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Reversible brain response to an intragastric load of L-lysine under L-lysine depletion in conscious rats

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Abstract

L-Lysine (Lys) is an essential amino acid and plays an important role in anxiogenic behaviour in both human subjects and rodents. Previous studies have shown the existence of neural plasticity between the Lys-deficient state and the normal state. Lys deficiency causes an increase in noradrenaline release from the hypothalamus and serotonin release from the amygdala in rats. However, no studies have used functional MRI (fMRI) to compare the brain response to ingested Lys in normal, Lys-deficient and Lys-recovered states. Therefore, in the present study, using acclimation training, we performed fMRI on conscious rats to investigate the brain response to an intragastric load of Lys. The brain responses to intragastric administration of Lys (3 mmol/kg body weight) were investigated in six rats intermittently in three states: normal, Lys-deficient and recovered state. First, in the normal state, an intragastric load of Lys activated several brain regions, including the raphe pallidus nucleus, prelimbic cortex and the ventral/lateral orbital cortex. Then, after 6 d of Lys deprivation from the normal state, an intragastric load of Lys activated the ventral tegmental area, raphe pallidus nucleus and hippocampus, as well as several hypothalamic areas. After recovering from the Lys-deficient state, brain activation was similar to that in the normal state. These results indicate that neural plasticity in the prefrontal cortex, hypothalamic area and limbic system is related to the internal Lys state and that this plasticity could have important roles in the control of Lys intake.

Key words: **L-Lysine: Depletion: Brain: Rats**

Most mammals, including human subjects and rodents, need a continuous intake of the essential nutrients that cannot be synthesised endogenously. To achieve the required intake, the brain monitors the body's nutrient state and controls the balance between ingestion and consumption of essential nutrients by modulating feeding behaviour and gastrointestinal function. In response to a diet lacking an essential nutrient (e.g. essential amino acids, vitamins or Na ions), animals and human subjects begin to desire that nutrient. This phenomenon appears to enforce the intake of these nutrients. This process is separate from addiction because further intake is stopped once the ingestion of the specific nutrient reaches adequate levels[1].

L-Lysine (Lys), an essential amino acid mainly present in wheat, maize and beans, is principally metabolised in the brain and in the liver. Circulating Lys is transported to the brain across the blood–brain barrier and metabolised to α-aminoadipic acid[2]. In the brain, Lys or its metabolites can interact with various neuronal receptors, including benzodiazepine and G-protein-coupled receptors[3–5]. Lys deficiency can induce an increase of serotonin in the amygdala, and dysregulation of the circadian release of noradrenaline in the hypothalamus[6,7].

Rats adapted to a Lys-deficient diet are sensitive to changes in dietary Lys concentrations as small as 0·01 %[8]. This finding indicates that neural modifications may occur in Lys-deficient rats to increase their sensitivity to Lys. The aim of the present study was to compare the brain response to intragastric Lys stimulation in the normal, Lys-deficient and Lys-recovered states in rats. We performed functional MRI (fMRI) on conscious rats (without anaesthesia) because it is difficult to exclude the effects of anaesthesia on blood oxygenation level-dependent (BOLD) signals[9]. Another advantage of conscious fMRI is that we could serially investigate brain responses in the same rats in the normal, Lys-deficient and Lys-recovered states.

Materials and methods

Animals

Six male Wistar rats (10 weeks old, 250–300 g at the time of surgery; Charles River Laboratories) underwent BOLD fMRI.

Abbreviations: BOLD, blood oxygenation level-dependent; fMRI, functional MRI; Lys, L-lysine; VOI, voxels of interest.

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They were housed individually in wire-mesh cages under controlled temperature (23 ± 0·5°C) and light (lights on: 01.00–13.00 hours) conditions, and were given free access to water and food throughout the study. All animal procedures for fMRI and animal care in the present study conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Surgery

To enable conscious fMRI, the rats underwent cranioplastic surgery and implant of a silicone tube under pentobarbital anaesthesia (50 mg/kg body weight, intraperitoneally), as previously described[9]. Cranioplastic acrylic cement was applied to the skull with two holes moulded on each side to serve as a receptacle for the four glass-fibre bars used for head fixation during the MRI session. For intragastric cannulation, one end of a silicone tube was passed from the abdomen under the skin on the back and held on the head. The other end of the silicone tube was inserted into the gastric fundus and ligated with silk thread. After surgery, the rats were allowed to recover for more than 1 week before carrying out further procedures.

