Auditory Sensory Gating in the Neonatal Ventral Hippocampal Lesion Model of Schizophrenia

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Key Words

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Abstract

Background/Aims: The neonatal ventral hippocampal lesion (NVHL) rat model shows biological and behavioral abnormalities similar to schizophrenia. Disturbed sensory gating reflects a consistent neurobiological abnormality in schizophrenia. Although of critical interest, sensory gating has not been evaluated in the NVHL model. **Methods:** The N40 rat analog of the human P50 was measured to assess sensory response and gating in NVHL and sham rats. Epidural electrodes recorded evoked potentials (EPs), from which amplitudes, latencies, difference scores (S1–S2) and gating ratios (S2/S1) were assessed. Power and phase locking were computed for evoked EEG activity, to test for frequency-specific abnormalities. **Results:** Prolonged S1 N40 latency was detected in the NVHL group, but amplitude and power measures did not differ. NVHL rats demonstrated disturbed phase-locked sensory gating at theta and beta frequencies, as well as reduced phase-locked gamma activity across stimuli, most robustly at S1. **Conclusions:** While measures of sensory gating obtained from the EP were relatively insensitive to the NVHL model, phase locking across trials was affected.

NVHL rats may have increased evoked response temporal variability, similar to patients with schizophrenia. This pattern of findings likely reflects core developmental NVHL disturbances in dorsal hippocampal circuits associated with temporal and frontal areas. Copyright © 2009 S. Karger AG, Basel

The P50 component of the auditory evoked potential (EP) is a positive deflection in the human electroencephalogram occurring approximately 50 ms following stimulation. The P50 has been extensively used to measure early auditory response and sensory inhibition, or gating, to repetitive stimulation. It is conventionally characterized by two measures: amplitude and latency, likely reflecting activation of large populations of neurons involved in sensory registration and sensory processing speed, respectively. P50 is most often elicited via a dual click procedure designed to test the strength of recurrent inhibitory mechanisms, activated by an initial click (S1) to suppress neural responsiveness to subsequent stimulation (S2) [1]. The most common dependent measures used to assess sensory gating are the P50 amplitude difference score (S1–S2) and gating ratio (S2/S1) [2].

While several neurobiological abnormalities have been observed in schizophrenia [3], an increased P50 gat-

ing ratio is among the most consistent observed in the disorder [3–6], with reports of deficits in at-risk, prodromal, first-episode and chronic schizophrenia [7–9]. Further, patients with schizophrenia often report increased sensitivity to sensory stimulation, distortions and distractibility [10, 11], which possibly index disturbed gating [12]. Increased S2/S1 gating ratios [13–21] in schizophrenia patients may be due in part to an attenuated S1 response [13, 14, 17, 19, 22, 23] or greater latency variability [2, 19, 22].

Scalp-recorded EP voltage changes are thought to result from synchronized extracellular currents produced by excitatory and inhibitory postsynaptic potentials [24, 25]. However, amplitude and gating measures index limited facets of the auditory response, sensitive to averaged EEG activity across trials, but less sensitive to phase variability between trials [24]. This signifies the need for differentially sensitive measures to clarify the understanding of EP generation and ultimately lead to improved interpretation of schizophrenia deficits. Reduced phase consistency, increased latency variability and altered frequency composition across trials of mid-latency EPs have been reported [19, 22, 26, 27]. Frequency decomposition of EPs in gating paradigms has shown that patients with schizophrenia have disturbed low- (0–20 Hz) [26, 27] and high-frequency (20–50 Hz) [28] responses. Recent reports suggest specific phase locking deficits at S1 in lower frequency bands, such as theta (4–6 Hz) and alpha (9–11 Hz) as well [29]. These findings together highlight the value of time-frequency analyses in characterizing sensory gating.

