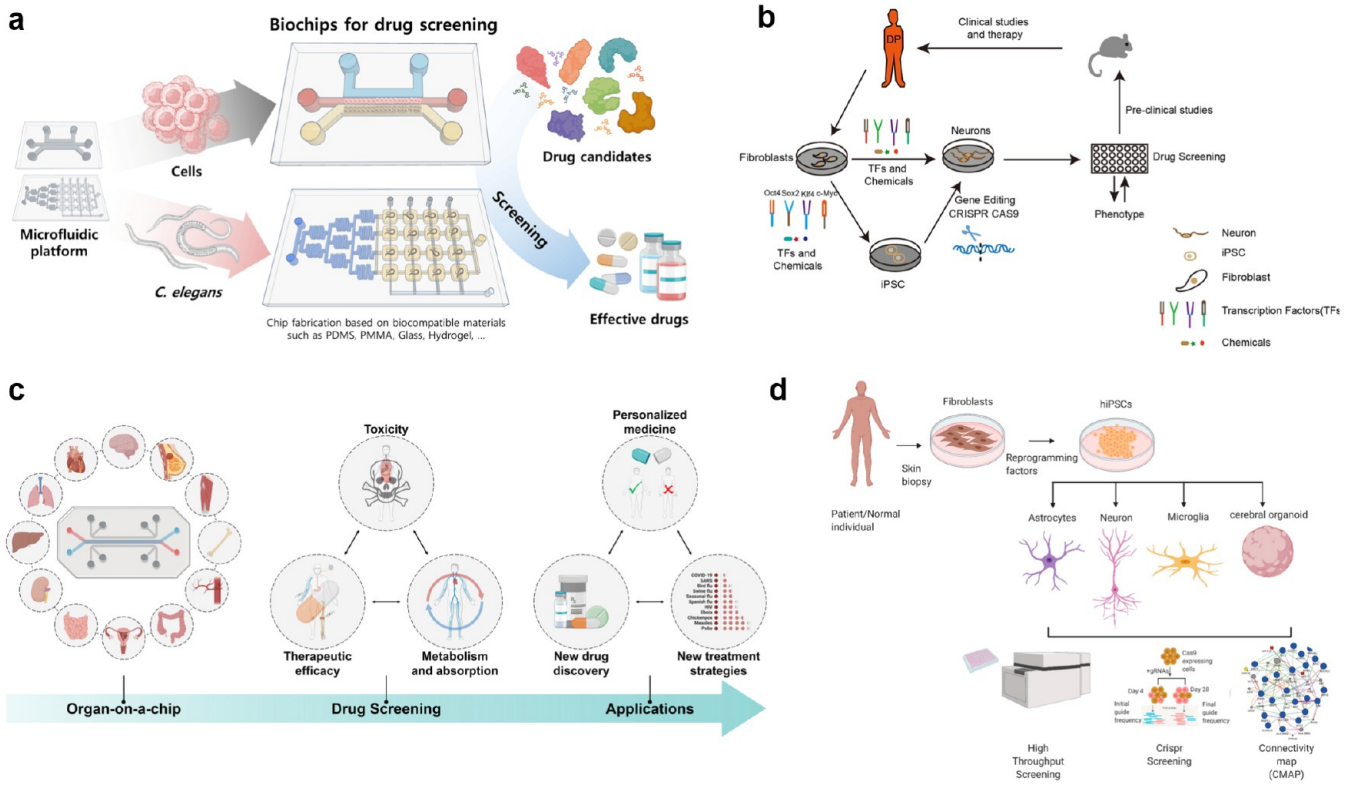

Figure 6. (a) Cell-based and model organism-based biochips are employed as platforms for efficient drug screening. Reproduced with permission from ref 75. Copyright 2024 MDPI. (b) iPSCs and iN for neurodegenerative drug discovery. Fibroblasts from patients are reprogrammed into neurons to model neurodegenerative diseases. Gene editing can be applied to induced pluripotent stem cells (iPSCs) to achieve a precise representation of disease. Drug screening identifies and evaluates potential candidates for efficacy and safety in preclinical studies, before clinical trials. Reproduced with permission from ref 76. Copyright 2020 Frontiers. (c) Schematic workflow of organ-on-a-chip systems applied to drug screening, from toxicity and therapeutic efficacy to ADME assessment and personalized medicine. Reproduced with permission from ref 77. Copyright 2023 Elsevier. (d) Schematic representation of hiPSC differentiation into various brain cell types and 3D brain organoids for drug discovery. HiPSCs can be directed to differentiate into neurons, astrocytes, and microglia. These cell types can be assembled into 3D brain organoids to model neurological disorders. Although not depicted, oligodendrocytes can also be generated from human-induced pluripotent stem cells (hiPSCs). Drug discovery strategies utilizing these models include high-throughput screening, CRISPR screening, and transcriptomic analysis with connectivity maps. Reproduced with permission from ref 78. Copyright 2021 MDPI.

polygenic risk and circuit-level pharmacology, laying the foundation for translationally robust drug discovery. Recent high-throughput CRISPRi screening in cerebral organoids derived from patients with schizophrenia has uncovered distinct gene-specific vulnerabilities.[80]

