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Store-Operated Calcium Entry in Peripheral Blood Mononuclear Cells as a Translational Biomarker of CNS Plasticity: A Mechanistic Hypothesis for Neuroplastogen Drug Programs

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Abstract

Background: *Psychedelic and neuroplastogen drug development programs require biomarkers capable of bridging intracellular pharmacology to clinically interpretable endpoints. Existing peripheral approaches, most notably plasma BDNF measurement, have demonstrated inconsistent results, with meta-analytic evidence reporting only a small, statistically marginal effect size in ketamine responders (SMD 0.26, 95% CI 0.03–0.48, p = 0.02) and no consistent correlation with symptom reduction. Critically, the peripheral biomarker field for neuropsychiatric disorders is characterised by high heterogeneity and systematic effect size inflation, placing a high evidential bar on any new candidate.*

Hypothesis: *We propose that store-operated calcium entry (SOCE), measured in patient-derived PBMCs using validated flow cytometric methods, may serve as a mechanistically specific pharmacodynamic readout of systemic 5-HT2A receptor engagement. We further propose that repeated sub-threshold 5-HT2A engagement produces oscillatory Ca2+ signals that engage the Ca2+-calcineurin-NFAT transcriptional feedback loop, progressively shifting the ORAI1:ORAI2 expression ratio and increasing thapsigargin-evoked SOCE amplitude as a cumulative readout of prior receptor engagement.*

Bipolar Disorder Precedent: *Critically, the logic of this assay has a direct published precedent in peripheral lymphocyte-derived cells. Thapsigargin-evoked SOCE is significantly elevated in lymphoblastoid cells from bipolar I disorder patients versus controls, and is attenuated by chronic lithium and valproate treatment but not by lamotrigine — providing drug-specific pharmacodynamic discrimination via peripheral lymphocyte SOCE. This body of evidence validates the principle that thapsigargin-SOCE in peripheral lymphocyte-derived cells reflects CNS disease state and is sensitive to mechanistically relevant psychotropic drug action.*

Proposed Methodology: *Thapsigargin-based passive ER store depletion followed by extracellular calcium readdition, measured by Indo-1 AM flow cytometry in freshly isolated human PBMCs, with cell-subset resolution using standard lymphocyte surface markers and companion ORAI1:ORAI2 mRNA quantification by qRT-PCR in sorted CD4+ naive T cells.*

Implications: *SOCE dynamics in patient-derived PBMCs represent a mechanistically grounded, technically validated, and currently underexplored candidate for exploratory pharmacodynamic endpoint development in neuroplastogen programs — particularly non-hallucinogenic programs where the psychedelic experience can no longer serve as a proxy for target engagement.*

Keywords: Store-Operated Calcium Entry, SOCE, ORAI, PBMCs, Biomarker, Neuroplasticity, Psychedelic, Psychoplastogen, Neuroplastogen, 5-HT2A, Translational Neuroscience

1. Introduction

Psychedelic-assisted therapies and emerging neuroplastogen programs have generated compelling early clinical signals across depression, PTSD, and substance use disorders. However, the evidentiary structure supporting these signals remains methodologically challenged. A systematic review and meta-analysis published in *JAMA Psychiatry* in 2026 demonstrated that psychedelic-assisted therapy was no more effective than open-label traditional antidepressants under equal unblinding conditions, confirming that psychedelic trials are effectively always open-label regardless of design intent [1]. If clinical benefit cannot be separated from expectancy effects through trial design alone, pharmacological target engagement must be demonstrable through objective biological readouts that exist independently of the therapeutic context.

The translational challenge is compounded at the cellular level. Preclinical studies consistently demonstrate that psychedelic and neuroplastogen compounds increase dendritic spine density, modulate synaptic proteins, and activate plasticity-related signalling cascades, supporting their description as psychoplastogens [2]. A molecular marker of psychedelic-induced neuroplasticity measurable across cell culture, rodent models, and human patients has not yet been validated [3]. The gap between cellular plasticity data and clinical outcomes — where the therapeutic mechanism likely resides — remains one of the most consequential open problems in the field. Calcium signalling dysregulation is increasingly recognised as a central feature of depression pathophysiology, with advances in Ca^{2+} signalling research identified as a promising avenue for both diagnostic biomarker development and therapeutic targeting [4]. The present paper proposes that store-operated calcium entry (SOCE) dynamics measured in patient-derived PBMCs may serve as an accessible, mechanistically grounded pharmacodynamic readout of the intracellular calcium architecture downstream of 5-HT2A receptor engagement. A critical distinction governs the proposed claim: the SOCE readout is framed as a pharmacodynamic biomarker of *systemic* serotonergic receptor engagement — not a CNS proxy. Peripheral lymphocytes express 5-HT2A receptors that are engaged by the same systemically distributed compound as their CNS counterparts; the peripheral assay reports that the compound has activated the receptor cascade downstream of 5-HT2A in a cell type where this is measurable, not that it has reached the brain specifically.

