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How the heart speaks to the brain:
neural activity during cardiorespiratory
interoceptive stimulation

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Prominent theories emphasize key roles for the insular cortex in the central representation of interoceptive sensations, but how this brain region responds dynamically to changes in interoceptive state remains incompletely understood. Here, we systematically modulated cardiorespiratory sensations in humans using bolus infusions of isoproterenol, a rapidly acting peripheral beta-adrenergic agonist similar to adrenaline. To identify central neural processes underlying these parametrically modulated interoceptive states, we used pharmacological functional magnetic resonance imaging (phMRI) to simultaneously measure blood-oxygenation-level dependent (BOLD) and arterial spin labelling (ASL) signals in healthy participants. Isoproterenol infusions induced dose-dependent increases in heart rate and cardiorespiratory interoception, with all participants endorsing increased sensations at the highest dose. These reports were accompanied by increased BOLD and ASL activation of the right insular cortex at the highest dose. Different responses across insula subregions were also observed. During anticipation, insula activation increased in more anterior regions. During stimulation, activation increased in the mid-dorsal and posterior insula on the right, but decreased in the same regions on the left. This study demonstrates the feasibility of phMRI for assessing brain activation during adrenergic interoceptive stimulation, and provides further evidence supporting a dynamic role for the insula in representing changes in cardiorespiratory states.

This article is part of the themed issue 'Interoception beyond homeostasis: affect, cognition and mental health'.

1. Introduction

Interoception refers to the process by which the nervous system senses and integrates information about the inner state of the body. It is multifaceted, covers both non-conscious and conscious levels of information processing, and encompasses physiological systems critical to body regulation, maintenance of homeostasis and survival. These include the cardiac, respiratory, gastrointestinal, genitourinary, humoral and immune systems [1–3]. Neuroanatomical evidence from animal and human species suggests a central role for the insular cortex in the integration and representation of interoceptive signals [4–7]. It is considered the principal cortical target receiving information about interoceptive body states, and functional neuroimaging studies have revealed it to be a consistently engaged region [8,9].

Various degrees of overlap in the representation of functions within the human insula have been described [10,11]. An anterior–posterior gradient has

been observed, with posterior regions activating during interoceptive stimulation and anterior regions activating during anticipation [12,13]. The human insula has been parsed functionally into different subregions based on the type of task (e.g. sensorimotor, cognitive, chemosensory and socio-emotional), with regions of the dorsal-anterior insula showing conjunction across most categories [14]. The dorsal anterior insula, predominantly on the right, has been subsequently included as part of a putative 'salience network', one of the functions of which is involved in the detection of incoming sensory information that is novel and relevant to the body [15]. A left–right asymmetry has also been proposed such that interoceptive information transmitted from peripheral body pathways arrives in the mid/posterior insula bilaterally, is integrated with other inputs and is then re-represented in the right anterior insula to yield a basis for self-awareness [9].

Numerous approaches exist for imaging interoceptive functions in humans. The most common methods measure brain activation when actively perturbing the body to stimulate body sensations, or under resting physiological conditions when attention is volitionally directed towards particular body sensations. Perturbation studies identify insula involvement during stimulation of the respiratory [16], gastrointestinal [17] and urinary [18] systems. Within the cardiac system, few stimulation studies exist. Most have assessed heartbeat interoception under resting physiological conditions. In each case, these resting studies have observed activation of the insula, although not always in the same regions. For example, tasks emphasizing interoceptive accuracy for heartbeat sensations tend to activate the anterior insula [19–21], whereas tasks emphasizing interoceptive attention tend to activate more posterior regions, specifically the dorsal mid-insula [22–24]. Although correlations between interoceptive accuracy and right insula activation have been observed in some studies, the fact that most individuals fail to accurately detect heartbeat sensations at rest [25] raises some uncertainty about the degree to which the observed insula activations directly reflect subjective experience.