In Alzheimer's disease (AD) models, brain organoids derived from familial AD iPSCs exhibit enhanced Aβ accumulation and tau pathology.[81] The regulation of amyloid-β (Aβ) and its medicinal products has been extensively studied using brain organoids derived from patients with AD. Efforts to screen patient-derived cells for Aβ levels using neurons produced from iPSCs found 27 small compounds that could reduce levels by up to 50%.[82]

Accelerating Drug Discovery Through Organoids: Case Studies and Impact. The introduction of 3D brain organoids represents a significant innovation in drug development, particularly in modeling neuropsychiatric and neurodegenerative diseases. These organoids further enhance the capabilities of hiPSCs, as they allow the cultivation of the human brain virtually and diseases, making them more accurate and efficient than other commonly used 2D cultures and animal models.[83] In the case of studies of AD, 3D organoids have been favorable for understanding the pathology of protein aggregates, such as tau and amyloid-β, which could be the possible reasons behind the disease's advancing mechanisms.[84] In addition, the possibility of expanding the organoid population has enabled their inclusion in automated systems for drug testing, highlighting their role in medicine through predictive modeling of drug treatment and toxicity. For instance, it is possible to use 3D models to enhance the reproducibility of assays in screening campaigns and to better understand the cellular and pathophysiological heterogeneity of different brain areas.[28] Similarly, the study of AD has employed the use of 3D organoids to investigate the processes of amyloid-β deposition and tau pathology, both classic features of the disease.[63]

High-Throughput Drug Screening. Advancements in neurotechnology have refined the identification of drugs for high-throughput screening in neurological applications. One potential therapeutic option arises from the combined libraries, where researchers can use iPSC-derived neuron model lines. For instance, in the case of Parkinson's disease, screening of human midbrain organoids has led to the discovery of neuroprotective agents such as LRRK2 inhibitors that rescued deficits in dopamine release by 40−50% in disease-specific screens.[85] In the case of neuroinflammation, coculture of iPSC-derived neurons and astrocytes showed that astrocytes can reduce cell death of neurons by 30−40% upon exposure to a

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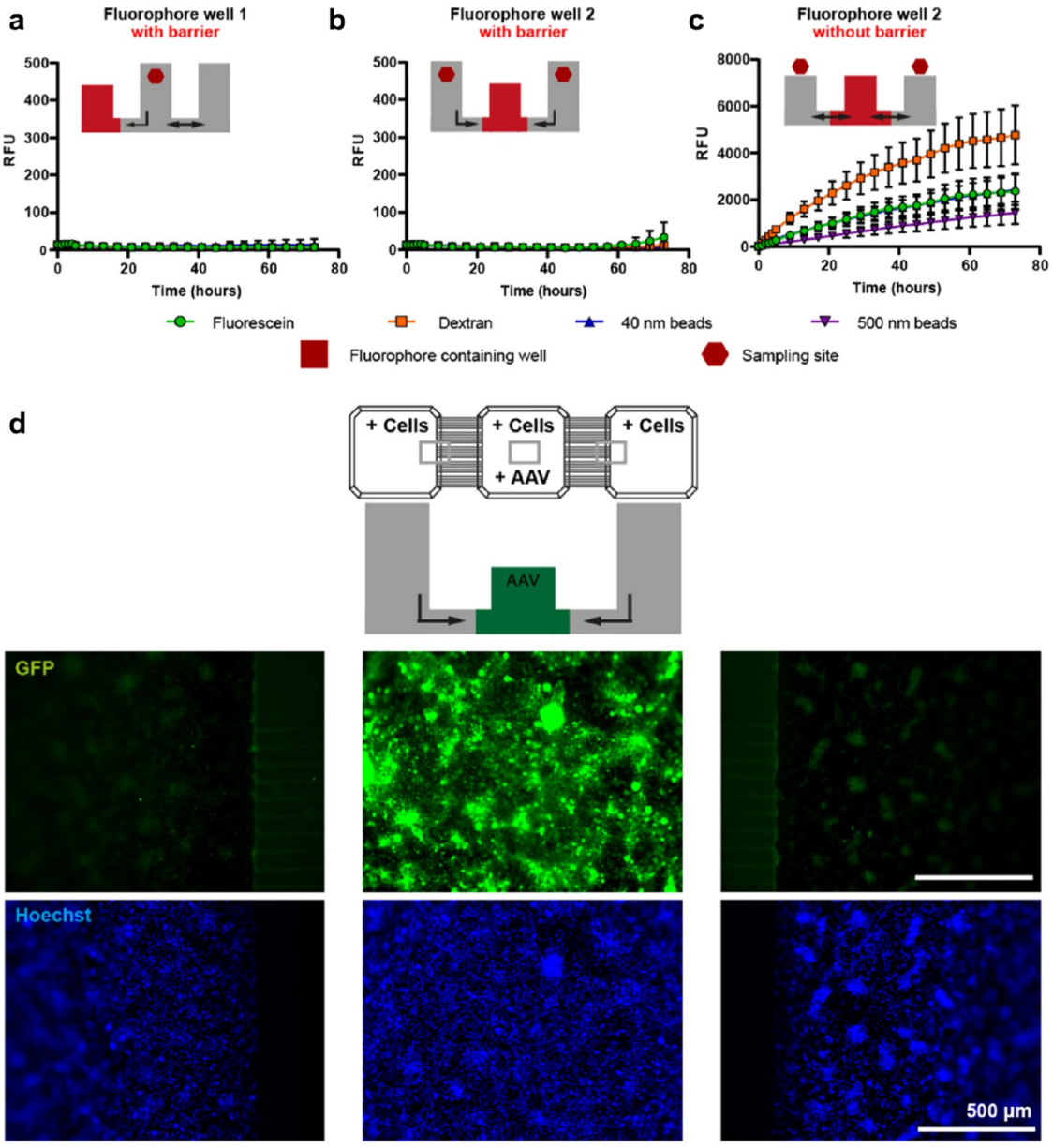

