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ORIGINAL ARTICLE

Effect of limb position change on capsaicin-evoked pain: Evidence of interplays between the vascular and nociceptive systems?

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Abstract

Background: This experiment aimed at confirming our incidental observation that, when capsaicin is applied on the volar forearm, raising the arm to a vertical position leads to a dramatic increase in capsaicin-evoked pain and to explore possible underlying mechanisms.

Methods: Twenty healthy volunteers received a 2% capsaicin patch on one forearm and a vehicle patch on the other. Patches were kept in place for 60 min. The perception caused by the patch was assessed repeatedly before, during and after patch application, both with the arm in horizontal resting position and raised vertically. In addition, capsaicin-induced secondary hyperalgesia was assessed using mechanical pinprick stimuli. Half of the participants were seated upright while the other half were lying supine, to assess whether the effect of limb position on capsaicin-evoked pain was due to gravity.

Results: After a few minutes of patch application, raising the capsaicin-treated arm (but not the vehicle-treated arm) led to a strong increase of the pain experienced at the patch. This effect of raising the arm did not differ between participants in the supine and seated groups and is therefore likely related to the position of the arm relative to the ground rather than to the body. Mechanical secondary hyperalgesia and the arm raising effect were strongly decorrelated at the last time point after patch removal, indicating different underlying mechanisms.

Conclusion: Our results indicate that capsaicin-evoked pain can be strongly modulated by limb posture and that this effect may be caused by an interplay between vascular and nociceptive systems.

Significance Statement: Capsaicin-evoked pain can be strongly modulated by limb posture and this effect may be caused by an interplay between vascular and nociceptive systems.

1 | INTRODUCTION

Within a few minutes, topical application of capsaicin leads to the perception of a strong burning pain (O'Neill et al., 2012). This phenomenon results from the effect of capsaicin on TRPV1 (a heat-sensitive cation channel), leading to a shift of its thermal activation threshold towards lower temperatures and, as a consequence,

to sustained activity at baseline skin temperatures in TRPV1-expressing heat-sensitive nociceptors (Voets et al., 2004). As some of these nociceptors are peptidergic fibres, their activation also leads to the development of a flare in and around the area of capsaicin application (Foreman et al., 1983). After some time, topical capsaicin also leads to mechanical hypersensitivity of the treated skin (primary allodynia and hyperalgesia) but also of a zone extending several centimetres beyond the application site (secondary allodynia and hyperalgesia) (Treede et al., 1992). Sensitization of the untreated skin is usually accepted to reflect a form of heterosynaptic potentiation of excitatory synapses between mechanosensitive primary afferents and second-order neurons in the dorsal horn of the spinal cord caused by the sustained activation of capsaicin-sensitive first-order nociceptors (Henrich et al., 2015; Treede & Magerl, 2000).

In a previous study during which we studied the temporal evolution of sensory alterations caused by topical capsaicin application in healthy volunteers, we accidently observed that raising the capsaicin-treated arm from a neutral to a vertical position led to a sudden and dramatic increase of burning pain (van Neerven & Mouraux, 2020). Changing the position of the untreated arm did not elicit this effect.

Our observation could be related to the report by Byas-Smith that arterial occlusion (using tourniquet constriction) led after a few seconds to a marked increase of the burning pain elicited by intradermal capsaicin injection in the volar forearm (Byas-Smith et al., 1999). Indeed, raising the arm can be expected to lead to a reduction of blood flow in the arm, not unlike (mild) tourniquet occlusion (Pappano & Gil Wier, 2013).

Interestingly, Byas-Smith also reported that this 'tourniquet allodynia' appeared only after pinprick secondary hyperalgesia had developed. This temporal correlation could hint at a shared mechanism. Indeed, the tourniquet allodynia and the arm raising effects could be hypothesized to result from the amplification of the excitatory input of vascular primary afferents (sensitive to changes in blood flow) to second order neurons, similarly to the amplification of mechanosensitive signals originating from the skin producing secondary mechanical hyperalgesia.

To further explore this matter, this preregistered study had several goals. First, to confirm our observation that raising the arm from a horizontal to a vertical position markedly increases capsaicin-induced burning pain at the treated arm (the arm-raising effect). Second, to confirm that this arm-raising effect is related to the position of the arm relative to the ground (effect of gravity on the vascular system) and not relative to the body. Third, to investigate whether the development of the arm-raising effect follows the same time course as capsaicin-induced secondary hyperalgesia (manifested as an increase in pinprick hypersensitivity).

2 | METHODS

All experiments were conducted according to the latest version (October 2013) of the Declaration of Helsinki. Approval for the conduction of the experiment was obtained from the local ethical committee (Comité d'Ethique Hospitalo-Facultaire CUSL-UCLouvain, protocol 2019/15MAI/215 HPoC) before the recruitment of participants started. This study was pre-registered and the preregistration record as well as data and analysis code can be found on the corresponding OSF repository (DOI 10.17605/OSF.IO/XJ8BY).

2.1 | Participants

Twenty healthy young volunteers (age range: 18–30, median age: 22, 10 males, 10 females, 5 left-handed, 15 right-handed) were included. They all satisfied the following inclusion criteria: being between 18 and 30 years old; not using drugs on a regular basis (i.e. less than once a week; anticonception not included) or having used drugs in the week preceding the experiment; not having a history of neurological or psychiatric condition; not having dermatological conditions or skin lesions on the forearms; not suffering of chronic pain or being in pain at the time of the experiment; not having a hobby that leads to over stimulation of the volar forearm (e.g. volleyball). All participants gave written informed consent before the beginning of the experiment.

Half of these participants were randomly assigned to the lying supine body position group and the other half to the sitting upright group (see Figure 1b).

2.2 | Capsaicin and vehicle patch application

Two patches were applied on each participant, one with capsaicin solution and the other with vehicle solution only. Capsaicin solution was prepared by diluting capsaicin powder (M2028, Sigma-Aldrich) in a vehicle solution consisting of 50% water and 50% ethanol absolute to a final concentration of 2% capsaicin. Each type of patch was obtained by dripping 1 mL of the corresponding solution on a 5 × 5 cm gauze pad (Sterilux ES, Hartmann) set on a 10 × 8.5 cm self-adhesive film (Opsite Flexifix, Smith and nephew).

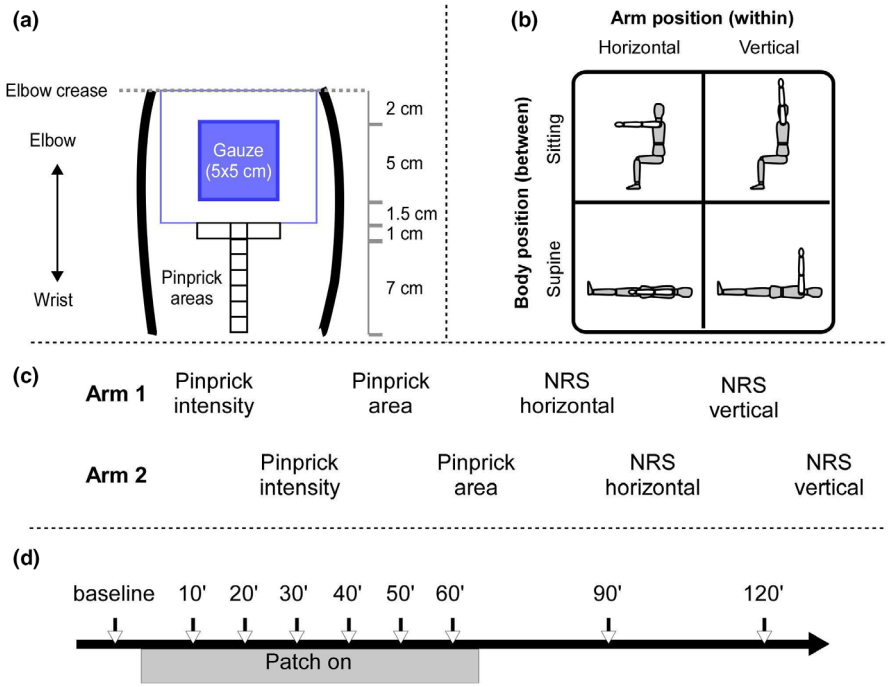

FIGURE 1 Experimental design (a) Placement of the patch and location of the pinprick stimulation areas. (b) Representation of the levels of between subject factor 'Body position' and within subject factor 'Arm position'. (c) Representation of one measurement set. (d) Representation of the timeline of one experimental session, showing the timing of the different measurement sets relative to patch application.

