Shank3-Deficient Rats Exhibit Degraded Cortical Responses to Sound

Crystal T. Engineer, Kimiya C. Rahebi, Michael S. Borland, Elizabeth P. Buell, Kwok W. Im, Linda G. Wilson, Pryanka Sharma, Sven Vanneste, Hala Harony-Nicolas, Joseph D. Buxbaum, and Michael P. Kilgard

Individuals with *SHANK3* mutations have severely impaired receptive and expressive language abilities. While brain responses are known to be abnormal in these individuals, the auditory cortex response to sound has remained largely understudied. In this study, we document the auditory cortex response to speech and non-speech sounds in the novel *Shank3*-deficient rat model. We predicted that the auditory cortex response to sounds would be impaired in *Shank3*-deficient rats. We found that auditory cortex responses were weaker in *Shank3* heterozygous rats compared to wild-type rats. Additionally, *Shank3* heterozygous responses had less spontaneous auditory cortex firing and were unable to respond well to rapid trains of noise bursts. The rat model of the auditory impairments in *SHANK3* mutation could be used to test potential rehabilitation or drug therapies to improve the communication impairments observed in individuals with Phelan-McDermid syndrome. ***Autism Res*** *2018, 11: 59–68.* © 2017 International Society for Autism Research, Wiley Periodicals, Inc.

Lay Summary: Individuals with *SHANK3* mutations have severely impaired language abilities, yet the auditory cortex response to sound has remained largely understudied. In this study, we found that auditory cortex responses were weaker and were unable to respond well to rapid sounds in *Shank3*-deficient rats compared to control rats. The rat model of the auditory impairments in *SHANK3* mutation could be used to test potential rehabilitation or drug therapies to improve the communication impairments observed in individuals with Phelan-McDermid syndrome.

Keywords: Phelan-McDermid syndrome; 22q13 deletion; autism; SHANK3-haploinsufficiency syndromes

Introduction

Individuals with Phelan-McDermid syndrome have severely delayed or absent speech, as well as motor impairments and developmental delays [Philippe et al., 2008; Phelan & McDermid, 2012; Soorya et al., 2013; Zwanenburg, Ruiter, van den Heuvel, Flapper, & Van Ravenswaaij-Arts, 2016]. Phelan-McDermid syndrome is a neurodevelopmental disorder that arises from deletion or single mutation in one copy of the *SHANK3* gene. *Shank3* is a scaffolding protein in the postsynaptic density of glutamatergic synapses [Jiang & Ehlers, 2013]. *SHANK3* deletions and mutations account for up to 1.7% of individuals with autism spectrum disorder [Leblond et al., 2014]. Interestingly, the neural responses evoked by sounds in individuals with Phelan-McDermid syndrome are distinct from the neural responses evoked in individuals with idiopathic autism [Wang et al., 2016]. While it is well-documented that individuals with *SHANK3* mutations have receptive speech processing problems [Soorya et al., 2013; Sarasua et al., 2014a; Wang et al., 2016], no studies have examined basic auditory processing in these individuals compared to typically developing individuals.

Rats are an excellent model of speech processing. Rats can accurately distinguish between most English consonant and vowel sounds [Reed, Howell, Sackin, Pizzimenti, & Rosen, 2003; Engineer et al., 2008; Perez et al., 2013]. In addition, rats are able to correctly generalize to different talkers, and accurately discriminate speech sounds presented in background noise or in a continuous speech stream [Shetake et al., 2011; Engineer et al., 2013; Centanni et al., 2016]. Speech discrimination ability in rats is well correlated with the

From the School of Behavioral and Brain Sciences, The University of Texas at Dallas, 800 West Campbell Road BSB11, Richardson, TX 75080 (C.T.E., K.C.R., M.S.B., E.P.B., K.W.I., L.G.W., P.S., S.V., M.P.K.); Texas Biomedical Device Center, The University of Texas at Dallas, 800 West Campbell Road BSB11, Richardson, TX 75080 (C.T.E., K.C.R., M.S.B., E.P.B., S.V., M.P.K.); Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, NY (H.H.-N., J.D.B.); Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY (H.H.-N., J.D.B.); Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY (J.D.B.); Fishberg Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY (J.D.B.); Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY (J.D.B.); The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, NY (J.D.B.)

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Address for correspondence and reprints: Crystal T. Engineer, The University of Texas at Dallas, 800 West Campbell Road, BSB11, Richardson, TX 75080. E-mail: novitski@utdallas.edu

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distinctness of the neural activity patterns evoked by the speech sounds [Engineer et al., 2008; Centanni, Engineer, & Kilgard, 2013; Perez et al., 2013]. While sounds that evoke similar neural patterns are difficult to discriminate, sounds that evoke distinct neural patterns are easy to discriminate. For example, the speech sounds 'rad' and 'lad' evoke very similar neural patterns, and are difficult for both rats and non-native English speakers to discriminate, while the speech sounds 'dad' and 'bad' evoke very distinct neural patterns, and are easy for rats to discriminate.

