Selective deletion of leptin receptors in adult hippocampus induces depression-related behaviours

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Abstract

Previous studies have demonstrated that leptin and its receptors (LepRb) in the central nervous system play an important role in regulating depression- and anxiety-related behaviours. However, the physiological functions of LepRb in specific brain regions for mediating different emotional behaviours remain to be defined. In this study, we examined the behavioural effects of LepRb ablation in the adult hippocampus using a series of behavioural paradigms for assessing depression- and anxiety-related behaviours. Targeted deletion of LepRb was achieved using the Cre/loxP site-specific recombination system through bilateral stereotaxic delivery of an adeno-associated virus expressing Cre-recombinase (AAV-Cre) into the dentate gyrus of adult mice homozygous for a floxed leptin receptor allele. AAV-Cre-mediated deletion of the floxed region of LepRb was detected 2 wk after injection. In accordance with this, leptin-stimulated phosphorylation of Akt was attenuated in the hippocampus of AAV-Cre injected mice. Mice injected with AAV-Cre displayed normal locomotor activity and anxiety-like behaviour, as determined in the elevated plus-maze, light–dark box and open field tests, but showed increased depression-like behaviours in the tail suspension, saccharin preference and learned helplessness tests. Taken together, these data suggest that deletion of LepRb in the adult hippocampus is sufficient to induce depression-like behaviours. Our results support the view that leptin signalling in the hippocampus may be essential for positive mood states and active coping to stress.

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Introduction

Leptin is a hormone synthesized by adipose tissue. Once released into circulation, leptin can be transported across the blood–brain barrier and bind to receptors on neurons in various brain areas to exert its biological functions. Several lines of evidence have suggested that leptin plays a role in depression-and anxiety-related behaviours. We have previously shown that administration of leptin produces antidepressant-like effects in rats and mice (Garza et al. 2011; Liu et al. 2010; Lu et al. 2006). The antidepressant-like properties of leptin have recently been confirmed independently by other investigators (Yamada et al. 2011). Also, we found that leptin stimulates adult hippocampal neurogenesis, a feature shared by antidepressants (Czeh & Lucassen, 2007; Jacobs, 2002; Malberg et al. 2000; Santarelli et al. 2003), under basal and chronic stress conditions, accompanied by mood improvement (Garza et al. 2008a). Moreover, we have demonstrated that acute leptin administration has anxiolytic effects (Liu et al. 2010). By contrast, leptin-deficient and leptin-receptor deficient mice display anxiogenic behaviour (Finger et al. 2010).

Among six isoforms of the leptin receptor (LepRa–f) that have been identified, the LepRb isoform is principally responsible for signal transduction events stimulated by leptin, leading to biological actions (Chua et al. 1996; Lee et al. 1996). The physiological...
roles of endogenous leptin receptor signalling in depression- and anxiety-related behaviours have been investigated in previous studies using conventional LepR knockout (Dinel et al. 2011; Sharma et al. 2010) or conditional LepRb knockout (Guo et al. 2012; Liu et al. 2011) mice. While selective deletion of LepRb in midbrain dopaminergic neurons causes increased firing of dopamine neurons and an anxiogenic phenotype (Liu et al. 2011), loss of LepRb specifically in forebrain glutamatergic neurons in the dorsal cerebral cortex and hippocampus induces depressive-like behaviours (Guo et al. 2012). These studies support the view that LepRb signalling in specific neuronal populations plays essential but distinct roles in regulating depression- and anxiety-related behaviours.

The hippocampus has been implicated in the pathophysiology of depression and anxiety disorders. Within the hippocampus, LepRb mRNA is primarily expressed in the dentate gyrus, with a lower level in CA3 (Guo et al. 2012; Scott et al. 2009). Although it has been previously shown that intra-hippocampal microinjection of leptin produces antidepressant-like effects (Lu et al. 2006), the physiological roles of hippocampal LepRb signalling in depression- and anxiety-related behaviours remain to be determined. In this study, the Cre-loxP system and adeno-associated viral (AAV) vectors were used to achieve site-specific deletion of LepRb in the hippocampus. AAV expressing Cre-recombinase was delivered to the dentate gyrus of adult LepRbfl/o mice. The effects of AAV-Cre mediated deletion of LepRb in adult hippocampus on depression- and anxiety-like behaviours were examined using a battery of behavioural tests.

