Regional brain responses associated with using imagination to evoke and satiate thirst

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In response to dehydration, humans experience thirst. This subjective state is fundamental to survival as it motivates drinking, which subsequently corrects the fluid deficit. To elicit thirst, previous studies have manipulated blood chemistry to produce a physiological thirst stimulus. In the present study, we investigated whether a physiological stimulus is indeed required for thirst to be experienced. Functional MRI (fMRI) was used to scan fully hydrated participants while they imagined a state of intense thirst and while they imagined drinking to satiate thirst. Subjective ratings of thirst were significantly higher for imagining thirst compared with imagining drinking or baseline, revealing a successful dissociation of thirst from underlying physiology. The imagine thirst condition activated brain regions similar to those reported in previous studies of physiologically evoked thirst, including the anterior midcingulate cortex (aMCC), anterior insula, precentral gyrus, inferior frontal gyrus, middle frontal gyrus, and operculum, indicating a similar neural network underlies both imagined thirst and physiologically evoked thirst. Analogous brain regions were also activated during imagined drinking, suggesting the neural representation of thirst contains a drinking-related component. Finally, the aMCC showed an increase in functional connectivity with the insula during imagined thirst relative to imagined drinking, implying functional connectivity between these two regions is needed before thirst can be experienced. As a result of these findings, this study provides important insight into how the neural representation of subjective thirst is generated and how it subsequently motivates drinking behavior.

thirst | dehydration | cingulate | insula | fMRI

Human neuroimaging has revealed a network of brain regions associated with the subjective experience of thirst. These regions include the cingulate cortex, insula, primary sensorimotor cortex, inferior frontal gyrus, and middle frontal gyrus (1–8). In previous studies, thirst has been evoked with administration of intravenous (i.v.) hypertonic saline solution (1–4, 6), through water and electrolyte loss caused by exercise-induced sweating (5), and by fluid deprivation (7, 8). These physiological manipulations produce changes in osmotic pressure, sodium concentration, and angiotensin II concentration that are detected by receptors within the circumventricular organs of the lamina terminalis (9), a region of the brain without a blood–brain barrier (10). The changes in blood composition detected by these receptors produce a physiological thirst stimulus, which leads to the generation of a thirst experience.

The state of thirst that arises from a physiological stimulus is critical to maintaining homeostasis, and thus survival, because it motivates goal-directed drinking. As a result of this behavior, the fluid deficit produced by dehydration is corrected. While the relationship between physiological stimulus and subjective thirst is clearly important for survival, previous evidence suggests the subjective experience is not tightly coupled to the stimulus (11). This inference is supported by previous work from our group, in which a significant relationship between thirst ratings and fluid loss has not been observed (12, 13). We therefore questioned whether a physiological stimulus is indeed necessary for thirst to be experienced. Since the intensity of subjective thirst may not reflect the underlying fluid deficit, the two phenomena could be dissociable. This raises the possibility that subjective thirst can be evoked while in a fully hydrated state, an outcome that represents a complete dissociation of the conscious experience from the underlying physiological state.

Indirect evidence for this possibility has been provided in recent animal studies in which optogenetic stimulation of thirst-related neurons in the circumventricular organs produced robust drinking behavior in fully satiated mice (14, 15). In these studies, however, the experience of thirst could not be confirmed due to our inability to interrogate the subjective state of other species. Since a comparable optogenetic procedure in humans is not feasible, the use of imagination as a stimulus represents a viable alternative that has been exploited in several other studies of interoceptive experiences, including pain (16) and hunger (17). With this approach, an investigation can be undertaken in humans of subjective thirst and its underlying neural circuitry in the complete absence of a physiological stimulus.

It is also possible, using this approach, to cue the initiation and termination of imagined thirst. A state of imagined thirst can consequently be examined using functional MRI (fMRI) and brain

Significance

This study provides three important insights into the neural representation of thirst. First, the experience of thirst can be dissociated from a physiological stimulus produced by changes in blood chemistry. Second, the network of brain regions associated with subjective thirst may incorporate regions involved in drinking behavior. Third, functional connectivity between the insula and the anterior midcingulate cortex appears necessary to generate an experience of thirst. In the absence of a physiological stimulus, use of imagination to evoke thirst also provides a means of investigating this subjective state with fMRI.

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activity associated with imagined thirst and imagined satiation can be directly compared. The state of thirst produced by a physiological stimulus, in contrast, cannot be used in such a comparison as dehydration or infusion of hypertonic saline produces relatively slow changes in blood chemistry that are incompatible with the temporal dynamics of fMRI.

