Supplementary materials for Derbyshire et al.

Although we believe that the analyses provided in the main paper answer our primary questions satisfactorily, other data categorizations can yield additional insights. In this supplementary document, we examine the results when the data are 1) analyzed using a standard ANOVA instead of a linear mixed effects model (LMEM), 2) matched by trial length instead of step duration, 3) replotted to show only the matched timepoints in the primary analyses, 4) aligned across Step Type by matching steps that follow temperature increases, or 5) split by temperature group (Max44 and Max46, both for all timepoints within each step and for just the final timepoint of each step). The visualizations and analytical approaches are otherwise similar to those in the main document. All p-values are uncorrected for these supplementary explorations.

Alternative analysis: Standard ANOVA

For ordinal variables such as Step Number, one has to decide about treating the variable as either categorical or continuous. We chose the latter because we expected and wished to assess trends in pain ratings across temperature steps. Interactions between Step Number and other factors were also readily interpretable with this model specification.

Nevertheless, we conducted an alternative analysis for which Step Number was treated as categorical. Doing so led to far too many predictors in our LMEMs given the number of data points; indeed, the modified LMEMs often failed to converge. We therefore set up a standard ANOVA with pain ratings averaged for each step. The results were comparable to those from the LMEM. The only difference was a significant 3-way interaction instead of a significant Type x Duration interaction.

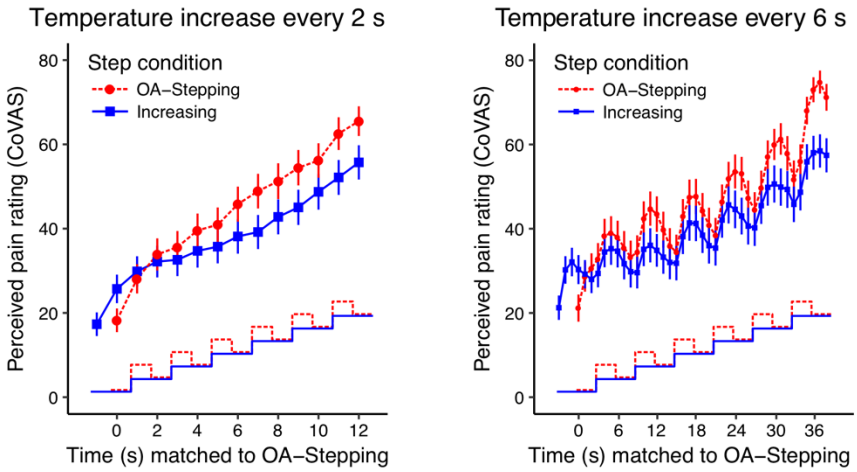
Supplementary Table 1. Results from ANOVA-based alternative to the main analyses of Step Type, Step Number, and Step Duration on CoVAS ratings. Greenhouse-Geiser correction has been applied to p-values where appropriate. Statistically significant effects are indicated in **bold**.

Effect	dfs	*F(dfs)*	*p*
Step Type	1, 47	1.56	.22
Step Number	**6, 282**	**200.61**	**<.001**
Step Duration	**2, 94**	**4.49**	**.017**
Type x Number	**6, 282**	**13.77**	**<.001**
Type x Duration	2, 94	1.52	.22
Number x Duration	**12, 564**	**5.72**	**<.001**
Type x Num x Dur	**12, 564**	**3.03**	**.013**

Alternative matching: Trial length

Instead of matching conditions by step duration, one can match them by the time between successive temperature increases. To do so, we aligned the Medium steps from the OA-Stepping condition to the Long steps from the Increasing condition. Note that OA-Stepping and Increasing trials were already aligned in this way for the Short steps condition (Figures 1 and 2).