2. The Peripheral Biomarker Landscape

2.1. Blood-Based Biomarkers and the BDNF Experience

Blood-based biomarkers have emerged as practical tools in CNS drug development. The most extensively studied peripheral biomarker of CNS plasticity induction is brain-derived neurotrophic factor (BDNF). Haile et al. (2014) demonstrated that plasma BDNF at 240 minutes post-ketamine infusion predicted antidepressant response in treatment-resistant depression [5]. However, the aggregate meta-analytic literature is considerably weaker: ketamine responders show small but statistically marginal BDNF increases (SMD 0.26, 95% CI 0.03–0.48, $p = 0.02$), with mixed results for the correlation between BDNF increases and symptom reduction [6]. Two independent meta-analyses confirm that peripheral BDNF does not change consistently after psychoplastogen administration in humans, with massive heterogeneity ($I^2 = 96.8\%$) precluding any reliable clinical use [7,8].

A systematic methodological analysis of 31 meta-analyses evaluating peripheral biomarkers in MDD identified high heterogeneity ($I^2 \geq 50\%$) in 29 of them, effect size inflation above the largest single study in 25, and excess statistically significant studies in 21. Carvalho et al. (2016) concluded that the peripheral psychiatric biomarker literature is systematically contaminated by publication bias and inflated effect sizes [9]. This finding applies to the SOCE hypothesis by extension, and makes a pre-registered, adequately powered validation design non-optional for any serious biomarker claim in this space. Large-scale Mendelian randomisation across 4,907 plasma proteins (Bhattacharyya et al., 2025, *JAMA Psychiatry*) identified 113 Bonferroni-corrected associations with schizophrenia, bipolar disorder, MDD, and cognitive performance [10]. Immune-related proteins showed pleiotropic effects across conditions. This defines the current proteomics frontier — genetically anchored causal inference — against which any functional assay-based approach must be positioned as mechanistically specific rather than discovery-grade.

2.2. PBMC Serotonergic Signalling: the Translational Basis

A converging body of evidence establishes that PBMCs express functional serotonergic machinery that reflects CNS disease state. Amidfar et al. (2017) demonstrated that 5-HT2A receptor mRNA is significantly upregulated in PBMCs of MDD patients ($Z = -3.875$, $p < 0.05$), with strong positive correlations with HDRS scores ($rs = 0.902$, $p < 0.001$) [11]. In late-onset Alzheimer's disease, 5-HTR2A, 5-HTR3A, and MAO-A mRNA expression were all significantly downregulated in patient PBMCs compared to age-matched controls, mirroring CNS serotonergic alterations in post-mortem tissue [12]. In schizophrenia, 5-HT2A receptor mRNA expression in PBMCs has been confirmed with peripheral blood lymphocytes proposed as indicators of CNS serotonergic receptor gene expression changes [13]. Beyond neuropsychiatric conditions, upregulation of 5-HT2A mRNA in PBMCs of asthmatic patients has been documented [14].

2.3. Peripheral Serotonin and Serotonin Receptor Signalling Regulate ORAI Expression

A mechanistically critical finding establishes direct regulation of ORAI channel expression by peripheral serotonin signalling. Horseman et al. (2014) demonstrated in tryptophan hydroxylase 1

knockout mice that peripheral serotonin deficiency causes decreased expression and disrupted basolateral membrane localisation of ORAI-1 in mammary epithelial cells, implicating PLCβ3, PKC, and ERK1/2 as mediators [15]. Zhou et al. (2025) demonstrated that elevated 5-HTR7 signalling in human megakaryocytes upregulates Orai1 expression via the PKA/ERK1/2 pathway [16]. Together, these findings establish that serotonin receptor signalling directly controls ORAI transcriptional output in peripheral non-immune cells, with relevance to the Gq-coupled and Gs-coupled pathways engaged by neuroplastogen compounds.