Although disturbed sensory gating is among the most robust and replicable neurobiological findings in schizophrenia [3], its substrates are not readily tested in humans. Therefore, animal models examining pathophysiological processes hypothesized to contribute to schizophrenia have been implemented. The neonatal ventral hippocampus lesion (NVHL) rat model [30, 31] entails delivery of an axon-sparing excitotoxin to the ventral hippocampus, an area that is implicated in the developmental pathogenesis of schizophrenia by cellular and morphometric [32] as well as neuroimaging [33] studies. NVHL rats demonstrate an emergence of a ‘schizophrenia-like’ behavioral syndrome upon aging to adulthood, including locomotive hypersensitivity, altered pharmacological responsiveness to psychomimetic drugs, prefrontal cortex molecular alterations [30, 31], deficits in working memory and spatial contextual learning [34] and impaired cue association learning [35]. Moreover, NVHL rats have impaired CNS inhibition [36] indexed

by sensorimotor gating [37], a phenomenon theoretically similar to P50 sensory gating.

Rodent EP components share fundamental characteristics with human P50 when elicited via the dual click procedure [20, 38–42]. The N40, a negative deflection in the EP approximately 40 ms after stimulus onset, is considered a rodent analog of P50 by many [14, 20, 43–46], but not all [41, 47] investigators. In rodents, as in humans, sensory gating is usually measured as the ratio of the response amplitude to the first compared to the second stimulus (S2/S1). This ratio is often impaired in pharmacological models of schizophrenia targeting dopaminergic [43, 48–51] and glutamatergic [38, 51] systems, with many studies suggesting that the increased ratio is due to a decreased S1 response [49–51]. Although sensory gating has been tested in pharmacological animal models of schizophrenia, to our knowledge it has not been examined in NVHL rats. Since sensory gating is thought to be mediated by hippocampal [52, 53] and frontal [54, 55] circuits, a developmental lesion affecting these areas would likely impact gating. Therefore, this study tested whether the sensory gating deficit observed in schizophrenia was present in the NVHL model. Two forms of signal analysis were applied: time domain averaged EPs and time-frequency response characterization. Time domain measures of N40 amplitude and latency obtained from averaged EPs to S1 and S2 were used for comparison with other animal and human studies. Like pharmacological animal models of, and patients with, schizophrenia, NVHL rats were expected to have decreased amplitude and increased latency variability to S1, a smaller difference score (S1–S2) and increased gating ratio (S2/S1), compared to sham lesion controls. The two time-frequency measures used were mean trial power (MTP) and phase locking factor (PLF). MTP reflects baseline-subtracted EEG activity following sensory stimulation, resulting from both stimulus-evoked and background EEG, which may be modulated by differing neural network properties or substrates. PLF is a measure of phase consistency, or reproducibility, of evoked EEG activity across trials at a given latency, or temporal interval, and frequency [24]. PLF decomposes EPs into phase-locked frequency band elements. PLF values can range from 0 (absent phase consistency) to 1 (perfect phase consistency). Based on previous findings in human schizophrenia [27–29], NVHL rats were expected to show decreased MTP and PLF, particularly in low-frequency bands (0–20 Hz) at S1. These findings would further support the validity of this model of schizophrenia and advocate for its use in future EP studies.

Methods and Materials

Animals

All procedures were in compliance with the Guide for the Care and Use of Laboratory Animals and were approved by the Indiana University Animal Care and Use Committee. Four pregnant Sprague-Dawley rats were obtained from Harlan Laboratories (Indianapolis, Ind., USA). Rats were individually housed for 7–14 days until birth. According to the protocol developed by Lipska and Weinberger [31], at postnatal day 7 (P7), pups were removed from their home cage and taken into a surgical room for 2–3 h. Pups were anesthetized by hypothermia and secured with tape to a stereotaxic platform to maintain head position. A horizontal incision across the dorsal surface of the head was made, and a 26-gauge needle was lowered through the thin level skull into the ventral hippocampal formation (AP –3.0 mm, ML $\pm$ 3.5 mm and VD –5.0 mm relative to bregma). Over a period of 135 s, each pup received either 3.0 μ g of ibotenate (Sigma, St. Louis, Mo., USA) delivered in 0.3 μ l artificial CSF, to produce an excitotoxic lesion to both ventral hippocampi (n = 16) or a sham lesion (n = 14), following the same procedure without the excitotoxin (artificial CSF only). The surgical wound was closed with veterinary wound cement, and the pups were monitored and warmed by a heating pad before being returned to home cages.

