The translationally relevant mouse model of the 15q13.3 microdeletion syndrome reveals deficits in neuronal spike firing matching clinical neurophysiological biomarkers seen in schizophrenia

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Abstract

Aim: To date, the understanding and development of novel treatments for mental illness is hampered by inadequate animal models. For instance, it is unclear to what extent commonly used behavioural tests in animals can inform us on the mental and affective aspects of schizophrenia.

Methods: To link pathophysiological processes in an animal model to clinical findings, we have here utilized the recently developed Df(h15q13)/+ mouse model for detailed investigations of cortical neuronal engagement during pre-attentive processing of auditory information from two back-translational auditory paradigms. We also investigate if compromised putative fast-spiking interneurone (FSI) function can be restored through pharmacological intervention using the Kv3.1 channel opener RE1. Chronic multi-array electrodes in primary auditory cortex were used to record single cell firing from putative pyramidal and FSI in awake animals during processing of auditory sensory information.

Results: We find a decreased amplitude in the response to auditory stimuli and reduced recruitment of neurones to fast steady-state gamma oscillatory activity. These results resemble encephalography recordings in patients with schizophrenia. Furthermore, the probability of interneurones to fire with low interspike intervals during 80 Hz auditory stimulation was reduced in Df(h15q13)/+ mice, an effect that was partially reversed by the Kv3.1 channel modulator, RE1.

Conclusion: This study offers insight into the consequences on a neuronal level of carrying the 15q13.3 microdeletion. Furthermore, it points to deficient functioning of interneurones as a potential pathophysiological mechanism in schizophrenia and suggests a therapeutic potential of Kv3.1 channel openers.

Keywords auditory stimuli, copy number variation, electrophysiology, interneurone, mouse, translational model.

The lack of valid animal models for schizophrenia has been a key problem for the exploration of underlying biological mechanisms and has hampered the development of novel drugs. Certain copy number variants (CNVs) in the human population have recently been shown to be associated with a high risk for developing schizophrenia (Stefansson *et al.* 2008, Levinson *et al.* 2011) and provide the basis for genetic schizophrenia

models with high construct validity. The rare 15q13.3 deletion CNV is associated with an about 10-fold increased risk for developing schizophrenia and highly increased risk of particularly epilepsy and mild intellectual disability (Dibbens *et al.* 2009, Malhotra & Sebat 2012). Recently, encephalography (EEG) recordings in a mouse model of the 15q13.3 microdeletion syndrome, *Df(h15q13)/+* mice, have been published (Fejgin *et al.* 2014), and the mice display reduced excitability and reduced capacity to generate cortical evoked gamma oscillations. This finding has been reproduced in an independent 15q13.3 deletion mouse line (Kogan *et al.* 2015). A possible cause for reduced cortical gamma oscillations is dysfunctional local cortical networks. One cell group that has attracted particular attention as a key modulator of local gamma oscillations is the gamma-aminobutyric acid (GABA)-ergic fast-spiking interneurones (FSIs) that express the Ca^{2+}-binding protein parvalbumin (PV+) (Nakazawa *et al.* 2012). Expression of FSI markers such as PV is reduced, and compromised interneurone function has been implicated in several psychiatric diseases including schizophrenia (Fuchs *et al.* 2007, Uhlhaas & Singer 2010, Carlén *et al.* 2012, Nilsson *et al.* 2016). FSIs are specifically adapted to fast firing by the expression of a unique selection of ion channels including rapid kinetics voltage-activated potassium channel Kv3.1 subtype (Espinosa *et al.* 2008, Gu *et al.* 2012, Yanagi *et al.* 2014), and the voltage-activated sodium Nav1.1 channels that have recently been suggested as a therapeutic target for cognitive disorders in schizophrenia (Jensen *et al.* 2014). The *Df(h15q13)/+* mouse line offers exciting possibilities to analyse the physiological characteristics of different cell types, including FSIs, within the cortical network.

Of interest is to study cortical network engagement during pre-attentive processing of sensory information that repeatedly has been reported to be altered in schizophrenia (Light *et al.* 2012). Deficits at this perceptual level can be probed by back-translational auditory stimulation paradigms, such as the 'paired click paradigm' or 'auditory steady-state responses (ASSR)' (Hamm *et al.* 2011). Studying cortical function by electrophysiology at the pre-attentive perceptual level is likely more translational between species, as very similar methodology can be used hereby minimizing assay differences between rodents and humans, for example related to behaviour.

In this study, we characterize the neurophysiological responses of single neurons in the auditory cortex in freely moving *Df(h15q13)/+* mice and demonstrate several aspects of cortical dysfunction in different cell types using two translational auditory assays. A computer model was adapted to replicate the electrophysiological findings, indicating compromised

interneurone function at high firing rates as a possible cause for deviating response patterns in *Df(h15q13)/+* mice. We therefore also investigated if disturbed response patterns could be restored through pharmacological intervention using the Kv3.1 channel opener RE1 (Alvaro *et al.* 2012, Taskin *et al.* 2015). The findings presented offer insights into some of the consequences of carrying the 15q13.3 microdeletion being a high-risk variant for schizophrenia and epilepsy.

Methods

Animals

The *Df(h15q13)/+* mouse line was generated by Taconic Artemis (Köln, Germany) (Fejgin *et al.* 2014). Mice were bred, genotyped and housed until the age of 8 weeks at Taconic MB A/S (Lille Skensved, Denmark). At 8 weeks of age, the mice were transferred to the Lundbeck facility. Food (Altromin 1323 pills; Brogaarden, Lynge, Denmark) and tap water were available *ad libitum*. All experimental procedures involving animals were conducted with permission from The Danish National Committee for Ethics in Animal Experimentation.

