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RESEARCH ARTICLE | *Sensory Processing*

Stepwise increasing sequential offsets cannot be used to deliver high thermal intensities with little or no perception of pain

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Derbyshire SW, Long VJ, Asplund CL. Stepwise increasing sequential offsets cannot be used to deliver high thermal intensities with little or no perception of pain. *J Neurophysiol* 122: 729–736, 2019. First published June 26, 2019; doi:10.1152/jn.00007.2019.—Offset analgesia (OA) is the disproportionate decrease in pain experience following a slight decrease in noxious heat stimulus intensity. We tested whether sequential offsets would allow noxious temperatures to be reached with little or no perception of pain. Forty-eight participants continuously rated their pain experience during trials containing trains of heat stimuli delivered by Peltier thermode. Stimuli were adjusted through either stepwise sequential increases of 2°C and decreases of 1°C or direct step increases of 1°C up to a maximum of 46°C. Step durations (1, 2, 3, or 6 s) varied by trial. Pain ratings generally followed presented temperature, regardless of step condition or duration. For 6-s steps, OA was observed after each decrease, but the overall pain trajectory was unchanged. We found no evidence that sequential offsets could allow for little pain perception during noxious temperature presentation.

NEW & NOTEWORTHY Offset analgesia is the disproportionate decrease in pain experience following a slight decrease in noxious heat stimulus intensity. We tested whether sequential offsets would allow noxious temperatures to be reached with little or no perception of pain. We found little evidence of such overall analgesia. In contrast, we observed analgesic effects after each offset with long-duration stimuli, even with relatively low-temperature noxious stimuli.

human; offset analgesia; psychophysics; temporal contrast; thermal perception

INTRODUCTION

Offset analgesia (OA) was first described by Grill and Coghill (2002) and was defined as a disproportionate decrease in pain experience following a slight decrease in heat stimulus intensity. In a typical OA experiment, three successive periods (T1, T2, T3) each contain a continuous noxious stimulus. The first and last stimuli are of equal intensity, but the middle stimulus is slightly more intense (e.g., 45°C, 46°C, 45°C). The OA effect is revealed by a greater fall in reported pain intensity following a step back to the original noxious stimulus temperature compared with delivery of a continuous noxious stimulus temperature (e.g., 45°C, 45°C, 45°C).

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A relatively large literature has developed around OA, and it is now broadly accepted that the OA technique can deliver robust analgesia (Derbyshire and Osborn 2008, 2009; Grill and Coghill 2002; Hermans et al. 2016; Honigman et al. 2013; Kobinata et al. 2017; Ligato et al. 2018; Martucci et al. 2012a, 2012b; Nahman-Averbuch et al. 2014; Naugle et al. 2013; Niesters et al. 2011; Nilsson et al. 2014; Olesen et al. 2018; Oudejans et al. 2015; Petersen et al. 2018; Szikszay et al. 2018; Yelle et al. 2008, 2009; Zhang et al. 2018). Many studies include a third condition involving a return to a nonnoxious baseline stimulus during the T3 period (e.g., 45°C, 46°C, 35°C), and at least some subjects report that the return to the first noxious stimulus (OA condition) feels indistinguishable from a return to baseline (Derbyshire and Osborn 2008; Grill and Coghill 2002). In their original study, Grill and Coghill speculated that such profound analgesic effects may extend still further. They informally reported that thermal intensities sufficient to produce tissue damage could be reached with little or no perception of pain via stepwise sequential increases of 2°C and decreases of 1°C (e.g., 42°C, 44°C, 43°C, 45°C, 44°C, 46°C, 45°C, ...). To our knowledge, however, that speculation has never been formally tested.

In this study we directly tested Grill and Coghill's speculation by performing the experiment they described. Healthy volunteers received trains of sequential stimuli involving either stepwise sequential increases of 2°C and decreases of 1°C (OA stepping) or direct stepwise increases of 1°C (increasing). Because the optimal length of these periods is unclear, we tested stimulus durations of 1, 2, 3, and 6 s across trials. We tested shorter durations foremost because it seemed unlikely that OA stepping would provide an entirely painless ascent. The OA experience is dependent on a period of increased noxious stimulation from an already noxious stimulus, which results in more intense pain, followed by a slight decrease in noxious intensity that is unexpectedly experienced as not painful. Overly long periods of increased noxious stimulation would likely exceed tolerance.