Rats were weaned normally (P25) and, at P49, paired and moved to plastic cages. Three days prior to EEG electrode implantation, rats were moved to single-house plexiglass cages. At all stages, rats were kept in a temperature-controlled room, maintained on a 12-hour light:12-hour dark cycle (7 a.m. to 7 p.m.), with food and water available ad libitum. To reduce undue stress, rats were handled daily until implantation of EEG electrodes and twice weekly thereafter.

Surgical Procedures: EEG Electrode Implantation

Upon reaching 350 g ($\pm$ 50 g; approx. P70), rats underwent surgery for EEG electrode implantation. Rats were anesthetized by an isoflurane/air mixture and positioned in a stereotaxic frame. A midline incision exposed the dorsal surface of the skull, and another incision reflected the right temporalis muscle, exposing the temporal bone. Stainless-steel screw electrodes with wire leads were implanted epidurally over the vertex (AP –4.0 mm, ML –1.0 mm), temporal cortex (AP –4.5 mm, DV –4.0 mm), cerebellum (ground) and frontal sinus (reference). Stainless-steel lead wires were connected to an Amphenol plug, and the entire assembly was secured to the skull with dental cement. All EP measures reported here were recorded from the vertex electrode, as the temporal electrode was implanted for a later study.

Recording Procedures

Recording commenced following 2 weeks of recovery from surgery (approx. P95). Rats were placed in a 5-sided plastic mesh cage in an electrically shielded chamber, where they were acclimated for 20 min prior to recording. A female connector, with flexible, insulated wires was attached to the Amphenol plug. Single trial EEG data was collected (band pass 0.1–300 Hz), digitized (1,000 Hz), displayed on a computer monitor and written to the hard drive. Each trial epoch was 899 ms, including a 160-ms prestimulus baseline. Stimulus presentation was performed using Presentation® software (version 0.70, www.neurobs.com). EPs were elicited via clicks (5 ms white noise pips, 1 ms rise/fall, 80

dB) delivered in pairs (S1 and S2), with an interclick interval of 500 ms. Click pairs (80) were presented every 8–10 s, from a speaker mounted on the front of the chamber. Time of testing was held constant for each rat, with either morning (7.00 a.m. to 12.30 p.m.) or afternoon (1.00 p.m. to 6.30 p.m.) assignment.

Horizontal and vertical movements were recorded. Horizontal movement was defined as the number of times the rat crossed a midline point in the recording chamber. Vertical movements were defined as the animal 'rearing', lifting its front paws from the recording chamber floor for a minimum of 5 s.

Histology

Following completion of recordings, rats were sacrificed by deep isoflurane anesthesia (ensured via decapitation), brains were sectioned (initially at 40 μ m and then cut every 10th rotation to 400 μ m) by a cryostat and Nissl stained. Lesions were assessed by two investigators (R.A.C. and S.B.) using a low-magnitude microscope, blind to physiological results. NVHL rats with either unilateral lesions or inappropriately placed lesions impacting extra-hippocampal structures and sham rats with needle track damage to the hippocampus or surrounding tissue were excluded from further analysis. Lesion damage for each section was recorded by hand in coronal section maps (Paxinos and Watson, 1998). Two sham lesion control and 6 NVHL rats were excluded. Data from the remaining rats (sham, n = 12; NVHL, n = 10) was then analyzed. Figure 1 shows histological results, with the greatest (black) and least (gray) extent of lesion damage allowed for rats included in the study.

Data Reduction

Amplitude, Latency, Difference Score and Gating Ratio

Data reduction was performed using Brain Vision Analyzer software® (Brain Products GmbH, www.brainproducts.com). After data segmentation and baseline correction, trials were averaged and the response to each click pair was high pass filtered (1 Hz; 12 dB/octave rolloff). The N40 component was then determined from the averaged responses to the first (S1) and second (S2) clicks as the maximum negative waveform in the 30- to 90-ms range relative to the P20 component, which was defined as the positive component that preceded the N40 or the maximum positive voltage in the 15- to 65-ms range. Peaks were semiautomatically detected and visually verified by two authors (J.L.V. and S.L.M.). Amplitude and latency of both the P20 and N40 peaks were quantified. EP peak-to-peak voltage values were calculated by subtracting N40 amplitude from P20 amplitude, a more stable measure of EP amplitude insensitive to baseline voltage shifts. Difference scores, calculated by subtracting the P20–N40 amplitude of S1 from the P20–N40 amplitude of S2, and gating ratios, calculated by dividing S2 P20–N40 amplitude by S1 P20–N40 amplitude, were used to examine N40 gating.

