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Relating Glutamate, Conditioned, and Clinical Hallucinations via 1H-MR Spectroscopy

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Background and Hypothesis: **Hallucinations may be driven by an excessive influence of prior expectations on current experience. Initial work has supported that contention and implicated the anterior insula in the weighting of prior beliefs.** ***Study Design:*** **Here we induce hallucinated tones by associating tones with the presentation of a visual cue. We find that people with schizophrenia who hear voices are more prone to the effect and using computational modeling we show they overweight their prior beliefs. In the same participants, we also measured glutamate levels in anterior insula, anterior cingulate, dorsolateral prefrontal, and auditory cortices, using magnetic resonance spectroscopy.** ***Study Results:*** **We found a negative relationship between prior-overweighting and glutamate levels in the insula that was not present for any of the other voxels or parameters.** ***Conclusions:*** **Through computational psychiatry, we bridge a pathophysiological theory of psychosis (glutamate hypofunction) with a cognitive model of hallucinations (prior-overweighting) with implications for the development of new treatments for hallucinations.**

Key words: conditioned hallucinations/predictive processing/schizophrenia/computational psychiatry/MR spectroscopy (MRS)

Introduction

The predictive processing account of psychosis posits that hallucinations—percepts without an external stimulus—arise when prior beliefs are over-weighted relative to sensory evidence.[1–3] If the incoming data are less precise (i.e. sensory uncertainty is overestimated), inference will conform to priors and hallucinations will result.[1] Participants who hallucinate tend to overweight prior beliefs in experimental tasks, e.g. they can be more readily conditioned to hallucinate an absent tone based on previous learning that visual stimuli predict tones.[4–6] Here we replicate and extend this observation, testing the hypothesis that glutamate signaling underwrites the association between hallucinations and strong priors.

Predictive processing accounts posit a hierarchy of inference that is recapitulated in the anatomy and physiology of the brain.[7] Forward connections promulgate prediction errors up the hierarchy via AMPA receptor signaling.[7] In contrast, backward connections mediate contextual effects—priors—downwards via NMDA receptors.[7] This division of labor is supported by primate data gathered from the visual system.[8] Blocking NMDA glutamate receptors—for example with the noncompetitive NMDA receptor antagonist ketamine—induces a state redolent of endogenous psychosis.[9] It may do so by decreasing glutamatergic neurotransmission via NMDA receptors (hence the NMDA hypofunction model) but in addition, NMDA blockade on GABAergic interneurons increases glutamate release[10] and thus, may increase transmission through AMPA receptors (hyper-glutamatergic accounts).[11–13]

Proton magnetic resonance spectroscopy (MRS) studies in patients with schizophrenia (SCZ) suggest low glutamate levels,[14] although patients with more severe symptoms may have higher glutamate concentrations.[15–18] A follow-up study found elevated glutamate levels in left superior temporal gyrus but reduced glutamate in anterior cingulate cortex in hallucinators compared to nonhallucinators.[19] We aimed toward to clarify the relationship between hallucinations, psychosis, and glutamate using methods from computational psychiatry.[20–22]

We invited patients with SCZ, some of whom hallucinate and some of whom do not, as well as healthy control

participants (CTR), to complete the conditioned hallucinations task.[4] On a separate occasion we measured glutamate levels in the auditory, anterior insular, anterior cingulate, and right dorsolateral prefrontal cortices. Previous work implicated left anterior insula in perceptual precision weighting and conditioned hallucinations: Powers and colleagues found that conditioned hallucinations engaged insula, while model-based parameters extracted from left insula were significantly greater in hallucinators, compared to nonhallucinators.[4] Based on this tripartite relation between insular activation, model parameters, and hallucination status, we predicted that an alteration in glutamate levels in left anterior insula might play a role in endogenous psychosis and prior overweighting. The other three voxels were chosen based on their established link with other tasks used in the project (presented elsewhere) and were analyzed in a purely exploratory fashion.

Methods

Participants

Thirty-six SCZ and 24 CTR participated in the study (see table 1 for participant demographics). They were recruited across two sites: the Connecticut Mental Health Center (CMHC), at Yale University and the Maryland Psychiatric Research Center (MPRC), at the University of Maryland School of Medicine. Participants were recruited via local advertisements or referred to the study through their clinician. Exclusion criteria included: 1) any neurological disorder or head trauma resulting in loss of consciousness or sustained deficits; 2) any recreational drug or alcohol use meeting criteria for at-risk drinking (>2 drinks/day or 14 drinks/week for males, >1 drink/day or 7 drinks/week for females) for one month prior to enrollment or substance use disorder for 12 months prior to enrollment; 3) left-handedness; 4) self-reported abnormal hearing or vision; 5) any contraindication for MRI scanning; and 6) pregnancy for female participants. All SCZ were taking antipsychotic medication (table 1). CTR had no family history of psychotic disorders, had not been previously diagnosed with a psychiatric disorder, and had never been treated with psychotropic medications. All subjects gave written informed consent prior to the study, in accordance with the declaration of Helsinki. All procedures were approved by institutional review boards at Yale University and the University of Maryland School of Medicine.

