

# *Fuzi* polysaccharide-1 produces antidepressant-like effects in mice



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## Abstract

Current antidepressants are clinically effective only after several weeks of administration. We show that *Fuzi* polysaccharide-1 (FPS), a new water-soluble polysaccharide isolated from *Fuzi*, which has been used to treat mood disorders in traditional Chinese medicine for centuries, increases the number of newborn cells in the dentate gyrus in adult mice, and most of these cells subsequently differentiate into new neurons. We also found that FPS administration reduces immobility in the forced swim test, and latency in the novelty suppressed-feeding test. Moreover, a 14-d regimen with FPS reverses avoidance behaviour and inhibition of hippocampal neurogenesis induced by chronic defeat stress. In contrast, imipramine, a well known antidepressant, reverses this avoidance behaviour only after 4 wk of continuous administration. Finally, acute treatment with FPS had no effect on brain monoamine levels in frontal cortex but significantly increases BDNF in the hippocampus, while the antidepressant effect and enhancement of cell proliferation induced by FPS administration were totally blocked by K252a, an inhibitor of trkB in a chronic social defeat depression model, suggesting that the neurogenic and antidepressant effects of FPS may involve BDNF signalling. In conclusion, our findings suggest that FPS could be developed as a putative antidepressant with a rapid onset of action.

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## Introduction

Depression is a devastating neurobiological illness, with 10–20% lifetime prevalence and is a leading cause of disability worldwide (Nemeroff & Owens, 2002). Significant progress has been made in the development of therapeutic agents, such as the serotonin and norepinephrine selective reuptake inhibitors, which function in the same manner as the prototypical tricyclic antidepressants (ADs), but with fewer side-effects. Unfortunately for patients and clinicians, the mood improvement starts only after 3–6 wk of AD treatment. In addition, ~35% of patients are resistant

to current drug therapies (Trivedi, 2003). More effective and faster therapeutic interventions are in urgent demand.

Evidence has shown that the hippocampus is able to generate new neurons (i.e. neurogenesis) throughout the lifespan of mammals, including humans, changing our opinion about mechanisms underlying AD effects of current drugs (Newton & Duman, 2004; Sahay & Hen, 2007). The subgranular zone (SGZ) of the dentate gyrus (DG) in the adult brain contains neuroprogenitor cells (NPCs) capable of generating thousands of new neurons per day (Cameron & MacKay, 2001). Recent findings have given rise to the hypothesis that AD effects of drugs and adult hippocampal neurogenesis may be causally related (Malberg & Schechter, 2005; Sahay & Hen, 2007). Chronic treatment with different classes of ADs, including serotonin/norepinephrine reuptake inhibitors, tricyclic ADs and monoamine oxidase inhibitors increases adult hippocampal neurogenesis

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and reverses stress-induced inhibition of hippocampal neurogenesis (Alonso *et al.* 2004; Czeh *et al.* 2001; Malberg *et al.* 2000; Malberg & Duman, 2003; Santarelli *et al.* 2003). Moreover, the time-course of maturation of newly generated neurons in the DG is generally consistent with the delayed onset of therapeutic effects of current ADs (Ngwenya *et al.* 2006). The most compelling evidence linking adult hippocampal neurogenesis with ADs comes from recent studies that demonstrate suppression of hippocampal neurogenesis, by localized X-ray irradiation, inhibits the actions of different ADs (Airan *et al.* 2007; Jiang *et al.* 2005; Santarelli *et al.* 2003). Based on the above studies, it has been suggested that stimulating hippocampal neurogenesis could provide novel avenues for the treatment of depression.

*Fuzi* (also known as aconite), the daughter root of *Aconitum carmichaeli* Debx, has been used as a main ingredient in prescriptions for treating mood disorders in traditional Chinese medicine for centuries (Zhang, 2006). However, little is known about the mechanism of its action. To date, research on the ingredients of *Fuzi* have mainly focused on a group of *Aconitum* alkaloids (Taki *et al.* 2003); little was known about the carbohydrate compounds in this herbal medicine. In 1985, four polysaccharides isolated from *Fuzi* exhibited prominent hypoglycaemic effects in normal and alloxan-induced hyperglycaemic mice (Hikino *et al.* 1985). This revealed another group of bioactive ingredients, which probably contribute to the actions of *Fuzi*. *Fuzi* polysaccharide-1 (FPS) is a new water-soluble  $\alpha$ -D-glucan isolated from *Fuzi* in 2006 (Zhao *et al.* 2006), but little is known about it.

In present study, we first assessed the neurogenic potential of FPS by using bromodeoxyuridine (BrdU) labelling in adult mice. Thereafter, the AD effects were examined by using various models, including the forced swim test (FST), novelty-suppressed feeding test (NSF) and chronic social defeat stress paradigms. Furthermore, the mechanisms for these AD effects were explored.

## Materials and methods

### Animals

Male C57BL/6J mice (aged 8–11 wk) were obtained from the Southern Medical University Animal Center (China) and CD1 retired breeder mice were purchased from Vital River Laboratory (China). Mice were housed in plastic cages (300 × 170 × 120 mm) at 23 ± 1 °C temperature and kept under standard laboratory conditions [12-h light/dark cycle (lights on

12:00 hours) with free access to food and water]. Behavioural studies were conducted during the light phase. All experiments were conducted in accordance with the Chinese Council on Animal Care Guidelines.

