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ARTICLE

Assessing the effects of antipsychotic medications on schizophrenia functional analysis: a postmortem proteome study

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Antipsychotic drugs (APDs) are effective in treating positive symptoms of schizophrenia (SCZ). However, they have a substantial impact on postmortem studies. As most cohorts lack samples from drug-naive patients, many studies, rather than understanding SCZ pathophysiology, are analyzing the drug effects. We hypothesized that comparing SCZ-altered and APD-influenced signatures derived from the same cohort can provide better insight into SCZ pathophysiology. For this, we performed LCMS-based proteomics on dorsolateral prefrontal cortex (DLPFC) samples from control and SCZ subjects and used statistical approaches to identify SCZ-altered and APD-influenced proteomes, validated experimentally using independent cohorts and published datasets. Functional analysis of both proteomes was contrasted at the biological-pathway, cell-type, subcellular-synaptic, and drug-target levels. In silico validation revealed that the SCZ-altered proteome was conserved across several studies from the DLPFC and other brain areas. At the pathway level, SCZ influenced changes in homeostasis, signal-transduction, cytoskeleton, and dendrites, whereas APD influenced changes in synaptic-signaling, neurotransmitter-regulation, and immune-system processes. At the cell-type level, the SCZ-altered and APD-influenced proteomes were associated with two distinct striatum-projecting layer-5 pyramidal neurons regulating dopaminergic-secretion. At the subcellular synaptic level, compensatory pre- and postsynaptic events were observed. At the drug-target level, dopaminergic processes influenced the SCZ-altered upregulated-proteome, whereas nondopaminergic and a diverse array of non-neuromodulatory mechanisms influenced the downregulated-proteome. Previous findings were not independent of the APD effect and thus require re-evaluation. We identified a hyperdopaminergic cortex and drugs targeting the cognitive SCZ-symptoms and discussed their influence on SCZ pathology in the context of the cortico-striatal pathway.

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INTRODUCTION

Schizophrenia (SCZ) is a devastating mental disorder that typically emerges in late adolescence or early adulthood and results in severe social and mental impairment [1]. SCZ pathophysiology involves altered functionality of different brain areas. However, the dorsolateral prefrontal cortex (DLPFC), an area crucial for verbal and working memory, has been of special interest due to evidence of SCZ-associated morphometric changes and neurotransmitter abnormalities [2–4]. Direct examination of protein expression in the DLPFC of SCZ postmortem tissue using high-throughput approaches has revealed several altered proteins and biological pathways. However, the main limitation of these high-throughput approaches is distinguishing the impact of antipsychotic drug (APD) treatment [5], which influences myriad cellular, subcellular, and molecular processes. Most postmortem studies do not have sufficient drug-naive and drug-treated SCZ subjects to compare with control groups, making it hard to infer the true molecular correlates of SCZ.

To address this challenge, several studies have performed expression profiling of nonhuman primates [6] or rodents [7, 8] that were chronically treated with APDs. While these animal-based studies identified APD signatures (relevant genes or proteins influenced by APDs), they heavily rely on unsustainable causal assumptions about SCZ pathophysiology and/or the APD mechanism of action (MOA). First, these animal-based studies focus on characterizing SCZ symptoms rather than understanding the molecular basis of the disease. Second, healthy animals exposed to pharmacological treatment or lesions to mimic SCZ symptoms might induce changes in brain structure or composition that might not reflect the disease pathology. Last, animal models metabolize APDs differently than humans, possibly altering different metabolic pathways. Nevertheless, specific signatures derived from these models are important for identifying genes and the related pathways involved in APD action.

The development of resources that provide drug-specific signatures now permits more sophisticated analyses of the APD effects on postmortem studies. Particularly useful are the comparative toxicogenomics database (CTD) [9] anchoring well-curated drug-associated features and the connectivity map (cmap) [10],

