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Anterior cingulate cortex modulates the affective-motivative dimension of hyperosmolality-induced thirst

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Neuroimaging studies have shown that the anterior cingulate cortex (ACC) is consistently activated by thirst and may underlie the affective motivation of drinking behaviour demanded by thirst. But direct evidence for this hypothesis is lacking. The present study evaluated potential correlations between ACC neuronal activity and drinking behaviour in rats injected with different concentrations of saline. We observed an increased number of c-Fos-positive neurons in the ACC after injection of hypertonic saline, indicating strong ACC neuronal activation under hyperosmotic thirst. Increased firing rates of putative ACC pyramidal neurons preceded drinking behaviour and positively correlated with both the total duration of drinking and the total amount of water consumed. Chemogenetic inhibition of ACC pyramidal neurons changed drinking behaviour from an explosive and short-lasting pattern to a gradual but more persistent pattern, without affecting either the total duration of drinking or the total amount of water consumed. Together, these findings support a role of the ACC in modulating the affective-motivative dimension of hyperosmolality-induced thirst.

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Introduction

Thirst is an essential homeostatic response to body fluid deficit and drives drinking behaviour that is crucial for survival. A set of brain regions underlie these homeostatic responses. Thirst is thought to originate within the lamina terminalis (Gizowski & Bourque, 2017). Neurons in the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT) are intrinsically osmosensitive and also receive ascending neural signals from peripheral baroreceptors (Babic *et al.* 2004; Ciriello, 2013). These interoceptive signals are

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further integrated in the median preoptic nucleus (MnPO) (McKinley *et al.* 2015). The SFO, OVLT and MnPO network monitors changes in blood osmolality, solute load and hormone circulation, and triggers coordinated homeostatic responses to maintain extracellular fluid homeostasis (Bourque, 2008; Zimmerman *et al.* 2016). These responses include both neural (the motivation to seek and consume water, and autonomic changes regulating blood pressure and heart rate) and endocrine (hormones regulating water and sodium retention) mechanisms (Leib *et al.* 2016; Gizowski & Bourque, 2017; Zimmerman *et al.* 2017).

In contrast to these well-established autonomic and neuroendocrine pathways, the neuronal basis for the affective-motivative dimension of drinking behaviour remains poorly defined (Leib *et al.* 2016). Many brain regions spanning the cortex, thalamus, midbrain and hindbrain either receive direct axonal projections from the lamina terminalis or functionally relate to thirst (McKinley *et al.* 2019). In particular, the medial thalamic anterior cingulate cortex (ACC) system receives thirst-relevant signals from the subcortical SFO, OVLT and MnPO network (Farrell *et al.* 2011). Activity in the ACC correlates especially well with the feeling of thirst (Vogt *et al.* 1996; Saker *et al.* 2014), as revealed from human neuroimaging studies using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). Thirst scores in volunteers with intravenous infusion of hypertonic saline correlate with regional cerebral blood flow in the ACC and the insular cortex (Egan *et al.* 2003; Saker *et al.* 2014), whereas increased functional connectivity between ACC and lamina terminalis is observed during thirst and drinking in humans (Farrell *et al.* 2011). Studies in animal models have also demonstrated the ACC's involvement in thirst. Autoradiographic metabolic trapping studies have shown that [^{14}C]glucose accumulates in the ACC of water-deprived rats, indicating metabolic activation of this region (Duncan *et al.* 1989). Decorticate rats, whose forebrains are separated from the brainstem, are unable to regulate water intake in response to osmotic stimuli (Robinson & Mishkin, 1968). Furthermore, water intake could be promptly evoked by delivering electric pulses through a stimulating electrode placed in the ACC of monkeys (Pastuskovas *et al.* 2003). These pieces of evidence consistently indicate a critical role of the ACC in the genesis of thirst perception. Research on pain and itch has associated ACC with affective motivation demanded by homeostatic changes (Craig, 2002, 2003). However, direct evidence supporting a similar role of the ACC in thirst or drinking is lacking. In addition, neuroimaging studies only reveal overall activity changes of the ACC, leaving obvious gaps in understanding the mechanisms of thirst or drinking behaviour at the single neuron level.

Increase in blood osmolality correlates well with the subjective feeling of thirst and is an important homeostatic signal for drinking (Leib *et al.* 2016; Zimmerman *et al.* 2016). In the present study, we performed *in vivo* electrophysiological recording and cell type-specific intervention in the ACC of rats injected with different concentrations of saline (Krause *et al.* 2011; Zimmerman *et al.* 2016) and evaluated potential correlations between ACC neuronal activity and drinking behaviour.

Methods

Animals

Adult male Wistar rats (280–300 g at the beginning of experiments) were purchased from Charles River (Beijing, China). Rats were housed in cohorts of four to six unless otherwise noted at a room temperature of ∼23°C under a reversed 12 h dark–light cycle with free access to food and water. The lights were on at 20.00 h and off at 08.00 h. Temperature and humidity were maintained within a narrow range (20–24°C and 40–60%, respectively). Rats had access to standard food pellets (Meilvzhou, Beijing, China) *ad lib* throughout all experimental conditions. The standard of feed was in accordance with regulation of Laboratory Animals Nutrients for Formula Feeds (GB 14924.3-2010, China). All experimental procedures were approved by the Animal Care and Use Committee of Peking University Health Science Centre.

