

Figure S1. Graph of emitted USV (**A**), heart rate (HR) (**B**) and freezing levels (**C**) of rats before conditioning (**A**, **B**, dots in **C**; with medians); and test freezing levels to context (5 min, squares) and context with cue (light; averaged three 20-s long exposures, triangles). Arrows in **A** show two copiously vocalizing rats which were not included in the final analysis. (**D**) Table of the values graphed and group effects (Kruskal-Willis). (**E**) Table of statistical comparisons of freezing between preconditional (pre) values and test values (Wilcoxon) as well as test values between groups (Mann-Whitney), with $p < 0.05$ values in bold.

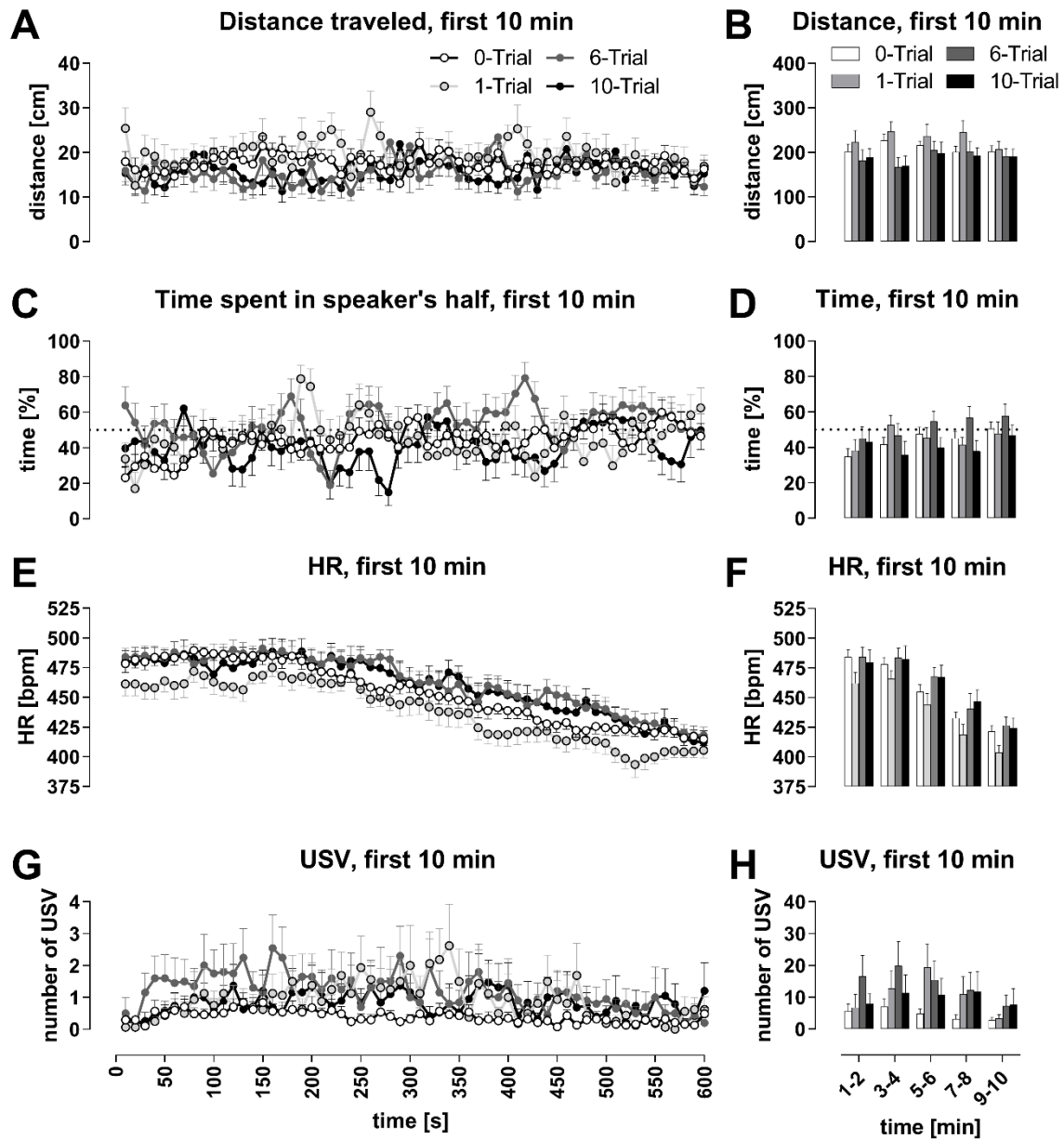


Figure S2. Assessment of locomotor activity, heart rate (HR) and the number of USV emitted of all animals during the first 10 min of silence in the experimental cage; (**A, B**) distance traveled (cm); (**C, D**) time (%) spent in a speaker's half of the cage with horizontal dotted line marking 50% chance level; results above it show more time in the speaker's half; (**E, F**) heart rate (bpm); (**G, H**) number of USV. Values are means with SEM. HR, initially stable, declined progressively in all four groups; however, HR in 1-Trial group was lower than in groups with zero, six and ten shocks. Mean values with SEM are presented for 10-s intervals in line graphs (**A, C, E, G**) and 2-min intervals in bar graphs (**B, D, F, H**); groups: 0-Trial, $n = 37$; 1-Trial, $n = 16$; 6-Trial, $n = 20$; 10-Trial, $n = 19$.

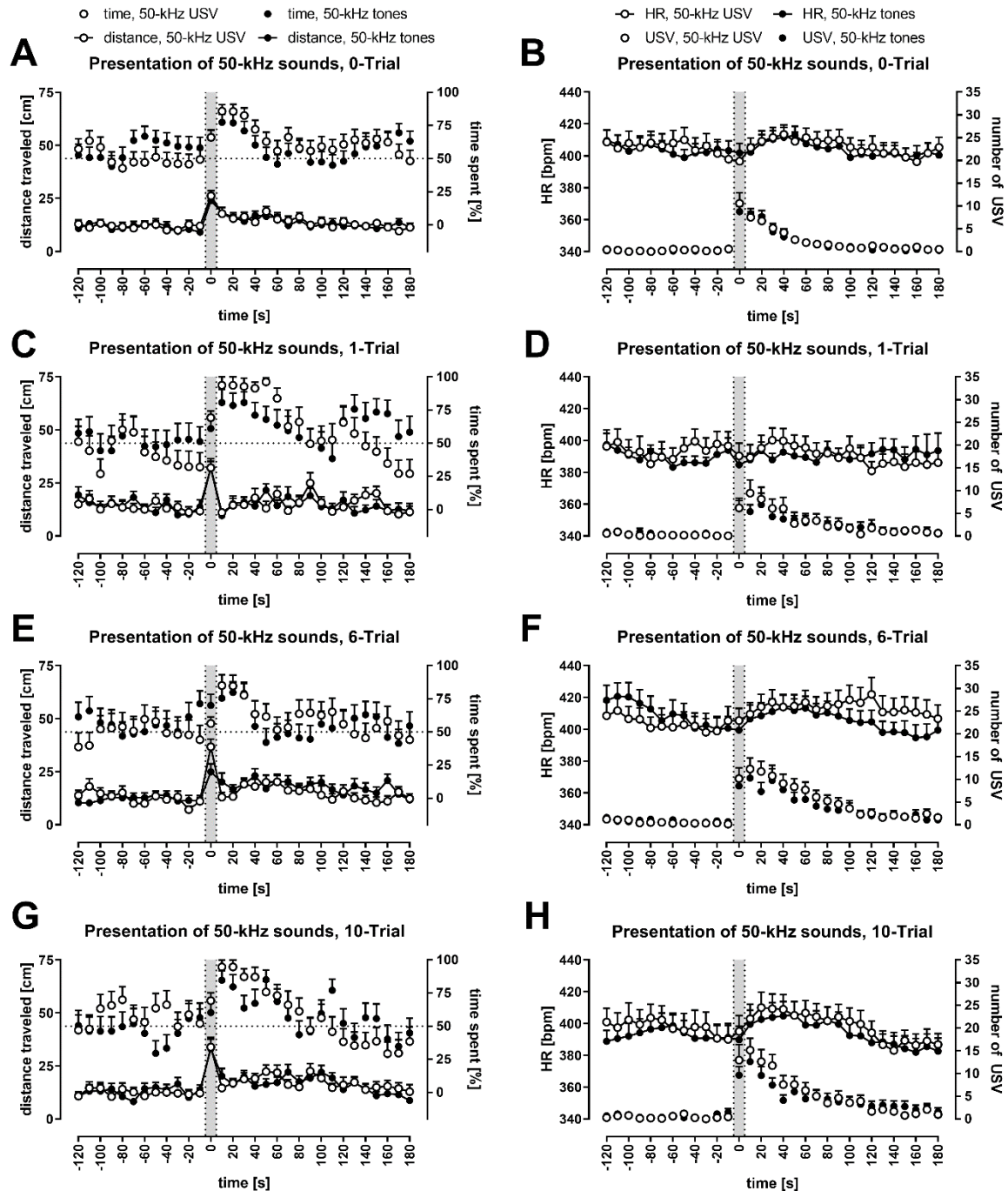


Figure S3. Effect of 50-kHz ultrasonic playback sounds on distance traveled, time spent in the speaker's half of the cage, heart rate (HR) and USV emission. Gray sections correspond to the 10-s-long ultrasonic presentation. Graphs depict responses after previous exposure to: no shock (A, B), one shock (C, D), six shocks (E, F), and ten shocks (G, H). In the left column (A, C, E, G), distance traveled is presented as connected dots (cm, left Y axis), percentage of time spent in the speaker's cage half – as not connected dots (% , right Y axis). In the right column (B, D, F, H), HR is presented as connected dots (bpm; beats per minute, left Y axis); the number of USV is presented as not connected dots (right Y axis). Each point is a mean for a 10-s-long time-interval with SEM. The dotted horizontal line marks a 50% chance value for time in a side of the cage. HR increased after playback in all groups except 1-Trial rats. There was also a clear USV response to the playback in all groups. The increase in locomotor activity to the playback was more pronounced in fear-conditioned (FC) animals than in 0-Trial rats; groups: 0-Trial, n = 37; 1-Trial, n = 16; 6-Trial, n = 20; 10-Trial, n = 19.