In addition, the precise mechanisms of OA itself remain uncertain, as do their potential contributions to an OA-stepping situation. One possibility is that OA events must sum temporally to produce an overall analgesic effect across increasingly noxious steps. If so, short-duration steps may be required, because typical stimulus durations (5–6 s) could allow anal-

gesic effects to dissipate before the next offset occurs. Conversely, short-duration steps might fail to induce robust OA, particularly if OA effects are due to adaptation, fatigue, or habituation of the primary afferent fibers. The temperatures used and the positioning of the probe in most OA studies mean that all nociceptor types could potentially impact the OA effect. Type I Aδ-fiber mechanoheat (AMH-I) nociceptors have a long response latency (~6 s) and then continue to respond for at least 30 s (Treede et al. 1995, 1998). AMH-II and C-fiber mechanoheat (CMH) nociceptors, in contrast, respond rapidly and habituate rapidly (LaMotte et al. 1984; Treede et al. 1995, 1998). The different transduction and conduction delays between those afferent fiber types may lead to variable offset effects with different stimulus timings. Alternatively, OA may be dependent on central mechanisms of descending inhibitory control, and so the different timings may affect temporal integration in higher order central nervous system (CNS) neurons that contribute to OA (Grill and Coghill 2002). As such, the present experiment is relevant to understanding the mechanisms of OA itself. Regardless, by testing different stimulus durations, we provided multiple opportunities for the OA-stepping condition to produce an analgesic effect that would result in the relatively painless ascent of temperatures that Grill and Coghill described.

MATERIALS AND METHODS

Participants. Fifty-three individuals participated in the experiment, although five terminated the experiment early because they were unable to tolerate the temperatures used. The data from 48 participants (mean age = 22 yr, range 19–26 yr, 27 women) were thus included in the final sample. All participants were healthy volunteers with no history of a neurological, psychiatric, or pain disorder and who had not taken any pain-relieving medication on the day of the study. Participants were recruited through an online platform for research volunteer recruitment hosted by the National University of Singapore (NUS). All participants provided written informed consent before any procedures. The NUS Institutional Review Board approved all procedures. Participants received S$10 remuneration for their participation.

Materials. The CHEPS (contact heat-evoked potential stimulator) PATHWAY system (Medoc Advanced Medical Systems, Ramat Yishia, Israel) fitted with a 27-mm-diameter Peltier thermode was used to deliver heat stimuli. Participant pain ratings were recorded using a computerized visual analog scale (CoVAS; Medoc Advanced Medical Systems) anchored at 0 ("no pain") and 100 ("maximum pain").

Experimental procedure. Following a standard explanation of the equipment and procedure, all participants received a series of test stimuli to acclimatize them to the heat stimuli and the CoVAS rating device. Once familiar with the probe and CoVAS, participants were presented with a single ascending limits trial to test whether they could tolerate the maximum target temperature of 46°C. The probe was heated from a baseline of 35°C at a rate of 0.5°C. Participants were instructed to press a button to return the probe to baseline when the heat pain reached their maximum point of tolerance. This test revealed that many participants had tolerance levels below the intended maximum of 46°C. To ensure that the sample remained representative of the population, we presented such individuals (n = 25, 15 women) with a maximum temperature of 44°C (Max44 group) during the main experiment. Individuals with a higher tolerance (n = 23, 12 women) were presented with a maximum temperature of 46°C (Max46 group).

Participants were then given a 5-min break while the experimenter prepared the experimental trials. These trials consisted of directly increasing stimulus trains (3), OA-stepping stimulus trains (3), and standard OA-relevant trials (9; not analyzed for this report). Each trial was preceded by 5 s of 35°C heat, stepped from the 32°C baseline (Fig. 1). All temperature transitions occurred at the CHEPS's maximum slew rate of 70°C per second for temperature increases and 40°C per second for decreases. For the Max46 group's increasing trials, each trial consisted of a train of seven stimuli that began at 39°C and

