Prokinetic meranzin hydrate from Chaihu-Shugan-San improves depression-like behaviors and hypomotility in rats via ghrelin and neurocircuitry

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Research

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Abstract

Background

Depression and functional dyspepsia (FD) are characterized by comorbidity, overlapping depression, and nausea. The pathogenesis of depression and FD is mediated by $\alpha 2$ -adrenoreceptor and/or ghrelin. Antidepressant (A) or prokinetic (P) agents are numerous, but few have been investigated in this context. Ancient Gan-zhu-shu-xie (GZSX), whose representative traditional Chinese medicine (TCM) is Chaihu-Shugan-San (CSS), may exert antidepressant effects with prokinetic meranzin hydrate (MH) via $\alpha 2$ -adrenoreceptors in the acute forced swimming (FS) test in rats. Therefore, the main aim of the study is to investigate the acute antidepressant and prokinetic effects of CSS and MH after acute FS on rats, and its possible mechanism.

Methods

FS rats were treated with CSS, MH, fluoxetine, ghrelin antagonist [D-Lys3]-GHRP-6, and take a series of behavior tests and gastrointestinal motility tests, and via 7.0 T fMRI-BOLD signal, compared with well-known mechanism of positive control.

Results

MH has similar effects to CSS-stimulated deactivation when compared to those of fluoxetine (4.02-fold for hippocampus and 1.45-fold for thalamus). The ghrelin antagonist [D-Lys3]-GHRP-6 synchronously inhibited A&P and BOLD HTB foci. Prokinetic mosapride had effects on the thalamus and basal ganglia but not the hippocampus. Within the HTB, the hippocampus is implicated in depression and FD.

Conclusion

These data show that on acute FS-stimulated DB&H, MH-induced rapid A&P, and ghrelin-related regulation coupled to BOLD signals in brain areas before, providing insight into a unified theory of depression pathogenesis and pharmacotherapy.

Background

Depression is a major cause of suicide and disability worldwide [1]. Functional dyspepsia (FD, a functional gastrointestinal disorder including especially upper abdominal fullness and nausea etc) significantly reduces quality of life in 5–40% of the global population [2]. The comorbid incidence of depression and FD (CDF) is 54.6–99.0% [3–5], and overlapping symptoms include depressed mood and nausea [3–5], similar to the dysfunction of Gan-zhu-shu-xie (GZSX) which implicated in mood and digestion etc recorded 2,200 years ago [6]. These conditions impact and aggravate each other via the brain-gut axis [7]. However, the one-disease-one-target-one-drug [8–10] dogma only targets half of CDF, posing a unique challenge for treatment [9, 11–13]. Although the brain-gut axis [2–7, 12–14] encompasses comorbidity and challenges prevailing dogma, this concept has not integrated any antidepressant or prokinetic strategies, the underpinning neural circuits remain unclear [9, 12–13, 15–28]. Tricyclic antidepressants (TCAs) and mirtazapine have prokinetic clinical applications [30], but TCAs have only been used in FD with ignoring their original antidepressant activity [31].

Antidepressant strategies [32–33] have overlooked the peripheral gut which implicates the brain-gut pathway. Currently, therapeutic strategies for nausea are lacking [4]. Patients with nausea are administered oral selective serotonin reuptake inhibitors (SSRIs), which inhibit gastrointestinal (GI) motor activities via the serotonin 2c-receptor (5-HT_{2c}) [34], but these are often discontinued [9]. Few antidepressants among 50 new therapies are superior to SSRIs. Ketamine has failed due to side effects of dissociation and nausea in clinical trials [9, 35–37], although it exerts rapid antidepressant effects within hours via glutamatergic signaling [38] and blocking burst firing of neurons [39]. Prokinetic metoclopramide may cause depressive symptoms [40]. All current unified conceptualizations of depression are incomplete, but involve norepinephrine, neurotrophic factors, circadian rhythms, glutamate, gamma-aminobutyric acid (GABA), serotonin, and dopamine systems [11].

Unlike predefined unified neural architecture [32] using the above paradigms, functional magnetic resonance imaging (fMRI) can capture the full map [8] of brain circuits underlying depression and FD. Blood oxygen level-dependent (BOLD) signal provides a robust and quantitative readout of depression and FD in the brain-gut pathway via neurovascular coupling [41]. To date, 19 circuits identified using BOLD have been implicated in depression or delayed gastric emptying including circuits involving the hippocampus, thalamus, and prefrontal cortex (PFC) [17–29]. Acute stress has typically been overlooked. Indeed, even a single stressor can induce neuropsychiatric disorders or contribute towards psychopathology [42]. Acute stress is a determinant of pro-adaptive to maladaptive trajectory of stress responses and can be used to study mechanisms of fast-acting antidepressants [42]. Acute forced swimming (FS) as a stressor can reveal neurobiological mechanisms underscoring antidepressant action in a rapid, low-cost, and reliable manner [42]. Immobility time and sucrose preference are considered measures of depression [42]. The hippocampus-thalamus-basal ganglia (HTB) circuitry has been implicated in depression and FD in different studies. No single locus of HTB fully reflects overlapping substrates of depression, FD, or their comorbidity [17–29, 43]. HTB regulates memory, reward, learning, and decision-making; these processes are related to depression and mediate intestinal transit, delayed gastric emptying, and gastric distention related to FD when the stomach is stimulated by agents or mechanical injury [12, 20, 22, 23, 25, 29, 44–45].

Ancient GZSX may be a clue to divulging substrates underlying brain-gut disorder. Disorders of GZSX are analogous to comorbidities of depression and FD in terms of symptoms. CSS effectively treats above comorbidities. Dualistic properties of GZSX and HTB in separate conditions have certain similarities. BOLD foci are spatially precise [46]. Sensory transduction of nutrient information from the gut to brain is precise [47]. GZSX may provide insight into FS-

induced DB&H, CSS-induced A&P [6, 48], which have α 2-adrenoreceptor (α 2-AR) as a common denominator between the two [49]. Similar to the effects of ketamine, the rapid antidepressant effects induced by MH are mediated via AMPA-ERK1/2-BDNF pathway [48–49]; these drugs have opposing roles in gut motor function [50]. Compared to brain-derived neurotrophic factor (BDNF), reactive oxygen species (ROS), and α 2-AR, ghrelin has been reported to have a strong influence on DB&H. We have also observed acute FS responses to ghrelin antagonists and ghrelin gene knockout (KO) mice [51], highlighting the role of ghrelin signaling in modulating brain and gut disorders [6, 48].

Here, we aimed to assess whether ghrelin could affect DB&H [6, 48] following acute FS and rapid A&P by MH and CSS. Further, we aimed to elucidate the unified neurocircuitry of depression and FD based on brain activation indicated by BOLD signal.