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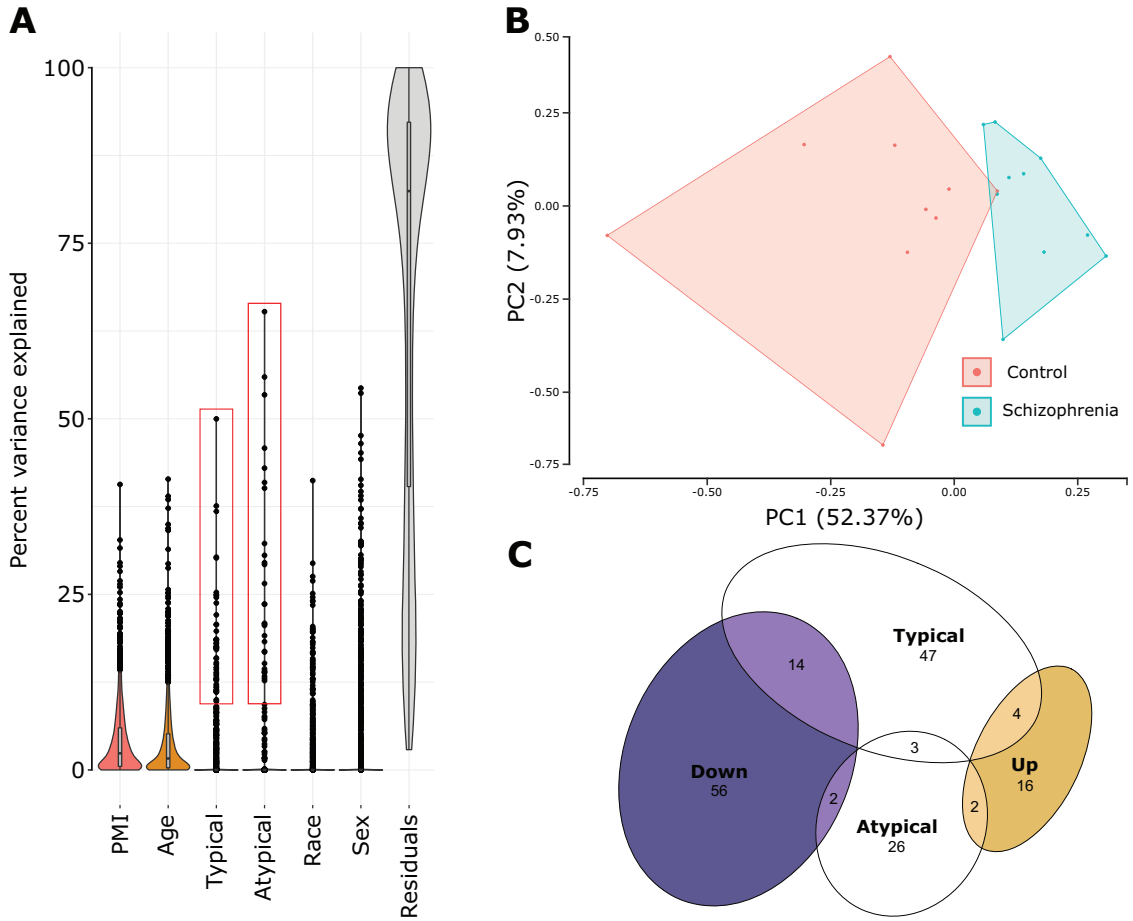

Fig. 1 SCZ-altered and APD influenced proteome. A A violin plot to sum up the proteome-wide trend and rank each variable's contribution. Age and PMI explained the highest percentage of variance in the data, followed by APDs, race, and sex. The influence of APDs was investigated using proteins that contributed more than 10% of the variables (red boxes). **B** Principal component analysis segregating the proteome profile of the control and schizophrenia subjects. **C** Venn diagram showing the intersection of up- and downregulated SCZ-altered and APD-influenced proteomes (red boxes in **A**). For more details on the proteome, see Supplementary Table 1.

anchoring experimentally derived drug-specific features. We predict that these resources, together with known gene ontologies and recent advances in generating cell-specific signatures, will provide an opportunity to generate informed hypotheses regarding drug and disease effects at the cellular level in SCZ. To this end, we performed systematic integration of these resources with liquid chromatography–mass spectrometry (LCMS)-based proteomics data to highlight alterations attributed to APD medications versus SCZ-driven pathophysiology in human postmortem DLPFC. We demonstrate unique proteins, biological pathways, and synaptic and cellular alterations differentially influenced by APD drugs and SCZ pathophysiology, as well as potential MOAs connected with each of these proteomes.

METHODS AND MATERIALS

Subjects and Tissue Preparation

DLPFC (Brodmann area 9) tissues from SCZ and nonpsychiatric control subjects were obtained from the Maryland and Alabama brain collections. There were two cohorts used in this study. A mass spectrometry cohort from SCZ ($n = 10$) and nonpsychiatrically ill controls ($n = 10$) (Supplementary Table 1). Second, a conformation study cohort of SCZ patients ($n = 23$) and nonpsychiatrically ill controls ($n = 23$) was included (Supplementary Table 1). Schizophrenia subjects were diagnosed based on the DSM-IV criteria. The medical records of the subjects were examined using a formally blinded medical chart review instrument and in person interviews with the subjects and/or their caregivers, as previously described [11]. The Institutional Review Boards of the Maryland and Alabama brain collections approved the study's ethical protocol, and informed written consent was obtained from all subjects' legal next of kin. Tissue sections (14 µm thick) were generated and stored at −80 °C until use. Frozen tissues were thawed, scrapped, and homogenized in 1 ml of 5 mM Tris HCl, 32 M sucrose pH 7.4, with 1% protease and phosphatase inhibitor (Halt, Thermo Fisher™). The protein concentration was measured with the Pierce BCA kit for the mass spectrometry and western blot experiments detailed in the supplementary information.

Further details on extracting SCZ-altered and APD-influenced proteomes, theme-centric pathway analysis, and drug-MOA/target-specific enrichment analysis are provided in the supplementary information.