### Drugs

FPS, isolated from *Fuzi*, as previously reported (Zhao *et al.* 2006), is a homogeneous  $\alpha$ -(1→6)-D-glucan, with a weight-average molecular weight of ~14 000 Da identified by high-performance gel permeation chromatography with a purity >99.8%. BrdU and imipramine were purchased from Sigma (USA), and K252a was purchased from Alomone Laboratories (Israel). All drugs were injected intraperitoneally and the injection volume was constant with 5 ml/kg body weight.

### BrdU labelling

Adult mice received a single injection of saline or FPS (5–400 mg/kg, dissolved in saline) ( $n=7$ ), followed by BrdU injection (75 mg/kg body weight) after 30 min. Twenty-four hours after the BrdU injection, all mice were anaesthetized with chloral hydrate (0.35 g/kg), and then sacrificed by intracardial perfusion with heparinized 0.9% saline followed by ice-cold 4% paraformaldehyde. The brains were removed and post-fixed with 4% paraformaldehyde overnight, incubated with 30% sucrose solution in PBS at 4 °C for 2 d, and 40- $\mu$ m coronal sections were cut on a microtome. Sections from every 240  $\mu$ m spanning the whole hippocampus (six sections) were selected for BrdU immunostaining (Tashiro *et al.* 2007). DNA denaturation was conducted by incubation for 2 h with 50% formamide/2 × SSC at 65 °C, followed by 30 min incubation in 2 N HCl at 37 °C, and rinsing in 0.1 M boric acid (pH 8.5) at room temperature (RT). After washing with PBS, sections were incubated with 1% H<sub>2</sub>O<sub>2</sub> in PBS for 15 min and then blocked with solution containing 2% normal goat serum and 0.3% Triton X-100 in PBS for 2 h at RT. Sections were then incubated with mouse monoclonal anti-BrdU antibody (2  $\mu$ g/ml; Roche, USA) overnight at 4 °C. Sections were treated with avidin-biotin-peroxidase solution (SABC kit, Boster, China) and cells were visualized with 3,3'-diaminobenzidine (DAB) kit (Boster, China).

For double labelling, FPS (100 mg/kg) or saline was injected daily to mice for seven consecutive days, and BrdU injections (4 × 75 mg/kg at 2-h intervals) were given on the last 2 d. Mice were sacrificed on day 28 after the last BrdU injection and brain sections were produced. After DNA denaturation, sections were

incubated with polyclonal sheep anti-BrdU (25 µg/ml, BioDesign, USA) and monoclonal mouse anti-neuronal nuclear antigen (NeuN) (1:100; Chemicon, USA) overnight at 4 °C. After washing, FITC-conjugated goat anti-mouse IgG and rhodamine-conjugated rat-absorbed donkey anti-sheep IgG (1:200; Jackson Immuno-Research, USA) were applied for 1 h at RT. Sections were visualized with confocal laser scanning system (Leica DM IRE2, Germany).

#### Quantification of BrdU labelling

A modified unbiased stereology protocol was used that has been reported to quantitate BrdU labelling successfully (Malberg *et al.* 2000; Tashiro *et al.* 2007). All six sections, obtained from the entire span of each hippocampus, were processed for BrdU immunohistochemistry. All BrdU-labelled cells in the granule cell layer and the hilus were counted from each section by an experimenter blinded to the study code. To distinguish single cells from clusters, all counts were performed under 400× and 100× lens of a light microscope (Olympus BX51, Japan), omitting cells in the outermost focal plane. A cell was counted as being in the SGZ of the DG if it was touching the border or was actually in the SGZ. Cells that were located more than two cells away from the SGZ were classified as hilar. The total number of BrdU-labelled cells per section was determined and multiplied by six to obtain the total number of cells per DG.

For double labelling, at least 50 BrdU<sup>+</sup> cells per animal were analysed using Z-plane sectioning (1-µm steps) to confirm the co-localization of both BrdU and NeuN.

#### FST

In the FST, ADs have been shown to reduce the duration of immobility when mice are placed in a container filled with water (Dulawa *et al.* 2004; Porsolt *et al.* 1977). Although this behavioural test works in subacute (30 min after injection) conditions, it does remain highly reliable in predicting the therapeutic potential of the tested compounds (Cryan *et al.* 2002; Dulawa & Hen, 2005). Following documented protocols, mice were singly injected with saline, imipramine (15 mg/kg) or FPS (50, 100 mg/kg) ( $n=10$  in each group). Thirty minutes after drug injection, mice were placed in a clear glass cylinder (height 45 cm, diameter 19 cm) filled to 23 cm with water at 22–25 °C for 6 min. The duration of immobility was recorded during the last 4 min by an investigator blinded to the study.

#### Open-field test

The open-field apparatus was a rectangular chamber (60×60×40 cm) made of grey polyvinyl chloride. A video camera, a loudspeaker providing masking noise, and a 25 W red light bulb placed 200 cm above the maze (illumination density at the centre of the maze, 0.3 lx) were positioned above its centre. The digitized image of the path taken by each animal was stored and analysed *post hoc*. After each trial, the apparatus was cleaned with water containing 0.1% acetic acid. The floor of the open field was divided into 144 rectangles (5×5 cm). Animals were placed on the centre square, and left to explore the arena for 5 min. The locomotion activity was registered as the distance in squares an animal moved.