Time Frequency Analyses

A short-time fast Fourier transform (STFFT) was applied on single trials of EEG data in order to obtain MTP and PLF. The STFFT of a single trial involved computing FFT on EEG segments that were extracted using an overlapping moving window which is displaced in time by a constant time step. The length of the moving window was 128 ms and the step size was 10 ms. A Hanning window (amplitude ranging from 0 to 1) was applied prior to computing the FFT on each window. The STFFT returns a

Fig. 1. Hemicoronal representations showing the extent of neonatal ibotenic-acid-induced lesions of the ventral hippocampal region. Solid black areas represent the largest lesion size (on either side) of subjects accepted for analysis ($n = 10$), and gray areas represent the smallest areas. The majority of rats had lesion sizes falling between these extremes. Numbers below sections indicate the distance posterior to the bregma based on coordinates of Paxinos and Watson.

complex number output $[\tilde{F}(f, t)]$ for each frequency (f) and time step (t) in each trial.

The MTP is the mean change in power across trials relative to baseline. Power is obtained by computing the squared absolute value of $\tilde{F}(f, t)$.

The MTP was obtained by first subtracting the mean value of power in the baseline period from the entire trial separately for each trial, then averaging this baseline subtracted power across trials. The MTP is the same as event-related spectral perturbation [56]. The MTP measures both phase-locked and non-phase-locked activity across trials. A 160-ms baseline period was used for computing MTP in this study. The PLF was obtained using the following formula:

$$PLF(f, t) = \left| \frac{1}{K} \sum_{i=1}^K \frac{\tilde{F}(f, t)}{|\tilde{F}(f, t)|} \right|$$

where K is the total number of trials. The PLF is a normalized average of the normalized complex output from the STFFT. The PLF measures phase synchronization of EEG activity across trials [57]. Since the FFT was applied using short time windows, PLF is obtained for each time of the window (t) and frequency (f).

MTP and PLF were calculated for further analysis within discrete frequency bands. In line with previous investigations, two methods for division of frequency windows were selected: (1) low-versus high-frequency band activity [31, 32] and (2) theta (3.92–7.84 Hz), alpha (9.8–13.72 Hz), beta (16.69–25.49 Hz) and gamma (29.41–41.16 Hz) bands. The second classification of smaller frequency bands is similar to other investigators [29] but necessarily differs due to the differences in exact frequency bins produced by the STFFT. For statistical analysis, average PLF values were computed within 10-ms intervals (5–15, 16–25, 26–35, 36–45, 46–55 ms) and then averaged across all 5 windows for comparisons (5–55 ms after stimulus onset). Average PLF values within the 10-ms windows were used to elucidate effects or interactions, as appropriate. Frequency intervals were exported corresponding to 2-Hz windows (2–4, 4–6 Hz, etc.) and were averaged according to the frequency band of interest.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows (version 14.0, 2007; SPSS Inc., Chicago, Ill., USA). Univariate analyses of variance (ANOVAs) were used to test the hypotheses that NVHL rats would demonstrate a decreased amplitude and prolonged latency at S1, decreased S1–S2 difference score and

Table 1. Mean amplitude (mV) and latency (ms) measures for the P20 and N40 components of the sensory gating EP following auditory stimuli (S1, S2)

	Sham		NVHL	
	P20	N40	P20	N40
Amplitude				
S1	0.87 (0.25)	-0.26 (0.49)	0.65 (0.50)	-0.26 (0.38)
S2	0.53 (0.17)	-0.25 (0.39)	0.49 (0.39)	-0.08 (0.34)
Latency				
S1	30.57 (5.50)	58.66 (9.17)	32.89 (5.17)	65.24 (11.93)*
S2	19.39 (3.69)	51.43 (9.92)	23.02 (7.26)	57.89 (13.36)