Clinical Procedures

Diagnoses were confirmed with the Structured Clinical Interview for DSM-5 (SCID-5). The Brief Psychiatric

Table 1. Participant Characteristics (mean (SD))

Variables	CTR (n = 24)	SCZH$^-$ (n = 17)	SCZH$^+$ (n = 18)	Statistic	P value
Age	34.8 (10.3)	31.3(9.6)	35.5(10.7)	F = 0.86	.43
Gender	10/14	11/6	13/5	χ^2 = 4.42	.11
Race(African American/Caucasian/other/missing)	3| 17| 3| 1	8| 9| 0| 0	4| 12| 1| 1	χ^2 = 8.94	.18
Participant Education	15.8 (1.9)	14.1(1.2)	14.5 (1.6)	**F = 6.42**	**.003**
Maternal Education	14.6 (4.0)	14.5 (2.4)	13.7 (5.5)	F = 0.31	.73
Paternal Education	14.5 (4.6)	14.0 (4.7)	14.0 (6.9)	F = 0.05	.95
Neurocognitive test results					
WTAR	114.2 (13.8)	101.2 (28.5)	112.4 (9.1)	F = 1.47	.39
MD Processing Speed	55.2 (14.0)	42.5 (14.4)	45.2 (16.2)	**F = 3.31**	**.04**
MD Working Memory	55.8 (7.9)	43.1 (15.2)	46.4 (16.6)	**F = 4.03**	**.02**
MD Verbal Learning	49.2 (6.3)	42.9 (14.5)	42.3 (15.7)	F = 1.81	.19
Clinical Ratings					
BPRS Hallucinatory Behavior	1.0 (0.0)	1.0 (0.0)	4.7 (1.2)	-	-
BPRS Delusions	2.0 (0.0)	3.6 (1.8)	5.8 (1.8)	**t = 3.53**[a]	**.001**[a]
BPRS Negative Symptoms	3.3 (1.1)	6.1 (2.4)	4.9 (1.7)	t = –1.72[a]	.09[a]
Duration of Illness (yrs)		8.4 (8.3)	13.4 (11.4)	t = 1.45	.16
Medication					
Antipsychotic(Atypical |Typical)		16|1	15|3	χ^2 = 1.00	.32
Antipsychotic medication: CPZ equivalents (mg)		278.4 (259.5)	388.47 (208.98)	t = 0.58	.57
Other psychotropic medication					
Antidepressant + Benzodiazepine + Mood stabilizer		1	0		
Antidepressant+ Benzodiazepine		0	3		
Benzodiazepine		0	3		
Antidepressant		4	4		
Mood stabilizer		3	0		

Note: WTAR, Wechsler Test of Adult Reading, measure of premorbid intelligence; MD Working Memory, Working memory domain (MATRICS); MD Processing Speed, processing speed domain (MATRICS); MD Verbal Learning, verbal learning domain (MATRICS); CPZ, chlorpromazine equivalent; BPRS Delusions, Suspiciousness+Unusual Thought Content; BPRS Negative Symptoms, Emotional Withdrawal+Motor Retardation+Blunted Affect;
[a]SCZH$^-$ vs. SCZH$^+$

Rating Scale (BPRS) was used to assess symptom severity (table 1). Item 12 of the BPRS was used to separate SCZ into hallucinators (SCZH$^+$) and nonhallucinators (SCZH$^-$). Hallucinators had a score larger than 1. The SCZ group comprised 18 hallucinators (11 patients had moderate-to-severe (BPRS-12 = 5) or severe (BPRS-12 = 6) hallucinations; the majority experienced auditory hallucinations) and 17 nonhallucinators (figure 1C). None of the CTR endorsed any hallucinations (BPRS-12 = 1).

Conditioned Hallucinations Task

Participants were instructed to listen for a 1 kHz tone, embedded in white noise, and presented concurrently with a flashed visual stimulus (light- and dark-gray checkerboard) on a black background (figure 1A). Stimulus presentation and data collection were controlled using Matlab 2017b (Mathworks, Natick, MA) and Psychtoolbox 3.0.12.

Threshold estimation was followed by 12 blocks of 30 trials. In each trial, a checkerboard flashed on the screen and a tone was played simultaneously at 25%, 50%, or 75% detection-likelihood tone intensity. Importantly, the tone was omitted on some trials (no-tone condition) (figure 1B, left). Trials were pseudorandomized within block. The likelihood of threshold tone presentation decreased nonlinearly over blocks. The presentation of subthreshold and no-tone trials increased (figure 1B, right). This distribution encouraged initial learning of audio-visual associations and then offered more opportunities for conditioned hallucinations (CH) later in the experiment. Participants were instructed to press one response button if they heard a tone and another button to indicate that they heard no tone. They also held the response button down longer to indicate higher confidence in their choice. More information about the task and the stimuli can be found in supplementary methods.

