Impaired cognitive discrimination and discoordination of coupled theta-gamma oscillations in Fmr1 knockout mice

SUPPLEMENTAL INFORMATION

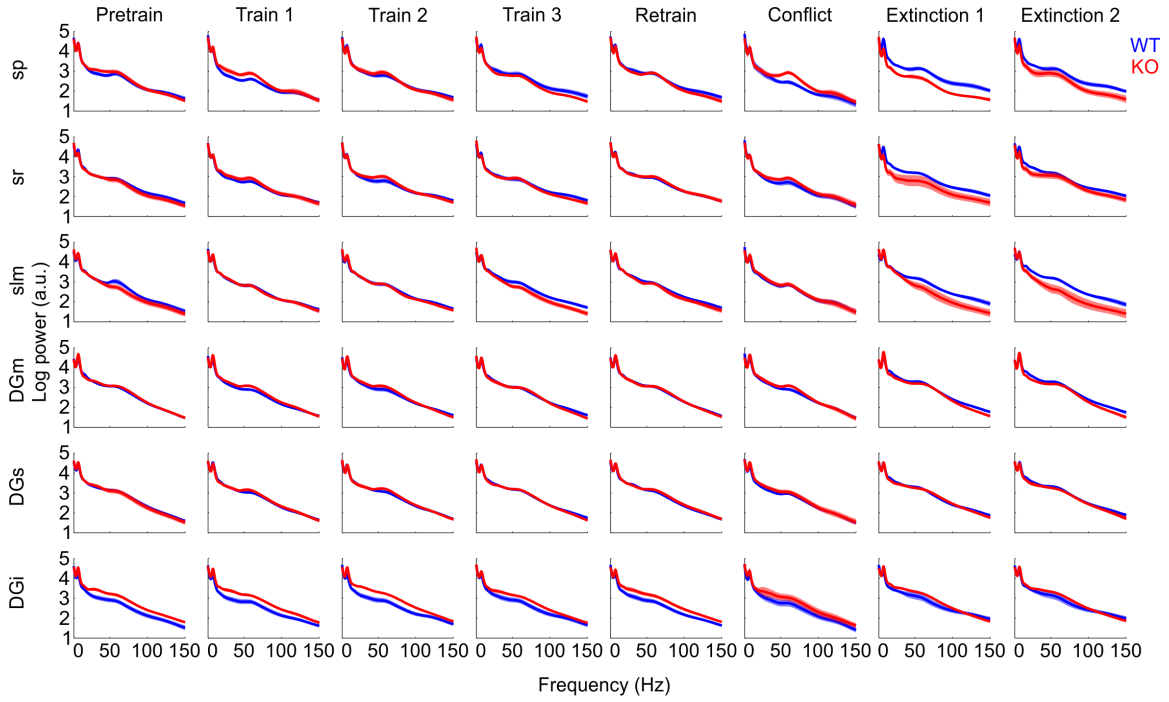

Fig. S1. Power spectra in wild-type and Fmr1 KO mice. The power spectra of LFPs extracted from the six electrode sites spanning a uniform distance between sp and DGi for both WT (blue) and Fmr1 KO (red) mice across the eight different behavioral sessions. The transparent band indicates ± SEM. The power spectra at each electrode site was computed from behavioral episodes when the mouse was moving faster than 3 cm/s. The power spectra are very similar for both KO and WT mice.