Surgical procedures

Fourteen male mice 10–14 weeks old [seven wild types and seven *Df(h15q13)/+*] divided on two different batches were used. Only male mice were used as this study is a mechanistic extension of the Fejgin *et al.* (2014) study. Mice were treated with prophylactic antibiotic and peripheral acting analgesia prior to surgery by the injection of 5 mg kg^{-1} Baytril vet® (SC, 50 mg mL^{-1} Enrofloxacin; Bayer, Leverkusen, Germany) and 1.5 mg kg^{-1} Rimadyl vet® (SC, 50 mg mL^{-1} Carprofen; Pfizer, New York, NY, USA) respectively. Mice were anesthetized with Sevoflurane (Abbott Scandinavia AB, Solna, Sweden) and placed in a stereotaxic frame (KOPF; David Kopf Instruments, Tujunga, CA, USA). Eye gel (Ophtha A/S, Gentofte, Denmark) was applied to avoid exsiccation of the cornea. Body temperature was maintained at 37 °C by means of an isothermal heating pad (CMA/150 temperature controller; CMA Microdialysis AB, Kista, Sweden). The top of the skull and both lateral ridges were exposed and cleaned with 15% H_2O_2; bregma was identified, and holes were drilled in the skull according to coordinates from The Mouse brain in stereotaxic coordinates, third edition (Paxinos & Franklin 2004).

Target area for the fixed 16 channel array electrode (Innovative Neurophysiology, Duhram, NC, USA) was primary auditory cortex (−2.5 mm posterior,

+4.0 mm lateral relative to Bregma and 1.0 mm below the cortex surface). A set of anchor screws were mounted on the skull, one over the cerebellum and one over frontal cortex. A small hole for ground wires was drilled adjacent to the cerebellum anchor screw. A micromanipulator mounted on the stereotaxic frame was then used to lower the electrode bundles into position. The electrodes were secured by means of dental cement (3M relyX Unicom self-adhesive universal resin cement, 3M, St. Paul, MN, USA).

After surgery mice were housed individually and treated once daily for 5 days post-surgery with 5 mg kg^{-1} Baytril vet$^{®}$ (SC, 50 mg mL^{-1} Enrofloxacin; Bayer) and 1.5 mg kg^{-1} Rimadyl vet$^{®}$ (SC, 50 mg mL^{-1} Carprofen; Pfizer). Mice were allowed a minimum of one-week post-surgical recovery. The circadian rhythm of the mice was reversed (12 h of light starting at 18:00) to allow electrophysiological experiment to be performed during the dark phase in awake mice.

Signal acquisition

Single- and multi-unit activities were recorded using a multi-channel recording system (Plexon, Dallas, TX, USA). LFPs were filtered 0.1–200 Hz and digitized at 2000 Hz, whereas action potentials were filtered at 300–9000 Hz and digitized at 40 kHz. Threshold for storage of spiking events was set to three SDs of the mean of peak height histogram of the filtered signal. The activity of a large number of individual neurones during freely behaving states was recorded. Artefact waveforms were removed, and the action potential waveform minima were aligned using the Offline Sorter 3.2 software (Plexon), which resulted in more tightly clustered waveforms in principal component space.

Auditory sensory gating in paired pulse paradigm

During recording sessions, the mice were placed in a dark recording camber in a Macrolon (type III) cages with standard sawdust bedding, and their behaviour was monitored via an infrared digital camera to allow exclusion of recordings during motor activity. They were allowed to habituate to the recording camber for 40 min prior to recording sessions. Auditory stimuli (white noise, 82 dB, 10 ms duration with 1 ms rise and fall) were presented in click pairs with an inter-stimulus interval of 500 ms and an interpair interval of 5 s. A transistor–transistor logic (TTL) pulse 50 ms prior to the sound was used as event mark and saved together with Single unit (SU) data in the Plexon system. Single unit activity was recorded simultaneously with the auditory paradigm for 10 min. No startle response was observed during the recording sessions.

Auditory steady-state response (ASSR)

Auditory stimuli were presented in 1.8- to 2-s pulse trains composed of 40–144 pulses of 3 ms white noise, an intertrain interval of 4 s and a sound intensity of 82 dB. ASSR were elicited at 20, 40, 60 and 80 Hz. A TTL pulse was sent to the Plexon system 50 ms prior to the pulse train and was used as an event mark. No startle response was observed during the recording session. Each mouse went through the whole test battery twice with at least a week between recording sessions. At the end of experiments, the mouse was anesthetized and a small amount of current was applied to two channels in the array to mark the positioning of the electrode bundles. The brain was quickly removed, frozen and cut on a microtome (Leica Biosystems, Wetzlar, Germany) to confirm the electrode positions.

Spike sorting and classification

Activity of single neurones was identified by sorting action potentials manually into unit clusters using Offline Sorter (Plexon). Waveform features used for separating the units were the first three principal components of the sampled waveforms for a given data set. A cluster was classified as SU when <0.3% of the action potentials in a defined cluster occurred within the refractory period (set to 2 ms). In total, 713 neurones in the wild type and 745 neurones in the *Df (h15q13)/+* were identified. To further group the units in putative cell types, we extracted three features from the average waveform: valley full width at half maximum, peak full width at half maximum and peak-to-valley time (Fig. 1e). Fuzzy *k*-means clustering (Duda *et al.* 2012) was used to classify the units as pyramidal cells (PCs) or interneurones (Barthó *et al.* 2004, Halje *et al.* 2012). If the probability of cluster membership for a unit did not exceed 0.7 for any of the clusters, then unit was excluded from further analysis.