2.4. Functional 5-HT2A–SOCE Linkage in Peripheral Cells

Direct functional evidence for 5-HT2A-SOCE coupling in peripheral cells was provided by three independent experimental systems. Machida et al. (2017) demonstrated in vascular smooth muscle cells that sarpogrelate — a selective 5-HT2A antagonist — completely inhibited 5-HT-induced Ca^{2+} increase and partially but significantly inhibited Ca^{2+} influx upon extracellular calcium readdition in calcium-free conditions, consistent with 5-HT2A-specific SOCE [17]. Linden et al. (2016) demonstrated that autocrine 5-HTR2A-Gq-PLCβ signalling amplifies ORAI1-mediated SOCE during platelet activation in serotonin transporter knockout mice, measured using thapsigargin-evoked SOCE [18]. Kanda et al. (2016) demonstrated that clozapine — a 5-HT2A antagonist at therapeutic concentrations — bidirectionally modulates SOCE in 5-HT2A-expressing cells [19].

2.5. Lymphocyte SOCE as a Pharmacodynamic Readout: the Bipolar Disorder Precedent

The most direct published precedent for the proposed assay comes from the bipolar disorder calcium literature, a body of evidence predating the neuroplastogen field by two decades. Kato et al. (2003) demonstrated that thapsigargin-evoked cytosolic Ca^{2+} responses are significantly elevated in transformed lymphoblastoid cell lines (LCLs) from bipolar I disorder patients compared to controls ($p < 0.05$) [34]. Critically, the enhanced response was abolished in Ca^{2+}-free buffer — confirming that the difference resides in SOCE specifically, not in ER calcium content. This is the first published demonstration that thapsigargin-SOCE in peripheral lymphocyte-derived cells carries a CNS disease-relevant signal. Wasserman et al. (2004) extended this finding pharmacologically: chronic treatment with therapeutic concentrations of lithium (0.75 mM, 7 days) significantly attenuated thapsigargin-evoked SOCE in bipolar I disorder LCLs ($F = 8.36$, $p = 0.007$), alongside LPA-stimulated Ca^{2+} responses. Acute lithium exposure had no effect, confirming that the pharmacodynamic signal requires sustained drug exposure to emerge — a temporal profile directly analogous to the cumulative transcriptional readout argument for the ORAI1:ORAI2 ratio proposed here [35]. Perova et al. (2010) demonstrated that chronic valproate treatment similarly attenuated thapsigargin-evoked SOCE (−10% to −19%, $p < 0.05$) in bipolar I disorder LCLs.

Critically, chronic lamotrigine treatment — an anticonvulsant mood stabiliser whose mechanism does not involve Ca^{2+} homeostasis directly — did not affect SOCE [36]. This drug-specific pharmacodynamic fingerprint — lithium and valproate attenuate peripheral LCL SOCE, lamotrigine does not — establishes that thapsigargin-SOCE in peripheral lymphocyte-derived cells can discriminate between mechanistically distinct CNS drugs. This is the closest published precedent for the pharmacodynamic application proposed here. An important cell-type caveat must be noted: these precedent studies used EBV-transformed B lymphoblastoid cell lines, not primary peripheral T lymphocytes. Immortalisation may alter SOCE properties, ORAI expression ratios, and drug sensitivity relative to primary cells. The degree to which primary naive $CD4^+$ T cells show analogous SOCE pharmacodynamics is a tractable experimental question that constitutes the primary pre-clinical validation objective (see Validation Pathway).

2.6. Electrophysiological and Network-Level Approaches

Electrophysiological biomarker strategies have gained traction in parallel with peripheral molecular approaches. Skosnik et al. (2023) reported that EEG theta power doubled in amplitude two weeks after a single psychedelic dose of psilocybin, with improvements in depression correlating with theta power increases [20]. At the network level, a precision functional mapping study published in *Nature* (2024) demonstrated that psilocybin massively disrupted functional connectivity across cortex and subcortex, with persistent suppression of hippocampal-DMN connectivity proposed as a pro-plasticity neuroanatomical correlate [21]. The peripheral molecular readout proposed here is not an alternative to these approaches but a mechanistically grounded cellular substrate that anchors functional readouts to specific pharmacological targets.