MRS Data Acquisition

Twenty-one CTR, 17 SCZH$^-$ and 16 SCZH$^+$ of our initial CH sample also completed (on a different day)

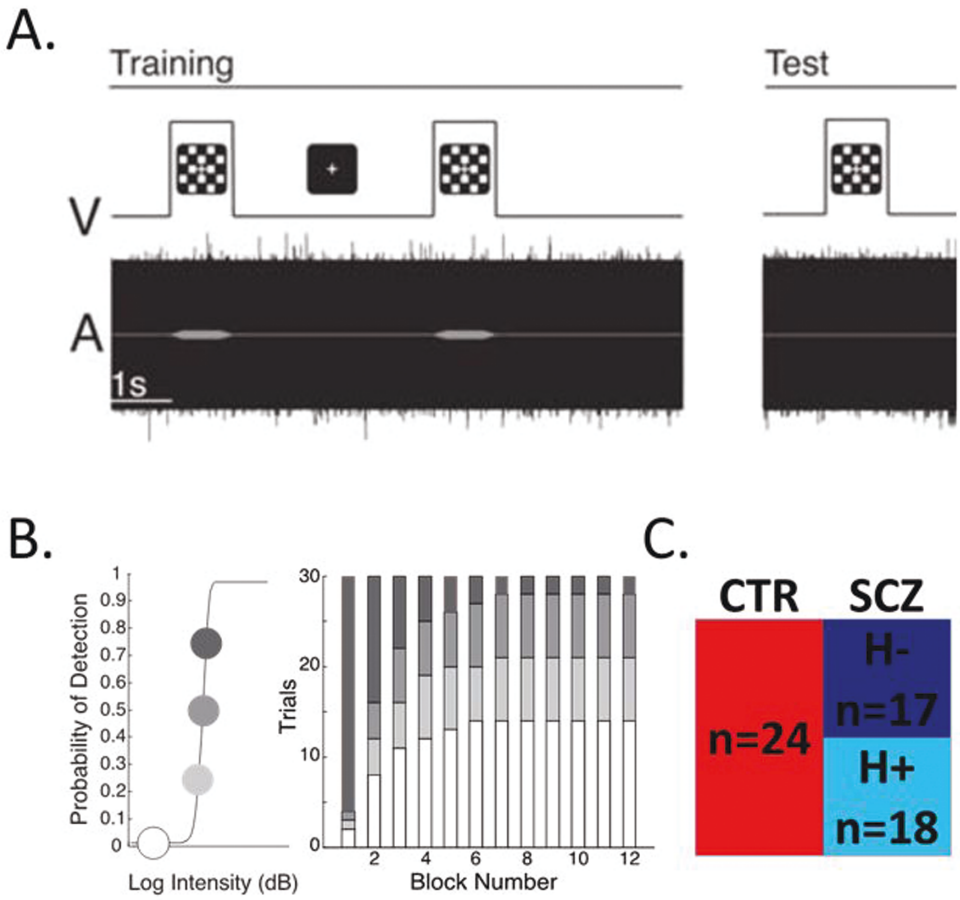

Fig. 1. The conditioned hallucinations task. (A.) Participants worked to detect a tone embedded in noise and presented concurrently with a visual checkerboard (B.) We estimated individual psychometric curves for tone detection and varied tone intensity over 12 blocks of 30 trials (left). High-intensity tones were more likely early and low-intensity and absent tones were more likely later (right). (C.) Our sample consisted of 3, demographically-matched groups: 24 healthy controls (CTR), 17 schizophrenia-patients without hallucinations (SCZH$^-$), and 18 schizophrenia-patients with hallucinations (SCZH$^+$).

a 1H-MRS session (supplementary table S1) at the Yale Magnetic Resonance Research Center or the University of Maryland Center for Brain Imaging Research. Participants were asked to stay awake and keep their eyes closed. Spectra were acquired from four voxels encompassing the anterior cingulate cortex (ACC), left anterior insula (LIN), right dorsolateral PFC (rdlPFC), and left auditory cortex (LAC) (see figure 4A–D) We focused on "Glx" (Glutamate and Glutamine) which is reliably quantified with PRESS at 3T. More details about MRS can be found in the supplementary methods.

Statistical Analysis

The main variables of interest were the CH rate and confidence in CH. We also computed the number of and confidence in "Yes" responses for trials where the tone was present (25%, 50%, and 75%), as well as the confidence ratings when responding "No". No-response trials were discarded from further analysis. All the analyses were performed using the Statistics toolbox of MATLAB R2019b. Details about the statistical analysis can be found in the supplementary methods.

Hierarchical Gaussian Filter

To probe the computational mechanisms underlying conditioned hallucinations, we fitted variants of the Hierarchical Gaussian Filter (HGF)[23,24] to the behavioral data.[4,6] The HGF toolbox (v.5.3.2) is freely available for download in the TAPAS package at https://translationalneuromodeling.github.io/tapas.