Firing rate modulations

In the sensory gating paradigm ('paired pulse'), peristimulus time histograms (PSTH) were constructed for each spike train with a bin width of 1 ms. After smoothing (Gaussian window; sigma = 4 bins) and baseline subtraction (baseline interval: −1 to −0.4 s relative to the first pulse) of each unit's PSTH, the average PSTH across units were calculated. In the ASSR paradigm, PSTHs were constructed in the same way, but with a sigma of 10 bins. To compare the responses of the two pulses in the paired pulse paradigm, the average of the first response (0–100 ms) was divided by the average of the second response (500–600 ms).
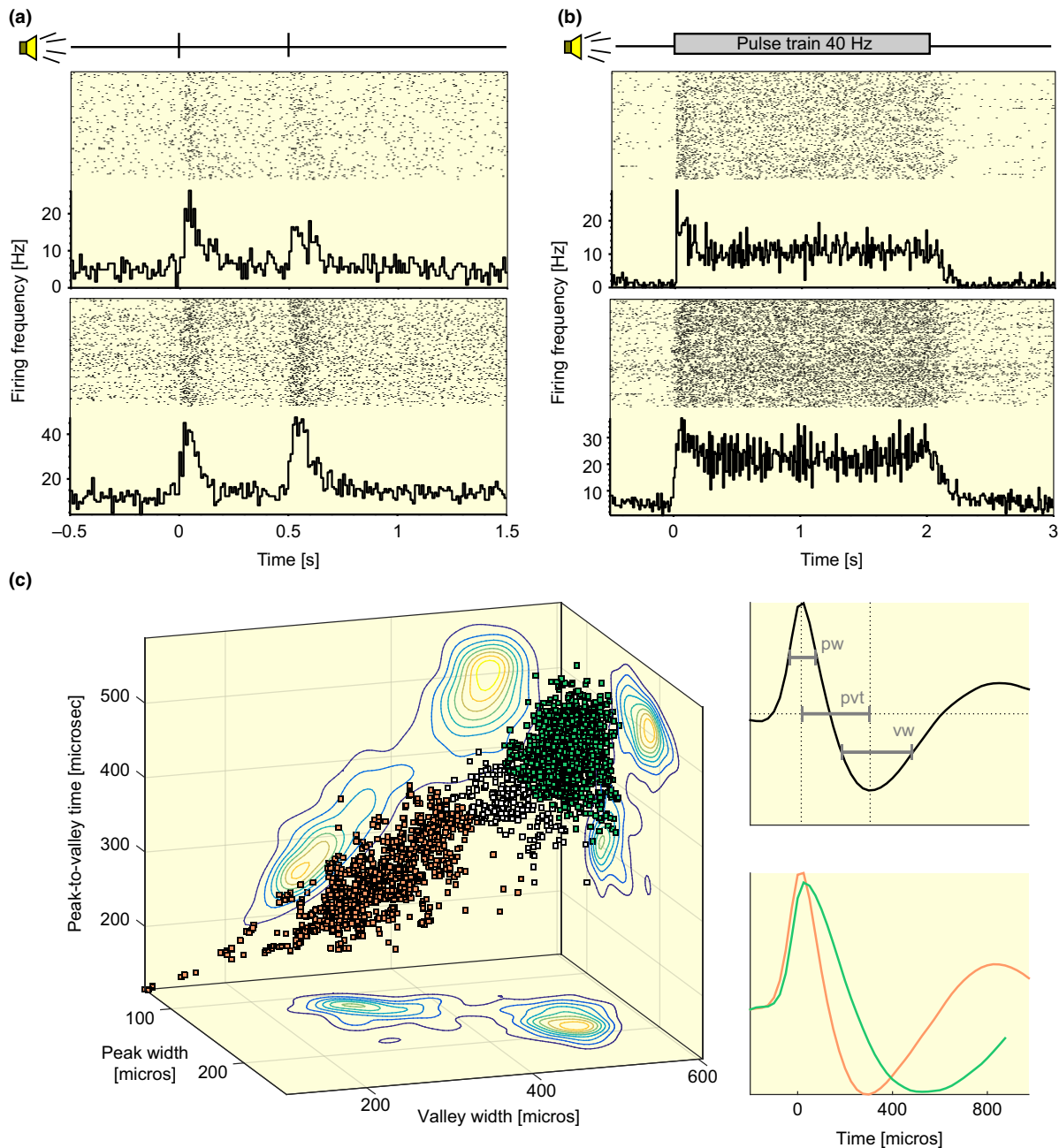
Figure 1 Auditory evoked responses obtained through intracortical recordings in Df(h15q13)/+ mice. (a) A pyramidal neurone (top) and putative interneurone (below) in A1 fire in response to paired auditory pulses with the expected delay of direct sensory input from the thalamus. Each dotted line represents one event. (b) A pyramidal neurone (top) and putative interneurone (below) in A1 fire in response to a 2s 40 Hz auditory steady-state stimuli. Each dotted line represent one 2s train. (c) Waveform characteristics of the recorded cells separate into two groups. Putative interneurones, characterized by a shorter action potential, cluster in the lower left corner. The small panels to the right show the different measures used in clustering the neurones, and the lower panel exhibits the mean wave form for each of the cell classes, green pyramidal neurones and orange putative interneurones.

Interspike interval distributions

In the ASSR paradigm, the interspike interval (ISI) histograms were calculated for each spike train with a bin width of 1 ms. Only intervals occurring during the pulse train were included except the first 200 ms of each pulse train that were excluded in the analyses to allow network activity to settle to a steady state.

The average ISI probability distributions were calculated by adding ISI histograms of SUs and dividing the sum by the total number of action potentials. Only units with a significant firing rate modulation to single auditory pulses (average z-score > 3 on the interval 7–9 ms after the first pulse in the paired pulse paradigm) were included in the average.

To compare stimulus-driven spiking to background spiking, one exponential $y = a \times \exp(b \times x)$ was fitted to the peaks of the average ISI distributions (at multiples of the stimulus interval $\pm$ 1 ms), while another exponential was fitted to the times between peaks. The best fit was found with a nonlinear least squares method (Matlab fit function). Intervals smaller than 10 ms were excluded from fitting to avoid refractory behaviour that clearly violated the implicit assumption of an underlying Poisson process.

Probabilistic model of ISI distributions

To test whether the observed ISI distributions could be explained by a Poisson process with two states, simulated spike trains were generated with a Bernoulli process (i.e. repeated 'coin flipping') with a firing probability that cycled between a high state P_{hi} with duration T_{hi} and a low state P_{low} with duration $T_{low} = T_{stim} - T_{hi}$, where T_{stim} is the interstimulus time (Fig. 4b). The sample rate was set to 4096 Hz. After spike train generation, a refractory period was simulated by removing any spikes that occurred earlier than 2 ms after a spike. For each set of model parameters, P_{low}, P_{hi} and T_{hi} spike trains were generated for 200 000 stimulus cycles and the model ISI distribution was compared to the real ISI distribution by calculating the sum of the squared residuals (SSR). Intervals smaller than 10 ms were excluded from the SSR. A search for a set of parameters that minimized the SSR was performed on a supercomputer (3328 cores; Swedish National Infrastructure for Computing, SNIC, Uppsala University, Uppsala, Sweden) in the following parameter space: 2 Hz < P_{low} < 36 Hz at 2 Hz resolution; 10 Hz < P_{hi} <250 Hz at 10 Hz resolution; 0.2 ms < T_{hi} < 10 ms at 0.1 ms resolution.