Figure 7. Hydrodynamic-barrier strategy enables selective compartment dosing in the MC plate. (a) Schematic of the 3-compartment experimental unit and medium-height differential (Δh = 5 mm; ΔP = 49 Pa). (b) Fluorescein intensity in the untreated compartment remained at baseline for 72 h when the barrier was applied; mean ± SD (n = 3). (c) Without a barrier, unrestricted diffusion equilibrates within 10 h. (d) AAV8-shRNA-GFP added exclusively to the center well stays confined, verifying fluidic isolation in a live neuronal culture. Reproduced with permission from ref 86. Copyright 2024 American Chemical Society.

neurotoxic environment, demonstrating that they can protect the central nervous system.[56]

Incorporating robotic automation, advanced imaging, and electrophysiological measurements into high-throughput drug screening platforms enables the rapid assessment of thousands of compounds. For example, a scalable platform utilizing 96-well midbrain organoids enables the screening of over 100 compounds within a week, with interbatch reproducibility exceeding 90%. Full automation now pushes 3D throughput into the realm of small-molecule libraries. Renner et al. processed 2160 compounds in 96-well midbrain plates within 72 h, achieving a synaptic Z'-factor of 0.56 and an interbatch CV below 9%.[10] A follow-up microfluidic system introduced a hard-plastic microchannel microtiter plate that scales compartmentalized neuronal cultures to a screening format. The device maintains the external footprint of a standard ANSI/SLAS 384-well plate yet internally links the wells into triads, yielding 96 independent compartmentalized culture circuits per plate, with each circuit containing three wells bridged by microchannels. The microchannels were hot-embossed to 5 μm × 10 μm ($W \times H$) and 900 μm long, dimensions that block somata and dendrites while permitting axonal growth; 30 parallel channels per bridge were selected as the best compromise between axonal throughput and fluidic isolation (Figure 7).[86]

Compatibility with neuronal function was demonstrated by FLIPR-style optical electrophysiology: all 24 analyzed wells (12 circuits) showed spontaneous Ca^{2+} transients (100% engagement), and 5 of 12 circuits exhibited synchronous oscillations across the microchannel bridge. Signal variability remained low, falling well within industrial HTS limits.

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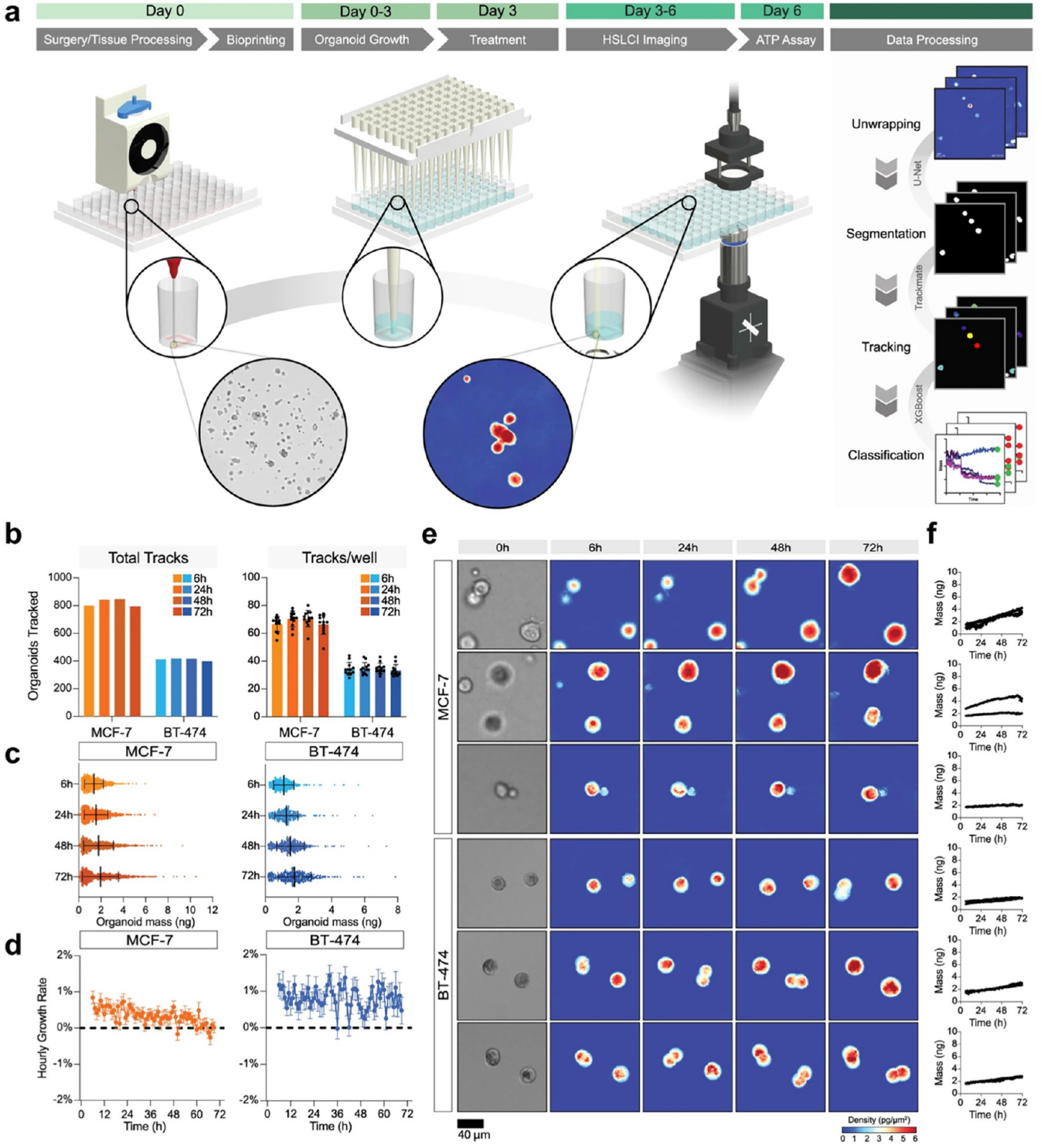