Numbers in parentheses indicate standard deviations. The NVHL rats demonstrated prolonged latency at S1 (* $p < 0.01$) relative to sham controls. No other group differences were detected.

increased S2/S1 gating ratio. The aforementioned comparisons were repeated using square-root transformations, attenuating the positive skew to which the gating ratio is particularly prone. Transformations did not affect results and are not reported. Repeated-measure ANOVA, with a within-subject factor response amplitude to each stimulus (S1, S2) and between-subject factor group (sham, NVHL), was also used to test for potential gating effects. In the frequency domain, repeated-measure ANOVA, with the within-subject factor stimulus and frequency band (low, high), and between-subject factor group were performed. To more fully characterize response pattern and potential gating effects, repeated-measure ANOVAs were performed for a number of frequency bands (theta, alpha, beta and gamma), with the within-subject factor stimulus and between-subject factor group. Independent-sample t tests were used to explicate effects and interactions, as appropriate. Effects and interactions were considered statistically significant at $p < 0.05$. Effect size, as measured by partial eta squared (η_p^2), was calculated to reflect strength of association [58]. η_p^2 is the proportion of the effect variance to the effect plus error variance: $\eta_p^2 = SS_{\text{factor}} / (SS_{\text{factor}} + SS_{\text{error}})$. Higher η_p^2 values reflect stronger effects.

Results

Concerning amplitude and latency, N40 and P20 components, compared with sham lesion controls, the NVHL rats did not show reduced S1 amplitude at N40, P20 or the P20-N40 complex. However, the NVHL group did show prolonged N40 latency [$t(20) = -3.04$, $p < 0.01$] at S1. No lesion effects were detected for EP component amplitude or latency at S2. Table 1 lists the mean amplitude and latency measures for the N40 and P20 components of the sensory gating EP following each auditory stimulus (S1, S2).

For difference score (S1–S2) and gating ratio (S2/S1), gating was observed in both groups of rats. No group dif-

ferences were detected for difference score (S1–S2) or gating ratio (S2/S1) measures.

Concerning MTP, comparing its average, no group differences were found in either low- (0–20 Hz) or high-frequency (20–50 Hz) bands.

When further divided into more specific frequency bands, effects of stimulus were detected across groups, with S1 having larger MTP than S2 in the alpha [9.8–13.72 Hz; $F(1, 22) = 15.56$, $p = 0.001$, $\eta_p^2 = 0.41$], beta [16.69–25.49 Hz; $F(1, 22) = 66.09$, $p < 0.001$, $\eta_p^2 = 0.75$] and gamma [29.41–41.16 Hz; $F(1, 22) = 7.32$, $p = 0.01$, $\eta_p^2 = 0.25$] frequency bands, within the first 55 ms after stimulation. No group by stimulus interaction was found, suggesting that, like EP voltage measures, MTP did not detect the expected S1 deficit in the NVHL group.

The PLF for sham ($n = 12$) and NVHL ($n = 10$) rats is illustrated in figure 2. After computation of the PLF, stimulus by frequency band by group ANOVA demonstrated main effects for stimulus [$F(1, 22) = 16.30$, $p < 0.001$, $\eta_p^2 = 0.40$], frequency band [$F(1, 22) = 47.30$, $p < 0.001$, $\eta_p^2 = 0.65$] and a stimulus by group interaction [$F(1, 22) = 5.28$, $p = 0.03$, $\eta_p^2 = 0.17$]. While PLF for low-frequency (0–20 Hz) band activity did not differ between NVHL and sham groups, the high-frequency (20–50 Hz) band [$t(22) = -2.28$, $p = 0.03$] PLF differed at S1. To further explicate the high-frequency band (20–50 Hz) effect, independent-sample t tests were performed between groups after S1, for each 10-ms time window average included in the 55-ms analysis window (5–15, 16–25, 26–35, 36–45, 46–55 ms, after S1 and S2 presentation). The groups differed in PLF between 5 and 15 ms [$t(22) = 3.51$, $p = 0.002$] and between 15 and 25 ms [$t(22) = 2.73$, $p = 0.01$] after S1, with NVHL rats having reduced phase locking compared to sham animals. No S2 effects were found. These data

