Supplemental information
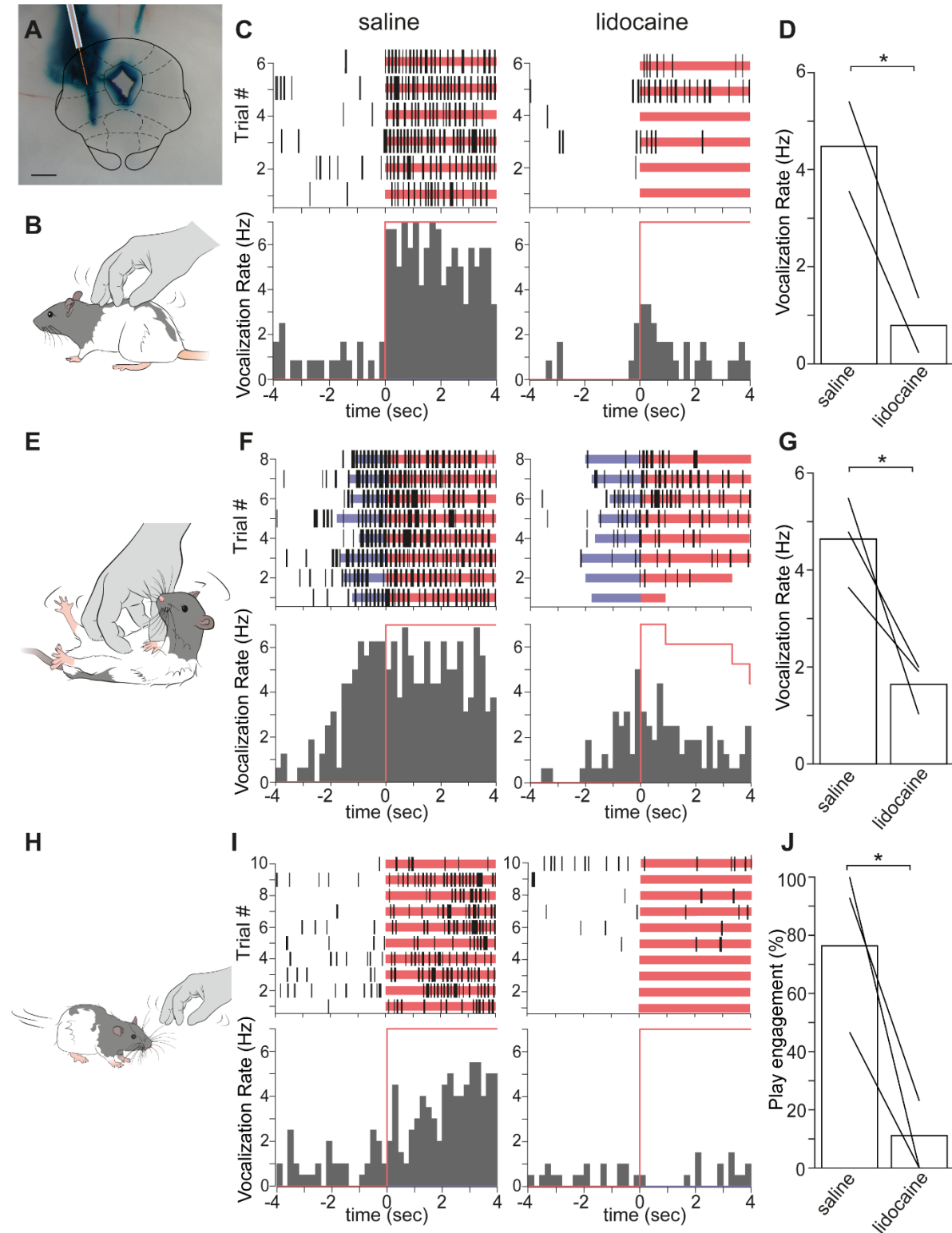
Play and tickling responses map to the lateral columns of the rat periaqueductal gray

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Supplementary Figure 1. Lidocaine injection into PAG disrupts ticklishness and playfulness

Supplementary Figure 1. Lidocaine injection into PAG disrupts ticklishness and playfulness, related to Figure 1

(A) Image of guiding cannula tract with DiR visualizing injection site. Scale bar: 0.5 mm.