Materials and method

Animals

Adult male LepRbfl/o mice (McMinn et al. 2004) were housed in groups of four or five at 22°C on a 14 h light/10 h dark cycle (lights on 07:00 hours) with ad libitum access to food and water. Mice were genotyped using PCR-based genotyping with the following primers: 5′-ATGCTATCGACAGCAGACATGA-3′ and 5′-CAGCGTTGACAGCAGACAAACAC-3′. The presence of the LoxP sites was verified by digesting the PCR products with Hind III. All animal procedures were conducted in accordance with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

Generation of viral vectors

The AAV-Cre-green fluorescent protein (GFP) vector was constructed by generating an expression cassette consisting of the Cre coding sequence with a 5′ nuclear localization signal preceded by the cytomegalovirus (CMV) promoter. Downstream of the Cre coding sequence, an internal ribosomal entry sequence (IRES) was used to express GFP followed by a 3′ simian virus 40 polyadenylation signal [SV40 poly(A)]. The construct was inserted into an AAV2 vector. The control AAV-GFP vector was constructed similarly by cloning the GFP coding sequence with the SV40 poly(A) signal under the control of the CMV promoter into the AAV2 vector. As previously described (Garza et al. 2008b), the viral vectors were generated by co-transfection of AAV vector plasmid and pDC2 helper plasmid (provided by Mark Kay, Stanford University) into HEK-293 cells by calcium phosphate transfection. The pDC2 helper plasmid was designed to include the AAV2 rep and cap genes as well as adenoviral helper functions necessary for AAV packaging. Viral vectors were purified by three cycles of cesium chloride density gradient centrifugation and concentrated using a nanosep centrifugation column (Pall Life Sciences, USA). The AAV virion titer was determined by infecting HEK-293 cells with serial dilutions of the purified virus and counting the number of GFP-positive cells.

Stereotoxic surgery

Mice were anaesthetized with an i.m. injection of a cocktail containing 60 mg/ml ketamine, 1 mg/ml acepromazine and 8 mg/ml xylazine (0.1 ml/kg i.m.) and mounted onto a stereotoxic frame. AAV-Cre-GFP vectors and control AAV-GFP vectors (with titers of 1 × 10^13 infectious units/ml) were injected bilaterally into the dentate gyrus of hippocampus (coordinates relative to bregma: anterior–posterior = −2.1 mm; medial–lateral = ±1.5 mm; dorsal–ventral = −2.1 mm) according to the mouse brain atlas (Paxinos et al. 2001). A volume of 1.0 µl AAV vectors was delivered into the dentate gyrus bilaterally with a slow injection rate (1.0 µl/10 min) through a 33-gauge stainless steel microinjor attached to a digital stereotoxic arm and connected to an infusion pump. After injection was completed, the injector was left in place for an additional 5 min to minimize backflow while withdrawing the injector. All of the behavioural experiments were conducted 14 d after AAV vector injection to allow sufficient time for viral vector transduction and Cre expression. For verification of injection sites, brains were sectioned at a thickness of 20 µm.

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using a cryostat and the presence of GFP was inspected with a fluorescent microscope.

**RT–PCR**

Tissue was dissected from the hippocampus and hypothalamus of Lepr{\textsuperscript{flox/flox}} mice and total RNA was extracted using Trizol reagent (Invitrogen, USA). SuperScript{\textsuperscript{TM}} II reverse transcriptase (Invitrogen) was used to generate cDNA using the oligo(dT), as the template primer. The resulting cDNA was used for PCR amplification of LepR exon 17 or β-actin with Accuprime {\textsuperscript{TM}} Pfx Supermix (Invitrogen). The PCR conditions consisted of an initial denaturing stage at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min followed by a final incubation at 72 °C for 10 min. The primer sequences used to amplify each product are as follows: LepR forward: 5′-GGGACGATGTTCCAAACCCCA-3′ and reverse: 5′-AGGCTCCAGAAGAAGAGGACC-3′; β-actin, forward: 5′-AGCCATGTACGTAGCCATCC-3′ and reverse: 5′-TGTTGGTGGAAGCTGTAGC-3′. The PCR products were analysed on a 1% agarose gel stained with ethidium bromide.

