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REVIEW ARTICLE

From Drug Response to Functional Rescue in Alzheimer's Disease Brain Organoids: Evidence and Validation

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Abstract

Human brain organoids provide experimentally accessible systems for investigating Alzheimer's disease (AD) pathology and evaluating candidate interventions. This review examines intervention studies to distinguish pathological attenuation, functional modulation, protection during an insult, and functional rescue. For each, we ask what disease process was represented, when treatment began, and how the measured domains relate to one another. Comparisons across genetic, patient-derived, and experimentally induced models reveal both concordant and divergent outcomes: reduced pathological burden can coexist with persistent synaptic abnormalities, and functional improvement can be partial and restricted to particular activity measures. We discuss how complementary functional measurements each capture a distinct facet of treatment response and why they cannot be interpreted in isolation. Their interpretation depends on compound exposure and tissue maturation, the recording interface, and replication across samples and donors. Endpoint-specific response profiles offer a practical basis for comparing candidate interventions. Within such profiles, functional rescue is the most demanding claim: it requires an interpretable improvement of a defined pretreatment deficit, reproduced across appropriate biological samples. Such profiles aim to strengthen organoid-based drug evaluation while preserving the distinction between an experimentally useful response and the prediction of clinical benefit. Framed this way, functional rescue becomes a claim that can be measured, compared, and improved across studies.

Keywords

Alzheimer's disease; brain organoids; drug response; functional rescue; multielectrode arrays; synaptic dysfunction; phenotypic screening; assay validation.

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1. Introduction

Alzheimer's disease (AD) develops through interacting pathological processes that affect protein handling, lipid metabolism, immune responses, synapses, and neuronal circuits. Amyloid-β (Aβ) deposits and pathological tau define important aspects of the disease, but their presence alone provides an incomplete account of neuronal dysfunction. For drug evaluation, this incompleteness has a concrete consequence: a compound can change a biochemical measure of these pathologies without producing an equivalent change in the ability of a neural circuit to respond to input. Conversely, a treatment acting on excitability may alter circuit function over a timescale too short for substantial changes in accumulated pathology. This distinction makes the choice and interpretation of experimental outcomes central to preclinical drug evaluation [1, 2, 3]. In a phase 3 trial, lecanemab reduced amyloid and modestly slowed clinical decline in early Alzheimer's disease [4]. The TRAILBLAZER-ALZ 2 trial showed donanemab reduced amyloid plaque burden and slowed clinical progression in early symptomatic Alzheimer's disease [5].

Human stem-cell-derived brain organoids have expanded the experimental settings in which these relationships can be investigated. Their value lies in combining human genetic backgrounds with three-dimensional cellular organization and an accessible culture environment. Early engineered neural cultures reproduced linked amyloid and tau abnormalities, while patient-derived self-organizing tissues demonstrated pathological responses to pharmacological treatment [6, 7]. Subsequent studies have examined APOE-associated degeneration, multicellular inflammatory processes, synaptic abnormalities, and altered electrical activity [8, 9, 10]. These preparations support questions about how an intervention acts within a defined human cellular system, including questions that are difficult to isolate in patients or to reproduce across species.

The growing range of available outcomes also creates an interpretive problem. In familial AD organoids with excessive firing and synchrony, a reduction in activity can represent correction of an abnormal state [11]. In an Aβ-exposure preparation with suppressed activity, greater firing after treatment can indicate preservation of function [12]. Neither direction is intrinsically beneficial. Similar ambiguity arises across measurement domains: inhibiting selected pathological pathways in serum-exposed organoids did not restore synaptic puncta, whereas a cholesterol-stress study found partial functional and structural responses without corresponding reductions in all disease-associated protein measures [13, 14]. The meaning of a response therefore emerges from the relationship between the model, the intervention, and the outcome.

Recent reviews have described the breadth of AD organoid models, their cellular limitations, and their applications to therapeutic discovery [15, 16, 17, 18]. The existing benchmarking framework for AD and related tauopathies already includes pathological, neuronal, functional, and pharmacological measures [19]. The task addressed here is therefore to examine how those domains are connected within intervention experiments. A study that reports pathology, electrophysiology, and survival may use different organoid ages, treatment schedules, or tissue cohorts for each measurement. Establishing these relationships is essential to deciding whether the evidence describes several parallel responses or a coherent improvement in a particular disease state.

This narrative review focuses on human AD-related brain organoid interventions, with selected engineered three-dimensional cultures, assembloids, and non-AD systems providing mechanistic or methodological context. We first define the model state and treatment claim, then compare what intervention experiments demonstrate. We subsequently examine functional measurements, competing explanations, and the conditions for reproducible candidate evaluation. Organoid intelligence (OI) [20] and task-based phenotyping receive particular attention as emerging approaches to probing neural transformations. Throughout, functional rescue is treated as an experimentally grounded claim whose meaning depends on the deficit and observation period, rather than as a universal property of a compound.

2. Defining the disease state and the intended treatment claim

2.1 *Matching the model to the pathological question*

Different AD-related processes require different experimental preparations. The early association of APOE ε4 with AD and its biochemical relationship with Aβ, the identification of abnormal tau phosphorylation, and the demonstration that human-brain-derived Aβ assemblies can disrupt synaptic plasticity established distinct lines of investigation [21, 22, 23]. These findings connect genetic susceptibility, protein pathology, and neuronal function, but they do not specify a single experimental phenotype that every model expresses. In organoid studies, the relevant question may concern production of soluble Aβ, formation of aggregates, altered tau species, inflammatory injury, or network dysfunction. The treatment outcome becomes more interpretable when it is aligned with the process the preparation actually represents.

Familial mutation models provide a relatively direct route to investigating genetically driven abnormalities. Amyloid precursor protein (APP) duplication and presenilin variants can generate measurable changes in Aβ handling and neuronal activity in human-derived systems [7, 10, 11]. Their experimental strength is the ability to examine a defined perturbation within a controlled environment. Patient-derived models extend this approach to backgrounds containing multiple sources of susceptibility. APOE4 organoids, for example, have been used to investigate synaptic loss, apoptosis, and tau-related abnormalities, with isogenic APOE4-to-APOE3 conversion attenuating selected phenotypes [8]. The comparison helps identify a genotype-related contribution while preserving the distinction between a risk background and a fully specified disease state. More recent preparations sharpen these comparisons: PSEN1/PSEN2-mutant organoids revealed intertwined developmental and degenerative phenotypes [24], chimeric segregation apportioned neuronal versus astrocytic APOE4 contributions to tau pathology [25], and isogenic APOE3-Christchurch substitution alleviated Aβ, tau pathology, and neurodegeneration in APP NL-G-F organoids [26]. In a separate engineered tauopathy model, adeno-associated virus (AAV)-mediated expression of the frontotemporal-dementia-associated P301L tau variant induced tau fibrils in control and familial-AD forebrain organoids; an APP-Seoul (V669L) model supported BACE1-inhibition testing [27, 28].

Experimentally induced preparations answer complementary questions. Aftin-5 shifts soluble Aβ production in organoids derived from a non-AD line, whereas exogenous Aβ exposure intro-

duces a defined extracellular challenge [12, 29]. Human serum exposure models consequences of contact with circulating factors, and AD brain extracts introduce a mixture capable of inducing several pathological features in a multicellular preparation [9, 13]. These routes offer control over the onset of exposure but differ in the processes they initiate. An intervention that limits toxicity during exogenous Aβ exposure addresses a different causal setting from one that alters endogenous APP processing in a mutation-bearing organoid. Endogenous regulatory states can likewise shift the same pathway: phosphoglycerate dehydrogenase (PHGDH) drove BACE1 translation and Aβ production through RNA-binding activity in a sporadic-AD brain organoid model, showing that induced and genetically driven routes can converge on shared nodes [30].

2.2 *Tissue identity, maturation, and cellular composition*

The biological organization of the preparation affects both its pathology and its measurable response. An engineered neural culture embedded in a matrix, a self-organizing cerebral organoid, a regionally patterned organoid, and an assembloid containing connected regions provide different cellular and spatial conditions. The original engineered three-dimensional AD model used modified neural precursor cells to support amyloid and tau pathology [6]. More recent integrated systems combine separately differentiated neuronal, glial, and vascular components, allowing selected interactions to be examined directly [31]. Describing these distinctions explains which biological relationships are available to a drug, including interactions involving microglia, astrocytes, or vascular-lineage cells. A growing set of more complex preparations extends this cellular and spatial scope; representative advances in multicellular and vascularized systems are summarized in Table 4.

Developmental state is equally important. Reprogramming through pluripotency can remove age-associated features retained by directly converted neurons, so donor age and cellular age represent different attributes [32]. Organoid differentiation also introduces variation in cell composition and tissue organization. Single-rosette approaches, morphological quality assessment, and controlled fluid dynamics address aspects of this variability through different mechanisms [33, 34, 35]. These advances can improve experimental consistency, but consistency is most useful when it supports the particular phenotype being tested. Uniform morphology alone does not determine whether a preparation has the synaptic organization or disease-associated vulnerability needed for an intervention experiment. Consistent with this distinction, brain organoids directly converted from APOE4 patient fibroblasts captured disease features without passage through a pluripotent stage [36].

2.3 *Establishing the deficit and its reference state*

Functional abnormalities are observed against a developing biological background. Network organization, responsiveness, and connectivity change as organoids mature, while differentiation conditions influence the populations contributing to the recording [37, 38]. APOE4 itself can affect developmental cell composition and network formation in cortical and ganglionic-eminence systems [39]. Thus, a difference between genotypes may contain developmental as well as degenerative components. The age at measurement, regional identity, and cellular mixture help determine whether an apparent treatment effect reflects modification of a dis-

ease-associated process, altered maturation, or both. Longitudinal multielectrode-array (MEA) recording illustrates the point: in PSEN1-A246E organoids, transient hyperexcitability and hypersynchrony preceded activity decline while tracking Aβ accumulation, so the measured deficit depends on where in that trajectory it is sampled [40].

A useful reference design combines several comparisons. A healthy or isogenic group establishes the relationship to a reference state; a disease vehicle group describes the untreated trajectory; and a pretreatment measurement determines the starting condition of a recorded organoid. These comparisons address different sources of variation. Repeated recording of the same organoid can reveal a treatment-associated change even when baseline activity differs substantially between samples. A time-matched vehicle group then helps establish whether that change exceeds the trajectory expected during culture. For destructive measurements, matched pretreatment cohorts provide a population-level baseline, although they cannot establish the exact starting pathology of each subsequently treated organoid [41, 42]. Culture-level variability makes these controls more than formalities: in AD-model cerebral organoids, inter-batch variation was a major confound for reproducing APOE-related phenotypes [43].

2.4 *From response to protection and rescue*

We use drug response to describe a measured change following exposure and functional modulation to describe a change in a functional outcome. Protection refers to prevention or attenuation of dysfunction when treatment precedes or accompanies the damaging condition. Functional rescue refers to improvement of a deficit established before treatment in the system being evaluated. This vocabulary connects the treatment claim to experimental timing. It also accommodates compounds with useful acute actions: a reversible correction of excessive activity can be pharmacologically valuable even if the effect depends on continued exposure.