Materials And Methods

Preparation of CSS extract

CSS consisted of seven medicinal plants (Radix Bupleuri, Dried Tangerine Peel, Fructus Aurantii, Rhizoma Ligustici Chuanxiong, Rhizoma Cyperi, Radix Paeoniae Alba, Liquorice Root) purchased from Xiangya Hospital of Central South University and identified by Dr. Xi Huang of Nanjing University of Chinese Medicine. First, 750 g of CSS was weighed according to the ratio (4:3:4:3:3:4:4) of the aforementioned herbs. A volume of 7,500 mL distilled water was added, and contents were soaked for 30 minutes. After soaking, contents were brought to a boil and continuously heated for 40 minutes, after which they were filtered with gauze. Decoctions were collected twice and lyophilized to obtain the powdered form of CSS, stored at 4°C until use. The yield of lyophilized powder of CSS was about 20.5% (w/w). In addition, the lyophilized powder was evenly dispersed in distilled water at dosage of CSS (30 g/kg) before the experiment.

Drugs and reagents

All reagents used in this research were purchased as follows: meranzin hydrate (MH) (Di-ao Co. Ltd., Chengdu, China); ghrelin, [D-Lys3]-GHRP-6 (Ji-er Biological Co. Ltd., Shanghai, China); fluoxetine (Sigma Aldridge Co. Ltd., America); mosapride (Jiangsu Haosen Pharmaceutical Co. Ltd., Lianyungang, China), isoflurane (Ruiwode Technology Co. Ltd., Wuxi, China); and dexmedetomidine (Yuanye Technology Co. Ltd., Shanghai, China). Albiflorin, naringin, hesperidin are purchased from Changsha kanglong biological products Co. LTD. (Changsha, China). Ferulic acid, paeoniflorin, liquiritin, ammonium glycyrrhizinate, α -cyperone and glycyrrhetic acid are purchased from National Institute for Control of Pharmaceutical and Biological Products (Beijing, China), merazin hydrate purchased from Di-ao Co. Ltd. (Chengdu, China). Neohesperidin, isoliquiritigenin purchased from Chengdu sikehua biological Co. Ltd (Chengdu, China). The purity of all reference compounds was > 98%.

Animals

Adult male Sprague Dawley rats weighing 180–200 g were obtained from Jiangning Qinglongshan Animal Cultivation Farm (Nanjing, China). Wild-type and GHSR KO male mice were purchased from Shanghai Model Animal Center. Rats were group-housed two per cage, and mice were group-housed four per cage in standard laboratory conditions (temperature, 23 $\pm$ 1°C; humidity, 50% $\pm$ 10%), under a 12-h light-dark cycle (lights on from 7 a.m.) with ad libitum access to water and food. All studies on animals were approved and conducted according to the institutional guidelines of the Animal Care and Use Committee at Nanjing University of Chinese Medicine. All animals were acclimatized 7 days before experimentation.

Grouping and drug administration

The rats were divided into seven groups (8 per group) in behavioral test: the sham group, vehi group, MH group (MH, 9.18 g/kg), CSS group (CSS, 30 g/kg), fluoxetine group (Flou, 20 mg/kg), MH + Dlys group (MH, 9.18 g/kg + [D-Lys3]-GHRP-6, 0.5 mg/kg) and Dlys ([D-Lys3]-GHRP-6, 0.5 mg/kg) group. The rats were divided into eight groups (8 per group) in GI motility test: the sham group, vehi group, MH group, CSS group, mosapride (Mosa, 10 mg/kg), fluoxetine group, MH + Dlys group and Dlys group. The wild-type and GHSR KO mice were divided into four groups in behavioral test: WT group, MH group (MH, 9.18 g/kg), GHSR group, and MH + GHSR group (MH, 9.18 g/kg). The rats were divided into three groups in western blot analysis: the vehi group, MH group and MH + Dlys group. The rats were divided into eight groups (8 per group) in fMRI: the sham group, vehi group, MH group, mosapride, fluoxetine group and MH + Dlys group.

Except the sham group, all animals were forced to swim 15 min to establish a comorbidity model for inducing depressive-like behaviors and hypomotility. Vehicle, Sham, WT and GHSR group refer to 0.9% saline (10 mL/kg).

Determination of 12 (ABCs) in CSS

Chromatographic conditions: use the ACQUITY BEH C18 as the column; Mobile phase: acetonitrile (A) and 0.5% acetic acid water (B); Gradient elution: 5%A: 95%B 10 min 15%A: 85%B 20 min 30%A: 70%B 30 min 50%A: 50%B 40 min 70%A: 30%B 45 min. On the PRD; Velocity: 0.5 ml/min, column temperature: 40°C; Detection wavelength: 190–480 nm; Injection quantity: 3 μ l.

Quantification of MH in SD rats' hippocampal and intestinal by UPLC

Selective depletion of meranzin hydrate from CSS. A hippocampal and intestinal sample of a rat with depression taken 30 min after oral administration of 30.0 g/kg CSS. MH were detected by UPLC (Waters Acquity UPLC system, Waters Micro mass Quattro Premier).

Behavioral test

Forced swimming test (FST)

Animals were placed in a cylinder (20 cm in diameter and 46 cm in height for rats; 10 cm in diameter and 25 cm in height for mice) containing 30 cm of water (23–25°C). Twenty-four hours before testing, animals were individually placed into the cylinder for 15 min FS. Test sessions (5 min in duration) were recorded with a videocamera. Periods of passive floating in the water and exerting minimal activity except for respiration were scored as immobility time [52–53].

Open field test (OFT)

The size of the open box was 50 × 50 × 40 cm, which was divided into 16 grids. Rats were placed in the center of the open box. Behavior was recorded for 10 minutes using a video camera 24 h following acute stress [53].

Tail suspension test (TST)

Tape was used to suspend mice in a hanging box (25 × 35 × 60 cm). The entire 6-min session was recorded using a video camera, and immobility was analyzed. The mobility bouts of front limbs with momentum-induced oscillations and pendulum swinging were not regarded as mobility [53].

In vivo measurements of GI motility

The scheme was designed according to previous reports [54–55]. First, CSS, MH, fluoxetine, saline, or mosapride was administered; 30 min later, animals were gavaged with 1.5 mL Evans blue (50 mg/mL in 0.9% NaCl with 0.5% methylcellulose [Sigma Chemical Co. St. Louis, USA]). Gastric emptying (GE) rates and intestinal transit (INT) were measured [55].

Western blot analysis

Animals were placed in a cylinder (20 cm in diameter and 46 cm in height for rats; 10 cm in diameter and 25 cm in height for mice) containing 30 cm of water (23–25°C) for 15 min FS. After 30 min, MH or MH + Dlys was administered. Animals were anesthetized 30 min later, and the hippocampus was removed.