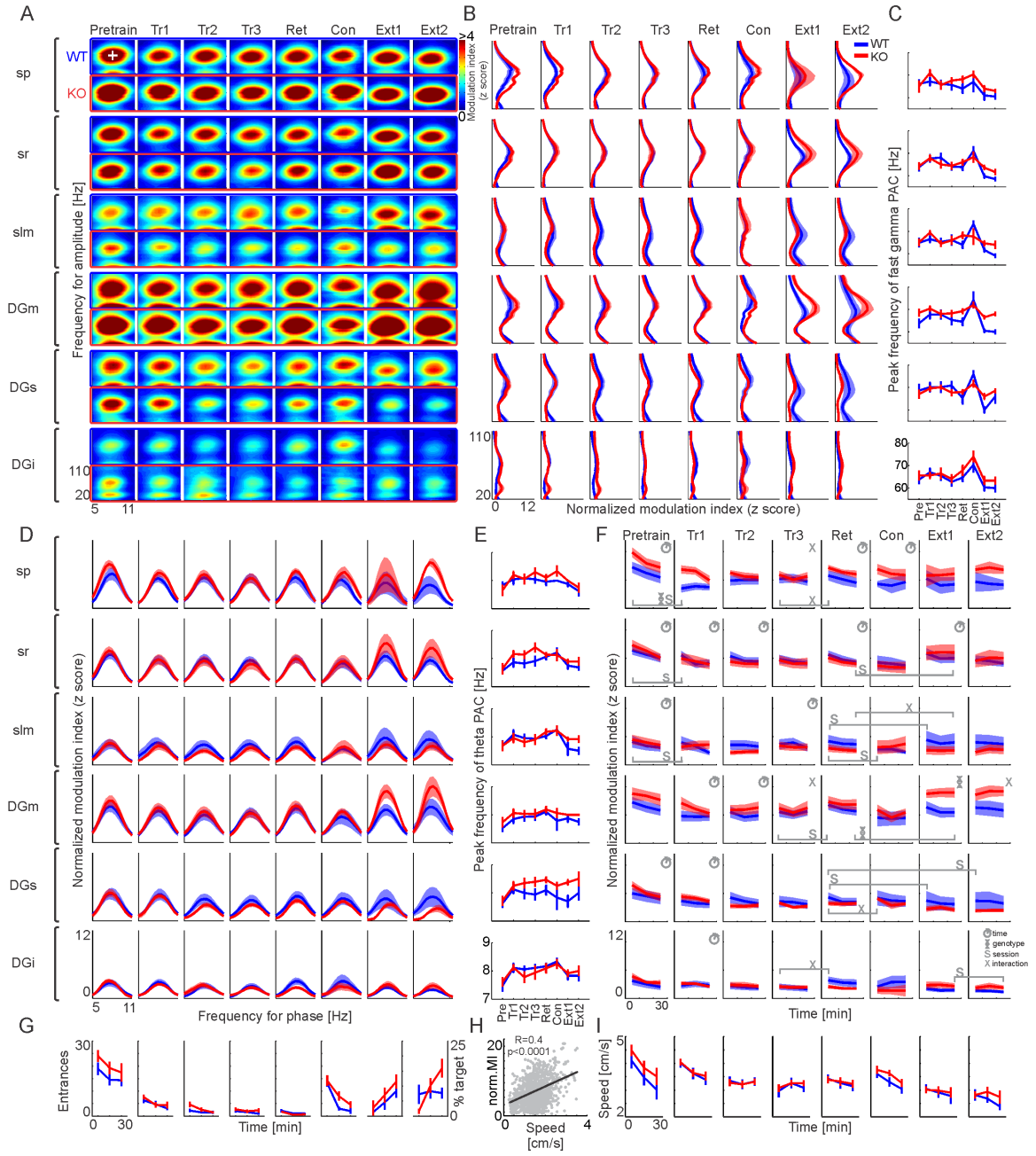

Figure S2. Discoordinated theta-gamma phase amplitude coupling in Fmr1 KO mice. (A) Average PAC comodulograms computed for all frequency combinations between the 5-11 Hz theta band and the 20-110 Hz gamma band across experimental sessions (columns) and electrode locations (rows) when the mice were moving faster than 3 cm/s. Each row includes data from both wild type and Fmr1 KO mice marked by blue and red rectangles respectively. Color corresponds to the normalized modulation index (z score).

(B) Vertical (gamma band) comodulogram profile taken at the peak theta frequency of the theta-fast gamma modulation (white cross in A top, left). Blue traces correspond to the wild type mice, red traces to the Fmr1 KO mice. Standard error is shown as a transparent area. (C) Peak fast gamma PAC frequency across electrode locations and experimental sessions. (D) Horizontal (theta band) comodulogram profile taken at the peak gamma frequency of the theta-fast gamma modulation. (E) Peak theta PAC frequency across electrode locations and experimental sessions. (F) The normalized modulation index (z score) extracted from the three 10-min epochs of each trial across electrode locations and experimental sessions. (G) Replot of the behavior data from Figure 2 for the same 10-min epochs as used in F. (H) Correlation between modulation index and speed computed from all experimental sessions combined. (I) Average animal speed computed from the same 10-min epochs as used in F. Legend: Significant ANCOVA effects indicated by the symbols helix – genotype, S – session, clock – 10-min session epoch.

DISCUSSION OF FIGURE S2

To measure theta-gamma coupling we computed average session- and electrode-specific comodulograms for each genotype (Fig. S2A). There is typically a single prominent blob indicating significant phase-amplitude coupling (PAC) between ~8 Hz (theta) and 60-80 Hz (fast gamma). Each blob in the comodulogram is characterized by three potentially independent features, the frequency of the fast modulated oscillation, the frequency of the slow modulating oscillation, and the strength of the phase-amplitude modulation. Each is analysed in turn in what follows.