The HGF is a generic hierarchical Bayesian model for inference in volatile environments. It consists of hierarchically organized states in which learning at higher levels determines learning at lower levels, by adjusting their learning rate. The lower levels, in turn, send precision-weighted prediction errors to the higher levels, updating posterior means on a trial-by-trial basis. Our generative model consists of three hidden states (X_1, X_2, X_3) and an observation (U). The hidden states represent a low-level perceptual belief (X_1), audio-visual associations (X_2), and the volatility of those associations (X_3) – how rapidly they change over time. Second- and third-level states evolve as hierarchical-coupled Gaussian random walks, controlled by parameters ω_2 and ω_3 respectively (audio-visual association and volatility evolution rates). X_2–X_3 coupling parameter κ and initial beliefs μ_{02} and μ_{03} were fixed (supplementary table S3).

Our three-level perceptual model was paired with a Softmax decision model:

$$P(''Yes'' \mid X_{tone}) = f(X_{tone}) = \frac{1}{1+\exp(-\beta X_{tone})} \quad (1)$$

A sigmoid function wherein β is a positive real number that corresponds to the inverse decision temperature (determining the slope of the sigmoid) and it is a free parameter and X_{tone} is the posterior probability of a tone being present given the subject's prior belief and the sensory input (intensity of tone). Formally, this can be described as the posterior mean of a beta distribution:

$$X_{tone} = p + \frac{1}{1+\nu}(s-p) \quad (2)$$

where s is the expected detection rate (depends on the trial-type: 0.25, 0.5, or 0.75) and p is the prior expectation regarding the presence of a tone and relies on the audio-visual association that the subject has learnt so far (it is equal to $\hat{\mu}_1$[23]). Crucially, the relative contribution of prior$p$, compared to sensory input$s$, was controlled by parameter ν (an index of prior weighting, akin to Kalman gain[25]), and was specific to each subject. Based on the strong-prior theory of hallucinations, we expected higher ν values in the SCZH$^+$ group.

Eight variants of the HGF model were fitted to the behavioral data ("Yes"/'No' responses), each with different sets of free/ fixed parameters (supplementary table S3). The models were inverted at the subject-level, using trial-by-trial tone intensities and participant responses. The best-fitting model was detected through a Bayesian model comparison procedure based on the protected exceedance probability.[26]

Results

Sample Characteristics

Participant demographics are presented in table 1. The groups were matched for age and sex, but there was a significant difference in years of education (F(2) = 6.42, P = .003), mainly due to a difference between patients and controls (CTR vs SCZH$^-$: P = .002; CTR vs SCZH$^+$: P = .03; SCZH$^-$ vs SCZH$^+$: P = .36). Hallucinators and nonhallucinators were also matched for duration of illness as well as severity of negative symptoms.

Behavior

Detection threshold estimates did not differ between groups (CTR: –17.77±2.85 dBSNR; SCZH$^-$: –19.29± 4.69 dBSNR; SCZH$^+$: –17.91±4.66 dBSNR; P = .90). There was also no difference between groups in the number of missed trials (P = .11).

In agreement with previous reports,[4,6] we found a significant effect of group on CH ($x^2(2)$ = 7.68, P = .022), with SCZH$^+$ endorsing more CH than SCZH$^-$ (z = 2.23, P = .026) and CTR (z = 2.53, P = .011) (figure 2A). No difference was observed between CTR and SCZH$^-$ (P = .86), or between CTR and SCZ (collapsed SCZ$^+$ and

SCZ⁻ groups) (P = .11), indicating that the effect is specific to SCZs who hallucinate. Importantly, we also found a significant positive correlation between the percentage of endorsed CH and hallucination-severity in the whole sample (r_S = 0.341, P_S = 0.008; remained significant after controlling for site ($r_{S,site}$ = 0.326, $P_{S,site}$ = 0.013) and diagnosis ($r_{S,diagnosis}$ = 0.273, $P_{S,diagnosis}$ = 0.038)) and in patients (r_S = 0.33, p_S = 0.05) (figure 2B).

Beyond no-tone trials, no significant differences were observed in the number of hits ("Yes" responses) at the 75% (P = .65) and 50% detection conditions (P = .42), while at the 25% detection condition a significant difference was evidenced only between SCZH⁺ and SCZH⁻, with SCZH⁺ achieving more hits (z = 2.05, P = .041) (supplementary figure S1). Additionally, we found no differences in reported confidence between groups, but observed the predicted profile across conditions (increase in reported confidence in "Yes" responses (decrease in "No" responses), from no-tone to 75% detection condition) (supplementary figure S2). Finally, we found a positive correlation between CH and confidence ratings related to CH (r_S = 0.310, P_S = 0.018; $r_{S,site}$ = 0.285, $P_{S,site}$ = 0.032; $r_{S,diagnosis}$ = 0.295, $P_{S,diagnosis}$ = 0.026): subjects who endorsed more CH also reported more confidence in their CH (supplementary figure S3).