Drugs

RE1 (Alvaro *et al.* 2012) was synthesized by the Department of Medicinal Chemistry (H. Lundbeck A/S) and was dissolved in 100% PEG400 and dosed to mice by *per oral* (PO) route in a volume of 10 mL kg^{-1}.

Statistics

All tests on firing rates and firing rate modulations were carried out with nested ANOVA (Matlab 2016a, 'anovan' function; cf. Aarts *et al.* 2014): To test for differences

in spontaneous absolute firing rates between genotypes, we used an ANOVA with factors genotype and animal, where animal was nested in genotype. To test for differences in evoked response amplitudes in the paired pulse paradigm, we used an ANOVA with factors genotype, animal, cell and pulse order (1st or 2nd), where cell was nested in animal and animal was nested in genotype. In the ASSR paradigm, we used an ANOVA with factors genotype, animal and pulse frequency (20, 40, 60 or 80 Hz), where animal was nested in genotype. When an omnibus effect of genotype was found, pairwise comparisons were carried out for each frequency with nested ANOVA (factors genotype and animal, not adjusted for multiple comparisons).

The corresponding tests for drug effects on firing rates and firing rate modulations were carried out differently because we could follow the same neurone across treatments and thereby compare rates directly for each neurone. In the paired pulse paradigm, we used an ANOVA with factors treatment (drug or vehicle), animal, cell and pulse order, where cell was nested in animal. In the ASSR paradigm, we used an ANOVA with factors treatment, animal, cell and pulse frequency (40 or 80 Hz), where cell was nested in animal.

To test for genotype differences in the ISI distributions, we used an ANOVA with factors genotype and animal, where animal was nested in genotype. The dependent variable was the decay coefficient of the exponential function fitted to the peaks of the ISI distribution.

In testing for drug effects on the ISI distributions, individual units were first tested for significant entrainment to the stimulation pulse train. This was performed by fitting exponentials to both the peaks and the time between peaks as described in the Methods section, but with the difference that the fitting was performed on individual units. If the parameter a of the peak exponential was significantly higher than the a of the between-peaks exponential, the unit was labelled as entrained. The two a parameters were determined to be significantly different when their 95% confidence intervals (estimated with the Matlab confint function) were non-overlapping. To test for a significant increase in the a parameter as a result of drug treatment, we counted the number of units with a significantly increased a in the treated condition and used the binomial cumulative distribution function to calculate whether the observed number of units was significant at the 5% level.

Results

Recording of evoked cellular responses in the primary auditory cortex

In order to understand the contribution of different cell types to the previously reported aberrant EEG

responses in Df(h15q13)/+ mice (Fejgin *et al.* 2014), which reflect synchronous occurrence of post-synaptic potentials in several thousand neurones, a closer assessment of the actual firing activity of individual neurones is needed. We therefore focused the analyses on the activity of single neurones in the intermediate cortical laminae of primary auditory cortex (A1) which is a region that has been implicated in deficient processing of auditory information. As expected, a large fraction of the recorded neurones responded to auditory stimuli (70% of both the 293 units recoded in seven wt mice and of the 415 units recorded in seven Df(h15q13)/+ mice). In general, cells in A1 were activated in response to auditory pulses (Fig. 1a, b), with the expected 7–10 ms delay of direct sensory input (Guo *et al.* 2012, Joachimsthaler *et al.* 2014). The fact that the fraction of neurones that respond to auditory stimuli and the onset latency is the same between genotypes, indicating that the otoacoustic emissions and the auditory pathways are unaffected by the mutation. By inspection of the waveform characteristics of the recorded cells, it became evident that at least two different classes of waveforms were present. Based on previously reported data, using different technologies (Barthó *et al.* 2004, Sparta *et al.* 2014), these two groups of cells were assigned as being either putative PCs or putative interneurones (FSIs; Fig. 1c). Responses to experimental manipulations were therefore henceforth analysed separately for these two putative cell classes.

Neuronal responses in the auditory paired pulse testing paradigm

We first studied cell firing responses to paired click stimulation. In this experimental paradigm, the first sound pulse referred to as condition stimulus (C) is followed, 500 ms later, by the test stimulus (T). In EEG recordings from patients with schizophrenia, several differences compared to healthy subjects have been observed in this test paradigm (Juckel *et al.* 2003, Park *et al.* 2010). These include a decreased amplitude of auditory evoked potentials (AEP) at certain latencies following each pulse as well as deceased ratio of the second to the first pulse response (*T/C* ratio).

Reduced AEP response in Df(h15q13)/+ mice has previously been shown in a similar paradigm with EEG measurements (Fejgin *et al.* 2014, Kogan *et al.* 2015), and we found that this difference is also present at the level of single cell responses. When averaging the evoked responses of all recorded cells in A1 in terms of increase in firing rate from baseline average rate, a reduced response amplitude was observed in the Df(h15q13)/+ mice. The response was compared

to wt mice during the first 100 ms of each pulse in order to match the known latency of the P1-N1-P2 AEP complex in rodents (Siegel *et al.* 2003, Fejgin *et al.* 2014, Witten *et al.* 2014), and a significant genotype difference was observed for both PCs and interneurones (73% and 71% of controls, ANOVA main effect of genotype, $P < 0.001$, $F = 16.55$ and $P < 0.001$, $F = 12.23$ respectively; Figure 2a, b). In accordance with clinical EEG observations using a similar sensory gating paradigm (Light *et al.* 2012), we observed a response suppression of the second pulse (ANOVA main effect of pulse order, $P < 0.001$, $F = 12.61$ and $P < 0.001$, $F = 11.91$ for PCs and interneurones respectively). However, there was no significant difference in sensory gating between genotypes ($P > 0.05$ for both cell types for the ANOVA interaction effect between genotype and pulse number; Fig. 2a, b). Besides the reduced firing rate response of A1 cells during the AEP in the paired click paradigm, it was also noted that the average baseline firing rate of PCs was reduced in mutant mice also in absence of auditory stimuli (3.9 ± 2.9 Hz compared to 5.3 ± 5.4 Hz; ANOVA main effect of genotype, $P < 0.01$, $F = 7.86$), while interneurones showed a tendency towards reduced firing rate (6.0 ± 6.6 Hz compared to 8.9 ± 7.7 Hz; $P = 0.051$, $F = 3.86$).