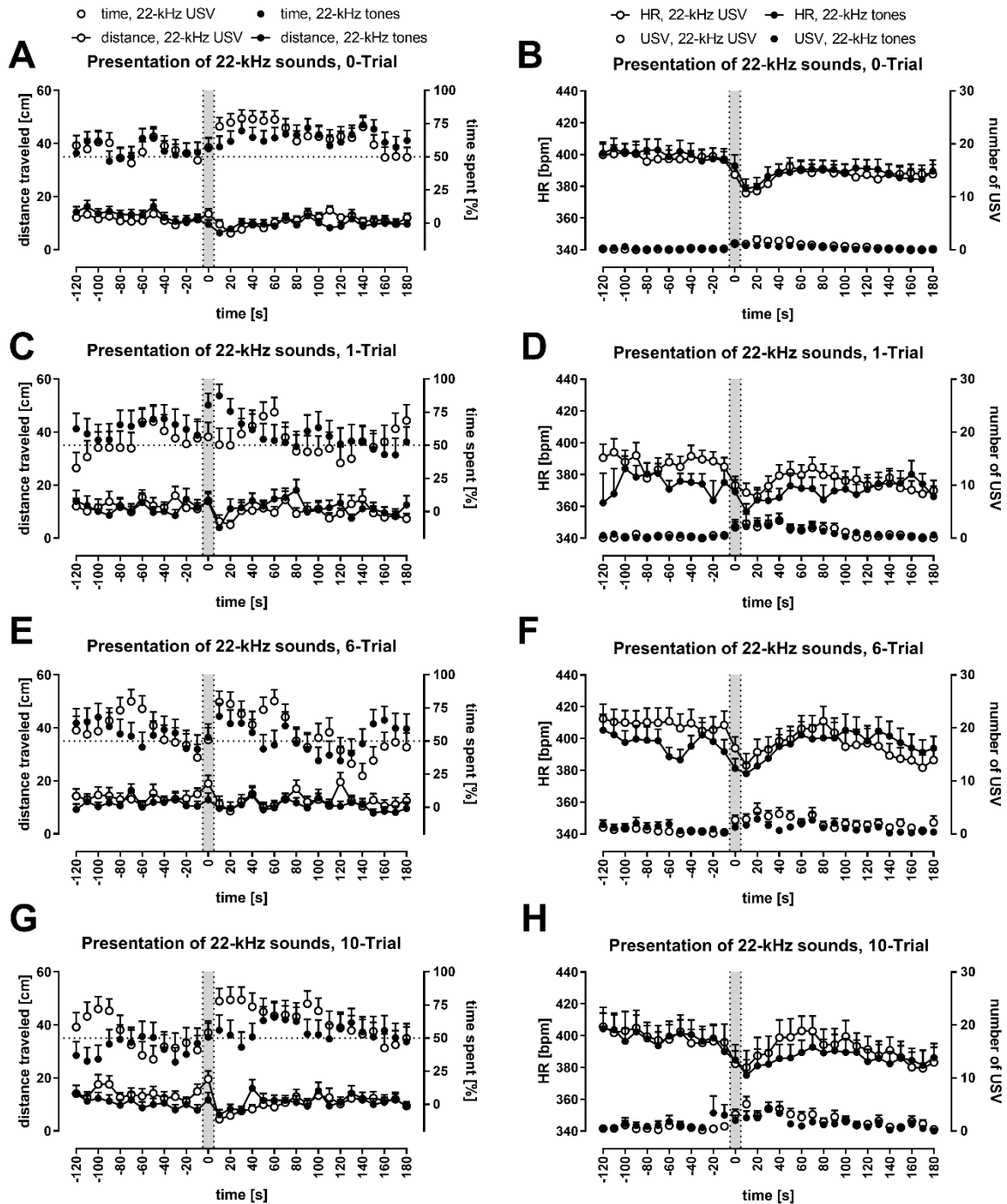


Figure S4. Effect of 22-kHz ultrasonic playback sounds on distance traveled, time spent in the speaker's half of the cage, heart rate (HR) and USV emission. Gray sections correspond to the 10-s-long ultrasonic presentation. Graphs depict responses after previous exposure to: no shock (A, B), one shock (C, D), six shocks (E, F), and ten shocks (G, H). In the left column (A, C, E, G), distance traveled is presented as connected dots (cm, left Y axis), percentage of time spent in the speaker's cage half – as not connected dots (% , right Y axis). In the right column (B, D, F, H), HR is presented as connected dots (bpm; beats per minute, left Y axis); the number of USV is presented as not connected dots (right Y axis). Each point is a mean for a 10-s-long time-interval with SEM. The dotted horizontal line marks a 50% chance value for time in a side of the cage. Playback exposure results in an HR drop in all four groups. There was also a clear USV response to the playback, especially in FC groups. The decrease in locomotor activity after the playback was more pronounced in FC animals than in 0-Trial rats; groups: 0-Trial, $n = 37$; 1-Trial, $n = 16$; 6-Trial, $n = 20$; 10-Trial, $n = 19$.

time-interval [s] / group	0-Trial	1-Trial	6-Trial	10-Trial	all rats	0-Trial	1-Trial	6-Trial	10-Trial	all rats
# of rats	n = 37	n = 16	n = 20	n = 19	n = 92	n = 37	n = 16	n = 20	n = 19	n = 92
distance										
	50-kHz USV					22-kHz USV				
-120 to 180	0.0000	0.0049	0.0000	0.0001	0.0000	0.0012	0.0073	0.5023	0.0018	0.0000
-60 to 60	0.0000	0.0035	0.0000	0.0001	0.0000	0.0001	0.0010	0.1131	0.0002	0.0000
-10 to 10	0.0000	0.0001	0.0001	0.0007	0.0000	0.1635	0.0152	0.0150	0.0004	0.0000
0 to 30	0.0009	0.0000	0.0000	0.0134	0.0000	0.0001	0.0005	0.0037	0.0002	0.0000
-120 to -100	0.3485	0.4724	0.7047	0.1426	0.7457	0.9733	0.7788	0.1572	0.6918	0.5153
-30 to -10	0.0855	0.6454	0.0907	0.2683	0.0461	0.1923	0.7788	0.3499	0.8539	0.6545
	50-kHz tone					22-kHz tone				
-120 to 180	0.0000	0.0019	0.0001	0.0000	0.0000	0.0000	0.1346	0.2252	0.0943	0.0000
-60 to 60	0.0000	0.0012	0.0028	0.0007	0.0000	0.0004	0.0130	0.1252	0.0397	0.0000
-10 to 10	0.0000	0.0016	0.0260	0.0029	0.0000	0.0306	0.0053	0.0863	0.0120	0.0000
0 to 30	0.0172	0.0005	0.0905	0.1657	0.0000	0.0846	0.0023	0.0043	0.0207	0.0000
-120 to -100	0.4820	0.1738	0.7047	0.6227	0.8404	0.7841	0.4437	0.5220	0.6918	0.7959
-30 to -10	0.9221	0.6456	1.0000	0.4308	0.9575	0.6489	0.4724	0.3867	0.6918	0.9892
	50-kHz sounds					22-kHz sounds				
-120 to 180	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	0.0014	0.0676	0.0025	0.0000
-60 to 60	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0261	0.0014	0.0000
-10 to 10	0.0000	0.0002	0.0001	0.0002	0.0000	0.0306	0.0072	0.0743	0.0009	0.0000
0 to 30	0.0005	0.0000	0.0000	0.0010	0.0000	0.0017	0.0027	0.0072	0.0004	0.0000
-120 to -100	0.4692	0.2691	0.5220	0.6918	0.8682	0.8276	0.6456	0.7047	0.5318	0.9267
-30 to -10	0.8276	0.4437	0.3867	0.2683	0.4237	0.0275	0.5698	0.5488	0.9487	0.2023

Table S1. Evaluation of changes in **distance traveled** around playback of ultrasounds (50-kHz USV, 50-kHz tones, 22-kHz USV, 22-kHz tones; with averaged results for USV and tones, i.e. sounds) presented during the 0 s time-interval (from -120 s to 180 s, -60 s to 60 s, -10 s to 10 s), as well as during the control intervals before playback (from -120 s to -100 s, -30 s to -10 s) observed in control rats (0-Trial), and rats which underwent fear-conditioning with different number of shocks (1-Trial, 6-Trial, 10-Trial) as well as all the rats analyzed together, all Friedman test, with $p < 0.05$ values in bold. Please note $p > 0.05$ values for -120 to -100 s and -30 to -10 s intervals.