#### NSF

Mice were randomly divided into two groups ( $n=10$  in each group), injected daily with saline or FPS (100 mg/kg) for seven consecutive days. Behavioural tests were conducted 24 h after the last injection. The NSF was performed during a 5-min period as previously described (Santarelli *et al.* 2003). The testing apparatus consisted of a plastic box, 50×50×20 cm. The floor was covered with ~2-cm-thick wood shavings. Food was withheld from the mice for 24 h before the test. At the beginning of the test, a single pellet of food was placed on a white paper platform positioned at the centre of the box. A mouse was placed in a corner of the maze and a stopwatch was immediately started. The scoring for measure of interest did not begin until the mouse reached for the food with its forepaws and began eating. Immediately after the test, mice were transferred to their home cages and the amount of food consumed in the next 5 min was measured (home-cage food consumption).

#### Chronic social defeat stress

Social defeat and avoidance testing were performed according to published protocol (Berton *et al.* 2006; Tsankova *et al.* 2006; Krishnan *et al.* 2007). Briefly, C57BL/6J test mice were exposed to a different CD1 aggressor mouse each day for 10 min over a total of 10 d. After the 10 min of contact, test mice were separated from the aggressor: the test mice were placed in an adjacent compartment of the same cage, separated by a plastic divider with holes, where they were exposed to chronic stress in the form of threat for the next 24 h. Control test mice were housed in equivalent cages but with members of the same strain which changed daily. Twenty-four hours after the last

session, all mice were housed individually and received daily injections of saline, imipramine (20 mg/kg), FPS (100 mg/kg) or K252a (25 µg/kg, dissolved in 1% DMSO)+FPS (100 mg/kg) for 7, 14 or 28 d. The day after the last injection, the long-term behavioural consequences of the chronic defeat stress were tested by using a measure of interaction and avoidance towards one of the aggressors used during the defeat procedure (Berton *et al.* 2006; Tsankova *et al.* 2006). All behavioural tests were conducted in darkness. Mice were placed in a new arena with a small animal cage at one end, and their movement was tracked for 2.5 min in the absence of the aggressor, followed by 2.5 min in the presence of the caged aggressor. The duration in the interaction zone and other measures were obtained using Ethovision XT (Noldus, USA) software. After each trial, the apparatus was cleaned with a solution of 70% ethanol in water to remove olfactory cues.

#### HPLC-EC analysis of monoamines

Serotonin hydrochloride (5-HT), dopamine hydrochloride (DA), and norepinephrine hydrochloride (NE) were of analytical grade and purchased from Sigma-Aldrich. HPLC grade water and acetonitrile were obtained from Fisher Scientific (USA).

For analysis of monoamines, mice were randomly divided into four groups ( $n=6$  in each group) and were singly injected with saline, imipramine or FPS (50, 100 mg/kg). All mice were sacrificed by cervical dislocation 45 min after the injection and frontal cortices were rapidly dissected and weighed and then homogenized in 100 µl ice-cold solution containing 0.1 M perchloric acid, 0.1 mM sodium metabisulfite and 0.1 mM EDTA per 10 mg wet weight. Homogenates were centrifuged at 12000 *g* for 15 min at 4 °C. Supernatants were filtered through 0.22 µm pore size polyvinylidene fluoride (PVDF) syringe-driven membrane filters (Millipore Corp., USA) and immediately frozen and stored at -80 °C until required.

HPLC analysis was performed with an ESA Model 5600A CoulArray<sup>®</sup> system (ESA Inc., USA), an ESA Model 582 pump, and an ESA Model 542 refrigerated autosampler. Chromatographic separation was achieved by autoinjecting 20-µl sample aliquots at 5 °C onto a Develosil ODS-UG-5 column (150 × 4.6 mm i.d., Nomura Chemical, Japan) with an ESA guard column (4.0 × 3.0 mm i.d., Phenomenex, USA). The mobile phase used for compound separation consisted of 75 mM monobasic sodium dihydrogen phosphate, 2.0 mM SDS, 25 mM EDTA, and 100 µl triethylamine and 10% acetonitrile (pH 3.0). A flow rate of 0.8 ml/min and analysis time of 45 min was used for all

experiments. System control and data acquisition/processing were performed using ESA CoulArray software (version 1.02). Electrochemical detection was designed using two-way electrode potentials (passage I -150 mV, passage IV +450 mV). All samples were processed in technical triplicate and median values used for statistical analysis.

#### Brain-derived neurotrophic factor (BDNF) ELISA

Mice were singly injected with saline or FPS (100 mg/kg) and were sacrificed by cervical dislocation at 2, 6 or 12 h after the injection ( $n=6$  in each group). Bilateral hippocampi were rapidly dissected and weighed and lysed in 2 ml/g tissue of protein lysis buffer (137 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1% Tergitol type NP40, 10% glycerol, 1 mM PMSF, 10 µg/ml aprotinin, 1 µg/ml leupeptin, and 0.5 mM sodium vanadate, and then were sonicated followed by centrifugation at 16000 *g* for 30 min at 4 °C. The supernatant was then collected and assayed for BDNF, using BDNF Emax<sup>®</sup> ImmunoAssay System (Promega, USA) according to the manufacturer's protocol.

