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Reversal of a treatment-resistant, depression-related brain state with the Kv7 channel opener retigabine

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Abstract

Neuroinflammation is associated with increased vulnerability to diverse psychiatric conditions, including treatment-resistant major depressive disorder (MDD). Here we assessed whether high fat diet (HFD) induced neuroinflammation may be suitable to model a treatment-resistant depressive-like brain state in mice. Male and female mice were fed a HFD for 18 weeks, followed by quantitation of glucose tolerance, inflammatory markers of brain tissue (TNF α , IL-6, IL-1 β , Iba-1), neural excitability in the prelimbic cortex (PLC), as well as assessment of emotional reactivity and hedonic behavior in a battery of behavioral tests. In addition, we assessed the behavioral responsiveness of mice to fluoxetine, desipramine, ketamine, and the Kv7 channel opener and anticonvulsant retigabine. HFD exposure lead to glucose intolerance and neuroinflammation in male mice, with similar but non-significant trends in females.

Neuroinflammation of males was associated with anxious-depressive-like behavior and defects in working memory, along with neural hyperexcitability and increased I_h currents of pyramidal cells in the PLC. The behavioral changes were largely resistant to chronic treatment with fluoxetine and desipramine, as well as ketamine. By contrast, retigabine (also known as ezogabine) normalized neural excitability and I_h currents recorded from slices of HFD-treated animals and significantly ameliorated most of the behavioral impairments, without effects in control diet exposed animals. Thus, treatment resistant depressive-like brain states that are associated with chronic neuroinflammation may involve hyperexcitability of pyramidal neurons and may be effectively treated by retigabine.

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Keywords

antidepressant; ketamine; neuroinflammation; neuronal hyperexcitability; obesity; high fat diet

INTRODUCTION

Major depressive disorder (MDD) is a common and phenotypically heterogeneous psychiatric syndrome with a lifetime prevalence of 17% worldwide (Kessler et al., 2003) that affects approximately 4.4% of the world's population at any given time (World Health Organization, 2017). About one third of patients are treatment resistant, meaning they fail to respond to two or more currently available antidepressant drug treatments (Fava, 2003; Fava and Davidson, 1996; Mrazek et al., 2014; Thomas et al., 2013). More than half of these patients show rapid and lasting improvements following treatment with ketamine, a rapidly acting experimental treatment that is increasingly available to otherwise treatment-resistant patients (Murrigh et al., 2013a; Wan et al., 2015). However, even ketamine treatment sometimes fails to ameliorate symptoms of depression (Han et al., 2016; Kiraly et al., 2017; Singh et al., 2016).

The biology of MDD remains poorly understood but is noteworthy for its enormous complexity (Thomas et al., 2013). Based on twin studies, approximately 40% of the variation in liability of MDD has been attributed to additive genetic factors (Rice et al., 2002; Viktorin et al., 2016), indicating that most of the vulnerability to MDD is determined environmentally. Among diverse environmental factors, chronic forms of stress have been widely recognized as key contributors (Duman, 2014). Consistent with stress as a causal factor, chronic stress-based animal models reproduce various aspects of the pathology of MDD, including reduced brain volumes of the hippocampus and medial prefrontal cortex (Delgado y Palacios et al., 2011), dysregulated HPA axis function (Checkley, 1996), reduced complexity of neural dendrites (Liu and Aghajanian, 2008; Magarinos et al., 1999), heightened anxiety and anhedonia, and reduced self-care (Surget et al., 2011), as well as limited cognitive deficits (Willner and Belzung, 2015). The detrimental effects of stress can be attenuated and reversed by both conventional monoaminergic antidepressants (Bessa et al., 2009) as well as ketamine (Brachman et al., 2016; Donahue et al., 2014; Hare et al., 2017). However, while these studies confirm that stress plays a key role in MDD, they also suggest that stress by itself does not play a distinctive role that is specific to treatment-resistant depression.