group:	0-Trial	1-Trial	6-Trial	10-Trial	all rats	0-Trial	1-Trial	6-Trial	10-Trial	all rats
# of rats	n = 37	n = 16	n = 20	n = 19	n = 92	n = 37	n = 16	n = 20	n = 19	n = 92
time in speaker's half vs. 50%										
time-interval [s]	50-kHz USV					22-kHz USV				
0 s (playback)	0.0089	0.0013	0.1259	0.0079	0.0000	0.3694	0.6051	0.9851	0.6580	0.1173
10 s to 30 s	0.0000	0.0023	0.0117	0.0002	0.0000	0.0009	0.6233	0.0090	0.0401	0.0000
10 s to 60 s	0.0001	0.0005	0.0333	0.0001	0.0000	0.0000	0.2775	0.0100	0.0586	0.0000
-120 s to -100 s	0.2516	0.5695	0.4781	0.8405	0.7807	0.8268	0.5180	0.3507	0.1119	0.1279
-30 s to -10 s	0.7400	0.0980	0.7938	0.7172	0.3831	0.3694	0.7564	0.6274	0.7475	0.9101
	50-kHz tone					22-kHz tone				
0 s (playback)	0.0115	0.2343	0.0383	0.2954	0.0001	0.4644	0.0174	0.7369	0.8092	0.0310
10 s to 30 s	0.0003	0.0038	0.0012	0.0048	0.0000	0.0738	0.0299	0.1672	0.8721	0.0058
10 s to 60 s	0.0123	0.0061	0.0366	0.0017	0.0000	0.0499	0.0557	0.2180	0.6292	0.0056
-120 s to -100 s	0.9339	0.9588	0.3317	0.8092	0.7377	0.2078	0.4080	0.1730	0.0910	0.2949
-30 s to -10 s	0.2454	1.0000	0.2043	0.7782	0.1379	0.8504	0.2553	0.9702	0.3981	0.8993
	50-kHz sounds					22-kHz sounds				
0 s (playback)	0.0004	0.0125	0.0249	0.0279	0.0000	0.3219	0.0294	0.8753	0.7564	0.0531
10 s to 30 s	0.0000	0.0007	0.0017	0.0003	0.0000	0.0003	0.0830	0.0072	0.1005	0.0000
10 s to 60 s	0.0002	0.0004	0.0206	0.0002	0.0000	0.0003	0.0438	0.0075	0.0840	0.0000
-120 s to -100 s	0.3037	0.4899	0.6580	0.8261	0.4755	0.1102	0.9750	0.1788	0.7983	0.0810
-30 s to -10 s	0.4331	0.3343	0.6701	0.6192	0.6486	0.7613	0.3627	0.6603	0.4229	0.9534

Table S2. Evaluation **time spent** in the speaker's half values in **comparison with 50% chance level** during playback of ultrasounds, i.e. 50-kHz USV, 50-kHz tones, 22-kHz USV, 22-kHz tones, averaged USV and tones (sounds), i.e. during the 0 s time-interval, and immediately after (from 10 s to 30 s, 10 s to 60 s) as well as during the control intervals before playback (from -120 s to -100 s, -30 s to -10 s) observed in control rats (0-Trial), and rats which underwent fear-conditioning with different number of shocks (1-Trial, 6-Trial, 10-Trial) as well as all the rats analyzed together, all Wilcoxon test, with $p < 0.05$ values in bold. Please note $p > 0.05$ values for all -120 to -100 s and -30 to -10 s intervals.

time-interval [s] / group	0-Trial	1-Trial	6-Trial	10-Trial	all rats	0-Trial	1-Trial	6-Trial	10-Trial	all rats
# of rats	n = 37	n = 16	n = 20	n = 19	n = 92	n = 37	n = 16	n = 20	n = 19	n = 92
time										
	50-kHz USV					22-kHz USV				
-120 to 180	0.0000	0.0000	0.0384	0.0000	0.0000	0.0000	0.0790	0.0000	0.0000	0.0000
-60 to 60	0.0000	0.0000	0.0034	0.0004	0.0000	0.0000	0.1591	0.0002	0.0000	0.0000
-10 to 10	0.0001	0.0001	0.0070	0.0007	0.0000	0.0012	0.9623	0.0023	0.0000	0.0000
0 to 30	0.0003	0.0001	0.0000	0.0012	0.0000	0.0017	0.6957	0.0035	0.0033	0.0000
-120 to -100	0.5163	0.0162	0.2725	0.1629	0.8995	0.9520	0.1677	0.9608	0.0839	0.2259
-30 to -10	0.7903	0.8777	0.8700	0.6483	0.9931	0.1499	0.8465	0.3932	0.8777	0.2111
	50-kHz tone					22-kHz tone				
-120 to 180	0.0021	0.0930	0.0948	0.0016	0.0000	0.0719	0.0492	0.1855	0.3230	0.1396
-60 to 60	0.0055	0.0800	0.0628	0.0001	0.0000	0.0882	0.3111	0.3943	0.2053	0.0028
-10 to 10	0.1283	0.0458	0.4493	0.0138	0.0000	0.2927	0.0098	0.0762	0.3093	0.0007
0 to 30	0.0407	0.0112	0.6933	0.0092	0.0000	0.0325	0.1061	0.0803	0.7074	0.0250
-120 to -100	0.9810	0.6065	0.5890	0.9556	0.7463	0.7309	0.6188	0.5258	0.9661	0.8310
-30 to -10	0.9355	0.9592	0.0949	0.8574	0.6065	0.9200	0.8984	0.1188	0.0366	0.4939
	50-kHz sounds					22-kHz sounds				
-120 to 180	0.0000	0.0000	0.0193	0.0000	0.0000	0.0000	0.0072	0.0000	0.0074	0.0000
-60 to 60	0.0000	0.0000	0.0012	0.0000	0.0000	0.0000	0.7520	0.0008	0.0041	0.0000
-10 to 10	0.0004	0.0005	0.0093	0.0008	0.0000	0.0005	0.1869	0.0002	0.0025	0.0000
0 to 30	0.0008	0.0011	0.0037	0.0008	0.0000	0.0006	0.9224	0.0006	0.0047	0.0000
-120 to -100	0.6286	0.0522	0.5097	0.6361	0.6023	0.4766	0.8975	0.8357	0.5669	0.4390
-30 to -10	0.7221	0.7463	0.0414	0.4700	0.1639	0.2775	0.4124	0.1637	0.1870	0.2750

Table S3. Evaluation of changes in **time spent** in the speaker's half **around playback of ultrasounds** (50-kHz USV, 50-kHz tones, 22-kHz USV, 22-kHz tones; with averaged results for USV and tones, i.e. sounds) presented during the 0 s time-interval (from -120 s to 180 s, -60 s to 60 s, -10 s to 10 s), as well as during the control intervals before playback (from -120 s to -100 s, -30 s to -10 s) observed in control rats (0-Trial), and rats which underwent fear-conditioning with different number of shocks (1-Trial, 6-Trial, 10-Trial) as well as all the rats analyzed together, all Friedman test, with $p < 0.05$ values in bold. Please note $p > 0.05$ values for -120 to -100 s and -30 to -10 s intervals.

group:	0-Trial	1-Trial	6-Trial	10-Trial	all rats	0-Trial	1-Trial	6-Trial	10-Trial	all rats
n	n = 37	n = 16	n = 20	n = 19	n = 92	n = 37	n = 16	n = 20	n = 19	n = 92
time in speaker's half										
time-interval [s]	50-kHz USV					22-kHz USV				
before vs. playback	0.0148	0.0031	0.3760	0.0356	0.0000	0.2122	0.4413	0.6002	0.1520	0.0623
before vs. after	0.0000	0.0059	0.0557	0.0027	0.0000	0.0058	0.9594	0.0171	0.0121	0.0000
playback vs. after	0.0046	0.0342	0.0065	0.0125	0.0000	0.0250	0.7353	0.0186	0.0051	0.0003
	50-kHz tone					22-kHz tone				
before vs. playback	0.2414	0.4631	0.1579	0.3003	0.0356	0.5098	0.0663	0.6784	0.0929	0.0239
before vs. after	0.0938	0.0277	0.1627	0.1089	0.0009	0.0534	0.1386	0.1361	0.2604	0.0051
playback vs. after	0.1908	0.0192	0.7333	0.1337	0.0054	0.0926	0.4990	0.1549	1.0000	0.1315
	50-kHz sounds					22-kHz sounds				
before vs. playback	0.0390	0.0084	0.1365	0.0347	0.0000	0.2301	0.0745	0.5695	0.0413	0.0079
before vs. after	0.0010	0.0025	0.0279	0.0042	0.0000	0.0018	0.5076	0.0139	0.0413	0.0000
playback vs. after	0.0072	0.0076	0.0126	0.0131	0.0000	0.0094	0.7794	0.0042	0.0342	0.0001

