Overdrinking, swallowing inhibition, and regional brain responses prior to swallowing

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In humans, drinking replenishes fluid loss and satiates the sensation of thirst that accompanies dehydration. Typically, the volume of water drunk in response to thirst matches the deficit. Exactly how this accurate metering is achieved is unknown; recent evidence implicates swallowing inhibition as a potential factor. Using fMRI, this study investigated whether swallowing inhibition is present after more water has been drunk than is necessary to restore fluid balance within the body. This proposal was tested using ratings of swallowing effort and measuring regional brain responses as participants prepared to swallow small volumes of liquid while they were thirsty and after they had overdrunk. Effort ratings provided unequivocal support for swallowing inhibition, with a threefold increase in effort after overdrinking, whereas addition of 8% (wt/vol) sucrose to water had minimal effect on effort before or after overdrinking. Regional brain responses when participants prepared to swallow showed increases in the motor cortex, prefrontal cortices, posterior parietal cortex, striatum, and thalamus after overdrinking, relative to thirst. Ratings of swallowing effort were correlated with activity in the right prefrontal cortex and pontine regions in the brainstem; no brain regions showed correlated activity with pleasantness ratings. These findings are all consistent with the presence of swallowing inhibition after excess water has been drunk. We conclude that swallowing inhibition is an important mechanism in the overall regulation of fluid intake in humans.

thirst | drinking | swallowing | inhibition | fMRI

Fluid depletion leads to drinking, an important evolutionary behavior that satisfies the physiological need to replenish lost fluid. The motivation to begin drinking is normally provided, in humans at least, by the presence of a subjective state of thirst. At some point after drinking has commenced, the sensation of thirst disappears and is replaced by the experience of satiation, along with the cessation of drinking. Studies performed in humans and animals indicate the regulatory mechanisms that have evolved to govern the cessation of drinking appear to be tightly calibrated, with the amount of fluid ingested commensurate with the degree of fluid depletion, even though some variation occurs between species regarding the time taken to conclude drinking (1–3).

Several factors have been implicated in the regulation of fluid intake, with the majority relating to thirst and the initiation of drinking. These include signals produced by osmoreceptors in the lamina terminalis (4–6), which respond to cellular dehydration and the resulting increase in sodium concentration within the cerebral spinal fluid (2), and signals produced in response to extracellular dehydration, such as those associated with the renin–angiotensin system, which is activated as a result of changes in vascular pressure and volume (7, 8). In comparison, the mechanisms responsible for terminating drinking are less well understood. Oropharyngeal metering related to the swallowing reflex is implicated in dogs (9) and humans (10), along with changes in mouth dryness during drinking in humans (11). Esophageal (12), gastric (13–15), and intestinal (16) factors also appear to play a role in terminating drinking in a variety of nonhuman species, although to what extent these factors influence cessation of drinking in humans is uncertain (10).

Recently, the results of a fMRI study by our group implicated swallowing inhibition as a potential factor contributing to the cessation of drinking in humans (17). If the presence of this inhibition could be directly demonstrated, it would provide confirmation of an important mechanism that regulates fluid intake. Humans, with their capacity to report subjective experience, represent an ideal species in which to investigate putative constraints on the act of swallowing. Ratings of "swallowing effort" can be obtained under varying conditions, with a systematic increase in ratings serving as a proxy for the presence of inhibition. Furthermore, although the factors that regulate fluid intake have been extensively investigated in animal studies (e.g., refs. 2, 5, 6, 12, and 18–20), they have received relatively little attention in humans despite their relevance to dysfunctional drinking behavior. This behavior includes the binge drinking associated with drinking alcohol (21), which is particularly prevalent among young adults (22), and the polydipsia linked to schizophrenia (23), which is associated with a higher mortality rate in the clinical population (24).

In our earlier study (17), regional brain responses were investigated at the time of swallowing. During this period, increased activation in bilateral sensorimotor cortex was revealed when individuals continued to drink after satiation relative to when they were thirsty. This finding was interpreted as the additional motor activity required to overcome the putative inhibition of swallowing so that drinking could continue after satiation. For the present study we reasoned that, if swallowing inhibition were present, an increase in goal-directed activity would also be required before

Significance

Drinking represents a crucial behavior that subserves the survival of species by maintaining fluid balance within the body. The present study confirms in humans the presence of swallowing inhibition after excess liquid has been drunk, revealing a mechanism important for the regulation of fluid intake. The findings presented here support the view that swallowing inhibition is probably a "hard-wired" process that aids maintenance of fluid balance within the body, thereby avoiding the detrimental effects of overdrinking that can cause water intoxication and eventually death.

