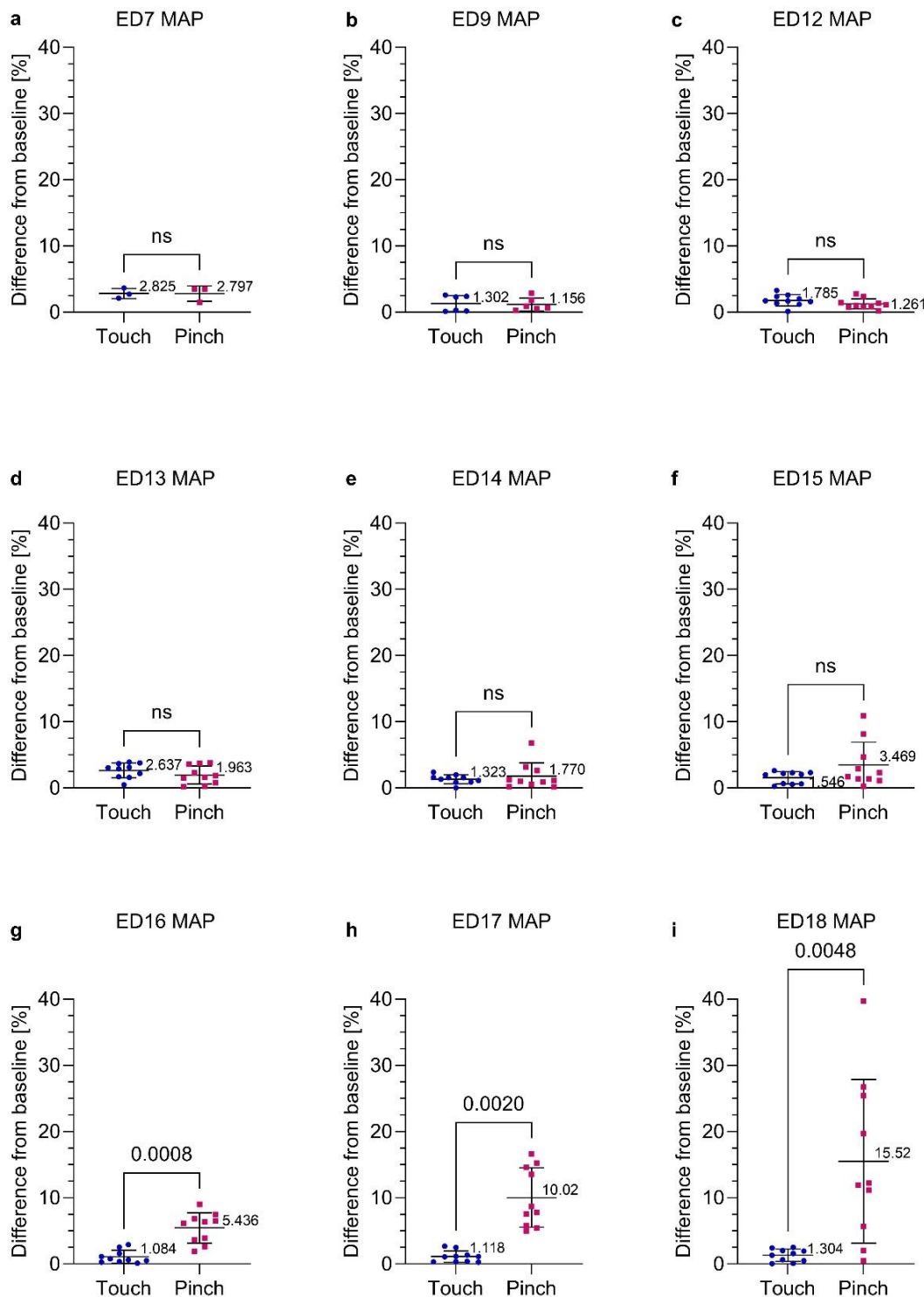
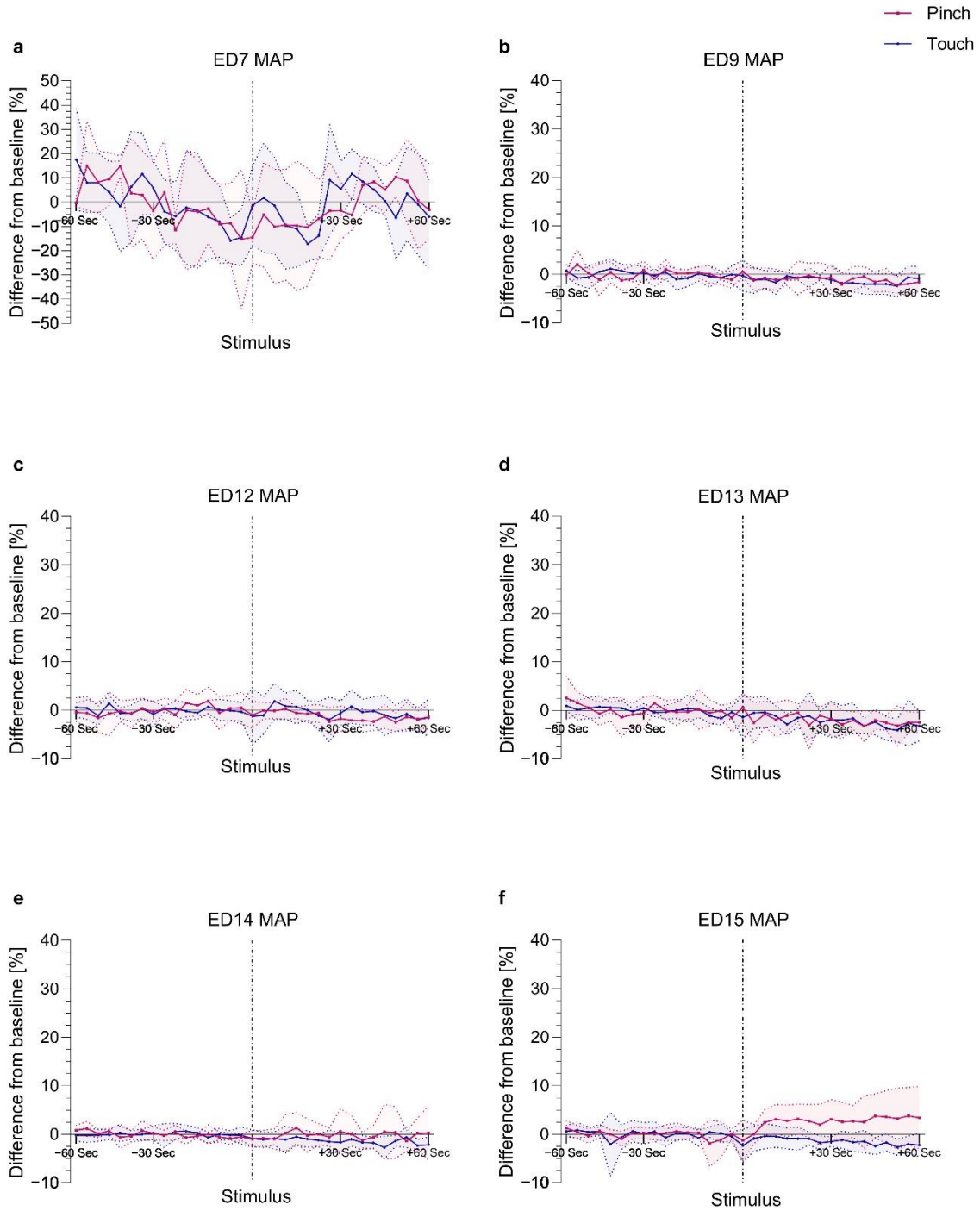


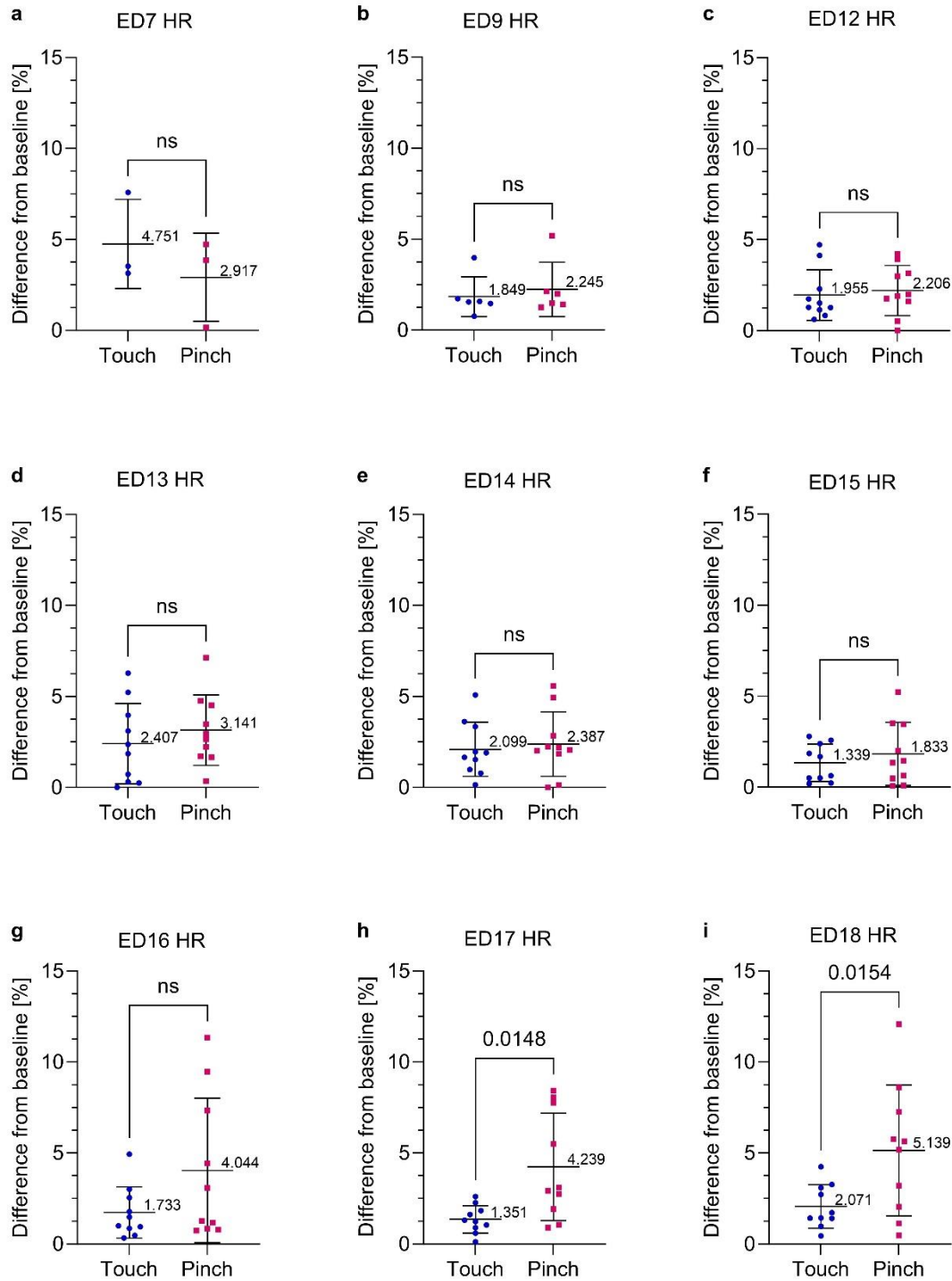
# Supplementary information



**Figure S1. Percent change in MAP post Touch and Pinch.** a-i Embryos at embryonic day (ED) 7 (n=3), ED9 (n=6) and EDs 12 to 18 (n=10) received a mechanical noxious stimulus (*Pinch*) and a light touch as control (*Touch*) at the base of the beak in randomized order. Displayed as the mean ± standard deviation. Paired t-test (normally distributed: c, d, g and i) or Wilcoxon signed-rank test (not normally distributed: a, b, e, f and h). Mean and p values shown; g:  $p=0.0008$ , h:  $p=0.0020$  and i:  $p=0.0048$ ; ns = no significant difference between the groups.