One noteworthy stimulation study used continuous infusions of isoproterenol to target brain regions responding to cardiorespiratory stimulation during positron emission tomography neuroimaging [26]. Isoproterenol peripherally stimulates beta-1 and beta-2 adrenergic receptors in the heart and lungs, resulting in stronger and faster heartbeats and bronchial airway dilation. In this between-subjects design, regional glucose metabolism was evaluated in response to sustained elevations in heart rate (120 beats per minute for 30 minutes). The main findings were increased cardiorespiratory sensations in the isoproterenol group and activation in the truncal region of somatosensory cortex, followed by insula activation in subgroup and region of interest analyses. There were no within-subject comparisons in this study, no parametric modulations of activity, no correlations between brain signals and subjective reports, and there have been no follow-up studies of this type in the functional magnetic resonance imaging (fMRI) environment to date.

Despite the prevailing focus on the insula, additional brain systems are also commonly implicated in interoceptive experiences. For example, fMRI studies of interoceptive stimulation identify the involvement of other body-sensitive brain regions including the primary and secondary somatosensory cortices, anterior cingulate cortex, fronto-parietal operculum, prefrontal cortex, caudate, thalamus, cerebellum and brainstem [27–30]. Lesion studies corroborate a role for several of these neural regions including the insula, but also the somatosensory

afferent pathways, basolateral amygdala and others [31–36]. These findings reinforce the notion that there might be multiple pathways contributing to interoceptive processing, as well as the possibility that a network of brain regions is involved [31].

Isoproterenol's peripheral receptor targets, minimal blood–brain barrier passage and rapid half-life [37,38] seem to indicate that it might produce few direct effects on the brain and be suitable for investigating brain activity during fMRI. However, introducing pharmacological agents into fMRI can potentially disrupt neurovascular coupling and in so doing, make results more difficult to interpret [39]. Isoproterenol is known to lower diastolic blood pressure through vasodilatory effects on arterial smooth muscle, and it is possible that this could perturb cerebral blood flow resulting in disrupted fMRI signals. On the other hand, the magnitude of this blood pressure decrease is small, and the cerebrovasculature has an intrinsic ability to maintain cerebral blood flow constant over a wide range of blood pressures [40]. We therefore found it reasonable to investigate this method in the fMRI environment. Given these uncertainties, for this initial fMRI study of isoproterenol's effects on the brain we elected to concurrently measure two signals on different sides of the vascular bed: arterial and venous side using arterial spin labelling (ASL) and blood-oxygenation-level dependent (BOLD), respectively.

Taking these considerations into account, we hypothesized that it would be feasible to record brain activation during interoceptive stimulation with isoproterenol. We hypothesized that the insula would exhibit activation during periods of cardiorespiratory sensation induced by isoproterenol. Given the available lesion and functional neuroimaging evidence, we also considered the possibility that sensory regions beyond the insula might also demonstrate activation.

2. Material and methods

(a) Participants

Twenty-one healthy individuals (15 female, 20 right handed) between the ages of 18 and 58 years (mean = 23.8, s.d. = 8.5) participated in this study. These participants were of normal body weight (body mass index = 22.4, s.d. = 4.9), did not have any life-long history of neurological, psychiatric, diabetic, cardiovascular or respiratory disorders, all were unmedicated, and demonstrated normal vital signs and 12-lead electrocardiograms.

(b) Experimental design

During the fMRI scan session participants received four intravenous bolus infusions containing either isoproterenol or saline, in a single blinded manner, and under constant physician observation. Isoproterenol was administered in three different doses (0.25, 1 and 2 µg) in order to parametrically modulate cardiorespiratory state and subsequently assess cardiorespiratory sensations [25,41]. A saline infusion was used as a control. The order of saline and isoproterenol infusions was pseudorandomized via a Latin square design to minimize the effects of dose order on the group-level results. During each infusion scan, which lasted 240 s, participants were asked to 'pay attention to your ongoing experience of body sensations and emotions', while keeping their eyes open and fixating on a white cross (figure 1a). This instruction was purposefully vague in order to avoid biasing subjects' attention towards a specific bodily focus or feeling state. There was no other task and no motor responses were required. Forty seconds into the infusion participants heard a verbal prompt ('get ready') followed by an auditory countdown to infusion. At 60 s, the countdown ended and the

Figure 1. (a) Experimental design for the concurrent BOLD/ASL isoproterenol infusion study. (b) Mean heart rate changes during the saline, 0.25, 1 and 2 mcg infusion scans. (c) Interoceptive detection rates for each infusion. (d) Body map showing the proportional location of heartbeat sensations across study participants at 2 mcg. The chest showed the greatest overlap.

infusion was delivered. Infusions were delivered a minimum of 5 min apart to allow for clearance of isoproterenol from the circulation. Cardiac and respiration waveforms were acquired concurrently during all scans at 1 kHz via MRI compatible pulse-oximeter and respiratory belt (Biopac MP150). In addition to infusion scans, there were two 300 s eyes-open resting-state scans, one before infusions and one after, which were conducted as a part of another study. Vital signs were recorded pre- and post-scan for safety monitoring.