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swallowing to overcome the inhibition. As goal-directed activity is reflected in cognitive control exerted by the prefrontal cortex (25, 26), we performed fMRI during the period immediately prior to swallowing (hereafter the "preswallow period") to test the hypothesis that activity in the prefrontal cortex increases following satiation and is correlated with the effort required to swallow. This would provide corroborating evidence for swallowing inhibition as well as providing additional insight into the cortical brain processes involved in the regulation of swallowing (27–31).

The results of our previous study also revealed complicit drinking after satiation was accompanied by a subjective state of unpleasantness, and that variance in unpleasantness ratings was related to regional brain responses during swallowing (17). Because of the absence of swallowing effort as a behavioral measure, it was not possible in this earlier study to identify the relative contributions of hedonic responses and swallowing effort to brain activation associated with drinking. It was therefore important, in the present study, to examine the extent to which brain activity is associated with hedonic attributes as well as with swallowing effort and whether the two were related. We consequently included ratings of pleasantness regarding liquid taste and used 8% (wt/vol) sucrose added to water (hereafter sugar solution) as a second stimulus in addition to water, reasoning that the rewarding properties of a nutrient such as sugar would lead to additional variation in hedonic responses. With this approach, we could potentially reveal if effort and pleasantness/unpleasantness were tightly coupled or if the two sensations varied independently.

Beginning with the same experimental protocol as our previous study (17), we modified procedures to examine swallowing effort and associated regional brain responses (Fig. S1). Two physiological conditions with opposing states of hydration were induced. First, exercise-related dehydration produced a thirst state conducive to drinking, before compliance with experimental protocol led to an excess intake of water and resulted in a second "oversated" state incompatible with further drinking. Participants were scanned during both conditions, and, while in the scanner, they periodically received (in random order) 5-mL volumes of either stimulus, which they briefly held in their mouth before swallowing. The participants subsequently rated the pleasantness of the taste of the liquid along with the effort required to swallow it. We hypothesized that drinking in the oversated state would be associated with a significant increase in swallowing effort, and that effort ratings would predict regional brain responses during preparation for swallowing, independently of any variance related to ratings of pleasantness/unpleasantness.

Results

Exercise-Related Temperature, Weight, and Thirst. At the beginning of the experiment, 20 healthy participants (13 male; age range, 23–45 y; mean age, 30.0 ± 1.5 y) had an average aural temperature of 36.9 ± 0.1 °C and reported an average thirst rating of 3.1 ± 0.4 (scale range, 0–10; 0 indicates no thirst; 10 indicates maximum thirst). Following 60 min of exercise at 60% of heart rate reserve on a stationary cycle, participants' average aural temperature had increased by 0.4 ± 0.1 °C [$t(19) = 4.0$; $P < 0.001$] and their average rating of thirst had increased to 6.2 ± 0.5 [$t(19) = 9.6$, $P < 0.001$]. As a result of exercise, participants lost 0.6 ± 0.06 kg [$t(19) = 11.0$; $P < 0.001$], or 0.9 ± 0.06% of body weight. During a subsequent 60-min cool-down period, participants' aural temperature decreased [−0.5 ± 0.1 °C; $t(19) = 6.5$; $P < 0.001$], whereas no significant change occurred in their average thirst rating (7.0 ± 0.4).