(B) Schematic of dorsal tickling.

(C) Experiment showing USV raster plot (top) and histogram (bottom) of USV response to dorsal tickling after saline and lidocaine injection. Red bars represent behavior of interest. USVs shown in black marks. Time point 0 indicates onset of behavior. Plots show a 4 second baseline and the first 4 seconds after behavior onset. Note that the behaviors can be of variable lengths and typically lasted longer than 4 seconds. Left: USV raster plot and corresponding histogram in control condition. Right: USV raster plot and corresponding histogram of dorsal tickling after lidocaine injection. Histogram bin size: 200 ms.

(D) Mean USV rate during dorsal tickling after saline and lidocaine injection ($p = 0.03$, paired t-test).

(E) Schematic of ventral tickling.

(F) Raster plot and histogram of USVs during ventral tickling trials after saline (left) and lidocaine (right) injection. Blue bars in raster plot indicate the flip of the animal before ventral tickling.

(G) Mean USV rate during ventral tickling after saline and lidocaine injection ($p = 0.032$, paired t-test).
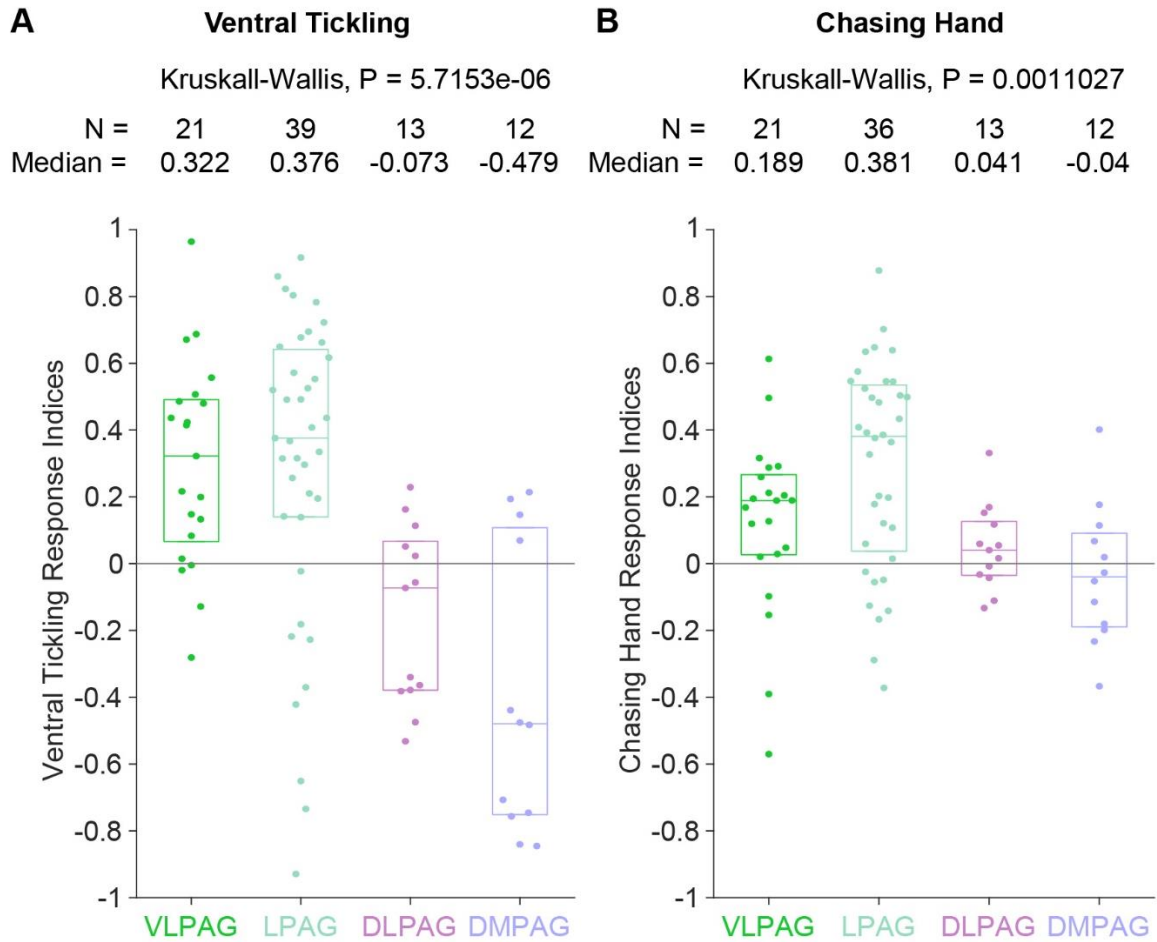
(H) Schematic of chasing hand.