Because the spatiotemporal fidelity of neural processing is critical for speech processing, many genetic and environmental factors that degrade neural responses also impair speech discrimination. Many patient populations suffer from difficulties processing speech sounds due to impaired cortical responses to sounds, including individuals with autism spectrum disorders [Moore & Shannon, 2009; Lai, Schneider, Schwarzenberger, & Hirsch, 2011; Anon, 2014; Nittrouer, Sansom, Low, Rice, & Caldwell-Tarr, 2014]. Cortical responses to sounds are often both weaker and delayed in individuals with autism compared to typically developing individuals [Bomba & Pang, 2004; Russo, Zecker, Trommer, Chen, & Kraus, 2009; Gandal et al., 2010; Roberts et al., 2011]. The extent of the auditory cortex impairment is correlated with language impairment [Kuhl et al., 2013]. Auditory cortex responses and speech discrimination ability are also impaired in both genetic and environmental rodent models of autism [Gandal et al., 2010; Liao, Gandal, Ehrlichman, Siegel, & Carlson, 2012; Engineer et al., 2014a, 2014c].

A *Shank3* deficient rat model has recently been developed that exhibits behavioral and neural deficits that resemble those observed in individuals with *SHANK3* mutation [Harony-Nicolas, De Rubeis, Kolevzon, & Buxbaum, 2015; Harony-Nicolas et al., 2017]. In an effort to identify whether sensory processing abnormalities could contribute to the receptive language problems observed in this population, we assessed the neural response characteristics to sounds in multiple auditory cortical fields in the novel *Shank3* genetically modified rat model. We hypothesized that the auditory cortex response to sounds would be altered, as seen in other rodent models of disorders that include receptive language deficits [Gandal et al., 2010; Liao et al., 2012; Kim, Gibboni, Kirkhart, & Bao, 2013; Centanni et al., 2014a, 2016, Engineer et al., 2014a, 2014c, 2015a].

Methods

Shank3 *Rat Model*

Male and female *Shank3* heterozygous and wild-type rats were obtained from Joseph Buxbaum at Mount Sinai. SAGE Labs (Boyertown, PA) used zinc-finger nucleases on the outbred Sprague-Dawley background to target the *Shank3* ANK domain (exon 6). A 68 base-pair deletion was introduced that produced a stop codon in exon 6 [Harony-Nicolas et al., 2017]. Heterozygous breeder rats were paired to generate the rats used in this study. Since no gender bias has been reported in Phelan McDermid syndrome, both male and female rats were included in the current study [Soorya et al., 2013; Sarasua et al., 2014b; Wang et al., 2016]. Eight heterozygous *Shank3* Sprague-Dawley rats (4 female and 4 male) and eleven control Sprague-Dawley rats (6 female and 5 male) were used in this study. Rats were housed two per cage, and were on a reversed 12 hr dark light cycle. All recording procedures were performed in adult rats over the age of 3 months. All procedures were approved by The University of Texas at Dallas Animal Care and Use Committee.

Sound Stimuli

The speech sounds used in this study included the words: 'bad', 'chad', 'dad', 'dead', 'deed', 'dood', 'dud', 'gad', 'sad', 'shad', and 'tad'. Each of these sounds was spoken in isolation by a female native English speaker, as in our previous studies [Engineer et al., 2008; Perez et al., 2013]. Speech sounds were randomly interleaved, and each speech sound was presented 20 times with an interstimulus interval of 2 sec. All speech sounds were frequency shifted up an octave using the STRAIGHT vocoder to shift the sounds into the rat hearing range [Kawahara, 1997]. Speech sounds were approximately 500 ms in duration, and were presented so that the loudest 100 ms of the vowel was 60 dB. The noise burst trains used in this study were presented at 7, 10, 12.5, and 15 Hz. Each train consisted of six 25 ms noise bursts. The noise bursts were white noise consisting of frequencies between 1.5 and 30 kHz, and were presented at 60 dB. The noise burst trains were randomly interleaved, and each of the 4 train speeds was presented 20 times with an interstimulus interval of 2 sec. The 1,440 tones used in this study ranged in frequency from 1 to 48 kHz in 0.0625 octave steps and intensity from 0 to 75 dB in 5 dB steps. All tones were 25 ms in duration and were randomly interleaved and presented with an interstimulus interval of 500 ms.

Auditory Cortex Physiology

Auditory cortex recordings were obtained from the right auditory cortex in 11 control rats and 8 heterozygous *Shank3* rats. Recordings were collected from four auditory cortical fields: anterior auditory field (AAF; $n = 230$ naïve AAF sites and $n = 158$ *Shank3* AAF sites), primary auditory cortex (A1; $n = 298$ naïve A1 sites and $n = 182$ *Shank3* A1 sites), ventral auditory field (VAF; $n = 132$ naïve VAF sites and $n = 113$ *Shank3* VAF sites), and posterior auditory field (PAF; $n = 100$ naïve PAF

Table 1. The Distribution of Characteristic Frequencies Was Matched between *Shank3* Heterozygous Rats and Control Rats