#### Western blotting analysis

Mice injected with saline or FPS (100 mg/kg) ( $n=5$  in each group) were sacrificed 6 h after the injection. Bilateral hippocampi were rapidly dissected and homogenized in ice-cold buffer [20 mM Hepes (pH 7.5), 1.5 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 0.1 M NaCl] supplemented with 0.2 mM dithiothreitol, and protease inhibitors cocktail (P8340, Sigma, USA). Subsequently, NaCl was added to a final concentration of 0.45 M, and the homogenate was centrifuged at 10000 *g* for 30 min. Supernatants were collected and mixed with an equal volume of homogenizing buffer containing 40% (v/v) glycerol before being stored at -80 °C. Samples were subjected to electrophoresis in SDS-10% polyacrylamide gel and transferred to PVDF membranes by standard procedures. The membranes were blocked with 5% non-fat milk powder and incubated overnight at 4 °C with polyclonal rabbit anti-BDNF (1:500; Chemicon, USA) or monoclonal mouse anti-β-actin (1:1000; Boster, China.). This was followed by incubation with secondary horseradish peroxidase-conjugated antibodies and enhanced chemiluminescence detection (Amersham, USA) and detected by the Quantitative Imaging System FluorChem<sup>™</sup> SP (Alpha Innotech, USA). To quantitate protein abundance, bands on Western blots were analysed with FluorChem<sup>™</sup> SP software. Optical densities (ODs) were normalized to OD values for the corresponding β-actin on the same membranes.



### Data analysis

Data are presented as means  $\pm$  S.E.M. Differences between mean values were evaluated using one-way ANOVA followed by *post-hoc* LSD test and the Student's *t* test was also used to compare the difference in values between two groups.  $p < 0.05$  was considered statistically significant.

### Results

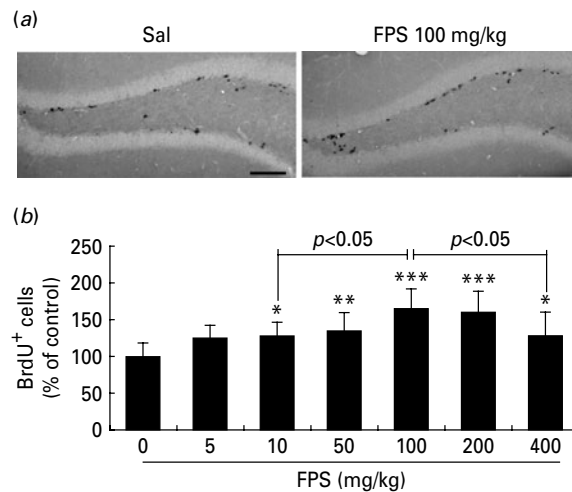
#### FPS stimulates neurogenesis in adult DG

The effect of FPS administration on the proliferation of NPCs was first investigated and BrdU was used to label the proliferating cells. A single injection of FPS clearly increased the total number of BrdU<sup>+</sup> cells in the DG ( $F_{6,42} = 5.697$ ,  $p = 0.001$ ), although the increase at 5 mg/kg concentration was not statistically significant. The largest effect was obtained at a concentration of 100 mg/kg, when the number of BrdU<sup>+</sup> cells increased by 64.3% relative to the saline group (*post-hoc* LSD,  $p < 0.001$ ). However, FPS, at the concentration of 400 mg/kg significantly decreased the number of BrdU<sup>+</sup> cells compared to that at 100 mg/kg (*post-hoc* LSD,  $p < 0.05$ ) (Fig. 1), suggesting the neurogenic effect of FPS has a plateau effect.

Newly generated cells in the DG differentiate into neurons within 28 d after their birth (Kempermann *et al.* 2003). To determine whether the newborn cells induced by FPS administration differentiated into new neurons, we selected a subchronic regimen of FPS administration and NeuN was employed as a marker for mature neurons (Mullen *et al.* 1992). The majority of BrdU-labelled cells dispersed into the granular cell layer and showed size and morphology indistinguishable from their neighbouring granule neurons when mice were sacrificed 28 d after the last BrdU injection (Fig. 2a). Seven-day treatment of FPS significantly increased the number of NeuN<sup>+</sup>/BrdU<sup>+</sup> cells in DG when compared to the saline-treated group (Student's *t* test,  $p < 0.05$ ,  $n = 7$ ) (Fig. 2b). Markedly, the ratio of NeuN<sup>+</sup>/BrdU<sup>+</sup> cells to total BrdU<sup>+</sup> cells was increased in FPS-treated mice relative to the saline group (Student's *t* test,  $p < 0.05$ ,  $n = 7$ ) (Fig. 2c), suggesting that newborn cells in the DG induced by FPS administration preferentially differentiated into neurons. Together, these results indicate that FPS administration promotes neurogenesis in adult hippocampus.

