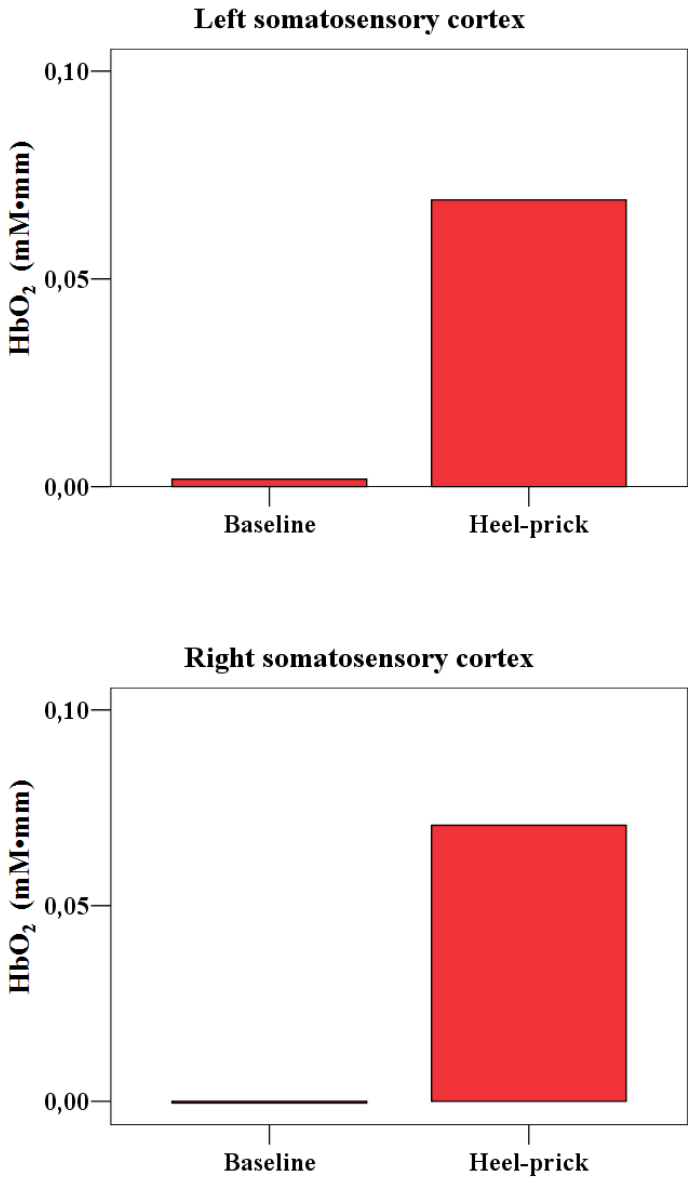
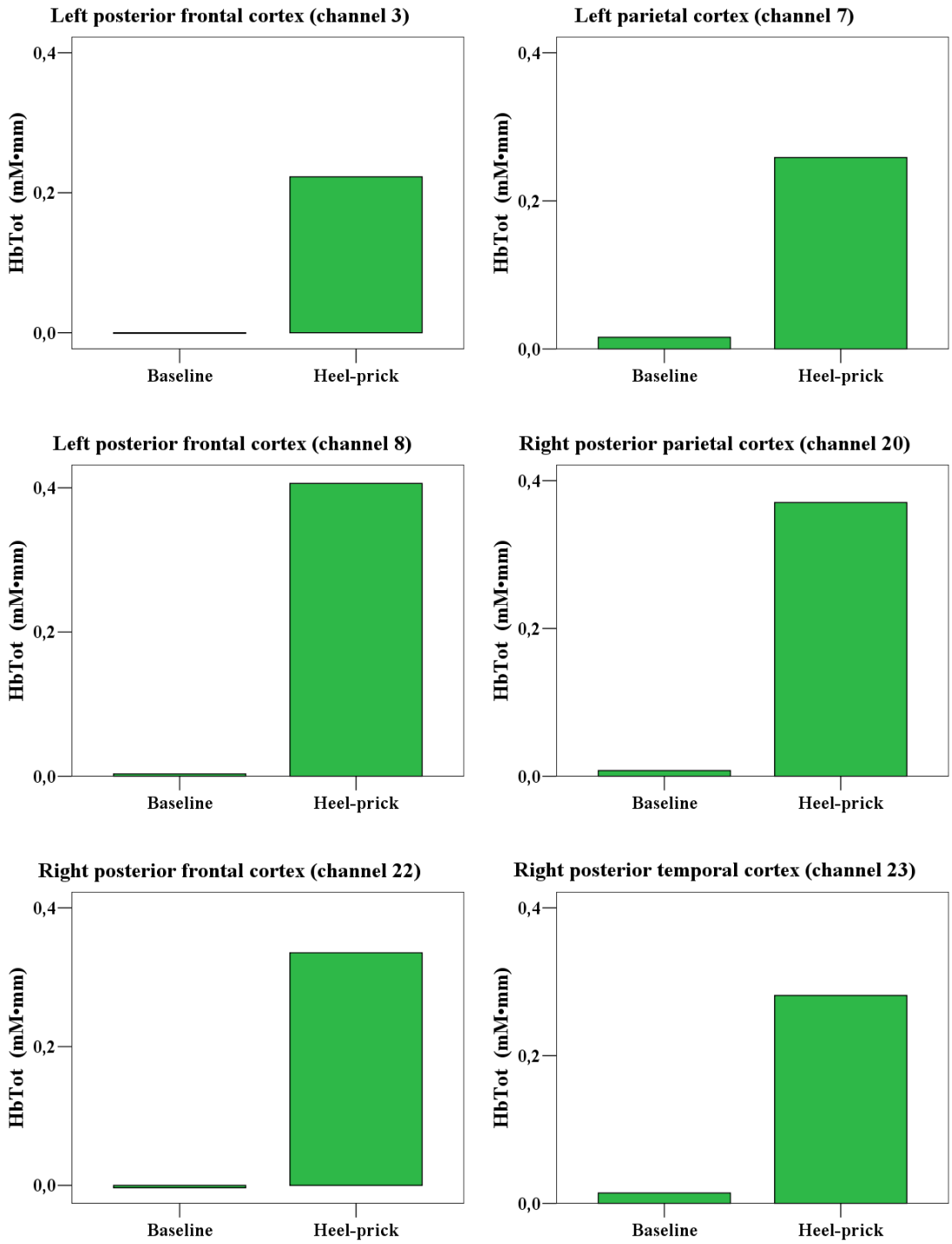
Supplemental Figure 1