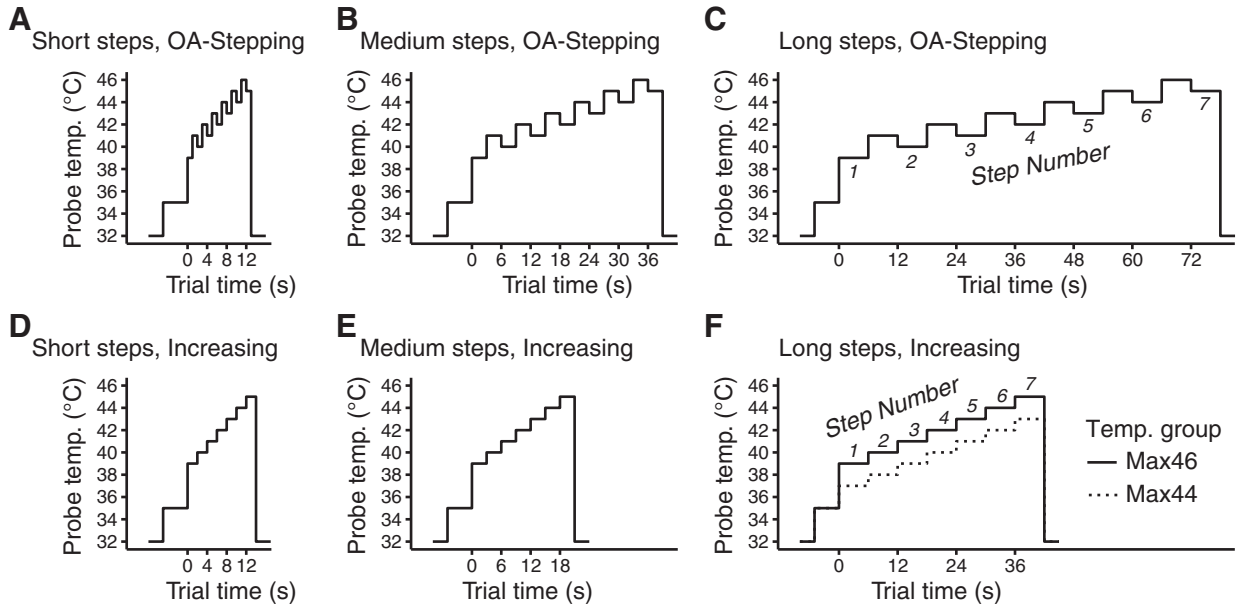

Fig. 1. Temperature delivery during each trial type. *A–C*: step type (short, medium, long), offset analgesia (OA) stepping. *D–F*: step type (short, medium, long), increasing. Step duration conditions are shown across columns. Matched step numbers are indicated for long steps (*C* and *F*). *F* also represents the temperatures for groups presented with a maximum temperature of 46°C (Max46) and 44°C (Max44). For clarity, *A–E* show only the Max46 group's temperatures. Note that the trial durations are the same for short steps (*A* and *D*), and trial durations are matched across medium steps, OA stepping (*B*) and long steps, increasing (*F*). In contrast, step durations are matched within medium (*B* and *E*) and long steps (*C* and *F*). (See main text for details on the two matching approaches.) Temp., temperature.

increased directly by 1°C until the final stimulus at 45°C. Stimulus durations of 2, 3, or 6 s were delivered in separate trials. For the Max46 group's OA-stepping trials, each trial consisted of a train of 13 stimuli that also began at 39°C but then increased by 2°C before decreasing by 1°C. This pattern was repeated until the penultimate stimulus at 46°C and the final stimulus at 45°C, thus providing temperature-matched steps for each of the seven steps in the increasing condition (step number). Stimulus durations of 1, 3, or 6 s were delivered in separate trials. At the conclusion of each trial, the presented temperature fell to 32°C before the probe was removed. Each participant experienced a total of six trials relevant to this study, each a different combination of step type (increasing, OA stepping) × step duration (short, medium, long). Participants in the Max44 group were presented the same trains of stimuli but with 2°C lower temperatures; i.e., stimuli were between 37°C and 44°C. Participants rated their pain experience throughout each train of stimuli, using the arm not being stimulated to move the CoVAS slider.

Note that this design affords two different ways of matching trials across step type (OA-stepping and increasing). They can be matched by the number of seconds per step (OA-stepping to increasing for medium or long steps) or by the number of seconds between temperature increases (OA-stepping to increasing for short steps, medium OA-stepping to long increasing). We chose to analyze and show a mixture of these two approaches so we could test a wide range of duration timings, thereby giving the OA-stepping approach the best chance of showing analgesic effects. The results of matching by time between temperature increases alone can be found in the Supplemental Materials (https://doi.org/10.6084/m9.figshare.8143445).