		Mean (kHz)	Median (kHz)	Minimum (kHz)	Maximum (kHz)	Range (kHz)
AAF	**Control**	18.4 ± 1.6	16.4	3.7 ± 0.5	41.6 ± 1.6	37.9 ± 1.5
	Shank3	16.9 ± 1.4	13.2	4.1 ± 1.1	39.7 ± 2.9	35.6 ± 3.5
A1	**Control**	11.4 ± 1.1	7.8	1.7 ± 0.2	35 ± 2.1	33.4 ± 2.1
	Shank3	14.1 ± 1.2	12.4	1.9 ± 0.2	37.4 ± 2.9	35.5 ± 2.8
VAF	**Control**	22.6 ± 2.6	21.6	7.8 ± 2.2	38.9 ± 2.5	31.1 ± 3.0
	Shank3	20 ± 3.4	18.7	9.1 ± 3.2	35.9 ± 3.8	26.8 ± 3.1
PAF	**Control**	10.2 ± 1.4	7.7	4 ± 0.7	23.9 ± 4.8	19.9 ± 4.9
	Shank3	8.6 ± 1.4	7.3	3.5 ± 0.6	20.2 ± 5.0	16.7 ± 4.9

sites and $n = 81$ *Shank3* PAF sites). There was no significant difference between the distribution of characteristic frequencies of the recorded neurons between *Shank3* heterozygous rats and control rats (Table 1, Mean $U = 668$, $z = -0.15$, $P = 0.88$, Mann-Whitney U test; Median $U = 644$, $z = -0.41$, $P = 0.68$; Minimum $U = 661$, $z = -0.23$, $P = 0.82$; Maximum $U = 678$, $z = -0.04$, $P = 0.97$; Range $U = 656$, $z = -0.28$, $P = 0.78$). At each recording site, multi-unit responses were obtained in response to speech sounds, trains of noise bursts, and tones. Rats were initially anesthetized with sodium pentobarbital (50 mg/kg), and they received supplemental doses of dilute pentobarbital (8 mg/mL) throughout the experiment as needed. A tracheotomy was performed to ease breathing and a cisternal drain was performed to reduce swelling. A craniotomy and durotomy were performed to expose the right hemisphere auditory cortex. Four Parylene-coated microelectrodes (1.5–2.5 MΩ, FHC, Bowdoin, ME) were used to record auditory cortex responses at a depth of approximately 600 μm, which corresponds to layer IV/V in experimentally naïve rats. Individuals with Phelan-McDermid syndrome typically have normal brain MRIs, although some individuals exhibit cerebellar or corpus callosum abnormalities [Philippe et al., 2008; Aldinger et al., 2013]. There is no evidence suggesting alterations in cortical thickness in rodent *Shank3* models or individuals with Phelan-McDermid syndrome [Philippe et al., 2008; Jiang & Ehlers, 2013; Wang et al., 2016]. Tucker-Davis Technologies (Alachua, FL) hardware and software were used for sound presentation and data acquisition. Sounds were presented from a free-field speaker (TDT, FF1) located 10 cm from the left ear. All recording procedures were identical to our previous studies [Centanni et al., 2014b; Engineer, Centanni, Im, & Kilgard, 2014b; Engineer et al., 2014c; Engineer, Rahebi, Buell, Fink, & Kilgard, 2015b].

Data Analysis

Each analysis technique was performed using recordings separated into individual auditory cortex fields [Polley, Read, Storace, & Merzenich, 2007; Puckett, Pandya, Moucha, Dai, & Kilgard, 2007; Takahashi, Yokota, Funamizu, Kose, & Kanzaki, 2011; Centanni et al., 2013; Engineer et al., 2014a]. AAF was distinguished by short response latencies and distinct low frequency to high frequency tonotopy in an anterior to posterior direction. A1 was distinguished by short response latencies and distinct low frequency to high frequency tonotopy in a posterior to anterior direction. VAF was distinguished by longer response latencies, no distinct tonotopy, and a location between AAF and A1. PAF was distinguished by late response latencies, no distinct tonotopy, and a location posterior to A1.

All analysis was performed using MATLAB software. The response threshold was defined as the lowest tone intensity that evoked a response. The driven rate was defined as the number of driven spikes evoked. The spontaneous firing rate was the rate of firing evoked during silence. For tones, the spontaneous firing rate was calculated across the duration of the trial (400 ms) across all 90 tone frequencies, when presented at an amplitude of 0 dB. For speech sounds, in order to calculate the driven rate by subtracting the spontaneous firing rate, 100 ms of silence collected before the presentation of a sound was used to calculate the spontaneous firing rate. The bandwidth was defined as the range of frequencies that evoked a response 40 dB above the threshold response. Only sites with a bandwidth value greater than 0 were included in the bandwidth analysis in order to account for high threshold sites without data 40 dB above the threshold (the highest tone intensity was 75 dB). For the noiseburst sounds, the number of spikes evoked by the second noise burst was defined as the number of driven spikes evoked during a 25 ms window for the second noise burst. For speech sounds, the response strength was defined as the number of driven spikes evoked during the 40 ms onset of the speech sound. The peak response latency was the time point when the largest number of spikes occurred.