For the present study, we compared and contrasted brain activation in an fMRI block design in which trained hypnosis practitioners, and thus individuals who use their imaginative ability regularly, imagined thirst in one condition ("imagine thirst") and imagined satiating their thirst by drinking in another condition ("imagine drink"). While previous studies have been unable to directly contrast these two states, drinking in response to thirst has been shown to activate a similar network of brain regions as the experience of thirst itself (12, 18). By comparing two subjective states with similar activation patterns but with only one of the states specifically associated with the experience of thirst, we aimed to reveal unique characteristics of the neural network that underlies the thirst experience. Using a psychophysiological interaction (PPI) approach (19, 20), we also aimed to investigate the degree of correlated activity, or functional connectivity, between brain regions during each of the imagined conditions to further elucidate and clarify the network underlying the experience of thirst. Based on previous reports that have emphasized consistent activation in the anterior midcingulate cortex (aMCC) with respect to thirst and drinking (18), the PPI analyses examined functional connectivity between the aMCC and the rest of the brain.

Behavioral Results

Mock Scanner. To test whether a thirst experience could indeed be evoked by imagination, each participant initially performed the experimental protocol in a mock scanner. The mean thirst ratings of participants were 3.6 ± 0.5 prior to entering the scanner and 2.8 ± 0.3 after exiting the scanner (scale range, 0 to 10; 0 indicates no thirst; 10 indicates maximum thirst). These pre- and postscanner ratings were not significantly different from each other [t (19) = 1.5; P = 0.14]. During mock scanning (Fig. 1), the mean thirst rating for all participants in the imagine thirst condition was significantly higher than the corresponding ratings for baseline (imagine thirst, 5.4 ± 0.5; baseline, 1.9 ± 0.6; [t (19) = 6.6; $P < 0.001$]) and imagine drink (imagine thirst, 5.4 ± 0.5; imagine drink, 1.8 ± 0.3; [t (19) = 6.4; $P < 0.001$]). Thirst ratings for baseline and imagine drink, in contrast, were not significantly different from each other [t (19) = −0.64; P = 0.53]. As these results supported the hypothesis that thirst could be evoked by imagination, they were used as justification for investigating brain activation and functional connectivity in relation to imagined thirst.

MRI Scanning. Prior to entering the MRI scanner for the first of two functional scans, the mean thirst rating of participants was 1.8 ± 0.3. After exiting the scanner at the conclusion of the second functional scan, this rating was 2.1 ± 0.3. These pre- and postscanner ratings were not significantly different from each other [t (19) = −0.863; P = 0.40].

Prior to scanning, participants also reported the degree to which their mouth felt dry and how full their stomach felt, and were offered ad libitum access to water followed by a further thirst rating. During this period, mean mouth dryness was 5.9 ± 0.5 (scale range, 0 to 10; 0 indicates completely dry; 10 indicates completely wet); mean stomach fullness was 5.9 ± 0.4 (scale range, 0 to 10; 0 indicates completely empty; 10 indicates completely full); mean volume of water drunk was 84 ± 17 mL; and mean thirst rating following ingestion of water was 0.20 ± 0.09. At the conclusion of the second functional scan, participants exited the scanner and rated the same set of behavioral measures, along with being offered ad libitum access to more water.