(c) Behavioural data acquisition

Upon exiting the scanner participants retrospectively reported if they had detected any changes in their cardiorespiratory sensations during each infusion. They also rated heartbeat, breathing and anxiety intensity experienced during the 2 mcg infusion, and traced the location of perceived heartbeat sensations on a manikin. To evaluate whether infusions in the pharmacological functional MRI (phMRI) environment had a prolonged effect on anxiety levels, we measured state anxiety immediately before and after scanning [42] in a subset of participants ($n = 18$). We also assessed a trait measure of interoceptive self-report in this subset [43].

(d) MRI data acquisition

MRI imaging was performed on a 3 T MRI scanner (Siemens Trio), while participants lay supine. Head movement was minimized with foam padding on both sides of the head and by securing tape to the forehead. fMRI data based on BOLD contrast and ASL perfusion contrast were simultaneously acquired using a pseudo-continuous ASL (pCASL) sequence with dual-echo gradient echo planar imaging [44]. Fundamental imaging parameters were as follows: repetition time (TR) 3.5 s, echo times (TE1 and TE2) 10 and 25 ms, flip angle 90° , 64×64 matrix size, 18 slices

(recorded sequentially from bottom to top), field of view 220 mm, voxel size $3.4 \times 3.4 \times 5 \text{ mm}^3$ and GRAPPA acceleration factor 2. For spin tagging, the tagging plane was positioned 90 mm inferior to the centre of the imaging slab with a labelling duration of 1500 ms and post-labelling delay of 1000 ms. A T1-weighted MP-RAGE (magnetization-prepared rapid gradient-echo; field of view $250 \times 250 \text{ mm}$, flip angle 9° , slice thickness 1 mm, TR 1900 ms, TE 2.26 ms, voxel size 1 mm^3) provided detailed brain anatomy for registration with functional data.

(e) Pharmacological functional magnetic resonance imaging data analysis

For each subject and each run, all functional volumes were realigned using a six parameter rigid-body spatial transformation to the first volume, and then spatially smoothed using a three-dimensional Gaussian kernel with full-width half-maximum of 6 mm in AFNI [45]. The first four volumes were discarded to allow the MR signals to reach steady state. Time locked fluctuations in the respiratory and cardiac frequencies and their first harmonics were removed using the RETROICOR procedure applied separately on tag and control images using a custom Matlab code [46,47]. Slice acquisition times were considered in the RETROICOR analysis, and signal mean, linear and quadratic trends were removed. No additional temporal band-pass filtering was performed. Perfusion images were calculated using pair-wise subtraction between control and label images [48]. Subject-level maps of brain responses to parametric modulation of cardiorespiratory state were generated using a block averaging method. Five blocks were defined on the basis of the experimental design and observed group average heart rate changes during the isoproterenol infusions, including baseline (0–40 s), preparatory (40–60 s), anticipatory (60–80 s), peak (80–120 s) and recovery

(120–160 s) periods (figure 1b). Contrast maps for the peak versus baseline and recovery versus baseline periods were generated separately for each infusion run, at the single subject level. For the preparatory versus baseline and anticipatory versus baseline periods, to equate block duration, only the first 20 s of the baseline period were used and all four infusions were combined using a fixed-effects analysis. All the contrast maps were generated in the subject's native space and for the computation of group maps they were spatially transformed to the Montreal Neurological Institute (MNI) 152 atlas space using an affine transformation in FSL [49] and an in-house developed procedure. In this procedure, subject-specific anatomical scans were co-registered to the first volume of functional scans, brain masks were extracted using FSL [50], and then affine transformation parameters were calculated by registering subject-specific structural brain images to the T1-weighted MNI brain (2 mm voxel size). Group-level statistical maps of brain activation were generated by averaging contrast maps across subjects and estimating the variance using a random effects analysis. Statistical maps were thresholded at $p < 0.005$ (uncorrected). A cluster size analysis based on random field theory was performed to determine the statistical significance of above threshold clusters at $p < 0.05$ (corrected).