The whole hippocampus was lysed in RIPA buffer containing protease inhibitors and phosphatase inhibitors. Protein concentration was determined colorimetrically by BCA assay (Pierce, Rockford, IL, USA). Protein lysates were separated by 15% and 8% SDS-PAGE electrophoresis and were transferred onto polyvinylidene difluoride (PVDF) membranes. After blocking with 5% bovine serum albumin (BSA) for 1 hr, the membranes were incubated with anti-BDNF (SANTAN, 1:200), anti-PMTOR (CST, 1:1000) and anti-beta-actin (Proteintech, 1:4000) antibodies at normal atmospheric temperature for 6 hr followed by incubation with horseradish peroxidase-conjugated secondary antibodies for 2 hr. Then, the blots were visualized using the SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific Inc.). BDNF and PMTOR were normalized to beta-actin bands.

MRI acquisition and analysis

Rats were positioned in the scanner in a prone position after stress. Rectal temperature was maintained at 37.5°C with a temperature-controlled water blanket placed beneath the rats. Respiratory rate was monitored at 60–80 breaths/min continuously throughout the entire experiment using an MRI-compatible pulse oximeter. Head position was stabilized with a bite bar and two rods located on opposite sides of the temporal surface of the head. Rats were anesthetized using inhaled isoflurane (3% for induction and 1.5–2% for maintenance). Intraperitoneal injection of dexmedetomidine was performed 3 minutes later.

A 7.0 T animal MRI scanner at the Department of Neurology, Affiliated ZhongDa Hospital of Southeast University (PharmaScan, Bruker Biospin GmbH, Germany) with a quadrature surface RF coil was used to collect MRI data. Twenty-seven anatomic images which extended anteriorly from the cerebral-olfactory bulb to the caudal region of the cerebellum posteriorly were obtained via a turbo-rapid acquisition relaxation enhancement (RARE) T2-weighted sequence (repetition time (TR)/echo time (TE) = 3200/36 ms, slices = 27, field of view (FOV) = 2.5 × 2.5 cm, number of averages = 1, matrix = 384 × 384, slice thickness/gap = 1/0 mm, flip angle = 90°). A single-shot gradient-echo echo-planar-imaging (GE-EPI) sequence was utilized to acquire BOLD measurements and multiple slices of images. The parameters were: TR/TE = 2000/19 ms, FOV = 2.5 × 2.5 cm, slices = 27, matrix = 96 × 96, number of averages = 1, flip angle = 90°, slice thickness/gap = 1/0 mm, 100 volumes.

SpmratIHEP toolbox [56–58] of the statistical parametric mapping (SPM8) software (Wellcome Department of Imaging Science; <http://www.fil.ion.ucl.ac.uk/spm>) was employed to preprocessing and data analysis. The SPM8 software comprises an fMRI rat brain template and atlas in Paxinos & Watson space.

The main steps for preprocessing in spmrat IHEP are as follows. In order to compass magnetization equilibrium, first ten volumes of each individual were discarded. Slice timing was used to revise the differences of slice acquisition times of each individual. The temporal processed volumes of each subject were realigned to the first volume to remove the head motion, and a mean image was created over the 310 realigned volumes. All participants had less than 1 mm of translation in x, y, or z axis and 1° of rotation in each axis. Normalize the realigned volumes through the corresponding mean image and the rat brain EPI template, and spatially standardize it to Paxinos & Watson space. All the normalized images were resliced in 1.0 × 1.5 × 1.0 mm³ voxels. A Gaussian kernel of 2 × 4 × 2 mm³ FWHM (Full Width at Half-maximum) was employed to smooth the normalized functional series. Afterwards, use Data Processing Assistant for Resting-State fMRI (DPARSF, <http://rfmri.org/DPARSF>) to detrend and filter (0.01–0.08 Hz) the smoothed images.

Individual regional homogeneity (ReHo) maps were generated by calculating Kendall's coefficient of concordance of the time series of a given voxel with those of its nearest neighbor (26 voxels). Then, all ReHo maps were smoothed with an isotropic Gaussian kernel of 2mm³ FWHM. Then all the calculated ReHo maps were analyzed within SPM8 based on the framework of the general linear model. To identify the difference in ReHo between the patients and

the healthy controls, the two-sample t-test was performed using SPM8. Brain regions with significant ReHo changes in patients were yielded based on a voxel-level height threshold of $P < 0.005$ (uncorrected) and a cluster-extent threshold of 10 voxels.

Statistical analysis

Data were analyzed using SPSS software, version 25.0 (IBM SPSS, China) and values were presented as mean $\pm$ standard deviation (S.D.). The results were considered statistically significant if $P < 0.05$. ANOVA was applied by the LSD testing as post hoc analysis for further examination of group differences. The T-test was applied to the comparison between the two groups.

Results

The contents (mg/g) of 12 (ABCs) in CSS

According to the UPLC method used, the contents (mg/g) of 12 (ABCs) in CSS were as follows: albiflorin, 559.9 ± 4.35 ; ferulic acid, 113.3 ± 0.22 ; paeoniflorin, 276.9 ± 1.02 ; liquiritin, 883.9 ± 5.15 ; naringin, 5893.6 ± 24.9 ; hesperidin, 1606.5 ± 8.44 ; merazin hydrate, 180 ± 0.62 ; neohesperidin, 2934.2 ± 26.1 ; isoliquiritigenin, 6.46 ± 0.1 ; ammonium glycyrrhizinate, 209.6 ± 2.46 ; α -cyperone, 97.2 ± 0.32 ; glycyrrhetic acid, 46.4 ± 0.38 . The overall intra-day and inter-day variations were less than 10% for all 12 analytes. These results demonstrated that the developed method is reproducible with good precision. The accuracy tests were carried out using a recovery test. Recovery of all 12 tested bioactive compounds were $> 90\%$.

Quantification of MH in SD rats' hippocampal and intestinal by UPLC

MH was determined in healthy SD rats' hippocampus and intestine after administration of the CSS (Fig. 1.E-F), explain that the MH could be absorbed by the healthy SD rats' hippocampus and intestine.

Behavioral tests results

To validate the effect of CSS and MH on behavior performance of depression-like rat, FST and OFT were conducted. Compared to vehicle (9174.43 ± 40.36), MH9.18 (124.57 ± 26.61), CCS (125.63 ± 6.781), and fluoxetine (123.11 ± 29.49) induced similar decreases in immobility time (IT) (Fig. 1.H), [$F(4,35) = 7.069, P = 0.000$]. Compared to vehicle (91.75 ± 8.07), MH9.18 (140.63 ± 29.66), CCS (113.00 ± 4.209) increases in the number of crossing (CN) (Fig. 1.G), [$F(4,35) = 7.089, P = 0.000273$]. The effect of MH (124 ± 26.61) on IT was inhibited by [D-Lys3]-GHRP-6 (160.45 ± 26.99) ($P < 0.05$). (Fig. 2.A-B), [$F(2,21) = 5.478, P = 0.012$]. MH had little effect on GHSR KO mice in TST (Fig. 2.E) [$F(3,28) = 3.617, P = 0.025$] and FST. (Fig. 2.F) [$F(3,28) = 3.411, P = 0.031$].