Each participant was presented the trial types in a random order, with each of the 15 trial types (including 3 increasing and 3 OA-stepping trials) experienced once. The thermal probe was moved after each trial to minimize sensitization or habituation. Eight skin locations were used, with stimuli presented to each location in the following sequence: lower left dorsal forearm, upper left dorsal forearm, lower left volar forearm, upper left volar forearm, lower right dorsal forearm, upper right dorsal forearm, lower right volar forearm, and upper right volar forearm. Pain thresholds and tolerances have been found to differ little across different volar sites (Schaffner et al. 2008), across the left and right forearms (Schaffner et al. 2008), or across the dorsal hand and volar wrist (Hagander et al. 2000). OA has also been successfully induced on both volar and dorsal aspects of the forearm (Szikszay et al. 2018). The starting location was set randomly for each participant. During the course of the experiment, each location was used for two trials (15 experimental trials + 1 practice trial). The intertrial interval was ~30 s, representing the time to move the probe and set up the next trial program on the CHEPS. More importantly, there were ~15 min between stimulations at a given skin location.

Analytical approach. Data preparations, visualizations, and models for statistical inference were implemented in RStudio version 1.0.136 (R Foundation for Statistical Computing) running R version 3.2.4. We first binned and averaged the CoVAS values by second. The values were further averaged within each step for the medium (3 time points) and long (6 time points) step duration conditions. To compare across the increasing and OA-stepping conditions (step type) within each step duration condition, we next aligned the data by step number (Fig. 1). Specifically, the time points in the increasing condition were temporally aligned with the time points for each step that followed a 1°C decrease in the OA-stepping condition. Across step type conditions, each step was thus matched for temperature within each temperature group and for duration within each step duration condition. For clarity, visualizations and minor variations of this data preparation are reported in RESULTS.

To characterize the condition influences on the CoVAS responses, we constructed linear mixed-effects models. These models were fit using the lmer function in the lme4 package (version 1.1-17) (Bates et al. 2015) and assessed using the anova function in the lmerTest package (version 3.0-1) (Kuznetsova et al. 2017). Fixed-effects factors were the length of each step (duration: short, medium, or long; categorical), how the temperature changed across steps (type: OA-stepping or increasing; categorical), and the temperature-matched steps within each trial (number: 1–7; continuous, and mean-centered in the models). Because each factor had been manipulated within subjects, random by-subject slopes and by-subject intercepts were modeled for each factor. The type × number interaction also had its own random by-subject slopes, resulting in the maximal random effects structure that still allowed the models to converge (Barr et al. 2013). Main effects and interactions were assessed using type III analysis of variance (ANOVA) tables, with the *F* values' degrees of freedom calculated via Satterthwaite's method (Kuznetsova et al. 2017). Post hoc tests on means were conducted using the ls_means function in the lmerTest package (version 3.0-1) (Kuznetsova et al. 2017), whereas the glht function in the multcomp package (version 1.4-8) (Hothorn et al. 2008) was used to characterize trends, and the emtrends() function in the emmeans package (version 1.3.1) was used for post hoc trend comparisons. The results for all inferential tests in this report were corrected for multiple comparisons using false discovery rate (FDR) (Benjamini and Hochberg 1995).

RESULTS

Effects of step duration and step type. Reported pain ratings (CoVAS) over time and averaged across participants were examined by step duration and step type (Fig. 2). As described in METHODS, *Analytical approach*, to compare the two step type conditions, the time points in the increasing condition were temporally aligned with the time points for each step that followed a 1°C decrease in the OA-stepping condition. Only steps matched for step duration and step number across step types were included in the inferential statistical analyses (see Supplemental Material for a visualization, https://doi.org/10.6084/m9.figshare.8143445). By virtue of this matching, each step was also matched for temperature across step type, although only within each temperature group (Max44 and Max46). The results presented below include data aggregated from all participants ($n = 48$), although similar results were found when the Max44 and Max46 groups were analyzed separately (see Supplemental Material, https://doi.org/10.6084/m9.figshare.8143445).

As the plots make clear, the OA-stepping condition did not induce overall analgesic effects (Fig. 2). The full mixed-effects model (factors: step duration, step type, and step number) corroborated this observation (Table 1). The same results were obtained with a linear mixed-effects model with step number as an ordered categorical variable (necessitating a simpler random effects structure without a random by-subject step type × step number slope) or with a standard ANOVA (see Supplemental Material https://doi.org/10.6084/m9.figshare.8143445). Foremost, there was no main effect of step type. Pain ratings increased with increasing temperature across step number as expected, but the significant type × number interaction was driven by even more rapid CoVAS increases per step number in the OA-stepping condition (Table 2). The type × number interaction did not significantly differ across step durations (nonsignificant type × number × duration interaction).