2.7. PBMCs as a Window into CNS Biology: the PLCγ2 Precedent

Beyond serotonergic receptor expression, PBMCs have attracted interest as proxies for CNS pathological processes through shared molecular machinery. Phongpreecha et al. (2020) identified reduced PLCγ2 activation across multiple PBMC subsets as a distinguishing feature of Alzheimer's disease patients in a mass cytometric study of 132 participants, proposing enhanced PLCγ2 activity as a therapeutic target with a readily accessible pharmacodynamic biomarker in PBMCs [22]. PLCγ2 sits immediately upstream of IP3 production and therefore directly upstream of the ER calcium release cascade that triggers SOCE, establishing direct precedent for PBMC-based measurement of CNS-relevant calcium signalling dysfunction.

3. The Soce Hypothesis

3.1. Intracellular Calcium Architecture Downstream of 5-HT2A

5-HT2A receptor activation couples primarily to Gq/11 proteins, driving PLCβ activation and the consequent production of IP3 and diacylglycerol. IP3-mediated ER calcium release elevates cytosolic calcium, activating PKC, CaMKII, and calcineurin-dependent pathways. Parallel β-arrestin recruitment engages ERK1/2 and mTOR signalling, linking acute receptor activation to translational control of synaptic protein synthesis including PSD-95 and GluA1.

Recent evidence from intracellular 5-HT2A receptor localisation studies suggests that psychedelic-specific membrane permeability enables engagement of an intracellular receptor pool whose Gq-calcium signalling may be mechanistically distinct from surface receptor activation [23].

The initial IP3-mediated calcium transient is sustained and amplified by SOCE. ER calcium depletion activates STIM proteins, which gate ORAI channels at the plasma membrane, enabling sustained calcium influx that extends the cytosolic calcium plateau into a transcriptionally relevant duration. This SOCE-mediated amplification drives calcium-dependent activation of CREB, NFAT, and MEF2, alongside immediate early genes including c-Fos and Egr1. The amplitude and duration of this plateau — critically shaped by SOCE dynamics — determines whether signalling produces durable structural remodelling or remains transient. Published work from this laboratory is directly relevant. Agostini et al. (2020) demonstrated that ORAI2 downregulation potentiates SOCE and reduces amyloid-β42 accumulation in human neuroglioma cells, establishing that SOCE modulation has functional consequences for neuronal calcium homeostasis in a CNS disease context [24].

3.2. ORAI Isoform Biology: Oscillatory Versus Plateau Calcium Signals, and Calcineurin-Nfat Feedback

Yoast et al. (2020) established that ORAI1 generates sustained Ca^{2+} plateaus under near-complete ER store depletion, while ORAI2 and ORAI3 generate Ca2+ oscillations under physiological GPCR stimulation at sub-maximal concentrations, owing to their enhanced basal STIM1-binding affinity and heightened Ca^{2+}-dependent inactivation (CDI) [25]. NFAT1 requires the large ORAI1-mediated plateau for nuclear translocation; NFAT4 is activated by lower-amplitude oscillatory signals and is the isoform most sensitive to repeated sub-maximal stimulation (Liang et al. 2003). Non-hallucinogenic 5-HT2A agonists are defined by sub-threshold Gq efficacy and do not fully deplete ER stores at therapeutic doses. The resulting Ca^{2+} signal in 5-HT2A-expressing T cells under therapeutic dosing is therefore most likely oscillatory and ORAI2/3-mediated. Repeated oscillatory SOCE signals engage NFAT4 and the Ca^{2+}-calcineurin-NFAT positive feedback loop.

Direct experimental evidence that this pathway upregulates ORAI and STIM expression comes from Daskoulidou et al. (2015), who demonstrated that chronic calcineurin-NFAT signalling drives transcriptional upregulation of ORAI1–3 and STIM1–2, with upregulation prevented by cyclosporin A and NFATc3 siRNA [26]. The naive-to-effector T cell transition involves a well-established ORAI1:ORAI2 ratio shift: ORAI2 expression is highest in naive $CD4^+$ T cells and decreases as cells transition to effector state, while ORAI1 expression increases [27]. This reframing has a critical practical consequence for clinical trial design: the thapsigargin assay does not require precise timing relative to dosing. Blood can be drawn at any point in the dosing cycle, because the assay reports the integral of prior transcriptional responses — making PBMC SOCE considerably more feasible as a clinical trial pharmacodynamic endpoint than strategies requiring tight sampling windows around drug administration.