(I) Raster plot and histogram of USVs during chasing hand trials after saline (left) and lidocaine (right) injection.

(J) Mean play engagement during chasing hand after saline and lidocaine injection ($p = 0.02$, paired t-test).

Cluster / Column association	p values	cumulative p-values and significance	
Play / tickling ++ in LPAG	0.00011	0.00011	*
Play 0 / tickling -- in DMPAG	0.0013	0.0014	*
Play / tickling 0 in LPAG	0.0026	0.004	*
Play 0 / tickling -- in VLPAG	0.0046	0.0086	*
Play / tickling 0 in DLPAG	0.007	0.016	*
Play / tickling + in VLPAG	0.0099	0.025	*
Play / tickling ++ in DLPAG	0.064	0.089	ns
Play / tickling ++ in DMPAG	0.064	0.15	ns
Play / tickling + in DLPAG	0.17	0.32	ns
Play / tickling + in DMPAG	0.17	0.5	ns
Play 0 / tickling -- in DLPAG	0.18	0.67	ns
Play 0 / tickling -- in LPAG	0.33	1.0013	ns
Play / tickling 0 in VLPAG	0.33	1.33	ns
Play / tickling ++ in VLPAG	0.34	1.67	ns
Play - / tickling + in DLPAG	0.35	2.013	ns
Play - / tickling + in VLPAG	0.41	2.42	ns
Play - / tickling + in LPAG	0.72	3.15	ns
Play / tickling 0 in DMPAG	1	4.15	ns
Play / tickling + in LPAG	1	5.15	ns
Play - / tickling + in DMPAG	1	6.15	ns