Table S4. Evaluation of changes in time spent in the speaker's half **before** (i.e. average time spent from -30 s to -10 s time-intervals) vs. during **playback** (i.e. 0 s time-interval) vs. **after** the playback (i.e. average from 10 s to 30 s time-intervals); significant values ($p < 0.05$) marked in bold, all Wilcoxon test. Analyzed were the control rats (0-Trial), and rats which underwent fear-conditioning with different number of shocks (1-Trial, 6-Trial, 10-Trial) as well as all the rats together, which one day later were exposed to ultrasonic playbacks (50-kHz USV, 50-kHz tones, 22-kHz USV, 22-kHz tones; with averaged results for USV and tones, i.e. sounds). Please note more instances of significance in case of "sounds" in comparison with USV and tone playbacks.

time-interval [s] / group	0-Trial	1-Trial	6-Trial	10-Trial	all rats	0-Trial	1-Trial	6-Trial	10-Trial	all rats
# of rats	n = 37	n = 16	n = 20	n = 19	n = 92	n = 37	n = 16	n = 20	n = 19	n = 92
HR changes										
	50-kHz USV					22-kHz USV				
-120 to 180	0.0000	0.1092	0.0244	0.0009	0.0000	0.0000	0.0001	0.0000	0.0015	0.0000
-60 to 60	0.0000	0.1797	0.0047	0.0235	0.0000	0.0000	0.0020	0.0004	0.0007	0.0000
-10 to 10	0.0572	0.3798	0.4620	0.1956	0.1030	0.0000	0.0548	0.0023	0.0799	0.0000
0 to 30	0.0000	0.0009	0.2371	0.0998	0.0000	0.0007	0.1491	0.3470	0.2203	0.0002
-120 to -100	0.0974	0.3453	0.7736	0.5157	0.6169	0.7656	0.5506	0.9874	0.5203	0.8449
-30 to -10	0.2880	0.6514	0.6106	0.6918	0.5452	0.6547	0.4724	0.6065	0.4317	0.7644
	50-kHz tone					22-kHz tone				
-120 to 180	0.0834	0.8709	0.0000	0.0180	0.0000	0.0000	0.0000	0.0026	0.0000	0.0000
-60 to 60	0.0316	0.2341	0.0728	0.2995	0.0000	0.0000	0.0000	0.0000	0.0017	0.0000
-10 to 10	0.7613	0.2092	0.6376	0.2924	0.5068	0.0004	0.0498	0.0032	0.6850	0.0000
0 to 30	0.3566	0.0244	0.1444	0.2703	0.0011	0.0023	0.2490	0.0664	0.4954	0.0001
-120 to -100	0.3299	0.7396	0.5803	0.7762	0.5115	0.9529	0.3022	0.2972	0.1233	0.4441
-30 to -10	0.6259	0.8290	0.9120	0.8056	0.6967	0.4032	0.8628	0.2123	0.1105	0.0797
	50-kHz sounds					22-kHz sounds				
-120 to 180	0.0001	0.7374	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-60 to 60	0.0002	0.3155	0.0041	0.0227	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
-10 to 10	0.1563	0.5698	0.1102	0.1335	0.0146	0.0001	0.0033	0.0011	0.2058	0.0000
0 to 30	0.0006	0.0021	0.0771	0.0117	0.0000	0.0012	0.1035	0.0640	0.2564	0.0000
-120 to -100	0.2500	0.8932	0.7287	0.4385	0.9645	0.9221	0.3679	0.3035	0.6918	0.9442
-30 to -10	0.5289	0.6319	0.9512	0.9487	0.8737	0.5843	0.6457	0.8607	0.0979	0.3004

Table S5. Evaluation of changes in **heart rate (HR)** around playback of ultrasounds (50-kHz USV, 50-kHz tones, 22-kHz USV, 22-kHz tones; with averaged results for USV and tones, i.e. sounds) presented during the 0 s time-interval (from -120 s to 180 s, -60 s to 60 s, -10 s to 10 s), as well as during the control intervals before playback (from -120 s to -100 s, -30 s to -10 s) observed in control rats (0-Trial), and rats which underwent fear-conditioning with different number of shocks (1-Trial, 6-Trial, 10-Trial) as well as all the rats analyzed together, all Friedman test, with $p < 0.05$ values in bold. Please note $p > 0.05$ values for all -120 to -100 s and -30 to -10 s intervals.

group:	0-Trial	1-Trial	6-Trial	10-Trial	all rats	0-Trial	1-Trial	6-Trial	10-Trial	all rats
n	n = 37	n = 16	n = 20	n = 19	n = 92	n = 37	n = 16	n = 20	n = 19	n = 92
heart rate (HR)										
time-interval [s]	50-kHz USV					22-kHz USV				
before vs. playback	0.0750	0.1183	0.5135	0.7628	0.2406	0.0007	0.0386	0.0057	0.0269	0.0000
before vs. 10 s	0.4417	0.2775	0.2180	0.2514	0.2008	0.0001	0.0038	0.0111	0.0074	0.0000
before vs. 10-30 s	0.0475	0.8160	0.0894	0.0429	0.0027	0.0000	0.0027	0.0366	0.0582	0.0000
before vs. 10-60 s	0.0190	0.8767	0.0479	0.0298	0.0003	0.0004	0.0494	0.0930	0.5595	0.0000
-10 s vs. playback	0.7186	0.1406	0.5595	0.3341	0.9494	0.0059	0.0783	0.0007	0.0298	0.0000
-10 s vs. 10 s	0.0797	0.2013	0.5016	0.0702	0.0607	0.0001	0.0140	0.0064	0.0166	0.0000
playback vs. 10 s	0.0047	0.7564	0.4898	0.1531	0.0117	0.0089	0.3259	0.1454	0.8092	0.0031
playback vs. 10-30 s	0.0004	0.3942	0.0967	0.0510	0.0000	0.0052	0.4380	0.6274	0.7022	0.0318
playback vs. 10-60 s	0.0001	0.2553	0.0826	0.0702	0.0000	0.1184	1.0000	0.6580	0.1842	0.9795
	50-kHz tone					22-kHz tone				
before vs. playback	0.9932	0.0703	0.7938	0.7782	0.3971	0.8327	0.2443	0.0012	0.0382	0.0027
before vs. 10 s	0.8741	0.7761	0.5016	0.1474	0.3012	0.0002	0.0106	0.0005	0.0242	0.0000
before vs. 10-30 s	0.1818	0.7564	0.2514	0.1221	0.0187	0.0000	0.0174	0.0008	0.1313	0.0000
before vs. 10-60 s	0.0733	0.7764	0.0366	0.1213	0.0020	0.0011	0.0627	0.0313*	0.1978	0.0000
-10 s vs. playback	0.7933	0.1118	0.9553	0.8617	0.5176	0.8741	0.1550	0.0106	0.5595	0.0338
-10 s vs. 10 s	0.9000	0.6909	0.4666	0.1474	0.4965	0.0002	0.0019	0.0021	0.1119	0.0000
playback vs. 10 s	0.8505	0.3011	0.2959	0.1387	0.1408	0.0002	0.0736	0.3411	0.0976	0.0000
playback vs. 10-30 s	0.1006	0.1148	0.0702	0.1262	0.0016	0.0076	0.1406	0.7369	0.5197	0.0118
playback vs. 10-60 s	0.0128	0.0666	0.0187	0.1262	0.0001	0.8327	0.4851	0.0124	0.7475	0.6375
	50-kHz sounds					22-kHz sounds				
before vs. playback	0.3231	0.0591	0.7089	0.8563	0.2266	0.0660	0.0787	0.0004	0.0166	0.0000
before vs. 10 s	0.7514	0.2220	0.1913	0.1165	0.2343	0.0000	0.0019	0.0027	0.0141	0.0000
before vs. 10-30 s	0.0535	0.8767	0.0793	0.0329	0.0022	0.0000	0.0023	0.0046	0.0586	0.0000
before vs. 10-60 s	0.0304	0.9588	0.0169	0.0242	0.0002	0.0001	0.0174	0.0366	0.1842	0.0000
-10 s vs. playback	0.8628	0.0832	0.5135	0.6791	0.6551	0.1597	0.0299	0.0006	0.0475	0.0000
-10 s vs. 10 s	0.3832	0.3011	0.3165	0.0872	0.1587	0.0000	0.0031	0.0007	0.0294	0.0000
playback vs. 10 s	0.1239	0.8160	0.1305	0.0684	0.0062	0.0011	0.0879	0.0872	0.3760	0.0001
playback vs. 10-30 s	0.0018	0.1337	0.0124	0.0329	0.0000	0.0040	0.1550	0.6274	0.9679	0.0077
playback vs. 10-60 s	0.0004	0.0557	0.0036	0.0329	0.0000	0.0635	0.6051	0.1474	0.3341	0.7470

Table S6. Evaluation of changes in heart rate (HR) around the time of ultrasonic playback, i.e. between **before** (i.e. average time spent from -30 s to -10 s time-intervals) or **immediately before** (at -10 s time-interval) vs. during **playback** (i.e. 0 s time-interval) vs. **after** the playback (i.e. at 10 s time-interval, vs. average from 10 s to 30 s or from 10 s to 60 s time-intervals); significant values ($p < 0.05$) marked in bold, all Wilcoxon test. Analyzed were the control rats (0-Trial), and rats which underwent fear-conditioning with different number of shocks (1-Trial, 6-Trial, 10-Trial) as well as all the rats together, which one day later were exposed to ultrasonic playbacks (50-kHz USV, 50-kHz tones, 22-kHz USV, 22-kHz tones; with averaged results for USV and tones, i.e. sounds). Please note the prevalence of significant values in the group of all rats; * – not significant when two outlying rats were included.