3.3. Mitochondrial Calcium Coupling

Calcium released from the ER is partially transferred to mitochondria through microdomain coupling at mitochondria-associated membranes. The mitochondrial calcium uniporter (MCU) regulates calcium entry into the matrix, stimulating TCA cycle dehydrogenases and enhancing ATP production. Critically, mitochondrial calcium uptake prevents premature calcium-dependent inactivation of CRAC channels, sustaining SOCE duration and extending the transcriptionally relevant calcium signal. This ER–mitochondria–SOCE feedback architecture has been directly demonstrated in T lymphocytes using imaging flow cytometry [28].

3.4. Why PBMCs: the Convergent Evidence Argument

The rationale for using PBMCs as a pharmacodynamic readout of 5-HT2A-driven SOCE rests on four converging lines of evidence. First, the canonical SOCE machinery — STIM1/2 and ORAI1/2 — was originally characterised in T lymphocytes and mast cells before its neuronal functions were established [29]. Targeted disruption of Orai1 in mice results in substantially decreased Ca2+ influx and functional defects in T cell-mediated immunity, establishing the physiological importance of ORAI1-mediated SOCE in peripheral lymphocytes [30]. Second, multiple independent studies confirm that 5-HT2A receptors are expressed and disease-state-regulated in human PBMCs across depression, Alzheimer's disease, and schizophrenia [11-14]. Third, peripheral serotonin availability directly regulates ORAI-1 expression, and 5-HT2A receptor activation drives SOCE in peripheral vascular and blood cells [15-19]. Fourth, the PLCγ2 PBMC findings in Alzheimer's disease establish that upstream disruption of the IP3-generating cascade is detectable in patient peripheral blood. And fifth — critically — the bipolar disorder lymphoblast literature establishes that thapsigargin-SOCE in peripheral lymphocyte-derived cells is sensitive to CNS disease state and responds pharmacodynamically to clinically relevant psychotropic drug treatment [22,34-36].

3.5. Multi-Receptor Convergence on SOCE

The SOCE biomarker retains mechanistic relevance across the full landscape of serotonergic drug development. The 5-HT2 family (2A, 2B, 2C) operates through canonical Gq–PLCβ–IP3 coupling, directly depleting ER stores and activating STIM1/ORAI1. The 5-HT1A receptor modulates SOCE through Gβγ-mediated PLCβ2/β3 activation generating IP3 via a non-canonical route, through reduced cAMP/PKA-mediated alteration of ORAI1 CDI kinetics and STIM1 phosphorylation state (Thompson & Shuttleworth 2015), and through indirect effects on ER filling via VGCC inhibition [31,33]. The 5-HT7 receptor transcriptionally upregulates ORAI1 via the PKA/ERK1/2 axis [16]. Critically, the thapsigargin SOCE assay does not require knowledge of which upstream receptor was engaged. By bypassing receptor-mediated store depletion entirely, the assay reports accumulated CRAC channel capacity: the ORAI1:ORAI2 ratio, STIM1 expression

level, and post-translational modification state shaped by the totality of prior serotonergic engagement. For mixed agonists with activity at multiple receptor subtypes, the SOCE readout captures the net pharmacodynamic signal. The companion qRT-PCR endpoint (ORAI1:ORAI2 mRNA ratio in sorted naive T cells) provides mechanistic resolution, distinguishing transcriptional from post-translational SOCE modulation.

4. Proposed Methodology

4.1. Rationale for Thapsigargin-Based SOCE Isolation

Thapsigargin (TG), a specific SERCA pump inhibitor, depletes ER calcium stores without activating IP3 receptors directly, providing clean pharmacological isolation of the SOCE component. This approach has been validated in human PBMCs by flow cytometry: TG at 50 ng/mL significantly elevated mean intracellular Ca^{2+} from 18.4 to 164.5 nM in the PBMC population, with downstream activation of NFAT-dependent gene programs [37]. Importantly, in human B cell subsets the plateau phase of the Ca^{2+} flux curve — which represents SOCE-mediated sustained entry — selectively activates the NFAT pathway, while peak amplitude activates NFκB and c-Jun pathways, with these differences quantifiable by the FacsKin mathematical fitting algorithm applied to kinetic flow cytometry data [38]. A practical note on assay design: anti-IgD labeling during B cell surface staining pre-activates naive B cells and substantially blunts subsequent SOCE. By analogy, the potential for surface labeling antibodies to pre-activate T cells via TCR or CD4 crosslinking should be evaluated during assay optimisation and mitigated by sequential rather than concurrent labeling where necessary.