Fig. 2. PLF for sham (**a**, $n = 12$) and NVHL (**b**, $n = 10$) rats. The figure shows time (0–600 ms) on the x-axis and frequency (0–100 Hz) on the y-axis. PLF (PLF units, 0–1) is indicated by color, with hot colors (reds and oranges) indexing more activation and cool colors (blues and greens) indexing less activation. Stimulus onset occurs at 0 ms (S1; band of activation on the left) and 500 ms (S2; band of activation on the right). Within the first 55 ms after the onset of stimulation, the sham group had reduced theta band activity at S2, relative to S1, while the NVHL group showed the opposite pattern, with average PLF in this time window increased at S2, relative to S1 ($p < 0.05$). Within the first 25 ms after stimulation, PLF in the high-frequency range (20–50 Hz) was significantly reduced in NVHL rats, relative to sham lesion controls ($p < 0.05$) at both S1 and S2. The average PLF windows used to detect these effects are outlined in red for theta (3.92–7.84 Hz), green for beta (16.69–25.49 Hz), black for gamma (29.41–41.16 Hz) and dashed black for the high-frequency band (20–50 Hz).

Fig. 3. Frequency bands reported at S1 (**a**) and S2 (**b**) are highlighted by color band and labeled with the corresponding Greek letter: theta (3.92–7.84 Hz), alpha (9.80–13.72 Hz), beta (16.69–25.49 Hz) and gamma (29.41–41.16 Hz). PLF stimulus by group interactions are observed by decreased S2 in the sham lesion

group and increased S2 in the NVHL group in the theta frequency ranges. While sham rats had robust reduction of S2 beta activity, NVHL rats demonstrated the same pattern to a lesser extent. NVHL rats had decreased phase locking in the gamma frequency band, especially at S1 ($p < 0.05$).

Fig. 4. Average PLF at S1 and S2 for sham ($n = 12$) and NVHL ($n = 10$) groups. Low-frequency bands theta (**a**, 3.92–7.84 Hz) and alpha (**c**, 9.80–13.72 Hz), and high-frequency bands beta (**b**, 16.69–25.49 Hz) and gamma (**d**, 29.41–41.16 Hz), and stimuli (S1, S2) are presented on the x-axis and PLF on the y-axis. Within the first 25 ms after stimulation, theta comparisons illustrate disturbed NVHL sensory gating, with decreased S2 phase locking in the sham group and increased S2 phase locking in the NVHL group, relative to S1. Reduced beta range PLF sensory gating, and an ef-

fect of stimulus, is illustrated. The NVHL group had decreased S1 PLF and relatively less S2 reduction compared to sham rats. In the gamma frequency band, NVHL rats had decreased phase locking at S1 and S2, with the S1 reduction being more pronounced. Note that S1 PLF was consistently reduced in NVHL relative to sham animals. Effects were considered significant when $p < 0.05$. Alpha was reduced at S1, but this interaction was only marginally significant ($p = 0.06$).

suggest that NVHL rats have a decreased S1-phase-locked high-frequency response within the first 25 ms after stimulation.

When PLF was calculated within smaller frequency bands, group differences and interactions were detected. NVHL rats had sensory gating deficits in theta and beta frequency bands, and a gamma deficit at both S1 and S2. Figure 3 shows the frequency distribution (0–100 Hz) at S1 and S2, for each group, with the relevant frequency bands highlighted. Stimulus by group interactions revealed that the sham group had decreased S2 average phase locking relative to S1, while the NVHL rats had less average phase locking at S1 than S2 in the theta [$F(1, 22) = 4.92$, $p = 0.04$, $\eta_p^2 = 0.18$] frequency band. Figure 2 illustrates that the increased S2, relative to S1, phase locking in the NVHL group was due to more consistent phase locking across the 55-ms window used for the analysis. At S1, early phase-locked activity is reduced while at S2 phase locking was more stable across the examined window, therefore resulting in increased average phase lock-