Immunostaining

For c-Fos immunostaining, rats were injected subcutaneously with 1 ml of 0.15 or 2.0 M NaCl. Each injection was mixed with 0.5% lidocaine to minimize pain and irritation. Ninety minutes later, rats were anaesthetized with 1% pentobarbital sodium and intracardially perfused with 4% paraformaldehyde (PFA, in 0.1 M phosphate buffer, pH 7.4). The brain was post-fixed with 4% PFA for 12 h, and cryoprotected in 20% and 30% sucrose solutions in turn. Sections of 35 μm were sliced coronally using a cryostat microtome (Model 1950, Leica Instrument Co., Ltd) throughout the entire ACC. Free-floating sections were washed in phosphate-buffered saline (PBS), blocked with a buffer containing 3% bull serum albumin and 0.3% Triton X-100 for 1 h, and incubated with the following primary antibodies in 4°C for 24 h: rabbit anti-c-Fos antibody (1:500, 2250S, Cell Signaling Technology, Danvers, MA, USA) and mouse anti-NeuN antibody (1:200, ab104224, Abcam, Cambridge, MA, USA). Sections were then washed in PBS and incubated with secondary antibodies at room temperature for 90 min: Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:500, ZF-0511, ZSGB-BIO, Beijing, China) and Alexa Fluor 647-conjugated goat anti-mouse IgG (1:500, ab150115, Abcam).

For virus verification, brain sections were obtained as described above. Free-floating sections were washed in the PBS, blocked with a buffer containing 3% bovine serum albumin and 0.3% Triton X-100 for 1 h, and incubated with the following primary antibody in 4°C for 24 h: goat anti-glutamate transporter (EAAC1) antibody (1:1000, AB1520, Merck Millipore, Burlington, MA, USA). Sections were then washed in PBS and incubated with the secondary antibody at room temperature for 90 min: Alexa Fluor 488-conjugated donkey anti-goat IgG (1:500, A11055, Thermo Fisher Scientific, Waltham, MA, USA).

Images were taken by a laser scanning confocal microscope (model FV1000, Olympus Co., Ltd, Tokyo, Japan).

In vivo electrophysiological recording

Electrode implantation for *in vivo* recording was based on a custom, 3D-printed design. Six tetrodes of tungsten wires (diameter 20 μm, California Fine Wires Company, Grover Beach, CA, USA) were used for recording multi-unit activities in the ACC (anterior–posterior (AP): +1.5 mm; medial–lateral (ML): −0.5 mm from bregma; dorsal–ventral (DV): −1.5 mm from brain surface).

The animals were initially anaesthetized with isoflurane at 3% gas volume. The animal's head was then shaved using electric clippers. Once the animal was stably anaesthetized, it was fitted with ear bars and mounted in the stereotaxic frame. The percentage of isoflurane was gradually decreased throughout surgery, stabilizing at 1.0–1.5%. Electrodes were fixed on the skull by dental acrylic and protected by a custom, 3D-printed shell for the longitudinal recording. The reference and ground wires were set on the cerebellum. After surgery, the rat was single-housed and allowed to recover for at least 1 week before further experiments.

Each rat was handled by the experimenter for 30 min per day for three consecutive days and adapted to the recording chamber with video recording before recording sessions. Electrophysiological data were acquired using the 32-channel Intan system (Intan Technologies, Los Angeles, CA, USA). An electrode interface board was connected to a headstage on the head of the rat, and headstages were connected to the system amplifiers with cables.

The recording experiment was performed in a Latin square design. Four recording sessions (0.15, 1, 2 and 3 M NaCl solutions) were conducted for each rat in a random sequence. The interval between two sessions was 4 days. The rat was injected subcutaneously with 1 ml of NaCl (0.15, 1, 2 or 3 M) mixed with lidocaine and immediately placed into the recording chamber without water access. After 30 min, a bottle of water was made available for the rat. The total duration of recording was 90 min, when drinking behaviour was most apparent as revealed from our pilot experiment.

Behavioural analysis

Each rat was handled by the experimenter for 10 min per day for three consecutive days before behavioural testing. Experimenters were blind to animal grouping. Behavioural tests and electrophysiological recording were conducted during the rodents' active period. The recording cages were located in a quiet 30 lux-illuminated room. On the test day, water bottles were removed at 10.00 h, and rats were administered 0.15, 1.0, 2.0 or 3.0 M NaCl as described. Thirty minutes later, the water bottle was placed back in the recording cage and made accessible to the rats.

To visualize the temporal distribution of drinking behaviour, we divided the whole experimental session into 30 s bins and measured the drinking time in each bin for each rat. The binned drinking time was then averaged for each group with different saline concentrations.