Neuronal responses in the auditory steady-state testing paradigm

We next examined the single cell responses to a train of auditory pulses using the experimental paradigm typically applied when assessing ASSRs in patients with schizophrenia (Rass *et al.* 2012, Nakao & Nakazawa 2014). Interestingly, also in this testing paradigm, the response amplitude of single cells, in terms of relative change in mean firing rate, showed a significant decrease in Df(h15q13)/+ compared to wild-type mice (Krishnan *et al.* 2009). However, while PCs in Df(h15q13)/+ mice displayed a clear reduction in mean firing rate during the steady-state phase for the frequency interval tested (20–80 Hz), interneurones only showed a tendency towards reduced mean firing which did not quite reach significance (mean firing rate for PCs and FSIs in mutants was on average: 45.5% and 94.5% of those in wt mice; ANOVA main effect of genotype, $P < 0.001$, $F = 20.13$ and $P = 0.0586$, $F = 3.59$ respectively; post hoc test showed a significant genotype difference for PCs for all frequencies except 20 Hz; Fig. 3a–d).

Given this difference in the sustained response to steady-state stimuli, we next investigated the evoked firing in response to each of the pulses in the stimulus train in further detail. Exploring ISIs of single neurones, we found that PCs rarely generated action
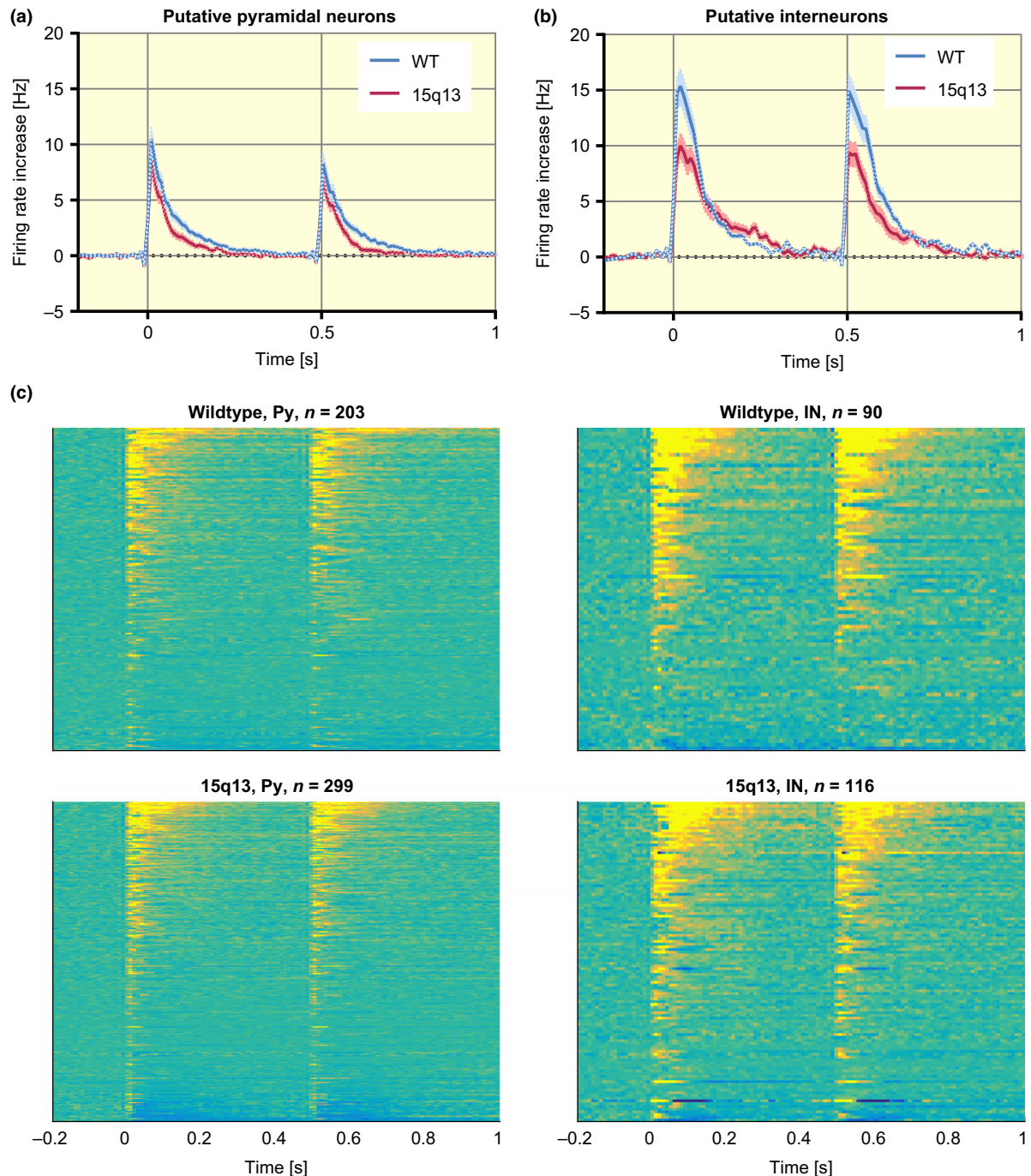
Figure 2 Increased firing rate during pulse responses in A1 is reduced in Df(h15q13)/+ mice, 100 ms bin. (a) Putative pyramidal neurones response were significant lower in Df(h15q13)/+ (*n* = 299) mice compared to wild type mice (*n* = 203). (b) In putative interneurones (*n* = 116), Df(h15q13)/+ mice showed a decreased response to the auditory stimuli, which was also significantly shortened compared to wild-type mice (*n* = 90). (c) Heat plots over neuronal responses for the complete population of neurones during the paired pulse paradigm. Neurones that increase their response the most are on the top whereas inhibited neurones are at the bottom with blue colour indicating inhibition. Note the diversified firing patterns. Thick lines denote mean; shaded area show SEM.