These categories are most informative at the endpoint level. A study may demonstrate pathological attenuation in one cohort, protection of neuronal survival in another, and modulation of network activity in a third. Their joint interpretation depends on how closely the cohorts and exposure conditions correspond. We therefore propose describing each intervention as a response profile containing pathological, functional, and tissue-condition findings, together with their sampling relationship. This approach retains informative disagreement between outcomes and provides a basis for deciding which experiment would most improve understanding of a candidate intervention. These definitions are summarized in Box 1.

Box 1. Working definitions for the response categories used in this review.

Term	Working definition
Functional rescue	Intervention improves a predefined functional endpoint from a deficit established before treatment toward an appropriate non-AD or isogenic reference state; complete normalization is not required.
Functional protection	Endpoint values are preserved when treatment precedes or accompanies the insult; no pretreatment deficit is required.
Functional modulation	A measured change in a functional endpoint after exposure, interpreted relative to the model baseline; by itself, it does not establish protection or rescue.
Pathology attenuation	Reduced molecular or histological disease markers; does not by itself establish functional improvement.
Endpoint-specific response profile	The pattern of pathological, functional, and tissue-condition outcomes for an intervention, including favorable, adverse, absent, or uncertain responses, with treatment timing and sampling relationships specified.

Cellular composition can also determine whether a proposed mechanism is experimentally available. In a study of astrocyte priming, the contribution to Aβ clearance depended on interactions with microglia and differed with APOE background [44]. Such findings make the presence and identity of supporting cells part of the pharmacological question. A preparation containing only one of the relevant populations may be appropriate for testing a cell-autonomous action but poorly suited to evaluating a mechanism mediated through another population. The practical objective is therefore sufficient biological complexity for the proposed action, together with enough experimental control to distinguish that action from changes introduced by the additional cells. The relationship between treatment timing, the measured deficit, and the resulting claim is summarized in Figure 1.

3. What intervention studies actually demonstrate

3.1 *Pathological responses in early three-dimensional systems*

The engineered culture developed by Choi and colleagues established that human neural cells could express amyloid and tau abnormalities within a common three-dimensional environment. Inhibition of Aβ generation with β- or γ-secretase inhibitors reduced amyloid-related measures and attenuated tau pathology; glycogen synthase kinase 3 (GSK3) inhibition provided an additional way to interrogate the relationship between these processes [6]. The principal advance was pharmacological access to linked human-cell pathological features. Because the preparation used engineered precursor cells and a supporting matrix, its contribution is best understood as an antecedent to later self-organizing organoid studies, with a particular strength in manipulating disease-associated mechanisms.

Raja and colleagues extended compound testing to self-organizing neural tissue derived from

familial AD induced pluripotent stem cells (iPSCs). Combined β- and γ-secretase inhibition began at day 30, followed by pathological measurements after 30 or 60 days of treatment [7]. Amyloid aggregate counts were reduced at the earlier endpoint, whereas phosphorylated tau (pTau) immunoreactivity showed a treatment-associated reduction only at the later endpoint. The temporal separation is informative: two pathological outcomes did not respond on the same schedule, even within an experiment designed around APP processing. The design demonstrates sustained pathological attenuation during early treatment, while the fixed-tissue endpoints provide no direct measurement of network restoration.

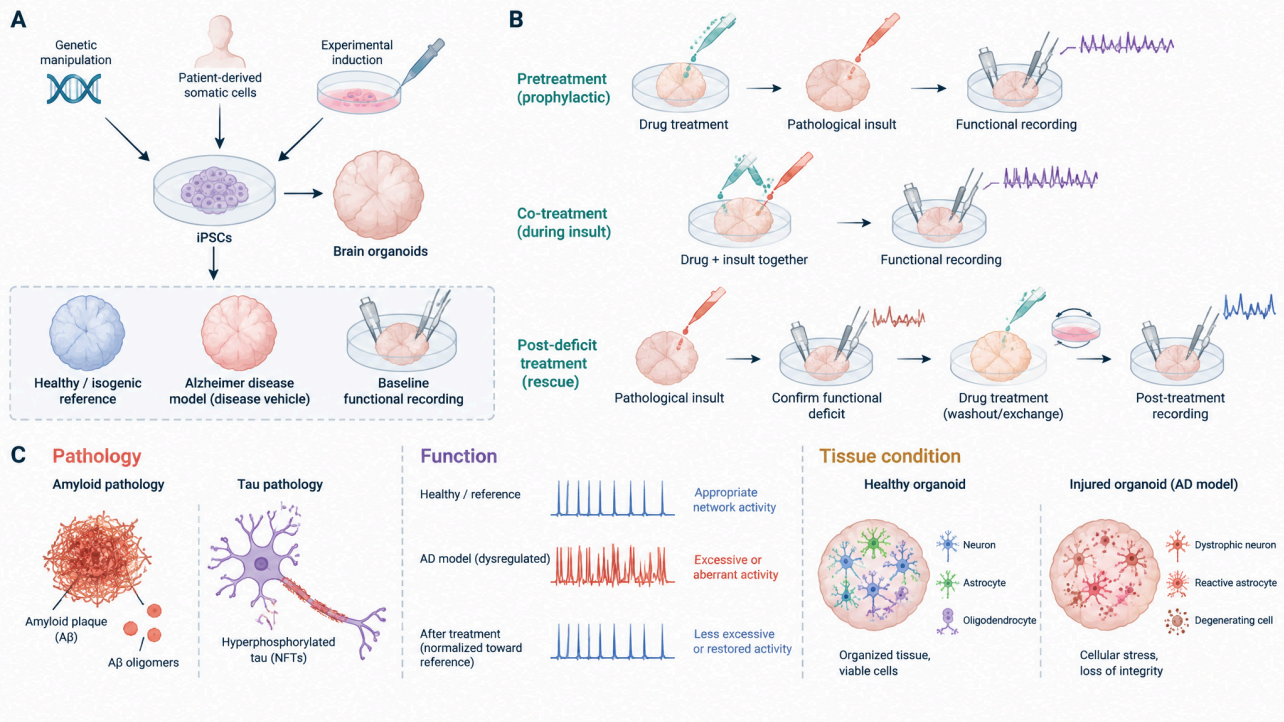

Figure 1. Treatment timing and endpoint-specific interpretation of drug responses in Alzheimer's disease organoids. (A) Genetic, patient-derived, and experimentally induced preparations provide distinct starting states for intervention studies. A healthy or isogenic reference, a disease vehicle group, and a pretreatment functional measurement address different comparisons. (B) Treatment before or during an insult tests protection, whereas treatment after a demonstrated functional deficit tests improvement of that deficit. Exposure onset alone does not establish a functional deficit. On-treatment and post-washout observations define different temporal claims. (C) Pathology, neural function, and tissue condition form complementary response domains; improvement in one does not imply improvement in the others. Reduced firing can be favorable in a hyperactive preparation, while increased firing can be favorable after a suppressive insult. The illustrated trajectories are conceptual and do not represent experimental data. Examples informing these distinctions include NitroSynapsin, oxytocin, and CCT020312 experiments [11, 12, 41]. AD, Alzheimer's disease; Aβ, amyloid-β; pTau, phosphorylated tau.

Structured screening approaches have since connected candidate selection to larger organoid datasets. Park and colleagues integrated a molecular regulatory-network model with pathological features of patient-derived and isogenic cerebral organoids [45]. Predicted perturbations were used to nominate single and combination treatments, which were then exam-

ined through high-content Aβ and tau measurements and tissue-viability assays. The resulting workflow separated computational nomination from experimental validation. Responses varied with organoid background, combination, concentration, and pathological endpoint. This variability is more useful for candidate prioritization than treating a favorable result at one concentration as a general property of the drug or the entire platform. Induced and infection-linked preparations extend the same logic: HSV-1-infected cerebral organoids recapitulated multicellular Aβ-associated pathology that was attenuated by antiviral treatment [46], while additional compound examples, including galangin co-treatment during Aβ exposure, are compared in Table 1.

3.2 *Direct intervention on abnormal network activity*

Ghatak and colleagues investigated a different therapeutic level by studying excessive neuronal activity in familial AD preparations [11]. Cerebral organoids carrying PSEN1 or APP variants showed increased firing, network burst frequency, and synchrony relative to isogenic reference cultures. In the organoid comparison, NitroSynapsin reduced these abnormalities, with a smaller effect on wild-type activity, whereas equimolar memantine did not produce the same reduction under the tested conditions. The experiment therefore links an established network phenotype to a compound-dependent electrophysiological response. It is an important example of evaluating a functional state directly rather than inferring it from a pathological marker.

The same report also included two-dimensional neuronal assays and animal experiments, each contributing a different part of the biological explanation [11]. Receptor-level effects and calcium signaling in neuronal cultures help characterize pharmacological action; synaptic findings in mice address an additional tissue context. Together, these experiments motivate a mechanistic interpretation, but the organoid-specific treatment outcome remains the measured correction of abnormal network activity. Its duration, selectivity across physiological responses, and relationship to accumulated pathology are separate questions. Keeping the experimental systems distinct preserves the value of their convergence without assigning every finding to the organoid intervention.

3.3 *Protection and multimodal treatment responses*

Protection is most clearly established when the intervention is tested before or during a defined insult. In Aβ-challenged human cerebral organoids, oxytocin was administered before the challenge and maintained during exposure [12]. Treated preparations showed lower Aβ-associated immunoreactivity and cleaved-caspase-3 signals; functional experiments also found greater calcium activity, spike counts, and mean firing rates than in Aβ-exposed organoids without oxytocin. Increased activity is informative here because the challenge suppresses activity. The treatment schedule therefore supports preservation during injury, rather than recovery from a functional deficit demonstrated before treatment.

The cellular environment can also influence whether neural tissue is protected. Incorporating microglia into long-term adhesion brain organoids increased synaptic density and reduced p-tau and cell-death measures [47]. This study connects glial support with several aspects of tissue condition, but the intervention is sustained co-culture rather than drug treatment of an established AD phenotype. It provides a ra-

tionale for retaining relevant cell interactions in screening models, while leaving open whether a candidate compound can reproduce the protective effect or improve neuronal function.

Recent compound studies broaden the range of pathological responses observed in human organoids. Semaglutide reduced Aβ and phosphorylated tau in human AD brain organoids, whereas the learning and memory benefits in the same report were measured in APP/PS1 mice [48]. Chrysin and its analogue reduced Aβ and p-tau in chimeric cerebral organoids [49]; abemaciclib reduced tau phosphorylation in AD patient-derived organoids [50]; and taurine attenuated Aβ accumulation and tau phosphorylation in patient-derived cerebral organoids [51]. These findings support pharmacological effects on disease-associated protein measures. Whether those effects restore organoid function requires a corresponding physiological measurement, even when animal experiments provide behavioral evidence.