Spike sorting, classification and analysis

Spike sorting was carried out in KlustaKwik (http://klustakwik.sourceforge.net/). Using principal component analysis (PCA), a rough separation of units from ACC pyramidal neurons and interneurons was mainly based on their differences in spike wave shapes and mean baseline firing rates. Putative interneurons had a high firing rate (>10 Hz) and a narrow peak-to-valley width (<0.4 ms), whereas putative pyramidal neurons showed a lower mean firing rate (<10 Hz) and a wide peak-to-valley width (>0.5 ms) (Barthó *et al.* 2004).

To calculate firing rate variation over time, we analysed the spike firing sequence from −600 s to 1500 s relative to water access. The spike firing sequence was divided into 35 bins (60 s per bin), and the firing rate of each bin was normalized to the baseline period (600 s before water access) by the formula:

$$\text{Firing rate}_{\text{normalized}} = \frac{\text{Firing rate} - \text{Baseline}_{\text{mean}}}{\text{Baseline}_{\text{SD}}}$$

where $\text{Baseline}_{\text{mean}}$ and $\text{Baseline}_{\text{SD}}$ are the average and standard deviation, respectively, of the neuronal firing rate of the bins in the baseline period.

Chemogenetics

AAV5-CaMKIIα-hM4D(Gi)-mCherry (1×10^{12} virus particles/ml) was packaged and purchased from the OBIO Technology (Shanghai, China). The rat was anaesthetized with 1% sodium pentobarbital (0.1 g/kg, I.P.) and positioned in a stereotaxic frame (RWD, Shenzhen, China). The virus solution was injected bilaterally into

ACC (AP +1.0/2.0 mm; ML ± 0.5 mm from bregma; DV −1.5 mm from brain surface) with 0.5 µl/hole and two holes per side, at a speed of 0.1 µl/min. Needles were left *in situ* for an additional 5 min after injection.

A guide cannula (O.D. 0.48 mm/I.D. 0.34 mm, center to center 1.2 mm, RWD) was implanted 1.0 mm above the ACC (AP +1.5 mm; ML ± 0.5 mm from bregma; DV −1.5 mm from brain surface). Four skull screws were used for securing the guide cannula to the skull surface with dental acrylic. The matching cap (0.5 mm below the guide cannula, RWD) was inserted into the guide cannula. After 3 weeks for virus expression and recovery from surgery, behavioural tests and electrophysiological recording were performed.

Clozapine *N*-oxide (CNO; Tocris, Bristol, UK) was dissolved in artificial cerebrospinal fluid at a concentration of 0.8 mM. A volume of 0.5 µl per hemisphere was micro-injected into the ACC over a 5 min period using the polyethylene catheter (PE-10) connected to a 1 µl syringe needle (RWD). The needle was kept for 5 min to maximize drug diffusion. Behavioural tests started 30 min after CNO injection. Our pilot experiment had shown that the inhibitory chemogenetic effect was prolonged (>8 h) and outlasted the behavioural testing (90 min).

Statistics

Data are expressed as means ± SEM. Group comparisons were made using one-way analysis of variance (ANOVA) followed by the Bonferroni *post hoc* test. Single variable comparisons were made with a two-tail unpaired Student's *t* test. The Wilcoxon signed-rank test or the Kruskal–Wallis test with Dunn's *post hoc* test was used to compare *in vivo* neuronal firing rates. The Kolmogorov–Smirnov two sample test was used to analyse cumulative probability. All statistics were calculated using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA) software or MATLAB (The MathWorks Inc., Natick, MA, USA). $P < 0.05$ was taken as statistically significant.

Results

Hypertonic saline activates ACC neurons

To examine whether ACC neurons were recruited by thirst, we performed c-Fos (a marker for neuronal activity) mapping after hypertonic saline injection. The expression of c-Fos was sparse in the ACC after injection of isotonic saline (0.15 M) (Fig. 1*A*). Injection of hypertonic saline (2 M), by contrast, significantly increased the number of