Differences in heart rate (HR) values following 50-kHz vs. 22-kHz playback															
time	50-kHz USV vs. 22-kHz USV					50-kHz tones vs. 22-kHz tones					50-kHz sounds vs. 22-kHz sounds				
groups	0-Trial	1-Trial	6-Trial	10-Trl.	all rats	0-Trial	1-Trial	6-Trial	10-Trl.	all rats	0-Trial	1-Trial	6-Trial	10-Trl.	all rats
-120	0.4924	0.9176	0.5067	0.6580	0.9322	0.1065	0.0703	0.3803	0.0910	0.1121	0.1971	0.2775	0.5379	0.2684	0.3198
-110	0.9219	0.6233	0.8789	0.7782	0.9423	0.6151	0.1706	0.1403	0.1446	0.3116	0.7514	0.2343	0.3604	0.3958	0.4286
-100	0.5923	0.8767	0.7172	0.8092	0.9023	0.6887	0.5695	0.1615	0.2684	0.4873	0.4699	0.7960	0.4330	0.4939	0.5607
-30	0.4110	0.5180	0.9359	0.7939	0.4914	0.5362	0.1089	0.8248	0.6435	0.4824	0.2363	0.2343	0.8083	0.6165	0.3937
-20	0.7860	0.3259	0.9839	0.5328	0.8184	0.1843	0.0525	0.8519	0.6165	0.2057	0.1843	0.0386	0.7089	0.4094	0.2922
-10	0.8268	0.2553	0.8373	0.6726	0.8274	0.3515	0.1406	0.6951	0.9839	0.1225	0.4783	0.0929	0.7228	0.8405	0.2515
0	0.1353	0.0744	0.0793	0.2684	0.0021	0.1080	0.1272	0.0731	0.5732	0.0064	0.0882	0.0465	0.0276	0.0949	0.0002
10	0.0001	0.0980	0.0160	0.0186	0.0000	0.0002	0.0008	0.0124	0.0294	0.0000	0.0000	0.0106	0.0080	0.0249	0.0000
20	0.0000	0.0090	0.0363	0.0559	0.0000	0.0000	0.0048	0.0152	0.1119	0.0000	0.0000	0.0016	0.0080	0.0534	0.0000
30	0.0004	0.0174	0.0038	0.0559	0.0000	0.0006	0.0072	0.0290	0.0347	0.0000	0.0002	0.0052	0.0051	0.0269	0.0000
40	0.0005	0.0627	0.0400	0.3760	0.0000	0.0004	0.0016	0.0570	0.0836	0.0000	0.0004	0.0131	0.0008	0.1712	0.0000
50	0.0071	0.0832	0.0582	0.7172	0.0011	0.0002	0.0664	0.1851	0.0346	0.0000	0.0002	0.0703	0.0479	0.2049	0.0000
60	0.0069	0.1089	0.1084	0.9039	0.0014	0.0103	0.0309	0.1354	0.1650	0.0001	0.0007	0.0262	0.1084	0.4813	0.0000
70	0.0038	0.3520	0.2250	0.8721	0.0068	0.0143	0.0664	0.3507	0.2049	0.0010	0.0008	0.2243	0.3048	0.3838	0.0003
80	0.0079	0.1477	0.5016	0.5732	0.0055	0.0696	0.0004	0.3604	0.1071	0.0002	0.0045	0.0072	0.3317	0.2352	0.0001
90	0.0005	0.0787	0.1560	0.4460	0.0002	0.0085	0.0186	0.3905	0.3760	0.0006	0.0003	0.0174	0.2043	0.3547	0.0000
100	0.0008	0.0262	0.0303	0.9199	0.0001	0.0855	0.0557	0.8519	0.7172	0.0357	0.0026	0.0140	0.1354	0.7782	0.0004
110	0.0027	0.1406	0.0894	0.5732	0.0004	0.1115	0.0083	0.8519	0.4688	0.0161	0.0045	0.0200	0.3317	0.4939	0.0006
120	0.0012	0.2775	0.0894	0.8721	0.0012	0.0955	0.0229	0.4222	0.7771	0.0087	0.0030	0.1272	0.1169	0.5869	0.0005
130	0.0056	0.1148	0.1506	0.8248	0.0025	0.0750	0.0362	0.7228	0.4326	0.0343	0.0047	0.0703	0.5135	0.8092	0.0038
140	0.0201	0.0468	.0438*	0.7628	0.0020	0.0169	0.1089	0.9839	0.6009	0.0088	0.0039	0.0437	0.2396	0.7782	0.0007
150	0.1049	0.0879	0.0152	0.9679	0.0015	0.0177	0.1406	0.8228	0.8789	0.0277	0.0210	0.0929	0.1560	0.8405	0.0025
160	0.2032	0.0105	0.0349	0.5197	0.0034	0.0050	0.0664	0.8373	0.9679	0.0102	0.0069	0.0319	0.2043	0.6292	0.0009
170	0.0499	0.0214	0.0251	0.1474	0.0001	0.0006	0.0832	0.6677	0.4688	0.0006	0.0016	0.0299	0.1005	0.2954	0.0000
180	0.0814	0.1089	0.0522	0.7172	0.0033	0.0424	0.0557	0.6813	0.2954	.0464*	0.0202	0.0200	0.1506	0.8721	0.0012

Table S7. Statistical evaluation of comparisons between the effects of **50-kHz vs. 22-kHz playback** of USV, tones, as well as of USV and tones averaged (sounds) presented during 0 s time-intervals **on heart rate (HR)** – as reported for time-intervals between -120 s and 180 s; significant values ($p < 0.05$) marked in bold, all Wilcoxon test. Analyzed were the control rats (0-Trial), and rats which underwent fear-conditioning the day before – with different number of shocks (1-Trial, 6-Trial, 10-Trial), as well as all the rats together. Please note the onset of significant values to coincide with the time of the playbacks; * – not significant when two outlying rats were included.

	50-kHz USV-playback					50-kHz tones-playback					50-kHz sounds-playback				
groups:	0-T	6-T	10-T	6-10-T	other	0-T	6-T	10-T	6-10-T	other	0-T	6-T	10-T	6-10-T	other
HR changes	vs. 1-Trial rats					vs. 1-Trial rats					vs. 1-Trial rats				
before vs. 10 s	0.1909	0.0885	0.1046	0.0569	0.0799	0.6281	0.5037	0.2397	0.2950	0.4011	0.3137	0.1519	0.0792	0.0693	0.1223
-10 vs. 10 s	0.0485	0.1810	0.0547	0.0638	0.0371	0.6989	0.4639	0.1496	0.2177	0.3698	0.2185	0.1225	0.0507	0.0456	0.0781
before vs. 10-30 s	0.2334	0.1114	0.0821	0.0569	0.0911	0.4556	0.5665	0.2968	0.3589	0.3646	0.1909	0.1660	0.0314	0.0453	0.0704
-10 vs. 10-30 s	0.0532	0.1864	0.0608	0.0693	0.0411	0.5788	0.3385	0.1798	0.1914	0.3044	0.1211	0.1346	0.0240	0.0329	0.0444
before vs. 10-60 s	0.1601	0.0484	0.0851	0.0345	0.0541	0.2570	0.2934	0.2083	0.1882	0.1822	0.1211	0.0561	0.0314	0.0199	0.0343
-10 vs. 10-60 s	0.0254	0.0746	0.0637	0.0379	0.0186	0.4335	0.2934	0.1277	0.1432	0.2167	0.0551	0.0667	0.0341	0.0233	0.0221

Table S8. Comparisons of **heart rate (HR) changes**, i.e. differences in HR value between two periods, in **1-Trial group** (the rats received one electric shock the day before) **vs. other groups** (control, 0-Trial/0-T group; the rats which received 6 shocks, 6-Trial/6-T; 10 shocks, 10-Trial/10-T; the 6-Trial and 10-Trial rats combined, 6-10-T, and all non-1-Trial rats combined, other); significant values ($p < 0.05$) marked in bold, all Mann-Whitney test.