There is mounting evidence that resistance to antidepressant drug treatment is associated with vulnerabilities that differ from stress (Willner et al., 2014). In particular, heightened vulnerability for MDD and treatment resistance may involve a state of heightened neuroinflammation (Alcocer-Gomez et al., 2016; Barnes et al., 2017; Colpo et al., 2018; Furtado and Katzman, 2015; Menard et al., 2017; Miller et al., 2009; Setiawan et al., 2018; Wohleb et al., 2016). MDD is comorbid with pathological conditions that involve chronic inflammation, such as autoimmune diseases of the CNS (Colasanti et al., 2016), neurodegenerative disorders (Dobos et al., 2010; Roos et al., 2016) and obesity (Pereira-Miranda et al., 2017). Obesity-associated adipocytes serve as a major peripheral source of

inflammatory cytokines (Lumeng and Saltiel, 2011) that can promote neuroinflammation (Guillemot-Legrís and Muccioli, 2017; Schachter et al., 2018). Furthermore, pro-inflammatory cytokines are elevated in patients with depressive symptoms (Al-Hakeim et al., 2015; Franklin et al., 2018; Lanquillon et al., 2000; Raison et al., 2006; Shelton et al., 2011), whereas agents that inhibit inflammatory cytokines can ameliorate symptoms of depression (Kappelmann et al., 2018; Raison et al., 2013). Lastly, inflammation can be causal for depression as evidenced by antiviral therapy with interferon- α , which results in depressive symptoms as typical side effects (Conversano et al., 2015; Kraus et al., 2003; Renault et al., 1987). Importantly, depressed patients with signs of chronic inflammation often do not respond to current antidepressants (Lanquillon et al., 2000; Maes et al., 1997; O'Brien et al., 2007; Yoshimura et al., 2009). Moreover, emerging evidence suggests that even the efficacy of ketamine may be compromised by inflammation (Király et al., 2017). Collectively, the data suggest that neuroinflammation plays a key role in treatment resistance to both monoaminergic antidepressants and ketamine.

A causal link between obesity and psychiatric syndromes has been established in model animals, with altered gut microbiota (Hassan et al., 2018; Schachter et al., 2018) and neuroinflammation as likely key mediators (Almeida-Suhett et al., 2017; Arnold et al., 2014; Dutheil et al., 2016; Valdearcos et al., 2017; Zemdegs et al., 2016). Corresponding links have also been described in patients (Buckman et al., 2014; Jacka et al., 2014; Jacka et al., 2010; Schachter et al., 2018; Shelton et al., 2015; Thaler et al., 2012). Moreover, obesity is associated with treatment resistance of depression (Dennehy et al., 2015; Kloiber et al., 2007; Oskooilar et al., 2009; Papakostas et al., 2005; Raison et al., 2013; Rizvi et al., 2014), which is consistent with a role of inflammation.

Neuroinflammation involves increased production and release of microglia and astrocyte-derived pro-inflammatory cytokines such as TNF- α , IL-6, IL-1 β , which ultimately act to increase the ratio of glutamatergic to GABAergic synaptic transmission (Pribiag and Stellwagen, 2013a), which is predicted to increase neural excitability. Conversely, agents that enhance the function of K⁺ channels reduce neuronal excitability. In particular, the Kv7 channel opener and anticonvulsant retigabine (also known as ezogabine) is of interest as it has recently been shown to exhibit antidepressant activity in both patients (Tan et al., 2018) and animals (Friedman et al., 2016).

Towards elucidating possible mechanisms of treatment resistant MDD, we here employed a chronic high fat diet (HFD) induced model of neuroinflammation that results in depressive-like behavior of mice, to assess differential antidepressant drug responses associated with neuroinflammation. HFD exposure of male mice resulted in glucose intolerance, elevated expression of inflammatory cytokines, microglia activation, an anxious-depressive-like behavioral phenotype and working memory deficits, consistent with result by others (Almeida-Suhett et al., 2017; Arnold et al., 2014; Dutheil et al., 2016; Isingrini et al., 2010; Zemdegs et al., 2016). By contrast, HFD exposure of female mice resulted in only marginal inflammation, as also reported by others (Pettersson et al., 2012). This brain state was associated with overt hyperexcitability of pyramidal cells. Moreover, the HFD-induced behavioral changes were largely resistant to treatment with three functionally distinct classes of antidepressants [desipramine (a prototypical norepinephrine uptake inhibitor), fluoxetine

(a prototypical selective serotonin reuptake inhibitor), and ketamine (NMDA receptor channel blocker)], suggesting that HFD-induced alterations in brain physiology are fundamentally distinct from those of stress-induced depressive-like brain states. By contrast, subchronic treatment of mice with retigabine normalized neural excitability and significantly ameliorated HFD-induced changes in most of the behavioral tests. Our data suggest that HFD-induced inflammation leads to increased intrinsic neuronal excitability and a largely treatment-resistant depressive-like brain state that can be ameliorated by Kv7 channel openers such as retigabine.