Supplementary table 1. Series of Fisher's exact tests assessing the nonrandom distribution of specific clusters into specific PAG columns, related to Figure 4
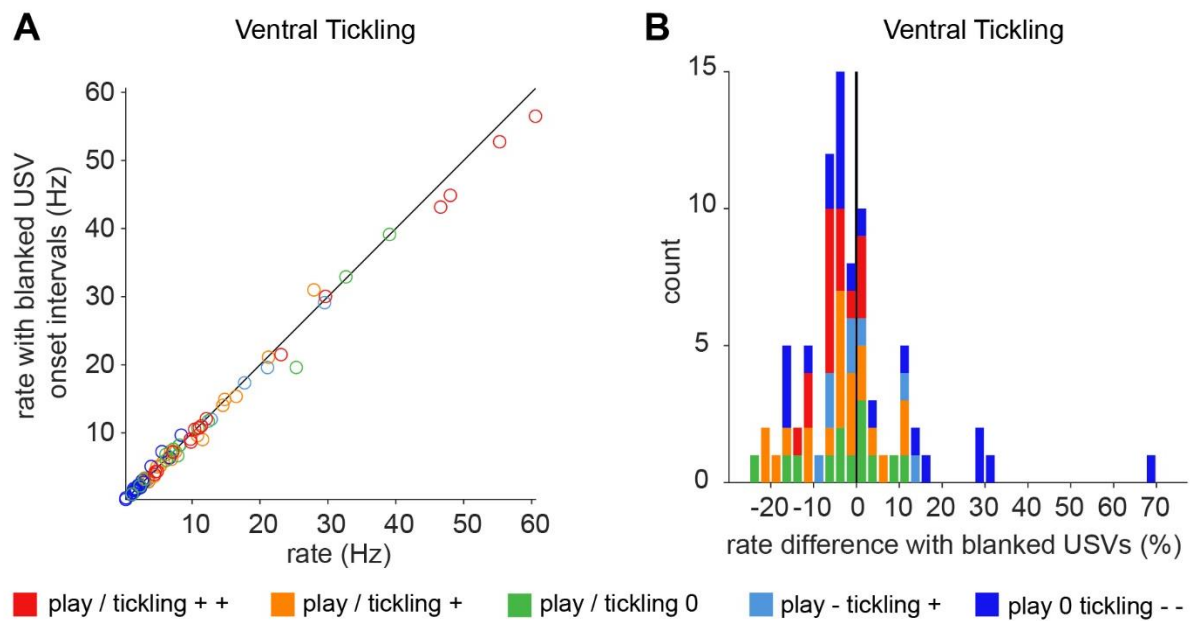We generated a 2x2 contingency table, where the tested cluster was isolated and opposed to all the other combined and the tested column was isolated and opposed to all the other combined. A Fisher's exact test was then performed and the p-value stored. Every possible combination was tested, and eventually 20 Fisher's exact tests were performed. This table shows the tests results performed on the different combination (column 1) ranked by p-values (column 2). A cumulative p-value was calculated, and the value had to remain below 0.05 to be significant. This method merely approximates the distribution of clusters into the PAG columns and allowed us to identify the 6 most significant non-random associations.

Supplementary Figure 2. Responses to ventral tickling and play (chasing the experimenter's hand) in different periaqueductal gray columns, related to Figure 4

(A) Response indices (RI) for neuronal response to ventral tickling in different periaqueductal gray columns; each dot represents one neuron.

