Differential responses to breath-holding, voluntary deep breathing and hypercapnia in left and right dorsal anterior cingulate

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Abstract
The role of subcortical structures and cerebral cortex in the maintenance of respiratory homeostasis in humans remains poorly understood. Emerging evidence suggests an important role of the anterior cingulate cortex (ACC) in respiratory control. In this study, local field potentials (LFPs) from dorsal ACC were recorded in humans through implanted deep brain electrodes during several breathing activities, including voluntary activities of breath-holding and deep breathing, and involuntary activities of inspiration of varying concentrations of carbon dioxide (1%, 3%, 5% and 7%). We found that the breath-holding task induced significant unilateral left-sided ACC changes in LFP power, including an increased activity in lower frequency bands (3–5 Hz) and decreased activity in higher frequency bands (12–26 Hz). The respiratory task involving reflex increase in ventilation due to hypercapnia (raised inspired CO2) was associated with bilateral changes in activity of the ACC (again with increased activity in lower frequency bands and reduced activity in higher frequency bands). The voluntary breathing task with associated hypocapnia (deep breathing) induced bilateral changes in activity within low frequency bands. Furthermore, probabilistic diffusion tractography analysis showed left-sided connection of the ACC with the insula and frontal operculum, and bilateral connections within subsections of the cingulate gyrus and the thalamus. This electrophysiological analysis provides direct evidence for a role of the ACC in respiratory control in humans.

KEYWORDS
anterior cingulate cortex, deep brain stimulation, local field potential, respiratory control

1 | INTRODUCTION

Control of respiration at the subcortical level involving midbrain and brainstem has been relatively well-studied albeit primarily in animal models (Smith et al., 2013). Relatively little is known about the interaction between these subcortical structures and the cerebral cortex in the maintenance of respiratory homeostasis. Such interactions have been suggested in the context of perception of dyspnoea with external...
resistive loads; one suggestion is that the periaqueductal grey (PAG) may down-regulate insular cortex activity in asthmatics (von Leupoldt et al., 2009). Specifically regarding hypercapnia, brainstem regions that respond to increasing levels of inspired carbon dioxide include the dorsal rostral pons, inferior ventral pons, dorsal medulla and lateral medulla (Pattinson et al., 2009). In addition, hypothalamus activity has shown CO₂ sensitivity (Williams et al., 2007). Higher areas that may be indirectly involved in respiratory control include the insula, frontal cortex (including cingulate cortex), thalamus and basal ganglia, which have been shown to increase in activity during voluntary breath-holding. It is uncertain whether the response is related to the ‘central command’ facilitating breath-hold as opposed to a response to the physiological changes accompanying the breath-hold (McKay et al., 2008).

The cingulate cortex is divided functionally into anterior and posterior parts. The anterior cingulate cortex (ACC) surrounds the frontal part of the corpus callosum and is further divided into dorsal and ventral components, Brodmann’s areas 24 and 32 respectively. The ACC has a role in regulating functions as diverse as emotion (Giuliani et al., 2011), attention (Yen et al., 2009) and motor activity (Morecraft & Van Hoesen, 1998). These functions correlate with anatomical models of the cingulate cortex, with evidence of afferent connections from limbic areas, and efferent connections to motor areas (Morecraft & Van Hoesen, 1998). A role for the ACC in respiratory control is supported by evidence of increased activity in this brain area during ‘air hunger’ (Evans et al., 2002). Inspiratory breathing load is an aversive experience associated with activations of brain areas including bilateral insula, dorsolateral prefrontal cortex and ACC (Paulus et al., 2012; Stewart et al., 2015). Furthermore, the degree of brain activation in ACC and left anterior insula positively correlates with the subjective ratings of unpleasantness due to breathing load (Paulus et al., 2012).

Deep brain stimulation (DBS) of the dorsal ACC is a relatively new treatment for chronic neuropathic pain (Boccard et al., 2014) and affords the opportunity to record local field potentials (LFPs) during a number of respiratory tasks. LFPs offer certain advantages over functional magnetic resonance imaging (fMRI). They present an opportunity to record neuronal activities with millisecond temporal resolution and recording whilst performing respiratory tasks without the constraints of a scanner. In addition, the blood oxygenation level dependant (BOLD) signal in fMRI studies may be affected independently by changes in CO₂ concentration and so observed signal change may not truly reflect changes in neuronal activity (Cohen et al., 2002). Unlike imaging techniques, LFP recording is limited by its ability to only study a single defined brain area, although it is possible to investigate connectivity between individual electrodes and distant brain areas using tractography techniques. This allows investigation of coupling and directionality of observed activity (Tass et al., 2010).