Gut motility results

Compared to healthy rats ($79.55 \pm 5.31\%$), the vehicle-treated group ($39.87 \pm 4.96\%$) showed a significant decline in GE (Fig. 1.I), [$F(5,42) = 80.227, P = 0.000$]. Compared to vehicle, MH9.18 ($61.36 \pm 7.37\%$), CSS ($72.18 \pm 7.27\%$), and mosapride ($50.59 \pm 9.48\%$) promoted GE ($P < 0.05$ or 0.01). In intestinal transit, compared to healthy rats ($79.55 \pm 5.31\%$), the vehicle-treated group ($57.49 \pm 6.25\%$) showed a significant decline in INT (Fig. 1.J), [$F(5,42) = 30.416, P = 0.000$]. MH9.18 ($66.35 \pm 6.79\%$), CSS ($71.93 \pm 2.93\%$), and mosapride ($70.42 \pm 4.82\%$) promoted INT ($P < 0.05$ or 0.01). Further, fluoxetine (GE: $29.82 \pm 5.27\%$, INT: $49.16 \pm 6.50\%$) dramatically slowed motility of FS rats ($P < 0.05$). [D-Lys3]-GHRP-6 ($51.52 \pm 6.51\%$) antagonized the prokinetic effect of MH ($61.36 \pm 7.37\%$) (Fig. 2.C) [$F(2,21) = 22.916, P = 0.000$].

Western blot analysis

Increased excitatory synaptic transmission dependent on mTOR signaling may contribute to more rapid antidepressant action. The p-mTOR levels in the hippocampus were significantly increased in the MH group ($81.75 \pm 6.14\%$) compared to those in the vehicle group ($44.00 \pm 7.17\%$) (Fig. 3.B) [$F(2,21) = 88.117, P = 0.000$], in the same way BDNF levels were increased in the MH group ($76.13 \pm 6.36\%$) compared to those in the vehicle group ($35.38 \pm 9.12\%$) (Fig. 3.A) [$F(2,21) = 56.660, P = 0.000$]. [D-Lys3]-GHRP-6 ($45.50 \pm 8.19\%$, $70.00 \pm 3.55\%$) prevented the increase in BDNF and p-mTOR levels in the MH group ($P < 0.01$).

Functional MRI results

Increased BOLD activation foci were observed in the hippocampus, thalamus, and basal ganglia of the vehicle group. FS-induced increased BOLD activation foci were reversed to varying degrees following MH, MH + [D-Lys3]-GHRP-6, and fluoxetine treatment. Compared with that in the fluoxetine group, a greater decrease in amplitude of BOLD activation was observed in the HTB circuit in the MH group. Comparison of MH and MH + [D-Lys3]-GHRP-6 groups further demonstrated the effects of ghrelin, a shared regulatory molecule. Administration of [D-Lys3]-GHRP-6 into the hippocampus of the MH group was performed. The mosapride group only demonstrated activation in the basal ganglia and thalamus (Fig. 4, Table 1).

Discussion

FS performance from dysfunctional GZSX [6, 59–61]) can be divided into DB&H [6, 48–49], exhibiting dual gut-brain disorders in an acute stressor as shown in Fig. 1.A-D. This dichotomy also includes MH $\rightarrow$ P&A and ghrelin $\rightarrow$ above regulation with antagonists, revealed in three different counterparts. The dualism of gut-brain disorder, P&A, and their regulation implicated the HTB circuit based on BOLD activation foci. These results are causalities within and between counterparts 1–3, different from depressive comorbidity with somatic disease whose dichotomy is equivocal [11–13, 32–33] in homogeneous studies. To date, an in vivo localized 1H-MRS study at 4.7 T without BOLD signal only coupled acute FS and rapid antidepressant desipramine [62]. We observed BOLD activated hippocampal responses to traditional Chinese medicine using first-line antidepressant fluoxetine as a control [27] and a connection from activated foci of the insular lobe, cingulate gyrus, and left amygdala cortex using subacute FS-induced visceral

hyperalgesia in ovariectomized rats [63]. As described, it simultaneously induced DB&H (IT of 55.6%↑, NC of 20.8%↓, GE 39.1%↓, and InT 40.3%↓) compared with sham. MH affected IT, NC, GE, and INT (28.6%↓, 53.2%↑, 53.9%↑ and 25.1%↑ vs vehicle). P&A actions by MH were attenuated in acute FS after pretreatment with [D-Lys3]-GHRP (ghrelin antagonist). The regulatory effects of ghrelin in dual gut-brain disorder in homogeneous studies differ from its orthodox role in regulation of feeding reported since 1999 and effects on depression-like behavior reported since 2008 [64–65]. Further, prokinetic MH increased mTOR phosphorylation in parallel with increased GluA1 expression after ketamine administration [66] and activated the AMPA–ERK1/2–BDNF pathway [49], suggesting rapid antidepressant effects. MH-induced antidepressant effects were almost absent in GHSR KO mice, suggesting ghrelin as a shared mediator of gut-brain disorder [51]. BOLD activation foci of the HTB circuit respond to stimuli within or between counterpart(s). Inside each counterpart, FS, MH, and ghrelin are bisected as gut-brain disorder and P&A, with shared regulation and interaction within the HTB circuit. Counterparts 1–3 represent FS→ pathophysiology, MH→therapy, and ghrelin→shared mechanisms, as shown in Fig. 3. The inside/outside counterparts indicate causalities. Here, each HTB circuit comprising comorbid-like profiles is different from the 19 other circuits identified from BOLD activated areas related to pathogenesis, therapy, and pharmacology for single diseases [17–29]. FS is widely used as a behavioral paradigm to assess depressive behaviors [52], but hypomotility has been overlooked. Similarly, the widely used chronic social stress model has been overlooked in heart disorder apart from when arrhythmia co-occurs with anxiety behavior during social stress and is integrated by an agent with simultaneous anti-anxiety and cardioprotective effects [67]. To date, no single compound except ferulic acid and MH [6, 52] have been designed as a simultaneous A&P. Further, ghrelin and α2-AR [49] have been reported to commonly mediate A&P using distinct regulatory mechanisms [49, 64–65]. In mapping activated regions, the roles played by the intricate neurovascular couplings of BOLD signal are invaluable [68] but may be nonspecific or have poor sensitivity [69]. The latter indicates spatial non-specificity of BOLD contrast especially from gradient echo pulse sequences [69] and lower sensitivity by spin echo pulse sequences which has higher specificity [69]. 7.0 T fMRI BOLD foci can precisely discriminate activity of neural populations in the sensorimotor cortex at 1.5 mm scale with high spatial fidelity, similar to electrophysiological determination of activated foci [46]. We address which foci are activated based on antagonist studies, using stress and SSRIs as control [17–29]. FS, MH, and ghrelin antagonist [D-Lys3]-GHRP-6 were used for activation and inactivation. Pre-inhibition of HTB matched the BOLD response map in Fig. 3. The nature and signal amplitude (Ke, cluster size or number of voxels) of fluoxetine and mosapride differed from that of MH in the HTB. Mosapride only activated the thalamus and basal ganglia. BOLD signal intensity for MH was >4.0 and 1.4 times greater than that for fluoxetine in the hippocampus and thalamus, respectively. Fluoxetine-induced c-fos expression in the thalamus contradicts its effects on behavior [70–71]. [D-Lys3]-GHRP-6 inhibited MH-reduced signal intensity by 84.8% and 23.3% in the hippocampus and thalamus, respectively. According to the top three standard criteria following acute FS-stimulated BOLD foci ranking among 17 regions (Table.1), we selected the HTB circuit. Other regions affected by MH, [D-Lys3]-GHRP-6, fluoxetine, and mosapride lie in proximity to the HTB. The HTB differentially regulates depression and FD. The hippocampus is implicated in depression and FD. The thalamus plays a major role in FD and minor role in depression. The hippocampus is a well-known target for the effects of antidepressants. Gastrectasia, nausea, and delayed gastric emptying, which are related to FD, match foci of BOLD regions centered in the thalamus [72–73]. In the present study, mosapride as a prokinetic stimulated the thalamus but not hippocampus. The feeding inhibitor [D-Lys3]-GHRP-6 acts on the thalamus, consistent with ghrelin's prominent role in feeding behavior. Ghrelin antagonist [D-Lys3]-GHRP-6 inhibits BOLD-activated HTB foci by MH. These effects are different from the BOLD signal of areas stimulated by monistic nutrition regulated by ghrelin, including the thalamus, limbic areas, parahippocampal cortex, insula, and caudate [74–75].