Acclimation training

The acclimation training procedure is described in detail in the previous study[9]. The rats were trained for 5 d to allow them to adapt to the fMRI conditions 1 week after the surgery (Fig. 1(a)). Training was done at the same time each day (10.00–17.00 hours) to minimise the effects of circadian rhythm variations. During the first 3 d (days 1–3), a pseudo-MRI system consisting of a non-magnetic bore and a head positioner was used. The rats were anaesthetised for a short time with 2% isoflurane. Then they were left in the pseudo-MRI apparatus for 30 min on day 1 and for 90 min on days 2 and 3 after recovery from anaesthesia. For the next 2 d (days 4 and 5), the rats were placed in an MRI system under the same conditions as those used for the MRI measurements, except for intragastric infusion of physiological saline. We used physiological saline during the training sessions because this has minimal influence on forebrain activity[10]. Heart and respiration rates were measured using an magnetic resonance-compatible monitoring system (Model 1025; SA Instruments) throughout the training period. The respiration was monitored using a small pneumatic pillow attached on the abdomen. The heart rate was measured using two sub-dermal electrodes that were implanted in the right and left forepaws. By the end of the acclimation training, the respiration and heart rates were at normal levels during the training session (respiratory rate, 80–90 beats/min; heart rate, 400–450 beats/min). There were no significant differences in heart or respiratory rates during fMRI measurements in any of the nutritional states.

L-Lysine deficiency

The fMRI was first performed in rats fed a house-made normal diet (normal state, Table 1). On the day after fMRI in the normal state, the diet was switched to a Lys-deficient diet, which was continued for 6 d to induce Lys deficiency (Lys-deficient, Fig. 1(a) and Table 1). The difference between the normal and Lys-deficient diet is that in the Lys-deficient diet, Lys is replaced with L-glutamine. This diet has been confirmed to induce an Lys-deficient condition[7]. In the present study, we confirmed typical symptoms of the Lys-deficient state, i.e. ruffed, mangy tail and diarrhoea. After fMRI in the Lys-deficient state, the rats were provided with a normal diet containing Lys for 6 d, and the fMRI data were acquired again (Lys-recovered state, Fig. 1(a) and Table 1). The L-type amino acids were used in all the experiments. All the amino acids were obtained from Ajinomoto Company, Inc.

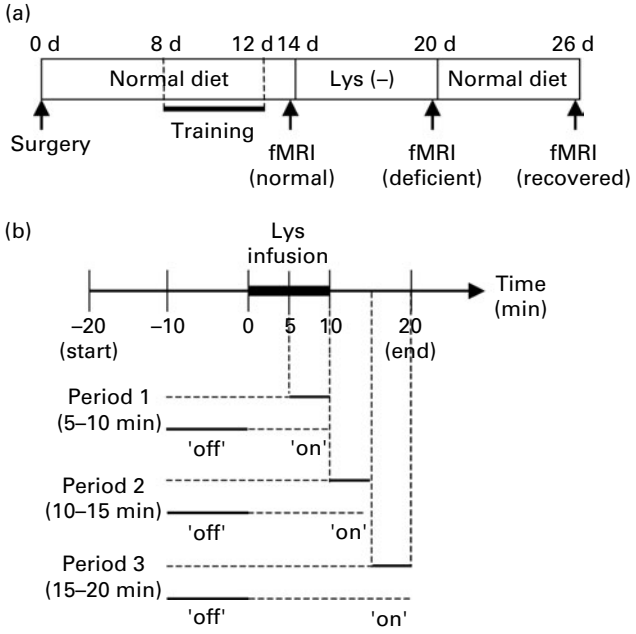

Fig. 1. Diagrams of the experimental and functional MRI (fMRI) procedure. (a) Experimental schedule. (b) fMRI procedure. Functional data were obtained at every 15 s for 40 min. The 'off' period corresponds to the 10 min before nutrient administration. To construct the boxcar function, the 'on' period corresponds to the time after starting L-lysine (Lys) administration and was divided into three 5-min periods, except for the first 5 min after starting nutrient administration (period 1, 5–10 min; period 2, 10–15 min; and period 3, 15–20 min after the start of the infusion of Lys). The bold line given here represents the 10-min infusion period.