2.3 | Assessment of perceptions elicited by the patch

During the experiment, participants were repeatedly asked to report the intensity of the perception elicited by the patches. To do so, they were asked to focus on the skin site corresponding to one of the two patches for 20 s and to give a rating on a 0 to 100 numerical rating scale (NRS), where 0 corresponded to '*no sensation*' and 100 to '*the most intense sensation you can imagine*'. They were also asked to provide descriptors of the perception elicited by the patch, in the form of epithet(s) from the following list and, if necessary, additional freely-chosen epithets: warm (*tiède*), hot (*chaud*), burning (*brûlant*), pricking (*piquant*), painful (*douloureux*), itchy (*démangeaison*), touch (*toucher*).

2.4 | Assessment of perceptions elicited by the pinprick stimuli

To assess the development of secondary mechanical hyperalgesia, a 128 mN pinprick stimulator (The Pinprick, MRC Systems) was used. At each time point, three pinprick stimuli were delivered within a 1 × 5 cm area located adjacent to the distal edge of the self-adhesive film (Figure 1a) and the participant was asked to rate and provide qualifiers for each of these stimuli using the procedures described in the previous section. The target of the pinprick stimulator was pseudo-randomly changed after each stimulus to avoid sensitization due to repetitive stimulation of the same skin spot.

2.5 | Assessment of the spatial extent of secondary hyperalgesia

We also attempted to quantify the spatial extent of secondary hyperalgesia. To do so, pinprick stimuli were delivered every cm on a 7 cm line starting at the distal edge of the self-adhesive film and following the axis of the forearm in the direction of the wrist (Figure 1a). Stimuli were delivered successively at each point of that line, twice in the proximal-to-distal direction (i.e. moving away from the self-adhesive film) and twice in the distal-to-proximal direction (i.e. moving towards the self-adhesive film). After each stimulus, participants were asked to indicate whether they felt the stimulus as clearly weaker (when stimuli were delivered in the proximal to distal direction) or stronger (when stimuli were delivered in the distal to proximal direction) as compared to the previous stimulus. The distance between the edge of the self-adhesive film and the stimulation site at which the perception was first reported as stronger/weaker was recorded, as it should have corresponded to the edge of the pinprick hyperalgesia area.

If valid, this measure should have resulted in a vast majority of 0 lengths for the vehicle patch (as it is not expected to elicit hyperalgesia) and >0 and approximately equal lengths (corresponding to the same 'border' location) for opposite direction trains. Whereas we observed this pattern in some participants (usually people who previously participated in experiments involving secondary hyperalgesia), it was not the case for all of them. We therefore decided not to analyse these length data as what

exactly was measured is unclear and likely variable across individuals.

Of note, this does not mean that capsaicin did not induce a zone of increased pinprick sensitivity around the patch (as evidenced by the pinprick rating results). Instead, it indicates that our procedure to evaluate its extent was not reliable.

2.6 | Experimental procedure

The experiment took place in a dimly lit and comfortably warm room. Before the beginning of the actual data collection, participants were explained and familiarized with the different measures. Then, participants had to practice the rating and description task on three 128 mN pinprick stimuli, three 256 mN pinprick stimuli (mimicking the perception produced by pinprick stimulation of sensitized skin), three 44°C stimuli and three 48°C stimuli (mimicking perception elicited by a capsaicin patch), applied to their hand dorsum. Noxious heat stimuli lasted 5 s and were delivered with the T06 thermode of the TCSII (Peltier effect thermode, QST. Lab; the active zone is ~3 × 3.5 cm^2), roughly matching in size the area of the patch. Finally, the area on which the patches would be applied was marked on the forearms, using an ethanol resistant marker.

Throughout the experiment, a standardized set of measurements was repeated at various time points (Figure 1c). First, perception elicited by pinprick stimuli were assessed as described previously for one forearm and then for the other. Second, the procedure described to quantify the spatial extent of secondary hyperalgesia was conducted for one forearm and then the other. Third, participants were asked to provide a rating for perceptions at the site of the (future) patch for one forearm and then the other. Finally, the participants had to raise one arm in a vertical position for 20 s and give a rating corresponding to the perceptions experienced at the site of the (future) patch during the arm lift. This was immediately repeated with the other arm.

Except for this last pair of ratings, all other measurements were performed with the arm in the horizontal resting position (Figure 1b). Since the stimulator relies on a weighted needle to deliver calibrated stimuli, it can only be applied vertically and perpendicular to the skin surface, which means that testing pinprick sensitivity on the arm in vertical position was not possible.

The side with which the measurement set started was the same for the whole experiment and was counterbalanced across participants (dominant & capsaicin, non-dominant & capsaicin, dominant & vehicle, or non-dominant & vehicle).

Actual data collection started with a first set of standardized measurements (see previous paragraphs and Figure 1c). Immediately after its completion, a capsaicin patch was applied on one of the participant's volar forearms and a vehicle patch on the other. As the two patches were applied by the same experimenter, they were not applied exactly at the same time but a few seconds apart. The side to which the vehicle and capsaicin patches were applied (dominant vs. non-dominant) was counterbalanced across participants. The patches were placed in such a way that the proximal edge of the gauze pad was located ~2 cm away from the elbow crease (Figure 1a). Whereas the participants were not told which of the patches had capsaicin and were therefore blinded to the conditions at the time of application, the experimenter prepared and applied the patches and was therefore aware of their content.

Six sets of measurements were taken while the patch was on, one every 10′ starting from 10′ after patch application (Figure 1d). After the end of the sixth measurement set (~65′ post patch application), the patches were removed and the skin was gently cleaned with tepid water and soap, to remove capsaicin and vehicle solution residues. Two additional measurement sets were taken at 90′ and 120′ post patch application (roughly 25′ and 55′ post patch removal).

To assess whether the position of the limb relative to the body (0° to ~180° shoulder flexion) or relative to the ground (~horizontal or ~vertical) was driving the arm raising-effect, half of the participants were lying on their back (supine position) and half of the participants were sitting in a comfortable chair during the experiment (with a high table in front of them to support and keep their arms horizontal).

2.7 | Data analysis

Data analysis was conducted in R using the RStudio interface (Allaire, 2012). In addition to base R, functions from the *tidyverse* package were used for data wrangling and plotting (Wickham et al., 2019), functions from the *ggeffects* package were used to construct plots (Lüdecke, 2018), functions from the *LmerTest* package were used to fit mixed models and to interpret their results as ANOVAs (Kuznetsova et al., 2017), and functions from the *emmeans* package were used to conduct post-hoc tests (Lenth et al., 2024).

For all tests, the alpha level was set to 0.05. For post-hoc tests, both uncorrected and Bonferroni corrected p-values are reported. Degrees of freedom were estimated using the Kenward-Rogers method.

2.7.1 | Patch ratings

To confirm that capsaicin induced burning perceptions and to evaluate whether these perceptions increased during arm elevation, an ANOVA based on a linear mixed model with a random intercept for each participant and fixed effect factors *time point* (9 levels), *arm position* (2 levels, horizontal vs. vertical), *body position* (2 levels, supine vs. sitting), *patch* (2 levels, vehicle vs. capsaicin), and all their interactions- was used to analyse the patch ratings. Based on our hypotheses, we expected ratings for the vehicle patch to remain stable (and close to 0) over time and arm position, whereas the ratings from the capsaicin patch would increase over time (and maybe plateau or start decreasing at some point) and would further increase during arm elevation. This would correspond to a main effect of patch, and interactions between patch, time point, and arm position.

Since the results showed an effect of the factor *patch*, we conducted post-hoc one-sided tests to determine whether patch ratings were overall larger than 0 for each type of patch. Since the results showed an effect of the factor *time point*, we conducted one-sided post-hoc tests (separately for each type of patch) to determine if ratings recorded during and after patch application were larger than ratings recorded at baseline (before patch application). As these initial post-hoc tests showed that vehicle patch ratings could not be distinguished from 0, all subsequent post-hoc analyses were conducted on capsaicin patch ratings only. To further characterize the effect of *time point*, we also conducted two-sided post-hoc tests assessing if capsaicin patch ratings were different at consecutive time points. Since the results showed an effect of factor *arm position*, we conducted one-sided post-hoc tests to confirm that ratings were higher in the *arm position vertical* condition. As the results indicated an interaction between factors *arm position* and *time point*, we conducted one-sided post-hoc tests to determine if the rating differences between the *vertical* and *horizontal arm positions* recorded during and after patch application (arm raising effect) were larger than the same difference recorded at baseline (before patch application). Finally, to further characterize this interaction we also conducted two-sided post-hoc tests comparing the magnitude of the arm raising effect at consecutive time points.