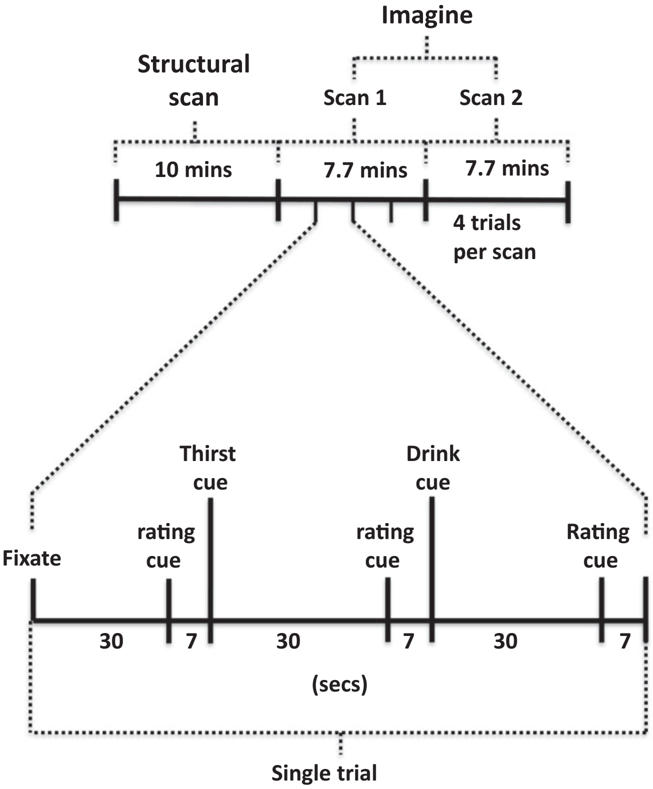

Fig. 1. Experimental protocol composed of one structural scan and two functional scans. Each functional scan was of 7.7-min duration and consisted of four trials. For each trial, "fixate" represented the baseline and involved fixating on three white crosses at the center of the screen. The "rating cue" represented a thirst rating that used a scale of 0 (no thirst) to 10 (maximum thirst) with participants indicating their rating with the appropriate number of fingers. Each rating cue lasted 7 s. The "thirst cue" represented the beginning of the imagine thirst condition and the "drink cue" represented the beginning of the imagine drink condition. The baseline, imagine thirst, and imagine drink events each lasted 30 s.

For this later set of ratings, mean mouth dryness was 5.6 ± 0.6; mean stomach fullness was 6.1 ± 0.4; mean volume of water drunk was 81 ± 15 mL; and mean thirst rating following ingestion of water was 0.3 ± 0.1. None of these postscanner ratings were significantly different from the set of ratings obtained prior to scanning.

Thirst ratings were also recorded during scanning, which was composed of two 7.7-min functional scans (230 volumes; repetition time, 2,000 ms). Each scan consisted of four trials, with every trial containing baseline, imagine thirst, and imagine drink events. Each event lasted 30 s, with thirst ratings obtained immediately afterward (Fig. 1). The mean thirst rating for all participants during imagine thirst was significantly higher than the ratings for baseline (imagine thirst, 4.9 ± 0.4; baseline, 1.7 ± 0.3; [t (19) = 7.9; $P < 0.001$]) and imagine drink (imagine thirst, 4.9 ± 0.4; imagine drink, 1.6 ± 0.2; [t (19) = 7.6; $P < 0.001$]). Thirst ratings for baseline and imagine drink were not significantly different from each other [t (19) = 0.71; P = 0.49], and no significant differences were found between thirst ratings for baseline, imagine thirst, and imagine drink in the real scanner compared with the thirst ratings for the corresponding conditions in the mock scanner.

Imaging Results.

Activation during imagine thirst and imagine drink. Compared with baseline, significant activations were observed during the imagine

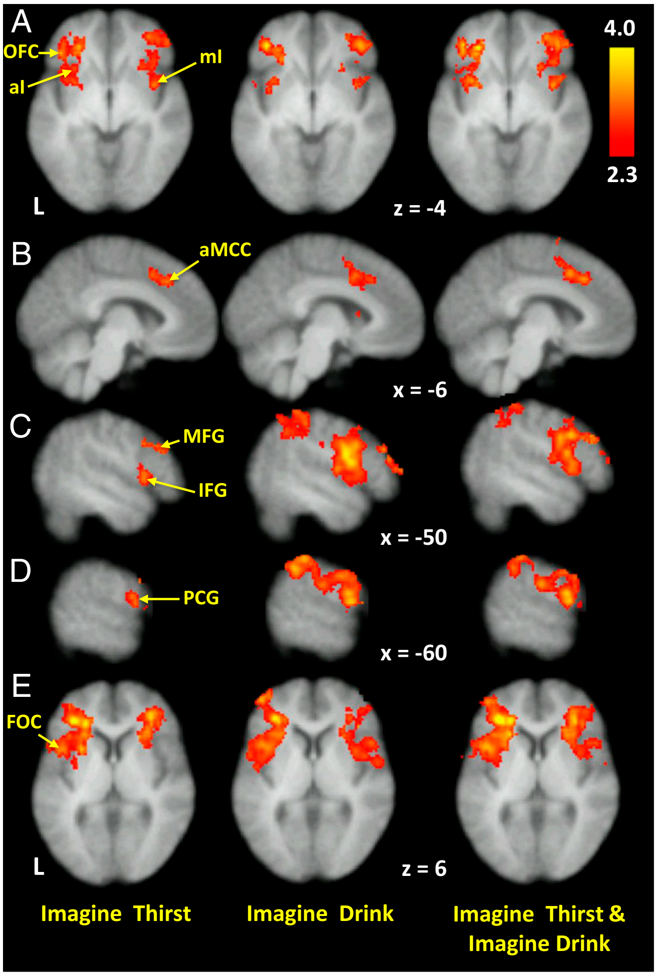

Fig. 2. Brain regions showing increased activation relative to baseline for imagine thirst, imagine drink, and the conjunction of imagine thirst and imagine drink. Corresponding brain images for each analysis have the same stereotactic coordinates to aid comparison. (*A*) Orbital frontal cortex (OFC); anterior insula (aI); mid insula (mI). (*B*) Anterior midcingulate cortex (aMCC). (*C*) Inferior frontal gyrus (IFG); middle frontal gyrus (MFG). (*D*) Precentral gyrus (PCG). (*E*) Frontal operculum cortex (FOC). L, left.