The analysis also revealed a main effect of step duration and a significant number × duration interaction. Post hoc comparisons showed that long steps had significantly lower CoVAS ratings than medium steps ($P = 0.001$), whereas the other pairwise comparisons were not significant (P values >0.18). In addition, pain ratings increased significantly more slowly for long steps as compared with either medium ($P = 0.005$) or

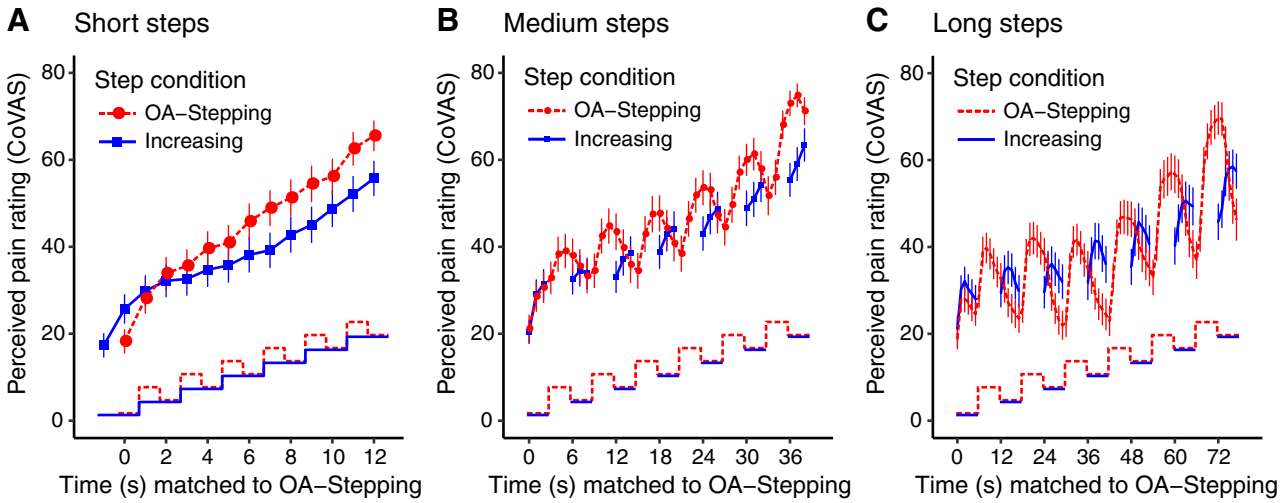

Fig. 2. *A–C*: reported pain ratings (computerized visual analog scale, CoVAS) and associated temperature steps for each step duration and step type averaged across participants. Stimuli within each trial were temporally aligned on the basis of matched step numbers in the increasing condition and the offset analgesia (OA)-stepping condition following each 1°C decrease. Note that the increasing trials were actually shorter than the OA-stepping trials for the medium and long steps (see Fig. 1), hence the discontinuities in their otherwise solid lines. Error bars are SE. For each data point, $n = 48$ participants.

short ($P = 0.026$). Medium and short step increases did not significantly differ ($P = 0.87$; see also Table 2). These effects are explored more below (see *OA effects within trials*). To better understand the effects within each step duration, we constructed separate models for each level (Table 1). Mirroring the results from the full model, results within each subset included a nonsignificant main effect of step type, a significant main effect of step number, and a significant type × number interaction. Within each subset, CoVAS values also increased more rapidly across step number in the OA-stepping condition (Table 2).

OA effects within trials. In addition to varying across steps, CoVAS ratings varied across the time points within the medium and long steps. During each matched step, the CoVAS rating largely decreased in the OA-stepping condition, whereas it rose and then fell slightly in the increasing condition (Fig. 2). The CoVAS decrease in the former condition likely reflects the 1°C temperature decrease that proceed each matched step (Fig. 1). If each of these offsets also induced OA, however, then the CoVAS decrease would reflect that influence, as well. In that case, one would predict lower CoVAS ratings during the OA-stepping condition compared with the increasing condition. Because of the lag between temperature changes and CoVAS ratings (due to perceptual or motor lags), we reasoned that the best time point at which to detect such an effect would be the final one of each step.

Accordingly, we conducted additional analyses focused on this final time point of each step. The analytical approach was otherwise identical to the one described above (see METHODS, *Analytical approach*, and RESULTS, *Effects of step duration and step type*). The results for these new analyses were similar. For the full model, there was no significant main effect of step type, but there were significant main effects of both step number and step duration (Table 3). Long steps were associated with significantly lower final CoVAS values compared with both medium ($P < 0.001$) and short steps ($P = 0.026$). The latter two conditions did not significantly differ ($P = 0.22$).