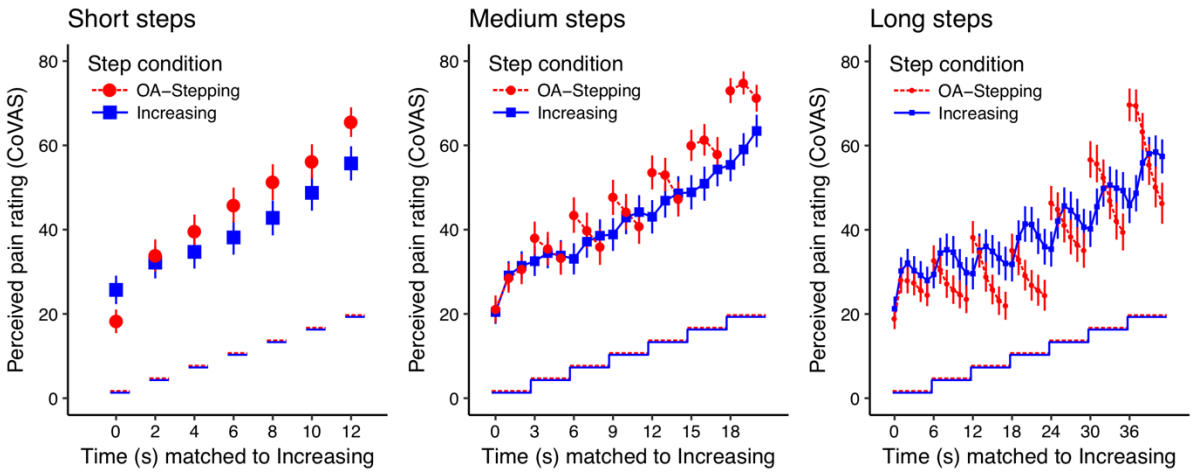
This alternative matching supports our primary conclusion that the OA-Stepping condition does not allow one to reach high temperatures without experiencing pain (Supplementary Figure 1). The replotted results also show CoVAS falling effects following each temperature increase. These effects are likely due to habituation in the Increasing condition, whereas they represent a mixture of habituation, decreased presented temperature, and offset analgesia in the OA-Stepping condition. Any offset analgesia effects, however, may be difficult to induce or measure due to the 3s step duration.



Supplementary Figure 1. Reported pain ratings (CoVAS) and associated temperatures matched by trial length. The trains of stimuli were aligned based on matched temperatures in the Increasing condition and the OA-Stepping condition following each 1 ^{0}C decrease as before, but now Long steps from the Increasing condition were compared to Medium steps from the OA-Stepping condition. Short steps were already aligned in this way, so the left panel is identical to the leftmost panel in Figure 2. Error bars represent standard errors of the mean (SEMs).

Alternative plotting: Matched timepoints only

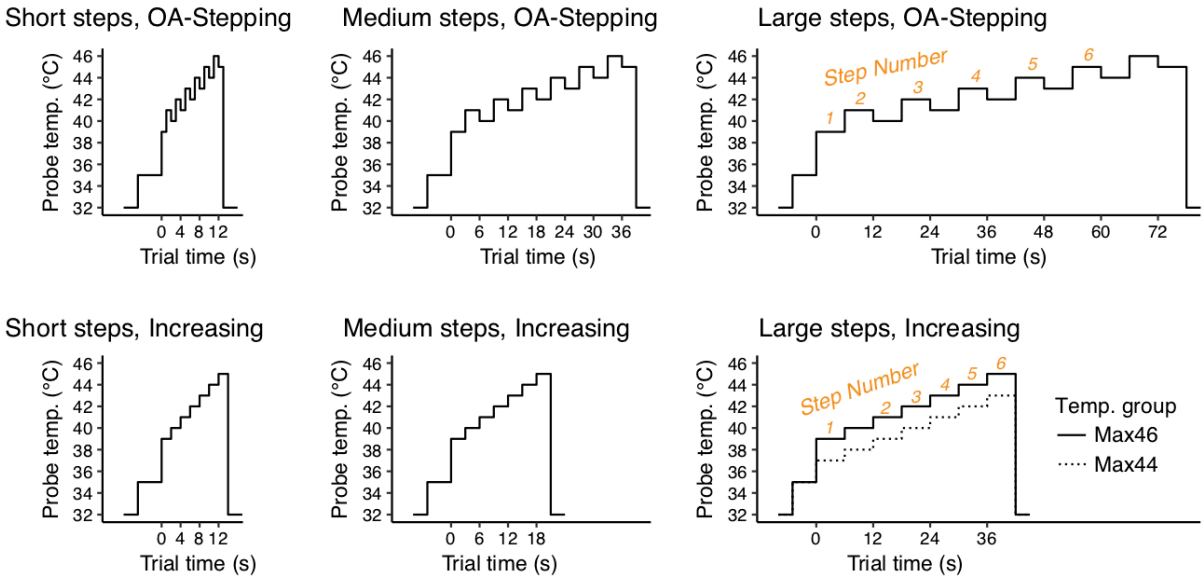
Figure 2 of the main manuscript showed all CoVAS rating data points across the six Step Duration x Step Type combinations. In that plot, the x-axis showed the time for the OA-Stepping conditions. Supplementary Figure 2 below shows only the mached data points, with the x-axis representing the Increasing condition time.