The aim of this study was to investigate the role of the dorsal ACC in respiratory control. We hypothesised that hypercapnia would elicit a change in activity in the ACC, in a dose-dependent manner. We also hypothesised that this response would differ from the effect of breath-holding such that breath-holding effects would not be solely due to the resultant hypercapnia. The basis for this hypothesis is that voluntary breathing tasks such as breath-holding require central command, that is, a top-down suppression of respiratory processes that precedes hypercapnia. In a similar manner, we hypothesised that the effect seen during a deep breathing exercise would also differ from those during hypercapnia and breath-holding.

2 | METHODS

2.1 | Ethical approval

The experiments planned and undertaken were done so with fully informed consent. The studies conformed to the standards set out in the Declaration of Helsinki, except for registration in a database. The study was approved by the Oxfordshire Research Ethics Committee (B:11/SC/0229).

2.2 | Data collection

This prospective cohort study involved seven patients with externalised bilateral electrodes in the anterior cingulate cortex, inserted to treat chronic neuropathic pain. The surgical technique for implantation of DBS electrodes has been previously described (Boccard et al., 2014). In brief, a pre-operative T2-weighted MRI scan (1 mm slice) was used for targeting. After application of a Cosman–Roberts–Wells base ring to the patient’s head (Radionics Inc., Burlington, MA, USA), a 1 mm slice computed tomography (CT) scan was then acquired and the two scans fused using Neuroinspire software (Renishaw plc, Bristol, UK). Insertion of the electrode was then planned to target the dorsal ACC, taken as 20 mm posterior to the frontal horn of the lateral ventricle (Figure 1), entry being gained by drilling a 2.7 mm, twist drill craniostomy. The position of implanted electrodes was inspected by a fused image of pre-operative MRI and post-operative CT. The contacts that were not in the ACC on imaging (Figure 1) were removed from further analysis.

New Findings

- What is the central question of this study?
- What is the role of dorsal anterior cingulate cortex (ACC) in respiration control in humans?
- What is the main finding and its importance?

Direct evidence is provided for a role of the ACC in respiratory control in humans. The neurophysiological responses in dorsal ACC to different breathing tasks varied and were different between left and right ACC.
Post-operatively (within 1 week) recordings were taken from the externalised electrodes with the participants at rest and during various voluntary and involuntary respiratory activities. Any medication being taken by the participants that might have altered respiratory function, such as opiates, was omitted on the day of data collection. All data collection was carried out between day 3 and 6 post-operatively, and in the morning. Voluntary activities included deep breathing and breath-holding. Involuntary activities included inspiration of varying concentrations of carbon dioxide. Exposures to different conditions and breathing tasks were randomised for each participant, to control for order effect. Not all participants were able to tolerate all tests (Table 1), as the patients often became fatigued and the task order was randomised.

Bipolar LFP recordings were taken from three adjacent pairs of deep brain electrode contacts (contacts 0–1, 1–2 and 2–3) with a common electrode placed on the surface of the mastoid. The LFPs were amplified using an isolated CED 1902 amplifiers (×10,000, Cambridge Electronic Design, Cambridge, UK), filtered between 0.5 and 500 Hz, and digitised using a CED 1401 mark II at a sampling rate of 2000 Hz, displayed on-line and saved onto a hard disk using Spike2 software (Cambridge Electronic Design).

A silicone full-face face-mask was attached to the participant and connected to a ventilator system. This allowed for the mixing of gases to specific inhaled concentrations of CO₂, room air and oxygen, and also to record end-tidal CO₂ concentrations. The mask incorporated a quick-release system, allowing the participant to remove the mask easily and quickly if found to be too uncomfortable or if the procedure was intolerable.