ing. In the beta band, a main effect of stimulus [$F(1, 22) = 11.05$, $p = 0.003$, $\eta_p^2 = 0.33$] and a stimulus by group interaction [$F(1, 22) = 4.74$, $p = 0.04$, $\eta_p^2 = 0.18$] were both detected. However, unlike the theta band, the beta stimulus by group interaction reflected decreased S2 PLF relative to S1 in sham rats, but similar S1 and S2 average phase locking in NVHL rats. Finally, PLF in the gamma band revealed only a main effect of lesion [$F(1, 22) = 4.29$, $p = 0.05$, $\eta_p^2 = 0.16$], illustrating a gamma deficit in the NVHL group across S1 and S2. Taken together, these data demonstrated clear gating (reduced S2 relative to S1 PLF) in both groups, but that NVHL rats had impaired phase-locked sensory gating in theta and beta frequency bands. NVHL rats also had a reduction in gamma activation across both S1 and S2 stimuli, relative to sham lesion rats. Figure 4 shows PLF for theta, alpha, beta and gamma frequency bands by group.

As for horizontal and vertical movement, no significant group differences were detected. Rats were freely moving during recording and movement trials were not

removed prior to analysis, therefore Pearson correlation coefficients (by group and collapsed across groups) were performed in order to detect any relationship between observed movement and electrophysiological measures. No correlations were detected between behavior and electrophysiological measures, suggesting that movement did not produce artifacts affecting EP measures.

Discussion

The aim of the present investigation was to test whether sensory gating deficits occurred in the NVHL rat model of schizophrenia analogous to those observed in patients. The time-frequency measure PLF revealed disturbed neural processing in NVHL rats, with a reduction in high-frequency band (20–50 Hz) activity within the first 25 ms after stimulation, and sensory gating disturbances in theta (3.92–7.84 Hz) and beta (16.69–25.41 Hz) frequency bands within the first 55 ms after stimulation. NVHL rats had reduced average theta PLF at S1 relative to S2, while the beta band gating deficit resulted from attenuated S1 and lack of S2 average PLF reduction. Gamma band (29.41–41.16 Hz) PLF was reduced at S1 and S2 in NVHL rats, but most robustly at S1. Averaged EP and MTP measures of sensory gating (reduced S2 relative to S1 responses) were not perturbed in the NVHL compared to sham rats.

The present finding that NVHL rats have disturbed PLF gating in theta and beta bands is consistent with disturbances seen in patients with schizophrenia, using similar time-frequency analyses. Reduced low-, but not high-frequency response at S1 has been reported [27]. NVHL beta and gamma disturbances are consistent with reduced high-frequency activity, which has been previously reported in schizophrenia by Johannesen et al. [28], who found that patients had impairments in both low- and high-frequency band sensory gating, due to a reduced S1 response [28]. The NVHL findings were partially consistent with a recent study that examined sensory gating disturbances in schizophrenia across multiple frequency bands (delta, theta, alpha, beta and gamma) [29]. In this study, Brockhause-Dumke et al. [29] did not find differences in beta and gamma bands, but did detect decreased phase locking in theta and alpha bands, most pronounced at S1. Findings from these studies are consistent with widely reported S1-mediated gating deficits reported in schizophrenia [5, 13–14, 17–19, 59] and rat models [39, 56, 48]. The S1-mediated sensory gating deficit likely reflects abnormal sensory registration and

maintenance of novel relevant information [60] in schizophrenia and pharmacological animal models of schizophrenia.

Theta, beta and gamma were the frequencies most sensitive to the NVHL model. Coupling of theta oscillations is thought to mediate hippocampal-cortical communication [61], and, therefore, developmental alterations in the hippocampus and associated structures may have resulted in disturbed PLF and gating. In terms of beta and gamma frequencies, NVHL rats had disturbed beta PLF sensory gating and reduced gamma PLF at both S1 and S2. PLF beta gating disturbances are consistent with a recent report suggesting that beta activity mediates sensory gating [62]. Reduced gamma PLF at S1 and S2 in NVHL rats is consistent with disturbed sensory registration [63] and altered function of the temporal cortex.