The CCT020312 study illustrates how pathological, structural, and functional evidence can be combined without treating them as interchangeable. Labra and colleagues examined familial AD cortical organoids using several treatment schedules [41]. Four-week treatment reduced selected amyloid and phosphorylated-tau measures, but the response depended on the tau species and assessment time: lower pT181 at an earlier endpoint was not accompanied by a significant reduction in the reported high-molecular-weight pT217 band. A separate six-week regimen in older PSEN1-mutant organoids altered the distribution of tau species and increased Synapsin-I abundance. The findings link protein handling to a synaptic marker while retaining the distinction between biochemical response and synaptic function.

The longitudinal electrophysiological component provides a more direct test of functional improvement. CCT020312 reduced elevated firing in PSEN1-mutant organoids, and the reduction remained detectable 24–72 hours after washout; wild-type organoids did not show an equivalent response [41]. This comparison supports correction of abnormal activity over the observed interval. Electrode impedance was monitored alongside activity, whereas conditioned-medium lactate dehydrogenase (LDH) was assessed in other experimental components. Thus, the study offers complementary evidence on physiology and tissue condition across related experiments, rather than demonstrating that every endpoint improved in the same organoid under one regimen.

Delivery and regenerative approaches require a similar separation of experimental systems. An exosomal small interfering RNA (siRNA) platform achieved neuronal gene silencing and attenuated serum-induced molecular and cellular damage in human cortical organoids; systemic treatment outcomes were evaluated separately in transgenic mice [52]. Nano-ERASER-mediated polypyrimidine tract-binding protein 1 (PTBP1) depletion was reported to generate functional neurons in human brain organoids, while cognitive improvement was demonstrated in AD mice [53]. The formation of functional neurons does not, by itself, establish recovery of a previously impaired organoid network. For both approaches, the treatment claim rests on the endpoint measured in each preparation and on whether its impairment was established before intervention.

3.4 *When pathological, structural, and functional outcomes diverge*

Serum-exposed organoids offer a particularly clear example of divergent outcomes. BACE inhi-

bition reduced Aβ-related measures but did not reduce pTau or restore SYN1-positive puncta under the tested co-treatment conditions [13]. In the same model, GSK3 inhibition with CHIR99021 reduced pTau without a corresponding reduction in Aβ or restoration of synaptic puncta. These findings place limits on the biological scope of each intervention within that preparation. They also establish an experimentally useful distinction between modifying a pathological feature and improving a structural correlate of synaptic integrity.

Experiments targeting PHGDH illustrate how another intervention can affect several features of a related serum-exposure model. NCT-503 reduced Aβ aggregate measures and apoptotic-cell readouts while increasing SYN1 expression [54]. Indole-3-propionic acid did not improve the three measured organoid phenotypes in that comparison. Treatment began after serum exposure had started, which differs from a purely prophylactic schedule, although tissue-level baseline pathology was established through the experimental design rather than repeatedly measured in each treated organoid. The organoid results concern pathological, structural, and injury-related outcomes; behavioral benefits were investigated separately in mice. The comparison broadens the range of biological responses available within an induced preparation.

A recent cholesterol-stress study adds direct functional information to this discussion. In forebrain organoids assessed at day 80, semaglutide reduced fragmented microtubule-associated protein 2 (MAP2)-positive branch density and normalized the elevated frequency of calcium events, while recovery of synchrony remained limited [14]. Organoid size and MAP2-positive area were not restored, and APP and p-tau217 did not show significant treatment-associated reductions at that endpoint. This response profile separates several forms of partial benefit that would be obscured by a single overall label. The preparation models metabolic injury with selected AD-related features, making it particularly relevant to questions about lipid stress and neuronal integrity.

Lecanemab treatment in vascularized neuroimmune organoids demonstrates a different type of divergence [9]. Following exposure to AD brain extracts, treatment reduced amyloid burden and increased observed microglial engulfment of Aβ. A separate APOE4-background experiment found increased vascular cell adhesion molecule 1 (VCAM-1) expression and a downward trend in VE-cadherin expression, implicating vascular inflammation-related responses. The model had demonstrated network impairment before pharmacological validation, but the antibody experiment primarily assessed pathological and vascular-related outcomes. This distinction focuses attention on a useful follow-up question: how does amyloid clearance in that multicellular environment relate to neuronal function and vascular-cell state over the same treatment period? Endpoint-selective effects of this kind, including the nanomaterial STB-MP, are compared in Table 1.

3.5 *Genetic context, molecular response, and experimental scale*

Additional patient-derived studies expand the mechanistic and genetic coverage of organoid pharmacology. C458 reduced intracellular Aβ42/Aβ40 and pTau/total-Tau ratios after a short exposure in AD-derived cerebral organoids [55]. W2A-28 altered histone/Wnt-related measures and reduced amyloid and tau-phosphorylation readouts in organoids representing APOE4-associated sporadic and PSEN1-associat-

ed familial backgrounds [56]. CI-994 was investigated in an AD-derived APOE4 preparation and, separately, a MAPT-P301L tauopathy preparation [57]. These studies support biochemical tractability across different mechanisms.

Tβ4 treatment similarly produced an endpoint-specific pattern. Early-treated APP-duplication organoids showed greater neuronal-marker density, reduced terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) signal, and lower Aβ immunoreactivity at a later endpoint, while cleaved-caspase-3 did not show the same treatment-associated reduction [58]. Electrophysiological intervention results in that study came from mice. Cell-based interventions introduce another level of comparison: human nasal turbinate stem cell (hNTSC) co-culture reduced injury-related responses during Aβ exposure in human brain organoids, whereas osteopontin (OPN)-related mechanistic tests and behavioral experiments were conducted in other systems [59].

Donor-dependent molecular responses also deserve attention. Boyd and colleagues generated serotonergic hindbrain organoids from 30 donors, including individuals with AD and controls without cognitive impairment [60]. Escitalopram exposure altered organoid and extracellular-vesicle proteomes, with a subset of treated samples clustering closer to untreated samples. This supports investigation of molecular response heterogeneity in a regionally relevant preparation. It does not by itself determine whether the corresponding neural network improved. Across these examples, the clearest contribution of organoid pharmacology is a richer description of what a candidate changes, in which biological setting, and with which unresolved relationship to function. A complementary hindbrain-organoid study found dose-dependent, donor-variable changes in extracellular serotonin after escitalopram exposure, extending response profiling to a pharmacodynamic endpoint without establishing rescue of network function [61].

A stimulus-based neuroimmune preparation further illustrates the dependence of pharmacology on how the pathological state is generated. Recombinant ApoE4 and Aβ were applied to planar, multicellular neural preparations, and responses to anti-inflammatory compounds were compared with those obtained under a lipopolysaccharide/interferon-γ (LPS/IFNγ) stimulus [62]. SB203580 reduced inflammatory and injury-related release measures together with microglial lipid accumulation in the rApoE4 condition, whereas the pattern across the other compounds differed from the comparator stimulus. These are pharmacological effects on a defined inflammatory and metabolic phenotype. Neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) release provide injury-related information, while the absence of a corresponding neural-recording outcome leaves the relationship to network function open.

Collectively, the intervention literature supports a more differentiated account of success than a list of compounds labelled effective. Some experiments establish that a pathological feature is pharmacologically tractable. Others show a selective correction of activity, preservation during an insult, or a combination of favorable and adverse changes. A negative result in one domain can be particularly informative when another domain changes under the same exposure. The serum-inhibitor and cholesterol-stress comparisons are useful in this respect [13, 14]. They help define what additional biological process remains to be addressed and prevent candidate selection from being driven solely by

the easiest marker to measure. Table 1 compares selected intervention experiments by preparation, treatment schedule, measured response, and the scope of the supported claim. Donor variation also affects the cell product used for treatment: nasal turbinate stem-cell preparations with higher SSEA3/CD105 positivity produced greater pathological improvements in AD organoids and therapeutic effects in 5XFAD mice [63].

Table 1. Selected intervention experiments and the scope of their organoid-specific outcomes.

Intervention and source	Preparation and timing	Measured response	Interpretation and sample boundary
Combined β- and γ-secretase inhibition [7]	Familial AD neural organoids; treatment from day 30 for 30 or 60 days.	Aβ aggregates decreased at the earlier endpoint; pTau reduction was observed after the longer treatment.	Pathological attenuation. Fixed-tissue endpoints came from separate samples; network restoration was not measured.
NitroSynapsin [11]	PSEN1- or APP-variant cerebral organoids with excessive activity; electrophysiology before and during exposure.	Excess firing, network burst frequency and synchrony decreased; equimolar memantine did not produce the same response.	Correction of abnormal activity during exposure. Mouse synaptic findings do not establish organoid synaptic rescue.
Oxytocin – pathology [12]	Aβ-challenged cerebral organoids; pretreatment followed by co-exposure.	Aβ-associated immunoreactivity and cleaved-caspase-3 signals decreased.	Protection in the tested exposure setting. This pathological cohort does not provide a same-organoid functional trajectory.
Oxytocin – physiology [12]	Separate functional experiments comparing Aβ organoids with and without oxytocin.	Calcium activity, spike count and mean firing rate were higher with oxytocin.	Functional preservation in an insult-associated low-activity state. Cohort ages and schedules cannot be collapsed into the pathological experiment.
CCT020312 – pathology [41]	Familial AD cortical organoids; four-week treatment.	Selected amyloid and pTau measures decreased; early pT181 and high-molecular-weight pT217 responses differed.	Species- and stage-dependent pathological response.
CCT020312 – synaptic protein [41]	Older PSEN1-mutant organoids; separate six-week treatment.	Tau-species distribution shifted and Synapsin-I abundance increased.	Structural/protein evidence. Synapsin-I abundance does not by itself demonstrate restored synaptic transmission.
CCT020312 – electrophysiology [41]	Separate longitudinal PSEN1-mutant/WT experiment with baseline, exposure and washout measurements.	Abnormally high firing decreased; reduction remained observable 24–72 h after washout.	Time-bounded functional improvement. Impedance supported interface assessment; all molecular and injury assays were not collected from the same recording cohort.
BACE inhibitor [13]	Serum-exposed brain organoids; co-treatment.	Aβ-related measures decreased; pTau and SYN1-positive puncta did not recover.	Pathology–structure divergence. Synaptic puncta are structural measures; post-treatment electrical rescue was not established.