Computational Modeling

Model 4 (free parameters: ω_2, β and ν; ω_3 was fixed; figure 3A) emerged as a clear winner (PEP_4 = 0.999; groups did not differ in model fit) (supplementary figure S4), consequently all the following results are with respect to this model, unless mentioned otherwise. Note that adding ν significantly increased log-model evidence, despite increasing the complexity of the model.

We found no significant differences in parameter estimates between groups (ω_2: P = .996; β: P = .20; ν: P = .30) (supplementary figure S5), except for a trend for lower β in hallucinators (SCZH⁺) as compared to nonhallucinators (CTR and SCZH⁻ collapsed) (P = .07). Nonetheless, we found significant correlations between CH and β(r_S = –0.72, P_S < 0.001; $r_{S,site}$ = –0.71, $P_{S,site}$ < 0.001; $r_{S,diagnosis}$ = –0.72, $P_{S,diagnosis}$ < 0.001) (figure 3D) as well as ν(r_S = 0.59, P_S < 0.001; $r_{S,site}$ = 0.56, $P_{S,site}$ < 0.001; $r_{S,diagnosis}$ = 0.57, $P_{S,diagnosis}$ < 0.001) (figure 3B). In other words, endorsement of more CH was related with more stochastic decisions and stronger prior weighting (the 2 parameters were not correlated r_S = –0.10, P_S = 0.45). Interestingly, although confidence ratings were not used to fit model parameters, we observed a correlation between confidence in reporting CH and ν(r_S = 0.46, P_S < 0.001; $r_{S,site}$ = 0.44, $P_{S,site}$ < 0.001; $r_{S,diagnosis}$ = 0.44, $P_{S,diagnosis}$ < 0.001) (figure 3C). Finally, with regard to belief trajectories, we found, that SZH⁺ had a tendency to update their perceptual (X_1) and contingency beliefs (X_2) less (X_1: a trend for elevated X_1 beliefs in SZH⁺ compared to CTR (P = .097); X_2: a trend for elevated X_2 beliefs in SZH⁺ compared to CTR (P = .069) and a significant block effect (t(409) = –2.15, P = .032); X_3: a significant block effect (t(409) = 4.18, P < .001)).

MR Spectroscopy

Glx levels were measured in four brain regions (LIN, ACC, LAC and rdlPFC) (figure 4A–D). We hypothesized that prior weights (ν) might be encoded in (or associated with) LIN.[4] The following datasets were excluded from further analyses, due to poor spectral quality: 1 from the LAC (1 SCZH⁻), 2 from the ACC (2 SCZH⁺) and 2 from the rdlPFC (2 SCZH⁻). Regional concentrations of Glx and spectral quality are presented in supplementary table S2. We found no differences in Glx in any of the four regions (or in total Glx), except for a trend for lower Glx

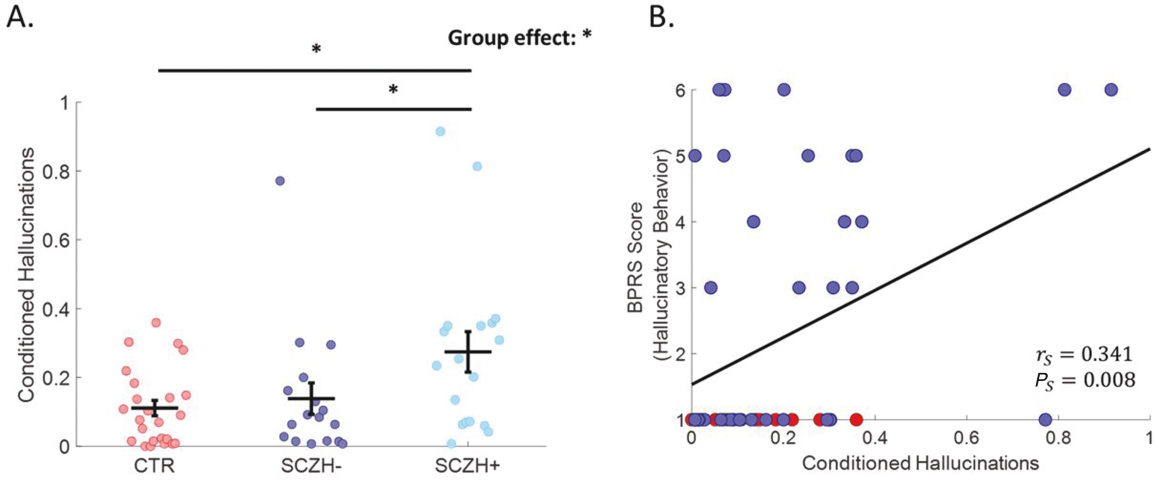

Fig. 2. Conditioned hallucinations and clinical hallucinations. (A.) Conditioned hallucinations rate was significantly elevated in SCZH⁺, compared to SCZH⁻ or CTR. Error bars represent ±1 SEM. (B.) The conditioned hallucinations rate correlated with clinical hallucination severity.