#### FPS produces AD effect in the FST

Having demonstrated the ability of FPS to promote neurogenesis in the hippocampus, we investigated for

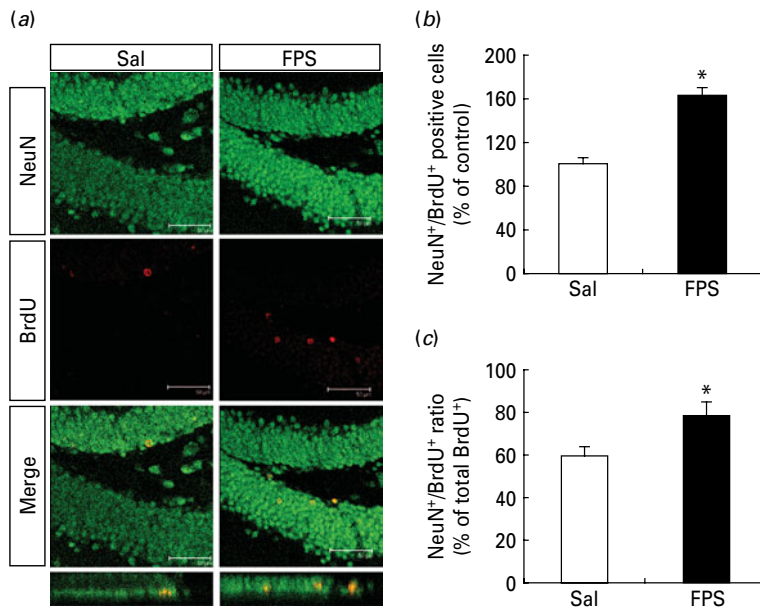


**Fig. 1.** FPS administration increased newborn cells in the dentate gyrus (DG) of adult C57BL/6 mice. (a) Representative photomicrographs of the DG showing BrdU<sup>+</sup> cells clustered or aggregated in the subgranular zone in mice that received an injection of saline (Sal) or FPS (100 mg/kg i.p.). Scale bar, 200  $\mu$ m. (b) Quantification of BrdU<sup>+</sup> cells in the DG. The results are expressed as the mean  $\pm$  S.E.M. ( $n = 7$ ); \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  when compared to the saline group by one-way ANOVA followed by *post-hoc* LSD test.

any AD effect related to its administration. To this end, FST was employed to test the AD effect of FPS and imipramine, a well known AD, was used as a positive control. Statistical analysis revealed a significant reduction of immobility in the Imipramine-treated mice (*post hoc* LSD,  $p < 0.01$ ). Interestingly, like imipramine, FPS significantly reduced the duration of the immobility at the concentrations of 50 and 100 mg/kg (Fig. 3a). To exclude the possible effect of FPS treatment on spontaneous locomotor activity which may contribute to immobility in the FST, naive mice treated as in the above schedule were exposed to the open-field apparatus for 5 min. There was clearly no difference in the number of squares an animal crossed in the four groups ( $F_{3,27} = 0.258$ ,  $p = 0.855$ ) (Fig. 3b), suggesting that FPS might have antidepressant-like effect.

#### AD effect of subchronic administration of FPS

Next, we measured the AD effect of FPS after repeated administration. In this experiment, we employed the NSF, previously used to assess chronic AD efficacy in rats and mice (Santarelli *et al.* 2003). In the NSF, rodents were presented with food in a novel and thus anxiogenic open field, and latency to feed was measured. As shown in Fig. 3c, a 7-d regimen of FPS



**Fig. 2.** FPS administration increased new neurons in the dentate gyrus (DG). After receiving daily injection of saline (Sal) or FPS (100 mg/kg i.p.) for seven continuous days and concomitant BrdU injections on the last 2 d, mice were sacrificed on day 28 after the last BrdU injection. (a) Representative confocal microscopic images show co-staining (yellow) of NeuN (green) and BrdU (red) in the DG. The majority of BrdU<sup>+</sup> cells are doubly labelled with the neuronal marker NeuN and located within the granule cell layer. Scale bars, 50  $\mu$ m. FPS significantly increased the number of NeuN<sup>+</sup>/BrdU<sup>+</sup> cells (b) and the proportion of NeuN<sup>+</sup>/BrdU<sup>+</sup> cells to the total number of BrdU<sup>+</sup> cells (c) in the DG. Error bars represent s.e.m. ( $n=7$ ); \*  $p < 0.05$  vs. saline group by Student's  $t$  test.

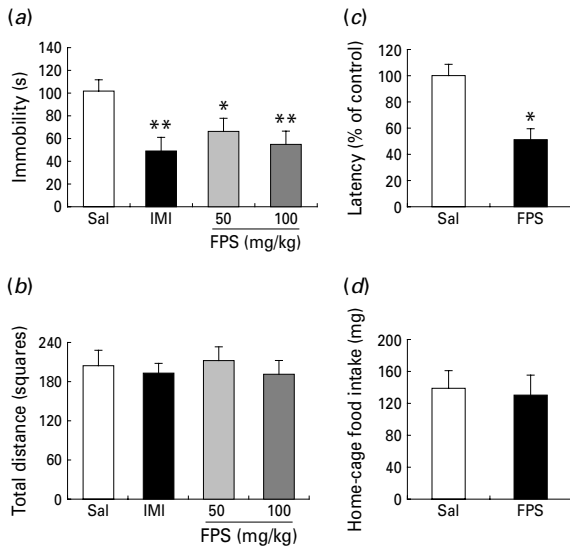
administration significantly shortened the latency to feed relative to the saline group (Student's  $t$  test,  $p < 0.01$ ,  $n=10$ ). Since ADs may alter appetite, we considered whether the reduction in latency to feed induced by FPS administration was due to an alteration in appetite. After the latency test, mice were immediately returned to their home cages and scored for food consumption. There was no significant difference in food consumption between saline- and FPS-treated mice (Fig. 3d). Thus, it is unlikely that the reduced latency from FPS treatment was due to a change in appetite. These results further indicate that FPS has AD effects.