3. Results

Isoproterenol elicited significant dose-dependent increases in heart rate (dose effect: $F_{3,80} = 93.6$, $p < 10^{-4}$; figure 1b). As expected, cardiorespiratory sensations were detected in a dose-dependent manner, with all participants correctly reporting increases at the 2 mcg dose (figure 1c). Heartbeat sensations for this dose were localized primarily to the chest, neck and head, but also to the abdomen and back (data available for 18 subjects; figure 1d). We did not observe any significant vital sign changes or prolonged anxiety following scan completion, though participants retrospectively reported experiencing increased anxiety during the 2 mcg infusion (electronic supplementary material, table S1).

We began our analysis at 2 mcg, as this was the only dose for which all participants reported increased sensation. A voxelwise whole brain analysis of the BOLD signal at this dose for the peak versus baseline contrast revealed a significant cluster of activation in the right mid-dorsal and posterior insula (voxelwise threshold at $p < 0.005$ and cluster size corrected for multiple comparisons threshold at $p < 0.05$; figure 2a; see the electronic supplementary material, table S2 for a complete list of clusters). Identical analysis of the ASL signal for the peak versus baseline contrast at 2 mcg also revealed increased activation in a similar region of the right insula, although this did not meet the same threshold for significance as the BOLD signal (electronic supplementary material, figure S1).

We also observed a general dose-related increase in the BOLD signal in the right mid-dorsal and posterior insula (figure 2b; electronic supplementary material, figure S2). However, at the 1 mcg dose we identified six individuals whose right insula signal was elevated at baseline, and excluded them from the analysis. Owing to the pseudorandomization order these participants received the 1 mcg dose after 2 mcg, and we speculated that they might have guessed the alternating order of low versus high doses leading to increased anxious anticipation during the baseline period. In support of this possibility, further investigation revealed that these subjects reported the highest palpitation ratings at 2 mcg (average 9.75 versus 8.26 for whole group) and physical anxiety (7.25 versus 6.13 for whole group; see 'Limitations' for further

Figure 2. (a) Voxelwise whole brain analysis reveals activation of the right mid-dorsal and posterior insula during cardiorespiratory stimulation with isoproterenol at 2 mcg (peak versus baseline; $p < 0.05$, corrected). Insula activation is shown in coronal, axial and sagittal views. (b) Differing patterns of BOLD and ASL signals were observed in this region during different doses. For BOLD, we observed dose-dependent increases, whereas for ASL the increase was only observed at 2 mcg.

discussion). No subjects were excluded from any other dose analysis. ASL insula responses did not show a dose-dependent relationship (figure 2b).

Maps of the anticipation period (anticipatory versus baseline contrast) revealed significant BOLD activations in more mid to anterior regions of the right insula (voxelwise threshold at $p < 0.001$ and cluster size corrected for multiple comparisons threshold at $p < 0.05$; figure 3). Significant clusters of activation were also observed in the right postcentral gyrus and left inferior parietal lobule (electronic supplementary material, table S3).

A time-series analysis of the BOLD signal in insula subregions revealed different response patterns for the left and right insula during the anticipatory and peak periods. To conduct this analysis, we first defined right insula subregions exceeding significance thresholds at 2 mcg for the peak and anticipation periods, and then identified the contralateral voxels in the left insula in the standard atlas. We then determined the unique as well as overlapping voxels, and graphed the group averaged time series for these regions during the 2 mcg infusion and saline for comparison. Only mid and anterior insular subregions on the right showed increased activation during the anticipation period. Right insula subregions showed increased activation during the peak period, whereas left insula subregions showed decreased activation (figure 4).

We performed several planned comparisons to explore the validity of the observed results. First, to evaluate whether physiological noise correction might have affected the observed brain activation responses, we analysed the data separately without and then with the RETROICOR procedure. Our results showed that the activation signal observed in the right insula was present in both cases, but

Figure 3. Cortical activity during the anticipatory period immediately after infusion administration, prior to onset of heart rate changes. Activation was observed in the right mid-dorsal and anterior insula, right postcentral gyrus and precuneus (voxelwise whole brain analysis, thresholded at $p < 0.001$, uncorrected). D, dorsal; V, ventral; L, left; R, right. Numbers indicate MNI coordinates for each axial slice.