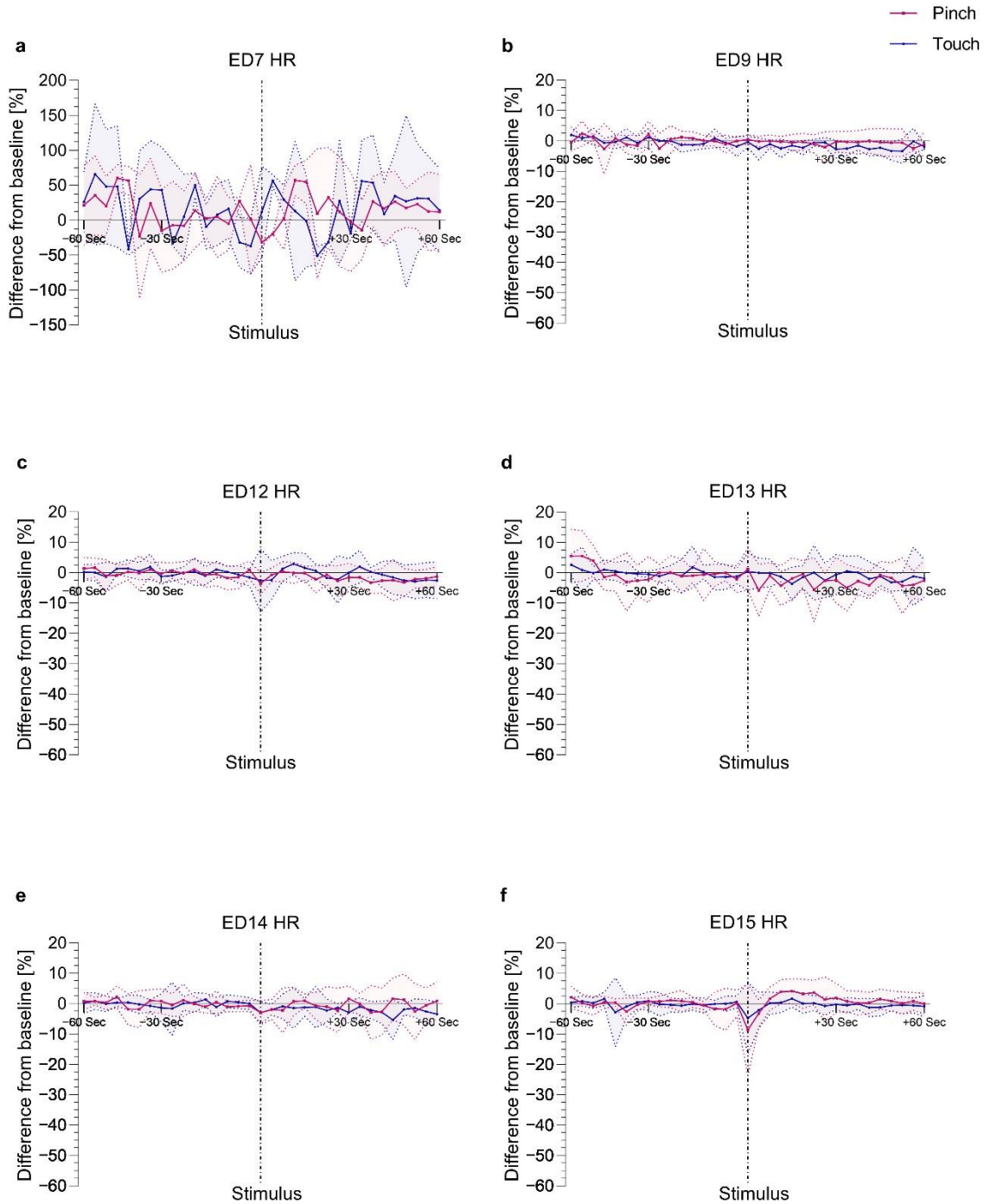


**Figure S2. Percent change from the baseline mean value in MAP over time.** a-f Embryos at ED7 (n=3), ED9 (n=6) and EDs 12 to 15 (n=10) received a mechanical noxious stimulus (*Pinch*) and a light touch as control (*Touch*) at the base of the beak in randomized order. Values were recorded every four seconds for one minute before and after stimulation (*Touch* and *Pinch*). Values are shown as the mean  $\pm$  standard deviation (shaded).



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14 **Figure S3. Percent change in HR post Touch and Pinch.** a-i Embryos at ED7 (n=3), ED9 (n=6) and EDs 12 to 18  
 15 (n=10) received a mechanical noxious stimulus (*Pinch*) and a light touch as control (*Touch*) at the base of the beak in  
 16 randomized order. Displayed as the mean  $\pm$  standard deviation. Paired t-test (normally distributed: a, d-f, h and i)  
 17 or Wilcoxon signed-rank test (not normally distributed: b, c and g). Mean and *p* values shown; h: *p*=0.0148 and i:  
 18 *p*=0.0154; ns = no significant difference between the groups.



**Figure S4. Percent change from the baseline mean value in HR over time.** a-f Embryos at ED7 (n=3), ED9 (n=6) and EDs 12 to 15 (n=10) received a mechanical noxious stimulus (*Pinch*) and a light touch as control (*Touch*) at the base of the beak in randomized order. Values were recorded every four seconds for one minute before and after stimulation (*Touch* and *Pinch*). Values are shown as the mean  $\pm$  standard deviation (shaded).