Data were initially recorded with the participant at rest, to give a recording of baseline activity. A 60-s sample of this recording was used for analysis. Sixty-second recordings were then also taken at varying (random order) levels of inspired CO₂ (1%, 3%, 5% and 7%), although at 7% CO₂ shorter recordings were made due to difficulties tolerating this condition (mean sample length 56.7 s). Recordings were also taken during the voluntary breathing tasks of ‘deep breathing’ and ‘breath-holding’. Deep breathing was targeted to 80% of vital capacity. Participants were asked to take maximal deep inspiration several times over a period of 90 s. A 30-s sample of this recording was used for analysis. For the breath-holding scenario, subjects were asked to hold their breath for as long as possible at the end of a normal expiration (i.e., at functional residual capacity). This was reflected in the end-tidal CO₂ recording. All data were recorded during a single session for each participant. The mean time between the samples used for analysis during the different conditions was 187.7 s (range 10–583 s).

2.3 Spectral analysis and statistical analysis

The selection of bipolar channels for further LFP analysis was on the basis of review of pre- and post-operative imaging and post-operative clinical programming by the surgical team. The selected LFPs were high-pass filtered at 2 Hz, low-pass filtered at 90 Hz, notch-filtered at 50 Hz, and down-sampled to 1000 Hz. Power spectra of the LFPs were calculated for all the patients in each condition using the windowed fast Fourier transform with 2 s of sliding-window and 50% overlap. The power spectra were normalised by dividing by total
| Participant | Age, sex | Diagnosis | Handedness | Past medical history | Medications | Smoker | Relevant respiratory history | Conditions and tasks involved |
|-------------|----------|-----------|------------|----------------------|-------------|--------|--------------------------|-------------------------------|
| A           | 65, M    | Post-stroke pain | Right | Osteochondroma, thalamic stroke | Pregabalin 225 mg BD, duloxetine 30 mg BD, amitriptyline 25 mg ON | No | No | √ | 1, 3, 5, 7% CO₂ | Breath holding | Deep breathing |
| B           | 50, M    | Post-stroke pain | Right | Thalamic stroke | Buprenorphine sublingual 800 µg BD, codeine 30 mg QDS, paracetamol 1 g QDS | Yes – 20/day | No | √ | √ | √ | √ | — |
| C           | 48, M    | Brachial plexus injury | Right | Brachial plexus injury, depression, asthma | Citalopram 20 mg OD, loperamide 2 mg QDS, tiotropium bromide inhaler 2 puffs/day, zopiclone 7.5 mg OD, pregabalin 300 mg BD, levetiracetam 2.5 mg/day, salbutamol 2 puffs QDS | Yes – 30/day | Yes | √ | √ | — | — |
| D           | 61, F    | Post-stroke pain | Right | Head trauma | Baclofen 30 mg TDS | No | No | √ | √ | √ | — | — |
| E           | 48, F    | Failed back surgery syndrome | Right | None relevant | Oxycontin 60 mg BD, oxynorm 60 mg every 5 h, diazepam 10 mg QDS, naproxen 50 mg BD, paracetamol 1g QDS, furosemide 40 mg/amiloride 5 mg BD, colecalciferol 800 IU OD, lactulose 20 ml BD | No | No | √ | — | — | — |
| F           | 44, F    | Bilateral leg pain following spinal cord injury, paraplegia | Right | Spinal cord injury | Morphine sulphate 120 mg BD, gabapentin 300 mg BD | No | No | √ | — | √ | √ | √ |
| G           | 55, M    | Cervical trauma and spinal cord injury, all over body pain | Right | Multiple trauma | Gabapentin 300 mg BD, amitriptyline 25 mg ON | No | No | √ | — | √ | √ |

Some subjects were unable to complete all tasks because of discomfort (√, completed; —, uncompleted).
power between 2 and 90 Hz. Relative rather than absolute power was analysed to allow comparison across subjects, as absolute power is more likely to be dependent on recording position and electrode impedance. Power spectra were calculated from the LFP recordings for well-recognised frequency bands: 1–3 Hz (delta), 3–5 Hz (low theta), 5–8 Hz (high theta), 8–12 Hz (alpha), 12–26 Hz (beta) and 26–90 Hz (gamma). In addition, the relationship between the power of LFPs and concentrations of CO₂ was quantified with correlation analysis.