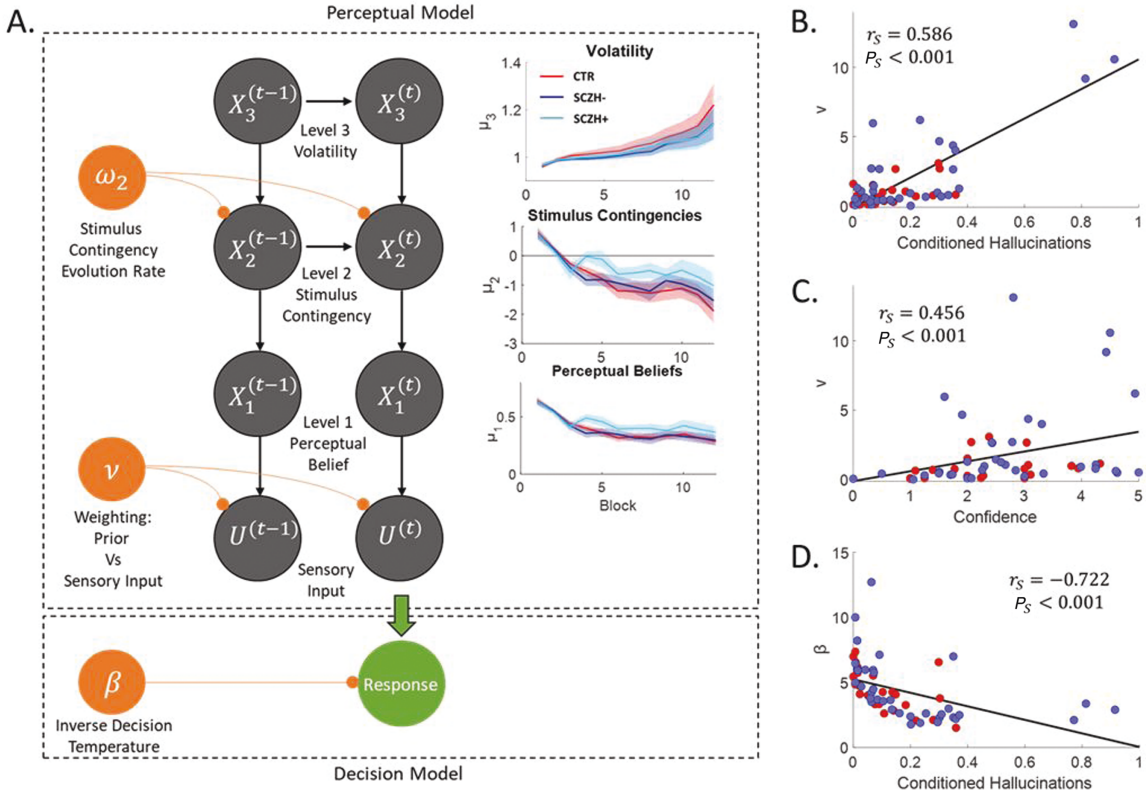

Fig. 3. Hierarchical Gaussian Filter. (A.) Illustration of the (best-performing) HGF model (perceptual and decision component) that was fitted to behavioral data (left). Nodes correspond to states (U: Sensory inputs; X_1: Probability of a tone being present; X_2:Audiovisual association; X_3: Volatility of the audiovisual association), parameters (ω_2;ν;β) and response/percept. SCZH+ showed a tendency towards reduced updating of their perceptual (X_1) and contingency (X_2) beliefs (right—middle and lower panels). We found no group-effect on volatility estimates (X_3; right upper panel). Shaded areas represent ±1 SEM. (B,C,D.). Prior weighting (ν) correlated with CH rate (B.) and CH-related confidence (C.). Inverse temperature (β) correlated only with CH rate (D.).

concentration in SCZH$^+$ in LIN (supplementary figure S6). Furthermore, in agreement with our hypothesis, we observed a significant negative correlation between Glx concentration in LIN and the ν parameter (r_S = –0.28, P_S = 0.043; $r_{S,age}$ = –0.30, $P_{S,age}$ = 0.027; but $r_{S,site}$ = –0.20, $P_{S,site}$ = 0.14), with stronger prior weighting (higher ν) being associated with lower Glx levels (figure 4E) (trend-wise correlation when controlling for diagnosis: $r_{S,diagnosis}$ = –0.25, $P_{S,diagnosis}$ = 0.068 or medication: $r_{S,medication}$ = –0.26, $P_{S,medication}$ = 0.056; but significant when we also took into account age: $r_{S,diagnosis,age}$ = –0.28, $P_{S,diagnosis,age}$ = 0.046; $r_{S,medication,age}$ = –0.29, $P_{S,medication,age}$ = 0.037). Crucially, ν did not correlate with Glx in any other region (ACC: r_S = –0.20, P_S = 0.15; LAC: r_S = –0.16, P_S = 0.24; rdlPFC: r_S = 0.16, P_S = 0.24; correlations remained nonsignificant after controlling for diagnosis and age) (supplementary figure S7) or with total Glx (r_S = –0.12, P_S = 0.39) and Glx in LIN did not correlate with β(r_S = 0.12, P_S = 0.37), CH (r_S = –0.21, P_S = 0.12; trend-wise negative correlation when controlling for age: $r_{S,age}$ = –0.24, $P_{S,age}$ = 0.081), BPRS-12 (r_S = –0.19, P_S = 0.18), age (r_S = –0.17, P_S = 0.22) or medication (r_S = –0.14, P_S = 0.32). Stepwise linear regressions with ν as dependent variable and the four Glx levels as potential predictors (with and without "site" as a predictor) or Glx in LIN as dependent variable and β, ν as potential predictors confirmed that the only significant predictor of ν is the Glx concentration in LIN ($F(53) = 4.2$, $P = .045$).