potentials in response to two subsequent auditory pulses, as would be expected because of their relatively lower firing rate compared to interneurones. In contrast, the interneurones were relatively often brought to spiking threshold by subsequent stimulation pulses even at the highest frequency used
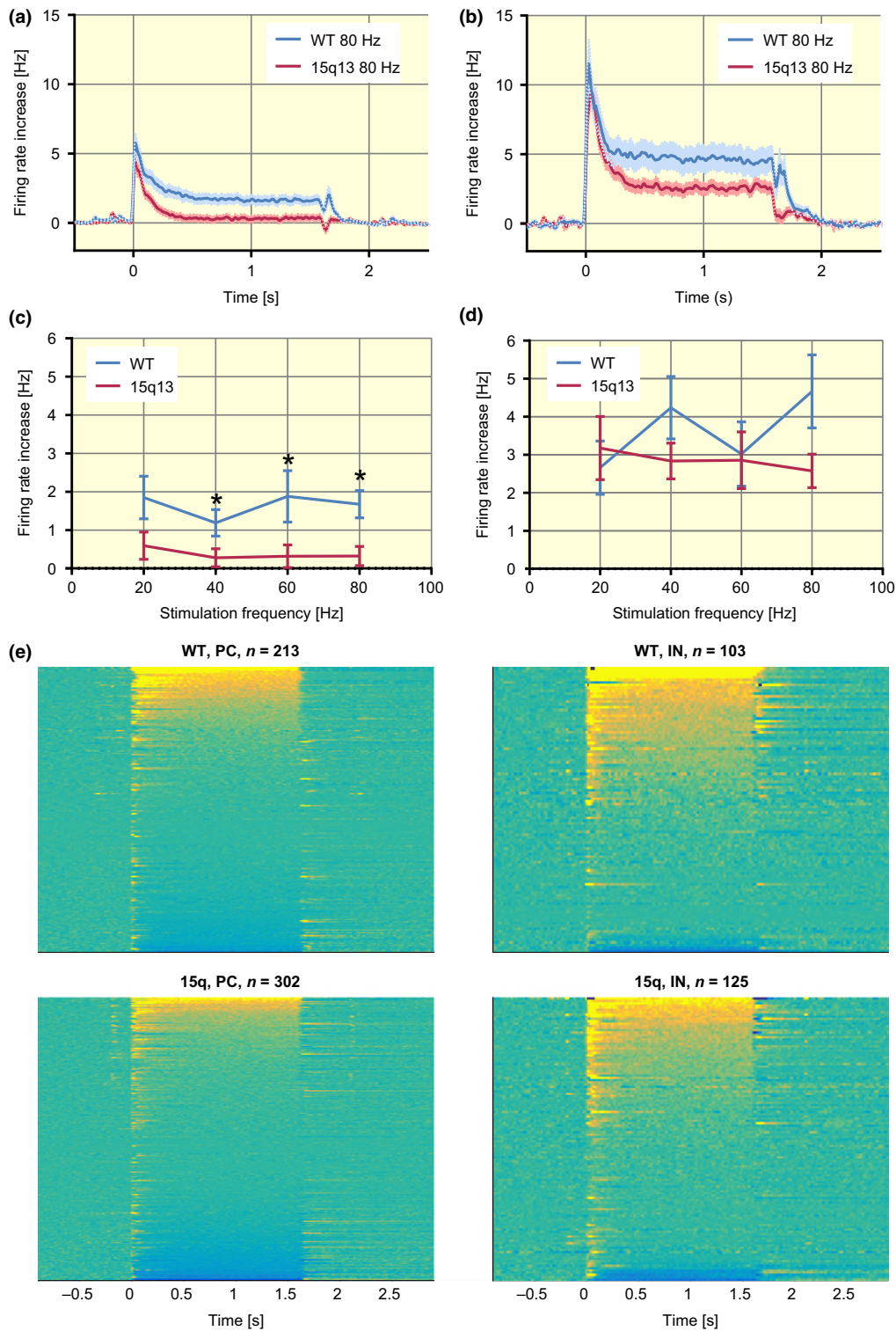
Figure 3 Steady-state response is lower in the Df(h15q13)/+ mice. (a, b) Average responses normalized to baseline firing rate of pyramidal cells (PCs) and interneurones in response to 80 Hz pulse trains. (c, d) Both putative pyramidal cells and putative interneurones exhibited a reduced mean firing rate, over different frequencies, during the steady-state phase of auditory pulse trains in Df(h15q13)/+ (n = 302) compared to wild-type (n = 213) mice. (e) Heat plots over neuronal responses for the complete population of neurone during the auditory steady-state responses (ASSR) paradigm (80 Hz). Neurones that increase their response the most during ASSR are on the top whereas neurones that is inhibited is at the bottom, note the blue colour indicating the inhibition. Note the diversified firing patterns. Thick lines denote mean; shaded area show SEM (*P < 0.05).

(80 Hz). More importantly however, the probability of putative interneurones to generate a new action potential in response to a short delay preceding spike clearly differed between Df(h15q13)/+ and wt mice. That is, in Df(h15q13)/+ mice, putative interneurones less often produced spikes in response to two pulses in a train with a brief interpulse interval (as indicated by the difference in decay coefficients for the two fitted exponential functions to the ISI distributions, ANOVA main effect of genotype, $P < 0.01$, $F = 10.32$; Fig. 4a). Given that interneurones can induce pronounced and rapid inhibition in neighbouring PCs, this deficiency should have directly consequences for network response properties. In particular, this response pattern corresponds to a shift in neuronal activity towards lower harmonics of the fundamental stimulation frequency, in agreement with the patterns seen with EEG in patients with schizophrenia (Kwon *et al.* 1999). Furthermore, by fitting simulated ISI distributions obtained from a simple spiking probability model to the observed data (where each cell is assumed to have two discrete activation probability states – high/low – corresponding to stimulation/silent periods of the pulse train), we could show that the duration and amplitude of the high-probability periods could explain the group difference (Fig. 4b, c). It can be noted that a reduced and shortened response to brief tone stimuli, as predicted by this model, also closely matches the reduced pulse response amplitude observed in the paired click paradigm (cf. Fig. 2b).