Conclusions

GZSX and CSS are implicated in CDF. Ancient wisdom provides clues on related entities and their dualistic properties, first by docking (in) activated HTB and integrating with neurocircuitry, as indicated via BOLD signals. If predefined by one-disease-one-target-one-drug dogma, half of the available comorbidity-like dualism properties would be overlooked. MH-stimulated BOLD changes were modulated by fluoxetine, mosapride, and [D-Lys3]-GHRP-6 in terms of the nature and intensity of signal foci. To conclude, we report on acute FS-stimulated DB&H, MH-induced rapid A&P, and ghrelin-related regulation coupled to BOLD signals in brain areas before, providing insight into a unified theory of depression pathogenesis and pharmacotherapy.

Abbreviations

FD, functional dyspepsia; GZSX, Gan-zhu-shu-xie; TCA, tricyclic antidepressant; SSRI, selective serotonin reuptake inhibitor; GI, gastrointestinal; GABA, gamma-aminobutyric acid; fMRI, functional magnetic resonance imaging; BOLD, blood oxygen level-dependent; PFC, prefrontal cortex; FS, forced swimming; A, antidepressant; P, prokinetic; MH, meranzin hydrate; DB&H, depressed behavior and hypomotility; HTB, hippocampus-thalamus-basal ganglia; BDNF, brain-derived neurotrophic factor; ROS, reactive oxygen species; KO, knockout; ABC, absorbed biological component; FST, forced swimming test; OFT, open field test; TST, tail suspension test; RARE, rapid acquisition relaxation enhancement; TR, repetition time; TE, echo time; GE-EPI, gradient-echo echo-planar-imaging; GE, gastric emptying; INT, intestinal transit; PVDF, polyvinylidene difluoride; BSA, bovine serum albumin; ANOVA, analysis of variance; CN, number of crossing; IT, immobility time

Declarations

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Authors contributions

C.K., L.X.F., Z.T., L.J.F., L.M.H., L.J., H.L.R., C.Y. and Z.J.L. planned herbs and animal breedings, decocted CSS extract, detected CSS of chemical contents and absorbed profile, determined behavior, gut motor and their mechanism. H.X., L.X.F., and Z.W. measured fMRI-BOLD signals. H.L.R., L.X.F., H.X. and S.X. cooperated understool and suggested BOLD datum of analysis. Z.W improved BOLD image. H.L.R., L.X.F., L.J.F. and S.X. manufactured data analysis. L.J.F. wrote manuscript's experiment part together with Z.T. and L.X.F., H.X. wrote manuscript theoretical part together with Z.T.. L.J.F. Prepared and submitted manuscript. H.X. and R.P. conceptualized, acquired funding and supervised the project.

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Availability of data and materials

The datasets used in this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The protocols were approved by the Animal Care and Use Committee at Nanjing University of Chinese Medicine. (No.201905A013).

Consent for publication

The manuscript is approved by all authors for publication.

Competing interests

The authors declare that they have no competing interests.