Table 1. (continued)

Intervention and source	Preparation and timing	Measured response	Interpretation and sample boundary
CHIR99021 [13]	Serum-exposed brain organoids; separate GSK3-inhibition experiments under co-treatment.	pTau decreased; Aβ and SYN1-positive puncta did not show corresponding improvement.	Endpoint-specific pathological response. Different assay samples must not be treated as repeated observations of one organoid.
NCT-503 [54]	Serum-exposed organoids; treatment started after serum exposure began.	Aβ aggregates and apoptotic-cell measures decreased; SYN1 expression increased.	Pathological, structural and injury-related benefit. Exposure onset is not a repeatedly measured functional baseline; mouse behavior is a separate outcome.
Semaglutide [14]	Cholesterol-stressed forebrain organoids; day-80 endpoint.	Calcium-event frequency normalized and fragmented MAP2-positive branch density decreased; synchrony recovery was limited.	Partial response in a metabolic-injury model. APP, p-tau217, organoid size and MAP2-positive area did not show corresponding recovery.
Lecanemab – amyloid [9]	Vascularized neuroimmune organoids after AD brain-extract exposure.	Amyloid burden decreased and microglial engulfment of Aβ increased.	Amyloid-directed response. Network impairment characterized in the model was not retested as an antibody-rescue endpoint.
Lecanemab – vascular markers [9]	Separate APOE4-background experiment.	VCAM-1 increased; VE-cadherin showed a downward trend.	Vascular inflammation-related molecular response. The trend is not a confirmed decrease; clinical vascular toxicity was not established.
Semaglutide [48]	Human AD brain organoids treated in culture; separate APP/PS1 mouse experiments.	Aβ and phosphorylated-tau measures reduced in organoids; cognitive improvement reported in mice	Pathological response in the organoid cohort; the functional outcome was measured in animals, not in organoid tissue.
Galangin [64]	Human brain organoids under 8-day Aβ exposure; galangin co-treatment during exposure.	Aβ-induced impairment of mitophagy and organoid growth was prevented.	Protection during amyloid challenge. Growth and mitophagy are tissue-condition endpoints rather than neural function.
STB-MP (mica nanoparticle) [65]	AD patient iPSC-derived cortical brain organoids.	Aβ accumulation diminished; p-tau was not inhibited.	Endpoint-selective response: amyloid reduction without a p-tau effect.

Note. Selected examples emphasize treatment timing, multidomain responses, and discordance rather than exhaustive compound coverage. Rows separate regimens or assay cohorts when their biological correspondence affects interpretation. "Not measured" or "not established" does not mean absence of an effect. Observed changes refer to the comparisons and exposure conditions described in each study; no common effect size or drug ranking is implied. Aβ, amyloid-β; AD, Alzheimer's disease; BACE, β-site APP-cleaving enzyme; GSK3, glycogen synthase kinase 3; MAP2, microtubule-associated protein 2; pTau, phosphorylated tau; SYN1, synapsin 1; WT, wild type.

4. Interpreting functional outcomes in biological context

4.1 *Activity measures and the sampled network*

Spontaneous firing is often the first functional outcome available in an organoid assay. Mean firing rate, spike count, and the fraction of active recording sites can identify robust changes during exposure, but each summarizes a different property. A lower mean rate may reflect reduced firing within active neurons, recruitment of fewer detectable units, or a changing distribution of activity across the sampled tissue. The interpretation also depends on the baseline state. Reduced activity in a hyperactive preparation and increased activity after a suppressive insult may both be consistent with improvement, as il-

lustrated by the NitroSynapsin and oxytocin studies [11, 12]. The reference condition supplies the biological meaning that the numerical direction alone lacks. Convergent standardization efforts now provide shared protocols and metrics for exactly this characterization problem, aiming to reduce the high inter-study variability of functional assessment [66].

The recording interface contributes to the phenotype being observed. Contact area, electrode distribution, tissue position, and the criteria used to identify active channels influence which neural events enter the analysis [37, 67]. In a shape-conformal interface study, restricting the electrodes used to sample the same organoid changed the extent of detected oscillatory and synchronous activity [68]. This is direct evidence that apparent network organization depends partly on coverage. Longitudinal drug experiments therefore benefit from separating changes in recording quality from changes in the tissue. A stable set of channels may simplify comparison, whereas a changing set can reflect genuine recruitment or loss of activity. Interface engineering continues to change what longitudinal and cellular-resolution designs can resolve; representative recording advances are summarized in Table 4.

4.2 *Burst structure, synchrony, and connectivity*

Burst measures retain temporal information that is lost in average firing. Burst duration, interval, participation, and propagation describe how activity is organized into episodes and distributed across the sampled network. An intervention may leave the total spike count relatively unchanged while altering the timing or spatial participation of these episodes. Conversely, reduced bursting can accompany generalized suppression. AD organoid studies showing increased burst frequency and synchrony provide an identifiable abnormal state against which such changes can be evaluated [10, 11].

Synchrony and connectivity add related but distinct information. Correlated activity describes coordination among recorded signals; inferred functional connections summarize statistical relationships under a particular analytical model. Organoid circuitry studies have shown that pharmacological manipulation can alter the distribution of inferred connections and the organization of firing patterns [37]. The resulting network representation is influenced by spike availability, sampling duration, and estimator assumptions. Accordingly, a treatment-related change in an inferred graph is strongest when supported by repeatable signal-level changes and complementary observations. This matters especially when treatments modify overall activity, because the amount of available spiking can itself affect the stability of connectivity estimates. Shared analysis pipelines now address part of that analytic variability directly—for example, MEA-NAP extracts functional connectivity, topology, and network dynamics from 2D and 3D organoid multielectrode recordings under a standardized workflow [69].

4.3 *Evoked responses and activity-dependent plasticity*

Defined stimulation probes make it possible to examine how a network transforms input. Response probability, latency, magnitude, spatial spread, and trial-to-trial consistency address properties that spontaneous activity cannot fully reveal. Repeated or patterned input can additionally test how the response depends on recent activity. In human neural organoid microphysiological systems, input-specific stimulation produced plastic changes at the level of neuronal units [70]. Importantly, these changes did not require every network-level measure to shift

in parallel. The distinction between unit-specific potentiation and an overall change in network firing is therefore biologically meaningful. The stimulation probe itself can also be chemical: bioelectronic delivery of ions and neurotransmitters sequentially raised and lowered neuronal activity in cortical organoids, offering a reversible perturbation for testing response dynamics [71].

Assembloid experiments provide more direct access to synaptic mechanisms. In human thalamocortical preparations, changes in evoked synaptic currents were used to characterize potentiation in each direction of the circuit [72]. Receptor dependence differed between thalamocortical and corticothalamic potentiation under the tested protocols. This demonstrates why the mechanistic interpretation of a plasticity assay depends on the circuit and induction method. For drug evaluation, a plasticity outcome is most useful when it addresses a plausible impairment or proposed action of the candidate, which complements measures of excitability and tissue integrity. Circuit complexity continues to scale in this direction: reciprocally axon-connected cerebral organoids generated more complex oscillations and exhibited short-term plasticity after stimulation of the connecting axons, extending circuit-level assays to connected organoid pairs [73].

4.4 *Task-based phenotyping and organoid intelligence*

OI approaches extend functional assessment by coupling biological responses to a defined input–output task. The relevant pharmacological question is whether a perturbation changes the transformation performed by the measured neural system. This differs from asking only how often neurons fire. A useful task can reveal separation between response strength and the capacity to distinguish inputs, integrate temporal information, or maintain a response across repeated stimulation. Such outcomes introduce additional experimental choices, including input encoding, response windows, channel selection, and digital decoding. Their biological interpretation depends on how these choices are held constant or explicitly incorporated into the comparison.

The Brainopheno preprint offers an AD-related example of this approach [74]. Evoked responses to spatial stimulation patterns were processed with a digital classifier to quantify pattern discrimination. Pharmacological perturbations in normal organoids produced different relationships between activity and classification: combined glutamatergic receptor blockade reduced both, whereas bicuculline increased activity while reducing classification performance. Sodium-channel blockade brought performance toward the task's chance level. These comparisons make a specific contribution to functional interpretation. They demonstrate that a larger activity signal need not provide a more discriminable representation of input, and that a task-related phenotype can be sensitive to changes in network organization beyond simple firing direction.

The same preprint compared APP-duplication organoids with healthy-donor organoids and examined the effects of monocytes from different donor groups [74]. APP-associated preparations showed poorer classification despite similar resting-state measurements for some conventional metrics. Monocyte co-culture was associated with donor-group-dependent task phenotypes. These experiments establish a connection between AD-related conditions and a measurable input–output property. The intervention experiments used to validate the readout, how-

ever, were distinct from therapeutic treatment of the AD-associated preparations. This leaves a focused experimental opportunity: testing whether a candidate alters the disease-associated task phenotype while preserving an interpretable relationship to pathology, tissue condition, and baseline activity.

A second preprint used paired organoids connected through guided axonal growth to investigate open-loop potentiation and closed-loop maze performance [75]. Repeated stimulation increased evoked responses under selected training conditions; brain-derived neurotrophic factor (BDNF) supplementation and N-methyl-D-aspartate (NMDA)-receptor blockade produced different effects on this response. In the maze experiment, improvement depended on the feedback condition and trophic support, and the reported training advantage did not persist into the subsequent memory phase under the tested protocol. The open-loop and closed-loop results therefore contribute different information: one concerns changes in evoked network response, while the other concerns performance within an experimentally configured feedback task. Their timescales and biological requirements remain separate properties of the preparation.

For drug evaluation, the most useful extension would compare conventional physiology and task performance under the same exposure schedule. A compound might reduce aberrant spontaneous activity while improving stimulus discrimination, or increase evoked amplitude without improving discrimination. Digital procedures are part of this comparison. Re-estimating a decoder after treatment can test how much information is recoverable from the new neural state, whereas a fixed decoder tests whether a previously established mapping remains useful. These are legitimate but different outcomes. The task can consequently enrich the response profile when its input, biological transformation, and readout are specified together. It becomes a candidate functional assay whose pharmacological sensitivity and repeatability can be evaluated alongside established measurements, rather than a general measure of cognition. Figure 2 places spontaneous, evoked, and task-based measurements in their biological and analytical contexts. The closed-loop precedent extends this comparison. In mouse cortical organoids embodied in a pole-balancing task, adaptive feedback improved performance during training, although gains did not persist after a 45-min rest [76]; dissociated cortical neurons similarly learned a simulated game under closed-loop stimulation (DishBrain), demonstrating the principle outside organoid preparations [77].

