Supplementary material

Methods

Subjects

Twelve healthy volunteers (6 women; mean age 41.7±9.6 years) with a nonobese body mass index (BMI <30 kg/m^2; mean 25.0±3.3) were recruited from the staff of Salford Royal NHS Foundation Trust and its local population. Persons with a history of gastrointestinal or psychiatric disease or taking medication that might affect the digestive system or central nervous system were excluded by self-report questionnaires.

Intragastric Nutrient Infusion

Free fatty acids of a chain length of 12 carbon atoms [dodecanoic acid (C12)] were selected for intragastric infusion because of reasons described extensively in previous publications, such as their ability to release CCK and water solubility (1-4). Moreover, a recent study by our group defined a lipid-induced, CCK-dependent brain activation matrix using intragastric C12 infusion (4).

To avoid awareness of subjects to the nature of the test meals and to eliminate the sensations of taste or smell, all were administered directly into the stomach by an orogastric tube. We chose a dose of C12 sufficient to release CCK, but known to be subthreshold for inducing aversive sensations, as in previous studies (1-4).

MR Image Acquisition

Images were acquired on a 3.0 T Philips Achieva MR System at the University of Manchester's Magnetic Resonance Imaging Facility, with a multislice, singleshot echoplanar imaging sequence to achieve whole brain coverage: 40 axial slices, TR = 5 seconds, TE = 35 milliseconds, 3.5-mm slice thickness, in-plane resolution 1.8 mm by 1.8mm. A SENSE 8-channel head coil was used for radio frequency transmission and reception.

Results & Discussion

Sex differences

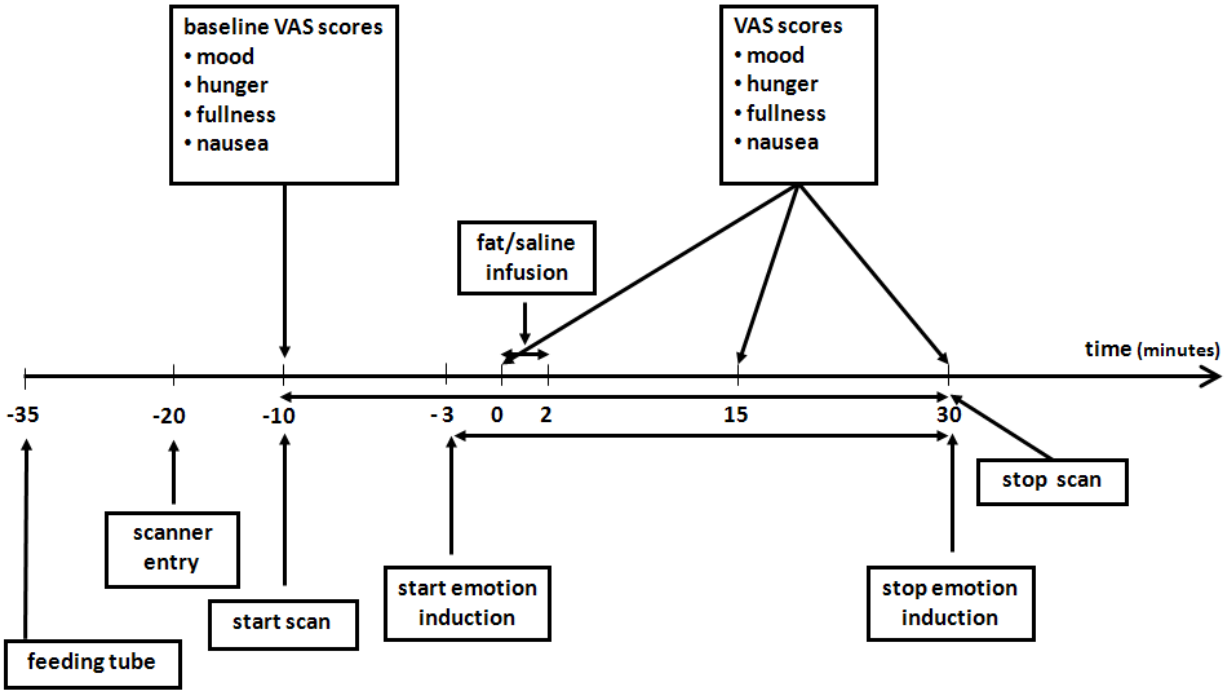
Although the study was not designed a priori to detect sex differences, we tested a potential effect of sex on both the behavioural and the brain imaging data, despite the small sample size. Sex had no significant effect on any of the behavioural measures when added as an independent variable to the mixed models, in addition to emotion, fat and the emotion-by-fat interaction. Sex did have a significant effect on the emotion-by-fat interaction in the fMRI data with males showing a greater interaction effect than females in most of the pre-hypothesized regions of interest. However, as this gender effect on the interaction was primarily driven by the effect of fat versus saline under the neutral mood condition we have to view this result in the context of an independent gender analysis collating data from other fat versus saline studies within our group (n=19 females; 18 males) (McKie et al, unpublished observations) which showed no significant gender differences on the fat versus saline fMRI signal.

Differential effect of fatty acid and vehicle on gastric emptying and interoceptive sensitivity

Fatty acid and saline vehicle have a differential effect on gastric emptying through a CCK-dependent mechanism (3), which may in turn lead to differences in gastric distension between both types of infusion. Therefore, stimulation of gastric mechanoreceptors may indeed play a role in the gut-brain signaling observed in this study, in addition to stimulation of duodenal chemoreceptors. However, as the volume of the infusion is rather low (250 ml), it is rather unlikely that this leads to important differences in gastric distension level. Furthermore, even if gastric distension would partially explain the main effect of fat, it is unlikely to play a role in the interaction effect which is the key finding of this study, as this effect tests a *differential* effect of fat versus vehicle in both emotional states. If gastric distension plays a role, it is conceivable that it is similar between both emotional states and is therefore subtracted out in the interaction effect.

Further, lipid infusion may increase interoceptive sensitivity in the stomach, as introduodenal lipid infusion has previously been demonstrated to alter stomach sensitivity to distension (5). However, this potential difference between fatty acid and saline infusion is equally likely to be subtracted out in the interaction effect.

Figure S1: Overview of fMRI scanning protocol



References to supplementary material

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