Supplementary Figure 2. Reported pain ratings (CoVAS) and associated temperature steps for each Step Duration and Step Type across participants. Stimuli within each trial were temporally aligned based on matched Step Numbers in the Increasing condition and the OA-Stepping condition following each 1 ^{0}C decrease. Only these matched temperatures and times within each trial are shown. The lack of connecting lines between datapoints indicate where unmatched data have been omitted. Error bars represent standard errors of the mean (SEMs).

Alternative step matching: Stepping up to the same temperature

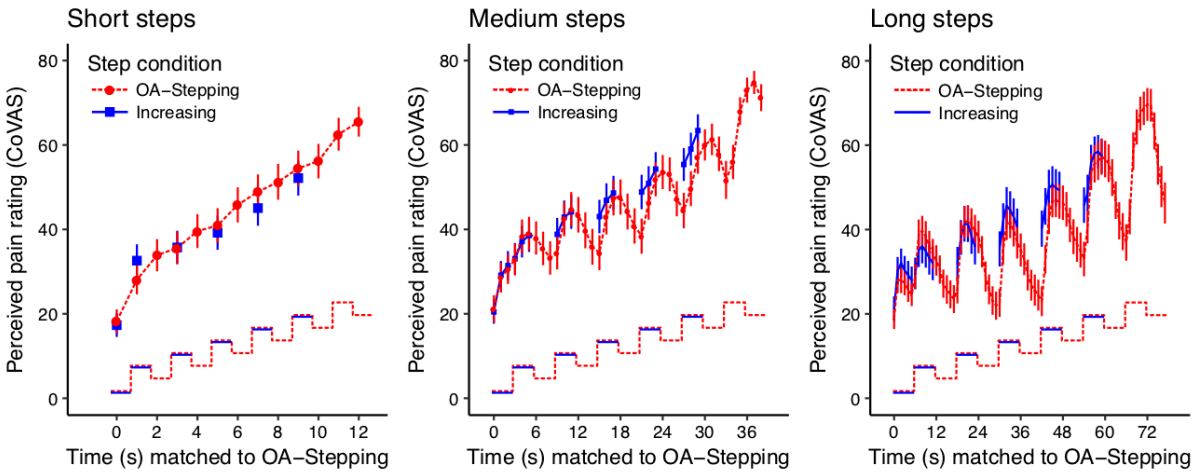
For our primary analyses, seven temperature steps across the Increasing and OA-Stepping conditions (Step Type) were aligned based on each 1 ^{0}C decrease in the OA-Stepping condition. An alternative is to match steps so that each step is preceded by a temperature increase, yielding six matched steps (Supplementary Figure 3). This step numbering approach is less sensitive to each step's individual offset effects and CoVAS rating delays. We were therefore able to test whether CoVAS ratings generally tracked presented temperatures in a similar way across Step Type conditions. As before, each step was matched for duration and temperature across Step Type, and within each Step Duration condition (Short, Medium, and Long) and Temperature Group (Max44 and Max46). Data across temperature groups were then aggregated based on Step Number.