RESULTS

Schizophrenia altered and APD-influenced proteomes

LCMS-based expression profiles for 1547 non-imputed proteins were obtained from postmortem DLPFC gray matter (layers 1 through 6) samples of the control (CTL) and SCZ subjects ($n = 10$/group; Supplementary Table 1). After regressing the effect of age and PMI (each accounting for > 5% overall variability in the data, Fig. 1A), we obtained 72 differentially expressed proteins (Supplementary Table 2; up = 16, down = 56, p *value* < 0.05, designated as the SCZ-altered proteome) that segregated the control and SCZ samples (Fig. 1B). The subjects in this study were treated either with typical or atypical APDs, which together accounted for <5% of the overall variability in the data (Fig. 1A). Since regressing this small effect from a limited number of samples is challenging [12, 13], we instead filtered the proteins that independently explained >10% of the variability

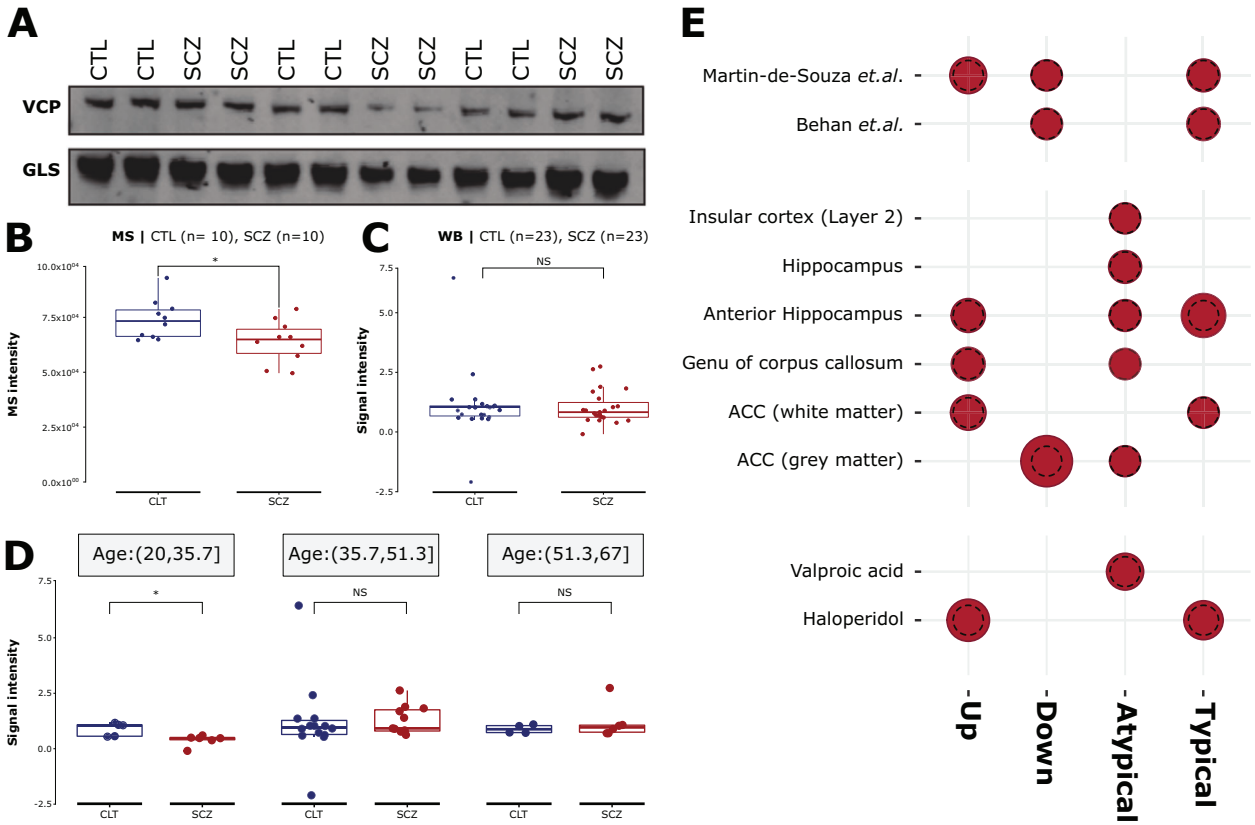

Fig. 2 Validation of SCZ alterations and APD-influenced proteomes. A A representative image of western blot analysis for *GLS* proteins associated with the SCZ-altered proteome in an independent cohort. Valosin-containing protein (*VCP*) was used as a loading control. **B** Significant (p-value < 0.02) downregulation of *GLS* in the MS-based analysis. **C** Western blot analysis of *GLS* in an independent cohort did not show any significant difference. However, the downregulation was significant (p-value < 0.03) for subjects within 20–37.5 years of age (**D**). **E** Hypergeometric overlap between the SCZ-altered and APD-influenced proteome with similar proteome studies of the DLPFC (top), other brain regions (middle) and drug-specific features (bottom). The size of the circles is proportional to the −log10 of p-values associated with the overlap. The black dotted circles represent the reference = −log10(p-value = 0.05).

associated with atypical and typical APDs using linear mixed modeling [14] (Supplementary Table 3; Fig. 1A, red boxes, see the methods). Regardless of the direction (i.e., up- or downregulated), 26 and 47 proteins were associated with atypical and typical APDs, respectively (designated the APD-influenced proteome). Except for the downregulated SCZ-altered and typical APDs, there was minimal overlap between the SCZ-altered and APD-influenced proteomes (Fig. 1C).