Normalizing firing properties through pharmacological interventions

The mechanistic insights relating to the firing characteristics of neurones in the Df(h15q13)/+ mouse open up for the possibility to pharmacologically modulate response properties in order to normalize interneurone firing patterns. Thus, after having established physiological response properties at the single cell level in the Df(h15q13)/+ mice that in many ways resemble previously reported results in patients with schizophrenia, we decided to evaluate whether a pharmacological manipulation aimed at normalizing the deficient fast-spiking properties in Df(h15q13)/+ mice could help normalizing the aberrant responses in the two testing paradigms. In order for a neural network to sustain spiking with high temporal precision, FSIs are thought to be particularly important (Wang & Gao 2010). For this reason, RE1, a substance that speeds up the opening kinetics of the voltage-dependent potassium channels Kv3.1, which are known to be highly expressed in FSIs, could potentially restore certain aspects of the deviating response patterns observed in Df(h15q13)/+ mice. To test this

hypothesis, six mice carrying the 15q13.3 deletion were first administrated saline (PO) and subjected to the two auditory testing paradigms (paired click and high-frequency ASSR, at 40 and 80 Hz). Following the acquisition of this control data set, RE1 was administrated (PO) and 30 min later the two testing paradigms were repeated once more. Using this experimental design, the same cells ($n = 108$) could be monitored throughout vehicle and drug treatment in all animals. Furthermore, there were no alterations in number of neurones before and after the treatment or big shifts in the PCA space of spike morphology indicating robust recording conditions. Interestingly, in both auditory testing paradigms, a partial normalization of certain aspects of the abnormal response patterns in the neurones was observed. To be precise, in the paired click experiments, there was a tendency towards normalization of the response amplitude (reaching significance for putative interneurones for the average summed response to each first pulse; ANOVA main effect of treatment, $P < 0.01$, $F = 12.89$). In the ASSR paradigm, RE1 did not restore reduced firing rates, but for the group of putative interneurones that were recorded in both conditions and showed significant entrainment to the pulses, a normalization of the flattened ISI distributions was in fact observed ($n = 12$; Fig. 5a, b; $P < 0.05$, tested with a binomial cumulative distribution function). While a more extensive study would be required to fully characterize all the physiological changes induced by manipulation of Kv3-channels, these experiments support the hypothesis that Kv3.1 channel modulation impact the function of FSIs *in vivo* in the intact awake brain.

Discussion

We have utilized our recently developed Df(h15q13)/+ mouse model (Fejgin *et al.* 2014, Nilsson *et al.* 2016) for detailed investigations of cortical putative pyramidal neurone and putative interneurone single cell firing in awake mice during pre-attentive processing of auditory sensory information. We find a decreased neuronal firing in response to auditory stimuli in the 'paired click paradigm' paradigm and reduced neuronal responses to fast 80 Hz steady-state auditory stimulation. Furthermore, the probability of putative interneurones to fire with low ISIs during 80 Hz gamma oscillations was reduced in Df(h15q13)/+ mice, an effect that was partially reversed by the Kv3.1-channel modulator, RE1.

The Df(h15q13)/+ mouse carrying the 15q13.3 microdeletion is a promising animal model of construct validity and may provide insight into psychiatric disorders like schizophrenia aetiology and ultimately
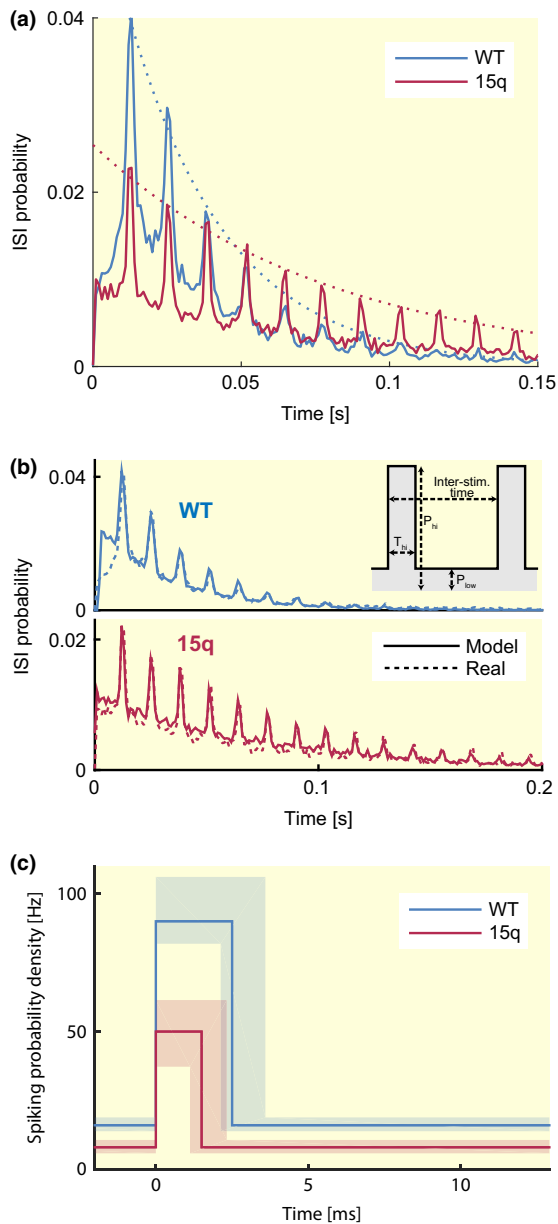
Figure 4 Interneuronal interspike interval (ISI) distributions are significantly altered in Df(h15q13)/+ mice in the auditory steady-state responses auditory testing paradigm (*n* = 12). (a) In mutant mice, putative interneurones less often produced spikes in response to two pulses with short ISI. Spiking probability distributions represent grand average of all interneurones in the two groups, and dashed lines denote the respective fitted exponentials. (b, c) Simulated ISI distributions show great similarities to the observed data after appropriate adjustments of the underlying spiking probability parameters, indicating a relatively reduced response amplitude and duration in Df (h15q13)/+ mice (red) compared to wild-type mice (blue). Shaded areas denoted 99% confidence intervals.