2.7.2 | Patch descriptors

An additional, not pre-registered analysis of the probability of describing the patch stimuli with the descriptors 'pricking' and/or 'burning' and/or 'painful' as a function of factors *time point* (9 levels), *arm position* (2 levels, horizontal vs. vertical), and *patch* (2 levels, vehicle vs. capsaicin) was performed, to check whether quantitative changes in the perception (ratings) were matched with qualitative changes. For this analysis, an ANOVA based on a logistic mixed effects model with a random intercept for each participant was used. Due to convergence problems, interaction effects were not estimated.

One sided post-hoc tests were used to determine if the directionality of the effects of significant factors aligned with our hypotheses (capsaicin>vehicle; difference between consecutive time points; arm vertical > arm horizontal).

2.7.3 | Pinprick ratings

To confirm that capsaicin treatment led to the development of secondary mechanical hyperalgesia, an ANOVA based on a linear mixed model with a random intercept for each participant and fixed effect factors *time point* (9 levels) and *patch* (2 levels, vehicle vs. capsaicin), and all their interactions- was used to analyse the pinprick ratings. Based on our hypotheses, we expected to observe stable and low pinprick ratings on the forearm that received the vehicle patch and to observe increasing (over time) pinprick ratings on the arm that received the capsaicin patch (possibly followed by a plateau and decay). This would correspond to a main effect of patch and time point, as well as an interaction between these factors. To satisfy the distributional assumption of the model (normality of the residuals), the ratings had to be log-transformed.

Since the results showed an effect of the factor *patch*, we conducted post-hoc one-sided tests to determine whether pinprick ratings were larger than 0 for each type of patch. Since the results showed an effect of factor *time point*, we conducted two-sided post-hoc tests (separately for each type of patch) to determine if pinprick ratings recorded during and after patch application were different from ratings recorded at baseline (before patch application). These post-hoc tests revealed significant differences from baseline both for the capsaicin and the vehicle patch, indicating that capsaicin induced pinprick secondary hyperalgesia should be assessed by comparing pinprick ratings at both arms for a given time point rather than comparing capsaicin arm ratings against baseline. As the results indicated an interaction between factors *patch* and *time point*, we conducted one-sided post-hoc tests to determine if the

rating differences between the *capsaicin* and the *vehicle* arms (secondary mechanical hyperalgesia) recorded during and after patch application were larger than the difference recorded at baseline (before patch application). To further characterize the temporal evolution of pinprick secondary hyperalgesia, we also used two-sided post-hoc tests to compare pinprick hyperalgesia values at consecutive time points.

2.7.4 | Pinprick descriptors

An additional, not pre-registered analysis of the probability of describing the pinprick stimuli with the descriptors 'pricking' and/or 'burning' and/or 'painful' as a function of factors *time point* (9 levels) and *patch* (2 levels, vehicle vs. capsaicin) was performed, to check whether quantitative changes in the perception (ratings) were matched with qualitative changes. For this analysis, an ANOVA based on a logistic mixed effects model with a random intercept for each participant was used.

One sided post-hoc tests were used to determine if the directionality of the effects of significant factors aligned with our hypotheses (capsaicin>vehicle; difference between consecutive time points).

2.7.5 | Spatial extent of pinprick hyperalgesia

As mentioned before, we decided not to analyse the spatial extent of pinprick hyperalgesia.

2.7.6 | Temporal association

The pre-registered measure of temporal association between the arm-raising effect and mechanical secondary hyperalgesia proved impractical to compute due to the imbalance between pinprick ratings (3 *per* condition combinations) and the patch ratings (1 *per* conditions combinations). Instead, we decided to use visual inspection of the marginal means (obtained from the previously described models) of the capsaicin patch rating in horizontal position (resting position), of the difference between the capsaicin patch ratings obtained in vertical and horizontal position (the 'arm raising' effect), and of the difference between the pinprick ratings obtained from the vehicle and capsaicin arms (secondary hyperalgesia). For ease of comparison, these marginal means were normalized by dividing them (for each variable separately) by the largest marginal mean, so that each time course saturated at 1.

3 | RESULTS

On average, the standardized measurements set took 5.23 ± 1.17 min to complete (baseline: 5.55 ± 2.09, time point 10: 6.30 ± 1.22, time point 20: 5.55 ± 0.94, time point 30: 5.37 ± 0.83, time point 40: 5.10 ± 0.64, time point 50: 4.80 ± 0.70, time point 60: 4.50 ± 0.51, time point 90: 5.10 ± 1.07, time point 120: 4.85 ± 0.88).

Right after removal of the capsaicin patch, a clear flare response was visible at the treated volar forearm, extending several centimetres outside the area of the patch. At the vehicle treated arm, no flare was detectable upon visual inspection.

3.1 | Patch perceptions

3.1.1 | Ratings

The estimated marginal means as well as the raw values for patch ratings stratified as a function of factors *time point*, *patch*, and *arm position* (all having significant effects, see Table 1) are represented in Figure 2.

Ratings at the vehicle patch were not significantly different from 0 (Table 2a), corresponding to no detectable perception. This absence of significant patch evoked perception appeared stable over time (Table 2e) and did not seem to be influenced by arm (Table 2c) or body position (Table 1).

The capsaicin patch evoked strong perceptions (Table 1, Table 2a) which increased quickly after patch application, plateaued around +20 min, and slowly decayed after patch removal (Table 1, Table 2d,f). Raising the arm while wearing the capsaicin patch produced a clear augmentation of these burning perceptions (Table 2b). This arm raising effect was significant at time points 30 to 90 (inclusive, Table 2g), corresponding to a period covering the second half of patch application and more than 30 minutes after removal. The comparison between the magnitude of the arm raising effect at consecutive time points (Table 2h) appeared slightly underpowered (difference of differences are hard to estimate) and it is therefore difficult to make strong statements about the temporal evolution of the arm raising effect (when does it plateau, when does it decay). We can however say that a significant reduction of the magnitude of the arm raising effect took place between time points 90 and 120 (30 and 60 min after patch removal, respectively).

Body position did not appear to influence the arm-raising effect (see Table 1; all factor combinations including 'body position' had $p > 0.05$ whereas all the other coefficients had $p \leq 0.001$), suggesting that it was the

TABLE 1 Results of the patch ratings ANOVA.

Factor	Sum Square	Mean square	Num DF	Den DF	*F* value	Pr (>F)
Time point	81275.894	10159.487	8	630	62.450	<0.001
Arm position	9709.381	9709.381	1	630	59.683	<0.001
Body position	20.522	20.522	1	18	0.126	0.727
Patch	370101.144	370101.144	1	630	2275.000	<0.001
Time point: arm position	4273.694	534.212	8	630	3.284	0.001
Time point: body position	215.950	26.994	8	630	0.166	0.995
Arm Position: Body Position	172.089	172.089	1	630	1.058	0.304
Time point: patch	81035.444	10129.431	8	630	62.265	<0.001
Arm position: patch	9679.996	9679.996	1	630	59.503	<0.001
Body position: patch	264.023	264.023	1	630	1.623	0.203
Time point: arm position: body position	170.061	21.258	8	630	0.131	0.998
Time point: arm position: patch	4158.800	519.850	8	630	3.196	0.001
Time point: body position: patch	247.878	30.985	8	630	0.190	0.992
Arm position: body position: patch	168.200	168.200	1	630	1.034	0.310
Time point: arm position: body position: patch	179.300	22.412	8	630	0.138	0.997