Next, we validated the proteomics approach and identified proteome sets (i.e., SCZ-altered and APD-influenced). First, we validated the proteomics approach in an independent cohort by performing western blot analysis of glutaminase (*GLS*; Fig. 2A), a protein that was differentially expressed in our MS-based proteomics analysis (Fig. 2B and Supplementary Table 2). We did not observe any difference in *GLS* expression between CTL and SCZ subjects (Fig. 2C). However, consistent with previous findings [15] and known downregulation of glutamate (a product generated by the catalytic action of *GLS*) in SCZ during early adulthood or late adolescence [16, 17], a significant downregulation was observed in the age range of 20–37 (Fig. 2D, $n = 6$/group, p-value < 0.02). Second, the SCZ-altered up- and downregulated protein-sets were validated using hypergeometric overlap analysis with other similar proteomics studies of DLPFC [18, 19] (Supplementary Table 5; Fig. 2E, top) and other brain areas including *insular-cortex* [20], *hippocampus* [21], *anterior-hippocampus* [22], *genu-of-corpus-callosum* [23] *and gray* [24] *and white* [25] *matter of anterior-cingulate-cortex (ACC)* (Supplementary Table 5; Fig. 2E, middle). The upregulated proteins overlapped with the studies from most other areas, while the downregulated proteins only overlapped with the ACC gray matter but consistently overlapped with DLPFC in the other studies. Notably, all studies overlapped significantly with features associated either with atypical or typical APDs. Finally, we validated the atypical and typical APD-influenced proteome using hypergeometric overlap analysis with drug-specific features available from either CTD or cmap (Fig. 2E, bottom). Consistent with their mechanism involving dopaminergic transmission, typical APD-associated proteins overlapped significantly with haloperidol, a dopamine receptor antagonist (and typical APD) [26]. Interestingly, *haloperidol* also overlapped significantly with the upregulated proteins and was administered to the studied SCZ samples (Supplementary Table 1), thus validating the APD-influenced proteome set. Atypical APD-associated proteins overlapped with *valproic acid*, which is often used as an adjunctive agent to treat SCZ [27].

Overall, our statistical analysis revealed SCZ-altered and APD-influenced proteomes. The SCZ-altered proteome is conserved across multiple brain areas and studies. However, as revealed by the overlap with the APD-influenced proteome, it was not independent of the medication effect. Both proteome sets were validated either experimentally or in silico, thereby building confidence to contrast the functional analysis performed around them.

Distinct functional changes associated with SCZ and APD

Next, to understand the functional changes associated with SCZ-altered and APD-influenced proteomes, we performed gene ontology (GO) analysis (Fig. 3). The identified pathways (*q-value*