Neural classifier accuracy was quantified using a PSTH-based nearest-neighbor classifier, as in previous studies [Engineer et al., 2008, 2014a; Centanni et al., 2013; Perez et al., 2013]. Classifier performance is highly correlated with behavioral discrimination ability

Table 2. Receptive Field Properties Were Altered in *Shank3* Heterozygous Rats Compared to Control Rats. All Property Values Are Presented as the Mean and Median. Bolded Numbers Marked with a Star Are Significantly Different Compared to Control Rats

			Threshold (dB)		Driven rate (spikes/tone)		Spontaneous rate (Hz)		Bandwidth (octaves)		Peak latency (ms)	
		Number of sites	Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median
AAF	**Control**	230	18.3	12.6	2.5	2.2	14.5	12.5	3.2	3.3	17.6	17.0
	Shank3	158	15.8	12.1	**2.0***	**1.7***	**10.0***	**8.1***	3.2	3.2	17.0	17.0
A1	**Control**	298	11.5	7.0	3.1	3.0	16.1	13.9	2.8	2.8	19.5	19.0
	Shank3	182	14.0	7.2	**2.6***	**2.5***	15.1	12.2	2.9	3.0	19.2	18.0
VAF	**Control**	132	18.7	12.5	2.6	2.1	20.0	17.2	2.6	2.9	23.8	21.0
	Shank3	113	16.9	11.6	2.5	2.2	**14.6***	**14.1***	**3.6***	**3.7***	29.1	21.0
PAF	**Control**	100	20.3	16.5	2.5	2.1	15.3	11.0	3.8	3.7	30.4	25.5
	Shank3	81	13.9	7.2	**1.8***	**1.3***	**8.6***	**7.8***	3.9	3.9	40.1	25.0

across multiple auditory fields, in both anesthetized and awake rats [Engineer et al., 2008; Centanni et al., 2013]. The classifier was provided the 40 ms onset response to pairs of speech sounds using 1 ms precision. Each of the 7 consonant onset sounds ('bad', 'chad', 'dad', 'gad', 'sad', 'shad', and 'tad') was compared to every other consonant onset, for a total of 21 consonant pairs. Each single trial response pattern was compared with the average response pattern evoked by each of the sounds. The similarity between the single trial response pattern and the average response patterns was quantified using Euclidean distance. The classifier assigned each single trial response pattern to the average response pattern that it was the most similar to. For two-group comparisons, the non-parametric Mann-Whitney *U* test was used to determine statistical significance. For multiple group comparisons, the non-parametric Kruskal-Wallis test was used to determine statistical significance. Bonferroni correction was used to correct for multiple comparisons.

Results

Shank3 *Deficiency Alters the Cortical Response to Tones*

Receptive field properties were significantly impaired across auditory cortical fields in *Shank3* heterozygous rats. The threshold response was unaltered in *Shank3* heterozygous rats compared to control rats ($U = 202143$, $z = -0.12$, $P = 0.91$, Mann-Whitney *U* test, Table 2). There was a significant difference in threshold response across the auditory fields ($H(3) = 40.7$, $P < 0.0001$, Kruskal-Wallis test).

The number of driven spikes evoked by tones was significantly weaker in *Shank3* heterozygous rats compared to control rats ($U = 175826$, $z = -4.09$, $P < 0.0001$, Mann-Whitney *U* test, Table 2). There was a significant difference in the number of driven spikes across the auditory fields ($H(3) = 47.13$, $P < 0.0001$, Kruskal-Wallis test). The number of driven spikes was 20% weaker in AAF in *Shank3* heterozygous rats ($P = 0.01$, Mann-Whitney *U* test, Table 2), 16% weaker in A1 ($P = 0.003$, Mann-Whitney *U* test), and 28% weaker in PAF ($P = 0.01$, Mann-Whitney *U* test).

Similarly, the spontaneous firing rate was also significantly weaker in *Shank3* heterozygous rats compared to control rats ($U = 168073$, $z = -5.27$, $P < 0.0001$, Mann-Whitney *U* test, Table 2). There was a significant difference in the spontaneous firing rate across the auditory fields ($H(3) = 44.74$, $P < 0.0001$, Kruskal-Wallis test). *Shank3* heterozygous rats exhibited a 31% decrease in the spontaneous rate in AAF ($P < 0.0001$, Mann-Whitney *U* test, Table 2), a 27% decrease in VAF ($P = 0.03$, Mann-Whitney *U* test), and a 44% decrease in PAF ($P < 0.0001$, Mann-Whitney *U* test).

Bandwidths quantified 40 dB above the response threshold were significantly different in *Shank3* heterozygous rats compared to control rats ($U = 133682$, $z = -4.16$, $P < 0.0001$, Mann-Whitney *U* test, Table 2). There was a significant difference in the bandwidth across the auditory fields ($H(3) = 111.15$, $P < 0.0001$, Kruskal-Wallis test). The bandwidth was 39% wider in VAF in *Shank3* heterozygous rats compared to control rats ($U = 2767$, $z = -5.67$, $P < 0.0001$, Mann-Whitney *U* test, Table 2).