Supplementary Figure 3. Temperature delivery during each trial type. Step Type OA-Stepping is shown in the top row, with Step Type Rise in the bottom row. Step Duration conditions are shown across columns. The alternative Step Numbering scheme is indicated in the Large steps panels, with the relevant text highlighted in gold. The bottom-right panel also represents the temperatures for the Max46 and Max44 groups. For clarity, other panels show only the Max46 group's temperatures.

As expected, CoVAS ratings for each Step Duration and Type largely tracked the presented temperature (Supplementary Figure 4). Inferential tests revealed the only main

effect of Step Number, though there were also significant interactions involving Step Duration (Supplementary Table 2). As before, these interactions were investigated by considering the Step Duration conditions separately, including the slope coefficients for Step Number (Supplementary Table 3). These slopes were generally lower in the OA-Stepping condition for Medium and Long steps. Such values explain the pattern of interactions and possibly reflect a lingering confound in the alternative step matching approach: The OA-Stepping condition increases by 2 ^{0}C before each step, whereas the Increasing condition rises by 1 ^{0}C. Accordingly, much of the Step Type differences across Step Number appear to be due to lower CoVAS ratings at the beginning of each step in the OA-Stepping condition (Supplementary Figure 4).



Supplementary Figure 4. Reported pain ratings (CoVAS) and associated temperatures for each step duration and type across participants. Stimuli within each trial were temporally aligned based on matched temperatures in the Increasing condition and the OA-Stepping condition following each temperature increase. Error bars represent standard errors of the mean (SEMs).

Supplementary Table 2. Type III Analysis of Variance table containing the results from the linear mixed effect model assessing the influence of Step Type, (alternative) Number, and Duration on pain experience ratings (CoVAS). Statistically significant effects are indicated in **bold**. dfs = degrees of freedom calculated via Satterthwaite's method.

				Models by Step Duration condition					
		Full model		Short steps		Medium steps		Long steps	
Effect	dfs	*F(dfs)*	*p*	*F(1, 47)*	*p*	*F(1, 47)*	*p*	*F(1, 47)*	*p*
Step Type	1, 47	1.10	.30	0.04	.84	1.08	.30	0.80	.37
Step Number	**1, 47**	**214.0**	**<.001**	**174.2**	**<.001**	**155.1**	**<.001**	**78.05**	**<.001**
Step Duration	2, 47	2.69	.078						
Type x Number	1, 47	2.55	.12	2.26	.14	**5.98**	**.018**	4.00	.051
Type x Duration	**2, 1434**	**4.18**	**.016**						
Number x Duration	**2, 1434**	**12.83**	**<.001**						
Type x Num x Dur	**2, 1434**	**5.26**	**.005**						

Supplementary Table 3. Linear mixed effects model slope coefficients, which represent CoVAS ratings as a function of Step Number when other effects are held constant.

	Short steps		Medium steps		Long steps	
Step Type	Slope	*95% CI*	Slope	*95% CI*	Slope	*95% CI*
OA-Stepping	7.11	[5.91, 8.31]	4.16	[2.95, 5.37]	3.70	[2.48, 4.92]
Increasing	6.14	[5.00, 7.28]	6.03	[5.06, 6.99]	4.97	[3.91, 6.04]