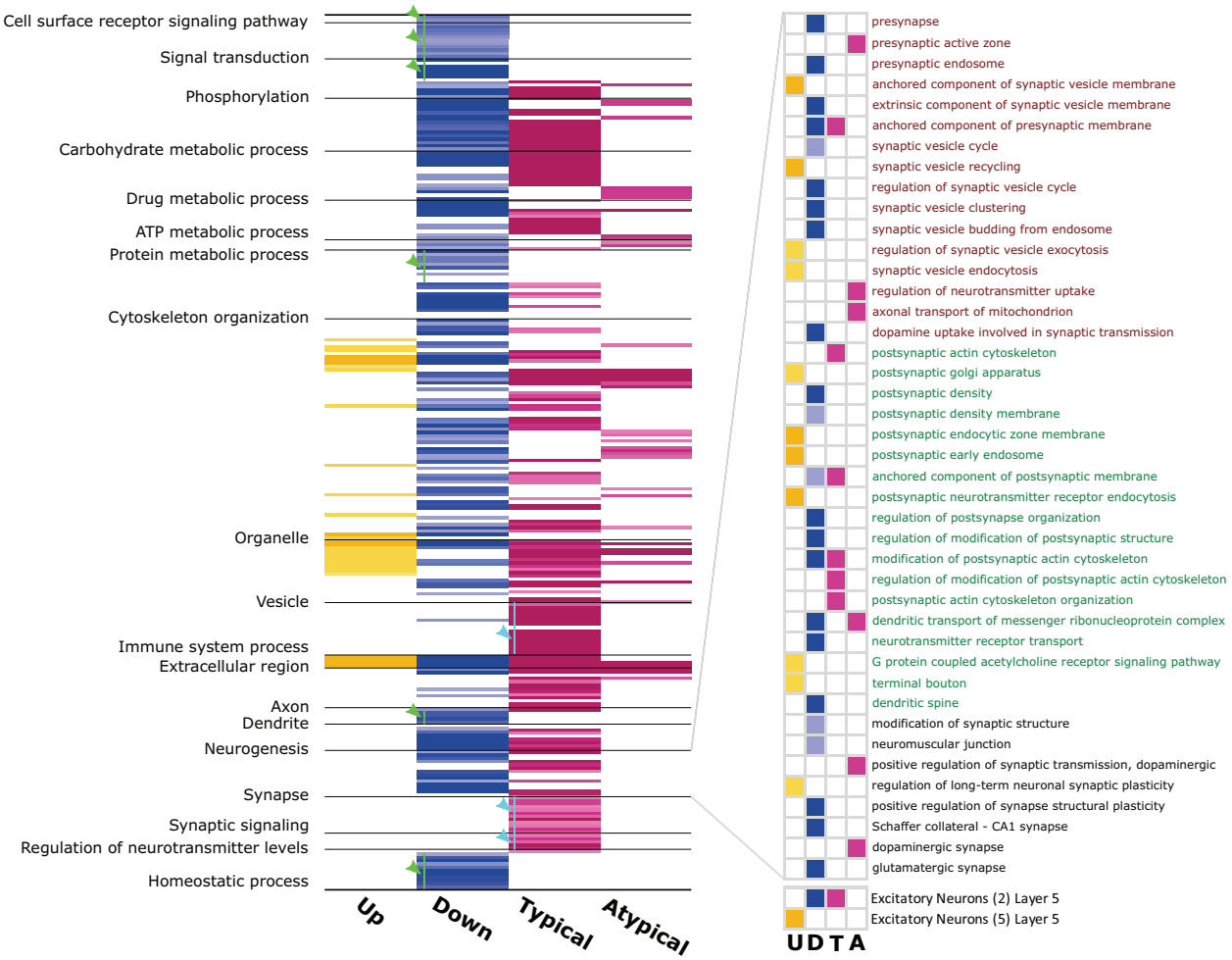

Fig. 3 Biological pathways, synaptic changes and cell types associated with SCZ alterations and APD-influenced proteomes. Changes associated with SCZ-altered up- and downregulated proteomes are shown in yellow and blue, respectively. Pathways associated with typical and atypical APD-influenced proteomes are shown in pink. The green arrowheads indicate pathways exclusive to the SCZ-altered downregulated proteome, whereas the cyan arrowheads indicate pathways specific to typical APDs. Right inset (top): The presynaptic (red), postsynaptic (green) and other synaptic pathways (black) studied using the SynGo database anchoring curated synapse-related ontology. Right inset (bottom): Cell-type-specific enrichment of SCZ-altered and APD-influenced proteomes studied using DLPFC-specific cell-type signatures. Lighter to darker shades of any color represent an increasing enrichment score represented by −log10 (*q*-value). See Supplementary Table 4 for details on each pathway.

< 0.05) were clustered into functional themes (Fig. 3, left labels). Upregulated pathways were involved in only a few themes related to *organelles*, *vesicles*, and *extracellular regions*. The *extracellular region* was the most prominently altered upregulated theme, as the majority of other pathways (Supplementary Table 4), including those involving *organelles* and *vesicles* (*extracellular exosome*, *extracellular organelle*, *extracellular vesicle* and *neurofilament*), also had extracellular functionalities. Notably, the majority of the upregulated pathways overlapped with those that were downregulated. Given that GO pathways represent a set of coordinated features [28–30] against different biological processes, the simultaneous enrichment of extracellular functionalities in the up- and downregulated feature sets reflects their aberrant coordination in SCZ.

Downregulated pathways were involved in almost all of the themes and overlapped considerably with the typical APD-associated pathways; however, there were some notable non-overlaps between the two profiles. For instance, the downregulated pathways showed exclusive enrichment of themes associated with *cell surface receptor signaling*, *signal transduction, dendrites, cytoskeleton organization* and *homeostatic* processes (Fig. 3, green arrowhead), whereas the typical APD-associated pathways showed exclusive enrichment of themes associated with *synaptic signaling*, *regulation of neurotransmitter levels* and *immune system processes* (Fig. 3, cyan arrowhead). The atypical APDs also showed fewer pathways and were mostly associated with themes involving *extracellular regions*, *vesicles*, *organelles*, *phosphorylation*, and metabolic processes involving ATP, drug, and carbohydrate metabolism. Unlike typical APDs, atypical APDs showed no association with immune system processes or other structural changes (*cytoskeletal organization, axons, dendrites, and synaptic signaling*).

Overall, the functional analysis revealed that, with the exception of the loss of homeostasis, signal transduction, dendritic, and cytoskeleton-related processes, APDs appear to compensate for the majority of the pathways disrupted in SCZ, but they alter synaptic signaling and the immunological process.

Distinct synaptic events and layer 5 pyramidal neurons are disrupted in SCZ

Disruptions of synaptic and cellular function play an important role in the complex network of events that underpin SCZ pathophysiology [31]. To focus on the enrichment of those events in the functional analysis, we used SynGO [32], a database of

synaptic ontology (Fig. 3, inset top) and cell-specific signatures from the human DLPFC [33] (Fig. 3, inset bottom). Synaptic enrichments (*p-value* < 0.05) were organized into presynaptic (Fig. 3 inset, red) and postsynaptic (Fig. 3 inset, green) groups of pathways.