Statistical significance of the differences of power in frequency bands was assessed between tasks. Before comparison, raw values of power in each state were examined for deviations from normality using the Kolmogorov–Smirnov test. The power was compared between different states using the Wilcoxon signed ranks test if the data were not normally distributed, or Student’s paired t-test if normally distributed. To correct for multiple comparisons, the resulting distributions of P-values were analysed with the false discovery rate (FDR) method (Benjamini & Hochberg, 1995). In order to compare the differences of power for multiple concentrations of CO₂, a one-way analysis of variance (ANOVA) and post hoc test was performed.

2.4 Electrode location and tractography analysis

Electrode identification was performed with the Oxford FSL toolbox (www.fmrib.ox.ac.uk/fsl). On the post-surgery CT scan, contacts were identified as the centres of CT-derived artefacts and saved as a mask. The BET algorithm was used to remove extracranial tissues from post-surgery CT scans and pre-surgery MRI scans. FLIRT was used to register the CT scan with the MRI, as well as the MRI with the Montreal Neurological Institute (MNI) standard brain. All patients’ tractographies could then be seen in a common space. These two transformations (CT to MRI and MRI to MNI space) were concatenated, and the resulting transformation was applied to the electrode masks. Coordinates of contacts in MNI space were then registered.

An empirical model for estimating the volume of activated tissue (VAT) developed for monopolar stimulation and validated for movement disorders (Madler & Coenen, 2012) was used in this study. The electric field distribution of bipolar stimulation between non-adjacent contacts is believed to produce a continuous VAT between the contacts, with reduced radial dispersion (Montgomery, 2010). Hence the electric field distribution was estimated by simulating the VAT for each of the four DBS electrode contacts, using a fixed value of 3 V and tissue impedance of 1003 Ω, followed by the concatenation of the resulting masks (Madler & Coenen, 2012).

For each subject, we ran probabilistic tractography using the DBS electrode VAT as a seed area and the ROIs included in the parcellation template as target areas (Behrens et al., 2003). Five thousand sample streamlines were seeded from each voxel of the seed region (VAT). To quantify the connectivity from the VAT to the regions of interest, we computed the mean intensity of non-zero voxels within the masks (anatomic parcellations) (Rozanski et al., 2014).

3 RESULTS

3.1 Breath holding

Mean respiratory rate during the breath-holding task was 3.4 breaths per minute (bpm). In the left ACC there was a significant decrease in activity between 5 and 8 Hz (−9.1%, P = 0.033) and 12–26 Hz (−15.9%, P = 0.043). On the right side, no significant differences in activity were observed (Figure 2).

3.2 Deep breathing

Mean respiratory rate during the deep breathing task was 6.0 bpm. At the 1–3 Hz frequency band there was a significant increase in activity in the left ACC (+14.4%, P = 0.012). At the 3–5 Hz and 5–8 Hz frequency bands, there were significant decreases in activity in the left ACC (−8.1%, P = 0.004; −17.4%, P = 0.004, respectively), which were not evident in the right (Figure 3).

3.3 Inspired CO₂ concentration

Respiratory rates were recorded at each CO₂ concentration. Mean rate at room air was 9.0 bpm, with 10.4 bpm at 1% CO₂, 9.8 bpm at 3% CO₂, 10.0 bpm at 5% CO₂ and 9.8 bpm at 7% CO₂.

Changes in activity were observed in both the left and the right ACC at different concentrations of CO₂. Significantly increased activity associated with increased concentration of CO₂ compared to rest was seen bilaterally for 3–5 Hz. Significantly decreased activity associated with increased concentration of CO₂ compared to rest was seen bilaterally for 12–26 Hz (Figure 4).

Correlation analysis for activity in relation to CO₂ concentration showed a trend for bilateral positive correlation at the 3–5 Hz band (left R = 0.69, P = 0.19; right R = 0.86, P = 0.06). There was also a trend for negative correlation bilaterally at the 12–26 Hz band (left R = −0.80, P = 0.10; right R = −0.85, P = 0.07) (Figure 5).

The observed significant changes in ACC activity during voluntary and involuntary respiratory activities are summarised in Figure 6.