MRI procedure

All MRI measurements were performed during the dark period after the rats had been food deprived for 12–15 h. The rats were then immediately placed into the correct head position by fixing four bars to the cranioplastic acrylic mount for painless fixation and their bodies were gently restrained with elastic bands. To reduce any possible stress induced by the scanning noise, we placed earplugs, which were made by cutting the human earplug to fit within the rat earhole, on the rats throughout the experiment. Scanning was then started. We used a Bruker Avance III system (Bruker Biospin) with a 4·7 T/40 cm horizontal superconducting magnet equipped with gradient coils (73 mT/m, 26 cm diameter). We obtained

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Table 1. Ingredients of the normal (with lysine) and lysine-deficient diet*

Ingredient	g/kg
Maize starch	560
Cellulose powder	40·0
Mineral mixture*	40·0
Vitamin mixture†	10·0
DL-α-Tocopherol acetate	0·100
Choline Cl^-	12·0
Maize oil	50·0
Wheat gluten	243
L-Thr	4·34
L-Val	5·62
L-Met	5·03
L-Ile	4·55
L-Leu	5·48
L-Tyr	1·75
L-Phe	1·44
L-Histamine	0·930
L-Arg	6·89
L-Trp	1·24
L-Gln	6·76
Lys or L-Gln‡	13·5

* Mineral mixture of AIN-93-MX (per kg diet): $CaCO_3$, 14·28 g; KH_2PO_4, 10 g; $K_3C_6H_5O_7.H_2O$, 1·12 g; NaCl, 2·96 g; K_2SO_4, 1·86 g; MgO, 0·96 g; $FeC_6H_6O_7$.-nH_2O, 0·24 g; $2ZnCO_3.3Zn(OH)_2.H_2O$, 66 mg; $MnCO_3$, 25·2 mg; $CuCO_3.Cu(OH)_2.H_2O$, 12 mg; KIO_3, 0·4 mg; Na_2SeO_4, 0·41 mg; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0·32 mg; $Na_2SiO_3.9H_2O$, 58 mg; $CrK(SO_4)_2.12H_2O$, 11 mg; H_3BO_3, 3·26 mg; NaF, 2·54 mg; $NiCO_3.2Ni(OH_2).4H_2O$, 1·27 mg; LiCl, 0·7 mg; NH_4VO_3, 0·26 mg. Purchased from CLEA Japan, Inc.

† Vitamin mixture of AIN-93-VX (per kg diet): nicotinic acid, 30 mg; calcium pantothenate,16 mg; pyridoxine-HCl, 7 mg; thiamin-HCl, 6 mg; riboflavin, 6 mg; folic acid, 2 mg; D-biotin, 0·2 mg; cyanocobalamine, 25 mg; all-*rac*-α-tocopheryl acetate, 150 mg; all-*trans*-retinyl palmitate, 8 mg; cholecalciferol, 2·5 mg; phylloquinone, 0·75 mg. Purchased from CLEA Japan, Inc.

‡ L-Glutamine was used instead of lysine in the lysine-deficient diet.

BOLD fMRI data using a T2*-weighted multi-slice, fast, low-angle shot sequence with the following parameters: repetition time = 345 ms, echo time = 12 ms, flip angle = 30°, field of view = 35 × 35 mm, acquisition matrix = 64 × 64, slice thickness = 1·3 mm and slice number = 17. Structural images were obtained by multi-slice rapid acquisition with a relaxation enhancement sequence using the following parameters: repetition time = 2500 ms, effective echo time = 60 ms, rapid acquisition with a relaxation enhancement factor = 8 and acquisition matrix = 128 × 128, four means. At 20 min after beginning the scanning procedure, 300 mmol/l (w/v in distilled water) of Lys (Ajinomoto Company, Inc.) solution was delivered to the stomach via the intragastric cannula at a rate of 1 ml/min per kg body weight over 10 min using a syringe pump (CVF-3200; Nihon Kohden) to reduce the effects of gastric expansion (Fig. 1).

Data analysis

We used SPM5 software (Wellcome Trust Centre for Neuroimaging) for pre-processing, including slice realignment, co-registration to structural images and spatial normalisation of functional data. Before pre-processing, we obtained template images co-registered with the Paxinos & Watson[11] rat brain atlas. Image sets containing motion artefacts were discarded.