#### *FPS reverses avoidance behaviour induced by chronic defeat stress*

We then considered the possibility that FPS could reverse depression in an animal model of depression – i.e. a chronic social defeat stress protocol, which mimics many symptoms of depression in humans (Berton *et al.* 2006; Tsankova *et al.* 2006; Krishnan *et al.* 2007). After a brief (10 min) daily exposure to a highly aggressive resident mouse for 10 d, the test mouse developed defeated and subordinate behaviour (data not shown). In the absence of an aggressor, all mice

spent similar amounts of time in the interaction zone when they were tested at the end of the treatments (Fig. 4a). Compared to control mice, chronically defeated mice spent about 60% less time in the interaction zone when an aggressor was introduced into the cage. The 28-d treatment of imipramine reversed this avoidance behaviour, increasing the interaction time so that it was close to that of the non-defeated mice (Fig. 4b). These results are in agreement with previous reports (Tsankova *et al.* 2006; Krishnan *et al.* 2007). Interestingly, a 28-d treatment of FPS significantly increased the interaction time relative to saline group ( $F_{3,36}=3.88$ ,  $p < 0.05$ ), very similar to that of imipramine-treated mice ( $p=0.14$  between two groups) (Fig. 4b), suggesting further that FPS produces AD effects.

Of further interest, our results indicated that the effect of FPS had a much more rapid onset of action than imipramine. We could not detect a significant increase in the interaction time in the defeated mice after a 7-d regimen of FPS (data not shown). However, after only 14 d treatment, the interaction time in the FPS-treated group was significantly higher than in the vehicle group ( $F_{3,36}=4.2$ ,  $p=0.0006$ ) (Fig. 4b). In contrast, the interaction time for imipramine- and vehicle-treated mice were very similar ( $p=0.604$ ).



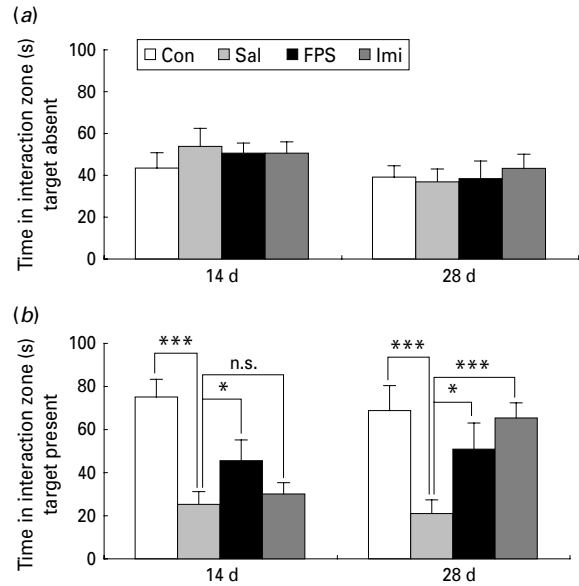
**Fig. 3.** FPS produced antidepressant effects. In the forced swim test, mice were singly injected with saline (Sal), imipramine (Imi, 15 mg/kg) and FPS (50, 100 mg/kg) ( $n=10$  in each group); behavioural tests were conducted 30 min after the injection. FPS decreased the immobility time in a dose-dependent manner (a) without affecting spontaneous locomotor activity in the open-field test (b). In the NSF, mice were injected daily with saline or FPS (100 mg/kg) for 7 d and behavioural tests were conducted 24 h after the last injection. FPS administration markedly decreased the latency to feed (c); there was no significant difference in food consumption between saline- and FPS-treated mice when they were tested in their home cages (d). The results are expressed as the mean  $\pm$  S.E.M ( $n=10$ ); \*  $p < 0.05$ , \*\*  $p < 0.01$  vs. saline group by one-way ANOVA followed by *post-hoc* LSD test or by Student's *t* test.

#### FPS has no effect on brain monoamine levels

To investigate the effect of FPS on modulation of the neurochemical system in adult mice, we performed HPLC-EC analysis of monoamine transmitters. As a positive control, imipramine significantly increased NE and 5-HT contents, without any effect on contents of DA in frontal cortex (Fig. 5a). This pattern is generally consistent with previous report (Wesolowska & Kowalska, 2008). However, FPS administration had no effect on the contents of 5-HT, NE and DA.