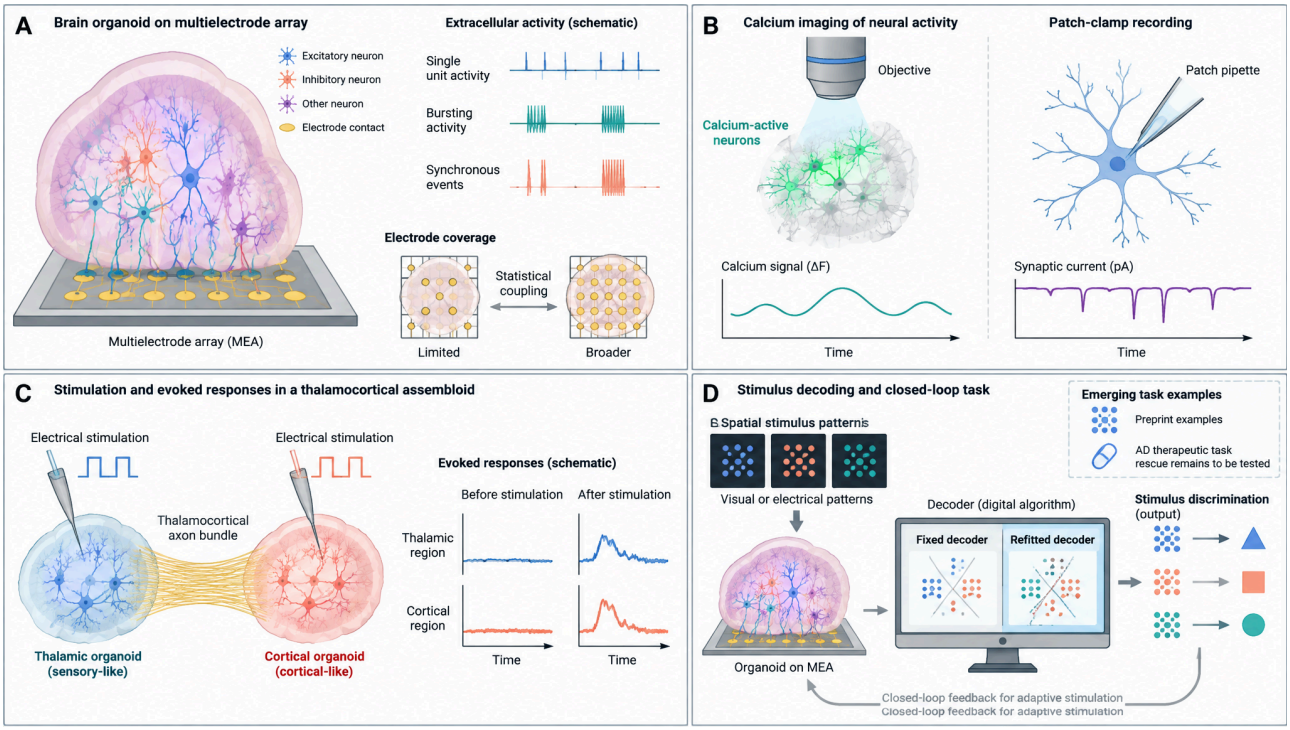

Figure 2. Biological scope and interpretation of functional readouts in brain organoids. (A) Organoid-electrode interfaces sample spontaneous firing, burst organization, and correlated activity. Electrode coverage and contact influence the network that is observed. (B) Calcium imaging and patch-clamp recording interrogate intracellular calcium dynamics and cellular or synaptic currents, respectively; these signals are complementary to extracellular spikes. (C) Defined stimulation probes response probability, timing, and activity-dependent plasticity. Connected regional tissues can provide access to direction-specific synaptic responses. (D) Task-based phenotyping couples an encoded input to an organoid response and a digital readout. Input discrimination and feedback-dependent performance depend on both biological activity and the specified analysis. Fixed and refitted decoders answer different questions. Illustrative traces and task symbols are schematic rather than reproduced data. The framework draws on organoid electrophysiology, calcium imaging, and plasticity studies [14, 37, 67, 68, 70, 72]. The task examples described in this review include preprints [74, 75]; assay sensitivity and disease-associated task differences do not by themselves establish therapeutic rescue or human-like cognition. MEA, multielectrode array.

4.5 *Connecting functional measurements to other response domains*

Combining electrophysiology with pathology is most informative when the measurements have a clear biological correspondence. The same-organoid design can connect a longitudinal electrical response to subsequent imaging or biochemical analysis. Matched parallel organoids can support comparison when fixation or tissue lysis makes repeated assessment impossible. Different ages or dosing schedules instead provide related evidence at the study level. The autophagy and oxytocin studies illustrate why these distinctions matter: multiple response domains are available, but their sampling windows require separate interpretation [12, 41]. Similarly, paired patch-clamp and proteomic measurements in individual derived neurons demonstrate a complementary strategy for linking cellular physiology to molecular state at a defined unit of analysis [78].

Concordance strengthens a particular interpretation when the measured changes are mechanistically compatible and appropriately matched. Discordance identifies a different problem. A pathological marker may respond before structural repair, a treatment may primarily alter excitability, or the measured domain may be insensitive to the relevant change. The serum-exposure and cholesterol-stress experiments show that these possibilities cannot be reduced to a universal expectation of parallel improvement [13, 14]. Establishing the explanation requires additional comparisons. The response profile is therefore both a summary of treatment effects and a means of identifying the next causal or temporal question.

The measurement modality also sets the scale of the inferred function. Calcium transients integrate intracellular signaling over a different temporal window from extracellular spikes, while a synaptic-current recording interrogates a more specific cellular interaction [14, 72, 78]. Agreement between these measurements can be valuable, but direct numerical equivalence is rarely the relevant goal. A treatment might change calcium-event frequency while leaving the precision of spike timing unresolved, or alter a synaptic response without producing a large change in population firing. Matching the modality to the proposed biological effect allows these observations to coexist within one interpretation. It also explains why adding a second functional assay is most useful when that assay resolves a distinct uncertainty. The readouts summarized in Table 2 address complementary biological questions and require distinct controls. Outside the AD field, neural progenitor engraftment in a sporadic Creutzfeldt–Jakob disease (sCJD) organoid model was associated with spike-rate and burst-spike-rate values comparable to uninfected controls at the later endpoint. Because the functional deficit was not demonstrated before engraftment, this is evidence of a favorable functional outcome rather than a confirmed example of rescue under the definition used here [79].

Table 2. Functional readouts and their appropriate biological interpretation.

Readout and sources	Biological question	Useful measurements	Main interpretive boundary
Spontaneous extracellular activity [11, 12, 37]	How active is the sampled neuronal population?	Firing rate, spike count, active-channel fraction.	Neither higher nor lower activity is intrinsically beneficial. Compare with the pretreatment state, time-matched vehicle and an appropriate reference.
Bursts and synchrony [10, 11, 37]	How is activity organized across time and recording sites?	Burst duration, interval, participation and synchrony.	Less bursting can reflect correction or generalized suppression. Interpretation depends on overall firing and the selected analysis window.
Inferred connectivity [37, 68]	Which recorded signals show reproducible statistical relationships?	Coupling estimates, graph organization and sensitivity to channel selection.	Inferred functional coupling is not proof of an anatomical synapse. Coverage, spike availability and estimator assumptions affect the graph.
Calcium imaging [12, 14]	How do intracellular calcium dynamics change across cells?	Event frequency, amplitude, synchrony and region-of-interest responses.	Calcium events and extracellular spikes have different temporal and biological meanings. Regions within one organoid are nested observations.
Evoked responses [70]	How does the preparation respond to a defined input?	Response probability, latency, magnitude and trial-to-trial consistency.	Input delivery, electrode geometry and response windows must be controlled; a larger response need not mean better discrimination.
Synaptic plasticity [70, 72]	Does prior activity change a subsequent cellular or circuit response?	Evoked-current or unit-response changes after induction; receptor dependence.	Mechanism depends on circuit direction and induction protocol. Unit potentiation need not coincide with a change in population firing.
Stimulus discrimination [74]	How distinguishable are neural responses to different encoded inputs?	Classifier performance relative to task chance; fixed versus refitted decoder.	Control data partitioning and decoder choice. Readout validation in normal tissue is distinct from treatment of an AD-associated task deficit.
Closed-loop performance [75]	Does feedback alter performance in a defined biological-digital task?	Performance across training and subsequent testing under specified feedback.	Separate feedback effects, trophic support and retention. Task improvement does not establish human-like cognition or AD therapeutic rescue.
Network connectivity and graph metrics [69]	How is population activity organized into network-level structure?	Functional connectivity, topology and burst-network dynamics from MEA recordings	Graph output depends on estimator choices, channel selection and analysis pipeline; a shared pipeline such as MEA-NAP makes these choices explicit and facilitates consistent analysis.
Single-cell electrophysiology (patch clamp) [80]	What are the membrane and synaptic properties of individual neurons within the preparation?	Evoked currents, membrane parameters and linked morphology	Cellular, not network-level resolution; sampling depth and tissue access constrain the population inference.

Note. Methods are complementary rather than an ordinal scale of functional sophistication. Signal generation, sampling and analysis are part of the measured phenotype. Fixed decoders test the stability of an established mapping, whereas refitted decoders test information recoverable from a changed state. The task examples in references [74, 75] are identified as preprints in the cited versions. AD, Alzheimer's disease.

5. Distinguishing functional rescue from competing explanations

5.1 *Treatment timing and effective exposure*

The model-state, timing, and reference distinctions defined in Section 2 are here converted into design controls and competing explanations. The sequence of insult, baseline assessment, and treatment determines the claim an experiment can support. Pretreatment examines whether a compound changes susceptibility to a subsequent challenge; co-treatment examines its effect during the challenge; treatment after a demonstrated deficit examines improvement from an impaired state. The oxytocin and serum-inhibitor studies address the first two settings, whereas the PHGDH intervention begins after serum exposure has started [12, 13, 54]. These designs can all inform drug development. Their distinction becomes especially important when the proposed clinical use involves established pathology, because a preventive effect and recovery of an existing deficit may depend on different biological processes.

Exposure in three-dimensional tissue adds another layer. The concentration placed in the culture medium is a boundary condition, while local availability can depend on diffusion, tissue size, matrix, cellular uptake, and fluid handling. Microfluidic organoid studies demonstrate how geometry and flow can produce different local exposures across chambers [81]. Culture engineering also changes tissue development and the environment through which a compound moves [35]. Comparisons are therefore improved by reporting concentration together with the medium, exchange schedule, tissue configuration, and duration. Bioorthogonal Raman microspectroscopy quantified diffusion and perfusion of deuterated dimethyl sulfoxide in neural stem-cell spheroids, illustrating a method that could be adapted to study local exposure in organoids [82].

Dose-dependent interpretation benefits from retaining each endpoint separately. In organoid screening experiments, different concentrations or combinations can affect Aβ and tau measurements differently, and the relationship can also vary across genetic backgrounds [45]. A concentration producing the largest marker change may therefore be less informative than a range in which the response is reproducible and tissue function remains measurable. This creates a practical distinction between finding an active concentration and identifying a useful experimental window. The latter requires consideration of variability, exposure, and complementary outcomes, especially when greater suppression of electrical activity could be mistaken for a stronger therapeutic response.

5.2 *Acute action, sustained effects, and reference-tissue responses*

An on-treatment improvement and a persistent post-treatment change answer different pharmacological questions. Rapid modulation of excessive activity may be desirable for a drug intended to act continuously, while lasting effects after removal may support a different mechanism or dosing strategy. Repeated baseline, exposure, and washout measurements in the CCT organoid study provided evidence of reduced abnormal firing over a defined post-washout interval [41]. Such a design is more informative than assigning a general label of durability. The interval actually observed, the completeness of washout, and the behavior of the vehicle group determine what persistence means in that experiment. In a non-AD study using organoids from healthy donors and patients with depres-

sion, esketamine produced concentration- and time-dependent changes in firing and metabolism. These observations characterize drug-associated modulation but do not establish persistence after drug removal [83].