Thirst, Weight, and Subjective Ratings of Water and Sugar Solution During Drinking. The average thirst rating decreased to 4.2 ± 0.5 [$t(19) = 7.3$, $P < 0.001$] as a result of participants drinking, in random order, ten 5-mL volumes of water and ten 5-mL volumes of sugar solution (80 g sucrose per liter) during scanning under the thirsty condition. The first two fMRI scans, each of 8-min duration [240 volumes; repetition time (TR) = 2,000 ms], were acquired during this thirsty condition. On average, participants rated the 5-mL drinks during the scans as pleasant (water, 1.9 ± 0.4; sugar solution, 1.8 ± 0.4; scale range 0–5; 0 indicates neutral; 5 indicates extremely pleasant) and of minimal effort to swallow (water, 1.4 ± 0.4; sugar solution, 1.7 ± 0.5; scale range, 0–10; 0 represents no effort; 10 represents extreme effort). Neither rating differed significantly between the water and sugar solutions [pleasantness $t(19) = 0.2$, $P = 0.9$; effort $t(19) = 0.7$, $P = 0.5$]. When they had been removed from the scanner, participants were given ad libitum access to water along with encouragement to overdrink as much as could be comfortably tolerated; their average thirst rating then decreased to 0.1 ± 0.1 [$t(19) = 8.5$, $P < 0.001$].

The participants drank an average volume of 1.18 ± 0.13 L during ad libitum access and overdrinking. Participants reentered the scanner 15.7 ± 5.5 min after concluding the first period of scanning. Two fMRI scans were acquired during the oversated condition, in which drinks of ten 5-mL volumes of water and ten 5-mL volumes of sugar solution in random order were rated on average as unpleasant (water, 1.3 ± 0.3; sugar solution, 0.5 ± 0.4; scale range 0–5; 0 indicates neutral; 5 indicates extremely unpleasant) and of moderate effort to swallow (water, 4.7 ± 0.5; sugar solution, 4.1 ± 0.5; scale range 0–10; 0 represents no effort; 10 represents extreme effort). The pleasantness ratings did not differ significantly between the water and sugar solutions [$t(19) = 1.5$, $P = 0.2$], whereas the contrast for effort approached significance [$t(19) = 2.1$, $P = 0.054$]. During the entire scanning period, with ad libitum drinking and overdrinking included, participants drank an average of 1.38 ± 0.13 L. Their corresponding average weight increase was 1.34 ± 0.12 kg [$t(19) = 11.6$; $P < 0.001$].

The ratings of pleasantness/unpleasantness significantly decreased after overdrinking for water and sugar solution [$F(1,19) = 60.3$, $P < 0.001$) whereas effort ratings significantly increased for the same contrast [$F(1,19) = 45.1$, $P < 0.001$; Fig. 1]. Stimulus was not a significant factor for either rating [effort $F(1,19) = 0.3$, $P = 0.6$; pleasantness/unpleasantness $F(1,19) = 0.5$, $P = 0.5$]. Despite the absence of significant differences between ratings of water and sugar solution at any of the individual time points (before and after oversatiation, as detailed earlier), there were significant interactions between the effects of overdrinking and stimulus for both pleasantness/unpleasantness [$F(1,19) = 21.7$, $P < 0.001$] and effort [$F(1,19) = 4.7$, $P < 0.05$]. Post hoc tests showed an increased degree of change in ratings of water compared with sugar solution after oversatiation for effort [change scores, water, 3.3 ± 0.5; sugar solution, 2.4 ± 0.5; $t(19) = 2.2$, $P < 0.04$] and pleasantness/unpleasantness [change scores, water, 3.3 ± 0.4; sugar solution, 2.4 ± 0.4; $t(19) = 4.7$, $P < 0.001$]. The correlations between pleasantness ratings and effort ratings during the thirsty condition were not significant (water, $r = -0.4$, $P = 0.1$; sugar solution, $r = -0.3$, $P = 0.2$), along with the correlations between pleasantness ratings and effort ratings during the oversated condition (water, $r = -0.2$, $P = 0.3$; sugar solution, $r = -0.3$, $P = 0.2$).

Brain Activity During the Thirsty Condition Relative to the Oversated Condition. We examined regional brain responses during the preswallow period, when the brain processed the hedonic properties of

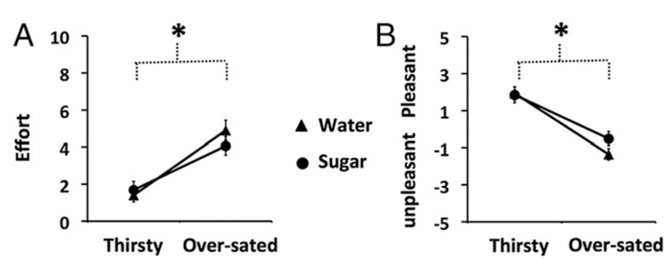

Fig. 1. Behavioral results for ratings of swallowing effort and pleasantness of liquid taste. (*A*) For effort ratings, water and sugar solution showed an effect of condition: both were associated with a significant increase in effort during the oversated condition compared with the thirsty condition (*$P < 0.001$). (*B*) An effect of condition for both liquids was also present for pleasantness ratings: a significant decrease in pleasantness was associated with water and sugar solution during the oversated condition compared with the thirsty condition (*$P < 0.001$).