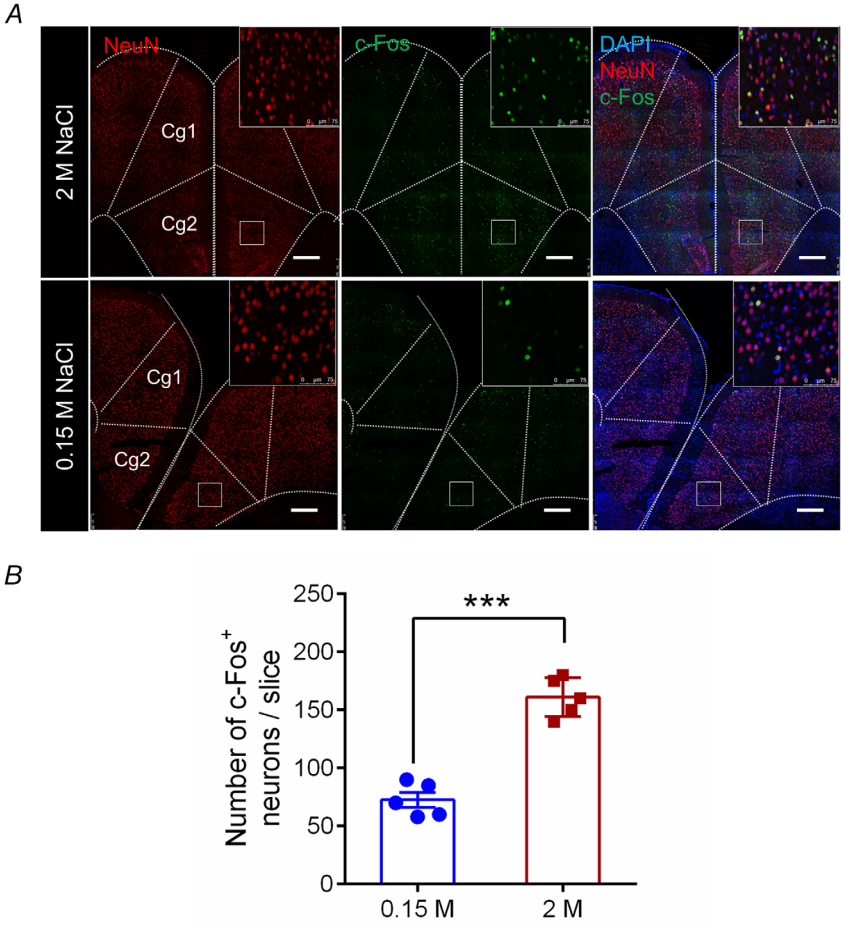

Figure 1. Enhanced activation of ACC neurons in rats with infusion of hypertonic saline
A, representative immunofluorescence images showing c-Fos-positive neurons in the ACC of rats with isotonic (0.15 M) or hypertonic (2 M) saline injection. Left row: NeuN (red); middle row: c-Fos (green); right row: merge. Four slices from each rat, five rats in each group. Scale bars: 500 µm. *B*, quantitative analysis of *A*. Hypertonic saline injection induced robust c-Fos expression in the ACC. $n = 5$ in each group. ***$P < 0.001$, 0.15 M *vs.* 2 M, unpaired *t* test. [Colour figure can be viewed at wileyonlinelibrary.com]

c-Fos-positive neurons in the ACC ($t_{(8)} = 8.94$, $P < 0.001$, unpaired t test; Fig. 1*A* and *B*). These results indicate that ACC neurons are activated by hypertonic saline and are potentially involved in the process of thirst.

ACC neuronal activation correlates with drinking behaviour

To address whether ACC neuronal activity correlated with drinking behaviour, we recorded single-unit activity in the ACC in conscious rats injected with different concentrations of saline (Fig. 2). A total of 253 single units in the ACC were recorded, among which 224 were putative pyramidal neurons based on their waveform and firing properties (Barthó *et al.* 2004). Putative interneurons were excluded from further analysis considering their low proportion.

Drinking behaviour after saline injection differed with various concentrations of saline (Fig. 3). A higher concentration of saline induced more water intake, indicated by increases in both total drinking time ($F_{(3,20)} = 35.99$, $P < 0.001$, one-way ANOVA with Bonferroni's *post hoc* test; Fig. 3*B*) and total amount of water intake ($F_{(3,20)} = 7.76$, $P = 0.001$, one way ANOVA with Bonferroni's *post hoc* test; Fig. 3*C*). Drinking probability was high in the first 500 s when water became available, but gradually decreased over time.

We analysed ACC neuronal activities after saline injection. As shown in Fig. 4*A*, the average firing rate of putative ACC pyramidal neurons increased with increasing concentration of saline ($P < 0.001$, Kruskal–Wallis test with Dunn's *post hoc* test; Fig. 4*B*). The increase in firing rate was more prominent after water became available, but could already be detected before water access (Fig. 4*A*). Notably, the mean firing rate variation of putative ACC pyramidal neurons positively correlated with both total drinking time (Pearson $\gamma = 0.66$, $P = 0.008$, Pearson correlation test; Fig. 4*C*) and total amount of water intake (Pearson $\gamma = 0.49$, $P = 0.06$, Pearson correlation test; Fig. 4*D*).

These results indicate that increased activity of putative ACC pyramidal neurons precedes and correlates with hyperosmolality-induced drinking behaviour.

Inhibiting ACC neuronal activity changes drinking pattern

To causally evaluate how ACC neuronal activity contributed to drinking behaviour, we chemogenetically inhibited the activity of ACC pyramidal neurons (Fig. 5*A*) with AAV vectors containing hM4Di, an artificially designed receptor selectively activated by a designed drug, CNO (Fig. 5*B*). CNO delivery decreased the firing rate of ACC pyramidal neurons *in vivo* (vehicle: $P = 0.97$; CNO: $P = 0.009$, Wilcoxon test; Fig. 5*C*).