Statistical analyses were conducted using a program written in MATLAB (Mathworks, Inc.), as previously described[9]. Briefly, we identified the brain regions showing significant changes in BOLD signal intensity by applying boxcar functions. The 'off' period of the boxcar function was defined as the basal period from 10 min before starting nutrient administration until the time of nutrient administration (Fig. 1(b)). The 'on' period was defined as the period of potential activity from 5 min after the start of nutrient administration. Three post-administration 'on' periods were evaluated (period 1, 5–10 min; period 2, 10–15 min; and period 3, 15–20 min).

The first level (fixed effect) analysis was performed on data from individual rats. We constructed the regressor as described above (Fig. 1(b)), and then voxel-based regression analysis was performed on the individual data. Fixed-effects analysis allows inferences to be made at the subject level. To make inferences about the group data in each Lys state, second-level (random effect) analysis was conducted using the results of the first-level analysis in six rats. All voxels were calculated in the second-level analysis. We used the voxel-based *t* test for random-effects analysis in each Lys state. The activated areas were considered significant at $P<0{\cdot}01$, corrected for multiple comparisons using the false discovery rate procedure, with a cluster size greater than 49 pixels. We also used the results of the first-level analysis to compare the BOLD signal changes among Lys states (normal, Lys-deficient and Lys-recovered states). We applied two-way repeated-measures ANOVA between the Lys states in each voxel and then using the paired *t* test ($P<0{\cdot}01$, corrected for multiple comparisons using the false discovery rate procedure). We compared the regions that had significant BOLD signal changes by two-way repeated-measures ANOVA between the two states.

To analyse the mean BOLD signals in each brain region, voxels of interest (VOI) were drawn according to the rat brain atlas by Paxinos & Watson[11]. The percentage change in BOLD signal within a VOI was calculated as follows:

$$\%\ \text{changes in BOLD signal intensity} = \left(\frac{\text{Averaged BOLD signals within VOI in each period}}{\text{Averaged BOLD signals within VOI in basal period}} - 1\right) \times 100.$$

In this analysis, the basal period was defined as the 5 min before nutrient infusion. We calculated the changes in BOLD signal intensity during the infusion (period 1) and post-infusion (periods 2 and 3) periods. The statistical significance among the basal period (5 min before infusion), during (period 1) and after Lys infusion (periods 2 and 3) in each VOI was assessed using the Tukey–Kramer *post hoc* test after three-way repeated-measures ANOVA for the periods (basal period, before and after Lys infusion), Lys states (normal, Lys-deficient and Lys-recovered states) and VOI.

Results

Blood oxygenation level-dependent signal responses to intragastric L-lysine

In the normal state, the intragastric Lys load increased the BOLD signal in the ventral/lateral orbital cortex and the prelimbic cortex (Table 2). In Lys-deficient rats, the Lys load significantly increased the BOLD signal in several brain

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Table 2. Maximum *T*-value and those periods in each brain region showing significant increases in blood oxygenation level-dependent signals following intragastric administration of lysine in normal, lysine-deficient and lysine-recovered states*

	Normal state		Lys-deficient state		Lys-recovered state	
Brain region	Period (left/right)	Max *T*	Period (left/right)	Max *T*	Period (left/right)	Max *T*-value
Prelimbic cortex	3/3	7·8/7·8	–/–	–/–	3/3	7·8/7·8
Insular cortex	–/–	–/–	2/2	11/10	–/–	–/–
Ventral orbital cortex	3/–	8·2/–	–/–	–/–	3/–	8·5/–
Lateral orbital cortex	–/3	–/7·7	–/–	–/–	1/–	10/–
BNST	–/–	–/–	2/3	6·6/7·9	–/–	–/–
Nucleus accumbens	–/–	–/–	3/1	9·5/9·1	–/–	–/–
Ventral tegmental area	–/–	–/–	2/2	7·2/11	–/–	–/–
Amygdala	–/–	–/–	3/–	9·5/–	–/–	–/–
Hippocampus	–/–	–/–	3/2	12/9·1	–/–	–/–
PVN	–/–	–/–	3/3	6·5/7·3	–/–	–/–
Lateral hypothalamus	–/–	–/–	2/2	8·1/8·9	–/–	–/–
VMH	–/–	–/–	3/3	7·8/7·3	–/–	–/–
Raphe pallidus nucleus†	3	15	1	10	1	15
Medial preoptic area	–/–	–/–	2/2	9·1/13	–/–	–/–
Arcuate nucleus	–/–	–/–	1/1	9·2/8·5	–/–	–/–
NTS	–/–	–/–	–/–	–/–	–/–	–/–