Figure 8. Single-organoid drug response workflow enabled by HSLCI. (a) Timeline of the automated pipeline from bioprinting (Day 0) to interferometric imaging (Day 3–6); (b) total and per-well counts of tracked organoids at 6, 24, 48, and 72 h; (c) dry-mass distributions highlighting growth heterogeneity; (d) hour-by-hour growth-rate plots for vehicle-treated controls; (e) representative phase-shift images at successive time points; (f) individual mass-accumulation trajectories. Reproduced with permission from ref 93. Copyright 2023 Nature.

Collectively, these quantitative performance metrics confirm that the MC-plate supports structurally complex neuronal networks while delivering reproducible, high-content functional readouts, positioning it as a viable high-throughput screening platform for CNS drug discovery.[86]

Personalized Medicine and Precision Therapeutics. The ability to formulate iPSCs that are patient-specific and recreate the features of specific pathologies is a highly encouraging aspect of personalizing medicine in neuropsychiatry. Neuron-engineering techniques enhance in vitro patient genetic and phenotypical variability models to develop individualized therapeutic strategies.[87]

Not only can organoids recapitulate individual pathologies, but patient-derived cultures also enable n-of-1 trials in vitro.

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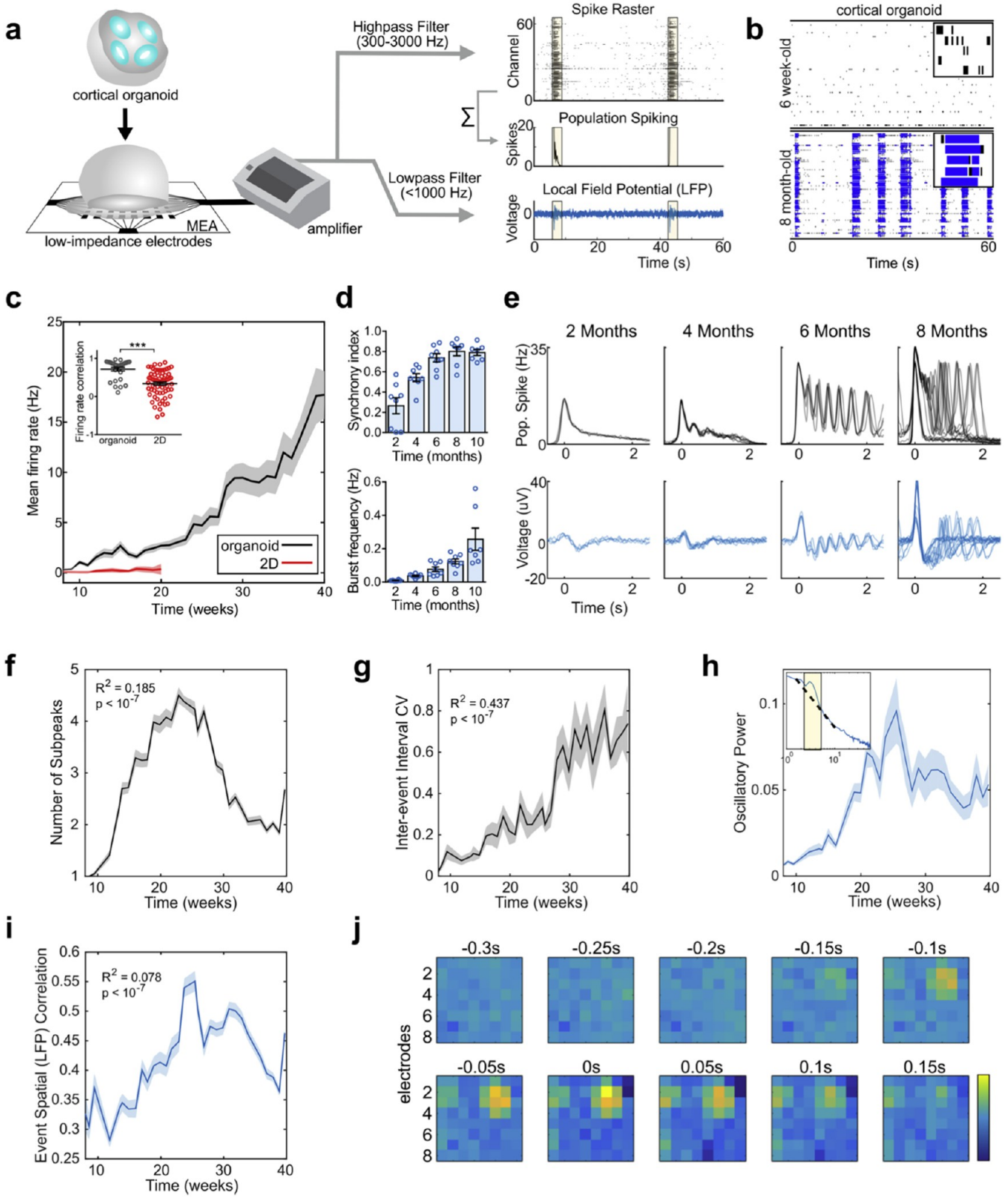