Additionally, the peak firing latency was unaltered in *Shank3* heterozygous rats compared to control rats ($U = 191135$, $z = -1.79$, $P = 0.07$, Mann-Whitney *U* test, Table 2). There was a significant difference in the peak latency across the auditory fields ($H(3) = 342.08$, $P < 0.0001$, Kruskal-Wallis test).

Shank3 *Deficiency Alters the Cortical Response to Rapid Trains of Sound*

Auditory cortex responses to trains of noise bursts were recorded to assess whether there was a deficit in the neural response to rapidly presented sounds in *Shank3* heterozygous rats. The auditory cortex response to noise burst trains was significantly impacted in *Shank3* heterozygous rats in multiple auditory fields (Fig. 1). In AAF, the number of spikes evoked in response to the

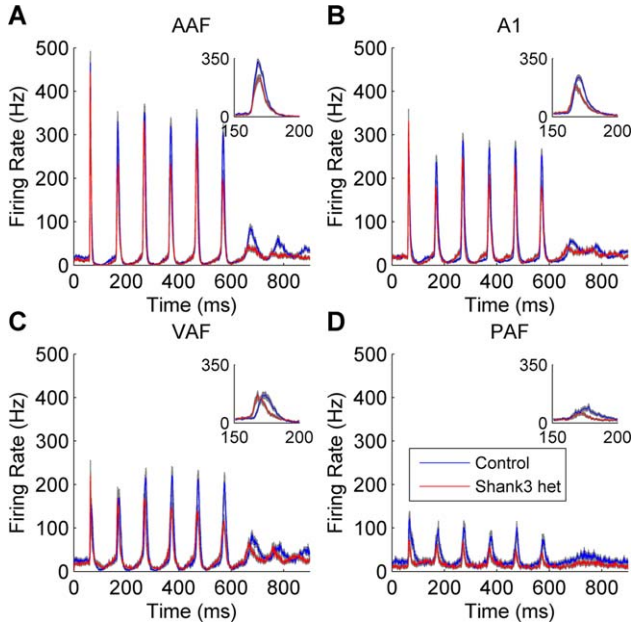

Figure 1. The auditory cortex response to rapidly presented noise burst trains was altered in *Shank3* heterozygous rats. The post-stimulus time histogram (PSTH) response to a train of 6 noise bursts presented at 10 Hz (interstimulus interval of 100 ms) is shown in (**A**) AAF, (**B**) A1, (**C**) VAF, and (**D**) PAF. The gray shading indicates SEM across recording sites. The figure inset displays a zoomed in version of the response to the second noise burst.

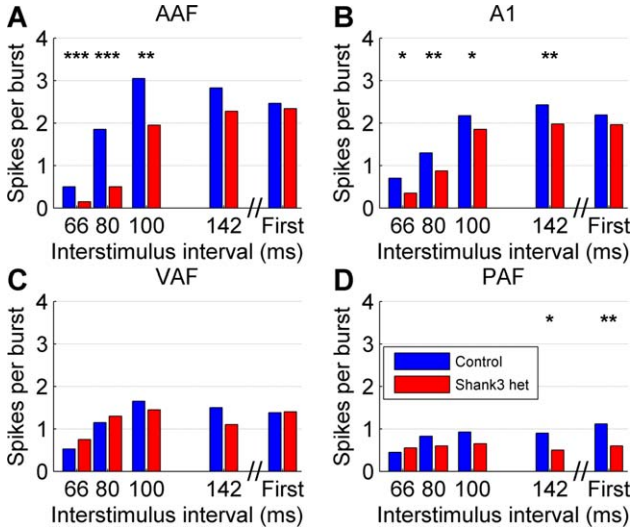

Figure 2. The median number of spikes evoked in response to the second noise burst was weaker in *Shank3* heterozygous rats compared to control rats in (**A**) AAF, (**B**) A1, and (**D**) PAF, but not (**C**) VAF. The interval between the onset of the first and second noise burst is shown on the x axis. The response to the first noise burst is plotted on the right. The stars indicate statistically significant differences between *Shank3* heterozygous rats and control rats (* $P<0.01$, ** $P<0.001$, *** $P<0.0001$, Mann-Whitney U test Bonferroni corrected for multiple comparisons).

second noise burst of the train was significantly weaker in *Shank3* heterozygous rats compared to control rats ($U=369355$, $z=-7.01$, $P<0.0001$, Mann-Whitney U test, Fig. 2a). There was a significant difference in the number of spikes evoked to the second noise burst across the presentation rates ($H(4)=313.12$, $P<0.0001$, Kruskal-Wallis test). The number of spikes evoked in response to the second noise burst was also significantly weaker in *Shank3* heterozygous rats in A1 ($U=595054$, $z=-5.03$, $P<0.0001$, Mann-Whitney U test, Fig. 2b), where there was also a significant difference in the number of spikes evoked to the second noise burst across the presentation rates ($H(4)=334.66$, $P<0.0001$, Kruskal-Wallis test). The number of spikes evoked in response to the second noise burst was unaltered in *Shank3* heterozygous rats in VAF ($U=184206$, $z=-0.36$, $P=0.72$, Mann-Whitney U test, Fig. 2c), but there was a significant difference in the number of spikes evoked to the second noise burst across the presentation rates ($H(4)=70.08$, $P<0.0001$, Kruskal-Wallis test). The number of spikes evoked in response to the second noise burst was also significantly weaker in *Shank3* heterozygous rats in PAF ($U=88663$, $Z=-3.22$, $P=0.001$, Mann-Whitney U test, Fig. 2d), where there was also a significant difference in the number of spikes evoked to the second noise burst across the presentation rates ($H(4)=11.44$, $P=0.02$, Kruskal-Wallis test). Collectively, these findings demonstrate that the *Shank3* heterozygous mutation alters auditory cortex processing.