–, NS ($P \geq 0{\cdot}01$, corrected); BNST, bed nucleus of the stria terminalis; PVN, paraventricular nucleus; VMH, ventromedial nucleus of the hypothalamus; NTS, nucleus of the solitary tract.
* Increases were deemed significant at $P < 0{\cdot}01$ (corrected, n 6) from second-level functional MRI analysis.
† Raphe pallidus nucleus has only one value because it is located in the medial part of the brain and is too small to distinguish left and right sides.

regions, including the ventral tegmental area, amygdala, hypothalamus, nucleus accumbens and the bed nucleus of the stria terminalis (Fig. 2 and Table 2). In contrast to the normal state, the BOLD signal was not increased in the ventral/lateral orbital cortex or the prelimbic cortex. Remarkably, several hypothalamic regions, including the lateral hypothalamus, paraventricular and arcuate nuclei, were activated in the Lys-deficient state (Fig. 2(b), (c)). Activation of these areas of the hypothalamus occurred after completing the infusion (i.e. in periods 2 and 3). Notably, after rescuing the Lys-deficient state with the normal diet, the BOLD signals were similar to those in the normal state (Fig. 3 and Table 2). The nucleus of the solitary tract was not activated in any state.

Blood oxygenation level-dependent signal changes by intragastric L-lysine in each brain region

We assessed the statistical significance among the averaged BOLD signal changes in basal period, during (period 1) and after (periods 2 and 3) Lys infusion by the Tukey–Kramer *post hoc* test after three-way repeated-measures ANOVA ($P < 0{\cdot}01$ for infusion periods). The mean BOLD signal intensity in the ventral tegmental area, nucleus accumbens, hippocampus and the hypothalamic area (including the dorsomedial, lateral and paraventricular nucleus) increased significantly in the Lys-deficient state, but not in the normal or recovered state (Fig. 4). Interestingly, in the ventral tegmental area and raphe pallidus nucleus, which are sources of dopamine and serotonin secretion in the forebrain, the intensity of the BOLD signal increased significantly during the infusion period. We did not observe any increases in BOLD signals in any other region during Lys infusion. The intragastric Lys load increased the BOLD signals in the ventral/lateral orbital cortex in the normal and Lys-recovered state, but not in the Lys-deficient state.

Discussion

In the present study, we showed for the first time that the rat brain response to ingested Lys is regulated by the body's internal Lys status. In the normal and Lys-recovered states, we found no increases in BOLD signal in response to the intragastric load of Lys, except in the raphe pallidus nucleus, the prelimbic cortex and the orbital cortex. In contrast, there were significant increases in BOLD signals in several regions in the Lys-deficient state, including the dopaminergic system, limbic system and the several regions of the hypothalamic area. These data indicate that dopaminergic and serotonergic systems play key roles in Lys sensing in the Lys-deficient state.

Yokawa *et al.*[12] previously showed that, using conscious fMRI of the hypothalamus, the medial and lateral hypothalamic areas were activated after intragastric infusion of Lys solution in Lys-deficient rats. Despite these findings, several questions remained. First, are other brain regions, such as the forebrain, mesencephalon or medulla, activated by Lys? Second, are the brain responses to intragastric Lys in the Lys-deficient state reversed by Lys rescue? The present data provide answers to both these questions. Other brain regions, including the limbic and dopaminergic system, were activated by Lys deficiency, but these regions were not active in the normal and Lys-recovered states. These data indicate reversible changes at the cellular or circuit levels integrating the internal state information with nutrient ingestion.