A key difference in the modified model's results is the presence of a significant three-way interaction (type × number × duration) but only a marginal type × number two-way interaction. As before, we further investigated such interactions by examining separate models for each step duration condition. The type × number interaction was significant only for short steps (Table 3), whereas the interaction's direction was numerically reversed in the condition for long steps (Table 4). This pattern accounts for the full model's three-way interaction. More

Table 1. *Type III analysis of variance results from linear mixed-effects models assessing the influence of step type, number, and duration on CoVAS ratings*

		Full Model		Models by Step Duration Condition					
				Short steps		Medium steps		Long steps	
	dfs	*F*(dfs)	*P*	*F*(1,47)	*P*	*F*(1,47)	*P*	*F*(1,47)	*P*
Step type	1, 47	1.56	0.32	1.84	0.22	2.27	0.19	0.51	0.52
Step number	1, 47	**309.5**	**<0.001**	**195.1**	**<0.001**	**294.0**	**<0.001**	**118.3**	**<0.001**
Step duration	2, 47	**6.07**	**0.010**						
Type × number	1, 47	**24.30**	**<0.001**	**21.74**	**<0.001**	**8.45**	**0.011**	**5.17**	**0.044**
Type × duration	2, 1722	**15.27**	**<0.001**						
Number × duration	2, 1722	**6.35**	**<0.001**						
Type × number × duration	2, 1722	0.78	0.22						

Data are ANOVA results from models assessing influence of step type, number, and duration on pain experience ratings (computerized visual analog scale, CoVAS). Statistically significant effects are indicated in bold. dfs, Degrees of freedom calculated via Satterthwaite's method.

Table 2. *Linear mixed-effects model slope coefficients representing CoVAS ratings as a function of step number*

	Short Steps		Medium Steps		Long Steps	
Step Type	Slope	95% CI	Slope	95% CI	Slope	95% CI
OA stepping	7.07	[6.14,8.01]	7.09	[6.01,8.18]	5.56	[4.33,6.78]
Increasing	4.69	[3.69,5.69]	5.08	[4.24,5.93]	4.11	[3.22,5.00]

Data are linear mixed-effects model slope coefficients representing computerized visual analog scale (CoVAS) pain experience ratings as a function of step number when other effects are held constant. Separate slopes and 95% confidence intervals (CI) were calculated for each step type condition within each step duration model. Slopes were significantly greater in the offset analgesia (OA)-stepping condition for each step duration, as indicated by the type × number interactions in Table 1. There was also a shallower overall slope for long steps compared with short or medium steps (see main text for details).

importantly, the full model's type × duration two-way interaction could be explained by a significantly lower final time point in each step of the OA-stepping condition compared with the increasing condition for long steps. In other words, there were significant OA effects following the 1°C temperature decreases, at least for long steps.

DISCUSSION

In their original OA report, Grill and Coghill (2002) presented the possibility that stepwise offset increases would allow the delivery of distinctly and increasingly noxious heat with little or no perception of pain. In this article we describe an effort to test this speculation. Overall, we did not find evidence to support it. There was no significant difference in pain reports with 1°C direct step increases compared with stepping 2°C up and then 1°C down (OA stepping), and there were greater pain increases over successive steps with OA stepping compared with comparable direct stepping. Otherwise, reported pain largely followed presented temperatures across time and step duration conditions.

A notable exception to this general correspondence was the presence of sequential offset effects. Analgesia is often induced following the reduction in noxious heat in the context of a previous increase in noxious heat, but OA is typically induced only once per trial (Derbyshire and Osborn 2008; Grill and Coghill 2002; Szikszay et al. 2018). Our study provided good evidence that such offset effects can be induced sequentially within a trial as well, at least when relatively long-duration stimuli (6 s) are used. There was little evidence for such effects with shorter (3 s) duration stimuli, however, and no evidence when very short-duration (1 s) stimuli are used. One caveat is that the trial length was longest for OA-stepping trials, so habituation may be a confounding factor. That said, sequential offsets often had substantially lower CoVAS ratings compared with the long-steps increasing condition, despite the fact that CoVAS ratings increased more quickly overall in the OA-stepping condition.