Reference-tissue treatment helps distinguish correction of an abnormal state from a broadly similar effect in all tissues. In the NitroSynapsin and CCT comparisons, the drug's effect on abnormal AD-associated activity could be considered alongside its effect on wild-type organoids [11, 41]. This comparison provides context for selectivity without requiring identical untreated baselines. Additional probes can determine whether a quieter network retains appropriate responsiveness. The balance between spontaneous and evoked outcomes is particularly useful for compounds acting on receptors or ion channels, where a large reduction in firing may coexist with either improved organization or excessive suppression.

5.3 *Tissue condition, maturation, and the recording interface*

Tissue viability changes the meaning of a functional response. Reduced activity accompanied by cell loss differs from reduced pathological hypersynchrony in a preparation with preserved tissue integrity. Injury-related proteins, cell-death assays, neuronal markers, and structural measurements offer complementary information, but their relevance depends on when and where they are collected. In the Tβ4 study, TUNEL and cleaved-caspase-3 did not show identical treatment responses [58]. In the CCT study, conditioned-medium LDH and electrode impedance contributed information in different experimental components [41]. These examples support a specific approach to tissue condition: interpret each assay's biological target and sampling context before using it to explain the electrical outcome.

Development continues during treatment. Changing cell composition, neurite organization, aggregation, and medium conditions can alter the same endpoints used to assess efficacy. Multi-line studies of dissociated organoid cultures demonstrate increasing network activity and connectivity alongside cell-line and batch variation [84]. Morphological and culture-engineering studies show additional ways that production history affects the resulting preparation [33, 34, 35]. Time-matched controls and repeated baselines make these changes visible. Their importance increases with long experiments, where a before-and-after difference can contain treatment effects, natural maturation, and changes in the tissue–electrode interface.

Recording and analysis choices can contribute further variation. Broader electrode coverage detects activity patterns that may be missed by sparse sampling, and pharmacological changes in firing can alter the units available for analysis [37, 68]. Comparing a prespecified primary analysis with a small number of biologically justified sensitivity analyses can reveal whether the conclusion depends on a narrow processing choice. The relevant outcome is stability of the treatment interpretation, not identical numerical values under every pipeline. If the effect reverses with modest changes in channel inclusion or response windows, the measurement itself becomes an important subject for further investigation. Culture engineering addresses a different source of variation—tissue production and maintenance; recent microfluidic and suspension-culture advances are summarized in Table 4.

5.4 *Mechanistic evidence and the scope of the conclusion*

Mechanistic support is strongest when different experiments address distinguishable parts of the proposed causal chain. Target engagement, changes in a downstream pathway, and improvement of a phenotype provide complementary information. The engineered AD culture experiments used pharmacological manipulation to examine relationships between Aβ generation and tau pathology, while APOE isogenic conversion examined the contribution of genotype to organoid abnormalities [6, 8]. More recent organoid studies have combined pathway perturbation with treatment responses, including manipulation of Wnt-related signaling during CI-994 exposure [57].

Parallel changes in pathology and function remain compatible with several mechanisms. A drug may act on a shared upstream process, alter one endpoint through another, or influence them independently. Temporal sampling and selective perturbations can help distinguish these explanations. The PHGDH study, for example, combined genetic and pharmacological approaches with pathological and injury-related outcomes, giving a broader basis for its mechanism than a single favorable marker change [54]. In other settings, the most defensible conclusion remains narrower: reduced abnormal synchrony during exposure, protection against an induced insult, or improvement of a specified pretreatment deficit. These statements retain the scientific value of an experiment while identifying the additional evidence needed for a broader therapeutic interpretation.

The same reasoning applies to molecular signatures associated with synaptic or cognitive processes. Changes in a gene set can identify a plausible pathway through which an intervention acts, but their functional significance depends on the experimental relationship to physiology. Proteomic measurements in serotonergic organoids reveal a donor-sensitive molecular response, while combined patch-clamp and proteomic measurements in derived neurons provide closer correspondence at the single-cell level [60, 78]. Table 3 links the main competing explanations to informative comparisons and the conclusions those comparisons can support.

Table 3. Controls that distinguish functional improvement from competing explanations.

Competing explanation	Informative comparison	What to record	Permissible interpretation
Protection mistaken for recovery [12, 13, 54]	Separate pretreatment, co-treatment and treatment after an established deficit.	Insult onset, functional baseline, exposure sequence and endpoint time.	A post-insult exposure is not sufficient to demonstrate rescue unless the relevant deficit was established before treatment.
General suppression mistaken for normalization [11, 41]	Treat reference tissue as well as disease tissue; pair spontaneous and evoked outcomes where feasible.	Baseline state, firing/burst changes and retained responsiveness.	An appropriate direction depends on the starting abnormality and reference response; quiescence alone is not restoration.
Acute action mistaken for persistence [41]	Measure before exposure, during exposure and over a specified washout interval.	Medium exchanges, sampling times, untreated trajectory and missing recordings.	Report persistence over the observed interval. Washout alone does not prove a permanent or disease-modifying effect.
Cell injury or contact loss mistaken for a functional response [41, 58, 67, 68]	Match tissue-condition measurements to the recording cohort; assess the interface separately.	Cell-death or injury measures, morphology, impedance and usable channels.	Impedance is not a viability gold standard. Distinguish neuronal loss, disengagement and altered firing.
Maturation or production variation mistaken for treatment [33, 34, 35, 84]	Use time-matched controls, repeated baselines and independent differentiations.	Culture age, regional/cellular identity, donor or line and batch.	A treatment-associated change must be interpreted against development and production history.
Nominal dose mistaken for equivalent tissue exposure [35, 45, 81]	Compare concentration ranges with matched geometry, medium handling and exposure duration.	Tissue size, matrix, exchange schedule, delivery conditions and endpoint-specific responses.	An active medium concentration does not establish equivalent local exposure or a validated therapeutic window.
Parallel outcomes mistaken for one mechanism [6, 8, 41, 54]	Identify same-organoid, matched-cohort and separate-regimen evidence; use selective perturbation.	Target engagement, downstream response, temporal order and sample correspondence.	Concordance supports a mechanism only to the extent that experiments distinguish plausible alternatives.
Technical abundance mistaken for biological replication [42, 85]	Model or summarize observations at their donor, batch and organoid levels.	Independent biological units, pooled tissue, repeated sessions and within-organoid measurements.	Many electrodes or imaging regions do not create additional independent organoids or donors.

Note. This table is a proposed interpretive framework, not a validated checklist or universal assay-acceptance standard. Comparisons should follow the biological question and preparation. Orthogonal evidence is most informative when its sampling and treatment schedule correspond to the functional measurement. Missingness, exclusions and pooled samples should be reported at the relevant biological level.

6. From individual experiments to reproducible drug evaluation

Section 5 showed how controls clarify a functional result within one experiment. That interpretation does not by itself establish when the result justifies advancing a candidate beyond that experiment. For the interventions examined in this section, three links address different risks to a rescue claim. A pretreatment deficit must be demonstrated because, without an initial abnormality, there is no functional deficit to rescue. Tissue exposure describes how much compound reaches cells throughout the tissue. Without that

information, a weak response can reflect inadequate exposure rather than biological inactivity. Tissue-condition measurements are also needed because injury can suppress activity and be mistaken for a beneficial reduction in abnormal firing. We argue that establishing these links is often more consequential for a rescue claim than adding another pathological marker [13, 14, 41]. Figure 3 mirrors this structure. Panel A accompanies Section 6.1 on model suitability, compound exposure, and functional readout analysis. Panels B and C accompany Section 6.2 on biological replication and longitudinal analysis. Panel D accompanies Section 6.3 on donor variation, transfer, and candidate prioritization.

6.1 *Model suitability, compound exposure, and functional readout analysis*

Here, a reproducible preparation is one for which independent differentiations yield comparable cell-type composition. That consistency supports a drug-evaluation claim only if the cells also represent the biological state through which the proposed effect would occur. Two cortical organoid studies show why composition reproducibility and fidelity to reference tissue must be assessed separately. Single-cell profiling of individual dorsal forebrain organoids compared cell populations across cell lines and differentiation batches and found consistent cortical cell-type composition [86]. A second study compared cortical organoids with primary human cortex and identified elevated stress pathways and impaired molecular subtype specification in the organoid cells [87]. The first study asked whether separate organoids resembled one another, whereas the second asked whether organoid cells resembled their primary counterparts. Consistency between organoids therefore does not establish how closely their cell states match developing cortex. Since the two studies used different preparations, the second is not a direct replication of the first. Together, they distinguish consistent cellular composition across production from fidelity of the resulting cell states to the reference tissue. A protocol can produce consistent composition without satisfying that fidelity requirement, leaving differences in maturity or stress state that affect the intended mechanism.

In AD models, the biological state also determines whether treatment addresses an AD-related process, the conditions used to induce the abnormality, or an interaction between them. In serum-exposed organoids, for example, BACE inhibition reduced amyloid-related measures under an experimentally imposed serum challenge [13]. That result supports a response within the serum-induced model. Extrapolation to endogenous disease processes requires a comparison connecting the induced state to those processes. Section 2.2 distinguishes the cellular interactions available in a preparation from the age-related state of its cells. A mechanism involving microglial responses requires the relevant cell interaction to be present [19, 31]. Without microglia, an improvement cannot be attributed to a microglia-mediated mechanism. And a claim about age-related vulnerability requires evidence that the modeled cells express the pertinent state because reprogramming through pluripotency can remove age-associated features (Section 2.2) [32].

Within a preparation selected for that biological question, low activity still has to be distinguished from failed tissue production or inadequate recording. Reduced firing in viable tissue can be a genuine disease phenotype. Poor electrode contact, however, can yield the same recording while the underlying network remains active. A low firing value therefore cannot distinguish a biological deficit from a recording failure

on its own. Distinguishing these possibilities requires measurements that fail in different ways. Time-matched reference and disease tissues establish the biological contrast expected before treatment. Tissue-condition assays determine whether injury could account for the recording. Interface measurements show whether neural activity was accessible to the electrodes. As one interface measurement, impedance can reveal a coupling problem, but it cannot establish neuronal viability [41, 42]. Viable tissue must therefore be assessed independently.

For tissue-condition assessment, a hierarchical framework in 60-day cortical organoids combined initial morphological and growth assessments with cellular organization and cytotoxicity measures, and its discrimination of tissue quality was evaluated using an oxidative-injury challenge [88]. That validation concerns tissue condition under the tested conditions, not the ability to detect AD therapeutic rescue. Morphological screening serves an earlier and coarser quality-control step. In unguided organoids, Feret diameter—the longest distance between two points on the organoid outline—was associated with expert quality assessment and the abundance of unintended mesenchymal cells [34]. This measure can help reject overtly unsuitable tissue, but it cannot establish that two accepted preparations are biologically comparable. Within one iPSC background, a microfluidic comparison used morphology, cytoarchitecture, transcript-expression consistency, and quality scores to assess production consistency and reported improvements in these outcomes [89].