Figure 9. Long-term cortical organoids develop structured oscillatory network dynamics. (a) Experimental workflow: individual cortical organoids are placed on low-impedance multielectrode-array (MEA) plates; high-pass filtering (300−3000 Hz) yields spiking rasters, whereas low-pass filtering (<1000 Hz) yields local-field potentials (LFPs). (b) Raster plots illustrate the transition from sparse, irregular spiking at 6 weeks to dense, synchronous bursting at 8 months (inset shows 3 s zoom). (c) Mean firing rate rises steadily over = 35 weeks and exceeds that of matched 2D monolayer cultures (inset, ***$P < 0.001$, unpaired t test). (d) Top: Network synchrony index increases with maturation (mean ± SEM, $n = 8$ wells). Bottom: Burst frequency follows a similar upward trend. (e) Population-spike histograms (black) and LFP traces (blue) at 2, 4, 6, and 8 months reveal the emergence of nested oscillations around 6 months. (f) The number of subpeaks within each network event peaks near week 25, following an inverted-U trajectory (quadratic fit, $R^2 = 0.185$, $P < 10^{-4}$). (g) Interevent-interval coefficient of variation (CV) rises monotonically,

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Figure 9. continued

indicating increasing temporal variability (linear fit, R^2 = 0.437, $P < 10^{-4}$). (h) Delta-band (1−4 Hz) oscillatory power grows until week 25 and then plateaus (quadratic fit, inset shows the power-spectral-density method). (i) The spatial correlation of LFPs across electrodes increases and then declines, reflecting a shift from highly stereotyped to more diversified propagation patterns (quadratic fit, R^2 = 0.978, $P < 10^{-4}$). (j) Sequential LFP heat-maps (−0.3 s to +0.15 s around burst onset) visualize a representative network event's spatiotemporal spread and extinction. Reproduced with permission from ref 22. Copyright 2019 Elsevier.

Combination therapies, which can yield synergistic efficacy or overcome drug resistance, are desirable for complex disorders. Organoid models provide a platform for systematically evaluating such synergistic effects. For example, neurons derived from a familial Alzheimer's patient were used in an in vitro trial screening of >1000 compounds. This effort identified 27 active hits, from which a personalized 3-drug combination was designed; remarkably, the cocktail reduced pathological Aβ levels by 50−60% in the patient's neurons. Such personalized organoid screens can pinpoint effective therapies tailored to the genetic background.[82,88] These results illustrate how personalized organoid screens can pinpoint effective therapies for specific genetic backgrounds.

Through cellular screening, drugs from patients are tested to assess their therapeutic efficacy in individual patients, estimate expected responses and outcomes after treatment, and inform treatment protocols. This approach lays the groundwork for truly precision neuropsychiatry, wherein a patient's own cells test and predict drug responses, guiding tailored therapy with maximal efficacy and minimal side effects.[89]

Target Identification and Validation. Neuronal engineering techniques have significantly contributed to the discovery and identification of new targets for treating neuropsychiatric conditions. Scientists can target signalers that participate in the regrowth of functional, resident-like cells in diseased tissues and inhibit those signals in neurons and organoids derived from iPSCs. Genome editing tools can manipulate specific genes or pathways, allowing researchers to assess their influence on disease-related characteristics or phenotypes. The ability to investigate the functional effects of genetic variations in controlled experimental environments has improved our understanding of target biology. This process also helps to validate the objectives of the new drugs for their subsequent development.[90,91]

Drug Repurposing and Combination Therapy. Combination therapy for neuropsychiatric disorders and drug repurposing are two areas in which neural engineering approaches show promise. To identify potential candidates for repurposing, researchers can utilize neural models derived from induced pluripotent stem cells to evaluate already approved drugs or compounds.[92] These models enable the assessment of drug effects on disease-specific traits and the identification of existing drugs with unexplored therapeutic potential. Using neural engineering techniques has opened up possibilities for research into drug interactions and the development of novel approaches to treat multiple disease processes simultaneously. In this regard, combination therapies have the potential to enhance treatment efficacy, overcome drug resistance, and provide synergistic effects, leading to improved outcomes in patients with neuropsychiatric disorders.