A recent review has commented on the wide variation in OA paradigms, analysis, and presentation of data (Szikszay et al. 2018). In this study we adopted what is broadly the standard approach, involving repetitive testing at multiple regions of the volar and dorsal forearm, using temperatures set according to individual tolerance (albeit grossly) and recording pain report with continuous ratings. The use of virtually continuous ratings generates many repeated measures and therefore analytical challenges, but such measures also allow for data visualizations that cogently illustrate the dynamic changes in pain experience. For example, Fig. 2 demonstrates the dynamic reduction in pain ratings that begins shortly after the offset step for the long steps. This reduction of pain experience disproportionate to that experienced when the same noxious heat is delivered continuously is the defining feature of OA. We confirmed that effect for long-duration stimuli by using a linear mixed-effects model based on each step's final pain rating after an offset, which is similar to the analytical approach used in earlier studies of OA that relied on pain reports at fixed intervals (Derbyshire and Osborn 2008; Grill and Coghill 2002).

Initially, we anticipated that a maximum temperature of 46°C would be readily tolerated by all our participants, but our initial testing indicated otherwise. Anecdotally, we have noticed that heat pain thresholds seem lower with Asian than with Western samples, and other groups have also commented on this difference (Kobinata et al. 2017; Rowell et al. 2011; Zhang

Table 3. *Type III analysis of variance results from linear mixed-effect models assessing the influence of step type, number, and duration on CoVAS ratings for the final time point of each step*

				Models by Step Duration Condition					
	Full Model			Short steps		Medium steps		Short steps	
	dfs	*F*(dfs)	*P*	*F*(1,47)	*P*	*F*(1,47)	*P*	*F*(1,47)	*P*
Step type	1, 47	0.27	0.63	1.84	0.22	0.01	0.92	**5.66**	**0.034**
Step number	**1, 47**	**284.8**	**<0.001**	**195.1**	**<0.001**	**260.2**	**<0.001**	**88.27**	**<0.001**
Step duration	**2, 47**	**14.27**	**<0.001**						
Type × number	1, 47	4.04	0.076	**21.74**	**<0.001**	2.85	0.14	1.32	0.30
Type × duration	**2, 1,722**	**32.35**	**<0.001**						
Number × duration	**2, 1,722**	**8.77**	**<0.001**						
Type × number × duration	**2, 1,722**	**8.48**	**<0.001**						

Data are ANOVA results from models assessing influence of step type, number, and duration on pain experience ratings (computerized visual analog scale, CoVAS) for the final time point of each step. (Note that the model for short steps is unchanged from Table 1.) Statistically significant effects are indicated in bold. dfs, Degrees of freedom calculated via Satterthwaite's method.

Table 4. *Linear mixed-effects model slope coefficients representing CoVAS ratings as a function of step number for the final time point of each step*

	Short Steps		Medium Steps		Long Steps	
Step Type	95% CI	Slope	95% CI	95% CI	Slope	95% CI
OA stepping	7.07	[6.14,8.01]	6.52	[5.37,7.67]	3.94	[2.61,5.28]
Increasing	4.69	[3.69,5.69]	5.25	[4.36,6.13]	4.86	[3.80,5.92]

Data are linear mixed-effects model slope coefficients representing computerized visual analog scale (CoVAS) pain experience ratings for the final time point of each step as a function of step number when other effects are held constant. Separate slopes and 95% confidence intervals (CI) were calculated for each step type condition within each step duration model. (Note that the model for short steps and its results are identical to those reported in Table 2.) The difference in slopes across step type was significant only for short steps (type × number interactions in Table 3). This pattern is reflected in the observed three-way interaction (see main text for details).

et al. 2018). This possible population difference is currently being explored formally using historic data from our group. For the present sample, separate analyses of the lower and higher tolerance groups do not indicate radically different effects from those observed in the combined sample (see Supplemental Material, https://doi.org/10.6084/m9.figshare.8143445). Examination of Supplemental Fig. S1 may lead to the impression of stronger sequential OA effects for the low-tolerance (Max44) group for both the long- and medium-duration steps, but that difference was not supported by formal analysis. The abbreviated thresholding procedure to determine the groups, the inevitable smaller samples within each group, and the between-group comparisons preclude any strong conclusions based on the delivered temperatures.

Overall, our results demonstrate stronger OA effects with the use of long-duration stimuli. One possibility is that the shorter stimulus durations provided participants insufficient time to experience or report OA effects following offsets. All OA effects were apparently extinguished by the 2°C increases regardless of step duration, but for the longer (6 s) duration stimuli, OA effects were reestablished sequentially before each 2°C increase. For the shorter duration stimuli, however, delivery of the next 2°C increase arrived just 1 or 3 s after each offset.