Notably, silencing ACC pyramidal neurons changed hyperosmolality-induced drinking behaviour from an explosive and short-lasting pattern to a gradual but more persistent pattern (Fig. 5*D*). After water became available, rats with CNO injection spent less time on drinking during 0–5 min, but more time on drinking during 10–60 min (0–5 min: $t_{(12)} = 2.26$, $P = 0.04$; 5–10 min: $t_{(12)} = 0.37$, $P = 0.72$; 10–60 min: $t_{(12)} = 2.23$, $P = 0.04$, unpaired t test; Fig. 5*D*). These results were further confirmed

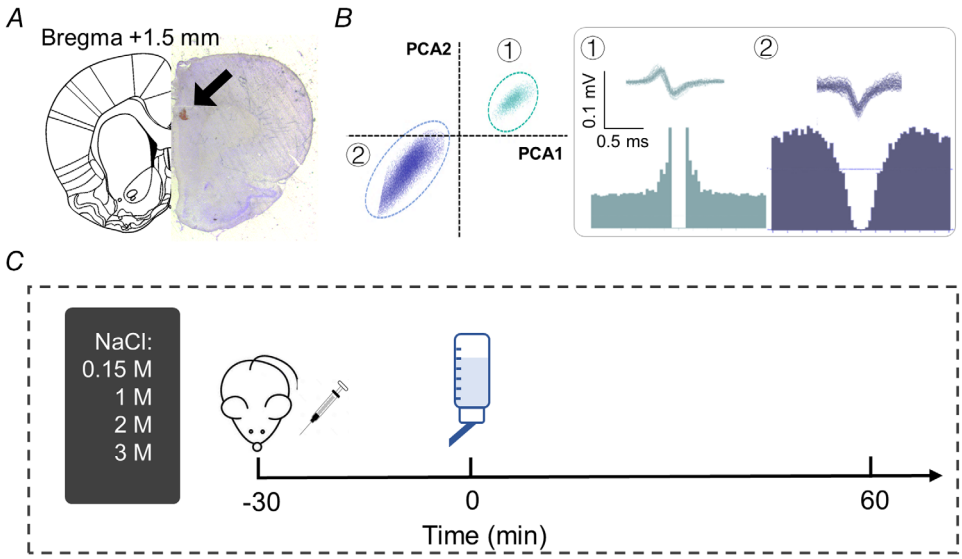

Figure 2. Methodology of *in vivo* electrophysiological recording
A, representative electrode track in the ACC. *B*, principal component analysis (PCA) of spikes yielding two single units (1 and 2); '1' represented a putative pyramidal neuron, and '2' represented a putative interneuron. Top, waveform. Bottom, autocorrelogram. *C*, diagram showing the recording procedure. [Colour figure can be viewed at wileyonlinelibrary.com]

by the rightward shift in the cumulative probability curve of drinking time after CNO injection ($P < 0.001$, Kolmogorov–Smirnov two sample test; Fig. 5*E*). By sharp contrast, inhibition of ACC pyramidal neurons affected neither the total duration of drinking ($t_{(12)} = 0.73$, $P = 0.48$, unpaired t test; Fig. 5*F*), nor the total amount of water intake ($t_{(12)} = 0.26$, $P = 0.26$, unpaired t test; Fig. 5*G*). These results suggest that pyramidal neuronal activity of the ACC is not necessary for thirst or drinking behaviour, but may modulate the affective motivation of drinking demanded by thirst.

Discussion

Thirst is a homeostatic response to changes in the blood: increases in plasma osmolality or decreases in plasma volume or pressure trigger the sensation of thirst, which motivates animals to seek and consume water and thereby restore these parameters to their physiological set-points. The aim of our present study was to investigate whether the ACC was associated with drinking behaviour during a state of thirst. We found that injection of hypertonic saline increased the number of c-Fos-positive neurons in the ACC, reflecting recruitment of this brain region (Fig. 1). This finding is consistent with previous human (McKinley *et al.* 2008; Freiria-Oliveira *et al.* 2015) and animal (Duncan *et al.* 1989; Pastuskovas *et al.* 2003) studies.

The ACC is a major brain region associated with affective motivation. Several basic homeostatic emotions such as pain, itch, hunger or thirst reflect an adverse condition within the body that prompts a behavioural response (Craig, 2002, 2003). Thirst, as an unpleasant state, reinforces drinking behaviour to eliminate the aversive feeling (Allen *et al.* 2017; Gizowski & Bourque, 2017). Indeed, thirst has been described as one of the primal or homeostatic emotions (Bourque, 2008), which induces an affective motivation in the ACC. It is interesting to note that inhibiting ACC pyramidal neurons alters the pattern of drinking behaviour, without affecting the total amount of water consumed (Fig. 5). In fact, several studies have shown that the consumption of water under dehydration is more reliable on the activity of several subcortical regions such as SFO, OVLT and MnPO (Gizowski & Bourque, 2017; Zimmerman *et al.* 2017). Activity in cingulate cortex has been shown to correlate well especially with the feeling of thirst in human fMRI studies (Saker *et al.* 2014). At the level of neural circuits, increased functional connectivity during a state of thirst has been demonstrated between the ACC and the lamina terminalis (Farrell *et al.* 2011). In addition, increased firing of putative ACC pyramidal neurons precedes water access (Fig. 4), indicating that the ACC does not directly drive drinking behaviour. These findings suggest that the motivation to drink, drinking *per se* and the total amount of water required are determined by separate mechanisms.