ADVANCED MODELING SYSTEMS FOR NEUROPSYCHIATRIC DRUG DISCOVERY

In Vitro Models for Drug Screening. 2D monolayer cultures are fast and cost-effective; however, their translational accuracy is limited. They flatten cell polarity and compress signaling gradients, thereby misscaling drug responses that depend on the cyto-architecture. In a head-to-head comparison of 22 anticancer compounds, 3D spheroid IC_{50} values explained 74% of in vivo tumor responses, whereas 2D cultures accounted for only 27%. These deficiencies motivate the shift toward 3D brain models that capture extracellular matrix tension, oxygen microgradients, and long-range neurite extension. Therefore, innovative methods such as 3D cultures and organoid systems are necessary to further translational neuroscience research because 2D cell models cannot capture the complexity of in vivo conditions. However, these models remain indispensable for preclinical research. Compatibility with real-time imaging and electrophysiological technologies requires the development of transparent, optically compatible culture systems that capture high-resolution, live organoid data. To date, high-speed live cell interferometry has been implemented to measure mass changes and dynamic responses in bioprinted tumor organoids, demonstrating the potential of real-time analysis of 3D structures.[93] Real-time single-organoid mass profiling (Figure 8) further illustrates how bioprinting, combined with interferometric imaging, captures heterogeneous drug responses that bulk assays often miss.

To overcome these obstacles, developments in automated liquid handling, image analysis platforms, and microfluidic systems have made it possible to precisely control the environment, scale effectively, and work with HTS methodologies. Further work is needed to achieve cost-effectiveness, scalability, and reproducibility for broader adoption.

Organoids represent a new frontier in in vitro human brain development and modeling of neurodegenerative diseases. Functional readouts further underscore this advantage. Multielectrode-array recordings of six-month cortical organoids reveal highly regular, nested network bursts with strong power and phase-amplitude coupling that closely resemble preterm EEG dynamics[93] (Figure 9). Brain organoids generated from human pluripotent stem cells model salient features of early organization, including diverse cell types, topological organization, and electrophysiological activity, which render them invaluable for drug discovery and disease modeling. Compared with traditional 2D cultures and animal models, they better model human brain structures and thus provide more accurate insights into human-specific neurodevelopmental processes and disease.[94,95]

Despite their promise, organoids face significant challenges, including heterogeneity, prolonged culture periods, and restricted maturation. Variability in organoid growth rates complicates reproducibility, and their relatively small size limits functional complexity.[96] Moreover, in the absence of microglia or vascular systems, the potential for in vivo interactions may

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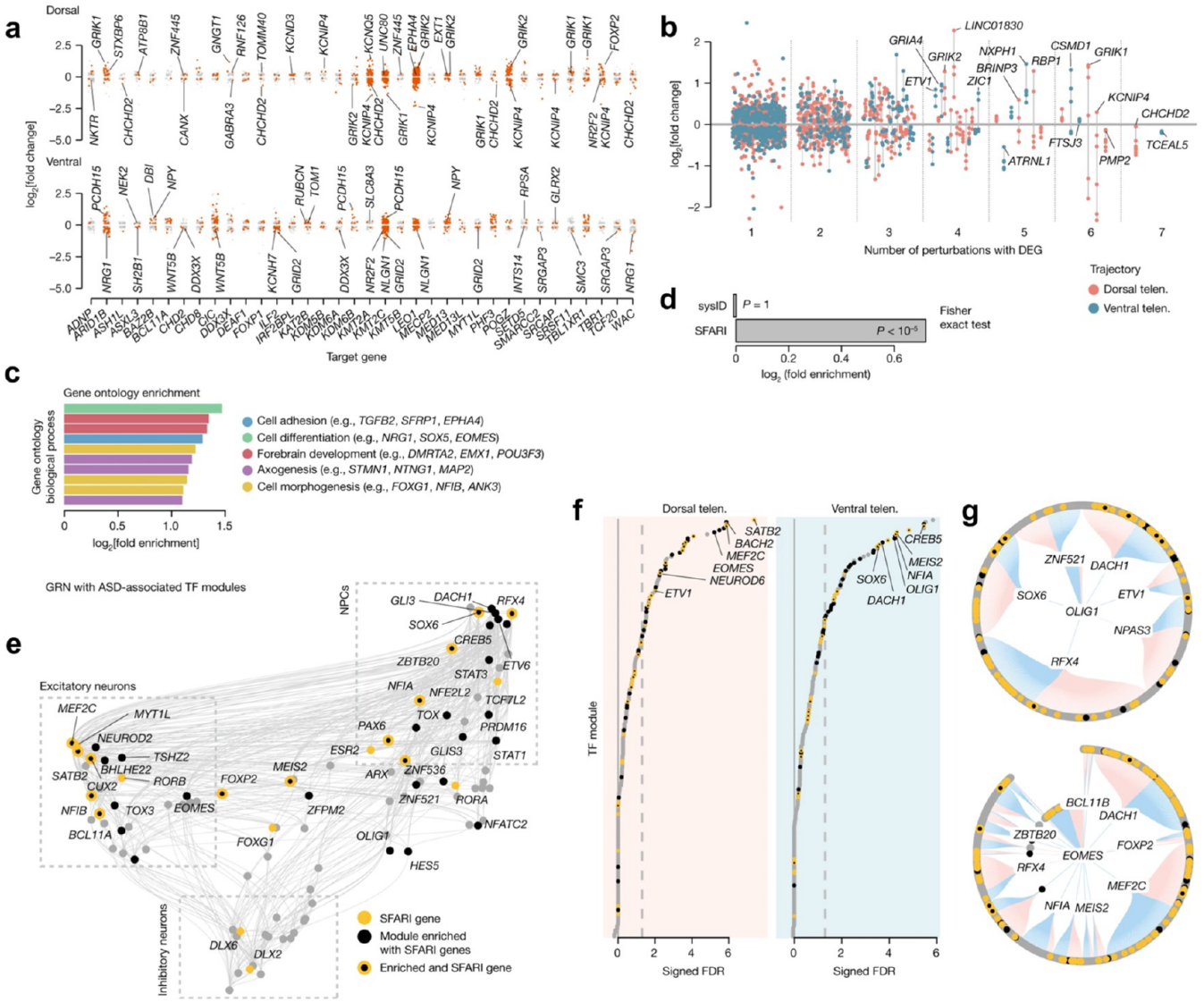