Effects of and by temperature group

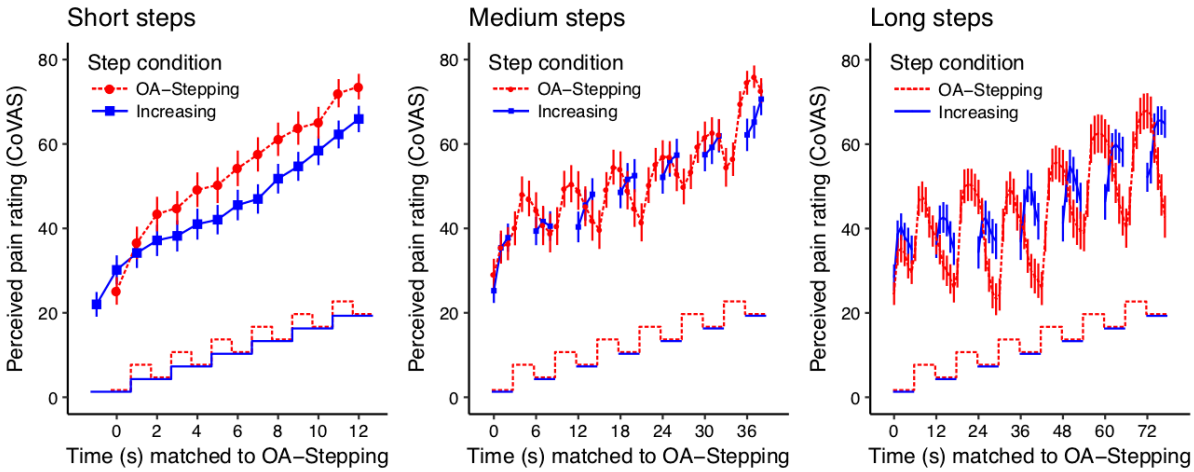
We found that roughly half of our participants reported being unable to tolerate temperatures of 46 ^{0}C or above. For these individuals (n=25), we reduced each trial's presented temperatures by 2 ^{0}C so that the maximum presented temperature was 44 ^{0}C (Max44 group). Everyone else (n=23) received the planned maximum of 46 ^{0}C (Max46 group). This procedural adjustment allowed us to achieve our pre-set sample size while ensuring that it remained representative of the population and its potentially lower pain thresholds.

For our main analyses, data from the two temperature groups were combined. We also conducted the same analyses with Temperature Group as a between-groups factor (Supplementary Table 4) and on the temperature groups separately (Supplementary Figure 5, Supplementary Tables 5A and 5B). The significant main effect of Temperature Group was due to higher CoVAS ratings overall in the Max44 group, perhaps because these individuals were more sensitive to noxious temperatures. There were no significant two-way interactions with Group, but each of the three-way interactions was significant. These interactions were further explored by examining each Temperature Group separately. (Examining each Duration separately instead revealed no significant two- or three-way interactions with Temperature Group, so these results have not been included here.)

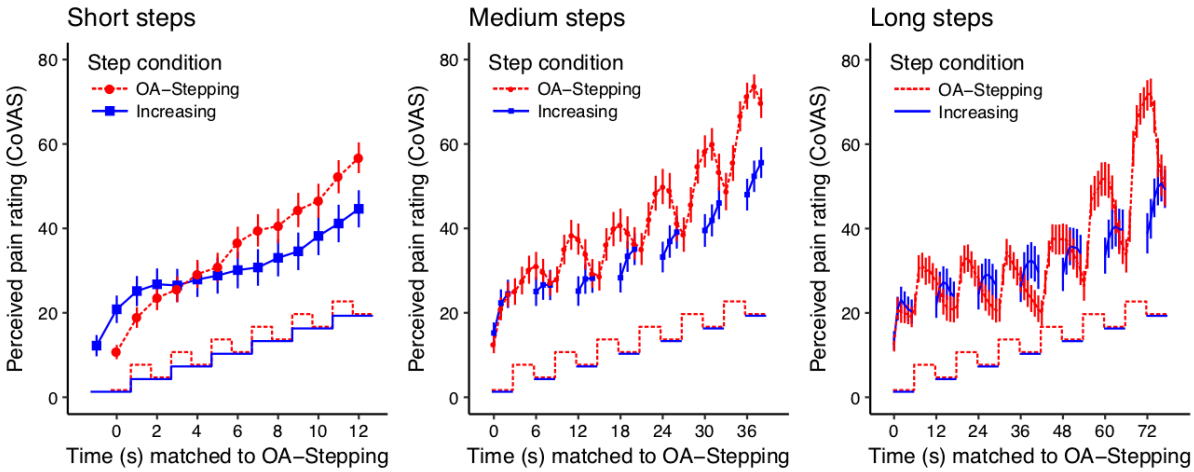
Supplementary Table 4. Type III Analysis of Variance table containing the results from the linear mixed effect model assessing the influence of Step Type, Number, Duration, and Temperature Group on pain experience ratings (CoVAS). Statistically significant effects are indicated in **bold**. dfs = degrees of freedom calculated via Satterthwaite's method.