liquid taste and prepared to initiate swallowing. Consistent with the behavioral results that revealed an absence of overall stimulus effect and absent pairwise differences between the stimuli in the individual thirsty and oversated conditions, contrasts between water and sugar solution in each condition revealed no differences in brain activity between the stimuli. Given the lack of differences for behavioral and imaging contrasts of the two stimuli, subsequent imaging analyses therefore amalgamated responses to water and sugar.

For the amalgamated analyses, the 7-s preswallow period (commencing with liquid being delivered to the mouth and terminating with a cue to swallow) revealed extensive activation in cortical and subcortical areas in the thirsty and oversated conditions (Fig. S2). An extensive network of regions including left parietal cortex, left motor cortex, left striatum, left thalamus, and lateral prefrontal cortex in both hemispheres showed greater activity during the oversated condition compared with the thirsty condition (Fig. 2 and Table S1). No brain region showed activity during the thirsty condition that was greater than activity during the oversated condition.

Brain Activity Correlated with Pleasantness and Swallowing Effort During the Oversated Condition. A regression analysis was used to investigate the capacity of the two behavioral ratings to independently predict brain activity before swallowing during the oversated condition. A distributed network of areas showed activity negatively associated with effort ratings (Fig. 3 and Table S2). These areas included the right orbital frontal cortex, bilateral amygdala, right inferior frontal gyrus, right frontal opercular cortex, right frontal pole, and ventral midbrain and pontine structures. The direction of the association indicates that participants with the greatest activity in these regions had the lowest ratings of swallowing effort. No brain regions showed activity that was independently predicted by pleasantness ratings.

Discussion

This study investigated the hypothesis that swallowing becomes inhibited when the volume drunk exceeds that required to restore fluid balance within the body. While in the scanner, participants periodically swallowed small volumes of water or sugar solution delivered in random order during two conditions: a thirsty condition conducive to drinking and an oversated condition incompatible with further drinking because of previous ingestion of excess water. Subjective ratings of pleasantness/unpleasantness associated with liquid taste replicated our earlier finding that drinking water in the oversated condition is unpleasant (17), whereas ratings of swallowing effort showed that swallowing liquid becomes more effortful during this condition. Regional brain responses during the preswallow period, when participants held liquid in their mouths in preparation for swallowing, were increased after overdrinking and these increases are best explained by swallowing effort rather than the hedonic attributes of liquid taste.

Behavioral Ratings During Thirsty and Oversated Conditions. The behavioral finding of a significant increase in average swallowing effort between the thirsty state and the oversated state (Fig. 1*A*) suggests a shift from relatively automatic instigation of swallowing during the thirsty condition to a moderate degree of volitional control during the oversated condition. This finding provides psychometric confirmation of the hypothesis that swallowing is inhibited during the oversated condition, as proposed in our previous study (17).

The shift in average pleasantness ratings, from pleasant during the thirsty condition to unpleasant during the oversated condition, replicates the outcome of our previous study (17) and is consistent with the findings of an earlier study that reported changes in the hedonic experience of water in the mouth during thirst and following satiation (32). Interestingly, the hedonic experience associated with sugar solution in the present study did not differ appreciably from that experienced with water in the thirsty or oversated conditions. It therefore appears that adding a rewarding nutrient such as sugar does not produce higher pleasantness ratings when individuals drink during these conditions. In the context of the present study, perturbation of fluid balance appears to exert a stronger influence over