In our previous study[9], we demonstrated the validity of the conscious fMRI protocol. Furthermore, other researchers[13] have used conscious fMRI to determine changes in the BOLD signal and showed that acclimation training helps rats to adapt to the MRI environment and thus minimises scanning stress. Although there is still the possibility that the Lys-induced increase in BOLD signal in the Lys-deficient state was due to physical effects, such as gastric expansion,

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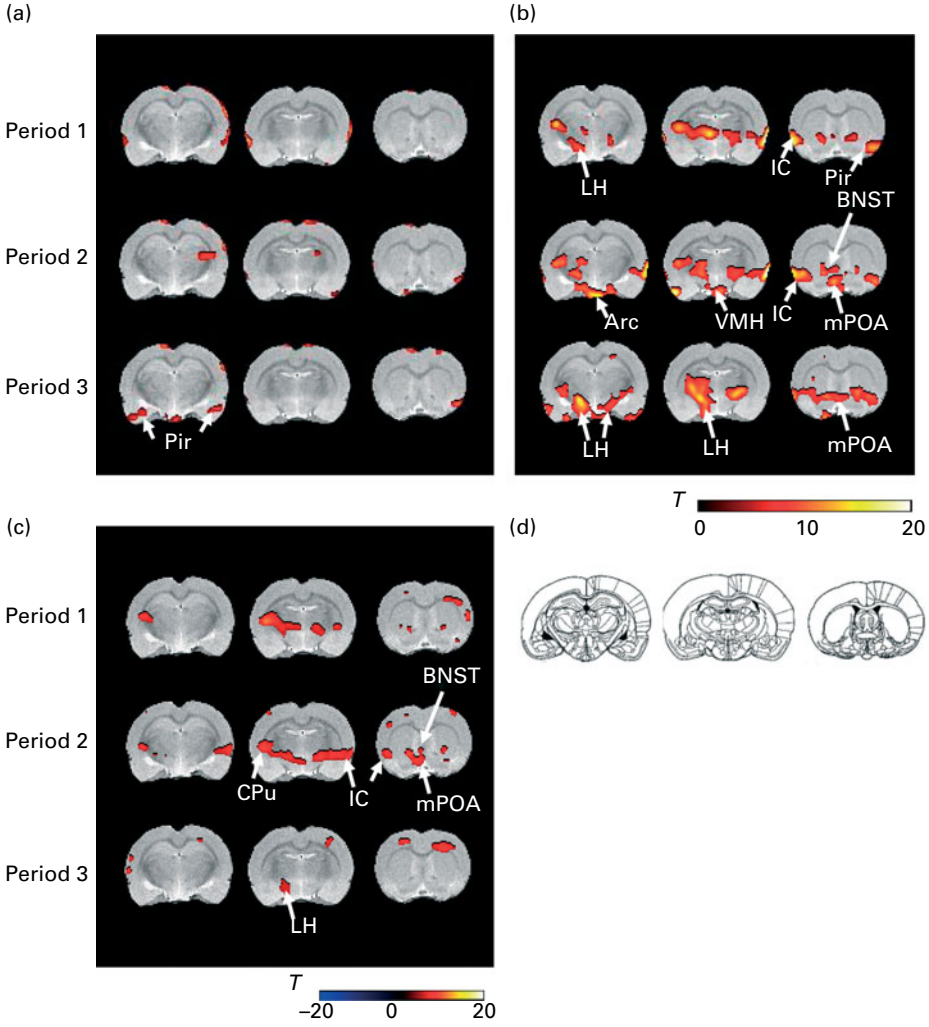

Fig. 2. Rat brain responses to a gastric infusion of L-lysine (Lys) in normal and Lys-deficient states. The *T*-map images in response to intragastric infusion of Lys in the (a) normal and (b) Lys-deficient states in rats (*n* 6). The *T*-map images depict the areas showing significant changes in blood oxygenation level-dependent (BOLD) signal intensity. The *T*-values show significant changes in BOLD signal intensity compared with the baseline period. (c) *T*-map images showing the areas with significant changes in BOLD signal intensity in the Lys-deficient state compared with the normal state (*n* 6). (d) The coronal figures of the Paxinos atlas at +0·7, −2·2 and −3·6 mm, from the bregma. Pir, piriform cortex; LH, lateral hypothalamus; IC, insular cortex; Arc, arcuate nucleus; VMH, ventromedial hypothalamus; BNST, bed nucleus of the stria terminalis; mPOA, medial preoptic area; CPu, caudate putamen.

we have already demonstrated that gastric expansion by intragastric infusion of physiological saline did not affect the BOLD signal[14]. Additionally, in the preliminary experiment, no regions were activated by the intragastric load of physiological saline in the normal or Lys-deficient states (data not shown). Accordingly, the results presented here indicate that the brain was activated chemically via Lys, not mechanically by gastric expansion. To remove the possibility of temporal effect, we scanned the same normal rats three times with the same fMRI protocol in the present study (second scan was at 6 d, third at 12 d after the first scan). We observed the BOLD signal increase in the same brain regions, as shown in normal rats in the present study (data not shown), indicating that different BOLD responses in Lys-deficient and Lys-recovered states were not temporal effects.