#### BDNF is necessary for the AD effects of FPS

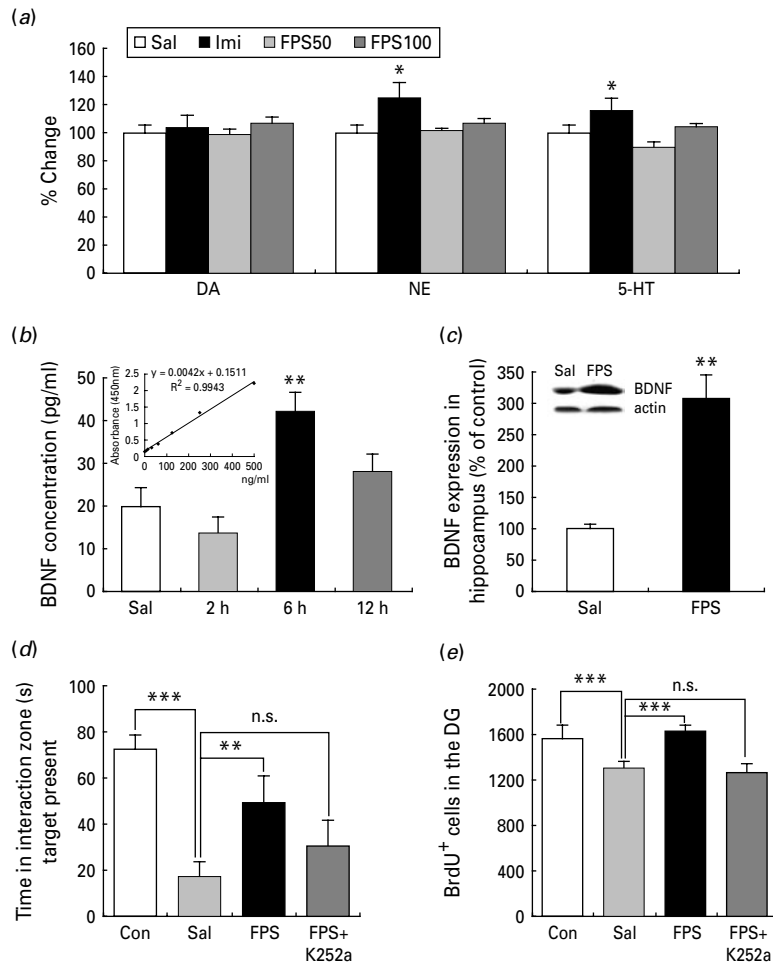
Several lines of evidence support an important role of BDNF in the response to ADs (Chen *et al.* 2001; Monteggia *et al.* 2007; Nestler *et al.* 2002; Nibuya *et al.* 1995; Shirayama *et al.* 2002; Smith *et al.* 1995). To examine the potential effect of FPS on inducing BDNF in adult hippocampus, we first performed BDNF



**Fig. 4.** FPS administration reversed avoidance behaviour induced by chronic social defeat stress. Mice were exposed daily to a highly aggressive resident mouse (10 min) for 10 d ( $n=10$  in each group). Twenty-four hours after the last session, mice were housed individually and received a daily injection (i.p.) of saline, imipramine (20 mg/kg), FPS (100 mg/kg) or K252a (25  $\mu$ g/kg) + FPS (100 mg/kg) for 14 d or 28 d. The day after the last injection, the long-term behavioural consequences of the chronic defeat stress were tested by using a measure of interaction and avoidance towards one of the aggressors used during the defeat procedure. (a) In the absence of an aggressor, all mice spent similar amounts of time in the interaction zone. (b) The time test mice spent in the interaction zone when an aggressor was introduced into the cage. \*  $p < 0.05$ , \*\*\*  $p < 0.001$  by one-way ANOVA followed by *post-hoc* LSD test; n.s., no significance.

ELISA analysis. FPS (100 mg/kg) significantly increased BDNF at 6 h after injection ( $F_{3,21}=8.033$ ,  $p=0.007$ ) (Fig. 5b). This result was further supported by using Western blotting method, which showed that FPS administration increased BDNF by 3-fold at 6 h after injection relative to the saline group ( $t_{1,8}=3.286$ ,  $p=0.005$ ) (Fig. 5c), suggesting that BDNF–trkB signalling could be involved in the neurogenic and AD effects of FPS.

To further determine whether BDNF–trkB signalling is necessary for the AD effects of FPS, chronic social defeated mice were co-injected with FPS (100 mg/kg) and K252a, a potent pharmacological inhibitor of the BDNF receptor trkB, for 14 d. Behavioural changes were examined 24 h after the last injection. As shown in Fig. 5d, K252a injections abolished the AD effect of FPS. Next, we examined whether K252a



**Fig. 5.** BDNF is necessary for the behavioural and neurogenic effects of FPS. (a) The effects of FPS on the contents of DA, NE and 5-HT in frontal cortices in adult mice ( $n=6$  in each group; Sal, saline; Imi, imipramine). (b) A single injection of FPS significantly increased BDNF in hippocampus at 6 h after injection by using ELISA analysis ( $n=6$  in each group). The inset indicates the BDNF standard curve. (c) Western blotting result of acute treatment of FPS ( $n=6$ ). The insets are the representative Western blots of BDNF and  $\beta$ -actin. In the chronic defeat stress depression model, the antidepressant effects (d) and the enhancement of cell proliferation (e) induced by FPS administration were blocked by co-injection with K252a, a potent inhibitor of trkB ( $n=10$  in each group). The results are expressed as mean  $\pm$  s.e.m.; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. saline group by one-way ANOVA followed by *post-hoc* LSD test; n.s., no significance.

injection blocked the neurogenic effect of FPS. To this end, immediately after the behavioural test, mice were given BrdU injections ( $4 \times 75$  mg/kg every 2 h) and sacrificed 24 h after the last BrdU injection. Chronic defeats resulted in about 17% reduction in the number of BrdU<sup>+</sup> cells in adult DG when compared to that in non-defeated mice. This result is in agreement with previous report (Yap *et al.* 2006). Interestingly, 14-d treatment of FPS totally counteracted this stress-induced neurogenic deficiency. There was no difference in the number of BrdU<sup>+</sup> cells between FPS and control groups, but a significant difference from that in

saline-treated defeated mice ( $F_{3,36} = 7.077$ ,  $p = 0.0002$ ). Most importantly, this neurogenic effect of FPS was fully blocked by K252a injections (Fig. 5e). Together, these results suggest that BDNF signalling is necessary for the neurogenic and AD effects of FPS.