guide development of novel treatments. Recent schizophrenia research has utilized the advantages of using pre-attentive neurophysiological endophenotypes

such as sensory gating in the 'paired click' paradigm (de Wilde *et al.* 2007, Light *et al.* 2012) or ASSR (Spencer *et al.* 2008, Brenner *et al.* 2009). Such electrophysiological measures can provide objective assessments and are potentially closely linked to neurobiological foundation of disease processes (Gottesman & Gould 2003). Consequently, pre-attentive electrophysiological measures are also less likely to be biased by differences in behavioural states between species than more traditional behavioural measures. While baseline gamma oscillations are reported to be state-dependent during resting non-controlled conditions (Palenicek *et al.* 2011), evoked oscillations controlled by auditory stimulation is thought to be much less state-dependent. We found that Df(h15q13)/+ mice have decreased neuronal firing during detection of auditory stimuli, as previously described by EEG measures (Fejgin *et al.* 2014, Kogan *et al.* 2015). Even though standard EEG recordings (frequency < 100 Hz) primarily reflect mass post-synaptic potentials rather than action potentials (Telenczuk *et al.* 2011), we found in the current study similar alterations on a neuronal level in the auditory cortex of Df(h15q13)/+ mice. This study further provides deeper understanding and confidence in the face validity of this model, because decreased AEP amplitudes have repeatedly been reported in patients with schizophrenia (Adler *et al.* 1998, de Wilde *et al.* 2007, Light *et al.* 2012). On a mechanistic level, we found deficient function of putative GABAergic FSIs during pre-attentive processing of auditory information. This is interesting, because growing evidence points to a deficient function of fast-spiking GABAergic interneurones (Hashimoto *et al.* 2003, Gonzalez-Burgos *et al.* 2010, Taylor & Tso 2014) as well as reduced capacity to generate gamma oscillations, as an important pathophysiological mechanism in schizophrenia (Uhlhaas & Singer 2010, Lewis *et al.* 2012). Moreover, using optogenetic tools in animals, it has recently been demonstrated that gamma oscillatory activity in the pre-frontal cortex controlled by FSIs is important for cognitive performance (Cho *et al.* 2015, Kim *et al.* 2016). To this end, studying induced gamma oscillations in the ASSR paradigm in the current study revealed a decreased capacity for FSIs firing, and importantly, the deficient firing was most pronounced at high frequency in Df(h15q13)/+ mice.

Fast-spiking interneurones are thought to be important to sustain spiking behaviour with high temporal precision during fast oscillatory activity (Kepecs & Fishell 2014). Kv3.1 channels are highly expressed in FSI where they play an important role in maintaining fast firing (Espinosa *et al.* 2008). Kv3.1 channels are functionally different from other
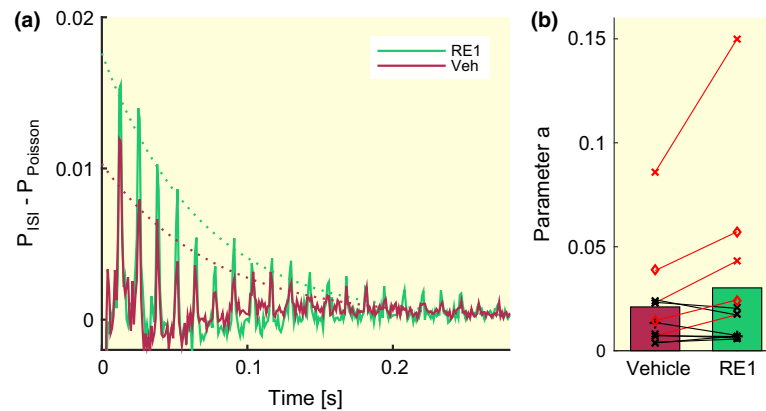
Figure 5 Pharmacological manipulation of Kv3.1 channels normalizes firing of putative fast-spiking interneurones (FSIs) and auditory steady-state responses in Df(h15q13) mice. (a) interspike interval (ISI) distributions (solid line) in putative FSIs show a normalization following RE1 treatment, as quantified by a faster decay of the fitted exponential (dashed line). To compensate for a general increase in firing rate following drug treatment, Poisson-like firing has been subtracted before curve fitting. (b) Decay coefficients of the normalized ISI distributions of individual neurones before and after RE1 treatment. Neurones with significantly altered coefficients are marked in red. The different symbols (plus, cross and diamond) represent different animals. The bars show the mean.

K^+ channels by having very rapid activation and deactivation kinetics (Rudy & McBain 2001). This unique biophysical characteristic of Kv3.1 channels makes them perfectly adapted to allow fast firing of interneurones by producing very fast repolarization with minimal duration of the effective refractory period (Espinosa *et al.* 2008). This is supported by the finding that application of the Kv3.1 activator RE1 to brain slices decrease the half-width of FSIs action potentials and enables interneurones to fire a train of action potentials (Rosato-Siri *et al.* 2015). In the Df (h15q13)/+ mice, we observed that RE1 increased spiking entrainment of FSIs to the auditory stimulus train in the high-frequency ASSR paradigm. Although subcortical mechanisms cannot be ruled out as the drug was administered systemically, this effect is consistent with the expected physiological action of this drug on FSIs. The facilitation of firing is also confirmed by the probabilistic dual-state model of the ASSR that revealed that in the Df (h15q13)/+ mouse, the putative interneurone population had reduced probability of spiking during the high state (immediately after a sound pulse), as well as a reduction of the time spent in the high state. These deficits revealed by the model could indeed be reversed by the Kv3.1 channel opener, which is known to shorten after-hyperpolarization time and thus decrease the effective refractory period (Rudy & McBain 2001, Espinosa *et al.* 2008).

Future larger studies should aim to substantiate our early findings that Kv3.1 channels are important for firing dynamics of FSIs *in vivo* during gamma oscillations and further aim to study the hypothesized effect on stabilizing cognitive function in animal models.

Nevertheless, current experimental data support the hypothesis that Kv3.1 channel modulation impact the function of FSIs and thus may constitute a potential target for pharmaceutical intervention in disorders with pathophysiological alterations in FSI such as schizophrenia.

In summary, freely moving *Df(h15q13)/+* mice display a decreased neuronal engagement during processing of auditory information in two translational auditory paradigms. Of particular pathological relevance to schizophrenia, *Df(h15q13)/+* mice display deficient function of putative FSIs, an effect that is partially normalized by a Kv3.1 channel opener, RE1. These findings offers insight into some of the mechanistic consequences of carrying the 15q13.3 microdeletion relevant to the schizophrenia and further points to deficient functioning of FSIs as a pathophysiological mechanism in schizophrenia and suggest a therapeutic potential Kv3.1 channel openers.

Conflict of interests

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