Figure 10. Multiomic CRISPR screen in cerebral organoids (CHOOSE) uncovers ASD gene-specific regulatory modules. (a) Jitter plots showing differentially expressed genes (DEGs) in dorsal versus ventral trajectories for each perturbation. (b) Frequency of DEGs across all perturbations separated into dorsal (orange) and ventral (blue) lineages. (c) Top Gene Ontology terms enriched among the top-30 DEGs per perturbation. (d) Enrichment of DEGs in intellectual disability (ID) and autism spectrum disorder (ASD) gene sets. (e) Gene-regulatory network (GRN) of 4-month organoids inferred from integrated single-cell RNA-seq and ATAC-seq data; ASD-associated TF modules are highlighted. (f) Lolliplots displaying CHOOSE-enriched TF modules in the dorsal (left) and ventral (right) lineages. (g) Subnetwork view of OLIG1 and EOMES primary/secondary targets with activating (red) and repressing (blue) edges. Reproduced from ref 79. Copyright 2023 Nature.

be limited, even with recent advancements that incorporate microglial cells to enhance immune functions in various organ types for studying neurodegenerative diseases. Tiny bioreactors or advanced vascularized organoid-on-chips enhance vascularization and nutrient diffusion, minimizing necrosis and hypoxic conditions.

These are further advanced into "assembloids", or fused organoid systems, which facilitate the examination of interactions among various brain regions, thereby offering a dynamic platform for investigating neuronal connections and circuit-level activities. Cortical and thalamic organoids have generated assembloids exhibiting dynamic neuronal migration and synaptic connections, essential for mimicking intricate neurodevelopmental disorders such as autism.[97] Contemporary high-throughput methods offer fully automated organoid creation and chemical screening in a scalable fashion, enhancing repeatability and enabling identification of novel therapeutic targets.[10]

While brain organoids have become progressively more developed, their applicability to clinical use remains limited due to the need for further advancements in molecular heterogeneity and differentiation to achieve higher physiological relevance. For instance, fused cortical-thalamic organoids form functional assemblies that recapitulate neuronal migration and synaptic connectivity, modeling complex features of neurodevelopmental disorders. Bioengineering approaches such as 3D-bioprinted scaffolds and microfluidic organ-on-chip devices are improving organoid perfusion and maturation, partially alleviating issues of necrosis and nutrient diffusion.[98] Comprehensively, biomaterial-assisted strategies, including degradable hydrogels and microarchitected scaffolds, enhance organoid maturation, reduce stress heterogeneity, and

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enable HTS-compatible formats.[99] Collectively, these studies confirm that scalable, stress-reduced organoid production, along with the inclusion of supporting cell types such as microglia, significantly enhances both biological fidelity and functional maturity, supporting the adoption of such models in drug discovery and disease modeling pipelines.

Multiomics Integration. Single-cell multiomics reveals how risk alleles perturb molecular networks across cell types.

A showcase of this approach is the CRISPR-human organoid-single-cell RNA-seq (CHOOSE) screen, which profiled 36 high-confidence autism-risk genes across more than 49,000 cells and reconstructed cell-type-specific regulatory modules (Figure 10).

In human ESC-derived microglia carrying Alzheimer's disease variants in CD33, INPP5D, SORL1, or TREM2, an integrated ATAC-seq/RNA-seq/proteomics atlas (91 data sets) showed that seemingly diverse mutations converge on an APOE-centered lipid-metabolism module, with 72% of differentially open chromatin peaks mapping to APOE-regulated enhancers.[100] Systems-level studies like this complement large-scale reviews that outline best-practice pipelines for combining genomics, transcriptomics, and metabolomics in neurodegeneration research.[101]

Microphysiological Systems. The advancement of microphysiological systems, often referred to as "organs-on-chips", presents exciting opportunities for neuronal engineering in drug screening and discovery. These microfluidic devices strive to replicate the structure and functionality of particular organs or tissues, including the brain.[102] Microfluidic chips recreate continuous perfusion, shear stress, and endothelial-glial cross-talk of the human blood-brain barrier (BBB). The most comprehensive BBB-on-chip survey to date documents trans-endothelial-electrical-resistance values up to 2000 $\Omega \cdot cm^2$ (approaching in vivo levels) and permeability coefficients for sodium fluorescein below 1×10^{-6} cm s^{-1}, while remaining compatible with 96-well plate automation.[103] Such organ-on-chip systems can complement brain organoids by modeling interfaces such as the blood-brain barrier, thus enabling pharmacokinetic screening of modalities (e.g., biologics, nanoparticles) that traditional Transwell assays cannot capture.