Shank3 *Deficiency Alters the Cortical Response to Speech Sounds*

The altered auditory cortex response in *Shank3* heterozygous rats was not specific to non-speech sounds. Similarly, the multi-unit auditory cortex response to speech sounds in *Shank3* heterozygous rats was weaker compared to control rats (Fig. 3). The onset response strength to speech sounds was significantly weaker in *Shank3* heterozygous rats compared to control rats ($U=174948$, $z=-4.23$, $P<0.0001$, Mann-Whitney U test, Fig. 4a). There was a significant difference in the response strength to speech sounds across the auditory fields ($H(3)=92.32$, $P<0.0001$, Kruskal-Wallis test). The response strength was significantly weaker in A1 in *Shank3* rats compared to control rats ($U=21055$, $z=-4.11$, $P<0.0001$, Mann-Whitney U test Bonferroni corrected for multiple comparisons, Fig. 4a).

The peak response latency to speech sounds was significantly delayed in *Shank3* heterozygous rats compared to control rats ($U=189297.5$, $z=-2.06$, $P=0.04$, Mann-Whitney U test, Fig. 4b). There was a significant difference in the response latency to speech sounds across the auditory fields ($H(3)=148.74$, $P<0.0001$,

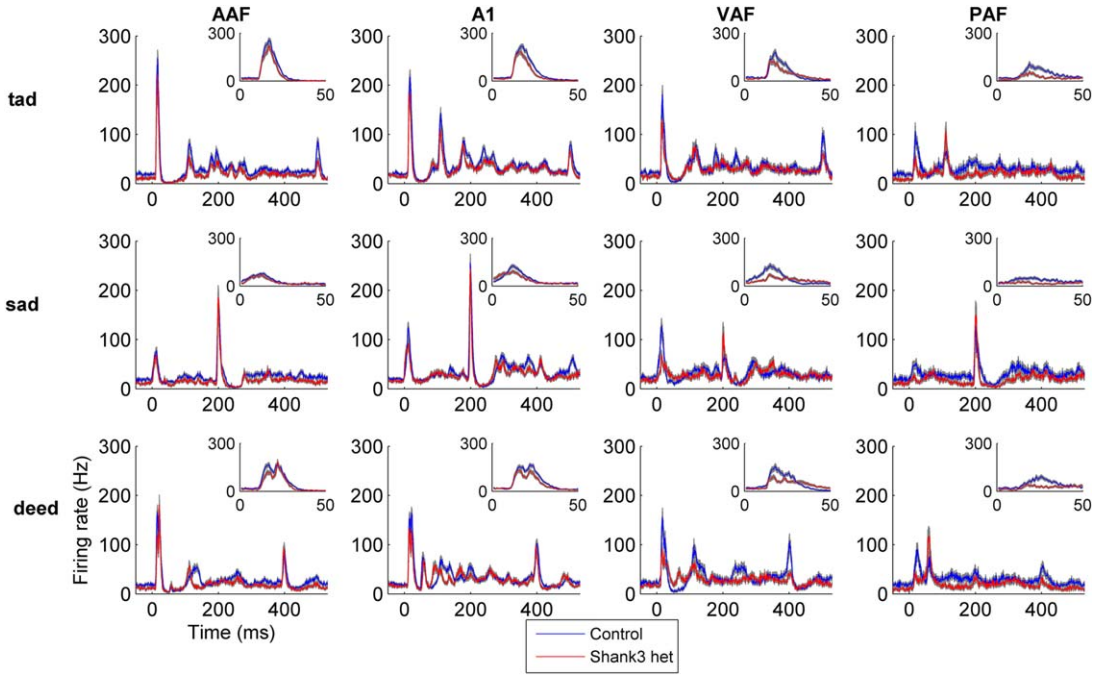

Figure 3. The auditory cortex multi-unit response to speech sounds was altered in *Shank3* heterozygous rats. The post-stimulus time histogram (PSTH) response to the speech sounds 'tad' (top row), 'sad' (middle row), and 'deed' (bottom row) in AAF, A1, VAF, and PAF. The gray shading indicates SEM across recording sites. The figure inset displays a zoomed in version of the response to the onset of the initial consonant.