3.4 Electrode location and tractography

Tractography analysis (Figure 7a) was performed for participants A, B, C, E and G, for whom diffusion tensor imaging (DTI) scans were available. We observed relatively consistent connectivity between different areas of the cingulate cortex. In addition, significant connectivity to other brain areas was observed on the left side.
between the electrodes and the frontal operculum and insula and bilaterally with the thalamus.

4 | DISCUSSION

In this study we found that the respiratory task most likely to be associated with generation of air hunger (breath-holding), produced unilateral left-sided changes in LFP activity in the ACC; this included decreased activity in lower frequency bands (5–8 Hz) and higher frequency bands (12–26 Hz). The respiratory task involving reflex increase in ventilation due to hypercapnia (raised inspired CO2) was associated with bilateral changes in activity of the ACC (again with reduced activity in higher frequency bands but increased activity in lower frequency bands). The voluntary breathing task with associated hypocapnia (deep-breathing) produced significant changes in low frequency bands in the left ACC. Connectivity analysis (DTI tractography) indicated (i) left-sided connection of the ACC with the insula and frontal operculum and (ii) bilateral connections within sub-sections of the cingulate gyrus and the thalamus.

The BOLD fMRI study of air hunger by Evans et al. (2002) found bilateral activation of the insula and the ACC. Similar bilateral activation has been shown in a study of fear processing by Makovac et al. (2018). However, in the current study, air hunger associated with breath-holding appears to involve just the left-side ACC, and it was only the left side that had significant connections to the insula. We speculate that these differences may be related to the different tasks involved in these studies. For example, in the air hunger stimulus in Evans et al., the CO2 was fixed at a hypercapnic level whilst tidal volume was repeatedly reduced, whereas in the current study, breath-holding involved absence of tidal breathing and an increasing CO2 signal.

We further speculate that the connectivity of the ACC with the frontal operculum may be related to the voluntary breath-holding task itself. Our data indicate that this motor element of breath-holding is likely to be predominantly controlled by the left hemisphere. This fits with our finding that the left ACC connects to the left frontal operculum, which is where Broca’s motor speech area is located; that is, for speech control the muscles concerned with speech need to be coordinated with the act of breathing and this would occur mainly on the dominant side, that is, the side of speech predominance. This is supported by studies that have shown that voluntary motor activity associated with expressions of mirth are elicited with electrical stimulation of the frontal operculum (Caruana et al., 2017), and the act of voluntary breath-holding may involve similar motor activation. Furthermore, brain imaging studies of breath-holding have shown...
activation of the frontal operculum (McKay et al., 2008; Pattinson et al., 2009).

With respect to the hypercapnia condition, it is notable that the bilateral changes in LFP activity are consistent with bilateral connectivity of the ACC to the thalamus and subsections of the cingulate gyrus. Furthermore, we would associate these bilateral changes in activity to the hypercapnia itself rather than the associated reflex ventilation because the deep breathing task did not produce similar bilateral changes in LFP activity. A caveat is that it is well known that hypercapnia and breath-holding increase sympathetic activity in humans (Somers et al., 1989; Busch et al., 2019). Furthermore, the ACC has been implicated in the sympathetic modulation of heart rate, dissociable from cognitive and motor-related activity in humans (Critchley et al., 2003). Therefore it is difficult to distinguish whether these changes are related to the hypercapnia itself or a correlate such as increased sympathetic activity (or indeed increased sympathetic drive as a response to the manoeuvres). The fact that the changes associated with breath-holding were unilateral in this study whereas ACC changes associated with increased sympathetic activity are bilateral in the aforementioned studies would tend to favour the former hypothesis.

Low theta activity has been shown to be associated with ‘survival’ activities such as ‘fight or flight’ reactions (Adhikari et al., 2010). This is consistent with the hyperventilation in the deep breathing task, the ‘panic’ response to hypercapnia in the CO₂ condition and the air hunger generation associated with the breath-holding task, all of which were associated with increases in the 1–5 Hz band albeit predominantly on the left. With regard to the bilateral decrease in LFP activity in the 12–26 Hz band (beta frequency) seen in the hypercapnia condition, we suggest that this is associated with the reflex increase in the drive to breathe that is elicited with the hypercapnia. This would be consistent with the treatment-related reversal of high resting beta activity in basal ganglia nuclei in patients with Parkinson’s disease that is associated with increased movement (Brown et al., 2001). Our assertion that it is the drive to breathe rather than the actual change in ventilation that mediates the reduced beta activity is supported by the fact that we found no reduction in beta activity for the deep breathing task, which involves a voluntary hyperventilation with hypocapnia that switches off the drive to breathe from the brainstem. It is interesting that we did pick up some reduction in beta activity for the breath-holding condition in which the drive to breathe will rise as the breath-hold progresses.