Effect	dfs	*F*	*p*
Step Type	1, 46	1.65	.21
Step Number	**1, 46**	**302.9**	**<.001**
Step Duration	**2, 46**	**5.91**	**.005**
Temperature Group	**1, 46**	**6.46**	**.014**
Type x Number	**1, 46**	**29.32**	**<.001**
Type x Duration	**2, 1716**	**14.98**	**<.001**
Number x Duration	**2, 1716**	**6.23**	**.002**
Type x Group	1, 46	0.90	.35
Number x Group	1, 46	0.03	.85
Duration x Group	2, 46	0.68	.51
Type x Number x Duration	2, 1716	0.79	.45
Type x Number x Group	**1, 46**	**8.31**	**.006**
Type x Duration x Group	**2, 1716**	**6.83**	**.001**
Number x Duration x Group	**2, 1716**	**4.49**	**.011**
Type x Number x Duration x Group	2, 1716	0.02	.98

Max44 temperature group (n=25)



Max46 temperature group (n=23)



Supplementary Figure 5. Reported pain ratings (CoVAS) and associated temperatures for each step duration and type across participants separated by temperature group, Max44 (top) and Max46 (bottom). Stimuli within each trial are temporally aligned based on matched temperatures in the Increasing condition and the OA-Stepping condition following each 1 ^{0}C decrease. Error bars represent standard errors of the mean (SEMs).

The effects within each Temperature Group were similar to those observed in the combined sample (Supplementary Tables 5A and 5B). Specifically, there was no main effect of Step Type, but there was a main effect of Step Number as CoVAS ratings increased with increasing temperature. These increases were similar across Step Type in the Max44 group (Supplementary Table 6A), whereas the Max46 group evidenced a significant Type x Number interaction. As it was in the full sample, this interaction was driven by faster increases in the OA-Stepping condition (Supplementary Table 6B).

Crucially, within each Temperature Group, there was no evidence of an overall analgesic effect in the OA-Stepping conditions. Aside from the main effect of Group, the group differences appeared to be most pronounced for temperatures above 44 ^{0}C, which only the Max46 group experienced.

Supplementary Table 5A. Max44 Temperature Group (n=25) results from the linear mixed effect models assessing the influence of Step Type, Number, and Duration on pain experience ratings (CoVAS).

				Models by Step Duration condition					
	Full model			Short steps		Medium steps		Long steps	
Effect	dfs	*F(dfs)*	*p*	*F(1, 24)*	*p*	*F(1, 24)*	*p*	*F(1, 24)*	*p*
Step Type	1, 24	0.05	.82	1.31	.26	0.10	.75	1.16	.29
Step Number	**1, 24**	**147.7**	**<.001**	**99.13**	**<.001**	**130.2**	**<.001**	**56.05**	**<.001**
Step Duration	2, 24	3.24	.057						
Type x Number	1, 24	2.54	.12	3.54	.072	0.65	.43	0.20	.66
Type x Duration	**2, 894**	**12.50**	**<.001**						
Number x Duration	**2, 894**	**5.91**	**.003**						
Type x Num x Dur	2, 894	0.34	.71						

Supplementary Table 5B. Max46 Temperature Group (n=23) results from the linear mixed effect models assessing the influence of Step Type, Number, and Duration on pain experience ratings (CoVAS).