We first investigated the frequency of the modulated oscillations in the fast gamma range (Fig. S2B). The modulated gamma frequency at each electrode location was

typically stable, as was the modulation strength, across the training sessions except, on the initial pretraining session, and interestingly, on the conflict and extinction sessions when the KO mice were impaired. During the conflict session, the frequency of the modulated oscillations tended to increase in a site- and genotype-specific manner (Fig. S2C). WT mice increased the modulated fast gamma frequency at all sites except sp, whereas the frequency was relatively constant in KO mice. Running speed was a covariate in all statistical evaluations. The genotype x session ANCOVAs on the site-specific peak frequencies confirmed an effect of genotype at the molecular layer of the dentate gyrus (DGm) site, which receives inputs from layer II of the entorhinal cortex ($F_{1,253}$ = 6.02; P = 0.01). These data indicate that WT mice adapt to the demand for flexible cognitive responses, potentially by adjusting the frequency of the theta-modulated gamma oscillations at the neocortical hippocampal inputs. Because Fmr1 KO mice are cognitively impaired and they fail to make these electrophysiological changes, these observations suggest that the genotypic differences in PAC are specific to the cognitive discrimination deficits that accompany loss of FMRP.

Next we examined the frequency of the modulating theta oscillations (Fig. S2D). The frequency was relatively constant across sessions but at some locations, relative to pretraining, it increased on the initial place avoidance session when shock was first encountered. The frequency increase tended to persist across training but could decrease on the extinction session when shock was turned off (Fig. S2E). The two-way genotype x session ANCOVA found significant effects of genotype at DGs input from ECII ($F_{1,194}$ = 10.8; P = 0.001). Thus, like the modulated gamma frequency, the modulating theta frequency was also sensitive to differences in genotype, consistent with the idea that PAC is sensitive to the cognitive information processing differences we observed.

We then considered the magnitude of the theta-gamma PAC, which is represented as a session average in Fig. S2A and as a function of time within the session in Fig. S2F. By inspection, PAC magnitude appeared to be modulated by genotype, session, and electrode location (Fig. S2D). The three-way ANCOVA confirmed main effects of session ($F_{7,930}$ = 2.23; P = 0.03) and location ($F_{5,930}$ = 48.9; P = 10^{-45}) but not genotype ($F_{1,930}$ = 0.85; P = 0.4). The interaction between location and genotype was also significant ($F_{5,930}$ = 5.19; P = 10^{-4}). Subsequent comparisons of genotype were location-specific and focused on session-specific questions that also took the time during a session into account.

To investigate the effect of introducing place avoidance training with shock, we compared PAC between pretraining and the first training session with shock, at each electrode location (Fig. S2F). To minimize within-session time effects we compared the first 10 min of each session by two-way genotype x session ANCOVA. There was an effect of genotype only at the sp site ($F_{1,12}$ = 9.68; P = 0.009) while an effect of session was observed at sp, sr and slm (F's > 7.0; P's < 0.02). Thus PAC was sensitive to the particular type of behavioral session, which we emphasize were physically identical except during the 500 ms when shock was delivered and thus primarily differed in cognitive demand and consequently, perhaps the cognitive effort.

Next, to investigate the effect of the place avoidance training, we compared the PAC in the first 10 min of the first and third training sessions on Day 2. PAC did not differ between the genotypes (F's < 3.5; P's > 0.09). Although PAC at the sp site changed within a session, we did not observe an effect of training at any site (F's < 1,9; P > 0.2), suggesting that PAC magnitude is not a robust estimate of across session place avoidance learning *per se*.

Then we examined the effect of the 24-h retention period by comparing the change in PAC during the first 10 min of the last training session and the retraining session. No genotypic differences were observed at any layer (F's < 0,9; P's > 0.3), consistent with similar memory retention in Fmr1 KO and WT mice (Fig. 2B). There was an effect of session localized to DGm ($F_{1,28}$ = 5.44; P = 0.03). The genotype x session interaction was also significant at sp and DGi electrodes (F's < 4.6; P's < 0.02). In contrast to within day training, PAC at the principal cell layers of hippocampus was sensitive to between day training.

We were next interested to examine how PAC in the WT and KO mice changed between the first 10 min of the retention and conflict sessions, since this is when the genotypes differed in cognitive performance (Fig. 2C). PAC in WT mice was relatively unchanged on the conflict session, whereas PAC was elevated and then declined in KO mice. This difference was specific to the sp electrode but not significant (genotype: $F_{1,8}$ = 2.68; P = 0.1). In contrast, there was a clear effect of session at the slm site ($F_{1,10}$ = 12.0; P = 0.006) and a genotype x session interaction at the DGs electrodes ($F_{1,8}$ = 11.6; P = 0.009).