References

1. Ledford H. If depression were cancer. *NATURE*. 2014;515:182–4. doi:10.1038/515182a.
2. Mahadeva S, Ford AC. Clinical and epidemiological differences in functional dyspepsia between the East and the West. *NEUROGASTROENTEROLOGY AND MOTILITY*. 2016;28:167–74. doi:10.1111/nmo.12657.
3. Afridi MI, Siddiqui MA, Ansari A. Gastrointestinal somatization in males and females with depressive disorder. *J Pak Med Assoc*. 2009;59:675–9.
4. Jamil O, Sarwar S, Hussain Z, Fiaz RQ, Chaudary RD. Association Between Functional Dyspepsia and Severity of Depression. *JCPSP-Journal of the College of Physicians Surgeons Pakistan*. 2016;26:513–6.
5. Adibi P, Keshteli AH, Daghighzadeh H, Roohafza H, Pournaghshband N, Afshar H. Association of anxiety, depression, and psychological distress in people with and without functional dyspepsia. *Advanced biomedical research*. 2016;5:195. doi:10.4103/2277-9175.190936.
6. Zhang Y, Huang X, Wang Y, Xie Y, Qiu X, Ren P, et al. Ferulic acid-induced anti-depression and prokinetics similar to Chaihu-Shugan-San via polypharmacology. *BRAIN RESEARCH BULLETIN*. 2011;86:222–8. doi:10.1016/j.brainresbull.2011.07.002.
7. Koloski NA, Jones M, Kalantar J, Weltman M, Zaguirre J, Talley NJ. The brain-gut pathway in functional gastrointestinal disorders is bidirectional: a 12-year prospective population-based study. *GUT*. 2012;61:1284–90. doi:10.1136/gutjnl-2011-300474.
8. Monteggia LM. Put therapies first. *NATURE*. 2014;515:200. doi:10.1038/515200a.
9. Chancellor D. The depression market. *NATURE REVIEWS DRUG DISCOVERY*. 2011;10:809–10. doi:10.1038/nrd3585.
10. Glynn LG. Multimorbidity: another key issue for cardiovascular medicine. *LANCET*. 2009;374:1421–2. doi:10.1016/S0140-6736(09)61863-8.
11. Hasler G. Pathophysiology of depression: do we have any solid evidence of interest to clinicians? *World Psychiatry*. 2010;9:155–61.
12. Mayer EA. Gut feelings: the emerging biology of gut-brain communication. *NATURE REVIEWS NEUROSCIENCE*. 2011;12:453–66. doi:10.1038/nrn3071.
13. Stevens BR, Goel R, Seungbum K, Richards EM, Holbert RC, Pepine CJ, et al. Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS and altered gut microbiome in anxiety or depression. *GUT*. 2018;67:1555–7. doi:10.1136/gutjnl-2017-314759.
14. Roze C. Neurohumoral control of gastrointestinal motility. *Reprod Nutr Dev*. 1980;20:1125–41.
15. 10.1136/gut.47.suppl_4.iv5
Wilhelmsen I. Brain-gut axis as an example of the bio-psycho-social model. *GUT*. 2000;47 Suppl 4:v5-7, v10. doi: 10.1136/gut.47.suppl_4.iv5.
16. Drossman DA, Tack J, Ford AC, Szegedy E, Tornblom H, Van Oudenhoove L. Neuromodulators for Functional Gastrointestinal Disorders (Disorders of Gut-Brain Interaction): A Rome Foundation Working Team Report. *GASTROENTEROLOGY*. 2018;154:1140–71. doi:10.1053/j.gastro.2017.11.279.
17. Swerdlow NR, GF K. Dopamine, schizophrenia, mania, and depression: Toward a unified hypothesis of cortico-striatopallido-thalamic function. *BEHAVIORAL AND BRAIN SCIENCES*. 1987;10:197–245. doi:10.1016/j.cell.2018.10.018.
18. Huang W, Heffernan ME, Li Z, Zhang N, Overstreet DH, King JA. Fear induced neuronal alterations in a genetic model of depression: an fMRI study on awake animals. *NEUROSCIENCE LETTERS*. 2011;489:74–8. doi:10.1016/j.neulet.2010.11.069.
19. Grandjean J, Azzinnari D, Seuwen A, Sigrist H, Seifritz E, Pryce CR, et al. Chronic psychosocial stress in mice leads to changes in brain functional connectivity and metabolite levels comparable to human depression. *NEUROIMAGE*. 2016;142:544–52. doi:10.1016/j.neuroimage.2016.08.013.

20. Lohani S, Poplawsky AJ, Kim SG, Moghaddam B. Unexpected global impact of VTA dopamine neuron activation as measured by opto-fMRI. *Mol Psychiatry*. 2017;22:585–94. doi:10.1038/mp.2016.102.
21. Akhrif A, Geiger MJ, Romanos M, Domschke K, Neufang S. Task Performance Changes the Amplitude and Timing of the BOLD Signal. *Translational Neuroscience*. 2017;8:182–90. doi:10.1515/tnsci-2017-0025.
22. Huang P, Dong Z, Huang W, Zhou C, Zhong W, Hu P, et al. Voluntary wheel running ameliorates depression-like behaviors and brain blood oxygen level-dependent signals in chronic unpredictable mild stress mice. *BEHAVIOURAL BRAIN RESEARCH*. 2017;330:17–24. doi:10.1016/j.bbr.2017.05.032.
23. Huang P, Gao T, Dong Z, Zhou C, Lai Y, Pan T, et al. Neural circuitry among connecting the hippocampus, prefrontal cortex and basolateral amygdala in a mouse depression model: Associations correlations between BDNF levels and BOLD - fMRI signals. *BRAIN RESEARCH BULLETIN*. 2018;142:107–15. doi:10.1016/j.brainresbull.2018.06.019.
24. Fettes P, Schulze L, Downar J. Cortico-Striatal-Thalamic Loop Circuits of the Orbitofrontal Cortex: Promising Therapeutic Targets in Psychiatric Illness. *Front Syst Neurosci*. 2017;11:25. doi:10.3389/fnsys.2017.00025.
25. Deisseroth K. Circuit dynamics of adaptive and maladaptive behaviour. *NATURE*. 2014;505:309–17. doi:10.1038/nature12982.
26. Rusconi F, Battaglioli E. Acute Stress-Induced Epigenetic Modulations and Their Potential Protective Role Toward Depression. *Frontiers in Molecular Neuroscience*. 2018;11:184. doi:10.3389/fnmol.2018.00184.
27. Gao L, Huang P, Dong Z, Gao T, Huang S, Zhou C, et al. Modified Xiaoyaosan (MXYS) Exerts Anti-depressive Effects by Rectifying the Brain Blood Oxygen Level-Dependent fMRI Signals and Improving Hippocampal Neurogenesis in Mice. *Front Pharmacol*. 2018;9:1098. doi:10.3389/fphar.2018.01098.
28. Bruinsma TJ, Sarma VV, Oh Y, Jang DP, Chang SY, Worrell GA, et al. The Relationship Between Dopamine Neurotransmitter Dynamics and the Blood-Oxygen-Level-Dependent (BOLD) Signal: A Review of Pharmacological Functional Magnetic Resonance Imaging. *Front Neurosci*. 2018;12:238. doi:10.3389/fnins.2018.00238.
29. Hui JJ, Xi GJ, Liu SS, Li XL, Geng LY, Teng GJ, et al. Blood oxygen level-dependent signals via fMRI in the mood-regulating circuit using two animal models of depression are reversed by chronic escitalopram treatment. *BEHAVIOURAL BRAIN RESEARCH*. 2016;311:210–8. doi:10.1016/j.bbr.2016.05.044.
30. Thorkelson G, Bielefeldt K, Szigethy E. Empirically Supported Use of Psychiatric Medications in Adolescents and Adults with IBD. *INFLAMMATORY BOWEL DISEASES*. 2016;22:1509–22. doi:10.1097/MIB.0000000000000734.
31. Tomita T, Oshima T, Miwa H. New Approaches to Diagnosis and Treatment of Functional Dyspepsia. *Curr Gastroenterol Rep*. 2018;20:55. doi:10.1007/s11894-018-0663-4.
32. Press J. Metapsychological and clinical issues in psychosomatics research. *INTERNATIONAL JOURNAL OF PSYCHOANALYSIS*. 2016;97:89–113. doi:10.1111/1745-8315.12334.
33. Banks WA. Denial versus dualism: the blood-brain barrier as an interface of the gut-brain axis. *ENDOCRINOLOGY*. 2006;147:2609–10. doi:10.1210/en.2006-0335.
34. Fujitsuka N, Asakawa A, Hayashi M, Sameshima M, Amitani H, Kojima S, et al. Selective serotonin reuptake inhibitors modify physiological gastrointestinal motor activities via 5-HT_{2c} receptor and acyl ghrelin. *Biol Psychiatry*. 2009;65:748–59. doi:10.1016/j.biopsych.2008.10.031.
35. Schatzberg AF. Development of New Psychopharmacological Agents for Depression and Anxiety. *Psychiatr Clin North Am*. 2015;38:379–93. doi:10.1016/j.psc.2015.05.009.
36. Schatzberg AF. Issues encountered in recent attempts to develop novel antidepressant agents. *Ann N Y Acad Sci*. 2015;1345:67–73. doi:10.1111/nyas.12716.
37. Reardon S. Party drug's power to fight depression puzzles scientists. *NATURE*. 2017;545:17. doi:10.1038/545017a.
38. Developing Effective Treatments for Psychiatric Diseases. *CELL*. 2016;165:1307–9. doi:10.1016/j.cell.2016.05.062.
39. Yang Y, Cui Y, Sang K, Dong Y, Ni Z, Ma S, et al. Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *NATURE*. 2018;554:317–22. doi:10.1038/nature25509.
40. Ingram J, Taylor H, Churchill C, Pike A, Greenwood R. Metoclopramide or domperidone for increasing maternal breast milk output: a randomised controlled trial. *Arch Dis Child Fetal Neonatal Ed*. 2012;97:F241-5. doi:10.1136/archdischild-2011-300601.
41. Perez-Cervera L, Carames JM, Fernandez-Molla LM, Moreno A, Fernandez B, Perez-Montoyo E, et al. Mapping Functional Connectivity in the Rodent Brain Using Electric-Stimulation fMRI. *Methods Mol Biol*. 2018;1718:117–34. doi:10.1007/978-1-4939-7531-0_8.
42. Musazzi L, Tornese P, Sala N, Popoli M. Acute or Chronic? A Stressful Question. *TRENDS IN NEUROSCIENCES*. 2017;40:525–35. doi:10.1016/j.tins.2017.07.002.
43. Mayer EA, Naliboff BD, Craig AD. Neuroimaging of the brain-gut axis: from basic understanding to treatment of functional GI disorders. *GASTROENTEROLOGY*. 2006;131:1925–42. doi:10.1053/j.gastro.2006.10.026.
44. Zhou T, Zhu H, Fan Z, Wang F, Chen Y, Liang H, et al. History of winning remodels thalamo-PFC circuit to reinforce social dominance. *SCIENCE*. 2017;357:162–8. doi:10.1126/science.aak9726.
45. Hampton T. Organoids Reveal Clues to Gut-Brain Communication. *JAMA*. 2017;318:787–8. doi:10.1001/jama.2017.11545.
46. Siero JC, Hermes D, Hoogduin H, Luijten PR, Ramsey NF, Petridou N. BOLD matches neuronal activity at the mm scale: a combined 7T fMRI and ECoG study in human sensorimotor cortex. *NEUROIMAGE*. 2014;101:177–84. doi:10.1016/j.neuroimage.2014.07.002.