Discussion

Using a conditioned hallucinations task to probe prior-weighting,[4,6] and MRS to measure glutamate levels we replicated and extended previous findings regarding the neurobiology of hallucinations. In a previous functional neuroimaging study insula cortex responses were associated with conditioned hallucinations and prior-weighting.[4] We found that patients who hallucinate endorse more CH in comparison to nonhallucinators and healthy controls, and CH rate correlated with ratings of hallucinations outside the laboratory, in agreement with previous reports.[4,6] Additionally, participants who endorsed more CH exhibited stronger prior weighting (higher ν) and more stochastic decisions (lower β), while hallucinators were more rigid with regard to their perceptual beliefs (flatter X_1, X_2 beliefs, i.e. reduced belief

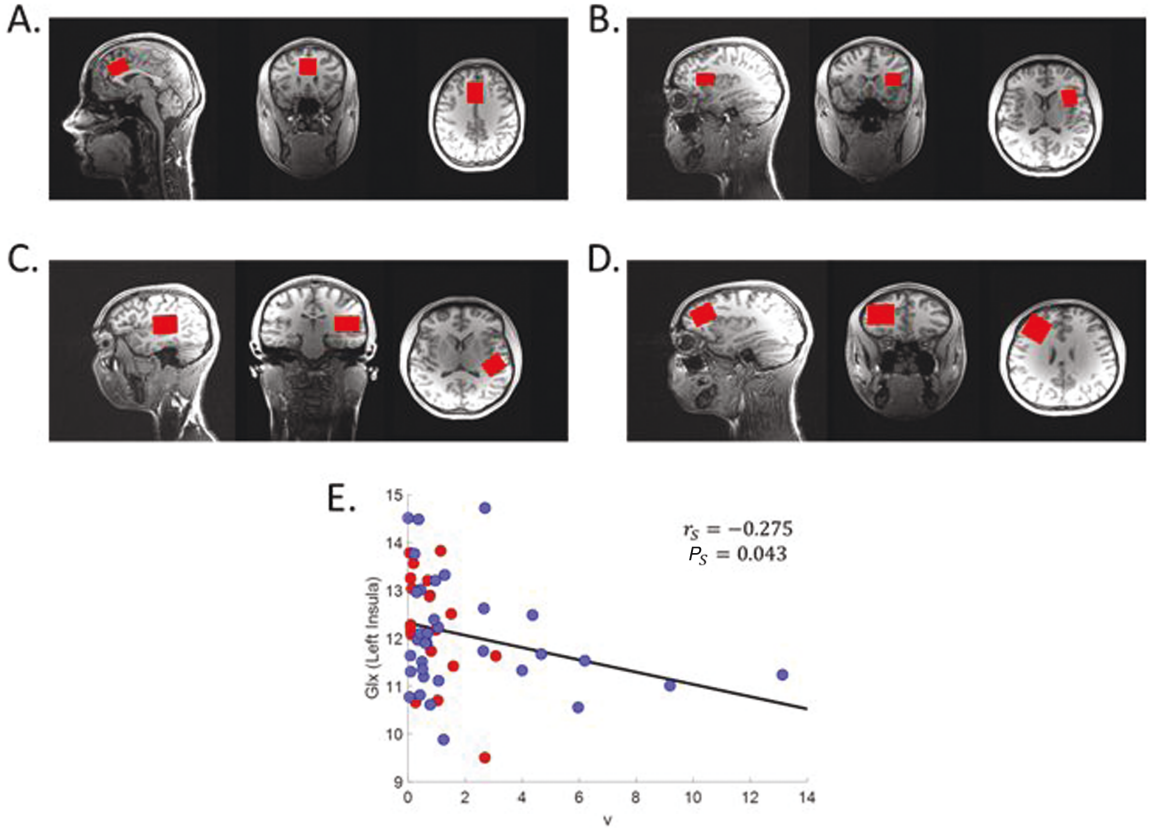

Fig. 4. MR Spectroscopy—voxels and correlation with model parameters. (A–D.) Illustration of MRS voxel placement: (A.) ACC, (B.) LIN, (C.) LAC, and (D.) rdlPFC. (E.) Correlation between the prior weighting and Glx level in LIN.

updating). Finally, we observed a negative correlation between prior-weighting and anterior insula Glx levels, but no relationship with sensory or association cortices.