We then investigated changes in PAC in the WT and KO mice when shock was turned off to evaluate extinction of the place avoidance. First, we compared the initial 10 min of the retention and following extinction session. PAC values did not differ between the genotypes or the two types of session, perhaps because the mice continued to avoid the former shock zone despite the absence of shock (Fig 2D; Fig. S2G). However, we observed an effect of time within the session localized to the slm and DGs sites (F's < 6.2; P's < 0.02). We then compared the last 10 min of the retention and the following extinction sessions when the mice expressed less avoidance in the absence of shock. Although the physical conditions were identical within each 30-min session, unlike the

initial 10-min periods when the genotypes did not differ, we now observed PAC was greater in the KO mice ($F_{1,12}$ = 9.84; P = 0.009). This genotypic difference was localized to the vicinity of the DGm electrode, where the neocortical input from ECII arrives. We also observed an effect of session that was localized to the sr electrodes ($F_{1,13}$ = 19.6; P = 10^{-4}) because PAC in both genotypes was greater at the end of the extinction session. There was also a significant genotype x session interaction localized to the slm site because the speed-corrected modulation index (MI) values were similar during retention but the WT values were greater at the end of extinction ($F_{1,12}$ = 6.41; P < 0.03). Together, these observations suggest that PAC is sensitive to internal, cognitive variables beyond the physical conditions of the task.

To further investigate the influence of internal cognitive variables we compared the changes in PAC between the genotypes across the two extinction sessions. The initial 10 min of the first extinction session was compared to the initial 10 min of the second session when only the WT mice showed reduced avoidance. There was no effect of genotype or session. Then the last 10 min of the first session was compared to the corresponding time of the second extinction session when the KO mice showed fully extinguished avoidance. Here, we observed an effect of session localized at DGi ($F_{1,10}$ = 8.04; P = 0.02). These findings demonstrate that when shock was turned off, neural coordination was reduced at the neocortical input DGm and intrahippocampal sr nodes of the perforant path in WT mice but the changes were asymmetric in KO mice since theta-gamma coordination was reduced only at the sr site.

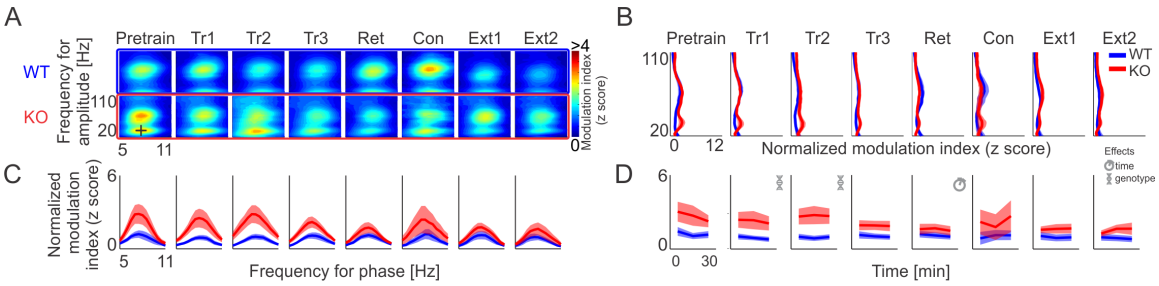

Figure S3. Abnormal theta-slow gamma PAC in the dentate gyrus of Fmr1 KO mice (A) Average PAC comodulograms computed from data when the mice were moving faster than 3 cm/s. (B) Vertical (gamma band) comodulogram profile taken at the peak theta frequency of the theta-slow gamma modulation (black cross in A bottom, left). (C) Horizontal (theta band) comodulogram profile taken at the peak gamma frequency of the theta-slow gamma modulation. (D) Normalized modulation index (z score) computed from the theta-slow gamma bands, extracted from three consecutive 10-min session epochs at the DGi electrode. Note: because a clear theta-slow gamma PAC peak was missing in the WT subjects, we instead computed the peak PAC value for each recording in the typical theta/gamma frequency range that was determined from the KO animals. Legend: Significant ANCOVA effects indicated by the symbols helix – genotype, S – session, clock – 10-min session epoch.

DISCUSSION OF FIGURE S3

KO mice showed clear theta-slow gamma PAC at the DGi site and WT mice did not so we examined PAC in the slow gamma range at the DGi site. Significant PAC in this range was observed in 7/10 KO mice and only 1/12 WT mice ($z = 7.13$; $P < 0.001$ test of proportions). The mean slow gamma PAC frequency in the KO recordings was 34 $\pm$ 0.4 Hz in the pretraining session, and this frequency was relatively constant across the

subsequent sessions. MI decreased during the pretraining session, mirroring the fast-gamma PAC changes. To compare genotypes, slow gamma was quantified by finding a peak in the 25-45 Hz frequency range. There were a number of significant effects of genotype and session when PAC was estimated in this range from the WT animals. Thus PAC in the slow gamma range was an additional factor that could potentially distinguish the WT and Fmr1 KO mice.