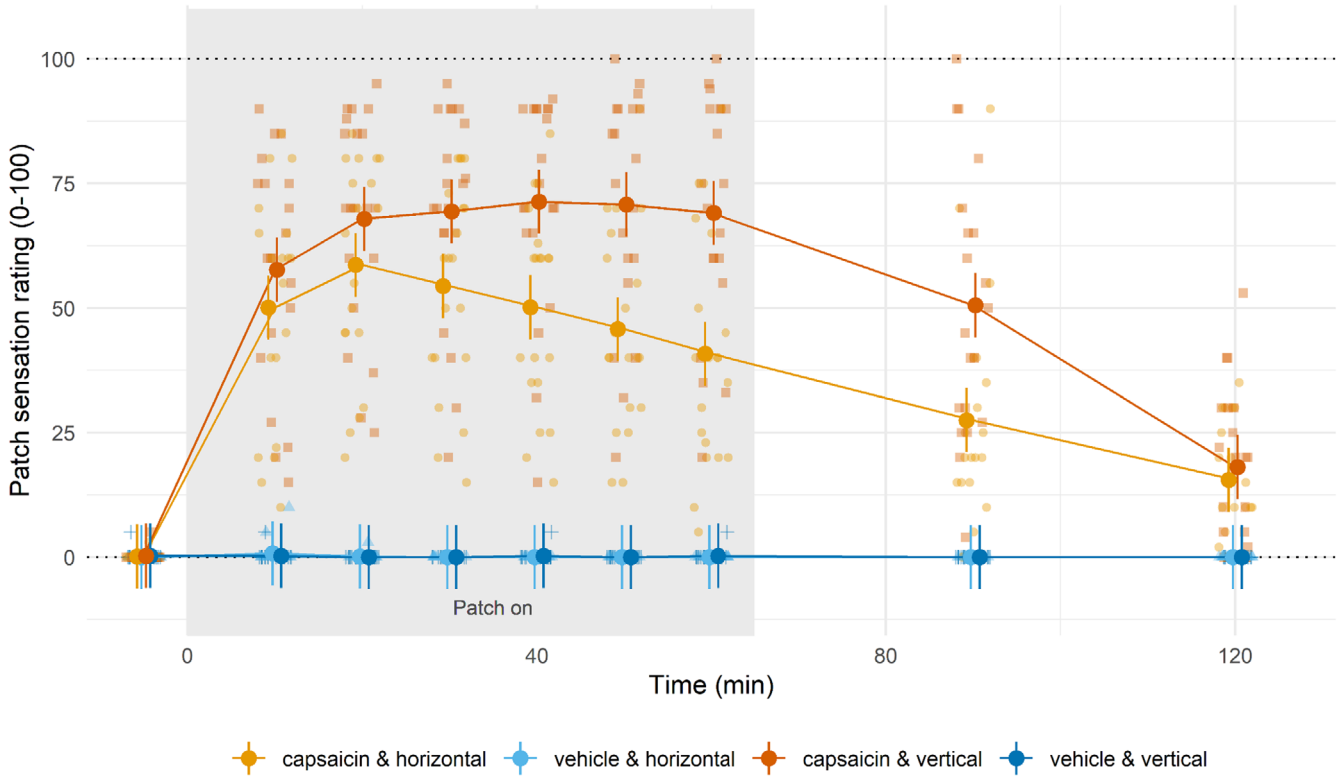

FIGURE 2 Perceived intensity of the perceptions elicited by the patches, over time and across conditions. Individual ratings are represented as translucent dots in the background. The solid dots and vertical lines represent the marginal means and 95% confidence intervals fitted by the model while averaging over the levels of 'body position' (as this factor had no significant effect on ratings). The solid lines connect the marginal means.

TABLE 2 Results of the patch ratings post-hoc tests.

PATCH RATINGS-POST HOC TESTS						
A. PR > 0						
Patch	**Est. M. Mean**	**SE**	**Df**	**T ratio**	***p* value $_{uncorrected}$**	***p* value $_{Bonferroni}$**
Capsaicin	45.450	1.755	21	25.898	<0.001	<0.001
Vehicle	0.106	1.755	21	0.060	0.476	0.953
B. PR vertical > PR horizontal					Patch: **Capsaicin**	
Contrast	**Estimate**	**SE**	**Df**	**T ratio**	**p value $_{uncorrected}$**	**p value $_{Bonferroni}$**
Vertical-horizontal	14.678	1.344	630	10.917	<0.001	<0.001
C. PR vertical > PR horizontal					**Patch: Vehicle**	
Contrast	**Estimate**	**SE**	**Df**	**T ratio**	***p* value $_{uncorrected}$**	***p* value $_{Bonferroni}$**
vertical-horizontal	0.011	1.344	630	0.008	0.497	0.994
D. PR T_t > 0				**Patch: Capsaicin**		
Contrast	**Est. M. Mean**	**SE**	**Df**	**T ratio**	***p* value $_{uncorrected}$**	***p* value $_{Bonferroni}$**
$T_{baseline}$	0.200	2.587	94	0.077	0.469	1.000
T_{10}	53.900	2.587	94	20.831	<0.001	<0.001
T_{20}	63.275	2.587	94	24.455	<0.001	<0.001
T_{30}	61.900	2.587	94	23.923	<0.001	<0.001
T_{40}	60.750	2.587	94	23.479	<0.001	<0.001
T_{50}	58.250	2.587	94	22.512	<0.001	<0.001
T_{60}	54.950	2.587	94	21.237	<0.001	<0.001
T_{90}	39.025	2.587	94	15.082	<0.001	<0.001
T_{120}	16.800	2.587	94	6.493	<0.001	<0.001
E. PR T_t > 0					**Patch: Vehicle**	
Contrast	**Est. M. Mean**	**SE**	**Df**	**T ratio**	***p* value $_{uncorrected}$**	***p* value $_{Bonferroni}$**
$T_{baseline}$	0.125	2.587	94	0.048	0.481	1.000
T_{10}	0.500	2.587	94	0.193	0.424	1.000
T_{20}	0.075	2.587	94	0.029	0.488	1.000
T_{30}	0.000	2.587	94	0.000	0.500	1.000
T_{40}	0.125	2.587	94	0.048	0.481	1.000
T_{50}	0.000	2.587	94	0.000	0.500	1.000
T_{60}	0.125	2.587	94	0.048	0.481	1.000
T_{90}	0.000	2.587	94	0.000	0.500	1.000
T_{120}	0.000	2.587	94	0.000	0.500	1.000
F. PR $T_t \neq$ PR T_{t-1}				**Patch: Capsaicin**		
Contrast	**Estimate**	**SE**	**Df**	**T ratio**	***p* value $_{uncorrected}$**	***p* value $_{Bonferroni}$**
T_{10}-$T_{baseline}$	53.700	2.852	630	18.829	<0.001	<0.001
$T_{20} - T_{10}$	9.375	2.852	630	3.287	0.001	0.009
$T_{30} - T_{20}$	−1.375	2.852	630	−0.482	0.630	1.000
$T_{40} - T_{30}$	−1.150	2.852	630	−0.403	0.687	1.000
$T_{50} - T_{40}$	−2.500	2.852	630	−0.877	0.381	1.000
$T_{60} - T_{50}$	−3.300	2.852	630	−1.157	0.248	1.000

TABLE 2 (Continued)

F. PR $T_t \neq$ PR T_{t-1}			Patch: Capsaicin			
Contrast	**Estimate**	**SE**	**Df**	**T ratio**	***p* value uncorrected**	***p* value Bonferroni**
$T_{90} - T_{60}$	−15.925	2.852	630	−5.584	<0.001	<0.001
$T_{120} - T_{90}$	−22.225	2.852	630	−7.793	<0.001	<0.001
G. PR (vertical-horizontal) T_t > PR (vertical-horizontal) $T_{baseline}$			**Patch: Capsaicin**			
Contrast	**Estimate**	**SE**	**Df**	**T ratio**	***p* value uncorrected**	***p* value Bonferroni**
T_{10}-$T_{baseline}$	7.500	5.704	630	1.315	0.095	0.756
T_{20}-$T_{baseline}$	9.150	5.704	630	1.604	0.055	0.437
T_{30}-$T_{baseline}$	14.900	5.704	630	2.612	0.005	0.037
T_{40}-$T_{baseline}$	21.100	5.704	630	3.699	<0.001	0.001
T_{50}-$T_{baseline}$	24.900	5.704	630	4.365	<0.001	<0.001
T_{60}-$T_{baseline}$	28.200	5.704	630	4.944	<0.001	<0.001
T_{90}-$T_{baseline}$	22.950	5.704	630	4.023	<0.001	<0.001
T_{120}-$T_{baseline}$	2.500	5.704	630	0.438	0.331	1.000
H. PR (vertical-horizontal) $T_t \neq$ PR (vertical-horizontal) T_{t-1}					**Patch: Capsaicin**	
Contrast	**Estimate**	**SE**	**Df**	**T ratio**	***p* value uncorrected**	***p* value Bonferroni**
T_{10}-$T_{baseline}$	7.500	5.704	630	1.315	0.189	1.000
$T_{20} - T_{10}$	1.650	5.704	630	0.289	0.772	1.000
$T_{30} - T_{20}$	5.750	5.704	630	1.008	0.314	1.000
$T_{40} - T_{30}$	6.200	5.704	630	1.087	0.277	1.000
$T_{50} - T_{40}$	3.800	5.704	630	0.666	0.506	1.000
$T_{60} - T_{50}$	3.300	5.704	630	0.579	0.563	1.000
$T_{90} - T_{60}$	−5.250	5.704	630	−0.920	0.358	1.000
$T_{120} - T_{90}$	−20.450	5.704	630	−3.585	<0.001	0.003

position of the arm relative to the ground (and not to the rest of the body) that drove the increase in perception while raising the arm (Table 1).