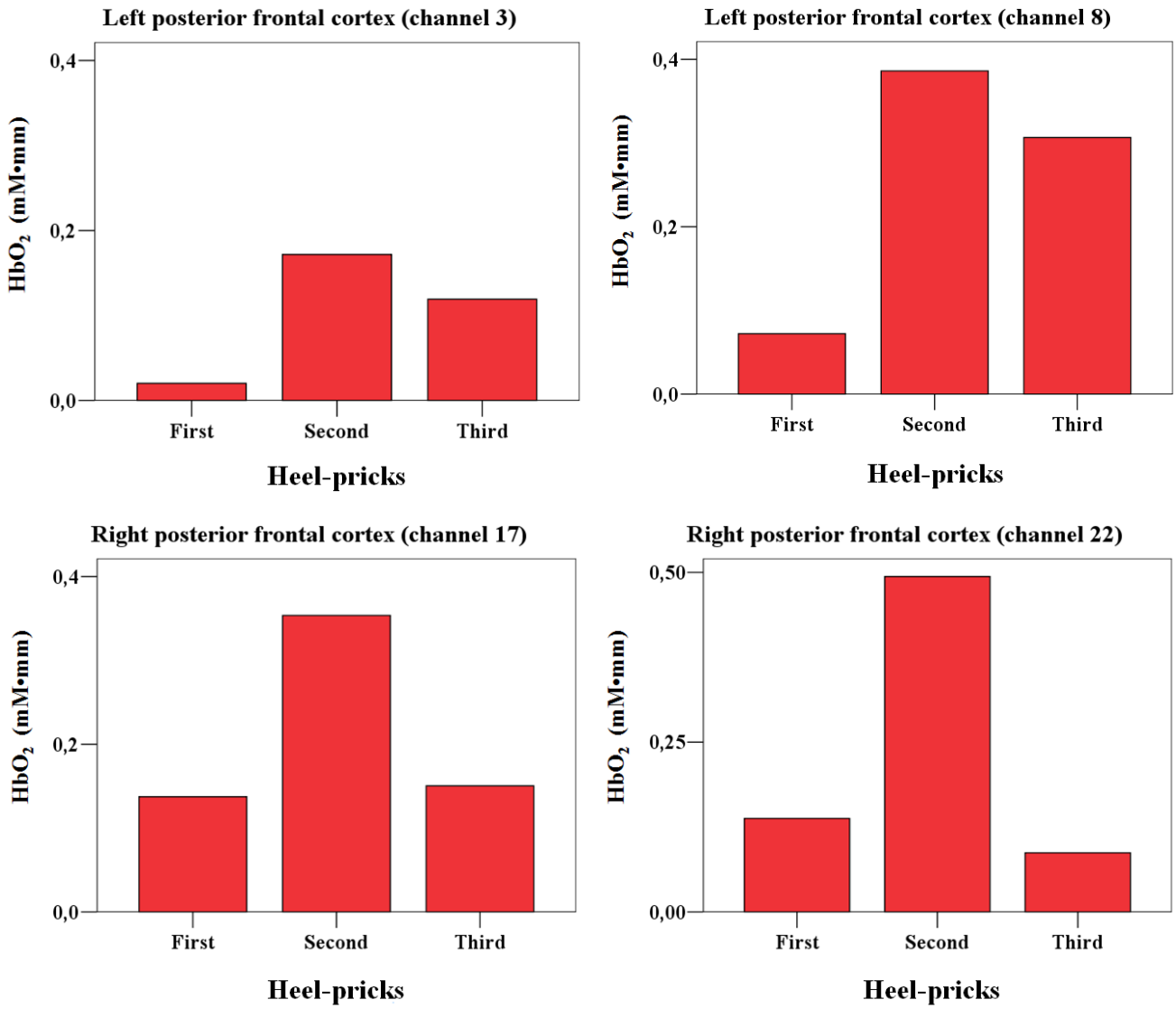
We did not find significant activation in any channel over the somatosensory cortex, unlike most previous NIRS research on cortical response to nociception in neonates [e.g., 5,40]. Consequently, we further analyzed our data. Specifically, we averaged oxy-haemoglobin (HbO_2) mean concentration detected, during baseline and heel-prick, in channels placed over left (4,5,6,7,9,10) and right (15,16,18,19,20,21) somatosensory regions, which are located posteriorly to the frontal channels we found activated (3,8,17,22). Then, by paired t test, HbO_2 mean concentration detected during baseline and heel-prick were compared, separately for the left and right somatosensory cortex. Both left and right somatosensory cortex showed a significant activation, associated with the noxious stimulation. HbO_2 variation is reported as millimolar per millimeter (mM•mm) of the optical path length.