Downregulated proteins were equally enriched in presynaptic and postsynaptic components. Postsynaptic components, however, exhibited greater structural variability (*actin-cytoskeleton, mRNA complexes, neurotransmitter receptor transport and anchored and membrane components of postsynaptic density*) than presynaptic components, which were primarily associated with the synaptic vesicle and its regulation. In addition to pre- and postsynaptic changes, *dopamine uptake* and *glutamatergic* synapse-associated pathways were also downregulated. Upregulated proteins, on the other hand, were more enriched in the postsynaptic components associated with the *Golgi apparatus*, *endocytic zone, endosomes*, and *G-coupled acetylcholine receptors*. Upregulated presynaptic components were associated with vesicle movements (*exocytosis, endocytosis*, and *recycling*). Long-term synaptic plasticity was also upregulated.

Among the synaptic changes associated with different APD signatures, atypical APD was mostly associated with presynaptic components, including dopaminergic synapses and neurotransmitter uptake. Mitochondrial-related pathways were also found in conjunction with atypical APDs, implying that these medicines impact bioenergetic functions. Typical APDs, in contrast, were primarily associated with postsynaptic components linked to the cytoskeleton and its organization.

Within the different cell-type-specific signatures obtained from DLPFC-specific single-cell studies [33], up- and downregulated proteins were enriched in a nonoverlapping set of layer-5 pyramidal neurons (PNs). Characterizing these neurons further (see Supplementary Table 6 notes) using GO revealed that the PN subsets were exclusively associated with the regulation of dopamine (excitatory neurons-(2)-layer-5: $q\text{-value} < 5.02 \times 10^{-5}$; excitatory neurons-(5)-layer-5: $q\text{-value} < 9.12 \times 10^{-4}$). Additionally, based on the enrichment of different deep-layer projection neuron markers [34], these neurons appear to be striatum projecting (excitatory neurons (2) layer 5: *p-value* < 0.08; excitatory neurons (5) layer 5: *p-value* < 0.09).

Overall, zooming in on cellular and synaptic changes reveals a balance of pre- and postsynaptic changes, as well as the influence of up- and downregulated proteins on distinct subsets of dopamine-regulated striatal projecting layer-5 PNs associated with distinct neuromodulatory and synaptic events.

Insights into the key MOAs associated with SCZ-specific signatures

To understand the molecular events (MOAs or targets) that precede the aforementioned functional events, we looked for enrichment of drug signatures associated with known MOAs/targets in SCZ-altered up- and downregulated proteins. Sixty-two and 18 drugs were enriched (*p-values* < 0.05) in up- and downregulated proteins, respectively (Fig. 4, Supplementary Table 7 notes).

MOAs/targets involved in upregulated events include neuromodulation (*dopamine, norepinephrine*, and *acetylcholine*), local immune response (*histamine*), and regulation of pain (*opioid receptors*). Contrary to the signature reversing principle [35], which assumes discordance between drug-disease signatures for a therapeutic effect, several known SCZ drugs were concordant with the disease signatures, confirming that the SCZ-altered proteome, as noted, is likely to be driven by the medications.

Among the MOAs/targets shared by up- and downregulated proteins, several neuromodulatory events (except dopaminergic), hyperpolarization events (*sodium channel blockers*), and cytochrome p450 activity were observed, implying that these targets are critical pharmacological nodes in SCZ pathophysiology. MOAs exclusive to downregulated events include both fast (GABA-A) and slow (GABA-B) inhibitory modulations; serotonergic and norepinephrine modulation by means of receptors and transporters; different cell-surface and cytoplasmic signal transduction events (*tyrosine-protein kinase LCK, serine/threonine-protein kinase*); enzymes involved in homeostasis (*carbonic anhydrases*), fatty acid oxidation (*carnitine palmitoyl transferase*), and anti-inflammatory activity (*cyclooxygenase*); hormonal receptor activity involving gender specificity (*androgen* and *progesterone*); cellular processes inhibiting depolarization by means of *acetylcholinesterase;* cellular processes associated with the DNA metabolic process (*DNA alkylation*, *DNA synthesis*); microtubule organization (*tubulin beta-1 chain*) and cellular detoxification events (*glutathione S-transferase kappa 1*) and gastrointestinal regulation (cholecystokinin B receptor).

Overall, SCZ-altered upregulated events were linked with a few MOAs/targets, most of which were neuromodulatory, whereas downregulated events were linked with a more diverse range of MOAs/targets outside of neuromodulation.

DISCUSSION

The majority of SCZ subjects receive lifelong APD treatment, which limits the inferences drawn from postmortem examinations. Here, to understand the effect of SCZ and APDs (in SCZ), we contrasted the statistically derived distinct SCZ-altered and APD-influenced proteomes using functional analysis focusing on GO, cell types, and subcellular synaptic changes. Using drug-specific signatures, we demonstrated that the majority of SCZ-altered changes were influenced by APDs. However, our approach could mitigate this issue in two different ways. First, the contrast between pathways associated with SCZ-altered and APD-influenced proteomes revealed that homeostasis-, signal transduction-, cytoskeleton-, and dendrite-related processes were generally not compensated for by APDs. The latter two processes (cytoskeleton and dendrite) are consistent with the increased neuronal density in nontreated postmortem SCZ-DLPFC, which is accompanied by a decrease in cell size (attributable to the cytoskeleton) and dendritic spines [36, 37].