thirst condition in the left aMCC, bilateral anterior and mid insula, left inferior frontal gyrus, left middle frontal gyrus, left precentral gyrus, left frontal operculum cortex, caudolateral orbital frontal cortex (OFC) in both hemispheres, bilateral frontal pole, left putamen, and left pallidum (Fig. 2 and *SI Appendix*, Table S1). The imagine drink condition, in comparison, produced significant activations in the superior frontal gyrus bilaterally, precentral gyrus bilaterally, left supramarginal gyrus, left supplementary motor area (SMA), bilateral aMCC, left middle frontal gyrus, left postcentral gyrus, bilateral frontal pole, bilateral central operculum, bilateral inferior frontal gyrus, bilateral anterior insula, left frontal operculum, and bilateral mid insula (Fig. 2 and *SI Appendix*, Table S2).

Comparison of imagine thirst and imagine drink. A conjunction analysis was performed to identify regions of the brain that were significantly active during both the imagine thirst and imagine drink conditions. This analysis revealed a network of activated regions that was similar to the network identified in the imagine thirst condition. These regions included the aMCC bilaterally, bilateral anterior and mid insula, bilateral inferior frontal gyrus, left middle frontal gyrus, bilateral precentral gyrus, bilateral frontal operculum, caudolateral OFC in both hemispheres, bilateral frontal pole, left putamen, left caudate, bilateral superior frontal gyrus, left postcentral gyrus, and left supramarginal gyrus (Fig. 2 and *SI Appendix*, Table S3). In contrast, while there appear to be differences between the imagine thirst and imagine drink conditions (Fig. 2 *C* and *D*), with a sample size of 20 the present study was unable to reveal any brain regions with greater activation for imagine thirst compared with imagine drink.

Functional connectivity during imagine thirst and imagine drink. A "seed" region located in the aMCC (Fig. 3*A*) showed significant functional connectivity with the right mid insula, right ventral anterior insula, right inferior frontal gyrus, right frontal pole, right caudolateral OFC, bilateral aMCC, and bilateral anterior cingulate cortex (ACC) during the imagine thirst condition compared with the baseline condition (*SI Appendix*, Table S4). In contrast, when the imagine drink condition was compared with the baseline condition, no significant functional connectivity was observed between the aMCC seed and other regions of the brain.

Comparison of functional connectivity during imagine thirst and imagine drink. The right ventral anterior insula, right temporal pole, and right caudolateral OFC showed greater functional connectivity with the aMCC seed in the imagine thirst versus baseline contrast relative to the imagine drink versus baseline contrast (Fig. 3 and *SI Appendix*, Table S5). Conversely, no regions showed greater functional connectivity in the imagine drink versus baseline contrast relative to the imagine thirst versus baseline contrast.

Discussion

Subjective Ratings of Thirst. During the imagine thirst condition, participants' reports of subjective thirst significantly increased relative to the imagine drink and baseline conditions. This provides behavioral evidence that the intended manipulation of subjective state by imagination occurred and, furthermore, that the subjective experience of thirst was successfully dissociated from the current state of fluid balance.

Brain Activation during Imagine Thirst. Relative to baseline, the imagine thirst condition showed significant activation in the aMCC, insula, precentral gyrus, inferior frontal gyrus, middle frontal gyrus, and frontal operculum. Activation was also observed in the vicinity of the claustrum. These activated regions correspond to those reported in previous studies that have used a physiological stimulus to investigate subjective thirst (1–8, 21). In those earlier studies, the physiological stimulus was produced by administration of i.v. hypertonic saline solution (1–4, 6), water and electrolyte loss due to exercise-induced sweating (5), and