**Western blot analysis**

Leptin-induced phosphorylation of Akt was determined by Western blot in the hippocampus of adult Lepr{\textsuperscript{floxflo}} mice 14 d after intra-dentate gyrus injection of AAV-Cre-GFP and AAV-GFP vectors. Animals were injected i.c.v. with 2 μg leptin in 1 μl artificial cerebrospinal fluid. The hippocampus was dissected out on ice 10 min after i.c.v. injection and immediately placed in liquid nitrogen and stored at −80 °C until further processing. The brain tissue samples were homogenized in lysis buffer (50 mM Hepes, pH 7.6, 1% Triton X-100, 150 mM NaCl, 20 mM sodium pyrophosphate, 20 mM β-glycerophosphate, 10 mM NaF) containing a mixture of phosphatase inhibitors (leupeptin, aprotinin, sodium orthovanadate, phenylmethylsulfonyl fluoride, Ser/Thr phosphatase inhibitor mixture, Tyr phosphatase inhibitor mixture). Total protein was extracted and the concentration was determined using the Bradford assay. A total amount of 40 μg protein was separated on an SDS-PAGE gel, transferred to a nitrocellulose membrane, blocked in a solution of 1% dried milk and 0.1% Tween 20 in 1× Tris-buffered saline and subsequently incubated in primary antibodies diluted in a solution of 1% bovine serum albumin and 0.1% Tween 20 in 1× Tris-buffered saline (anti-Akt, 1:1000; anti-phosphorylated Akt-Thr{\textsuperscript{308}}, 1:1000) overnight at 4 °C. Next, the membrane was washed and incubated in secondary antibody conjugated to horseradish peroxidase (1:10000) in blocking solution for 1 h. Western blot results were visualized using an electrogenerated chemiluminescence reaction and exposed to X-ray film.

**Food intake and body weight**

Mice injected with AAV-Cre-GFP or AAV-GFP were weighed on the day of surgery and at days 14, 21 and 46 after surgery. Food intake was measured for 5 consecutive days beginning 21 d after AAV injection.

**Behavioural procedures**

All behavioural tests were performed during the late light phase, except saccharin preference, which was measured every 24 h. On the test day, animals were individually housed in a new cage with some home cage bedding to avoid the stressful effect of sequential removal of the mice from the cage. Animals were transferred to the testing room and habituated to the room conditions for 3–4 h prior to the beginning of the experiments. After each individual test session, the apparatus was thoroughly cleaned with 20% alcohol to eliminate the odour and trace of the previously tested animal. We used three separate cohorts of male Lepr{\textsuperscript{floxflo}} mice for behavioural tests. One cohort of mice was sequentially subjected to the tail suspension test, forced swim test and learned helplessness test. A second cohort of mice was sequentially tested in the open field, elevated plus-maze, light/dark box and learned helplessness tests. The behavioural tests were spaced by 5–7 d. The learned helplessness data were pooled from two cohorts of mice. A third cohort of mice was used only for saccharin preference. All behaviours were scored by experimenters who were blind to the treatment of the animals.

**Locomotor activity**

On the testing day, the home cage lid was removed and replaced with clear Plexiglas{\textsuperset{R}} over the top of the cage to allow the animal’s activity to be recorded. The activity of the mice was recorded for 30 min and the Noldus EthoVision 3.0 system (Noldus Information Technology, The Netherlands) was used to determine distance travelled for the 30-min test in 2-min bins.

**Tail suspension test**

The apparatus consisted of a box (30 × 30 × 30 cm). The front of the box was open and a bar was placed
horizontally 1 cm from the top with an attached vertical bar hanging down in the centre. Mice were individually suspended by the tail to the vertical bar with adhesive tape affixed 2 cm from the tip of the tail. A camera positioned in front of the box was used to record the animals’ behaviour for a 6-min test session. Immobility in this test was defined as the absence of any limb or body movements, except those caused by respiration.