These outcomes concern preparation stability within that genetic background, not fidelity to primary tissue or pharmacological responsiveness. The timing of quality checks determines whether they prevent technical artifacts or introduce selection bias. Removing organoids because they deteriorated after treatment can erase a drug-associated adverse outcome and preferentially retain preparations that tolerated the candidate. Removing tissue that failed before exposure defines the population entering the comparison. Post-treatment deterioration must remain part of the response assessment unless an independently supported technical explanation justifies exclusion.

Even after tissue and recording failures have been addressed, a readout may be too variable or too restricted in range to resolve the improvement being sought. Untreated and vehicle trajectories establish how much activity changes over the relevant observation period without the candidate, including culture-related drift. Reference perturbations show how strongly the preparation and recording system respond under specified conditions [66, 70]. Together, these observations define the background variation and effective measurement range against which a proposed drug effect must be resolved. A response to a strong excitatory or inhibitory probe can show that the system responds under that condition, but it does not validate rescue measurement. Demonstrating rescue requires resolution of the disease-associated difference and movement toward its reference state.

Even a readout with adequate resolution can return a weak response because too little compound reaches the cells. The concentration added to the medium is a boundary condition, not a measurement of how much compound reaches cells throughout a three-dimensional tissue (Section 5.1). A small change can therefore reflect limited access rather than limited biological activity. Controlled-delivery platforms support comparisons across specified exposures [81]. Diffusion measurements in neural stem-cell

spheroids illustrate an approach to studying transport [82]. That study used deuterated dimethyl sulfoxide, so it establishes a way to measure small-molecule transport rather than tissue exposure to an AD therapeutic. A negative result becomes interpretable only within the exposure and measurement ranges actually examined.

Even with adequate tissue exposure, a task-based readout can resolve only what its decoding or classification procedure can distinguish. In the stimulus-discrimination assays described in Section 4.4, a classifier uses features of evoked neural responses to predict which stimulus pattern was presented [74]. These assays matter here because activity amplitude and stimulus discrimination can respond differently to pharmacological perturbation. Drug exposure can change the distribution of classifier features, so retaining and refitting a classifier answer different scientific questions rather than offering interchangeable technical options. A classifier fitted before treatment tests whether the original response-to-stimulus mapping remains usable after exposure. A lower score can indicate loss of useful information, but it can also occur when information is represented differently and the fixed classifier no longer matches it. Refitting the classifier instead measures how well post-treatment responses can be decoded under the selected model and training procedure.

To distinguish a genuinely better biological representation from adaptation to the training data, the refitted classifier should be evaluated on observations held out from fitting. The held-out observations then determine the scope of the result. Cross-session generalization can be assessed by training on one recording session and evaluating on a held-out session from the same organoid. Donor-held-out evaluation instead tests performance in backgrounds absent from model development, provided that preprocessing and hyperparameter tuning are performed within the training data only. The donor-held-out result therefore depends on variation between donors as well as on the neural representation of the input. A drug comparison must hold the classifier choice, preprocessing, fitting, and test scheme constant, so that an apparent treatment effect is not produced by changing the decoding or testing procedure.

6.2 *Biological replication and longitudinal analysis*

Fixing the readout and the analysis procedure still leaves open how many independent observations the experiment actually contains. A drug effect is supported by independently treated units, so counting measurements made within one such unit as further replicates makes the estimate appear more precise than the design warrants. Lazic's distinction between biological, experimental, and observational units defines the levels at which these values arise [90]. When the biological question concerns organoids, each organoid is a biological unit. If treatment is assigned to wells containing several organoids, the well is the experimental unit because its organoids share one treatment allocation. Cells or recording sites within them remain observational units. In an AD organoid study of the HDAC/Wnt modulator W2A-28, three organoids were pooled into each biochemical sample, with three samples per treatment group [56]. Pooling three organoids into one lysate removed any record of their individual responses. Each treatment group therefore supplied three measured sample values, not nine independently observed organoid values. Whether those samples also provide independent treatment or donor replication depends on their allocation and origin. In the cholesterol-stressed forebrain organoid study, 120 calcium-imaging regions were sam-

pled from three organoids across three independent differentiations, and statistical comparisons used the three biological replicates [14]. Treating the 120 regions as independent would give repeated observations within the same tissues the evidential weight of independently generated preparations. These two designs show why uncertainty must be estimated from the independently treated units rather than from the number of recorded values [85, 90].

Once recorded values have been traced to independently treated units, the next question is whether systematic differences arise at that level. Distributing treatment and control units across batches prevents treatment from being systematically paired with one production history. Allocating units at random removes systematic differences in assignment, although imbalance can still arise by chance in small experiments. Masking the person who scores or excludes samples limits the influence of expectations at the point where judgment enters, even though exposure itself usually cannot be concealed in an organoid experiment. These safeguards address different sources of bias, and none substitutes for replication at the level required by the claim. If the intended conclusion concerns donor backgrounds, repeated measurements from a few donors can characterize those donors precisely while leaving between-donor variation poorly estimated. Expanding the study should target the dominant source of uncertainty. Additional donors address background variation. Independent differentiations address production variation within backgrounds. Repeated recordings address measurement variability under comparable biological conditions.

Repeated measurements of independently treated units support a treatment interpretation when their change is evaluated against a comparable untreated trajectory. An organoid's baseline captures its starting activity, whereas a time-matched vehicle group describes changes expected during continued culture. If treatment and control groups are otherwise comparable, the difference between their changes helps separate the candidate-associated response from maturation over the same interval. Organoid-derived networks change with developmental stage as well as with line and batch [84]. A time-matched control trajectory is therefore required before any change can be attributed to the drug. In familial AD cortical organoids treated with CCT020312, longitudinal recordings extended this comparison beyond the exposure period [41]. PSEN1-mutant organoids had elevated firing, which decreased with treatment and remained lower during recordings obtained one to three days after washout. Wild-type preparations did not show an equivalent response. These recordings support a transient normalization of abnormal firing over the reported one-to-three-day post-washout window. They do not establish permanent recovery, and washout alone does not demonstrate complete removal of compound from tissue.

A trajectory difference represents the original treated and control samples directly only when their units remain observable. Missing records change which population a trajectory describes. If treatment-associated injury eliminates recordings, an analysis of surviving active organoids can overstate preservation of function. Loss of electrode contact leaves the underlying activity uncertain. An acquisition failure removes an observation whose relationship to tissue state may be unknown. A trajectory based on complete records therefore directly describes the retained subset, and extending it to the original population requires examining why records were lost. Confounding raises a different problem: whether

the effect can be identified at all. When all disease samples come from one batch and all controls from another, no model can separate the two influences because the design never varies them independently. Hierarchical or repeated-measures models represent dependencies within an informative design, but they cannot supply the missing comparison.

A credible trajectory still needs a specified comparison to carry the main treatment claim. The outcome, time window, and experimental unit together define the treatment effect being estimated. For example, the contrast might be change in abnormal firing relative to vehicle during exposure or persistence of that change during a defined washout period. Choosing the outcome, time window, or experimental unit after inspecting many endpoints risks selecting a favorable result whose evidential weight is overstated. Effect estimates and uncertainty intervals then indicate how precisely the chosen comparison is resolved [42, 85]. An interval spanning effects of practical benefit and harm leaves the decision open. An interval lying entirely on one side of zero may support a direction while remaining too broad to establish a useful magnitude. Molecular, structural, or tissue-condition measurements explain the functional result only when they come from the same samples at a comparable time. Otherwise the observations sit side by side without either accounting for the other. In cholesterol-stressed forebrain organoids, semaglutide normalized calcium-event frequency at the reported endpoint, while synchrony recovered incompletely and several structural or protein measures remained abnormal [14]. These outcome-specific differences define the conditions under which a response can be claimed and prevent the calcium result from standing for recovery of the whole preparation.

Sample matching across assays imposes a separate limit on interpretation. In the CCT020312 report, electrophysiology, synaptic proteins, and other tissue-condition assays included separate regimens or cohorts [41]. The resulting electrophysiological, synaptic-protein, and tissue-condition findings support related treatment effects, but they do not establish that all changes occurred together in the recorded organoids. Molecular measurements collected after recording can characterize those same tissues at harvest. Matched parallel samples support a group-level connection with less direct individual correspondence. Even in the same tissue, co-occurring changes support an association. Establishing that one mediates the other requires additional temporal or mechanistic evidence. Multiplexed single-cell approaches demonstrate a different correspondence because donor identities can be retained across sampled populations [91]. Because sampling is destructive, however, this approach cannot recover repeated observations of the same cell. Each endpoint should therefore be linked to its source samples, exposure schedule, and analysis, with planned comparisons and reasons for missing or excluded observations recorded alongside. Once effects have been connected across assays within a preparation, the next question is whether they recur across donor backgrounds.

6.3 *Donor variation, transfer, and candidate prioritization*

Within-preparation comparisons establish how molecular and functional effects correspond. A donor-related finding additionally requires the response to recur across independent differentiations rather than appearing in only one production run. Evidence from one production stage cannot simply be carried into the next. Analyses of donor genetic variation across starting iPSCs

have linked it to molecular and cellular phenotypes, including differentiation-related properties [92]. This establishes a source of variation before organoid generation, but it does not determine how much of an AD drug response it explains. The differentiation step can change the biological object being measured. AD patient-derived lines have generated organoids with different regional identities and neuronal responsiveness, limiting a direct attribution of their functional differences to disease status [93]. In serotonergic hindbrain organoids derived from 30 donors, escitalopram-associated proteomic changes varied across the cohort [60]. These proteomic results demonstrate heterogeneous molecular responses. Whether the variation reflects stable donor-related drug sensitivity depends on its recurrence across differentiations and on the corresponding cellular composition. Isogenic comparisons change genotype within one background, whereas comparisons across additional donors test background breadth, and repeated differentiations test persistence through tissue production. Each addresses a different source of uncertainty, so no single comparison can substitute for the other two. A response subgroup defined before analysis can be assessed against its stated criteria. A subgroup discovered by clustering the observed drug responses should be treated as exploratory, especially when technical or compositional features may drive the grouping. Neither form of organoid subgroup establishes clinical response stratification on its own. Clinical stratification instead requires evidence connecting the organoid response to outcomes in matched patients and evaluating the relationship beyond the samples used to define it.

A donor-associated response must next be evaluated under the conditions to which it is intended to transfer. Successful tissue production at another site does not establish transfer of the drug effect. In a coordinated study, three centers generated cortical organoids from the same PGP1 iPSC line and compared their phenotypes across differentiation replicates [94]. Cell-type proportions and cortical organization were relatively consistent, although organoid size and metabolic or stress-related expression differed between sites. These measurements support transfer of selected tissue properties under the harmonized protocol. That coordinated study did not test whether an AD candidate produced the same functional response across centers.