3.1.2 | Descriptors

Participants qualified the perception elicited by the capsaicin patch as 'warm', 'burning', 'pricking', or 'painful'. At the vehicle treated arm, the perception of the patch on the skin was reported by only a few participants. Frequencies of the different descriptors stratified by *time point*, *patch*, and *arm position* are reported in Table S1.

Reports of descriptors 'burning', 'pricking', and 'painful' were aggregated into a 'nociceptive' descriptor (1 if any of the three, 0 otherwise) and the frequency of nociceptive descriptors reports was modelled as a function of factors *time point*, *patch*, *body position*, and *arm position* using logistic regression. The estimated marginal means as well as the raw values stratified as a function of factors *time point*, *patch*, and *arm position* (all having significant effects, see Table S2) are represented in Figure 3.

The analysis revealed that participants were more likely to report 'burning', 'pricking', or 'painful' perceptions at the capsaicin patch site than at the vehicle patch site, that this probability quickly plateaued during patch application and slowly decreased after patch removal, and that this probability was higher when the arm was in the vertical rather than the horizontal position (Table S2 and S3). Body position did not appear to affect the probability of using one of these descriptors to describe patch perceptions (Table S2).

3.2 | Pinprick stimuli

3.2.1 | Ratings

For technical reasons, pinprick stimuli were only applied when the arms were in the horizontal resting position. The marginal means as well as the raw values for patch ratings stratified as a function of factors *time point* and *patch* (both having significant effects, see Table 3) are represented in Figure 4. Pinprick stimuli were clearly perceived on both

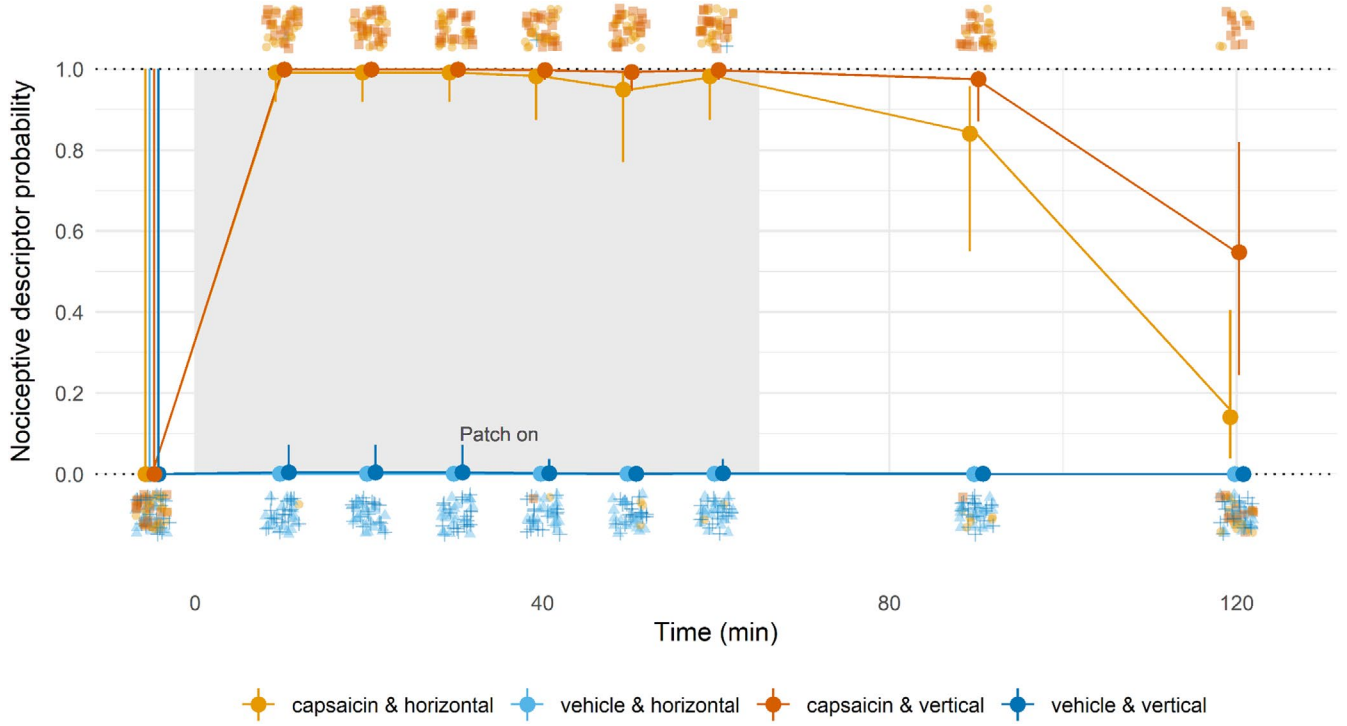

FIGURE 3 Qualitative assessment of perceptions elicited by patch: Probability of reporting a nociceptive descriptor (burning/pricking/painful). Individual responses are represented as translucent jittered dots in the background. The solid dots and vertical lines represent the marginal means and 95% confidence intervals fitted by the model. The solid lines connect the marginal means.

TABLE 3 Results of the pinprick ratings ANOVA.

Factor	Sum Square	Mean square	Num DF	Den DF	*F* value	Pr(>F)
Time point	18.253	2.282	8	990	13.748	<0.001
Patch	126.583	126.583	1	990	762.739	<0.001
Time point: patch	40.797	5.100	8	990	30.729	<0.001

forearms (Table 4a). Over time, ratings of pinprick stimuli delivered on the two forearms evolved in different directions: those recorded from the vehicle side tended to slightly decrease (Table 4c), whereas the capsaicin side ratings clearly increased (Table 4b). Secondary mechanical hyperalgesia, manifested by the difference of ratings observed between the arms, developed within the first 20′ of patch application and then plateaued and remained more or less stable until the end of the experiment (120′ past patch application, 60′ after patch removal; Table 4d,e).

3.2.2 | Descriptors

Participants qualified the perceptions elicited by the pinprick stimuli as 'touch', 'pricking', or in the case of stimuli delivered to the capsaicin treated arm- 'painful'. Frequencies of the different descriptors stratified by *time point* and *patch* are reported in Table S4.

Like for patch assessment, reports of descriptors 'burning', 'pricking', and 'painful' were aggregated into a 'nociceptive' descriptor (1 if any of the three, 0 otherwise) and were analysed using logistic regression. The estimated marginal means as well as the raw values stratified as a function of factors *time point* and *patch* (see Table S5) are represented in Figure 5.

The analysis revealed that participants were more likely to report 'nociceptive' descriptors for pinprick stimuli delivered on the capsaicin (rather than vehicle) treated arm both during and after patch application (see Table S5 and S6).