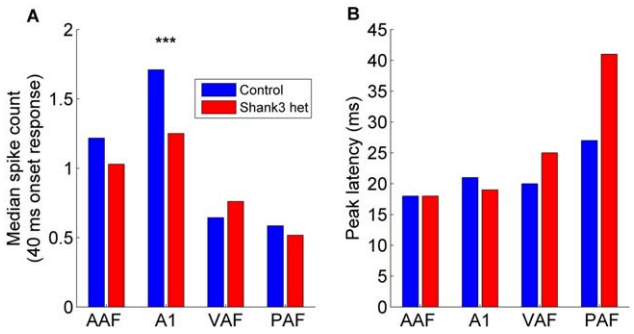

Figure 4. The auditory cortex response to speech sounds was both (**A**) weaker and (**B**) delayed in *Shank3* heterozygous rats compared to control rats. The stars indicate statistically significant differences between *Shank3* heterozygous rats and control rats ($P < 0.0001$, Mann-Whitney U test Bonferroni corrected for multiple comparisons). The bars indicate the median spike count and median peak response latency.

Kruskal-Wallis test). However, the response latency was not significantly delayed in any individual field in *Shank3* rats compared to control rats ($P > 0.0125$, Mann-Whitney U tests Bonferroni corrected for multiple comparisons, Fig. 4b). Together, these findings demonstrate that responses to sounds in multiple auditory cortex fields are disrupted in *Shank3* heterozygous rats.

Due to the weaker auditory cortex responses to the onset of speech sounds, we tested the hypothesis that a nearest-neighbor classifier would be less able to accurately discriminate between auditory cortex responses evoked by consonant pairs in *Shank3* rats compared to control rats. Using auditory cortex responses recorded from experimentally naïve rats, the nearest-neighbor classifier accurately predicts behavioral consonant discrimination accuracy [Engineer et al., 2008; Centanni et al., 2013; Perez et al., 2013]. Consonant pairs that evoke similar neural patterns are difficult for naive rats to discriminate, while consonant pairs that evoke distinct neural patterns are easy for rats to discriminate. Neural classifier accuracy was unimpaired in *Shank3* heterozygous rats compared to control rats ($U = 199333$, $z = -0.54$, $P = 0.59$, Mann-Whitney U test, Fig. 5 and Supplementary Figure 1). There was a significant difference in the classifier accuracy across the auditory fields ($H(3) = 150.91$, $P < 0.0001$). The weaker response strength observed in *Shank3* rats did not make the neural responses to consonant pairs less discriminable.

Discussion

This study was designed to identify whether abnormalities in the auditory cortex response to sounds could contribute to the speech processing problems observed in individuals with *SHANK3* haploinsufficiency. In this study, we used three distinct sound types to demonstrate

that cortical responses to sound are weaker in *Shank3* heterozygous rats. In addition, *Shank3* heterozygous rats were less able to follow rapidly presented sounds. Despite the well-documented receptive language deficits in individuals with *SHANK3* mutations [Phelan & McDermid, 2012; Soorya et al., 2013], the deficits in *Shank3* heterozygous rats do not appear to be speech-specific. Instead, auditory responses in general are degraded in these rats.

Only one previous study has examined the auditory cortex response to sounds in individuals with Phelan-McDermid syndrome [Wang et al., 2016]. They compared neural responses recorded using fMRI in children with Phelan-McDermid syndrome to responses in children with idiopathic ASD. Individuals with *SHANK3* haploinsufficiency exhibited primary auditory cortex activation in response to both communicative and non-communicative sounds, despite poor behavioral receptive language abilities in the same individuals. The current study also observed primary auditory cortex activation across a range of auditory stimuli. While the auditory cortex response to the onset of sounds was significantly weakened, there was a much weaker response to subsequently presented rapid noise bursts (Fig. 2). It is possible that although individuals with Phelan-McDermid syndrome respond to communicative vocalizations, they may exhibit more severely impaired neural responses to rapidly presented sounds [Russo, Hornickel, Nicol, Zecker, & Kraus, 2010]. Future work is necessary to determine whether individuals with Phelan-McDermid syndrome exhibit temporal processing problems that may explain the observed severe receptive language impairments.

Potential Functional Interpretation

In this study, the neural discrimination accuracy of isolated speech sounds was unaffected in *Shank3* heterozygous rats compared to control rats. Neural classifier accuracy is highly correlated with behavioral discrimination ability [Engineer et al., 2008; Centanni et al., 2013; Perez et al., 2013], so this finding suggests that behavioral discrimination of isolated speech sounds is likely to be unimpaired in *Shank3* heterozygous rats. Impaired neural and behavioral discrimination of speech sounds has been observed previously in other rat models of communication disorders [Centanni et al., 2014a; Engineer et al., 2014b, 2014c]. Future experiments are necessary to determine the behavioral consequences of altered auditory cortex responses in these rats. While the neural responses to isolated words are largely intact in *Shank3* heterozygous rats, the observed decreased ability to follow rapidly presented noise burst trains suggests that neural responses to speech sounds presented in rapid speech streams may be impaired. Wild-type rats can accurately discriminate