Transfer of a drug effect requires agreement on the aspect relevant to its intended use. Direction may suffice for confirming an initial activity signal, whereas quantitative candidate comparison also depends on magnitude and precision under comparable exposures. A background-specific claim additionally requires agreement within the stated donor group. Common reference preparations and perturbations help interpret platform differences by showing whether baseline measurements or responses to the same probe shift between systems. They cannot reveal behavior specific to a particular candidate on a particular platform, or to a particular donor-platform combination. For example, electrode coverage changes which activity is sampled [68]. A shared analysis pipeline makes processing choices comparable across sites [69]. Even when the medium concentration matches, differences in culture conditions and tissue geometry can produce different local exposures. Observed agreement therefore has to be tied to the preparation, exposure, recording interface, and analysis actually compared. A residual discrepancy should remain in the estimated uncertainty and in the stated scope of the result. Removing it by normalization without examining its source would

obscure precisely the variation that the transfer study was intended to assess.

Candidate advancement therefore requires a specific next claim rather than a general judgment that the candidate looks promising. We propose judging that claim from the response profile, the reliability of its main comparison, and the backgrounds and settings in which the effect recurs. The most useful next observation is the one likely to change the decision about advancing the candidate on that basis. In serum-exposed organoids, BACE inhibition reduced amyloid-related measures while phosphorylated tau and synaptic puncta failed to recover [13]. This result supports further investigation of a pathological action, but it cannot carry a claim of restored synaptic function. For a synaptic-rescue claim, the missing functional recovery matters more than another favorable amyloid assay. The semaglutide cholesterol-stress example reaches a different point: calcium-event frequency improved, yet synchrony and other outcomes limited the extent of recovery [14]. Independent confirmation of that specific functional response and investigation of the persistent deficits are the appropriate next steps. CCT020312 provides a defined post-washout observation interval [41]. Its one-to-three-day post-washout observations make confirmation of persistence over that interval and correspondence with tissue condition appropriate next steps. Establishing recovery beyond this interval would require longer post-washout observations. A hypothetical increase in activity accompanied by worsening viability would represent an adverse pattern rather than evidence of benefit. Selective survival or tissue injury could then alter the recorded population, so the adverse finding needs to be resolved before the activity change is advanced as a useful benefit. These examples follow the relationships shown in Figure 3 without assigning a common score to heterogeneous endpoints. Follow-up comparisons should be motivated by the identified mechanism or temporal gap and tested independently, so that searching additional endpoints does not become a way to preserve a preferred candidate. The Figure 3 framework supports decisions about further preclinical evaluation. Any decision thresholds subsequently adopted, and any ability to predict patient benefit, require dedicated validation for the intended clinical population and outcome.

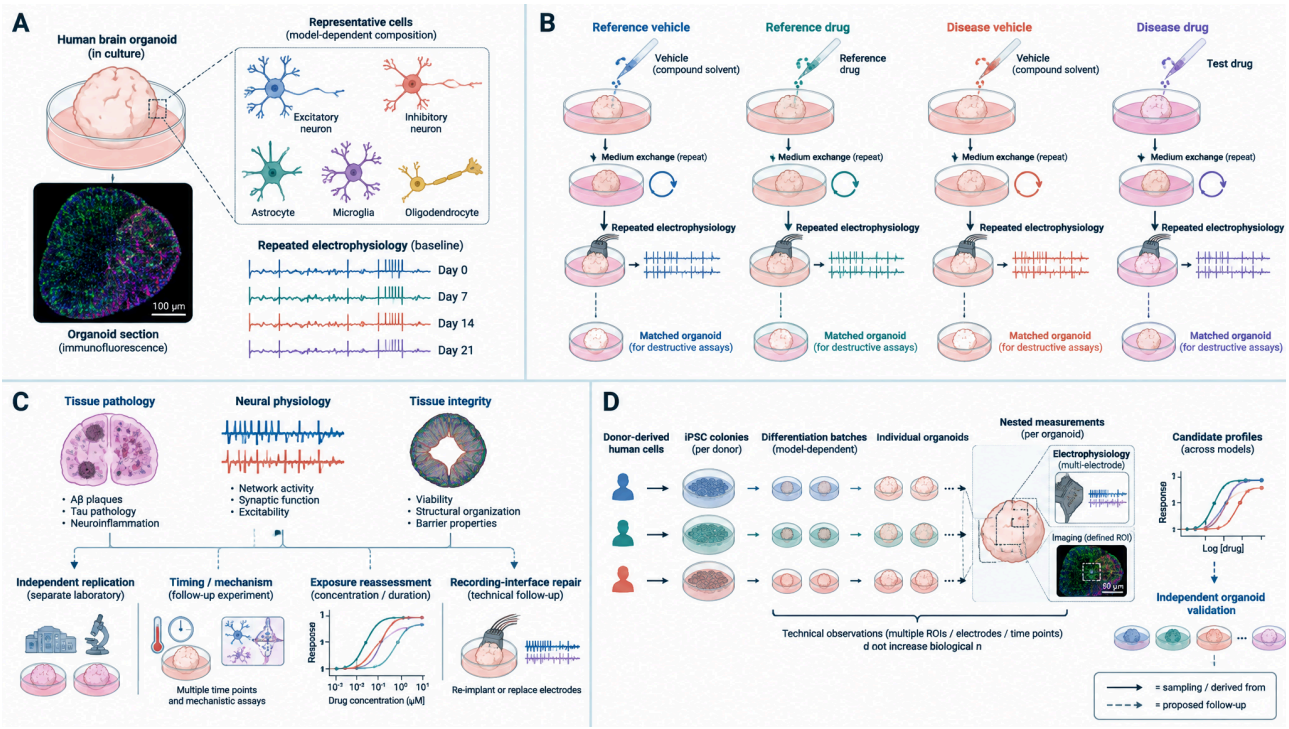

Figure 3. A proposed framework for evaluating organoid drug-response evidence, organized around the three stages of Section 6. (A) The first stage—model suitability, compound exposure, and functional readout analysis—links tissue identity, cellular composition, the disease-associated state, and baseline measurements to the intended research question (Section 6.1). (B) The second stage—biological replication and longitudinal analysis—specifies exposure, tissue geometry, and observation times through matched vehicle and candidate comparisons. Longitudinal recording and destructive endpoint assays require explicit sample correspondence (Sections 5.1 and 6.2). (C) The second stage also relates pathology, neural function, and tissue condition to the main functional comparison and the cohorts from which each result was obtained (Section 6.2). Concordant, discordant, adverse, and technically incomplete outcomes support different follow-up questions. (D) The third stage—donor variation, transfer, and candidate prioritization—uses replication across independently treated units, differentiation batches, donors, and sites to define the scope of candidate advancement (Sections 6.2 and 6.3). Observations within a shared experimental unit remain dependent. The schematic presents the authors' interpretive proposal. Decision thresholds and clinical predictive performance require validation for the intended use [34, 41, 42, 45, 81, 84, 85]. AD, Alzheimer's disease.

Table 4. Recent methodological advances relevant to interpretable organoid pharmacology.

Methodological advance	Representative capability (sources)	Variability or artifact addressed	Relevant section
Multicellular and vascularized preparations	Microglial and monocyte interactions in neuroimmune preparations; a genome-wide CRISPR screen in iPSC-derived oligodendrocytes followed by myelination assessment in brain organoids; APOE3-Christchurch microglial effects; vascularized assembloids and blood-brain barrier (BBB)-like structures [95, 96, 97, 98, 99, 100, 101]	Cell-type-specific pathology and cell-cell interactions become measurable intervention targets	§2.2, §3
Chronic and cellular-resolution recording	Kirigami electronics for suspended organoids; whole-cell patch clamp with morphological characterization in intact organoids [80, 102]	Recording-interface access, longitudinal sampling depth, single-cell resolution	§4.1, §6.3
Controlled culture engineering	Dynamic three-dimensional microfluidic perturbation; standardized microfluidic culture; uniform-shear suspension organoids-on-a-chip [89, 103, 104]	Core necrosis and culture non-uniformity; reported batch consistency is preparation-specific and requires validation across donor lines.	§5.3, §6.1
Open neuroplatforms	Remotely accessible platform for electrophysiological wetware-computing experiments [105]	Remote access and automated longitudinal recording; cross-site assay reproducibility remains to be established.	§4.4

Note. Entries are representative rather than exhaustive; section numbers indicate where each advance is relevant; the preparations and validation depth differ across studies. BBB, blood-brain barrier; CRISPR, clustered regularly interspaced short palindromic repeats.

7. Conclusions and research priorities

Human brain organoid studies have established several forms of pharmacological response relevant to AD. Early work demonstrated that amyloid and tau-related abnormalities could be modified in human three-dimensional preparations. Subsequent studies have connected interventions to abnormal network activity, synaptic markers, tissue injury, inflammatory responses, and donor-dependent molecular variation. The evidence is strongest when these outcomes are attributed to the specific model, treatment schedule, and measurement domain in which they were obtained. The resulting picture is heterogeneous but informative: organoids already support experimental comparison of drug actions, while the relationship between those actions and functional rescue remains a question that can be tested with increasing precision.

Three experimental priorities follow from the comparison. First, pathological, functional, and viability-related outcomes should be linked more closely within matched treatment schedules. This would help determine whether their divergence reflects biological separation, different response times, or differences between sampled tissues. Second, experiments that compare treatment before an insult with treatment after a measured deficit would clarify the relationship between protection and recovery. Third, promising effects require replication across independent biological preparations with enough infor-

mation to distinguish donor, batch, and recording variation.

Task-based phenotyping offers an additional opportunity within this programme. The emerging evidence shows that input discrimination, evoked potentiation, and feedback-dependent performance can reveal properties not captured by mean activity alone [70, 72, 74, 75]. Their next contribution to AD pharmacology will depend on experiments connecting a disease-associated task deficit to a controlled intervention and complementary biological measurements. Such studies could identify compounds whose effects on spontaneous activity and information transformation diverge, or determine whether changes in a conventional marker predict changes in a defined task. The choice of task should follow the functional question and the biology of the preparation.

Functional rescue is ultimately an endpoint-specific relationship between an established deficit and an interpretable treatment effect. Its value does not depend on a universal score or on making every outcome move in the same direction. A response profile that preserves pathology, physiology, tissue condition, and experimental timing gives researchers a clearer account of what an intervention accomplishes. By connecting that account to reproducible measurements and targeted follow-up experiments, brain organoid studies can make a more precise contribution to candidate evaluation and to understanding how AD-related pathology becomes neuronal dysfunction.

This contribution can be valuable before a preparation predicts individual treatment response in patients. An organoid experiment may help distinguish mechanisms, identify a vulnerable cellular interaction, or decide which concentration and outcome warrant testing in a more complex setting. Clinical translation then becomes a further empirical question involving exposure, disease stage, and outcomes beyond the culture system. Maintaining that sequence allows organoid pharmacology to develop through demonstrable gains in explanation and experimental decision-making, with functional rescue defined by the evidence accumulated for each use.

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