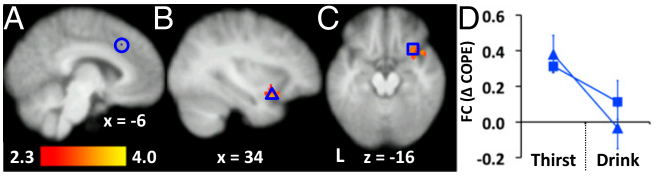

Fig. 3. Location of the anterior midcingulate cortex seed used for the functional connectivity analyses and the brain regions showing increased functional connectivity with the aMCC seed during imagine thirst compared with imagine drink. (*A*) Sagittal view of the brain showing the aMCC location used to seed the PPI analyses. (*B* and *C*) Regions showing increased functional connectivity with the seed during imagine thirst compared with imagine drink: (*B*) right ventral anterior insula; (*C*) right OFC. (*D*) Blue shapes in the graph correspond to blue shapes in the brain images (*B* and *C*) that denote regions of interest (ROIs): blue triangle, right ventral anterior insula; blue square, right OFC. The graph shows the average change in functional connectivity (FC) for the ROIs between imagine thirst and imagine drink as indicated by the contrast of parameter estimates (COPEs) derived from the PPI analysis. ROIs were selected according to the criteria Z statistic > 3.1 and >50% gray matter according to FSLView, a visual imaging tool available in the FSL brain imaging software used to analyze the fMRI data (53).

fluid deprivation (7, 8). Despite these different methods of induction, the anterior/midcingulate cortex has shown consistent activation in relation to thirst (1–8). Reliable activation has also been reported in the insula (3–5, 7, 8), inferior frontal gyrus (3–6), middle frontal gyrus (3–6), and primary sensorimotor cortex (3, 4, 6, 8), while regions with inconsistent activation include the OFC (2, 5), SMA (4, 6), premotor cortex (6), and operculum (4). The correspondence between regions implicated in the imagine thirst condition and the regions activated in earlier studies suggests that imagining thirst did indeed activate the same cortical network as a physiological thirst stimulus. This correspondence therefore provides evidence, based on the blood oxygenation level-dependent (BOLD) fMRI signal, that a physiological stimulus is not required in order for subjective thirst to be experienced.

Comparison of Brain Activation during Imagine Thirst and Imagine Drink. In the present study, it was possible to directly contrast a state of imagined thirst with a state of imagined drinking using fMRI. Such a comparison had previously been impossible due to an incompatibility between the physiological stimulus used to produce a state of thirst and the temporal dynamics of fMRI. The present results therefore provide insight into our understanding of the neural organization of thirst by revealing the network of brain regions associated with subjective thirst is likely to incorporate regions also involved in drinking behavior.

This finding is consistent with the view that the experience of thirst consists of a thirst sensation accompanied by an intention to drink (22). At the neural level, this intention could manifest as preparation for drinking, in which regions involved in the cortical control of swallowing are activated in anticipation of drinking behavior. As the pharyngeal and esophageal phases of swallowing are automatic and controlled by nuclei in the medulla (23), it is likely that these cortical regions regulate the initial oral phase of swallowing (24). This oral phase is volitional and involves water being manipulated toward the pharynx, where it triggers the automatic pharyngeal phase (25). It is therefore possible that the cortical regions in the present study are activated in anticipation of the oral phase and, consistent with this notion, when water is held in the mouth in expectation of swallowing, evidence of this anticipatory activation is indeed observed (13).

The brain regions activated in the imagine thirst and imagine drink conditions are similar to regions activated in previous fMRI studies of physiologically induced thirst and swallowing. While the regions principally implicated in physiologically induced thirst have been outlined above, those consistently implicated in swallowing are the primary sensorimotor cortex (26–28), insula (26–28), anterior/midcingulate cortex (26, 27), and frontal operculum (26). Other brain regions with inconsistent activation in relation to swallowing include the inferior frontal gyrus, middle frontal gyrus, SMA, and basal ganglia (26). A remarkable correspondence therefore exists between those regions activated during physiologically evoked thirst and those activated during swallowing. As similar regions were activated during the imagine thirst and imagine drink conditions, this reinforces the view that the same network of brain regions was activated in both of the imagined conditions. It should be acknowledged, however, that apparent differences between the two imagined conditions were noted in the parietal cortex, middle frontal gyri, inferior frontal gyri, and precentral cortex. This suggests a more extensive motor component may exist for the imagine drink condition compared with the thirst condition. Given this difference cannot be ruled out with the current study design, further inquiry with a larger sample size is therefore needed to confirm whether such a motor component does indeed exist.