EXPERIMENTAL PROCEDURES

Animals

All animal experiments were approved by the Institutional Animal Care and Use Committees (IACUC) of the Pennsylvania State University and conducted in accordance with guidelines of the National Institutes of Health (NIH). C57BL/6J mice were purchased from the Jackson Laboratory (Stock #000664, Bar Harbor, ME) and bred in-house. All mice were kept under a 12:12 h light-dark cycle with food and water available *ad libitum*. Starting at five weeks of age, separate groups of mice were fed a high fat diet (HFD) with 59% of calories from fat, 15% from protein and 26% from carbohydrates (F3282, BioServ, NJ) or a matched control diet (CD) with 16% of calories from fat, 21% from protein and 63% from carbohydrates (F4031, BioServ). All animals were kept on special diet for 18 weeks before the start of testing and throughout testing. They were group housed until one week prior to the start of testing.

Drugs

Desipramine (Sigma-Aldrich, St. Louis, MO) and fluoxetine (Dr. Reddy's Laboratories, Princeton, NJ) were administered in drinking water at 608 mg/L (desipramine) and 280 mg/L (fluoxetine), for empirically determined daily doses of 40 mg/kg and 18 mg/kg, respectively (desipramine, 38.96 ± 2.68 mg/kg/day; fluoxetine, 17.42 ± 0.97 mg/kg/day, data not shown) that were within the range of doses that we and others have shown to be effective as chronically administered antidepressants in mice (David et al., 2009; Shen et al., 2010; Yalcin et al., 2007). Drug treatment began in the 19th week of special diet exposure, with behavioral testing starting in the 22nd week. Ketamine (Ketaset®, Zoetis, Parsippany, NJ) was diluted freshly in 0.9% saline and injected at a dose optimized for male mice (6 mg/kg, i.p.) (Nosyreva et al., 2013), 8 h prior to behavioral testing. Retigabine (Alomone Labs, Israel) was administered in 1% DMSO and 0.9% saline and dosed at 1 mg/kg/day (i.p.) for 8 days and continued during subsequent behavioral testing, as described (Friedman et al., 2016).

Glucose Tolerance Test

The test was adapted from (Wang et al., 2014). Mice were singly housed and food deprived for 8 h (*ad lib* access to water). Single blood drops obtained by tail nick were read with a OneTouch® Ultramini® meter (LifeScan, CA) between 0 and 160 min after glucose injection (2 g/kg, in saline, i.p.). Mice on HFD with low body weight (identified as outliers in boxplot) were excluded from analysis.

Behavioral Analyses

All behavioral experiments were performed under red light during the dark cycle, starting 1 h after the lights were shut off. An Open Field Test (OFT) was used to assess locomotor activity in a novel environment. The mice were allowed to explore an arena of $50 \times 50 \times 20$ cm with opaque Plexiglass walls and a transparent floor underlined with white reflective paper, under red light (6 lux) for 10 min. The behavior was analyzed using an Ethovision XT video tracking system (Noldus Information Technologies, Leesburg, VA). The Novelty Suppressed Feeding Test (NSFT) (Fuchs et al., 2017) was used to assess anxiety-like behavior and anxiolytic-like effects of antidepressants. The test assesses an inhibitory response that is reduced by chronic but not acute antidepressant drug treatment (Santarelli et al., 2003; Suranyi-Cadotte et al., 1990). Mice were food deprived for 18 h and placed into a novel opaque Plexiglass arena ($50 \times 50 \times 20$ cm) for 15 min. A single food pellet (corresponding to CD or HFD) was placed on top of a white cotton nesting square ($6 \times 6 \times 5$ cm, Ancare Corp., Bellmore, NY) and the latency to feed was scored manually, with trials stopped after 15 min if no feeding occurred. Home Cage Feeding (HCF) as a measure of appetite was assessed at least one week after the NSFT. The mice were singly housed and familiarized for 48 h in their home cage to a Petri dish that was later used as a food container. They were then food-deprived for 18 h before measurement of the amount of food consumed from the Petri dish over 15 min. The Sucrose Splash Test (SST) (Nollet et al., 2013) was used to assess self-care. The mice were singly housed for at least 24 h and then transferred to an empty cage where they were sprayed on their backs with two sprays of 10% sucrose solution and immediately returned to their original cage. The total duration and frequency of grooming behavior over five min was recorded manually by an experimenter blinded to drug treatment. The Female Urine Sniffing Test (FUST) (Malkesman et al., 2010) was used to assess anhedonia. Grooming behavior in this test is reduced by chronic stress exposure and reversed by antidepressant drug treatment, indicating that it has predictive validity for antidepressant drug efficacy (Nollet et al., 2013). Male mice were singly housed for at least 24 h and then exposed to a sterile cotton-tip applicator (Patterson Veterinary Supply, Saint Paul, MN) for 30 min in their home cage. During the test, the mice were first exposed to a water-soaked cotton-tip for three min. After 45 min they were exposed to a fresh female-urine-soaked cotton-tip for another 3 min. The duration spent sniffing the cotton tip during a 3-min time windows was analyzed using Ethovision XT. The Y-Maze test (Sarnyai et al., 2000) was used to assess a form of working memory that is dependent on functional interactions between the mPFC and ventral hippocampus, two brain regions implicated in cognitive defects of MDD (Griffin, 2015; Xia et al., 2019). The apparatus consisted of three identical arms ($33 \times 9 \times 16$ cm) made of black Plexiglass and joined in the center to produce a Y-shape. The mice were placed randomly into the end of one arm and allowed to explore the maze for 5 min. Subjects that urinated or defecated during the test were excluded from analysis to rule out interference by odor cues (Douglas, 1966). The number and the sequence of arms entered were analyzed for percent alternation [(number of correct alternations/total entries-2) $\times$ 100%]. The Forced Swim Test (FST) was used as a rodent behavioral assay with predictive validity for antidepressant drug activity (Dulawa et al., 2004; Lucki, 1997). The mice were placed into a 5 L glass beaker containing 3 L water equilibrated to 24 ± 1 °C and video recorded for 6 min using Ethovision XT. The total time spent immobile during the last 4 min of each trial was quantitated.