The pathways that convey nutrient information from the gut to the brain are broadly classified into two categories: the neural and the humoral pathways[15]. Recent studies have revealed that intragastric infusion of a high-energy meal is signalled from the gut to the brain via changes in blood glucose concentrations[16,17]. An intragastric load of glutamate solution activates the brain via the vagus nerve[18,19]. However, it is unclear whether the vagus nerve mediates brain activation following intragastric Lys loading because the nucleus of the solitary tract, the terminus of the vagus nerve, was not activated in the present study (Table 2). Instead, the ingested Lys is absorbed from the gastrointestinal tract and transported to the brain via the circulatory system. We observed an increase in the BOLD signal, not a decrease, in the Lys-deficient state, but such changes were not observed in the normal state. Additionally, the blood Lys concentration is decreased in the Lys-deficient state and therefore Lys or its metabolites could be increased after Lys intake in the Lys-deficient state rather than the normal or recovered states[20,21]. Furthermore, Lys-deficiency could change the amount of the released neurotransmitters such as noradrenaline and serotonin in the

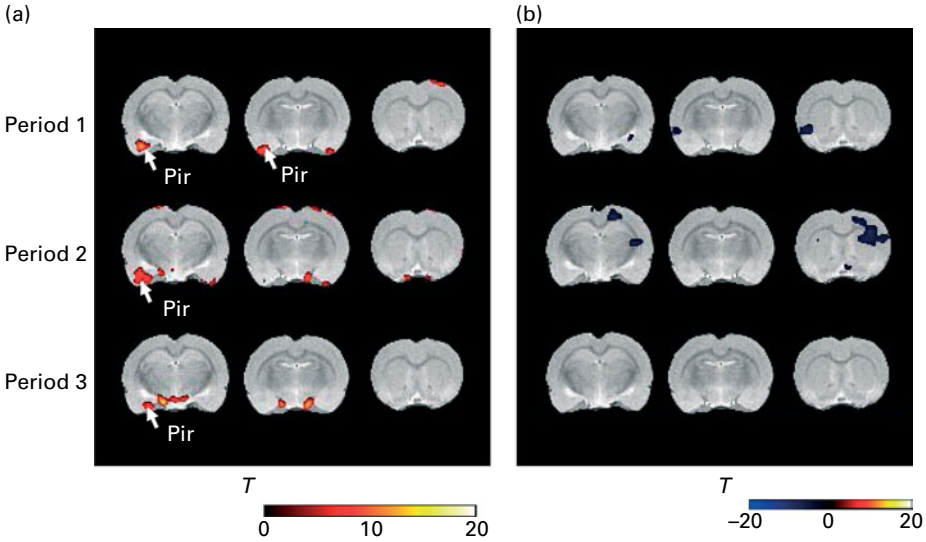

Fig. 3. Rat brain responses to a gastric infusion of L-lysine (Lys) in recovered states. (a) Areas of the rat brain responding to Lys solution following Lys rescue. The *T*-values show significant changes in blood oxygenation level-dependent (BOLD) signal intensity compared with the baseline period (*n* 6). (b) *T*-map images depict the areas showing significant changes in BOLD signal intensity in the rescued state compared with the normal state (*n* 6). Pir, piriform cortex.

amygdala and hypothalamus[6,7]. Taken together, it seems likely that changes in the blood Lys concentration and amount of the neurotransmitter could be related to reversible brain activation in the Lys-deficient state. However, the signalling pathway transmitting Lys information from the gut to the brain is still unclear. Further study is needed to clarify the roles of the vagus nerve, blood Lys or metabolites.