Supplemental Figure 2



When total haemoglobin (HbTot) mean concentrations during baseline and heel-prick were compared by *t* test, six channels resulted significantly activated (p < FDR 0.05). They were located on left posterior frontal cortex [channel 3 ($t_{(63)}$ = -3.178; p = 0.001), channel 8 ($t_{(63)}$ = -3.490; p = 0.0005)], left parietal cortex [channel 7 ($t_{(61)}$ = -2.794; p = 0.0035)], right posterior parietal cortex [channel 20 ($t_{(58)}$ = -3.194; p = 0.0015)], right posterior frontal cortex [channel 22 ($t_{(61)}$ = -3.112; p = 0.0015)] and right posterior temporal cortex [channel 23 ($t_{(62)}$ = -3.991; p = 0.0005)]. Analysis on deoxy-haemoglobin showed no significant result. HbTot variation is reported as millimolar per millimeter (mM•mm) of the optical path length.

Supplemental Figure 3



Within-subjects comparisons were performed on oxy-haemoglobin (HbO_2) mean concentration during heel-prick, during NICU stay. Since all participants had at least three measurements, we arbitrarily decided to include, in such analysis, the first, second and third monitored heel-pricks. Only channels showing a cortical activation were considered. No significant results were observed (channel 3: $F_{(2,30)} = 0.642$, $p = 0.53$; channel 8: $F_{(2,28)} = 1.833$, $p = 0.18$; channel 17: $F_{(2,30)} = 0.992$, $p = 0.38$; channel 22: $F_{(2,26)} = 2.289$, $p = 0.11$). The time span of the three measurements was rather wide (e.g., between 29 and 31 PMA weeks in one infant and between 32 and 34 weeks in another infant). The possible influence of maturation could not be taken into account by a within subjects analysis. Moreover, participants differed in the number of heel-pricks experienced between the three tests analyzed. Although similar trends emerge in all channels (first HbO_2 increases, then decreases), multiple regression analysis may be a more appropriate statistics for our study design. HbO_2 is reported as millimolar per millimeter (mM•mm) of the optical path length.