The ACC has also been associated with sensory stimulation, motor planning and voluntary but not automatic motor execution related to swallowing (Martin *et al.* 2001; Toogood *et al.* 2005; Lowell *et al.* 2008).

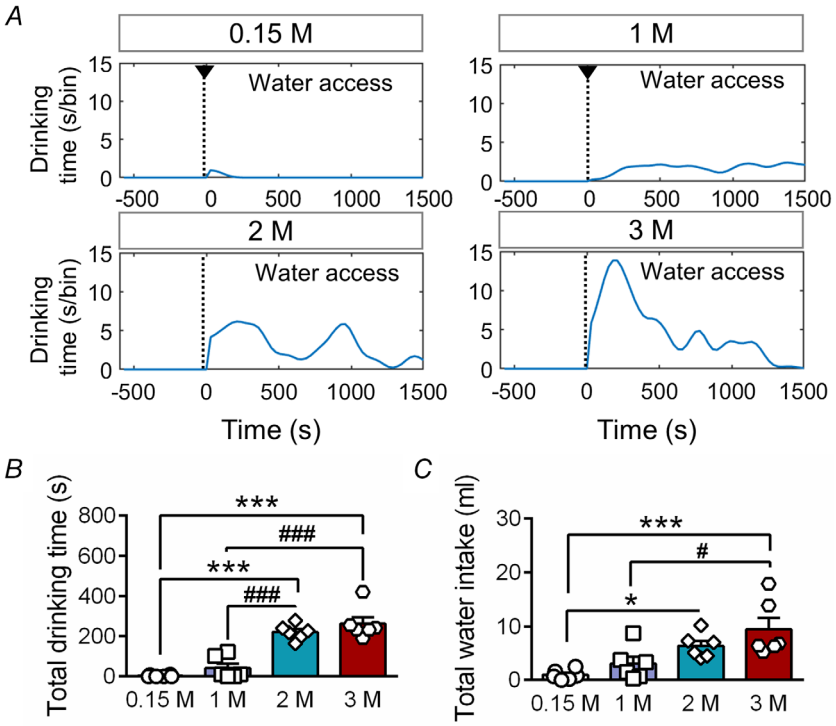

Figure 3. Hypertonic saline induces drinking behaviour
A, drinking curves after 0.15, 1, 2 or 3 M saline injection. *B*, increased total drinking time after injection of hypertonic saline. $n = 6$ in each group. $^{***}P < 0.001$ *vs.* 0.15 M; $^{\#\#\#}P < 0.001$ *vs.* 1 M, one-way ANOVA with Bonferroni's *post hoc* test. *C*, increased total water intake after injection of hypertonic saline. $n = 6$ in each group. $^{*}P < 0.05$, $^{***}P < 0.01$ *vs.* 0.15 M; $^{\#}P < 0.05$ *vs.* 1 M, one-way ANOVA with Bonferroni's *post hoc* test. [Colour figure can be viewed at wileyonlinelibrary.com]