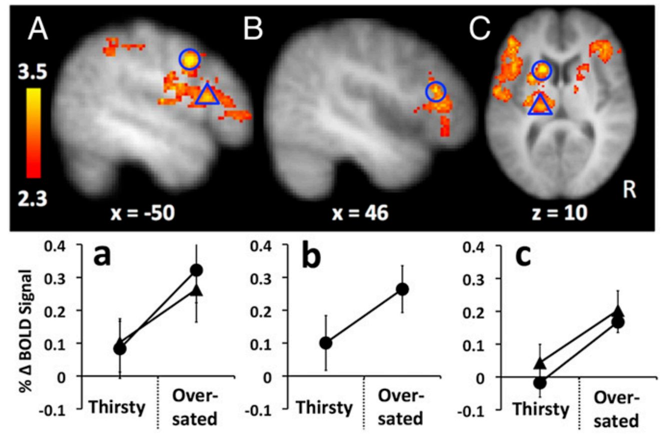

Fig. 2. Brain regions showing increased activation in the oversated condition compared with the thirsty condition during the preswallow period. Open blue circles and triangles in brain images represent regions of interest (ROIs). All ROIs were selected according to two criteria: *z* statistic >3.5 and >50% gray matter (based on probabilities provided by the Harvard–Oxford Cortical Structural Atlas, an atlas tool provided in FSLView). Each black circle or triangle in a graph (*a*–*c*) represents the average BOLD signal percentage change from the thirsty condition to the oversated condition from the corresponding ROI in the image above (*A*–*C*). All five ROIs show an increase in BOLD activity during the oversated condition relative to the thirsty condition. (*A*) Parietal, motor, and prefrontal regions in the left hemisphere. Blue circle marks the precentral gyrus and middle frontal gyrus; blue triangle marks the inferior frontal gyrus. (*B*) Prefrontal regions in the right hemisphere. Blue circle marks the inferior frontal gyrus. (*C*) Bilateral prefrontal regions and subcortical regions in the left hemisphere. Blue circle marks the caudate; blue triangle marks the thalamus.

hedonic experience than the perception of sweet taste, a subjective state that has genetic and experiential components (33) that lead to differences between childhood, adolescence, and adulthood (34, 35) and between different races/ethnicities (36). As plasma osmolality during the oversated condition may not accurately reflect the amount of water ingested [because of the absorption of fluid delaying the return of plasma osmolality to baseline, a process that can take as long as 50 min (10)], an allostatic mechanism (37) that influences behavior in anticipation of future contingencies may be involved. Such a mechanism could favor maintenance of correct fluid balance over the hedonic properties of ingested liquid.

Brain Activity During Thirsty and Oversated Conditions. Regional brain responses during the preswallow period revealed a distributed network of regions with increased blood oxygen level-dependent (BOLD) signal change for the oversated condition compared to the thirsty condition (Fig. 2 and Table S1). The identified network includes regions such as the lateral prefrontal cortex, motor cortex, striatum, and thalamus previously implicated in the regulation of goal-directed behavior (38–40). As processing in the prefrontal cortex has been associated with "mental effort" (41) and volition (42, 43), increased activity and regulation of prefrontal and motor regions during the oversated condition is consistent with the increase in volitional effort required to instigate goal-directed drinking observed behaviorally (Fig. 1*A*). Such increases in activity and regulation may be associated with working memory, one of the core executive functions performed by the prefrontal cortex (44, 45). Working memory is responsible for maintaining goal-related information for short periods of time, with prefrontal activity shown to correlate with working memory load (46). In the context of the oversated condition the observed increases in frontal activity may thus reflect increased difficulty in maintaining the goal of preparing to swallow before the swallow cue.

Previous studies of swallowing (27–29, 31), including our earlier study (17), have identified the lateral precentral gyrus, lateral postcentral gyrus, insula, operculum, cingulate cortex, and superior