4.2. Assay Protocol

- **PBMC Isolation:** Freshly isolated PBMCs from venous blood via Ficoll-Paque density gradient, processed within 2 hours of collection.
- **Calcium Dye Loading:** Indo-1 AM (1–2 µM, UV excitable, ratiometric) is preferred for flow cytometry. Fluo-4 AM is an acceptable alternative for standard 488 nm laser systems. Load with 0.02% Pluronic F-127, 30–45 minutes at 37°C in the dark, wash twice, rest 15–20 minutes to complete de-esterification.
- **Baseline Recording:** 60 seconds acquisition in calcium-free external solution (EGTA 500–600 µM).
- **ER Store Depletion:** Thapsigargin (50–200 nM) in calcium-free medium, minimum 10–15 minutes for complete passive store depletion. This phase must not be shortened.
- **SOCE Measurement:** Readdition of external calcium (2 mM $CaCl^2$). Record for 720 seconds post-readdition. Primary analytical parameters: peak SOCE amplitude, area under the curve, and rate of SOCE activation (slope over first 30–60 seconds).
- **Maximum Response Normalisation:** Ionomycin addition (1 µM) following SOCE recording to normalise for inter-individual differences in dye loading efficiency.
- **Cell Subset Resolution:** Co-staining with CD3-PE, CD4-APC, CD8-FITC, CD19-PerCP, CD56-PE-Cy7. Gating on $CD3^+CD4^+CD45RA^+CCR7^+$ naive T cells is specifically prioritised.
- **Companion Molecular Endpoint:** FACS sorting of $CD3^+CD4^+CD45RA^+CCR7^+$ naive T cells (minimum 50,000 cells), followed by RNA extraction and qRT-PCR with validated ORAI1, ORAI2, and STIM1 primer sets normalised to GAPDH. A SOCE amplitude increase accompanied by a shifted ORAI1:ORAI2 ratio is attributable to the serotonergic transcriptional pathway rather than non-specific PBMC activation.
- **Controls Required:** Positive control: ionomycin to confirm cell Ca^{2+} responsiveness. SOCE specificity control: 2-APB (50–100 µM) or CM4620 to confirm SOCE-dependence. Unstimulated baseline and ER store content measurement (initial TG transient in Ca^{2+}-free solution).

5. Translational Endpoint Framework

5.1. Context of Use

The proposed SOCE assay is framed as an exploratory pharmacodynamic biomarker appropriate for Phase 1 and Phase 2a studies where primary objectives include target engagement demonstration and dose-response characterisation. In the context of non-hallucinogenic neuroplastogens, the subjective experience — which has historically served as the most reliable indicator of central 5-HT2A engagement — is no longer available as a pharmacodynamic proxy. SOCE measurement in PBMCs is proposed not as a standalone endpoint but as a mechanistic molecular layer complementing functional electrophysiological readouts and network-level imaging.

5.2. Specific Testable Predictions

Prediction 1 (expression-level): Human T lymphocytes from participants receiving a 5-HT2A-engaging neuroplastogen for 7–14 days will show a shifted ORAI1:ORAI2 mRNA ratio compared to baseline, measurable by qRT-PCR on sorted $CD4^+$ naive T cells.

Prediction 2 (functional-cumulative): Thapsigargin-evoked SOCE amplitude in PBMCs from dosed participants will be increased compared to baseline, reflecting the ORAI1:ORAI2 ratio shift.

Prediction 3 (CNS-peripheral correlation): If Predictions 1 or 2 are confirmed, the magnitude of the ORAI ratio shift or SOCE amplitude increase will correlate with a clinical or functional outcome measure.

5.3. Validation Pathway

Prior to deployment as a clinical trial endpoint, the SOCE assay requires a staged validation pathway: (i) *technical pre-validation* — demonstration that primary naive CD4+ T cells produce a detectable SOCE response to thapsigargin and that this response is modulated by serotonergic agonism in single-cell imaging and flow cytometric settings; (ii) *in vivo expression validation* — ORAI1:ORAI2 mRNA ratio in naive T cells from animals receiving a 5-HT2A-engaging compound versus vehicle or antagonist co-treatment; (iii) *in vivo functional validation* — thapsigargin-evoked SOCE amplitude measurement with correlation against mRNA ratio and CNS structural readout; (iv) *human exploratory*

deployment — in Phase 1 clinical cohort with correlation analysis against pharmacokinetics, functional electrophysiology, and outcomes. A companion research proposal detailing this staged pre-clinical validation programme is available.

