Supplemental Figures



Supplemental Figure 1. **BMS-191011 treatment alters the effects of SS administration but does not alter baseline ASR functions. (A)** The percent reduction in startle amplitude by a silent gap, or %Gap PPI, displayed for each carrier frequency for the data in Figure 1D. The %Gap PPI was measured at baseline (black), after SS administration (orange), and after SS+BMS-191011 (blue; see Figure 1A for study details). When each carrier frequency was entered individually, a two-way RM ANOVA indicated a main effect of treatment (F(2, 10) = 13.80; p<0.005), but not frequency (F(4, 20) = 0.78). Post-hoc analysis showed a significant decrease in %Gap PPI following SS administration (orange) that was returned to baseline levels (black) by co-treatment with BMS- 191011 (blue) for the 12 kHz carrier. N=6 per group, $p\le 0.05$, p< 0.01. (**B**) ASR functions were measured in a separate study assessing the effects of BMS-191011 treatment alone. Assessments were made during a baseline block (black) and again during a BMS-191011 treatment block (green). Startle amplitude is plotted as a function of SES intensity. A two-way RM ANOVA showed a main effect of intensity (F(6, 84) = 32.72; p<0.0001). There was no significant effect of BMS-191011 treatment alone (green) on baseline (black) startle amplitudes (F(1, 14) = 0.003). (**B**) The percent reduction in startle amplitude by a silent gap, or percent Gap PPI, displayed for several carrier frequencies. A two-way RM ANOVA showed that there was not a significant effect of treatment (F(1, 7) = 0.06). N=8 per group.



Supplemental Figure 2. **BMS-191011 treatment does not alter the effect of SS administration on ABR thresholds. (A)** A plot of ABR thresholds as a function of frequency. A two-way RM ANOVA of ABR thresholds showed a main effect of treatment (F(1.233, 9.866) = 38.98; p<0.0001) and frequency (F(1.681, 13.45) = 52.26; p<0.0001). Post-hoc analyses showed that thresholds increased relative to baseline following SS treatment, but were not further altered by BMS-191011 treatment. Orange and blue asterisks indicate a significant difference from baseline for the SS and SS+BMS-191011 groups, respectively. (**B**) Plots show the quantification of P1 latencies for 12, 16, 20 and 24 kHz tones presented at varying intensities. Stimulus intensity is expressed relative to the threshold intensity so that 0 dB SPL represents threshold for each animal in each condition (baseline, SS, SS+BMS-191011). Two-way ANOVAs indicated main effects of intensity (12 kHz: F(5, 154) = 52.49; p<0.0001; 16 kHz: F(5, 152) = 49.99; p<0.0001; 20 kHz: F(5, 143) = 53.87; p<0.0001; 24 kHz: F(5, 102) = 5.870; p<0.005, but not treatment (12 kHz: F(2, 154) = 2.557; 16 kHz: F(2, 152) = 2.515; 20 kHz: F(2, 143) = 1.659; 24 kHz: F(2, 102) = 5.870). N=8-12 per group, *p<0.05, **p<0.01, ***p<0.001, ***p<0.001.



Supplemental Figure 3. BMS-191011 treatment does not suppress the increased gain in early auditory structures caused by SS treatment. (A) Plots show the quantification of P1, P2, and P4 amplitudes as a function of stimulus intensity for 6 kHz tones. Two-way ANOVAs indicated a significant effect of intensity for P1 and P2, but not P4 (P1: F(3, 60) = 4.795, p < 0.005; P2: F(3, 60) = 4.795; (55) = 4.902, p < 0.005; P4: F(3, 62) = 0.664). There is also a main effect of treatment (P1: F(2, 60)) = 7.864, p < 0.001; P2: F(2, 55) = 10.52, p < 0.0001; P4: F(2, 62) = 5.446, p < 0.01). Post-hoc analyses indicated that when SS was administered alone or with BMS-191011, P1-P4 amplitudes were reduced relative to baseline at multiple intensities (orange and blue asterisks, respectively). BMS-191011 treatment did not significantly alter the effect of SS treatment. (B) Plots show the quantification of P1, P2, and P4 amplitudes as a function of stimulus intensity for 24 kHz tones. As for 6 kHz tones, two-way ANOVAs indicated a main effect of intensity for P1 and P2, but not P4 (P1: F(3, 71) = 9.45, p < 0.0001; P2: F(3, 94) = 18.19, p < 0.0001; P4: F(3, 102) = 0.622). There was also a main effect of treatment (P1: F(2, 71) = 73.55, p < 0.0001; P2: F(2, 94) = 22.47, p < 0.0001; P4: F(2, 102) = 12.30, p < 0.0001). Post-hoc analyses indicated that when SS was administered alone or with BMS-191011, P1-P4 amplitudes were reduced relative to baseline at multiple intensities (orange and blue asterisks, respectively). BMS-191011 treatment did not significantly alter the effect of SS treatment. (C) Graph of the amplitude of P2 relative to P1 for 60 dB tones show the effect of treatment on gain in the auditory brainstem both near the behaviorally-probed tinnitus frequency region (12-20 kHz) and farther from the tinnitus frequency region (6 and 24 kHz). Two-way ANOVA showed a main effect of frequency (F(4, 111) = 3.04; p < 0.05) and treatment (F(2, 111) = 15.85; p < 0.0001). Post-hoc analysis indicated that SS administration (orange) significantly increased the P2/P1 ratio relative to baseline (black) near the tinnitus frequency region, and that this was unaltered by co-treatment with BMS-191011 (blue). In contrast, SS administration did not significantly alter P2/P1 ratios at frequencies farther from the tinnitus frequency region. (D) Graph of the amplitude of P4 relative to P1 for 60 dB tones. Similar to the P2/P1 ratios, there was also a main effect of frequency (F(4, 112) = 5.61; p < 0.0005)

and treatment (F(2, 112) = 18.00; p<0.0001). Post-hoc analyses indicated that SS administration (orange) significantly increased the P4/P1 ratio relative to baseline (black) for 12-24 kHz tones, and that this was unaltered by BMS-191011 treatment (blue). SS administration did not alter the P4/P1 ratio at 6 kHz. Dotted lines on the P2/P1 and P4/P1 graphs demarcate equal amplitudes (ratio = 1). *N*=8-12 per group, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



Supplemental Figure 4. Local administration of BMS-191011 alone modifies spontaneous activity for high-frequency, but not mid- or low-frequency midbrain neurons. (A) Total excitatory receptive fields (eFRAs) were recorded at baseline (black) and again 2 hours following local BMS-191011 administration over the IC (green) to determine best frequencies (BFs). Dotted lines indicate 12 and 24 kHz. Representation of high frequency BFs increases following BMS-191011 administration. (B) Plot of mean spontaneous activity rates measured at baseline (black), and again after BMS-191011 administration (green). A *t*-test showed a decrease in spontaneous activity for high-frequency neurons (t(105)=3.627; p=0.0004), but no effect of treatment for mid-(t(174)=0.7912; n.s.) and low-frequency (t(113)=1.319; n.s.) neurons.