**Forced swim test**

Mice were placed in a clear Plexiglas cylinder (25 cm high; 10 cm in diameter) filled to a depth of 15 cm with 24 °C water. A camera positioned directly above the cylinder recorded the 6-min swim session. For each test session, the first 2 min served as a habituation period. The immobility of the mice was measured during the last 4 min of the test. Immobility in this test was defined as the absence of any movement except for that required for keeping the animal’s head above water.

**Saccharin preference**

Mice were habituated to drinking from two bottles of water for 1 wk prior to testing. To measure the preference for saccharin solution, the animals were singly housed and given access to one bottle of water and one bottle of 0.01% saccharin for 4 consecutive days. Water and saccharin intake was measured daily and the position of the two bottles was switched every day to avoid any side preference. Saccharin preference was calculated as the volume of saccharin intake over the total volume of fluid intake.

**Learned helplessness test**

The learned helplessness test was performed in a shuttle cage divided equally into two chambers with an auto-controlled guillotine door between the two chambers. Learned helplessness was induced in mice by administering 200 scrambled, inescapable foot shocks (0.3 mA shock amplitude, 2-s duration, 16-s average interval) over a 1 h session. Control animals were placed in the apparatus for the same period of time but did not receive foot shocks. Escape performance was tested 24 h later in the same shuttle cage. Each mouse was given 30 shuttle escape trials with 25 s maximum duration and 30-s intervals. On the first five trials, a sound cue and the shock took place at the same time as the door to the safe compartment opened. For the remaining trials, the door opened 2 s after the shock was delivered. Each trial was terminated when the mouse crossed into the non-shock compartment. The latency to escape and the number of escape failures were recorded automatically by Graphic State software (Coulbourn Instruments Inc., USA).

**Elevated plus-maze**

The elevated plus-maze was constructed with white acrylic and had four arms (30 cm long and 5 cm wide) arranged in the shape of a ‘plus’ sign and elevated to a height of 70 cm from the floor. Two arms had no side or end walls (open arms). The other two arms had side and end walls (12 cm high) but were open on top (closed arms). The open and closed arms intersected, having a central 5 × 5 cm square platform giving access to all arms. The mice were placed in the central square facing the corner between a closed arm and an open arm and allowed to explore the elevated plus-maze for 5 min. Their activity on the elevated plus-maze was recorded. The time spent on the open and closed arms and the numbers of entries made into each arm were measured. Entry was defined as all four paws being positioned within one arm. The degree of anxiety was assessed by calculating the percentage of open arm entries (entries into the open arms/total entries into all arms) and percentage of open arm time (time spent in the open arms/total time spent in all arms).

**Open-field test**

The apparatus consisted of a 60 × 60 cm open arena with 40 cm high walls. The entire test arena was adjusted to even illumination. Mice were placed in the centre of the arena and their activity was recorded for 5 min. For analysis, a 3 × 3 grid was placed over the video and the centre square was defined as the central zone, in which the animal’s activity is usually regarded as a measure of anxiety. The distance mice travelled in the central zone over total distance travelled in the open arena was also quantified using the Noldus EthoVision 3.0 system. The total distance travelled was used as a measure of overall motor activity.

**Light–dark test**

The apparatus consisted of two equally sized compartments (17.8 × 17.8 × 30.5 cm) divided by a wall with an open door between the two compartments. One compartment (light compartment) was illuminated to a light intensity of 700 lx while the other compartment (dark compartment) was black-walled.
For each test, the mouse was placed in the centre of the light compartment facing away from the opening and behaviour was recorded for 5 min. The number of transitions between the two compartments and time spent in the light compartment were measured.

**Statistical analysis**

Results are expressed as means ± S.E.M. Statistical analyses were performed using one-way ANOVA with repeated measures on body weight gain and locomotor activity, two-way ANOVA on Western blot assays, escape latencies and number of failures to escape in the learned helplessness test. Bonferroni’s/Dunn’s or Tukey/Kramer (for unequal n) post-hoc comparisons followed ANOVAs. Two-tailed Student’s t test was used for statistical analysis of the rest of the experimental results. p < 0.05 was considered statistically significant.