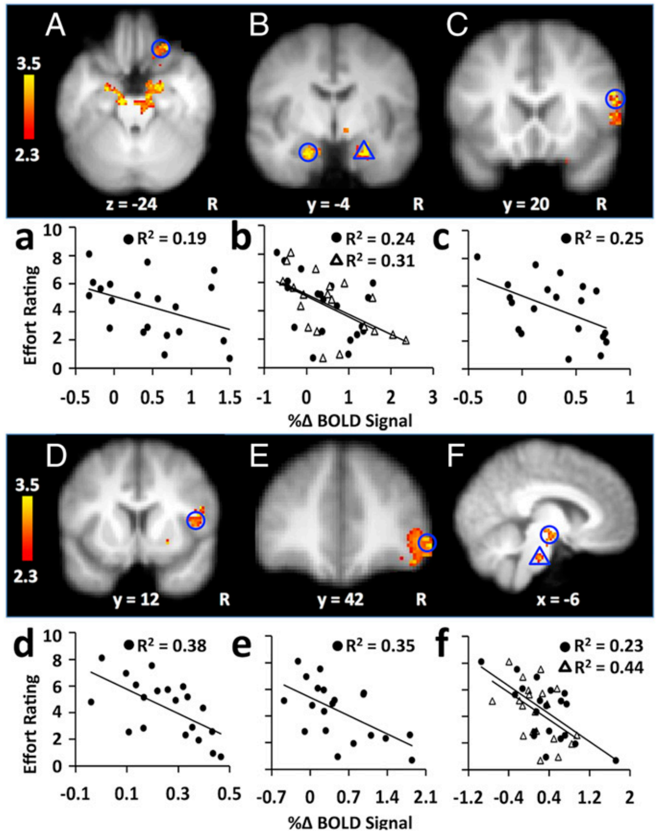

Fig. 3. Brain regions showing activity correlated with effort ratings in the oversated condition during the preswallow period. Open blue circles and triangles in images represent ROIs: (*A*) right orbital frontal cortex, (*B*) bilateral amygdala, (*C*) right inferior frontal gyrus, (*D*) right frontal opercular cortex, and (*E*) right frontal pole; (*F*) blue circle marks the ventral midbrain; blue triangle marks the mid-pons. All ROIs were selected according to two criteria: *z* statistic >3.0 and >50% gray matter (based on probabilities provided by the Harvard–Oxford Cortical Structural Atlas, an atlas tool provided in FSL View). Lowercase letters representing graphs (*a*–*f*) correspond to uppercase letters (*A*–*F*) representing brain images. Each black circle or open triangle in a graph represents the correlation between a participant's effort rating and the participant's BOLD signal percentage change derived from the ROI with the same shape in the corresponding image. All eight ROIs show activity negatively correlated with effort ratings.

temporal gyrus as regions involved in the cortical regulation of swallowing. A crucial difference between those studies and the present study, however, is that brain images for the present study were acquired while liquid was held in the mouth rather than while it was being swallowed. Despite this difference, the lateral precentral gyrus (Fig. 2*A*) and frontal operculum (Table S1) still showed an increase in BOLD signal for the oversated condition relative to the thirsty condition. In humans, both regions have been implicated in swallowing and tongue movement (47), and, in primates, stimulation of the two regions has been shown to induce swallowing (48), along with tongue (49) and jaw (50) movements associated with mastication. These movements contribute to the oral preparatory phase of swallowing responsible for transporting liquid to the pharynx, where the sensory properties of the liquid can subsequently trigger initiation of swallowing (51). The increased BOLD signal in the two regions may thus represent an increase in the difficulty of manipulating the liquid to a position where swallowing can be instigated during the oral preparatory phase of swallowing.

When subjective ratings of swallowing effort and pleasantness were regressed against brain activity during the oversated condition, only swallowing effort independently predicted brain activity (Fig. 3). This confirms that, during the preswallow period while liquid was still present in the mouth, the increase in frontal activity observed in the oversated condition relative to the thirsty condition was concerned with preparation for swallowing and not the hedonic properties of liquid taste. Indeed, two regions in the right inferior frontal gyrus and a region in the right frontal pole showed activity that was correlated with ratings of swallowing effort while also showing increased activity during the oversated condition relative to the thirsty condition (Fig. S3). Finally, for the identified lateral prefrontal regions, the negative direction of the correlation reveals participants with the greatest increase in BOLD signal had the lowest ratings of swallowing effort. This is consistent with these people being the most successful at recruiting the executive functions (44) performed by the lateral prefrontal cortex (52) to overcome the presence of swallowing inhibition.