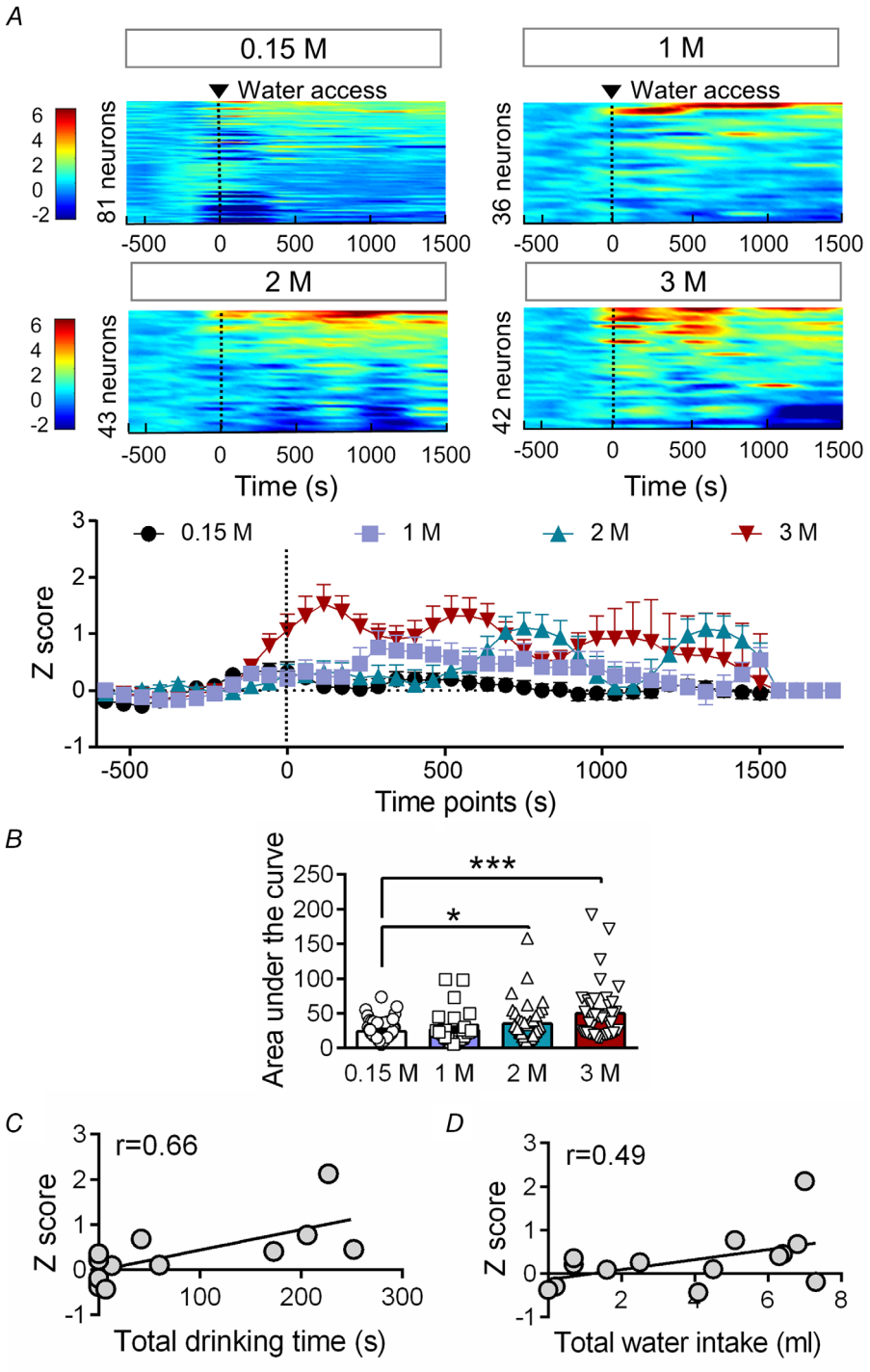

Figure 4. Activation of ACC neurons correlates with drinking behaviour
A, putative pyramidal neuronal responses in the ACC after 0.15, 1, 2 and 3 M saline injection. Top, heatmap rows represent the *Z*-score-transformed peri-stimulus time histograms (PSTH) for individual units, and columns represent time bins relative to water access (30 s width). Bottom, a plot showing the averaged *Z*-score of putative pyramidal neurons in the ACC under different conditions. *B*, ACC neuronal activity was quantified by area under the curve (AUC) of *A*. Data from 7 rats. Each point represents one firing unit. $^{*}P < 0.05$, $^{***}P < 0.001$, Kruskal–Wallis test with Dunn's *post hoc* test. *C*, scatter plot showing positive correlation between the total drinking time and the *Z*-score of ACC neuronal activity. Data from all animals. *D*, scatter plot showing positive correlation between the total amount of water intake and the *Z*-score of ACC neuronal activity. Data from all animals. [Colour figure can be viewed at wileyonlinelibrary.com]

Increased neuronal activity of the ACC is triggered by the state of thirst (Fig. 1) and associated with hyperosmolality-induced voluntary drinking behaviour (Fig. 4), thus resulting in water consumption (Fig. 3). Therefore, the ACC appears to be involved in both the subjective state of thirst and the cortical control of swallowing, and thus represents a prime candidate for a region capable of integrating both processes.

Previous studies have demonstrated that rats consume more water during their active period (Siegel & Stuckey, 1947). The diurnal variation in drinking is believed to be influenced by a circadian rhythm of activity, a feeding rhythm and external lighting conditions (Ang et al. 2001). In the present study, all experiments were conducted during the active period for the rats to exclude potential influences from the 24 h rhythm.

Urination significantly affects drinking behaviour. A previous study has shown that ACC is activated during the voiding phase of micturition (Harvie *et al.* 2019) and during bladder filling (de Groat *et al.* 2015). ACC