**Results**

**Targeted deletion of LepRb in the hippocampus**

Targeted deletion of LepRb in a region-specific manner was achieved using AAV-mediated Cre recombine expression in Lepr-floxed (Lepr^{floxed/foxed}) mice, in which exon 17, a critical exon involved in LepRb signalling, is floxed (McMinn et al. 2004). In the AAV-Cre-GFP vector, GFP was linked to the CMV promoter-driven Cre via an IRES (Fig. 1a), allowing them to be expressed simultaneously and used for identification of injection sites (Fig. 1b). Adult male Lepr^{floxed/foxed} mice were stereotaxically injected bilaterally with AAV-Cre-GFP into the dentate gyrus where LepRb is predominantly expressed (Guo et al. 2012; Scott et al. 2009). Lepr^{floxed/foxed} mice were bilaterally injected with AAV-GFP as a control. The injection sites were verified in each animal after completion of the behavioural experiments. The behavioural data from those mice with missed injection were removed from statistical analysis.

To examine the efficiency of AAV-Cre-mediated excision of the floxed exon 17, we used RT-PCR to measure exon 17 mRNA levels in hippocampus at 2 wk after intra-dentate gyrus injection of AAV-Cre-GFP or AAV-GFP. Exon 17 mRNA in the hippocampus was drastically decreased in AAV-Cre-GFP injected mice in comparison to control AAV-GFP injected mice (Fig. 1c). Akt phosphorylation, a downstream target of LepRb, in response to i.c.v. injection of leptin, was determined to confirm the functional loss of LepRb. Western blot demonstrated that leptin stimulated phosphorylation of Akt in the hippocampus of AAV-GFP injected mice, but this effect was greatly attenuated by AAV-Cre-GFP injection (Fig. 1d). Taken together, these observations support the functional loss of LepRb induced by AAV-Cre-GFP.

**Food intake and body weight**

To determine whether deletion of LepRb in the adult hippocampus affects energy homeostasis, body weight was measured on days 1, 14, 21 and 46 after stereotaxic delivery of AAV-Cre-GFP or AAV-GFP into the dentate gyrus. ANOVA with repeated measures indicated that treatment had no significant effect on body weight (F_{1,68} = 1.084, p > 0.1). There was no difference in body weight between AAV-Cre-GFP and AAV-GFP groups at any of the time-points measured. Three weeks after intra-dentate gyrus AAV injection, food intake was measured for 5 consecutive days. No difference in food intake was found between AAV-Cre-GFP and AAV-GFP treated mice (Fig. 2).

**Locomotor activity**

Locomotor activity was examined in AAV-Cre-GFP and AAV-GFP mice for 30 min and the distance travelled in 2-min bins was measured for the duration of the test. ANOVA with repeated measures revealed no significant effect of treatment on locomotor activity (F_{1,186} = 3.96, p > 0.05 for the time-course of the distance travelled every 2 min; t_{18} = 2.016, p > 0.05 for the total distance travelled within 30 min; Fig. 3).

**Anxiety-related behaviours**

To assess whether deletion of LepRb in the dentate gyrus affects anxiety-related behaviour, three behavioural tests were performed, i.e. the elevated plus-maze, open field test and light/dark choice. In the elevated plus-maze, a reduction in time spent in the open arms and number of entries made into the open arms within the 5 min test are interpreted as indices of anxiety. AAV-Cre-GFP and AAV-GFP mice showed similar time spent in the open arms (t_{17} = 1.146, p > 0.1) and number of entries into the open arms (t_{17} = 0.209, p > 0.5; Fig. 4a). In the light/dark choice test, there was no difference in the amount of time spent in the light box (t_{17} = 0.494, p > 0.5) or the total number of transitions between the light and dark boxes (t_{17} = 1.557, p > 0.1; Fig. 4b). In an open field arena, a decrease in time spent in the central area of the arena is considered as an index of anxiety. Mice that received an intra-dentate gyrus injection of AAV-Cre-GFP or AAV-GFP
spent a similar amount of time in the central zone ($t_{18} = 0.011, p > 0.5$) and travelled similar total distances within the arena ($t_{18} = 1.404, p > 0.1$; Fig. 4c). These data indicate that anxiety-like behaviours are not affected by loss of LepRb in the dentate gyrus.