It is well known that hypothalamic areas are involved in homeostatic pathways and feeding behaviour. In an electrophysiological study, neural activity in the lateral hypothalamus

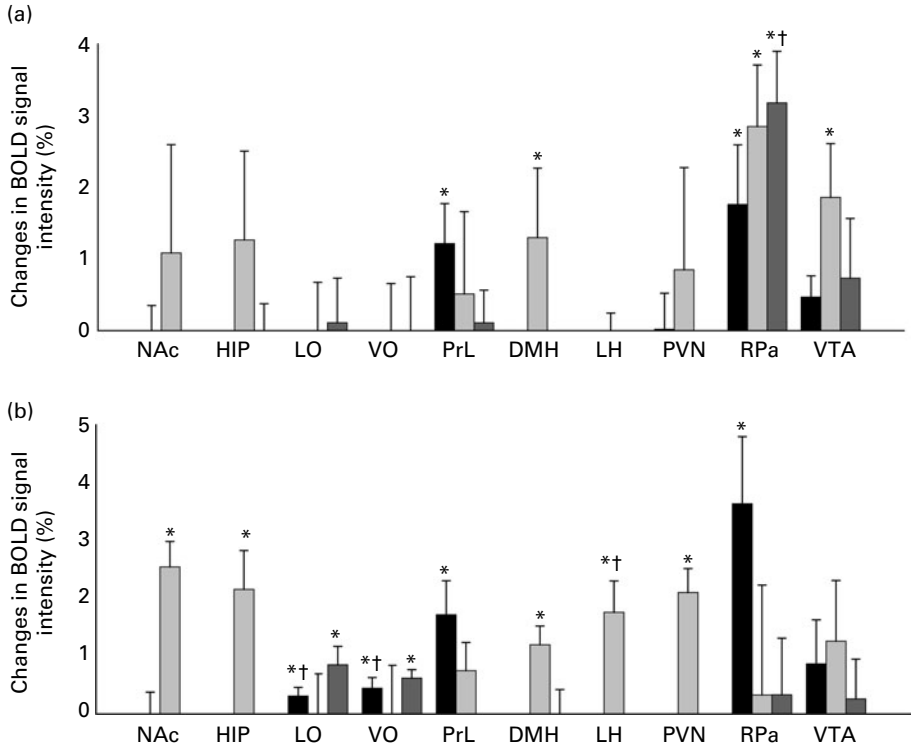

Fig. 4. Blood oxygenation level-dependent (BOLD) signal changes to L-lysine (Lys) infusion in three states in each voxels of interest (VOI). Changes in BOLD signals during (a; period 1) and after (b; periods 2 and 3) Lys infusion in normal (■), Lys-deficient (□) and recovered (▣) states in rats (*n* 6). *Mean values were significantly different compared with the baseline period in each brain region ($P<0{\cdot}05$). † Mean values were significantly different compared with during (period 1) or after (periods 2 and 3) the infusion using the Tukey–Kramer *post hoc* test following three-way repeated-measures ANOVA for infusion period, Lys state and VOI ($P<0{\cdot}05$). Values are means with their standard errors (*n* 6) for each state. NAc, nucleus accumbens; HIP, hippocampus; LO, lateral orbital cortex; VO, ventral orbital cortex; PrL, prelimbic cortex; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus; PVN, paraventricular nucleus; RPa, raphe pallidus nucleus; VTA, ventral tegmental area.

was increased in starved rats and was associated with activity in the orbitofrontal cortex during the hunger–satiety–hunger cycle[22]. These data are consistent with the present results.

The ventral tegmental area refers to a group of neurons in the mesencephalon that acts as a source of dopamine. It is very interesting that the dopaminergic system is activated in the Lys-deficient state because this system seems to be involved in the reward circuit[23]. Dopamine released from the ventral tegmental area acts on the nucleus accumbens, an important component of incentive salience[24–26] and food rewards after food deprivation[27,28]. Dopamine release in the lateral hypothalamus is also related to increased food intake. Taken together, these findings support the present explanation that the activation of the dopaminergic system is related to the reward value of Lys.

In conclusion, in the present study, we found that the changes in brain activity in response to Lys deficiency diminished after providing Lys, indicating that the dopaminergic response to Lys is dependent on the internal Lys state. These results indicate neural plasticity of the feeding system in response to changes in the internal nutrient state.

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