Two regions in the brainstem, the ventral midbrain (Fig. 3*F*, blue circle) and the mid-pons (Fig. 3*F*, blue triangle), showed activity that was also negatively correlated with ratings of swallowing effort. The locations of these regions is rostral to nuclei in the medulla known to control the swallowing reflex (53, 54). These medullary nuclei receive sensory input from the superior laryngeal nerve; stimulation of the nuclei (55), the nerve (55, 56), or regions innervated by the nerve, such as the epiglottis and larynx (57), initiate the swallowing reflex. The location of the pons activation instead corresponds approximately to the position of the facial nuclei and the oral part of the spinal trigeminal nucleus, two components of the central pattern generator (CPG) for mastication (58). The CPG contains neurons that, rather than swallowing, are primarily involved in rhythmic and repetitive orofacial movements involving the jaws and tongue (59). These movements also contribute to the oral preparatory phase of swallowing (51). Participants with the greatest BOLD signal in the identified pons region may thus have experienced less swallowing effort because, before swallowing, they were able to manipulate the liquid to a position where it was easier to trigger the swallowing reflex when the cue to swallow had appeared.

Although our results do not provide definitive insight into the neuroanatomical location of swallowing inhibition during the oversated condition, the location of the pons activation provides indirect evidence for the inhibition to influence brainstem nuclei involved in the oral preparatory phase. This is the first phase in the swallowing sequence; it is voluntary and can be interrupted at any time (53). The pharyngeal and esophageal phases of swallowing, in comparison, are automatic, and the pharyngeal phase is irreversible when it has been initiated (53). If the inhibition were to act on the CPG implicated in the pons activation, for example via GABAergic or glycinergic inhibitory premotor neurons (60, 61), it has interesting implications for the role of the prefrontal cortex during the preswallow period: does the frontal cortex modulate the CPG directly, as previous evidence implies (49), via projections to the facial (62) or peritrigeminal (59, 63, 64) regions? Or does it circumvent the influence of the inhibition by controlling the oral preparatory phase independently, via innervation of the orofacial region by the motor cortex (49, 50, 65, 66)? The two explanations are not mutually exclusive, and, if the frontal cortex did modulate the CPG directly, this response would represent an example of disinhibition rather than inhibition. It would therefore fundamentally differ from the function of response inhibition usually ascribed to the prefrontal cortex (26) and, in particular, the right inferior frontal gyrus (67), a region also implicated in our present findings (Fig. 3*C* and Fig. S3).

Our results also suggest that the amygdala may be involved in modulating swallowing inhibition (Fig. 3*B*). As activity in the amygdala during the oversated condition was negatively correlated with swallowing effort, this region could play a complementary role to the frontal cortex in regulating swallowing inhibition during the oversated condition. Any regulation of inhibition by the amygdala is likely to occur via the CPG for mastication located in the pons, which receives several direct and indirect projections from the amygdala (68–70), rather than via the motor cortex, which, in primates, is unlikely to receive afferents from the amygdala (71, 72).

Finally, it is important to note that the relationship between swallowing inhibition and other mechanisms implicated in the termination of drinking remains unclear. However, it is probable that inhibition is initiated in response to negative feedback provided by a combination of interoceptive factors (73), which, in humans, is likely to include oropharyngeal stimulation (10, 11) and possibly neural signals from a distended stomach (13–15), along with postgastric influences (16). Swallowing inhibition may thus represent the final common output pathway for this gestalt of factors implicated in providing the brain with a satiation signal.

Conclusion

The results of this study provide insight into the functional organization of drinking behavior by demonstrating the existence of a strong inhibitory influence that limits excess drinking in humans. Subjective ratings of swallowing effort provided psychometric confirmation of swallowing inhibition during the oversated condition. Regional brain responses during the preswallow period revealed an increase in frontal activity for the oversated condition compared with the thirsty condition, a result consistent with the recruitment of frontal cortex to overcome swallowing inhibition so that compliant drinking can continue. Additional evidence for this proposition was also provided by a regression analysis involving the oversated condition, which revealed that swallowing effort and not the hedonic evaluation of liquid taste predicted activity in the prefrontal cortex during this condition. Subjective ratings of swallowing effort, comparison of brain activity between physiological conditions, and the association of prefrontal activity with ratings of swallowing effort during the oversated condition therefore all provide converging evidence that swallowing becomes inhibited if more water has been drunk than is necessary to restore fluid balance.

