



Supplementary Figure 4: Persistent freezing to a loud tone in CB1^{-/-} is unrelated to contextual fear memory.

Naïve CB1^{-/-} and CB1^{+/+} (cf. Fig. 5) were exposed to a loud tone of 95 dB at two days (d1, d6). Here we show the freezing response before (open symbols; randomly chosen subset of animals) and during tone presentation at d6 (filled symbols; black bar indicates duration of tone presentation). As revealed by 2-way ANOVAs (*Genotype, Interval*) for repeated measures (*Interval*), CB1^{-/-} and CB1^{+/+} showed a similar freezing response to the test context before the second presentation of the loud tone (*Genotype*: $F_{1,15} = 0.9$, $p = 0.352$; *Genotype x Interval*: $F_{8,120} = 1.6$, $p = 0.128$). During tone presentation, in contrast, the freezing response was significantly more pronounced in CB1^{-/-} than in CB1^{+/+} (*Genotype*: $F_{1,15} = 5.2$, $p = 0.038$). A 3-way ANOVA (*Genotype, Tone, Interval*) for repeated measures (*Tone, Interval*) revealed significant effects of *Tone* ($F_{1,15} = 8.6$, $p = 0.010$), a significant *Tone x Genotype* interaction ($F_{1,15} = 5.8$, $P = 0.029$) and a significant effect of *Genotype* ($F_{1,15} = 4.5$; $p = 0.051$). Taken together, these data demonstrate that the first presentation of the loud tone at day 1 does not act as an aversive US that triggers contextual conditioning, which would become evident by an increase in pre-tone freezing at day 6. Moreover, pre-tone freezing appears to be unrelated to the sustained freezing response to the tone of CB1^{-/-}. Data were normalized to the 20-s observation intervals. * $P < 0.05$ vs. CB1^{+/+}