## Discussion

In the present study, we have demonstrated for the first time that FPS administration increases hippocampal neurogenesis and produces AD effects in adult mice. We found that FPS significantly increased



BrdU<sup>+</sup> cells and new neurons in the DG relative to the control group. Importantly, FPS has properties common to classical ADs in the FST. The reduction of immobility that we found in the test for single injection of FPS was not paralleled by an increase of motor activity, suggesting that FPS may have a potential interest for the treatment of depression. This hypothesis is further supported by the finding that mice receiving subchronic FPS treatment showed significant reduction in the latency to feed in the NSF without an alteration in their appetite. Moreover, like imipramine, a 28-d regimen of FPS reversed avoidance behaviour induced by chronic social defeat stress. Together, these results indicate that FPS could be developed as a novel AD.

The fast-acting AD potential of FPS was confirmed by several lines of evidences: (1) by using a protocol of BrdU labelling, which has been previously used to detect the effects of current ADs on the proliferation of NPCs (Malberg *et al.* 2000). The first result of this study indicates a single injection of FPS significantly increased the number of BrdU<sup>+</sup> cells in the DG, suggesting that FPS promotes the proliferation of NPCs. However, for the current ADs, up-regulation of the number of BrdU<sup>+</sup> cells in DG is observed only after chronic, not acute, treatment. (2) Hyponeophagia-based paradigms provide an anxiety-related measure that is sensitive to the effects of chronic, but not acute or subchronic, AD treatment and exhibit considerable potential as animal models for studying the neurobiology of the AD response (Dulawa & Hen, 2005). In the present study, subchronic treatment of FPS (a 7-d regimen) significantly decreased the latency to feed in the NSF without altering appetite, suggesting the onset of AD effects of FPS could be fast. (3) Most importantly, in a social defeat stress animal model, after 14 d treatment, the interaction time was significantly increased in the FPS group, although we could not find statistically significant effects of FPS on increasing interaction time at day 7 of treatment (data not shown). Meanwhile, on day 14 of treatment, the imipramine group was still not statistically different from the vehicle group. These comparisons strongly suggest that a 14-d treatment with FPS is sufficient to reverse the avoidance behaviour induced by chronic social defeat stress. In contrast, imipramine induced a reversion of avoidance behaviour only after 28 d of continuous administration. (4) Evidence has shown that BDNF plays a critical role in the activity of ADs in depressive patients and in animal models of depression (Chen *et al.* 2001, 2006; Newton & Duman, 2004; Shirayama *et al.* 2002; Smith *et al.* 1995). Moreover, only chronic, not acute treatment with ADs

up-regulates BDNF expression, consistent with the time-course for the therapeutic action of ADs, in adult hippocampus (Nibuya *et al.* 1995). In the present study, one single injection of FPS, unlike current ADs (H.-C. Yan, unpublished data), significantly increased BDNF level in hippocampus at 6 h after injection, detected by using ELISA and Western blotting methods. Most importantly, the effects of ADs and enhancement of cell proliferation induced by FPS administration were totally blocked by K252a injection, a potent inhibitor of the BDNF receptor, trkB, in a chronic defeat stress animal model, suggesting that BDNF signalling is involved in the AD and neurogenic effects of FPS. Together, these findings suggest that FPS could be developed as a putative class of ADs with a rapid onset of action.

Neurobiological evidences both in animals and humans have indicated the role of monoaminergic systems (catecholamines and serotonin) in the pathophysiology of mental depression (Elhwuegi, 2004). Most ADs exerted their action by elevating synaptic monoamine concentrations (Schloss & Henm, 2004). Consistent with this view, in this study, as a positive control, imipramine significantly increased NE and 5-HT levels in the frontal cortex. However, FPS administration had no effect on the contents of 5-HT, NE and DA, indicating FPS may act through molecular mechanisms distinct from conventional ADs. It is important to conduct additional tests to further identify the molecular and cellular mechanisms underlying the effect of FPS. It has been shown that some neuroprotective polysaccharides such as heparin derivatives are able to pass through the blood-brain barrier (BBB) (Leveugle *et al.* 1998; Ma *et al.* 2002). However, whether FPS is also able to pass through the BBB is beyond the scope of our study, but this certainly deserves further investigation.

In summary, polysaccharides play multiple roles, have wide-ranging biological effects and may reveal positive therapeutic indexes. Our results show FPS, a new water-soluble  $\alpha$ -D-glucan, produces antidepressant-like and neurogenic effects, suggesting that polysaccharides may represent a source of novel compounds endowed with potential AD activity.

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### Statement of Interest

None.

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