3.3 | Temporal association

As can be seen in Figure 6, the arm raising effect (on capsaicin patch ratings) and mechanical secondary hyperalgesia seemed to largely follow the same time course, which is very different from that of the capsaicin patch perceptions

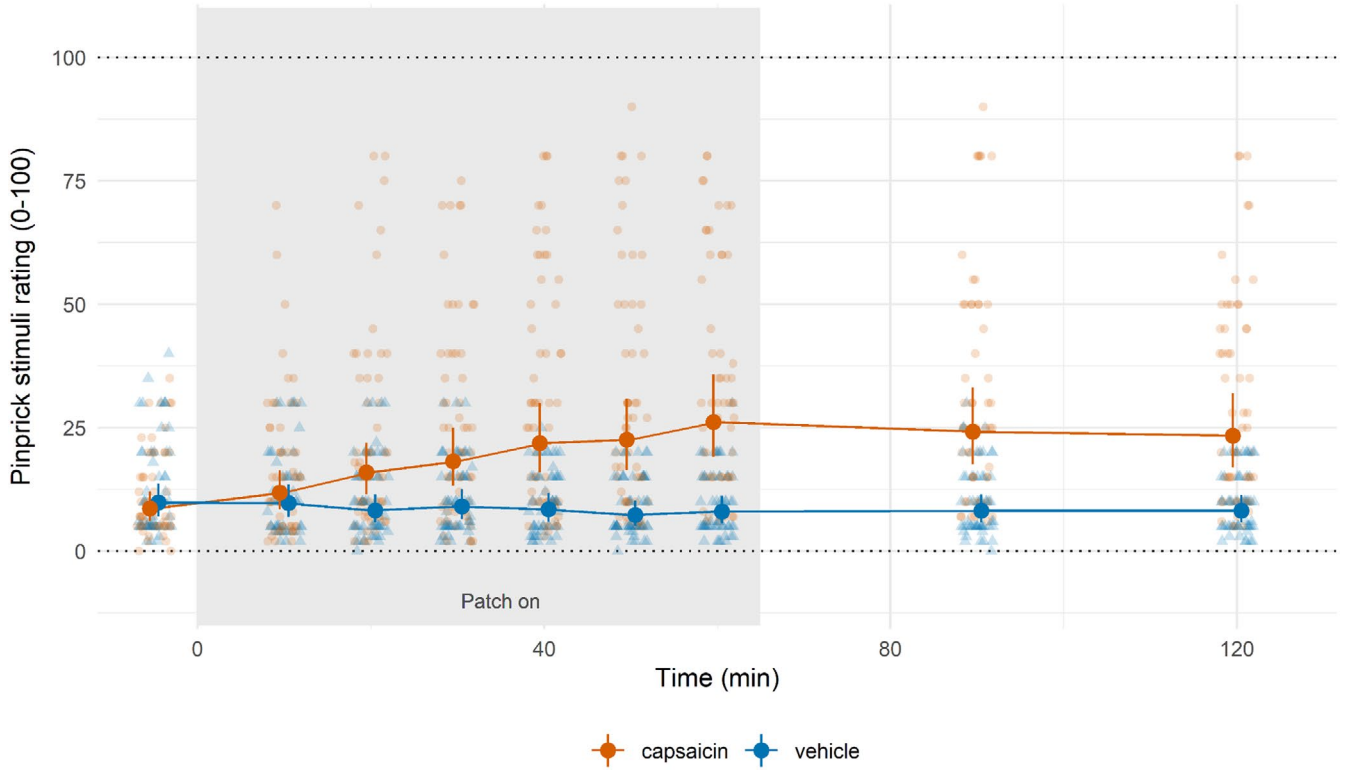

FIGURE 4 Perceived intensity of the pinprick stimuli, over time and across conditions. For technical reasons, pinprick stimuli were only applied when the arms were in the horizontal resting position. Individual ratings are represented as translucent dots in the background. The solid dots and vertical lines represent the marginal means and 95% confidence intervals fitted by the model. The solid lines connect the marginal means.

recorded in the horizontal resting position. However, the last set of measurements revealed that the arm raising effect and secondary hyperalgesia time courses were in fact distinct, as they dramatically diverged at this later time point. Indeed, whereas virtually no difference could be observed between the 90 and 120 time points for pinprick hyperalgesia, the arm raising effect, which was near maximal at 90 min post patch application, had (almost) completely disappeared at 120 min (and was not significant anymore, Table 2g).

4 | DISCUSSION

The present data confirms that raising the arm during topical capsaicin treatment leads to a significant increase of the pain experienced at the site of the patch (arm raising effect). This effect appears to be related to the position of the arm relative to ground rather than to the body and is therefore likely caused by gravity. In parallel and as expected, we also observed the development of an increased sensitivity to pinprick stimuli (mechanical secondary hyperalgesia) in the skin area adjacent to the capsaicin patch. Inspection of their temporal evolution revealed that the 'spontaneous' capsaicin-evoked pain, the arm raising effect, and secondary mechanical hyperalgesia followed three distinct time courses.

4.1 | The arm raising effect is likely caused by gravity-induced changes in the vascular system

Raising the arm from a horizontal to a vertical position and maintaining that position involves changes in the activity of various muscles of the arm and back. However, it seems unlikely that these changes are causing the arm raising effect as the muscles which would be the most involved in holding the arm vertically (*triceps brachialis* for elbow extension, the muscles involved in shoulder flexion and stabilization) are not located close to the patch (Hamill & Knutzen, 2006). Similarly, the muscles situated directly below the treated skin are mostly involved in mobilization of the wrist/hand and are not particularly involved in maintaining the arm in raised position (Hamill & Knutzen, 2006).

Furthermore, preliminary data collected during piloting (reported as Supplementary Materials) shows that there is no difference in pain ratings when participants actively raise their arm vertically compared to when their arm is held in that position passively by the experimenter. Even though we

TABLE 4 Results of the pinprick ratings post-hoc tests.

PINPRICK RATINGS (LOG-TRANSFORMED)-POST HOC TESTS

A. PPR >0

Patch	Est. M. Mean	SE	Df	T ratio	*p* value $_{uncorrected}$	*p* value $_{Bonferroni}$
Capsaicin	2.958	0.146	18	20.326	<0.001	<0.001
Vehicle	2.255	0.146	18	15.498	<0.001	<0.001

B. PPR $T_t \neq$ PPR $T_{baseline}$ Patch: Capsaicin

Contrast	Estimate	SE	Df	T ratio	*p* value $_{uncorrected}$	*p* value $_{Bonferroni}$
T_{10}-$T_{baseline}$	0.288	0.076	990	3.770	<0.001	0.001
T_{20}-$T_{baseline}$	0.565	0.076	990	7.406	<0.001	<0.001
T_{30}-$T_{baseline}$	0.691	0.076	990	9.053	<0.001	<0.001
T_{40}-$T_{baseline}$	0.866	0.076	990	11.353	<0.001	<0.001
T_{50}-$T_{baseline}$	0.896	0.076	990	11.740	<0.001	<0.001
T_{60}-$T_{baseline}$	1.037	0.076	990	13.584	<0.001	<0.001
T_{90}-$T_{baseline}$	0.962	0.076	990	12.604	<0.001	<0.001
T_{120}-$T_{baseline}$	0.928	0.076	990	12.160	<0.001	<0.001

C. PPR $T_t \neq$ PPR $T_{baseline}$ Patch: Vehicle

Contrast	Estimate	SE	Df	T ratio	*p* value $_{uncorrected}$	*p* value $_{Bonferroni}$
T_{10}-$T_{baseline}$	−0.010	0.076	990	−0.134	0.893	1.000
T_{20}-$T_{baseline}$	−0.155	0.076	990	−2.036	0.042	0.336
T_{30}-$T_{baseline}$	−0.072	0.076	990	−0.940	0.348	1.000
T_{40}-$T_{baseline}$	−0.132	0.076	990	−1.733	0.083	0.668
T_{50}-$T_{baseline}$	−0.265	0.076	990	−3.475	0.001	0.004
T_{60}-$T_{baseline}$	−0.182	0.076	990	−2.391	0.017	0.136
T_{90}-$T_{baseline}$	−0.162	0.076	990	−2.118	0.034	0.275
T_{120}-$T_{baseline}$	−0.164	0.076	990	−2.145	0.032	0.257

D. PPR (capsaicin-vehicle) T_t >0

Contrast	Estimate	SE	Df	T ratio	*p* value $_{uncorrected}$	*p* value $_{Bonferroni}$
$T_{baseline}$	−0.117	0.076	990	−1.532	0.937	1
T_{10}	0.181	0.076	990	2.372	0.009	0.081
T_{20}	0.604	0.076	990	7.911	<0.001	<0.001
T_{30}	0.646	0.076	990	8.46	<0.001	<0.001
T_{40}	0.882	0.076	990	11.554	<0.001	<0.001
T_{50}	1.044	0.076	990	13.684	<0.001	<0.001
T_{60}	1.102	0.076	990	14.443	<0.001	<0.001
T_{90}	1.007	0.076	990	13.19	<0.001	<0.001
T_{120}	0.975	0.076	990	12.773	<0.001	<0.001