Another possibility is that the longer stimulus periods induced greater peripheral habituation, which resulted in lower firing when offsetting to the lower noxious temperature and reduced pain experience. AMH-I nociceptors have a long response latency (~6 s) to sustained stimulation and then continue to respond for at least 30 s. Conversely, AMH-II nociceptors respond almost immediately (~0.15 s) and then habituate rapidly (within a second) (Treede et al. 1995, 1998), response features they share with CMH nociceptors (LaMotte et al. 1984; Treede et al. 1995). The three receptor types differ in their response thresholds (with variability across individual receptors as well), ranging from 40–44°C for CMH nociceptors (Churyukanov et al. 2012; LaMotte et al. 1982, 1984; Treede et al. 1995; Yeomans et al. 1996) and 44–48°C for AMH-II nociceptors (Churyukanov et al. 2012; Treede et al. 1995, 1998; Yeomans et al. 1996) to over 53°C for AMH-I nociceptors (Treede et al. 1995, 1998). Importantly, these thresholds are for short-duration stimuli; with prolonged stimulation (~30 s), the AMH-I nociceptor thresholds reduce substantially to 43–45°C, and the AMH-II nociceptor thresholds drop a few degrees, as well (Treede et al. 1998). Therefore, each receptor type could potentially play a role in the observed OA effects. Their different transduction properties, however, might lead to variable offset effects with different stimulus timings. Specifically, if OA depends on AMH-II and CMH responses, then we might anticipate that short OA periods would induce an OA effect. In contrast, if AMH-I responses are critical, then we might anticipate that longer OA periods would be necessary to induce an OA effect. That latter outcome has more support from this study.

It is worth noting, however, that C-fiber mechanoheat activity begins before any experience of pain for most subjects, and pain sensation is longer than the duration of C-fiber activity by 2–4 s (LaMotte et al. 1984). Pain is therefore not strictly tied to peripheral activity and will involve integrations of mean activity over time by higher order neurons in the CNS. Several studies suggest that a central component in the temporal filtering is necessary for OA (Mørch et al. 2015; Yelle et al. 2008), and our study cannot directly disentangle central and peripheral effects. Nevertheless, the weaker OA effects with shorter stimulus durations does suggest that input from AMH-II fibers is less critical and that more slowly adapting or accumulated central effects are more critical. These possibilities will need to be pursued further in future studies.

In the present study we have demonstrated that stepping OA cannot be used to deliver increasingly noxious temperatures without increasing pain. We have also demonstrated that offset effects can be created sequentially, but only with longer duration stimuli. The mechanisms behind OA remain an uncertain mix of peripheral and central effects that will need to be explored in future studies.

Limitations. Previous OA studies have used fixed temperatures for delivery (Grill and Coghill 2002; Martucci et al. 2012b; Nahman-Averbuch et al. 2014) or have used temperatures modified according to individual thresholds (Derbyshire and Osborn 2008). In this study we chose to use fixed temperatures both for simplicity and because of the need to use several increasing steps of noxious stimulation. Consequently, we did not prioritize the thresholding procedure and only delivered a single ascending trial to assess tolerance. That single trial was mainly intended to familiarize the participants with the stimulus and resolve any anxiety about their tolerance of the heat pain. When it became apparent that many participants could not tolerate 46°C, we reduced the maximum temperature to 44°C for such individuals. Intriguingly, another group working with Japanese participants reported a very similar problem and reduced their highest delivered temperature from 49°C to 46°C accordingly (Kobinata et al. 2017). Asian populations may have a lower pain tolerance (Kim et al. 2017), and we intend to explore that possibility in further studies. Nevertheless,

our ad hoc reduction of the maximal temperature could mean that at least some of our participants received noxious stimuli notably below their tolerance. It seems unlikely that stimuli closer to tolerance would enable painless OA stepping, but more careful assessment of tolerance in future studies will likely better facilitate understanding of the mechanisms causing the OA phenomenon.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.W.G.D., V.J.E.L., and C.L.A. conceived and designed research; V.J.E.L. performed experiments; S.W.G.D., V.J.E.L., and C.L.A. analyzed data; S.W.G.D. and C.L.A. interpreted results of experiments; C.L.A. prepared figures; S.W.G.D. and C.L.A. drafted manuscript; S.W.G.D. and C.L.A. edited and revised manuscript; S.W.G.D., V.J.E.L., and C.L.A. approved final version of manuscript.

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