Figure 4. Time series showing the BOLD signal over time in the left and right insula following infusion onset. Middle panels: right insula clusters passing the significance threshold during the anticipatory period (red), peak period (green) and their overlap (yellow). For comparison, contralateral regions in the left insula are shown. The time course of BOLD signal change averaged across all 21 subjects is displayed for each set of clusters during the saline (left panel) and 2 mcg (right panel) infusions. This figure suggests the presence of different response patterns for the left and right insula subregions, with (i) increased anterior responses during anticipation, (ii) increased mid-dorsal and posterior responses on the right during stimulation and (iii) decreased mid-dorsal and posterior responses on the left during stimulation. Since the first 4 volumes were discarded to allow the MR signals to reach steady-state, the plots show BOLD activity from 14 to 240 s.

was increased using this procedure (electronic supplementary material, figure S3). Next, we evaluated whether the preparatory period involving auditory ready and countdown cues activated brain regions associated with auditory processing. We found activations localized mainly to bilateral regions of the superior temporal gyrus, consistent with primary auditory and auditory association cortex (electronic supplementary material, figure S4). Since the bolus infusions allow the

measurement of change offset as well as onset, we also evaluated brain activations during the recovery period. We observed similar activation in the right insula during the recovery versus baseline period: namely, increased BOLD activation in mid-dorsal and posterior regions of the right insula at 2 mcg, and dose-dependent increases in this region for BOLD but not ASL (electronic supplementary material, figure S5). To examine the response of the entire insula, we also performed a

region of interest analysis of the left and right insula activation time series in response to each isoproterenol dose and saline. This showed a similar pattern of increased right insula activation and decreased left insula activation during stimulation (electronic supplementary material, figure S6). Finally, we computed correlations between activated insula regions at the 2 mcg dose and the associated interoceptive intensity ratings, interoceptive self-report questionnaires and anxiety. BOLD activity did not correlate significantly with any of these measures, while ASL activity showed significant correlations with intensity ratings of palpitation, dyspnea and physical anxiety (electronic supplementary material, table S4).

4. Discussion

In the current study, we successfully demonstrated that the isoproterenol infusion method for stimulating cardiorespiratory sensations can be implemented within the fMRI environment. We observed dose-dependent increases in heart rate and cardiorespiratory sensation that were accompanied by increased BOLD activation of the right insular cortex, in a dose-dependent manner. We also identified different responses across insula subregions. During interoceptive anticipation, prior to induction of bodily changes, right insula activation increased in more anterior regions. During interoceptive stimulation, activation increased in more posterior regions of the mid-dorsal and posterior insula on the right, but decreased in the same regions on the left.

The main finding of right-sided insula activation is noteworthy in that it supports neuroanatomically based theories positing a key role for the right insula in instantiating subjective awareness [5,9]. The observed asymmetry between right insula regions and their contralateral counterparts is particularly striking as it corroborates a well-described animal literature demonstrating enhanced responsiveness of the right insula to sympathetic and the left insula to parasympathetic activation (for a comprehensive review, see [7]). It seems possible that adrenergic-induced changes in baroreceptor stimulation, which are linked to insula responding [51,52], might play a role in this process. However, it seems unlikely that baroreceptors would be the sole contributor, as we observed overlapping activation in this region during the anticipatory period prior to the onset of any isoproterenol effect.

The anterior to posterior gradient of insula activation during anticipation versus stimulation warrants consideration. The mid-dorsal region of observed activation during the peak and recovery periods appears consistent with a region of the insula activated during interoceptive attention [22–24]. This has been described as a neural ‘spotlight effect’ whereby focal attention on a perceptual feature amplifies activity in brain regions underlying that modality [53]. On the other hand, more anterior insula activations were observed immediately following the infusion administration, prior to onset of bodily changes. It seems plausible that this activation might reflect anticipatory signal processing in the brain. This anterior cluster is closer to regions observed previously during the expectation of interoceptive modulation [12,13], and interestingly, also to the location of insula activation during neuroimaging tasks focusing on heartbeat interoceptive accuracy [19–21]. How can these apparent differences be integrated? One possibility comes from recent active inference accounts of interoception suggesting that