**Depression-related behaviours**

To determine whether loss of LepRb in the dentate gyrus affects depression-related behaviours, four behavioural tests were performed. The tail suspension...
test and forced swim test have been widely used to assess ‘despair behaviour’ (Porsolt et al. 1977; Steru et al. 1985). Immobility in these two tests is referred to as ‘despair’ and a variety of antidepressants, as well as leptin, reduce this behaviour (Cryan et al. 2005; Garza et al. 2011; Liu et al. 2010; Lu et al. 2006; Petit-Demouliere et al. 2005). Mice with an intra-dentate gyrus injection of AAV-Cre-GFP or AAV-GFP vectors into the dentate gyrus were first evaluated in the tail suspension test. The immobility time was significantly increased in AAV-Cre-GFP injected mice compared to those injected with control AAV-GFP (\( t_{20} = 4.349, p < 0.01 \); Fig. 5a). In the forced swim test, the immobility time in AAV-Cre-GFP injected mice showed a tendency to increase but this did not reach statistical significance (\( t_{18} = 0.941, p > 0.1 \); Fig. 5b).

Anhedonia, a core symptom of human depression, was assessed using the saccharin preference test. Mice were habituated to two drinking bottles in their home cages and then allowed access to a choice of water or a saccharin (0.1%) solution for 4 d. The preference for the saccharin solution was significantly lower in AAV-Cre-GFP injected mice compared to control AAV-GFP injected mice (\( t_{20} = 2.706, p < 0.05 \); Fig. 5c).

Depression-like behaviour was also evaluated using the learned helplessness paradigm, which resembles the passive, withdrawn behaviour of human depression. In this test, AAV-Cre-GFP injected mice and AAV-GFP injected mice were exposed to inescapable foot shock and subsequently tested for a deficit in avoidance-escape performance. ANOVA revealed a significant treatment × shock interaction for escape latencies (\( F_{1,185} = 4.812, p < 0.05 \)) and number of failures to escape (\( F_{1,27} = 6.593, p < 0.01 \)). Mice injected with AAV-Cre-GFP showed increased escape latencies and number of failures to escape in comparison to AAV-GFP-treated control mice (Fig. 5d).
Discussion

In this study, we demonstrate that AAV-Cre mediated deletion of LepRb in the dentate gyrus results in depressive-like behaviours without significantly affecting locomotor activity and anxiety-related behaviours. These results suggest that disruption of LepRb in the dentate gyrus is sufficient to induce depressive-like symptoms.