E. PPR (capsaicin-vehicle) $T_t \neq$ PPR (capsaicin-vehicle) T_{t-1}

Contrast	Estimate	SE	Df	T ratio	*p* value $_{uncorrected}$	*p* value $_{Bonferroni}$
T_{10}-$T_{baseline}$	0.298	0.108	990	2.760	0.006	0.047
T_{20}–T_{10}	0.423	0.108	990	3.917	<0.001	0.001
T_{30}–T_{20}	0.042	0.108	990	0.389	0.698	1.000
T_{40}–T_{30}	0.236	0.108	990	2.187	0.029	0.232

TABLE 4 (Continued)

E. PPR (capsaicin-vehicle) $T_t \neq$ PPR (capsaicin-vehicle) T_{t-1}						
Contrast	**Estimate**	**SE**	**Df**	**T ratio**	***p* value $_{uncorrected}$**	***p* value $_{Bonferroni}$**
T_{50}–T_{40}	0.163	0.108	990	1.506	0.132	1.000
T_{60}–T_{50}	0.058	0.108	990	0.537	0.592	1.000
T_{90}–T_{60}	−0.096	0.108	990	−0.886	0.376	1.000
T_{120}–T_{90}	−0.032	0.108	990	−0.295	0.768	1.000

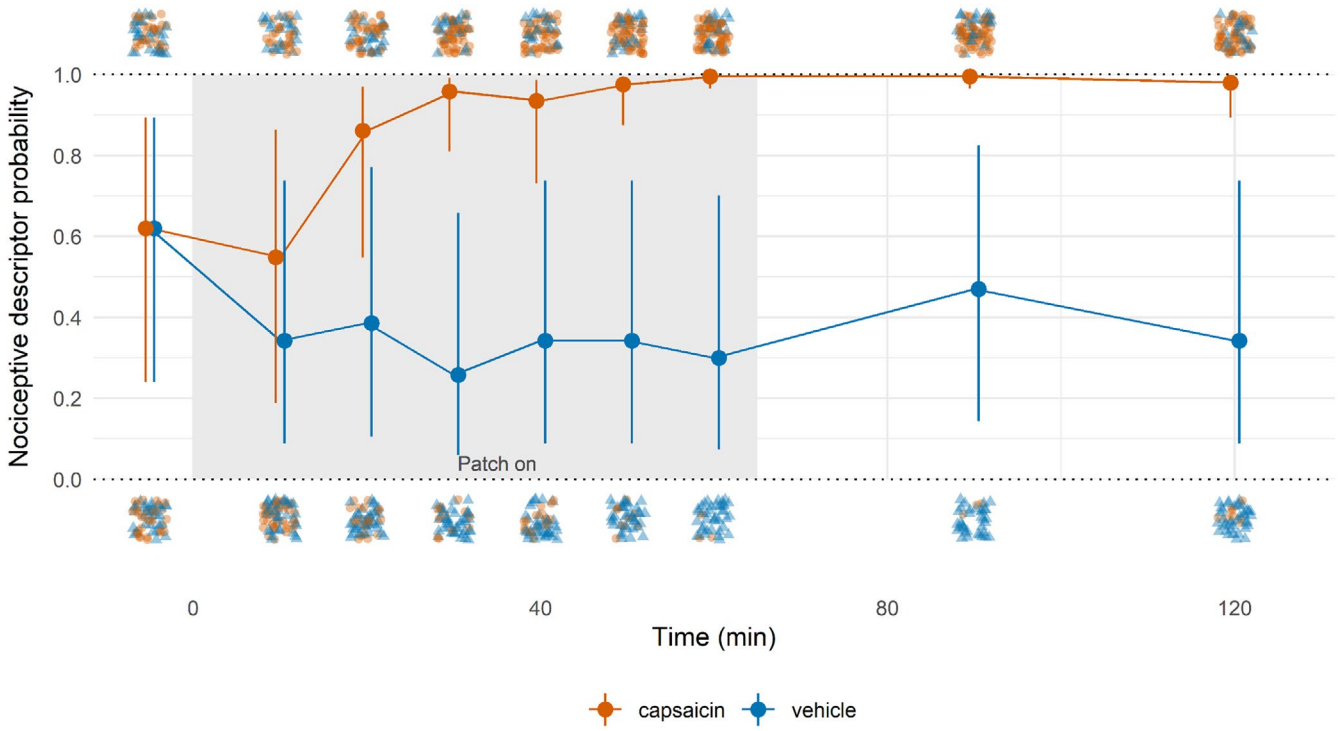

FIGURE 5 Qualitative assessment of perceptions elicited by pinprick stimuli: Probability of reporting a nociceptive descriptor (burning/pricking/painful). For technical reasons, pinprick stimuli were only applied when the arms were in the horizontal resting position. Individual responses are represented as translucent jittered dots in the background. The solid dots and vertical lines represent the marginal means and 95% confidence intervals fitted by the model. The solid lines connect the marginal means.

cannot rule out muscular activity in the passive mobilization condition (the reason why this manipulation was not included in the final study) this experimental manipulation should have led to more muscular activity in the active than the passive condition and, therefore, to changes in the pain ratings if the arm raising effect is indeed driven by muscular activity. This was not the case.

A more likely explanation is that the arm raising effect is related to changes in the vascular system. When the arm is raised, a large proportion of the blood contained in that limb is drained by gravity, superficial veins collapse and local blood pressure suddenly drops (Pappano & Gil Wier, 2013). Given the relatively modest volume of blood contained in a single upper limb, this is not sufficient to activate baroreceptors and trigger a systemic response (Low, 2004). Instead, the preservation of perfusion relies primarily on the veno-arteriolar reflex, a local mechanism countering changes in transmural pressure by adapting the degree of vasodilation/vasoconstriction of the blood vessels (Low, 2004). The fact that reducing blood flow by means of tourniquet constriction, rather than gravity, also leads to a sudden increase in the pain caused by capsaicin injection seems to corroborate this explanation (Byas-Smith et al., 1999).

4.2 | The arm raising effect is not another manifestation of the heterosynaptic LTP reflected by pinprick hypersensitivity of the skin

The development of the arm raising effect and of mechanical hyperalgesia appeared to be strongly correlated for