Human sensory gating is thought to be generated by neural circuitry with prominent involvement of temporal [53, 64] and frontal [64, 65] cortical structures, with the temporal cortex responsible for EP generation and the frontal cortex and hippocampus more involved in S2 gating [54, 66, 67]. Similarly, the rat N40 is likely generated by temporal cortex and hippocampal structures [53, 65, 67]. Therefore, deficits observed in the NVHL group likely involve multiple generators, with the S1 deficit suggestive of a temporal cortex abnormality [23] and the sensory gating deficit due to the developmentally compromised frontohippocampal circuits [68] of NVHL rats.

The lack of an increased averaged EP sensory gating ratio (S2/S1) in NVHL rats presents a discrepancy from many findings in the schizophrenia literature. It should be noted, however, that averaged EPs and MTP are affected by EP polarity and voltage differential, respectively. PLF, on the other hand, is a measure of phase consistency across trials. Computation of MTP involves the subtraction of the baseline period from the power, or magnitude, of the evoked response. The baseline period used in the present study was not ideal for this measure, as technical limitations allowed for only 160 ms. Given that the primary findings of this study were reduced PLF and PLF sensory gating, it is possible that the failure to detect MTP group differences was due to the short baseline length.

Another potential limitation of time-frequency analyses involves the inverse relationship between window length (time resolution) and frequency resolution, commonly referred as the uncertainty problem. While it is possible to modify the window size across frequencies, it would be difficult to compare events which occur in short

time intervals (e.g. 50 ms) across frequencies. As an alternative, a wavelet transform, which has variable time and frequency resolution, could have been used. However, wavelet transformation also has shortcomings, as it is dependent upon the type of wavelet and its parameters. The results of the current FFT analysis were compared with a wavelet transform (Morlet wavelet) and the results were qualitatively similar, thus suggesting that the primary results of the present study were robust enough to be captured using other time-frequency transformations. Notably, the time-frequency analyses implemented were hypothesis driven with predetermined frequency bands and time windows. Therefore, differences at other frequencies or temporal intervals could have been missed. For instance, NVHL rats have an extended high PLF (above 0.5) response (approx. 200 ms) after S1, relative to sham rats (approx. 100 ms). This finding may reflect an inability for NVHL rats to quickly desynchronize from external sensory stimulation. However, future investigations could be designed to specifically characterize and test such potential NVHL abnormalities.

Unlike the present investigation of NVHL rats, studies including patients with schizophrenia generally involve medication effect confounds, as nearly all human studies include patients medicated at the time of testing. No pharmacological manipulations were presently performed, allowing for a more realistic examination of underlying disturbances. A potential limitation of the present study was sample size, after histological exclusion for poor lesions. The NVHL group sample ($n = 10$) is smaller than some, but not all, human gating studies. A recent meta-analysis [6] cited 16 human P50 studies including 10 or fewer schizophrenia patients. Finally, given that this study utilized a neurodevelopmental animal model of schizophrenia, additional lesion manipulations at different developmental stages would have been informative. Future investigations of how developmental age of lesion could impact N40 measures will be an important direction for future studies.

Taken together, the most likely explanation for the failure to detect expected averaged EP gating ratio deficits, while still detecting PLF gating deficits, indicates differential disturbances in the network properties and neurotransmitter systems reflected by each technique (amplitude vs. phase consistency). Relevant pharmacological manipulations could serve to further explore these properties. Combining the NVHL model with pharmacological manipulations affecting neurotransmitter systems thought to be involved with schizophrenia or sensory gating generation (such as cholinergic, dopaminergic, GABAergic and glutamatergic systems) would provide a means to further explore neurobiological mechanisms. Pharmacological alternatives for the treatment of schizophrenia could also be explored.

In summary, time-frequency analysis allowed specification of auditory sensory processing and gating in the NVHL rat model of schizophrenia. Although less robust than anticipated, frequency response and sensory gating abnormalities, associated with schizophrenia, can be detected in the NVHL model. While PLF revealed disturbed gamma response and gating in theta and beta frequency bands, averaged EP amplitude and MTP measures did not capture these effects. These data are consistent with some previous reports in both human and rodent studies which suggest that sensory gating disturbances result from attenuated S1 activity. Moreover, these findings further support the validity of this model of schizophrenia and advocate its use in future EP studies.

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