				Models by Step Duration condition					
	Full model			Short steps		Medium steps		Long steps	
Effect	dfs	*F(dfs)*	*p*	*F(1, 22)*	*p*	*F(1, 22)*	*p*	*F(1, 22)*	*p*
Step Type	1, 22	2.80	.11	0.52	.48	**5.55**	**.028**	0.09	.76
Step Number	**1, 22**	**156.5**	**<.001**	**106.6**	**<.001**	**167.2**	**<.001**	**61.86**	**<.001**
Step Duration	**2, 22**	**3.70**	**.041**						
Type x Number	**1, 34.98**	**45.94**	**<.001**	**30.11**	**<.001**	**21.55**	**<.001**	**12.81**	**.002**
Type x Duration	**2, 844**	**8.91**	**<.001**						
Number x Duration	**2, 844**	**4.79**	**<.001**						
Type x Num x Dur	2, 844	0.55	.58						

Supplementary Table 6A. Max44 Temperature Group (n=25) linear mixed effects model slope coefficients, which represent CoVAS ratings as a function of Step Number when other effects are held constant.

	Short steps		Medium steps		Long steps	
Step Type	Slope	*95% CI*	Slope	*95% CI*	Slope	*95% CI*
OA-Stepping	7.16	[5.80, 8.51]	6.21	[4.62, 7.80]	4.66	[2.98, 6.34]
Increasing	5.73	[4.16, 7.31]	5.29	[3.91, 6.67]	4.21	[2.87, 5.55]

Supplementary Table 6B. Max46 Temperature Group (n=23) linear mixed effects model slope coefficients, which represent CoVAS ratings as a function of Step Number when other effects are held constant.

	Short steps		Medium steps		Long steps	
Step Type	Slope	*95% CI*	Slope	*95% CI*	Slope	*95% CI*
OA-Stepping	6.98	[5.68, 8.28]	8.06	[6.66, 9.45]	6.53	[4.79, 8.27]
Increasing	3.55	[2.52, 4.58]	4.85	[3.91, 5.80]	4.00	[2.82, 5.18]

Finally, we examined the effects of Temperature Group (and our other factors of interest) for the final timepoint of each step. The results were similar to when the entire step was included. As before, the significant main effect of Temperature Group was due to higher CoVAS ratings overall in the Max44 group. There were no significant two-way interactions with Group, but two of the three-way interactions with Temperature Group was significant. These interactions were further explored by examining each Temperature Group separately. (Examining each Duration separately instead revealed no significant two- or three-way interactions with Temperature Group, so these results have not been included here.)

Supplementary Table 7. Type III Analysis of Variance table containing the results from the linear mixed effect model assessing the influence of Step Type, Number, Duration, and Temperature Group on pain experience ratings (CoVAS) for the final timepoint of each step. Statistically significant effects are indicated in **bold**. dfs = degrees of freedom calculated via Satterthwaite's method.

Effect	dfs	*F*	*p*
Step Type	1, 46	0.23	.63
Step Number	**1, 46**	**278.2**	**<.001**
Step Duration	**2, 46**	**13.96**	**<.001**
Temperature Group	**1, 46**	**5.98**	**.018**
Type x Number	**1, 46**	**4.73**	**.035**
Type x Duration	**2, 1716**	**31.48**	**<.001**
Number x Duration	**2, 1716**	**8.64**	**<.001**
Type x Group	1, 46	0.99	.32
Number x Group	1, 46	0.02	.89
Duration x Group	2, 46	0.96	.39
Type x Number x Duration	**2, 1716**	**8.50**	**<.001**
Type x Number x Group	**1, 46**	**4.69**	**.036**
Type x Duration x Group	**2, 1716**	**6.94**	**<.001**
Number x Duration x Group	2, 1716	2.69	.068
Type x Number x Duration x Group	2, 1716	0.11	.90

The effects within each Temperature Group were similar to those observed in the combined sample (Supplementary Tables 8A and 8B). Specifically, there was no main effect of Step Type, but there was a main effect of Step Number as CoVAS ratings increased with increasing temperature.