6. Limitations

The central limitation of this proposal is that the specific prediction — that pharmacological 5-HT2A agonism in humans shifts the ORAI1:ORAI2 ratio and increases thapsigargin-evoked SOCE amplitude in patient T lymphocytes — has not been experimentally demonstrated. The hypothesis is mechanistically grounded and constructed from direct experimental evidence at each of its mechanistic nodes, but each precedent was established in cell types other than primary naive T lymphocytes, and direct demonstration in the clinical context remains required. The bipolar disorder lymphoblast precedent (Kato 2003, Wasserman 2004, Perova 2010) is the closest published analogy, but an important cell-type distinction applies: those studies used EBV-transformed B lymphoblastoid cell lines, not primary T lymphocytes. Transformation may substantially alter SOCE properties relative to primary cells [34-36]. Whether primary naive $CD4^+$ T cells show comparable SOCE pharmacodynamics is a tractable but currently unresolved experimental question. A direction-of-effect ambiguity must be acknowledged. The bipolar disorder data shows disease state *elevates* SOCE in peripheral lymphocytes. The neuroplastogen hypothesis proposes that drug engagement also *elevates* SOCE via NFAT-driven ORAI1 upregulation. In a depressed patient population receiving a neuroplastogen, both effects may operate simultaneously and in the same direction, creating a potential confound: elevated baseline SOCE due to disease state may reduce the sensitivity of the assay to detect drug-induced increments.

The BDNF experience provides a cautionary precedent: a biologically plausible peripheral biomarker hypothesis that failed to replicate with sufficient mechanistic specificity. A systematic methodological analysis of 31 meta-analyses of peripheral MDD biomarkers (Carvalho et al. 2016) identified systematic effect size inflation and publication bias across the entire field [9]. Any future validation study for SOCE must be pre-registered with specified effect size estimates, adequately powered, and include a pharmacologically specific control condition (e.g. ketanserin co-administration to block 5-HT2A in animal models). Without these safeguards, positive findings will be dismissed as another inflated peripheral biomarker result.

Additional open questions include whether 5-HT2A expression levels in resting peripheral lymphocytes are sufficient to produce a receptor-dependent oscillatory Ca^{2+} response under therapeutic dosing; whether SOCE dynamics in PBMC subsets vary between disease states in ways that are confounding; and whether inter-individual variability in baseline ORAI1:ORAI2 ratio is sufficiently low for pharmacodynamic signal detection in early-phase clinical cohorts.

7. Conclusion

Psychedelic and neuroplastogen drug development programs face a measurable gap between preclinical cellular pharmacology and clinically interpretable pharmacodynamic endpoints. The present hypothesis proposes SOCE dynamics in patient-derived PBMCs as a mechanistically grounded, technically validated, and currently underexplored candidate for this role. The scientific basis converges on six pillars: the established disease-state-regulated expression of 5-HT2A receptors and SOCE machinery in peripheral lymphocytes; published evidence of upstream calcium signalling dysfunction in patient PBMCs; direct experimental demonstration that peripheral serotonin regulates ORAI-1 expression; functional evidence that 5-HT2A receptor activation drives SOCE in peripheral cells; the author's own published experimental work on ORAI2/SOCE regulation in neuronal disease models; and critically — the bipolar disorder lymphoblast literature demonstrating that thapsigargin-SOCE in peripheral lymphocyte-derived cells carries a CNS disease-relevant signal and responds pharmacodynamically to CNS drug treatment with drug-specific discrimination [39].

The multi-receptor convergence analysis demonstrates that the SOCE biomarker captures pharmacodynamic information from engagement across the 5-HT2A, 5-HT2C, 5-HT2B, 5-HT1A, and 5-HT7 receptor subtypes through distinct but mechanistically convergent signalling routes, broadening the applicability of the proposed assay across the full serotonergic drug development landscape [40]. Experimental validation of the proposed correlation between PBMC SOCE dynamics and serotonergic pharmacology represents a tractable and high-value scientific objective. A companion staged pre-clinical validation programme is proposed separately. We invite collaboration from groups with access to appropriate preclinical models or early-phase clinical cohorts to test this hypothesis.

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