Of the regions implicated in the shared network between the two conditions, the anterior midcingulate cortex in particular appears to play an important integrative role in subjective thirst and the cortical control of swallowing. While studies of thirst and its neural representation consistently implicate the aMCC, this region has also been associated with tongue movements (29–31), preparing to swallow and clearing the throat (30), volitional swallowing (32, 33), and sensory stimulation, motor planning, and motor execution related to swallowing (34). Furthermore, in a recent study (18), evidence has also been presented that the aMCC facilitates swallowing during thirst through an interaction between the aMCC and regions involved in both the cortical and subcortical control of swallowing. The aMCC may thus integrate information regarding thirst with somatomotor information regarding preparation for a drinking response (18). This facilitation is consistent with a fundamental role for the aMCC in motivating drinking in response to thirst, and with evidence that points to a general integrative role for the midcingulate cortex concerning cognitive, somatomotor, and autonomic information (35).

Functional Connectivity during Imagine Thirst and Imagine Drink. The results of the functional connectivity analyses provide a potentially unique insight into the neural substrate underlying the experience of thirst. The principal finding of these analyses was greater functional coupling between the aMCC and the insula during the imagine thirst condition compared with the imagine drink condition. This is compatible with the findings of previous studies, which have demonstrated both anatomical connectivity and resting-state functional connectivity between the insula and the aMCC (36). Given a common neural network may underlie the experience associated with the imagine thirst and imagine drink conditions, enhanced neural signaling between the aMCC and the insula during the thirsty state may critically distinguish the experience of thirst from the experience of satiation due to drinking.

The insula is an important multimodal integration site implicated in a wide range of sensory, emotional, motivational, and cognitive functions (37–39). In addition to having extensive cross-modal connectivity, some sensory inputs to the insula are also organized topographically. These include the "visceral cortex," located in the dorsal midposterior insula, and the "gustatory cortex" or primary taste cortex, located in the ventral mid insula (38, 39). In the present study, both of these regions showed increased functional connectivity with the aMCC during the imagine thirst condition relative to baseline. The insula also has strong reciprocal connections with the limbic system, including the amygdala, OFC, and anterior/midcingulate cortex (37). The ventral anterior region of the insula, in particular, has been identified as a functionally distinct domain that processes emotion (39, 40) and, relative to both baseline and the imagine drink condition, this region also showed an increase in functional connectivity with the aMCC during the imagine thirst condition.

The relative increase in functional connectivity during the imagine thirst condition between the aMCC and insula regions dedicated to visceral and emotional processing is compatible with thirst being a "primordial emotion." Primordial emotions represent subjective states that consist of a sensation representing a physiological need and an intention that aims to satisfy that need (22). In the present study, the sensation of thirst could therefore be represented in the insula while the intention to drink is represented in the aMCC. The designation of the insula as the "limbic sensory cortex" and the anterior/midcingulate cortex as the "limbic motor cortex" (41) is consistent with this speculation.

Furthermore, the relative increase in functional connectivity between the ventral anterior insula and the aMCC during the imagine thirst condition relative to the imagine drink condition could explain the higher thirst ratings during imagine thirst. The activation of a similar neural network during both the imagine thirst and imagine drink conditions, together with the increase in thirst ratings reported only during the imagine thirst condition, suggests that enhanced functional connectivity between the ventral anterior insula and the aMCC may be necessary to distinguish the experience of thirst from the experience of satiation due to drinking.

Numerous animal and human studies implicate the OFC in learning and reversing associations between stimuli on the basis of changing reward or punishment values (42). A state of thirst signals the presence of a fluid deficit, which represents a threat to homeostasis and can therefore be considered punishing. Being fully hydrated corrects this deficit and, because it facilitates homeostasis, can be considered rewarding. To imagine a state of thirst while in a fully hydrated state, the reward value associated with the fully hydrated state may thus have to be either suppressed or changed to a punishment value so that it is compatible with the state of imagined thirst. As the OFC is likely to receive a representation of information processed within the anterior insula (41, 43), we speculate that the OFC receives information from the insula regarding the current hydration status of the body and is capable of suppressing or changing the value associated with this information so that it is compatible with current cognitive goals. Indeed, examples of this regulatory influence of the OFC over sensory information have previously been discussed with respect to emotion (44). Moreover, cytoarchitectonic evidence suggests the OFC and medial prefrontal cortex (which includes the aMCC) form separate networks that interact to link sensory and visceromotor functions, respectively (45). The functional connectivity observed between the OFC and the aMCC in the present study may therefore reflect output from the OFC that leads to the aMCC producing a visceromotor response compatible with a state of thirst. Given the aMCC appears to facilitate drinking during a state of thirst (18), this response may consequently provide motivation for drinking behavior, consistent with the view that the experience of thirst incorporates an intention to drink (22). While this explanation of OFC and aMCC function in relation to the present study is compatible with the role of these regions in cognitive control processes that modulate sensory responses in the insula (44), it should be acknowledged that functional connectivity between the OFC and the insula was not explicitly tested in the present study and so the proposed interactions between the OFC and insula remain conjectural.