Based on our findings, in order to determine a model of higher respiratory control that links the various stimuli to the bilateral changes in LFPs, we would need to perform further experiments that tease apart the effects of specific respiratory stimuli, for example, CO₂.
FIGURE 4  Power spectral analysis of the left and right ACC LFPs at rest and during breathing different concentrations of CO₂. (a, b) Power spectra of the left and right ACC LFPs, respectively, during rest and when breathing 1%, 3%, 5%, 7% CO₂ (mean (SD)). (c, d) Results of one-way ANOVA and post hoc test analysis between rest and breathing different concentrations of CO₂ at physiological frequency ranges for left and right ACC, respectively (mean (SD)). *P < 0.05, **P < 0.01

FIGURE 5  Correlation analyses investigating the relationship between activity in the left and right ACC in response to increasing levels of CO₂ with no change in ventilation or voluntary breathing with no change in CO₂. The connectivity suggested by the DTI in this limited number of subjects and a tentative model are summarised in Figure 7b. The ACC may act centrally, to process afferent signals from the periphery and brainstem. Whilst afferent signals may be projected to both the left and the right ACC, we hypothesise that our data provide evidence that it is the left side that is involved in the generation of efferent signals to affect a modulation in respiratory activity.

Recent studies have suggested roles for the stimulation of brain structures in the modulation of autonomic activity (Green et al., 2010; Hyam et al., 2012). Whilst stimulation of the ACC has been shown...
to be beneficial for the affective component of chronic pain (Boccard et al., 2014), it is likely that the diverse role of the ACC may present other therapeutic opportunities through deep brain stimulation. Indeed, there is already some evidence for the use of cingulotomy in the treatment of breathlessness associated with terminal disease (Pereira et al., 2014). Alongside the previously studied roles of the ACC in autonomic function (Critchley et al., 2003, 2005; Farrell et al., 2015), our work gives further evidence for the role of the ACC in modulating respiratory control.

Whilst the results above indicate a role for the ACC in the breathing activities as described, it is important to note a number of limitations of the data. The relative rarity of patients with implanted electrodes in the dorsal ACC means that conducting such experiments is difficult and done in relatively small numbers of subjects. The paucity of research on neurophysiology in the ACC in human related to respiration control reflects the rarity of our data. However such data may provide unique insights into the understanding of the role of ACC in respiratory control. In addition, the nature of some of the experiments undertaken was such that they were uncomfortable for subjects, and so the quantity of data was limited. Attempting to make recordings during inhalation of increasing concentrations of CO2 may be vulnerable to participants becoming aversive to the condition itself. In addition, the nature of the patients’ underlying conditions (chronic pain) makes conducting experiments requiring their concentration and compliance especially challenging. These factors should be taken into account when interpreting the results and reaching conclusions on ACC function.

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

FIGURE 7 (a) Tractography results for participants A, B, C, E and G. Mean values of connectivity have been used for illustration of connectivity between brain areas. (b) A proposed model for the involvement of the ACC in modulation of respiratory activity.

AUTHOR CONTRIBUTIONS
A.L.G. and D.J.P. conceived and designed the research; P.H., Y.H., N.F.A.B., J.A.H. and K.L.D. performed the experiments; P.H., Y.H. and S.B. analysed data; P.H., Y.H., S.H.M. and A.L.G. interpreted the results of the experiments; P.H., Y.H. and S.B. prepared the figures; P.H., Y.H., S.H.M. and A.L.G. drafted the manuscript; P.H., Y.H., N.F.A.B., S.B., J.A.H., D.J.P., K.L.D., T.Z.A., S.H.M. and A.L.G. edited and revised the manuscript. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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