47. Kaelberer MM, Buchanan KL, Klein ME, Barth BB, Montoya MM, Shen X, et al. A gut-brain neural circuit for nutrient sensory transduction. *SCIENCE*. 2018;361. doi:10.1126/science.aat5236.
48. Xie Y, Huang X, Hu SY, Qiu XJ, Zhang YJ, Ren P, et al. Meranzin hydrate exhibits anti-depressive and prokinetic-like effects through regulation of the shared alpha2-adrenoceptor in the brain-gut axis of rats in the forced swimming test. *NEUROPHARMACOLOGY*. 2013;67:318–25. doi:10.1016/j.neuropharm.2012.10.003.
49. Xie Y, Huang X, Hu SY, Zhang YJ, Wang Y, Qiu XJ, et al. The involvement of AMPA-ERK1/2-BDNF pathway in the mechanism of new antidepressant action of prokinetic meranzin hydrate. *AMINO ACIDS*. 2013;44:413–22. doi:10.1007/s00726-012-1347-2.
50. Grant IS, Nimmo WS, Clements JA. Lack of effect of ketamine analgesia on gastric emptying in man. *Br J Anaesth*. 1981;53:1321–3.
51. Muller TD, Nogueiras R, Andermann ML, Andrews ZB, Anker SD, Argente J, et al. Ghrelin Molecular Metabolism. 2015;4:437–60. doi:10.1016/j.molmet.2015.03.005.
52. Bogdanova OV, Kanekar S, D'Anci KE, Renshaw PF. Factors influencing behavior in the forced swim test. *PHYSIOLOGY BEHAVIOR*. 2013;118:227–39. doi:10.1016/j.physbeh.2013.05.012.
53. Powell TR, Fernandes C, Schalkwyk LC. Depression-Related Behavioral Tests. *Curr Protoc Mouse Biol*. 2012;2:119–27. doi:10.1002/9780470942390.mo110176.
54. De Winter BY, Bredenoord AJ, De Man JG, Moreels TG, Herman AG, Pelckmans PA. Effect of inhibition of inducible nitric oxide synthase and guanylyl cyclase on endotoxin-induced delay in gastric emptying and intestinal transit in mice. *SHOCK*. 2002;18:125–31.
55. De Winter BY, De Man JG, Seerden TC, Depoortere I, Herman AG, Peeters TL, et al. Effect of ghrelin and growth hormone-releasing peptide 6 on septic ileus in mice. *Neurogastroenterol Motil*. 2004;16:439–46. doi:10.1111/j.1365-2982.2004.00564.x.
56. Nie B, Hui J, Wang L, Chai P, Gao J, Liu S, et al. Automatic method for tracing regions of interest in rat brain magnetic resonance imaging studies. *JOURNAL OF MAGNETIC RESONANCE IMAGING*. 2010;32:830–5. doi:10.1002/jmri.22283.
57. Nie B, Chen K, Zhao S, Liu J, Gu X, Yao Q, et al. A rat brain MRI template with digital stereotaxic atlas of fine anatomical delineations in paxinos space and its automated application in voxel-wise analysis. *HUMAN BRAIN MAPPING*. 2013;34:1306–18. doi:10.1002/hbm.21511.
58. Nie B, Liu H, Chen K, Jiang X, Shan B. A statistical parametric mapping toolbox used for voxel-wise analysis of FDG-PET images of rat brain. *PLoS One*. 2014;9:e108295. doi:10.1371/journal.pone.0108295.
59. Chen M, Men L, Ou L, Li T, Li M, Zhong X, et al. Effectiveness and safety of modified 'Huoxue Shugan' formulas on coronary heart disease combined with depression: protocol for a systematic review. *BMJ Open*. 2018;8:e22868. doi:10.1136/bmjopen-2018-022868.
60. Huang X, Wang Y, Zhang YJ, Ren P, HN Z. Brain-Smooth Muscle Axis: A Hypothesis and Its Interrelation with the Liver Visceral Manifestation Theory of Traditional Chinese Medicine or Comorbid Depression. *J World Science Technology/Modernization of Traditional Chinese Medicine Materia Medica*. 2011;13:1–5.
61. Wang Y, Fan R, Huang X. Meta-analysis of the clinical effectiveness of traditional Chinese medicine formula Chaihu-Shugan-San in depression. *JOURNAL OF ETHNOPHARMACOLOGY*. 2012;141:571–7. doi:10.1016/j.jep.2011.08.079.
62. Kim SY, Choi CB, Lee HS, Lee SH, Woo DC, Kim HY, et al. Reversal of myo-inositol metabolic level in the left dorsolateral prefrontal cortex of rats exposed to forced swimming test following desipramine treatment: an in vivo localized (1)H-MRS study at 4.7 T. *MAGNETIC RESONANCE IMAGING*. 2010;28:1461–7. doi: 10.1016/j.mri.2010.06.019.
63. Hubbard CS, Karpowicz JM, Furman AJ, Da SJ, Seminowicz DA, Traub RJ. Estrogen-dependent visceral hypersensitivity following stress in rats: An fMRI study. *Molecular Pain*. 2016;12. doi:10.1177/1744806916654145.
64. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *NATURE*. 1999;402:656–60. doi:10.1038/45230.
65. Lutter M, Sakata I, Osborne-Lawrence S, Rovinsky SA, Anderson JG, Jung S, et al. The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *NATURE NEUROSCIENCE*. 2008;11:752–3. doi:10.1038/nn.2139.
66. Carnevali L, Vacondio F, Rossi S, Callegari S, Macchi E, Spadoni G, et al. Antidepressant-like activity and cardioprotective effects of fatty acid amide hydrolase inhibitor URB694 in socially stressed Wistar Kyoto rats. *Eur Neuropsychopharmacol*. 2015;25:2157–69. doi:10.1016/j.euroneuro.2015.07.015.
67. Zhang K, Yamaki VN, Wei Z, Zheng Y, Cai X. Differential regulation of GluA1 expression by ketamine and memantine. *BEHAVIOURAL BRAIN RESEARCH*. 2017;316:152–9. doi:10.1016/j.bbr.2016.09.002.
68. Nunes D, Ianus A, Shemesh N. Layer-specific connectivity revealed by diffusion-weighted functional MRI in the rat thalamocortical pathway. *NEUROIMAGE*. 2019;184:646–57. doi:10.1016/j.neuroimage.2018.09.050.
69. Harmer J, Sanchez-Panchuelo RM, Bowtell R, Francis ST. Spatial location and strength of BOLD activation in high-spatial-resolution fMRI of the motor cortex: a comparison of spin echo and gradient echo fMRI at 7 T. *NMR IN BIOMEDICINE*. 2012;25:717–25. doi: 10.1002/nbm.1783.
70. Hirvonen J, Hietala J, Kajander J, Markkula J, Rasi-Hakala H, Salminen JK, et al. Effects of antidepressant drug treatment and psychotherapy on striatal and thalamic dopamine D2/3 receptors in major depressive disorder studied with [11C]raclopride PET. *JOURNAL OF PSYCHOPHARMACOLOGY*. 2011;25:1329–36. doi:10.1177/0269881110376691.
71. Zhang Y, Liu F, Xiao H, Yao X, Li G, Choi SR, et al. Fluorine-18 labeled diphenyl sulfide derivatives for imaging serotonin transporter (SERT) in the brain. *NUCLEAR MEDICINE AND BIOLOGY*. 2018;66:1–9. doi:10.1016/j.nucmedbio.2018.06.008.