Our findings are consistent with the results of previous functional imaging studies suggesting the engagement of anterior insula in CH[4] and in AVH.[27,28] We speculate that the negative correlation between priors and insular Glx might be driven by a hypofunction of glutamatergic (NMDA) receptors situated on inhibitory interneurons, resulting in disinhibition of the anterior insula (also observed with ketamine infusion in healthy volunteers[29]). Future studies are needed to further explore the exact nature of the underlying biophysical mechanisms.

Subtle alterations in glutamate transmission and excitation/ inhibition balance play a critical role in the pathophysiology of SCZ.[30–32] A mega-analysis suggested lower glutamate in SCZ, perhaps as a function of medication status, with elevations in those who were more severely ill.[17] Some MRS studies have compared hallucinators with nonhallucinators, with mixed results. In sensory areas, a recent study found higher glutamate and glutamine in SCZ compared to CTR[33] while another study found reduced Glu in the occipital cortex in SCZ and their healthy relatives, suggesting an association with illness liability.[34] Other studies found reduced Glx concentrations in the auditory cortex in patients compared to healthy controls, but elevated Glx in the same region in hallucinators compared to nonhallucinators.[18,19] Our study went a step further, suggesting a functional interpretation to those neurochemical observations. We suggest that glutamate levels in left anterior insula are associated with (and might encode) the precision of prior distributions which, combined with sensory inputs, give rise to percepts. Low glutamate concentrations are related to overly-precise priors and hallucinations.[1]

Prior weighting correlated with glutamate levels specifically in LIN, and not in any other voxel (LAC, ACC, or rdlPFC). Several studies have implicated insula in the pathophysiology and computational anatomy of hallucinations: Powers et al found that CH were associated with a stronger insula response in people who hear voices and had strong beliefs in the visual-auditory association during the task. Other studies have demonstrated a link between anterior insula and hallucinations, directly (e.g.[27,35–38]) or indirectly (e.g.[39]). That being said, other brain regions, not explored in the present study, might also be implicated in perceptual precision weighting. A good candidate is STS, for which a tripartite relation with CH and hallucinations has also been observed.[4] Importantly, the lack of correlation between ν and global Glx can be interpreted as evidence against a global cortical glutamate theory of psychosis/hallucinations (in line

with predominant glutamatergic theories positing a widespread cortical NMDA hypofunction) and more in favor of localized hypo-glutamatergia.

Some predictive processing theories of psychosis posit that strong high-level priors are paradoxically driven by weak lower-level priors (or strong prediction errors).[2] Such a conclusion appears incompatible with the present results. If high-level strong priors were driven by low-level weak priors, we would expect significant but opposite associations between prior strength and low-level (LAC)/high-level (LIN) glutamate concentrations. Instead, we only observed a negative correlation between insular Glx and ν,which is compatible with high-level prediction errors being driven either by within-insula aberrant prediction errors, likely tethered to neuromodulators (dopamine, acetylcholine, or serotonin)[2] or by abnormalities in a parallel hierarchy, e.g. weak egocentric corollary discharge signals.[40] For instance, low glutamate concentration might be the result of excessive striatal dopamine release, which has also been linked to overreliance on prior expectations[41] and increased detection of missing tones in a conditioned hallucinations task.[5]

One limitation is that patients were classified as hallucinators based solely on their current experiences and not on their history. A more careful examination of the psychotic symptoms' trajectory in a small subset of the participants (not presented here) revealed important variability even within subgroups, which might have obscured the most nuanced differences. This might explain why we did not find group differences in model parameters or in Glx levels (only trends), although medication and the moderate level of hallucinations (40% of hallucinators scored below 5 in BPRS-12) might have played a role too. Furthermore, three participants exhibited very high CH rates (~80%), a common behavior in CH tasks.[4,6] Despite thorough training and exhaustive debriefing, it is difficult to know for certain whether those outliers reflect extreme but meaningful cases of conditioned hallucinations or poor performance. Finally, it is important to highlight the tentative nature of our MRS results: our hypothesis that glutamate concentration in anterior insula (and not in any other region) might be related to prior weighting was entirely based on functional (fMRI) data[4] and our result, although significant, would not survive a correction for multiple comparisons. Consequently, our conclusions, as well as the precise mechanisms underwriting the suggested associations, warrant further empirical attention.

In summary, this study represents a preliminary effort to describe a multiscale explanation of hallucinations, spanning behavior (conditioned hallucinations), information processing (strong priors), and neurochemistry (glutamate in LIN). Although this work bears further replication and extension, it provides neurochemical evidence to the relevance of prior beliefs to psychotic symptoms.

Supplementary Material

Supplementary material is available at *Schizophrenia Bulletin* online.

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