In addition, the signatures of drugs with a known MOA revealed a potential mechanism involved in SCZ pathophysiology despite the confounding effect of drugs. For instance, in addition to the influence of drugs in our cohort, an upregulated therapeutic effect of dopamine receptor antagonists (Fig. 4) supports the known compensatory upregulation of dopamine receptors during SCZ pathophysiology [38]. Interestingly, the SCZ-altered proteome in the present study significantly overlapped with several previous SCZ-related findings across different studies and brain regions (Fig. 2E). Furthermore, functional analysis using these proteomes demonstrated several key SCZ-specific findings, such as the effect of the extracellular region [39], the association of layer-5 PNs with SCZ pathology [40] and its projection to the striatum [41], which are consistent with several previous molecular, anatomical, and functional imaging-based studies. While these consistencies highlight the data reproducibility, the influence of APDs in all of these studies calls into question the inferences about SCZ pathophysiology based on them.

Influence of SCZ-altered and APD-influenced proteomes in cortico-striatal pathways

DLPFC neurons (Fig. 5) project to the *dorsal striatum* related to associative functionality via cholinergic interneurons, which modulate dopaminergic input (to the striatum) through the nicotine receptors [42]. The output of the *dorsal striatum* includes direct and indirect projections to the basal ganglia (*globus pallidus internal*) via GABAergic medium spiny neurons (MSNs) [43]. MSNs projecting directly use D1 (excitatory) receptors, and those relaying indirectly via the *globus pallidus external* and *subthalamic*

Fig. 4 Drug and MOA/target associated with the SCZ-altered proteome. The orange and green boxes represent the enrichment (ES) of drugs with known MOA/targets in up- and downregulated proteomes, respectively. A lighter to darker shade of both orange and green represents an increasing enrichment calculated using −log10 (*p*-value *of enrichment statistics*). The upward-pointing orange arrow and downward-pointing green arrow represent the up- and downregulated drug signatures, respectively, as predicted by the cmap database. See Supplementary Table 7 for details on each drug.

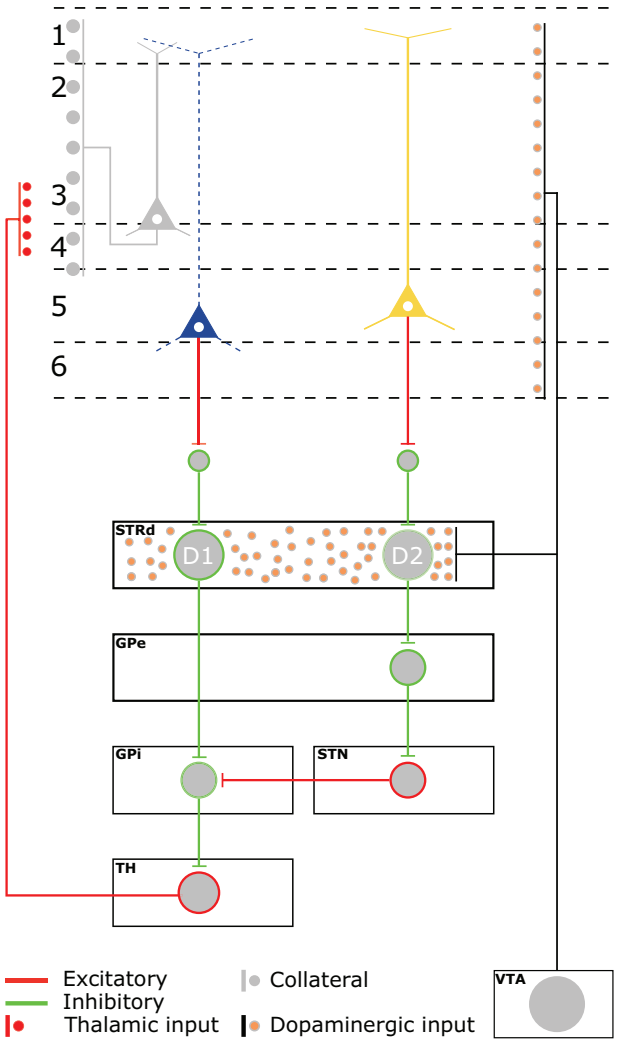

Fig. 5 Schematic summary of functional changes observed in the study in the context of cortico-stratum circuitry. Enrichment analysis of up- and downregulated SCZ-altered proteomes showed enrichment in two different subsets of layer 5 PNs, showing enriched dopaminergic signaling. The PNs enriched in upregulated events were also associated with D2 receptor antagonists. Together, these findings point toward selective association of the SCZ-altered up- and downregulated proteome with D1-PN and D2 PN of cortical layer 5, respectively. The data consistent with previous anatomical studies also suggests that the two neurons are striatum projecting, perhaps to D1- and D2-expressing MSN neurons. An order of excitatory and inhibitory events shown in red and green, respectively, sum up to modulate the excitatory thalamic input to the cortex. Unlike previous studies postulating a hypodopaminergic cortex, the functional analysis also suggests a hyperdopaminergic cortex and a potential selective and compensatory pruning of D1-PN dendrites (shown as a dashed blue line). STRd: dorsal striatum, GPe: Globus pallidus external, GPi: Globus pallidus internal, STN: Subthalamic nucleus, TH: Thalamus, VTA: Ventral tegmental area. See the text for more details.

nucleus use D2 (inhibitory) receptors. The *globus pallidus internal* projections hyperpolarize the thalamus, which projects back to layer-3, where the inhabitant PNs extend axon collaterals to other cortical areas [38]. Additionally, the DLPFC, similar to the dorsal striatum, receives input from dopaminergic neurons of the ventral mesencephalon [44].