Three potential objections may be raised regarding the interpretations presented above. The first concerns the participants' ability to comply with the instructions to imagine thirst and imagine satiation by drinking. It is indeed possible that the participants may have been unsuccessful in using mental imagery to evoke the intended states, and that instead the ratings simply reflect what participants believe is the correct response. While this possibility cannot be discounted, the likelihood of it happening was reduced by recruiting hypnosis practitioners who were experienced in using imagination to evoke particular subjective states. In addition, the brain activity associated with the imagine thirst and imagine drink conditions is consistent with activity reported in previous studies of thirst (1–8) and drinking (26–28). Such an outcome would be highly unlikely if participants were unable to imagine the relevant subjective state for each condition.

The second potential objection is the networks of regions activated by the imagine thirst and the imagine drink conditions may be similar because they represent activity associated with imagination rather than with thirst or drinking per se. Such an objection is countered by evidence reported in previous studies of mental imagery. This evidence reveals that the neural mechanisms of motor imagery, such as imagined drinking, show considerable overlap with the neural mechanisms of motor preparation and execution (46–48). The two have even been considered functionally equivalent except for the explicit production of movement, which is inhibited in motor imagery (48). Indeed, the presence of a common neural substrate readily explains how motor imagery improves motor performance for athletes, musicians, and rehabilitation (47). Common neural mechanisms are also considered to underlie sensory perception and mental imagery associated with the senses, such as imagined thirst, where mental imagery is regarded as a weak form of perception (49). The presence of such a common mechanism provides a compelling explanation for why visual imagery can enhance performance on a perceptual task (50), and why classical conditioning can be produced using visual imagery instead of perceptual stimuli (51). The correspondence between brain regions activated in previous studies of physiologically evoked thirst and drinking and those activated in the present study is therefore consistent with imagination recruiting the same neural network identified in these previous studies, rather than a separate network dedicated to imagination.

The third potential objection relates to the order of the imagined thirst and imagined drinking conditions. While the regular nature of this order has ecological validity, as it reflects the normal sequence of events that occurs in response to dehydration, it was nevertheless predictable and could therefore have produced anticipatory effects. Such effects should have been countered, however, if participants genuinely complied with the experimental protocol and focused their undivided attention on each task, irrespective of order. Furthermore, while it can be argued that brain responses during both conditions may be associated with anticipatory processes not directly related to thirst or drinking, such a possibility is ruled out by the central finding of enhanced functional connectivity between the insula and the aMCC for imagine thirst relative to imagine drink. This finding represents the critical demonstration of differences in brain connectivity between conditions; given each condition would be subject to similar anticipatory effects, the presence of these effects would have therefore been removed from the analysis.

Finally, the results of this study suggest future research will need to focus on the functional role of regions implicated in the neural representation of thirst. In particular, the respective roles of the aMCC and the insula need to be addressed. The outcomes of this study suggest the experience of thirst incorporates a motivational component associated with drinking behavior. This is consistent with the view of thirst as both a sensory experience and an intention to act (22). Furthermore, we have suggested here that the insula may be associated with the sensory experience while the aMCC is associated with the intention to act, and the two need to be functionally connected before thirst is experienced. This hypothesis provides a firm basis for future examination of the functional role of these two regions in relation to the subjective state of thirst.

Conclusion

In this study, the neural representation of thirst was investigated using fMRI and manipulating participants' imagination. With this methodology it was possible to directly compare a state of imagined thirst with a state of imagined satiation of thirst by drinking. The comparison of these states with fMRI had previously not been feasible because of the constraints imposed by a physiological thirst stimulus. Three outcomes have been presented that have important implications for the understanding of thirst. First, this subjective state can be dissociated from a physiological thirst stimulus. Second, the network of brain regions associated with subjective thirst is likely to incorporate regions involved in drinking behavior. Third, increased functional connectivity between the anterior midcingulate cortex and the insula may be necessary to generate a thirst experience. These findings have important implications for the neural understanding of subjective thirst and suggest clarification of the functional role of the aMCC and the insula is an important question needing to be addressed by future studies.