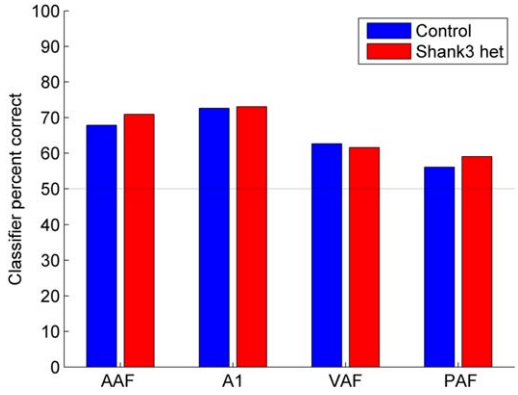

Figure 5. The neural classifier accuracy of consonant pairs was unimpaired in *Shank3* heterozygous rats compared to control rats. The neural classifier was provided the 40 ms onset response to pairs of consonants. The bars indicate the median percent correct. Chance discrimination performance is 50% correct, and is indicated by the dotted line.

speech sounds delivered in a speech stream at rates up to 6.7 syllables per second [Centanni et al., 2014b, 2016]. Our observations indicate that *Shank3* heterozygous rats exhibit relatively normal responses to isolated speech, but may have degraded neural and behavioral responses to speech sounds presented at rates that occur in conversational human speech. If future studies confirm this prediction, the *Shank3* heterozygous rat model may be useful for evaluating potential therapies to improve the well-documented receptive language deficits in individuals with Phelan-McDermid Syndrome.

Relationship to Other Genetic Disorders and Autism

Individuals with autism and genetic disorders, such as Rett syndrome, commonly exhibit receptive language deficits and cortical responses that are both weaker and slower [Bader, Witt-Engerström, & Hagberg, 1989; Stach, Stoner, Smith, & Jerger, 1994; Stauder, Smeets, van Mil, & Curfs, 2006; Gandal et al., 2010; Roberts et al., 2010]. Severe auditory cortex deficits have also been observed in the rodent *Fmr1* knockout model of fragile X syndrome, the rodent valproic acid model of autism, and the rat *Mecp2* knockout model of Rett syndrome [Gandal et al., 2010; Liao et al., 2012; Kim et al., 2013; Engineer et al., 2014a, 2014c, 2015a; Anomal et al., 2015]. The auditory cortex responses observed in the *Shank3* heterozygous rat model are weaker, which is consistent with other rat models of ASD. These findings are observed across auditory fields, as well as across different sound types, which suggests that responses to sound would likely also be impaired in other auditory regions. Future experiments are needed to determine if the differences observed in auditory cortical responses could be due to changes earlier in the auditory pathway. While hearing is typically reported to be normal

in individuals with Phelan-McDermid syndrome [Phelan & McDermid, 2012], it is possible that subcortical auditory areas, such as the inferior colliculus, could also exhibit altered responses to sounds, as seen in individuals with autism [Russo et al., 2008].

Future Avenues for Testing Auditory Processing Therapies

This novel model of the auditory processing impairments observed following *Shank3* mutation offers the unique opportunity to test drug or cognitive training therapies that could be used to treat patients with *SHANK3* mutation. For example, it is well known that intensive cognitive intervention therapy in individuals with autism can both improve behavioral outcomes and restore typical patterns of brain activity [McEachin, Smith, & Lovaas, 1993; Dawson et al., 2010, 2012; Russo et al., 2010]. Similar improvements in both speech discrimination ability and the auditory cortex response to speech have been documented in the rat *Mecp2* knockout model of Rett syndrome and the rat valproic acid model of autism [Engineer et al., 2014b, 2015a]. A recent study documented that neural and behavioral deficits can be rescued in *Shank3* mice, demonstrating that neural plasticity mechanisms can be activated in this model [Mei et al., 2016].

It would also be straightforward to evaluate the ability of potential drug therapies to improve auditory cortical responses in the *Shank3* rat model. *Shank3* mice have recently been used to document the reversal of both neural and behavioral deficits following IGF-1 treatment [Bozdagi, Tavassoli, & Buxbaum, 2013] or by inhibiting cofilin or activating Rac1 [Duffney et al., 2015]. IGF-1 treatment has also restored neural deficits in stem cells derived from individuals with Phelan-McDermid syndrome [Shcheglovitov et al., 2013] and improved social behaviors in children with Phelan-McDermid syndrome [Kolevzon et al., 2014; Costales & Kolevzon, 2015]. The *Shank3* rat model is ideal to quantify the improvements in auditory response strength using both these drug therapies and other promising potential therapies.

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Conflict of Interest

The funding sources had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. The authors report no conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Figure S1. The neural classifier accuracy of consonant pairs was unaltered in *Shank3* heterozygous rats compared to control rats in (**a**) AAF, (**b**) A1, (**c**) VAF, and (**d**) PAF. The neural classifier was provided the 40 ms onset response to pairs of consonants. Each of the 7 consonant onset sounds ('bad', 'chad', 'dad', 'gad', 'sad', 'shad', and 'tad') was compared to every other consonant onset, for a total of 21 consonant pairs. The bars indicate the median percent correct. Chance discrimination performance is 50% correct.