Our functional analysis revealed that the up- and down-regulated changes are enriched in two different subsets of striatum projecting layer 5 PNs, which, based on the exclusive enrichment of dopaminergic signaling, can be linked to D1- and D2-receptor-expressing cortical PNs. PN with upregulated events (Fig. 5, yellow) based on the association with *haloperidol*, which targets the D2 receptor [45], can be D2-PN, while PN with downregulated events (Fig. 5, blue) can be D1-PN. Under normal circumstances when dopamine is optimal, the network events sum up to relay the usual thalamic input to the cortex (Fig. 5, legend). However, during SCZ, the hyperactivity of the dopamine in the striatum assigns salience to unremarkable environmental stimuli [46] and relays it to cortical layer-3 PNs [47] (Fig. 5, gray PN), whose collaterals could cascade the salience signal to other cortical areas, leading to an inappropriate effect. Note that within this mechanism (i.e., a hyperdopaminergic striatum), previous studies have postulated a hypodopaminergic cortex [38, 48–51], but our functional analysis based on downregulated dopamine uptake (Fig. 3) and upregulated dopamine receptor antagonists (Fig. 4) suggests that the hyperdopaminergic effect leading to SCZ pathology might simultaneously initiate in the cortex and the striatum. A possible and perhaps important pathological implication of this could be a maladaptive association between working memory [DLPFC function [52]] and salience [striatum function [53]]. Note that under normal conditions, this association can be between working memory and reward [54]. There is also an association between stress—a prefrontal cortex (PFC)-related functionality—and SCZ. Supporting the latter, a recent study on mouse chronic unpredictable stress demonstrated that D1-and D2-PN subpopulations of the PFC undergo distinct stress-induced intrinsic and synaptic plasticity changes that may have functional implications for stress-related pathology [55]. One can argue that a hyperdopaminergic cortex, consistent with most medication-based mechanisms (e.g., *haloperidol*), is likely relevant to the positive and negative symptoms of SCZ (inappropriate affect) but does not involve the cognitive deficit related to DLPFC functionality. This, we conjecture, is largely because cognition (e.g., working memory) may be associated with intracellular signaling events [56, 57], while most SCZ-relevant therapeutic changes target the receptor events implicated in a circuit-wide phenomenon. We observed several downregulated kinase-related events in our MOA/target analysis that are involved in memory formation [58]. In this regard, a more rational design might look for adjuvants that target the kinase pathways suggested here (Fig. 4).

Further placing the functional analysis results in the context of a hyperdopaminergic cortex, the reduced dendrite and cell size (cytoskeleton, Fig. 3) seems to be selective for the D1-PNs (blue) and not the D2-PNs or other PNs (particularly layer-3), as postulated previously [38]. As the postulated hyperdopaminergic cortex by means of D1-PNs may also sum up to relay inappropriate affect from the thalamus, the selective pruning of these D1-PN dendrites and reduced cell size may be a compensatory mechanism that is further supported by the equally distributed pre- and post-synaptic events, a phenomenon often associated with a compensatory response [38].

Although our study focused only on the DLPFC, we observed conserved results across several brain areas, suggesting that a similar hyperdopaminergic mechanism might appear in all areas; however, depending upon the location, it can have different functionality. For instance, in the auditory or motor cortex and related areas in the striatum, inappropriate affect, instead of amplifying salience, can amplify inner speech, leading to thought echo, a phenomenon consistently observed in SCZ patients [43].

Limitations and future directions

This study does have some limitations. First, despite the fact that several variables, including sex, age, and race, play a significant role in SCZ pathophysiology [59], the data was not stratified or regressed for these variables, as doing so would have reduced the power of the analysis. Investigating the influence of these

variables on SCZ pathophysiology could be a promising future direction. Second, we acknowledge that the analysis of APDs related features is underpowered and should be interpreted as exploratory. Third, the functional predictions obtained through bioinformatics analyses should be interpreted as hypothesis-generating. Consistent with the medication-related effect of PN noticed in this study, in our recent single-nucleus transcriptomic study [60], we have demonstrated that disease and drug state are associated with to two distinct layer-5 PNs. However, the hyperdopaminergic functionality centered around these PNs as predicted in this study needs to be confirmed at the brain imaging level. Likewise, the kinase signaling specific drug discussed here need to be confirmed using human induced pluripotent stem cell derived neurons.

DATA AND MATERIALS AVAILABILITY

All analyzed data are available in the main text or the supplementary materials. The raw data are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

RS and RSA conceptualized the study and wrote the manuscript. JR, MAE, JM, and REM participated with RSA in LCMS data generation and processing. SMO and AJF participated with RSA in western blot analysis. All authors participated in writing the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

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