Methods

Protocol. The experimental protocol was approved by the Monash University Human Research Ethics Committee (Project no. 13134), and informed consent was obtained from participants before they commenced the study. Twenty healthy participants (14 female; age range, 27 to 72 y; mean age, 50.0 ± 2.7 y) were recruited from a database of students (provided by S.C.) who had previously demonstrated a high degree of hypnotic susceptibility. These participants were therefore likely to have a well-developed sense of imagination, thereby improving the probability they could evoke the experience of thirst. The participants were instructed to satiate their thirst 1 h before commencing the study and all participants confirmed they had complied with this instruction. Participants subsequently took part in two experimental sessions, one involving a mock scanner (which simulated the noises and physical confinement of the MRI scanning environment) and one involving an actual MRI scanner. Both sessions used the same experimental protocol. The set of behavioral measures obtained prior to scanning consisted of a thirst rating, a mouth dryness rating, a stomach fullness rating, volume drunk in response to ad libitum access to water, and a final thirst rating. Once the initial set of behavioral measures had been recorded, participants entered the scanner for a single fMRI scanning session (30 min; Fig. 1) that consisted of a structural scan followed by two functional scans. At the conclusion of scanning, participants exited the scanner and the same behavioral measures recorded prior to scanning were repeated.

MRI Scanning. The fMRI data were collected at the Monash Biomedical Imaging facility using a Siemens Skyra 3-T MRI scanner that incorporated a 20-channel head coil. For details of acquisition parameters for both structural and functional images, refer to ref. 13. Each functional scan was composed of 230 sequential BOLD contrast images and contained four trials, with each trial consisting of three 30-s cognitive events followed by three 7-s rating events (Fig. 1). The cognitive events consisted of baseline, imagine thirst, and imagine drink conditions. During the baseline condition, participants were instructed to let their mind wander and not to focus on anything thirst-related; during the imagine thirst condition, they were instructed to imagine the most intense thirst possible; and during the imagine drink condition, they were instructed to imagine satiating their thirst by drinking cool water. Participants rated the degree of thirst they experienced in the preceding condition by using their fingers on both hands to indicate their chosen rating. For this rating a 10-point scale was used in which 0 signifies no thirst and 10 signifies maximum thirst.

Brain Activation Analysis. Brain activity during each 30-s condition was analyzed together with the 7-s rating events that followed each condition. Preprocessing and analysis of the brain images were performed using FEAT (version 5.0.9), the functional imaging component of the FMRIB Software Library (FSL) suite of analytic tools used for analyzing brain imaging data. Five regressors were used in the general linear model, the first two representing brain responses to imagine thirst and imagine drink, and the remaining regressors representing brain responses to baseline, imagine thirst, and imagine drink rating events. Each of the imagined conditions was contrasted with baseline to produce a principal functional activation analysis. Contrasts between the two imagined conditions were also computed, with each contrast masked with the relevant principal functional activation analysis. To determine significant brain activations, clusters of activation were initially created using a single-voxel inclusion threshold of $Z > 2.3$ ($P < 0.01$). These clusters were then thresholded and corrected for multiple comparisons ($P_{\mathrm{corr}} < 0.05$) (52).

Location of Seed for PPI Analyses. The anterior midcingulate cortex has been consistently identified in both the thirst and drinking literatures (18). The aMCC is therefore an ideal seed region for the psychophysiological interaction analyses in the present study. To define the neuroanatomical location and extent of the aMCC seed, significant aMCC activations in both the imagine thirst and imagine drink conditions were used and the following voxel inclusion criteria applied: Z statistic > 2.8 and $>50\%$ gray matter probability. These probabilities were obtained using the Harvard–Oxford Cortical Structural Atlas tool found within FSLView, an analytic tool within FSL that enables brain images to be visualized.

PPI Analysis. To the general linear model used for the prior analyses of brain activation, three additional regressors were added: the time series of the aMCC seed, the PPI for imagine thirst, and the PPI for imagine drink. The imagine thirst PPI was generated from the imagine thirst regressor and the time series of the aMCC seed, while the imagine drink PPI was generated from the time series of the aMCC seed and the imagine drink regressor. Each of the imagined conditions was contrasted with baseline to produce a principal functional connectivity analysis. Functional connectivity between the two imagined conditions was then contrasted, with each contrast masked with the relevant principal functional connectivity analysis. To determine significant brain connectivity, a single-voxel inclusion threshold of $Z > 2.3$ ($P < 0.01$) was used to create clusters of connectivity that were then thresholded and corrected for multiple comparisons ($P_{\mathrm{corr}} < 0.05$) (52).

Data Availability Statement. All data necessary for replication have been included in this paper.

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