Experimental Procedures

Protocol. The experimental protocol was approved by the University of Melbourne Human Research Ethics Committee (no. 1341082), and informed consent was obtained from participants before they commenced the study. The study consisted of a single experimental session made up of three principal components: exercise on a stationary bicycle (60 min), cool-down (60 min), and MRI scanning (60 min; Fig. S1*A*). Ratings of thirst were made by using a 0–10 rating scale (with 0 indicating thirst and 10 indicating maximum thirst) according to a previously reported procedure (74). The degree of exercise was standardized by (*i*) calculating the heart rate reserve (HRR) for each participant before exercise [HRR = (220 − age in years) − resting heart rate (RHR)]; (*ii*) adding 60% of the HRR to the RHR; and (*iii*) ensuring that each participant maintained their heart rate at this value (RHR + 0.6*HRR) for the duration of the exercise period by using feedback on their heart rate provided by a telemetric device.

MRI Scanning. Four fMRI scans were acquired during the study (Fig. S1*B*). The first two scans were acquired ∼60 min after completion of exercise while participants experienced thirst (thirst condition). Participants were subsequently removed from the scanner, given ad libitum access to water, and asked to drink to satiation. Following satiation, they were instructed to drink more water, as much as they could comfortably tolerate. The participants then returned to the scanner, and two further scans were acquired (oversated condition). During each of the four scans, five 5-mL volumes of water and five 5-mL volumes of sugar solution were randomly delivered to the participant's mouth every 47 s. An investigator used a syringe connected to a plastic tube to administer each 5-mL volume of liquid. Participants imbibed 50 mL of liquid (25 mL water + 25 mL sugar solution) during each of the four functional scans (200 mL drunk in total during entire scanning period).

Behavioral Task. For each scan, participants completed 10 trials, with each trial consisting of a repeated series of cues and stimuli (Fig. S1*B*). Each trial began with a fixation cue, followed by a cue indicating that 5 mL of liquid was about to be delivered. Participants were instructed before entering the scanner that, when they had received the liquid, they were to hold it in their mouths until, 7 s later, a cue would be presented instructing them to swallow. Ten seconds after this instruction, participants were cued to rate the pleasantness of the liquid on a 0–10 point scale, with ratings of 0–4 representing decreasing degrees of unpleasantness, 5 representing a neutral rating (neither pleasant nor unpleasant), and 6–10 representing increasing degrees of pleasantness. The pleasantness cue was followed 5 s later by a cue to rate the effort required to swallow the liquid. This rating used a 0–10 point scale, with a rating of 0 representing no effort and a rating of 10 representing extreme effort. All cued instructions appeared visually on a screen at the foot of the scanner bed. Participants used the fingers on both hands to indicate their rating.

Image acquisition. Scanning was performed at the Murdoch Children's Research Institute (Melbourne, Australia) by using a Siemens Trio 3-T scanner and 32-channel head coil. Structural T1-weighted images were acquired in the sagittal plane [192 slices; 0.90 mm thickness; 0.84×0.84 mm^2 in-plane resolution; echo time (TE) 2.6 ms; TR, 1,900 ms; flip angle, 9.0°]. Echo-planar images (EPI) were acquired in the transaxial plane (33 slices; 4.5 mm thickness; 3.3×3.3 mm^2 in-plane resolution; TE, 35 ms; TR, 2,000 ms; flip angle 90°) during each of four 8-min acquisitions consisting of 240 sequential BOLD contrast images.

Analysis. Statistical analyses of the physiological parameters and thirst ratings were performed using SPSS 15.0 for Windows. A repeated-measures factorial ANOVA was used to test for differences in ratings of pleasantness and swallowing effort. The following tests were used: effect of stimulus (water, sugar solution), effect of condition (thirsty, oversated), drink order during scanning runs (from 1 to 10), scanning run (first or second in both conditions), and interactions between the principal factors. Preprocessing and analysis of functional images was performed using standard procedures with FEAT, version 4.1.9 (75). Regressors for rating, liquid in the mouth, and swallowing events were included in the model, which also incorporated the confound regressors related to head motion and physiological noise produced by respiratory maneuvers during swallowing (76, 77). A regression analysis was performed for the oversated condition by using the mean of each participant's effort and pleasantness ratings as independent regressors to explain brain activity during the preswallow period. Significant activations for all imaging analyses were determined by using a single voxel inclusion threshold of $Z > 2.3$ ($P < 0.01$) and a cluster level threshold of $P_{corr} < 0.05$ corrected for multiple comparisons (78).

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