Artificial Intelligence and Machine Learning. Integrating artificial intelligence (AI) and machine learning (ML) algorithms within neuronal engineering methodologies substantially enhances data analysis, model predictions, and decision-making processes. By adeptly identifying patterns and correlations within extensive data sets, these algorithms facilitate the unveiling of new connections between genetic variations, cellular traits, and drug responses. Utilizing these technologies enables researchers to expedite the discovery of potential therapeutic targets, refine drug-screening assays, and create predictive models for treatment outcomes. AI-driven approaches also offer the potential for in silico screening of compound libraries, thereby minimizing the time and expenses associated with experimental screening.[104] ML and deep learning (DL) algorithms are applicable across diverse drug discovery processes. Deep-learning classifiers trained on baseline clinical variables plus 19 EEG-derived features predicted selective serotonin reuptake inhibitor responses in drug-naive patients with major depressive disorder, outperforming logistic regression by 14%.[105] Convolutional neural networks can analyze thousands of cellular morphology features in real time from high-content imaging, potentially identifying neurite toxicity signatures before overt cell death occurs.

These technologies are utilized in ligand-based virtual screening, structure-based virtual screening, peptide synthesis, toxicity prediction, pharmacophore modeling, polypharmacology, drug monitoring, drug repositioning, and physicochemical activity assessment. They have played a significant role in advancing numerous facets of drug discovery. Additionally, unique data curation, mining, and management strategies play a crucial role in supporting the development of newly created algorithms for disease modeling.[106]

CONCLUSION

Neuronal engineering platforms, especially patient-derived iPSC organoids and neuron-glia cocultures, are enhancing the fidelity and scale of neuropsychiatric drug discovery by integrating human genetics with high-throughput, functional phenotyping. These systems reduce reliance on animal models and support circuit-level readouts relevant to psychiatric disease. Despite numerous advancements in this field, key limitations persist, including batch variability, incomplete maturation (e.g., vascularization/immune integration), cross-platform standardization, and the robust alignment of organoid readouts with clinical end points. Addressing these issues requires harmonized protocols, materials, and QC metrics across laboratories and vendors. The convergence of biomaterials, microphysiological systems, and AI-enabled analytics will enable digital-twin-organoid pipelines that prioritize compounds with higher translational probability. Through coordinated efforts across neuroscience, bioengineering, and computation, these advances can accelerate the development of precision therapies for neuropsychiatric disorders.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsptsci.5c00407.

> Platform categories for neuropsychiatric drug screening, including model types, key technologies, validated readouts/biomarkers, advantages/limitations, challenges, development stage, and indications (Table S1), and curated studies prioritized for screening, summarizing models/cohorts, assay families, readouts/biomarkers, drug classes, tiers, and translational relevance; list of abbreviations; supplementary refs (2−26) (Table S2) (PDF)

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Notes

The authors declare no competing financial interest.

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■ TABLE OF ABBREVIATIONS

2D	two-dimensional
3D	three-dimensional
AAV	Adeno-associated virus
Aβ	amyloid β
AD	Alzheimer's disease
AI	artificial intelligence
ANSI/SLAS	American National Standards Institute/Society for Laboratory Automation and Screening (plate format)
ASD	autism spectrum disorder
ATAC-seq	assay for transposase-accessible chromatin using sequencing
AUC	area under the ROC curve
BBB	blood−brain barrier
BrdU	5-bromo-2′-deoxyuridine
CCC	compartmentalized culture circuit (screening unit in MC plate)
CHOOSE	CRISPR-human organoid-single-cell RNA-seq (screen)
CRISPR	clustered regularly interspaced short palindromic repeats
CV	coefficient of variation
DEG	differentially expressed gene(s)
DL	deep learning
EEG	electroencephalography
ESC	embryonic stem cell(s)
FLIPR	(fluorometric) imaging plate reader (FLIPR-style calcium imaging)
GFP	green fluorescent protein
GRN	gene-regulatory network
GWAS	genome-wide association study
HCS	high-content screening
Hi-Q	high-quantity (organoid production workflow)
HSLCI	high-speed live cell interferometry
HTS	high-throughput screening
IC_{50}	half-maximal inhibitory concentration
ID	intellectual disability
iPSC	induced pluripotent stem cell
LC-MS	liquid chromatography−mass spectrometry
LFP	local-field potential
MC plate	microchannel microtiter plate (hard-plastic)
MDD	major depressive disorder
MEA	multielectrode array
ML	machine learning
MSC	mesenchymal stem cell(s)
NHP	nonhuman primate(s)
PD	Parkinson's disease
PSD-95	postsynaptic density protein-95 (DLG4)
QC	quality control
RNA-seq	RNA sequencing
scRNA-seq	single-cell RNA sequencing
SEM	standard error of the mean
shRNA	short hairpin RNA
SSRI	selective serotonin reuptake inhibitor
TEER	trans-endothelial electrical resistance
UMAP	Uniform Manifold Approximation and Projection
vGLUT1	vesicular glutamate transporter-1
Z′-factor	assay quality metric for screening robustness

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