time-interval [s] / group	0-Trial	1-Trial	6-Trial	10-Trial	all rats	0-Trial	1-Trial	6-Trial	10-Trial	all rats
# of rats	n = 37	n = 16	n = 20	n = 19	n = 92	n = 37	n = 16	n = 20	n = 19	n = 92
number of USV emitted										
	50-kHz USV					22-kHz USV				
-120 to 180	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
-60 to 60	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0106	0.0000	0.0000	0.0000
-10 to 10	0.0000	0.0002	0.0001	0.4967	0.0000	0.0073	0.2111	0.0084	0.3130	0.0000
0 to 30	0.0000	0.2299	0.8482	0.0001	0.0011	0.9963	0.5558	0.9420	0.0150	0.8622
-120 to -100	0.1869	0.5404	0.9078	0.1409	0.4755	0.3902	0.8669	0.2927	0.6918	0.2883
-30 to -10	0.0701	0.2319	0.6065	0.3679	0.1528	0.4459	0.1462	0.1738	0.1615	0.8348
	50-kHz tone					22-kHz tone				
-120 to 180	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0005	0.0318	0.0000	0.0000
-60 to 60	0.0000	0.0000	0.0000	0.0000	0.0000	0.0025	0.0042	0.0642	0.0000	0.0000
-10 to 10	0.0000	0.0001	0.0001	0.1942	0.0000	0.0454	0.8007	0.1778	0.0675	0.0044
0 to 30	0.0001	0.2660	0.1779	0.0001	0.0000	0.9558	0.3345	0.6676	0.3679	0.1597
-120 to -100	0.2276	0.2122	0.2276	0.5308	0.1738	0.3583	0.2574	0.2090	0.9394	0.8765
-30 to -10	0.0067	0.4937	0.8382	0.0441	0.1975	0.7047	0.1653	0.1561	0.8071	0.3189
	50-kHz sounds					22-kHz sounds				
-120 to 180	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-60 to 60	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
-10 to 10	0.0000	0.0000	0.0001	0.0266	0.0000	0.0065	0.1128	0.0012	0.1346	0.0000
0 to 30	0.0000	0.0705	0.4621	0.0000	0.0000	0.9974	0.7748	0.7665	0.0963	0.4743
-120 to -100	0.0921	0.2167	0.7491	0.0558	0.0291	0.7463	0.4046	0.1620	0.7548	0.8233
-30 to -10	0.0030	0.1466	0.8623	0.0320	0.1597	0.9775	0.2801	1.0000	0.2101	0.4878

Table S9. Evaluation of changes in the **number of USV emitted around playback** of ultrasounds (50-kHz USV, 50-kHz tones, 22-kHz USV, 22-kHz tones; with averaged results for USV and tones, i.e. sounds) presented during the 0 s time-interval (from -120 s to 180 s, -60 s to 60 s, -10 s to 10 s), as well as during the control intervals before playback (from -120 s to -100 s, -30 s to -10 s) observed in control rats (0-Trial), and rats which underwent fear-conditioning with different number of shocks (1-Trial, 6-Trial, 10-Trial) as well as all the rats analyzed together, all Friedman test, with $p < 0.05$ values in bold. Please note $p > 0.05$ values for -120 to -100 s and -30 to -10 s intervals.

group:	0-Trial	1-Trial	6-Trial	10-Trial	all rats	0-Trial	1-Trial	6-Trial	10-Trial	all rats
n	n = 37	n = 16	n = 20	n = 19	n = 92	n = 37	n = 16	n = 20	n = 19	n = 92
number of USV emitted										
time-interval [s]	50-kHz USV					22-kHz USV				
before vs. playback	0.0000	0.0027	0.0005	0.0003	0.0000	0.0168	0.2367	0.0060	0.0077	0.0000
before vs. 10 s	0.0000	0.0022	0.0008	0.0005	0.0000	0.0107	0.2249	0.0209	0.0029	0.0000
before vs. 10-30 s	0.0000	0.0015	0.0008	0.0004	0.0000	0.0025	0.0357	0.0022	0.0022	0.0000
before vs. 10-120 s	0.0000	0.0010	0.0004	0.0005	0.0000	0.0006	0.0093	0.0004	0.0004	0.0000
	50-kHz tone					22-kHz tone				
before vs. playback	0.0000	0.0019	0.0015	0.0002	0.0000	0.0284	0.1159	0.3980	0.3105	0.0024
before vs. 10 s	0.0000	0.0012	0.0015	0.0002	0.0000	0.0058	0.1159	0.0972	0.5541	0.0007
before vs. 10-30 s	0.0000	0.0014	0.0004	0.0002	0.0000	0.0035	0.0277	0.0099	0.0712	0.0000
before vs. 10-120 s	0.0000	0.0018	0.0002	0.0003	0.0000	0.0014	0.0044	0.0045	0.1330	0.0000
	50-kHz sounds					22-kHz sounds				
before vs. playback	0.0000	0.0008	0.0004	0.0002	0.0000	0.0137	0.1235	0.0157	0.0342	0.0000
before vs. 10 s	0.0000	0.0008	0.0005	0.0001	0.0000	0.0014	0.1159	0.0037	0.0108	0.0000
before vs. 10-30 s	0.0000	0.0008	0.0003	0.0002	0.0000	0.0012	0.0173	0.0018	0.0052	0.0000
before vs. 10-120 s	0.0000	0.0006	0.0002	0.0003	0.0000	0.0002	0.0037	0.0004	0.0229	0.0000

Table S10. Evaluation of changes in the **number of USV** around the time of ultrasonic playback, i.e. between **before** (i.e. average time spent from -30 s to -10 s time-intervals) vs. during **playback** (i.e. 0 s time -interval) vs. **after** the playback (i.e. at 10 s time-interval, vs. average from 10 s to 30 s or from 10 s to 120 s time-intervals); significant values ($p < 0.05$) marked in bold, all Wilcoxon test. Analyzed were the control rats (0-Trial), and rats which underwent fear-conditioning with different number of electric shocks (1-Trial, 6-Trial, 10-Trial) as well as all the rats together, which one day later were exposed to ultrasonic playbacks (50-kHz USV, 50-kHz tones, 22-kHz USV, 22-kHz tones; with averaged results for USV and tones, i.e. sounds). Please note the prevalence of significant values in all the groups.

Differences in the number of USV following 50-kHz vs. 22-kHz playback															
time	50-kHz USV vs. 22-kHz USV					50-kHz tones vs. 22-kHz tones					50-kHz sounds vs. 22-kHz sounds				
groups	0-Trial	1-Trial	6-Trial	10-Trial	all rats	0-Trial	1-Trial	6-Trial	10-Trial	all rats	0-Trial	1-Trial	6-Trial	10-Trial	all rats
-120	0.5698	0.0431	0.6566	0.6858	0.4427	0.8734	0.3452	0.6744	0.7263	0.9904	0.5360	0.0587	0.9063	0.8658	0.3298
-110	0.1599	0.0935	0.6726	0.3433	0.0661	0.8582	0.8551	0.6002	0.4755	0.7364	0.3454	0.4412	0.6356	0.3305	0.2070
-100	0.5098	0.3613	0.9645	0.3452	0.5098	0.6114	0.2851	0.7998	1.0000	0.4456	0.3233	0.0935	0.5761	0.4148	0.2257
-30	0.7089	0.1088	0.9326	0.1088	0.7938	0.6496	0.9326	NR	0.3452	0.6496	0.4072	0.0679	0.5541	0.1730	0.7775
-20	0.4859	0.2733	0.5930	0.5002	0.6359	0.7089	0.5930	0.7150	0.4772	0.9032	0.9569	0.5541	0.7150	0.7989	0.9569
-10	0.7282	NR	0.5294	0.5002	0.7282	0.9184	0.5294	0.1380	0.8886	0.8570	0.9937	0.1056	0.6744	0.7671	0.7996
0	0.0000	0.0597	0.0052	0.0065	0.0000	0.0000	0.0231	0.0019	0.0007	0.0000	0.0000	0.0202	0.0010	0.0009	0.0000
10	0.0000	0.0164	0.0008	0.0050	0.0000	0.0000	0.0277	0.0015	0.0003	0.0000	0.0000	0.0092	0.0007	0.0010	0.0000
20	0.0000	0.0207	0.0018	0.0050	0.0000	0.0000	0.1551	0.0033	0.0006	0.0000	0.0000	0.0360	0.0006	0.0008	0.0000
30	0.0000	0.1141	0.0015	0.0121	0.0000	0.0000	0.1549	0.0021	0.0979	0.0000	0.0000	0.0342	0.0007	0.0139	0.0000
40	0.0000	0.3863	0.0106	0.1055	0.0001	0.0000	0.8127	0.0015	0.3152	0.0000	0.0000	0.3824	0.0016	0.1075	0.0000
50	0.0011	1.0000	0.0052	0.0557	0.0027	0.0000	0.2300	0.0135	0.0086	0.0000	0.0000	0.7007	0.0009	0.0121	0.0000
60	0.0005	0.3505	0.0048	0.0787	0.0003	0.0000	0.1551	0.0120	0.0042	0.0000	0.0000	0.3454	0.0012	0.0060	0.0000
70	0.0055	0.5076	0.0288	0.2209	0.0128	0.0020	0.4990	0.1330	0.1467	0.0050	0.0006	0.5937	0.0229	0.0664	0.0017
80	0.0009	0.6726	0.0150	0.0935	0.0022	0.0006	0.2135	0.1159	0.0254	0.0016	0.0002	0.3863	0.0184	0.0199	0.0006
90	0.0056	0.7353	0.0113	0.2945	0.0132	0.0022	0.3454	0.3078	0.0454	0.0048	0.0007	0.6566	0.0186	0.0382	0.0017
100	0.0263	0.5286	0.1261	0.1361	0.0587	0.0001	0.0910	0.0593	0.0499	0.0001	0.0001	0.2860	0.0310	0.0258	0.0002
110	0.0080	0.5930	0.2845	0.0506	0.0169	0.0002	0.5937	0.1851	0.0185	0.0003	0.0001	0.5049	0.1005	0.0119	0.0001
120	0.0100	0.0910	0.4769	0.4755	0.0180	0.0019	0.3270	0.2411	0.0180	0.0033	0.0005	0.1235	0.3454	0.0150	0.0012
130	0.1110	0.5286	0.7671	0.6465	0.1696	0.0346	0.8339	0.7794	0.1688	0.0473	0.0154	0.7598	0.8385	0.2393	0.0305
140	0.0280	0.8927	0.2604	0.1823	0.0339	0.0000	NR	0.0117	0.0117	0.0000	0.0001	0.7874	0.0454	0.0076	0.0001
150	0.4545	0.6002	0.4069	0.9326	0.6959	0.0012	1.0000	0.0425	0.0663	0.0021	0.0143	0.5408	0.2026	0.1029	0.0360
160	0.0536	0.2249	0.4412	0.5076	0.0761	0.0007	0.0431	0.0251	0.0687	0.0004	0.0001	0.0910	0.0077	0.4420	0.0001
170	0.0125	0.1088	0.1235	0.5940	0.0174	0.0799	NR	0.2626	0.2076	.0323*	0.0030	0.1088	.0284*	0.2049	0.0011
180	0.0953	0.1088	0.9057	0.1775	0.0953	0.0520	1.0000	0.1730	0.0431	.0324*	0.0249	0.1441	0.6241	0.0910	0.0180