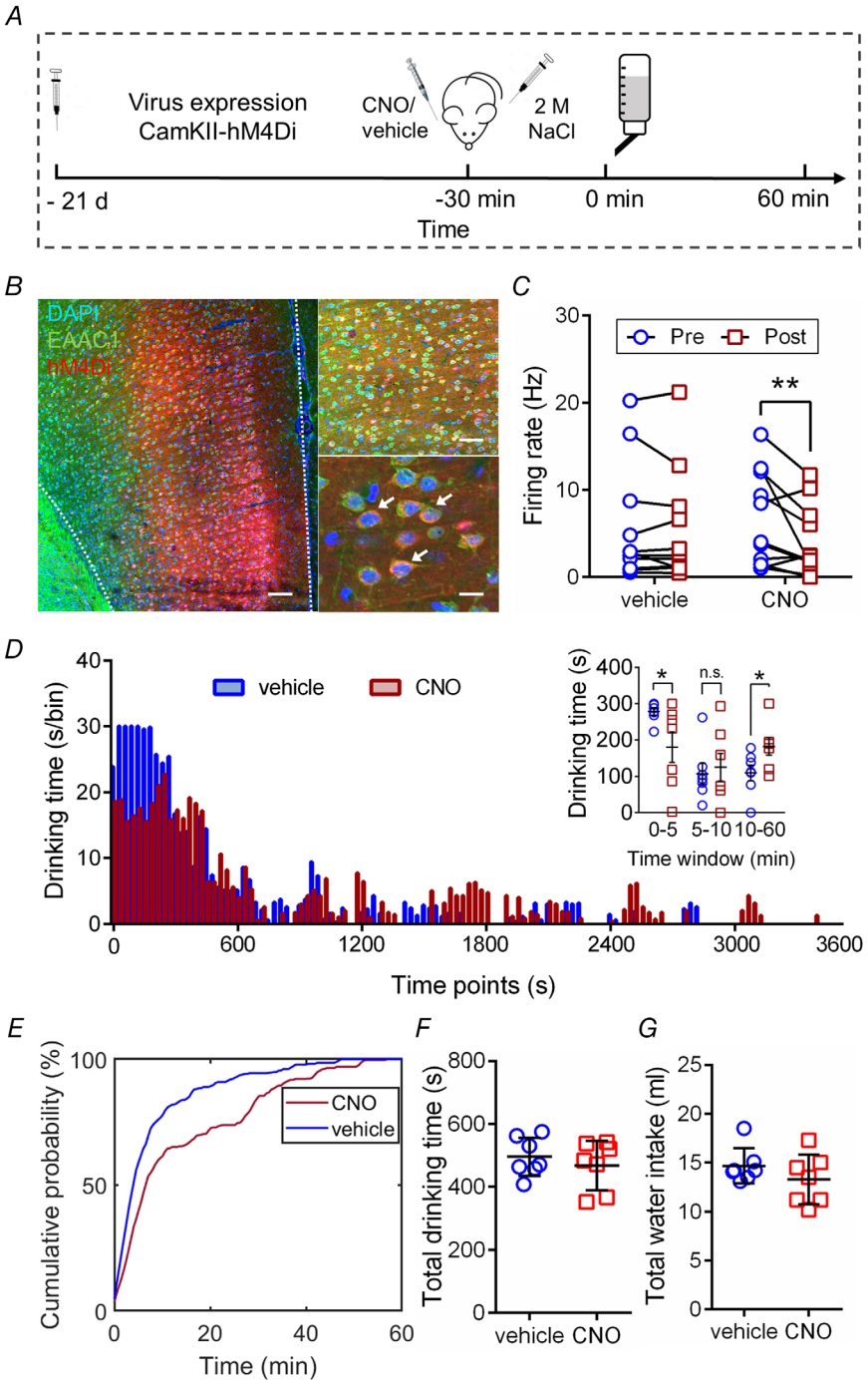

Figure 5. Inhibiting pyramidal neurons in the ACC alters drinking pattern, but not total water intake
A, diagram showing the experimental timeline. *B*, representative immunofluorescence images showing co-labelling of the majority (91.37 ± 4.57%) of hM4D(Gi)-mCherry$^+$ neurons (red) with EAAC1$^+$ neurons (green, a marker of glutamatergic neurons). Scale bars: left, 200 μm; right (top), 100 μm; right (bottom), 20 μm. *C*, decreased mean firing rate of ACC neurons following administration of CNO. Vehicle: $n = 11$ units; CNO: $n = 11$ units. $^{**}P < 0.01$, pre *vs.* post, Wilcoxon test. *D*, histogram showing averaged drinking time following water access. Column presented 30 s width. Inset shows cumulative drinking time during 0–5 min, 5–10 min and 10–60 min time windows following water access. $n = 7$ in each group. $^{*}P < 0.05$, vehicle *vs.* CNO, unpaired *t* test. *E*, cumulative probability of drinking time from rats injected with vehicle or CNO. $^{***}P < 0.001$, vehicle *vs.* CNO, Kolmogorov–Smirnov two sample test. *F* and *G*, no significant difference in the total drinking time (*F*) or the total amount of water intake (*G*) between vehicle and CNO groups. $n = 7$ in each group, vehicle *vs.* CNO, unpaired *t* test. [Colour figure can be viewed at wileyonlinelibrary.com]

activation results in increased thoracolumbar sympathetic discharge that causes detrusor relaxation and urethral sphincteric contraction (Griffiths & Fowler, 2013). In the present study, we observed little urination in both control and ACC inhibition groups, and no significant difference between groups during the experiment session (data not shown).

Together, our findings support a role of the ACC in modulating the affective-motivative dimension of hyperosmolality-induced thirst. We need to note that different subpopulations in the ACC may show distinct (excitatory *vs.* inhibitory, early *vs.* late, etc.) responses to thirst, thus representing distinct dimensions of drinking behaviour, which would be an interesting direction for future investigation.

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Additional information

Competing interests

The authors declare no conflict of interest.

Author contributions

L.Y.M., L.P.Y., Y.W. and M.Y. designed the research; L.Y.M., Y.Q.Z. and X.Y.Z performed behavioural experiments and electrophysiological recordings; L.Y.M. and L.P.Y. analysed behavioural and electrophysiological data; L.Y.M., S.C. and F.Y.L. performed the immunostaining; L.Y.M., L.P.Y. and M.Y. wrote 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.

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Keywords

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