The hippocampus has been implicated in both depression- and anxiety-related behaviours (Bannerman et al. 2004; Campbell & Macqueen, 2004; Engin & Treit, 2007; Soares & Mann, 1997). Our recent studies have shown that selective deletion of LepRb in forebrain glutamatergic neurons, specifically localized in the hippocampus and cerebral cortex, causes depression-like behaviours in a battery of behavioural tests (Guo et al. 2012). Infusion of exogenous leptin into the hippocampus targeting the dentate gyrus elicits antidepressant-like effects in the forced swim test (Lu et al. 2006). The results from the present study provide evidence that LepRb signalling in the dentate gyrus is physiologically relevant to mood regulation. Both the tail suspension test and forced swim test were used to assess behavioural ‘despair’ as measured by immobility time. Mice with deletion of LepRb in the dentate gyrus showed significantly increased immobility in the tail suspension test but not in the forced swim test. This could be due to different sensitivity of the mouse strain to these two tests (Liu & Gershenfeld, 2001; Lucki et al. 2001). One possible confounding factor in the ‘behavioural despair’ tests is altered locomotor activity. Mice that received intra-dentate gyrus injection of AAV-Cre-GFP showed no change in locomotor activity, suggesting that increased ‘behavioural despair’ in these mice is unlikely to be a result of general hypolocomotion. The ‘behavioural despair’ tests are commonly used to evaluate antidepressant efficacy. We further examined depression-like behaviour using sweet solution preference as a measure of anhedonia-like behaviour (Liu et al. 2011; Snaith, 1993; Willner et al. 1992). AAV-Cre mediated deletion of LepRb in the adult dentate gyrus resulted in a significant reduction of saccharin preference. Furthermore, we tested mice in the learned helplessness test, a paradigm that has been used as an animal model of depression (Seligman & Beagley, 1975; Seligman & Maier, 1967). Mice were exposed to inescapable shock stress and subsequently tested in an avoidance–escape task. AAV-Cre mediated deletion of LepRb in the adult dentate gyrus caused increased learned helplessness as indicated by increased escape latency and increased number of failures to escape. These behavioural deficits suggest that disruption of LepRb function in the adult hippocampus is sufficient to induce depressive-like behaviours under basal and stressed conditions. It was noticed that the depressive-like behaviours in conditional LepRb knockout mice induced by transgenic Cre targeting hippocampal and
cortical neurons appear more severe than those observed in mice injected with AAV-Cre-GFP in the adult dentate gyrus (Guo et al. 2012). This could be explained by incomplete deletion of LepRb in the adult hippocampus and possible involvement of LepRb in the prefrontal cortex.

The hippocampus is also a brain structure involved in the modulation of anxiety-related behaviours (Gray, 1982). Anxiety-like behaviours were assessed in three tests, i.e. the elevated plus-maze, light/dark box and open field. However, the performance on these behavioural tests was not significantly different between mice injected with AAV-Cre-GFP and control mice injected with AAV-GFP, suggesting that hippocampal LepRb may not participate in mediating anxiety behaviours. These results are in consistency with the findings in mice with selective ablation of LepRb in hippocampal and cortical glutamatergic neurons (Guo et al. 2012). A recent study reported that administration of leptin into the hippocampus reduces food intake and blocks the expression of a conditioned place preference for food (Kanoski et al. 2011).

However, we found no significant change in daily food intake and body weight gain in mice that received an intra-dentate gyrus injection of AAV-Cre-GFP compared to mice that received a control AAV-GFP injection. Similar findings were made in conditional LepRb knockout mice lacking LepRb in hippocampal and cortical neurons (Guo et al. 2012). The discrepancies between our studies using LepRb knockdown/knockout models and the studies by Kanoski et al. with intra-hippocampal infusion of leptin may reflect the differences between the effects of psychological vs. pharmacological treatments.

In addition, targeted deletion of LepRb in the hippocampus occurs in local neurons, whereas infusion of exogenous leptin into the hippocampus could activate LepRb located on both local neurons and presynaptic terminals originating from other brain regions.

Leptin stimulates multiple intracellular signalling pathways via LepRb, including the PI3-kinase-Akt, the signal transducer and activator of transcription-3 and the ERK signalling pathways (Ahima & Osei, 2004;...
Bjorbaek et al. 2001; Morris & Rui, 2009; Munzberg & Myers, 2005; Niswender et al. 2001; Xu et al. 2005; Zhang et al. 2004). In this study, we demonstrated that leptin-stimulated phosphorylation of Akt was attenuated in the hippocampus of mice with AAV-Cre mediated deletion of LepRb. Akt is an important negative regulator of glyceron synthase kinase-3β (GSK-3β; Cohen & Frame, 2001; Cross et al. 1995). Recent studies have reported alterations in Akt and its downstream target, GSK-3β, in depression and suicide (Dwivedi et al. 2010; Karege et al. 2007; Pandey et al. 2010; Yoon & Kim, 2010). A detailed analysis of the intracellular signalling pathways responsible for leptin action on depressive-like behaviours will be investigated in future studies.

In summary, we provide evidence that LepRb in adult hippocampus plays an important role in depression-related behaviours. Our results suggest that normal hippocampal LepRb activity may be required to maintain positive mood states and imply that dysfunction of hippocampal LepRb signalling may contribute to the pathogenesis or pathophysiology of depression.

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Statement of Interest

None.

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