Table S11. Statistical evaluation of comparisons between the effects of **50-kHz vs. 22-kHz playback** of USV, tones, as well as of USV and tones averaged (sounds) presented during 0 s time-intervals **on the number of emitted vocalizations (USV)** – as reported for time-intervals between -120 s and 180 s; significant values ($p < 0.05$) marked in bold, all Wilcoxon test. Analyzed were the control rats (0-Trial), and rats which underwent fear-conditioning the day before – with different number of shocks (1-Trial, 6-Trial, 10-Trial), as well as all the rats together. Please note the onset of significant values to coincide with the onset of the playbacks; NR – the test gave no results; * – not significant when two outlying rats were included.

groups	number of USV				parameters of 50-kHz USV		
USV	total USV	50-kHz USV	short 22-kHz USV	long 22-kHz USV	duration [ms]	n	MPF [kHz]
USV emitted to 50-kHz USV-playback (0–120 s time-intervals)							
0-Trial	41.2 ± 7.1	39.9 ± 6.9	0.9 ± 0.2	0.3 ± 0.3	27.0 ± 1.7	19	61.7 ± 2.2
1-Trial	59.1 ± 19.5	57.8 ± 19.3	1.3 ± 0.6	0.0 ± 0.0	26.9 ± 2.1	9	59.7 ± 1.6
6-Trial	86.4 ± 16.5	85.7 ± 16.5	0.8 ± 0.3	0.0 ± 0.0	29.3 ± 2.6	14	61.1 ± 0.6
10-Trial	97.5 ± 17.5	95.8 ± 17.2	1.7 ± 0.7	0.0 ± 0.0	30.1 ± 1.8	15	60.8 ± 0.9
all FC'ed	82.3 ± 10.3	81.0 ± 10.2	1.2 ± 0.3	0.0 ± 0.0	29.1 ± 1.3	38	60.7 ± 0.6
all rats	65.7 ± 7.1	64.5 ± 7.0	1.1 ± 0.2	0.1 ± 0.1	28.4 ± 1.0	57	61.0 ± 0.8
USV emitted to 22-kHz USV-playback (0–120 s time-intervals)							
0-Trial	13.5 ± 3.6 ###	12.9 ± 3.6 ###	0.6 ± 0.3	0.0 ± 0.0	21.9 ± 2.1		58.1 ± 1.9
1-Trial	14.5 ± 5.4 ##	13.4 ± 5.3 ##	1.1 ± 0.6	0.0 ± 0.0	20.2 ± 2.4 #		54.5 ± 3.9 #
6-Trial	32.2 ± 7.6 ###	31.9 ± 7.6 ###	0.3 ± 0.1	0.0 ± 0.0	29.4 ± 2.4		60.1 ± 0.7
10-Trial	30.9 ± 9.8 ##	29.9 ± 9.8 ##	1.1 ± 0.5	0.0 ± 0.0	23.8 ± 2.4 #		58.9 ± 1.8
all FC'ed	26.6 ± 4.7 ###	25.8 ± 4.7 ###	0.8 ± 0.3	0.0 ± 0.0	25.1 ± 1.5 ###		58.4 ± 1.1 ##
all rats	21.4 ± 3.2 ###	20.6 ± 3.2 ###	0.7 ± 0.2	0.0 ± 0.0	24.0 ± 1.2 ###		58.3 ± 1.0 ##
USV emitted to 50-kHz tone-playback (0–120 s time-intervals)							
0-Trial	41.4 ± 6.6	40.4 ± 6.5	1.0 ± 0.4	0.0 ± 0.0	27.0 ± 2.0	18	58.6 ± 0.9
1-Trial	49.3 ± 16.2	48.6 ± 16.1	0.8 ± 0.2	0.0 ± 0.0	23.8 ± 2.9	9	58.2 ± 1.8
6-Trial	76.0 ± 18.5	74.6 ± 18.4	1.4 ± 0.5	0.0 ± 0.0	32.8 ± 3.3	11	61.0 ± 0.6
10-Trial	76.3 ± 15.3	75.4 ± 15.2	0.9 ± 0.4	0.0 ± 0.0	31.0 ± 2.1	13	61.1 ± 0.5
all FC'ed	68.3 ± 9.7	67.3 ± 9.7	1.0 ± 0.2	0.0 ± 0.0	29.6 ± 1.7	33	60.3 ± 0.6
all rats	57.5 ± 6.5	56.5 ± 6.5	1.0 ± 0.2	0.0 ± 0.0	28.7 ± 1.3	51	59.7 ± 0.5
USV emitted to 22-kHz tone-playback (0–120 s time-intervals)							
0-Trial	6.6 ± 2.1 ###	6.1 ± 2.0 ###	0.5 ± 0.3	0.0 ± 0.0	18.2 ± 2.1 #		55.5 ± 2.0
1-Trial	16.0 ± 6.9 #	15.3 ± 6.9 #	0.7 ± 0.5	0.0 ± 0.0	28.5 ± 7.9		54.2 ± 3.0
6-Trial	18.1 ± 5.5 ###	17.8 ± 5.5 ###	0.4 ± 0.2	0.0 ± 0.0	28.7 ± 3.0		59.4 ± 0.6
10-Trial	23.3 ± 8.1 ###	23.2 ± 8.1 ###	0.1 ± 0.1 #	0.0 ± 0.0	25.1 ± 2.2 ##		58.3 ± 1.4
all FC'ed	19.3 ± 3.9 ###	18.9 ± 3.9 ###	0.4 ± 0.2 ##	0.0 ± 0.0	27.2 ± 2.5 ##		57.5 ± 1.0 #
all rats	14.2 ± 2.6 ###	13.8 ± 2.6 ###	0.4 ± 0.1 ##	0.0 ± 0.0	24.0 ± 1.8 ###		56.8 ± 1.0 ##
USV emitted to 50-kHz sound-playback (0–120 s time-intervals)							
0-Trial	41.3 ± 6.0	40.1 ± 5.9	1.0 ± 0.3	0.1 ± 0.1	27.7 ± 1.5	24	60.7 ± 1.1
1-Trial	54.2 ± 17.4	53.2 ± 17.2	1.0 ± 0.4	0.0 ± 0.0	24.0 ± 2.1	12	59.6 ± 1.2
6-Trial	81.2 ± 16.4	80.1 ± 16.3	1.1 ± 0.3	0.0 ± 0.0	29.8 ± 2.8	14	60.4 ± 0.6
10-Trial	86.9 ± 15.1	85.6 ± 15.0	1.3 ± 0.4	0.0 ± 0.0	29.2 ± 1.8	16	60.9 ± 0.7
all FC'ed	75.3 ± 9.4	74.2 ± 9.3	1.1 ± 0.2	0.0 ± 0.0	27.9 ± 1.3	42	60.4 ± 0.5
all rats	61.6 ± 6.3	60.5 ± 6.3	1.1 ± 0.2	0.1 ± 0.1	27.8 ± 1.0	66	60.5 ± 0.5
USV emitted to 22-kHz sound-playback (0–120 s time-intervals)							
0-Trial	10.1 ± 2.7 ###	9.5 ± 2.6 ###	0.6 ± 0.3	0.0 ± 0.0	18.4 ± 1.6 ##		58.3 ± 1.6
1-Trial	15.3 ± 4.7 #	14.3 ± 4.7 #	0.9 ± 0.4	0.0 ± 0.0	27.8 ± 6.8		51.9 ± 3.4 ##
6-Trial	25.2 ± 5.9 ###	24.8 ± 5.9 ###	0.3 ± 0.1 #	0.0 ± 0.0	28.3 ± 2.1		59.8 ± 0.6
10-Trial	27.1 ± 8.3 ###	26.6 ± 8.3 ###	0.6 ± 0.3	0.0 ± 0.0	23.2 ± 2.2 ##		58.0 ± 1.6 #
all FC'ed	23.0 ± 3.8 ###	22.4 ± 3.8 ###	0.6 ± 0.2 #	0.0 ± 0.0	26.1 ± 2.0 ##		57.1 ± 1.2 ###
all rats	17.8 ± 2.6 ###	17.2 ± 2.6 ###	0.6 ± 0.1 ##	0.0 ± 0.0	23.2 ± 1.5 ###		57.6 ± 0.9 ###