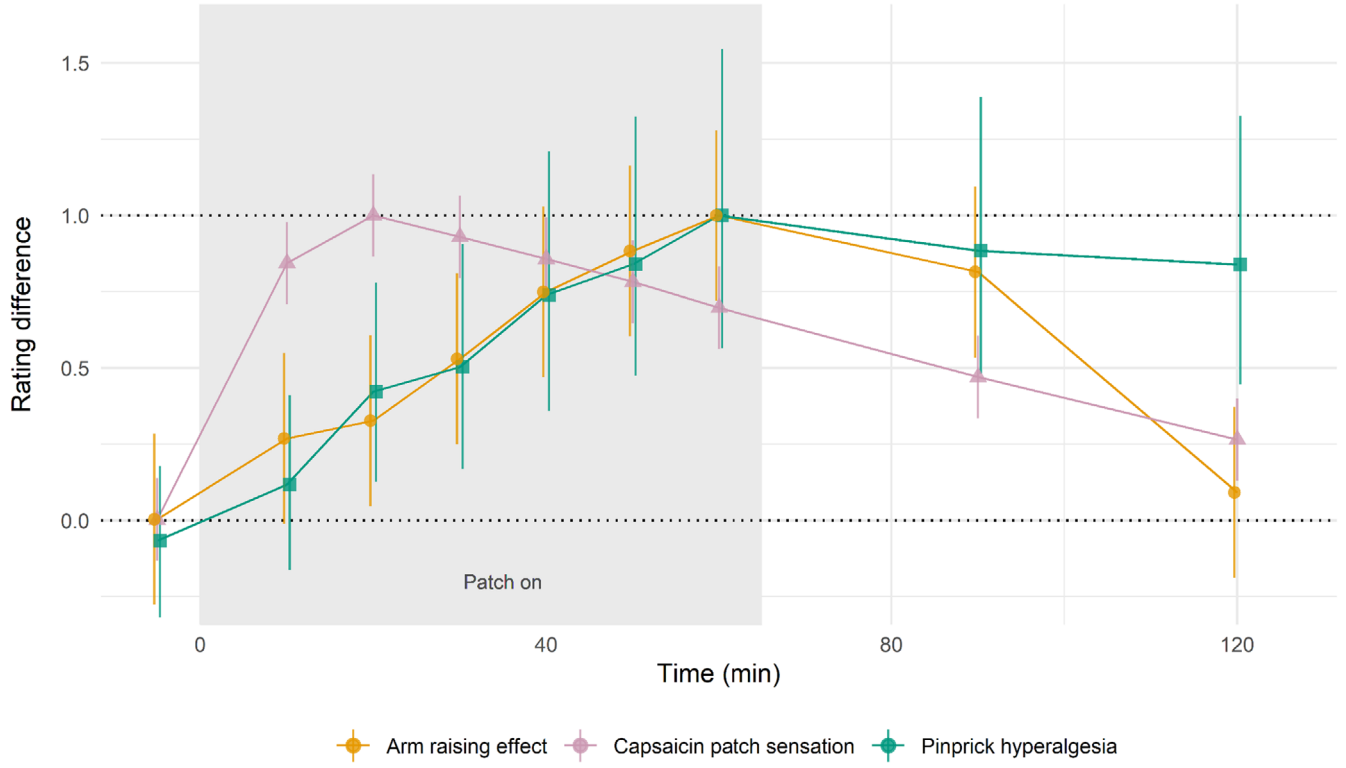

FIGURE 6 Normalized temporal evolution of the capsaicin patch perception, the arm raising effect on the capsaicin patch perception, and the capsaicin-driven mechanical hyperalgesia. The solid dots and vertical lines represent the marginal means and 95% confidence obtained from the 'patch rating' and 'pinprick rating' models. They were normalized so that the maximal marginal mean for each time course is equal to one. Solid lines connect the marginal means. The time course of the 'capsaicin patch perception' (pink) corresponds to the marginal mean for capsaicin patch ratings with the arm at rest (horizontal position), regardless of body position (shown not to affect these ratings). The 'arm raising effect' time course (orange) corresponds to the marginal difference between capsaicin patch ratings obtained in the two arm positions (vertical-horizontal), regardless of body position. The 'pinprick hyperalgesia' time course (green; only assessed on arms in the horizontal resting position) corresponds to the marginal difference between intensity ratings of pinprick stimuli delivered on the two forearms (capsaicin-vehicle). For ease of comparison, the time courses were normalized by dividing (for each variable separately) by the largest marginal mean, so that each time course saturated at 1.

the first hour (when the patch was on the skin), suggesting that the strong and sustained activation of epidermal nociceptors sensitized by capsaicin might be a common driving factor of both phenomena. This initial association is reminiscent of the observation by Byas-Smith et al. that tourniquet vascular occlusion led to an increase of capsaicin pain only after pinprick hyperalgesia had developed (Byas-Smith et al., 1999).

As secondary hyperalgesia is thought to reflect heterosynaptic LTP in the dorsal horn nociceptive pathways, Byas-Smith's observation led us to postulate that the arm raising effect could be another perceptual manifestation of this mechanism, amplifying the input of vascular sensory fibres in addition to that of mechanosensitive epidermal nociceptors (Henrich et al., 2015). This experiment was designed to test that hypothesis. The strong dissociation of time courses between the arm-raising effect (completely gone 60′ after patch removal) and secondary hyperalgesia at the skin (almost no decrease between patch removal and 60′ later) at later time points seems to rule out LTP as a mechanism. The relatively short half-life of the arm raising effect is reminiscent of the time course of dynamic mechanical allodynia (another form of heterosynaptic potentiation that can be induced by capsaicin) (Pfau et al., 2011). Unfortunately we did not measure it in this experiment and it is therefore hard to determine if they have the same time-course.

4.3 | Speculative mechanisms of the arm raising effect

One explanation of the arm raising effect could be that the decreased blood flow during arm raising leads to changes in the concentration of chemicals in the skin/perivascular milieu which would in turn modulate the activity of nociceptors and drive the increase in pain. For example, reduced blood flow could lead to a reduced

clearance and therefore accumulation of chemical compounds released in reaction to capsaicin application. Alternatively, it could lead to a local hypoxia to which certain cells react by releasing vasodilatory chemicals which could also be pronociceptive, such as nitric oxide (Holthusen & Arndt, 1994; Pappano & Gil Wier, 2013). However, such mechanisms relying on diffusional changes in chemical concentration in the extracellular milieu are bound to be rather slow and therefore cannot account for the large and quick pain increase observed upon arm elevation (sometimes within less than 2 s) and the similarly fast decrease in pain perception upon return to the resting position.

Another explanation could rely on the peripheral sensitization of nociceptive fibres innervating the perivascular space or blood vessels, which could be responsive to vascular changes caused by the arm raising procedure (e.g. vascular wall stretch) and therefore cause the increase in pain directly. Arndt and Klement have described polymodal nociceptors innervating vein walls which were sensitive to stretch and other physical stimuli (Arndt & Klement, 1991; Klement & Arndt, 1991, 1992). It has also been shown that the presence of capsaicin in the perivascular space leads to pain (Arndt et al., 1993). More recently, it has been suggested that not all small fibres innervating the arteriole-venule shunts are sympathetic but that some are nociceptors (Albrecht et al., 2013; Bowsher et al., 2009). Such nociceptors could be sensitized directly by capsaicin. In this case the delayed onset of the arm raising effect would be explained by the diffusion time necessary for capsaicin to reach deeper skin layers. The disappearance of the arm raising effect before spontaneous capsaicin pain completely fades away could similarly be attributed to a faster capsaicin clearance in the vicinity of the blood vessels (Babbar et al., 2009; Chanda et al., 2008).

Alternatively, sensitization of these nerve fibres could be caused by other chemicals released in reaction to the topical application of capsaicin (Braga Ferreira et al., 2020). However, to be compatible with the temporal evolution of the arm raising effect (i.e. delayed onset compared to 'spontaneous' burning perceptions and steady increase over time), the concentration and effect of these compounds would need to steadily increase until patch removal, a pattern which cannot be ruled out but does not appear likely.

One could also imagine that sensitization of vascular innervation (sensory or sympathetic) does not directly gives rise to the increased pain but does so by modulating the gating of nociceptive information coming from vascular and/or epidermal nociceptors (viscero-somatic convergence) (Jänig, 2014; Schwartz & Gebhart, 2014).

Finally, the involvement of vascular nociceptors is perhaps supported by the fact that, in the experience of the authors, the pain when the arm was raised felt deeper and more widespread than when the arm was at rest. This pain also did not seem to habituate and the quality of the pain perception was different in that it had a sharp, stabbing and pulsatile quality that the 'spontaneous' capsaicin pain did not have. These observations are reminiscent of the description of vascular pain provided by Arndt & Klement (Arndt & Klement, 1991).

Several of the aforementioned mechanisms rely on peripheral sensitization of nerve fibres. Interestingly, several non-neural cells also express TRPV1 which has been linked to the rapid regulation of myogenic tone (Jackson, 2022; Phan et al., 2022). In the skin, non-neural cells like Schwann cells and keratinocytes have been shown to contribute to the detection of noxious stimuli and pain perception (Abdo et al., 2019; Pang et al., 2015). It therefore could be possible that vascular smooth muscle cells or endothelial cells directly contribute to peripheral sensitization.

4.4 | Conclusion

In this study, we confirmed our previous observation that changes in limb position can dramatically modulate the pain perception caused by topical capsaicin. Even though the exact mechanism underlying this increase in pain perception is not entirely clear, it seems to involve nerve fibres innervating blood vessels. Knowledge of the sensory innervation of blood vessels and of possible crosstalks between the autonomic and nociceptive systems remains limited. In the future, the phenomenon described in this paper could prove a useful experimental model to investigate these topics.

AUTHOR CONTRIBUTIONS

ASC, AM and SGAvN designed the experiment. ASC and CK collected the data. ASC analysed the data and produced the figures. ASC drafted the manuscript. All authors discussed the results and commented on the manuscript. AM and ASC provided funding for the experiments.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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