the brain is constantly engaged in constructing predictions about what is going to happen to the body, in addition to continuously monitoring it [54,55]. The comparison between actual and expected changes is hypothesized to occur within the insular cortex along a posterior to anterior gradient, and across different cell layers [54] (see [56] for further discussion). From the current findings, perhaps more posterior activations of the insula reflect the ongoing mapping of a sympathetically enhanced bodily state, with the more anterior activations mapping the predicted (i.e. inference) state. This notion could be supported by the close proximity of these activations in the right insula across two different time periods (anterior during anticipation, posterior during stimulation), as well as by the regional overlap across both periods. Before firm conclusions can be drawn further experiments are needed. For example, investigating the impact of cued versus non-cued infusion delivery might help differentiate the role of more anterior regions in generating interoceptive inferences about the heart.

Consistent with our expectation, we found that other sensory brain regions were also active. The primary somatosensory cortex and precuneus were engaged during anticipation, and the caudate and midline cerebellum during stimulation. Somatosensory activations during interoceptive attention to the heartbeat have been previously demonstrated during resting heartbeat perception tasks [19–21] and somatosensory afferents have been implicated in isoproterenol-induced sensations [31]. Since most individuals show poor heartbeat perception at rest [25], it seems possible that the process of searching the brain for body signals via a neural spotlight effect might provide an integrated account of the somatosensory activations observed across these different paradigms. The precuneus shows strong functional connectivity with somatosensory regions and insula in monkeys [57], and its close proximity to truncal regions of primary somatosensory cortex might potentially indicate a role in the processing of heartbeat sensations localized to the chest. The mammalian cerebellum contains cutaneous body maps [58]. Caudate responses can occur during isoproterenol stimulation [59], although the significance of this is unclear.

(a) Limitations and alternative explanations

(i) Since participants retrospectively reported experiencing acutely increased anxiety during the peak period of the highest dose, we cannot exclude the possibility that the right insula activation was related to induction of a negative emotional state [60,61]. Anxiety changes do not necessarily explain the insula and somatosensory activations during the anticipatory period. (ii) It is possible that the exogenous induction of autonomic changes with isoproterenol obscured endogenous cardiorespiratory variability. (iii) While we observed correlations between ASL insula activity and cardiorespiratory intensity and anxiety ratings at the highest dose, we did not observe similar patterns for the BOLD signal. It seems possible that parametric brain–behaviour correlations might have been observed were they available for each dose. Given our use of retrospective data collection outside of the scanner, we felt confident only in obtaining such measures for the highest dose. (iv) Alternative explanations for the observed brain activations are possible. These include drug action on the vasculature causing signal contamination, residual movement artefact not addressed by standard motion correction, and

potential co-occurring changes in cerebral blood flow during respiratory stimulation. (v) While we observed a general dose-dependent increase in right insula activity, we removed six subjects from the 1 mcg analysis whose baseline insula BOLD signal was elevated. Although the exclusion of subjects from this dose potentially weakens the assertion of a dose-related activation of the insula, this pattern was observed for the remaining participants. Furthermore, dose-related insula activations were observed across the whole group at all other doses. Full randomization of dosing in future studies would solve potential pseudorandomization confounds. (vi) Finally, we did not observe corroborating dose-dependent increases in the ASL signal. The lower signal-to-noise ratio of ASL relative to BOLD, combined with our use of only one trial of each dose, might indicate that we did not have enough statistical power to detect a difference. Future studies could address this by including more trials.

5. Conclusion

This is the first study to demonstrate the feasibility of assessing interoceptive brain activation with fMRI during adrenergic stimulation. It provides key evidence supporting

a dynamic role for the insula in representing changes in cardiorespiratory states.

Ethics. Written informed consent was obtained for all participants as approved by the Institutional Review Board of the University of California Los Angeles.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions. S.S.K., J.D.F., D.J.J.W. and L.Y. designed the research; S.S.K., L.Y., M.S.H., R.C.L. and A.A. performed the research; M.S.H., L.Y. and S.S.K. analysed the data. S.S.K. and M.S.H. wrote the paper with input from all authors.

Competing interests. We have no competing interests.

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