Table S12. Comparison of **number of USV of different types** and **selected characteristics of 50-kHz USV** emitted during playback sessions, i.e. during the 10-s-long playback and 110 s afterwards, **in response to 50- vs. 22-kHz playback** (USV, tone and sounds) in control (0-Trial, n = 37), fear-conditioned (1-Trial, n = 16; 6-Trial, n = 20; 10-Trial, n = 19; all fear-conditioned/FC'ed), and all rats. MPF – mean peak frequency; categories of USV: 50-kHz (MPF >32 kHz), short 22-kHz (MPF of 18–32 kHz, duration <0.3 s), long 22-kHz (18–32 kHz, >0.3 s); significance levels are given for corresponding 50- vs. 22-kHz sounds, i.e. # p < 0.05, ## p < 0.01, ### p < 0.001, all Wilcoxon test. Please note, duration and MPF of 50-kHz USV were compared only in rats which emitted USV in response to both 50- and 22-kHz playback, n of animals are given. A single USV was eliminated as a long outlier (with > 6 x SD). USV emitted in reaction to 22-kHz playbacks were less numerous, shorter and of lower MPF.

groups	number of USV				parameters of 50-kHz USV		
USV	total USV	50-kHz USV	short 22-kHz USV	long 22-kHz USV	duration [ms]	n	MPF [kHz]
USV emitted to 50-kHz USV-playback (0–120 s time-intervals)							
0-Trial	41.2 ± 7.1	39.9 ± 6.9	0.9 ± 0.2	0.3 ± 0.3	26.6 ± 1.6	30	60.6 ± 1.6
1-Trial	59.1 ± 19.5	57.8 ± 19.3	1.3 ± 0.6	0.0 ± 0.0	24.8 ± 2.2	15	60.4 ± 1.1
6-Trial	86.4 ± 16.5	85.7 ± 16.5	0.8 ± 0.3	0.0 ± 0.0	29.5 ± 2.4	15	61.2 ± 0.6
10-Trial	97.5 ± 17.5	95.8 ± 17.2	1.7 ± 0.7	0.0 ± 0.0	30.6 ± 1.8	19	60.9 ± 0.7
all FC'ed	82.3 ± 10.3	81.0 ± 10.2	1.2 ± 0.3	0.0 ± 0.0	28.5 ± 1.2	49	60.8 ± 0.5
all rats	65.7 ± 7.1	64.5 ± 7.0	1.1 ± 0.2	0.1 ± 0.1	27.8 ± 1.0	79	60.7 ± 0.7
USV emitted to 50-kHz tone-playback (0–120 s time-intervals)							
0-Trial	41.4 ± 6.6	40.4 ± 6.5	1.0 ± 0.4	0.0 ± 0.0	27.5 ± 1.4		58.8 ± 0.8*
1-Trial	49.3 ± 16.2	48.6 ± 16.1	0.8 ± 0.2	0.0 ± 0.0	25.4 ± 2.2		60.6 ± 1.4
6-Trial	76.0 ± 18.5	74.6 ± 18.4	1.4 ± 0.5	0.0 ± 0.0	30.1 ± 2.9		60.1 ± 1.1
10-Trial	76.3 ± 15.3	75.4 ± 15.2	0.9 ± 0.4	0.0 ± 0.0	31.7 ± 2.2		60.6 ± 0.6
all FC'ed	68.3 ± 9.7*	67.3 ± 9.7*	1.0 ± 0.2	0.0 ± 0.0	29.3 ± 1.4		60.5 ± 0.6
all rats	57.5 ± 6.5	56.5 ± 6.5	1.0 ± 0.2	0.0 ± 0.0	28.6 ± 1.0		59.8 ± 0.5*
USV emitted to 22-kHz USV-playback (0–120 s time-intervals)							
0-Trial	13.5 ± 3.6	12.9 ± 3.6	0.6 ± 0.3	0.0 ± 0.0	23.5 ± 2.2	16	56.9 ± 1.9
1-Trial	14.6 ± 5.4	13.4 ± 5.3	1.1 ± 0.6	0.0 ± 0.0	18.8 ± 3.1	8	57.5 ± 2.2
6-Trial	32.2 ± 7.6	31.9 ± 7.6	0.3 ± 0.1	0.0 ± 0.0	31.6 ± 2.7	11	60.1 ± 0.7
10-Trial	30.9 ± 9.8	29.9 ± 9.8	1.1 ± 0.5	0.0 ± 0.0	25.7 ± 2.4	12	61.0 ± 0.6
all FC'ed	26.6 ± 4.7	25.8 ± 4.7	0.8 ± 0.3	0.0 ± 0.0	26.0 ± 1.7	31	59.8 ± 0.7
all rats	21.4 ± 3.2	20.6 ± 3.2	0.7 ± 0.2	0.0 ± 0.0	25.2 ± 1.4	47	58.8 ± 0.8
USV emitted to 22-kHz tone-playback (0–120 s time-intervals)							
0-Trial	6.6 ± 2.1**	6.1 ± 2.0**	0.5 ± 0.3	0.0 ± 0.0	19.3 ± 2.2		54.0 ± 2.1*
1-Trial	16.0 ± 6.9	15.3 ± 6.9	0.7 ± 0.5	0.0 ± 0.0	19.7 ± 3.7		58.3 ± 1.8
6-Trial	18.1 ± 5.5	17.8 ± 5.5*	0.4 ± 0.2	0.0 ± 0.0	28.7 ± 3.0		59.4 ± 0.6
10-Trial	23.3 ± 8.1	23.2 ± 8.1	0.1 ± 0.1	0.0 ± 0.0	26.3 ± 2.0		57.9 ± 1.5
all FC'ed	19.3 ± 3.9	18.9 ± 3.9	0.4 ± 0.2	0.0 ± 0.0	25.4 ± 1.7		58.5 ± 0.8
all rats	14.2 ± 2.6**	13.8 ± 2.6**	0.4 ± 0.1	0.0 ± 0.0	23.3 ± 1.4		57.0 ± 0.9

Table S13. Comparison of **number of USV of different types** and **selected characteristics of 50-kHz USV** emitted during playback sessions, i.e. during the 10-s-long playback and 110 s afterwards, **in response to USV- vs. tone-playback** in control (0-Trial, n = 37), fear-conditioned (1-Trial, n = 16; 6-Trial, n = 20; 10-Trial, n = 19; all fear-conditioned, all FC'ed), and all rats. MPF – mean peak frequency; three categories of USV are: 50-kHz USV (MPF >32 kHz), short 22-kHz (MPF of 18–32 kHz, duration <0.3 s), long 22-kHz (MPF of 18–32 kHz, duration >0.3 s); significance levels are given for corresponding USV- vs. tone-playbacks, i.e. * p < 0.05, ** p < 0.01, all Wilcoxon test. Please note, the duration and MPF of 50-kHz USV were compared only in rats which emitted USV in response to both USV- and tone-playback, and relevant n of animals are given. USV emitted in reaction to tone playbacks turned out to be less numerous and of lower MPF.

Differences in time spent in speaker's half following USV- vs. tone-playbacks										
group:	0-Trial	1-Trial	6-Trial	10-Trial	all rats	0-Trial	1-Trial	6-Trial	10-Trial	all rats
# of rats	n = 37	n = 16	n = 20	n = 19	n = 92	n = 37	n = 16	n = 20	n = 19	n = 92
time-interval [s]	50-kHz playback; USV vs tone					22-kHz playback; USV vs. tone				
0 s (playback)	0.7275	0.5936	0.0949	0.4631	0.9258	0.9757	0.0926	0.7782	0.9250	0.4763
10 s to 30 s	0.1005	0.1386	0.2787	0.0342	0.0020	0.2418	0.1141	0.3343	0.0528	0.1779
10 s to 60 s	0.0670	0.0231	0.1701	0.0627	0.0004	0.1215	0.6949	0.1701	0.0692	0.0236
-120 s to -100 s	0.3505	0.4900	0.0555	0.6791	0.7790	0.7704	0.2721	0.4777	.0309*	0.8076
-30 s to -10 s	0.2329	0.1730	0.1981	0.8971	0.0593	0.8824	0.6566	0.7939	0.6496	0.8710

Table S14. Differences in time spent in the speaker's half in response to USV- vs. tone-playbacks evaluated during playback (0 s time-interval), after playback (10 s to 30 s, 10 s to 60 s), as well as during the control intervals (from -120 s to -100 s, -30 s to -10 s), in control rats (0-Trial), and rats which underwent fear-conditioning with different number of shocks (1-Trial, 6-Trial, 10-Trial) as well as all the rats analyzed together, all Wilcoxon test, with $p < 0.05$ values in bold. Please note, USV-playback evoked more pronounced approach of the speaker, e.g. in "all rats" group; * – not significant when two outlying rats were included.