(B) Response indices (RI) for play (chasing hand) responses; convention as in (A).
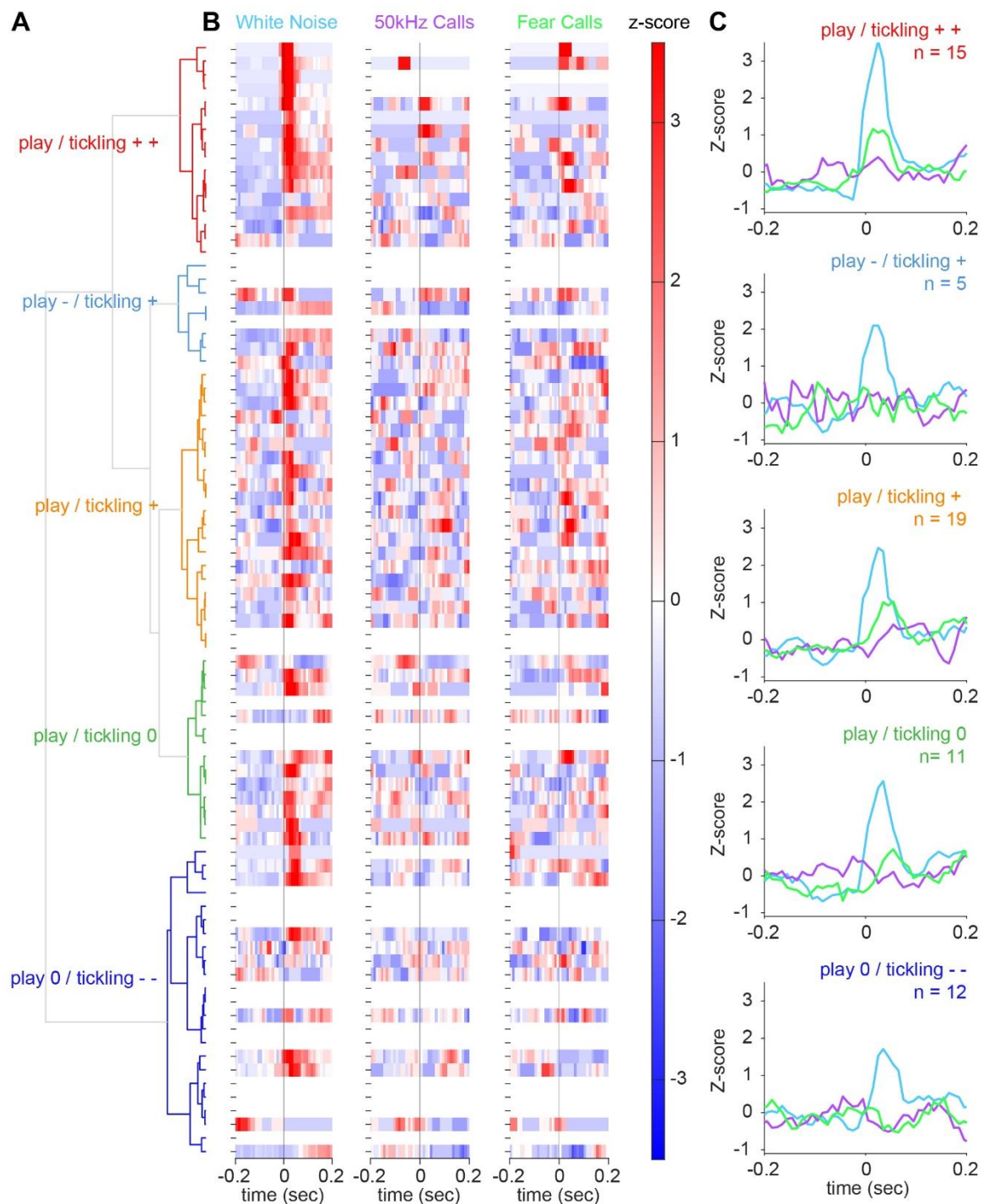
A Ventral Tickling

B Ventral Tickling

Legend:
- play / tickling + + (red)
- play / tickling + (orange)
- play / tickling 0 (green)
- play - tickling + (light blue)
- play 0 tickling - - (blue)

Supplementary Figure 3. Responses to ventral tickling are not merely a byproduct of ultrasonic vocalization responses, related to Figure 6

(A) Firing rate during ventral tickling with blanked peri-USV onset intervals as a function of firing rate during ventral tickling.

Rate with blanked peri-USV onset intervals: interval windows from 50 ms before and 50 ms after the onset of each USVs were discarded to calculate a firing rate "independent" from the USV generation. Each dot represents one neuron, the color scheme (legend at the bottom) refers to the response clusters defined in Figure 4 and the unity line is indicated in black. Response rates are very similar with and without inclusion of peri-USV intervals suggesting that tickling responses are not simply due to USV responses in these neurons.

(B) A histogram of rate differences imposed by excluding peri-USV intervals in the firing rate quantification. The firing rate changes imposed by excluding peri-USV intervals are minor.

Supplementary Figure 4. Play/tickling excited periaqueductal gray cells show strong responses to auditory stimuli, related to Figure 6

(A) Hierarchical cluster tree (dendrogram) obtained weighing chasing hand and ventral tickling responses. Five clusters were distinguished and named according to their activity patterns (see Figure 4 for details).

(B) Color-coded responses to auditory playback for each neuron.

(C) Line-drawing of average z-scored responses to playback in each cluster. White noise stimuli evoked responses in many periaqueductal gray neurons. Note that auditory responses were strongest in the strongly play/tickling excited cluster.