72. Liu P, Zeng F, Yang F, Wang J, Liu X, Wang Q, et al. Altered structural covariance of the striatum in functional dyspepsia patients. *Neurogastroenterol Motil.* 2014;26:1144–54. doi:10.1111/nmo.12372.
73. Lee IS, Wang H, Chae Y, Preissl H, Enck P. Functional neuroimaging studies in functional dyspepsia patients: a systematic review. *Neurogastroenterol Motil.* 2016;28:793–805. doi:10.1111/nmo.12793.
74. Jones RB, McKie S, Astbury N, Little TJ, Tivey S, Lassman DJ, et al. Functional neuroimaging demonstrates that ghrelin inhibits the central nervous system response to ingested lipid. *GUT.* 2012;61:1543–51. doi:10.1136/gutjnl-2011-301323.
75. Li J, An R, Zhang Y, Li X, Wang S. Correlations of macronutrient-induced functional magnetic resonance imaging signal changes in human brain and gut hormone responses. *AMERICAN JOURNAL OF CLINICAL NUTRITION.* 2012;96:275–82. doi:10.3945/ajcn.112.037440.

Table

Due to technical limitations, Table 1 is provided in the Supplementary Files section.

Figures

Fig2.

Figure 3

Effects of subcutaneous injection [D-Lys3]-GHRP-6 (0.5 mg/kg) 30 min prior to administration of MH (9.18 mg/kg) and 0.9% saline (10 mL/kg) on IT (s) (A-B), GE (C-D) of rats subjected to acute forced swimming. The effects of MH on immobility time of GHSR KO and wild-type mice in TST (E) and FST (F). Values are presented as mean $\pm$ S.E.M. of the immobility times of animals (n = 8). * $p \leq 0.05$, ** $p < 0.01$ vs vehicle (WT). \$ $P \leq 0.05$ vs MH.

Fig3.

Figure 5

MH (9.18 mg/kg) and MH (9.18 mg/kg)+[D-Lys3]-GHRP-6 (0.5 mg/kg) modulated the activities of hippocampus BDNF (A) and p-mTor (B) in rats 15 min after FS. Values are presented as mean ± S.E.M. (n=8). ** p < 0.01 vs vehicle; \$p<0.01 vs MH.

Figure 7

Statistical analysis of BOLD activities in the hippocampus, thalamus and basal ganglia of the vehicle versus sham, MH, MH+[D-Lys3]-GHRP-6, fluoxetine and mosapride. The voxel-level height threshold was $P < 0.005$ and the cluster-extent threshold were 10 voxels (uncorrected).

Supplementary Files

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