Crucially, within each Temperature Group, there was no evidence of an overall analgesic effect in the OA-Stepping conditions. It is also worth noting that the Max44 showed more convincing (and significant) sequential OA effects in the 6s Duration condition. These effects were not significantly different from the corresponding effects in the Max46 group. That lack of interaction likely reflects a lack of power. Indeed, it appears that the rapidly increasing CoVAS ratings for temperatures above 44 ^{0}C may have swamped the OA effects themselves. Future research will be needed to address this issue satisfactorily.

Supplementary Table 8A. Max44 Temperature Group (n=25) results from the linear mixed effect models assessing the influence of Step Type, Number, and Duration on pain experience ratings (CoVAS) for the final timepoint of each step.

				Models by Step Duration condition					
	Full model			Short steps		Medium steps		Long steps	
Effect	dfs	*F(dfs)*	*p*	*F(1, 24)*	*p*	*F(1, 24)*	*p*	*F(1, 24)*	*p*
Step Type	1, 24	1.05	.32	1.31	.26	0.24	.63	**5.53**	**.027**
Step Number	**1, 24**	**151.4**	**<.001**	**99.13**	**<.001**	**118.2**	**<.001**	**48.40**	**<.001**
Step Duration	**2, 24**	**9.12**	**.001**						
Type x Number	1, 24	0.00	.99	3.54	.072	0.23	.64	2.54	.12
Type x Duration	**2, 894**	**25.84**	**<.001**						
Number x Duration	**2, 894**	**6.40**	**.002**						
Type x Num x Dur	**2, 894**	**3.83**	**.022**						

Supplementary Table 8B. Max46 Temperature Group (n=23) results from the linear mixed effect models assessing the influence of Step Type, Number, and Duration on pain experience ratings (CoVAS) for the final timepoint of each step.

				Models by Step Duration condition					
	Full model			Short steps		Medium steps		Long steps	
Effect	dfs	*F(dfs)*	*p*	*F(1, 22)*	*p*	*F(1, 22)*	*p*	*F(1, 22)*	*P*
Step Type	1, 22	0.14	.71	0.52	.48	0.80	.38	0.74	.40
Step Number	**1, 22**	**127.8**	**<.001**	**106.6**	**<.001**	**140.8**	**<.001**	**39.41**	**<.001**
Step Duration	**2, 22**	**5.90**	**.009**						
Type x Number	**1, 22**	**10.60**	**.004**	**30.11**	**<.001**	**4.85**	**.038**	0.06	.81
Type x Duration	**2, 822**	**9.18**	**<.001**						
Number x Duration	**2, 822**	**4.78**	**.009**						
Type x Num x Dur	**2, 822**	**5.41**	**.005**						

Supplementary Table 9A. Max44 Temperature Group (n=25) linear mixed effects model slope coefficients, which represent CoVAS ratings as a function of Step Number when other effects are held constant.

	Short steps		Medium steps		Long steps	
Step Type	Slope	*95% CI*	Slope	*95% CI*	Slope	*95% CI*
OA-Stepping	7.16	[5.80, 8.51]	5.94	[4.30, 7.57]	3.21	[1.46, 4.96]
Increasing	5.73	[4.16, 7.31]	5.38	[3.95, 6.80]	5.18	[3.55, 6.82]

Supplementary Table 9B. Max46 Temperature Group (n=23) linear mixed effects model slope coefficients, which represent CoVAS ratings as a function of Step Number when other effects are held constant.

	Short steps		Medium steps		Long steps	
Step Type	Slope	*95% CI*	Slope	*95% CI*	Slope	*95% CI*
OA-Stepping	6.98	[5.68, 8.28]	7.15	[5.54, 8.76]	4.74	[2.71, 6.77]
Increasing	3.55	[2.52, 4.58]	5.11	[4.06, 6.16]	4.51	[3.17, 5.85]