Immunohistochemistry

The mice were deeply anesthetized with Avertin and perfused transcardially with ice-cold phosphate buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde (PFA) in PBS. The brains were postfixed for six hours in the same fixative at 4°C. Floating sections (40 μ m) were sliced with a Compresstome® VF-300-0Z (Precisionary Instruments LLC, Greenville, NC) and stained using rabbit anti-Iba-1 (1:800, 019-19741, Wako, Japan), and guinea pig anti-NeuN (1:2000, ABN90, Millipore, Burlington, MA) and developed using secondary donkey antibodies conjugated to Alexa 488 and Cy3 (1:800, Molecular Probes, Eugene, OR). Confocal images were captured using a Zeiss LSM5 microscope (Carl Zeiss Microscopy, Thornwood, NY) with a 20 $\times$ objective, and quantified using Image J. The percentage of areas of the regions of interest covered by Iba-1 immunofluorescent staining (4–5 sections/animal, 1–2 measuring areas per region of interest and section) was normalized to the average of the control group.

Brain Slice Electrophysiology

Conditions for slice recordings were as described (Crowley et al., 2016; Pleil et al., 2015). The animals were de-identified and the recordings performed with the experimenter blinded to diet and drug treatments. Mice were anesthetized under 5% isoflurane and rapidly decapitated. The brains were transferred to the electrophysiologist who was blinded to diet and the animal's body mass. Brains were placed in ice-cold oxygenated high-sucrose cutting solution (containing the following, in mM: 194 sucrose; 20 NaCl, 4.4 KCl, 2 CaCl₂, 1 MgCl₂, 1.2 NaH₂PO₄, 10 glucose, 26 NaHCO₃). Coronal slices (300 μ m) prepared on a vibratome (Leica Biosystems, Buffalo Grove, IL, USA) were transferred to oxygenated artificial cerebrospinal fluid (aCSF, in mM: 124 NaCl, 4.4 KCl, 2 CaCl₂, 1.2 MgSO₄, 1 NaPO₄, 10 glucose, 26 NaHCO₃), 31°C. For measurements of intrinsic excitability, cells were current-clamped first at the resting membrane potential (RMP) and then –70 mV using a potassium-gluconate based intracellular solution (in mM: 135 K-Gluc, 5 NaCl, 2 MgCl₂, 10 HEPES, 0.6 EGTA, 4 Na₂ATP, and 0.4 Na₂GTP). The RMP, rheobase (amount of current injected to produce an action potential), membrane potential at first action potential (AP), and APs fired during a voltage $\times$ current plot protocol were assessed at both RMP and –70 mV. For retigabine experiments, intrinsic excitability experiments were conducted in normal aCSF. Retigabine (100 μ M in aCSF) was bath applied for 15 min, and intrinsic excitability experiments were repeated in the same neurons. I_h currents were elicited using 10 mV steps (–70 mV to –120 mV) as described (Bonin et al., 2013; Cao et al., 2010; Friedman et al., 2014). I_h Data was digitized (10 kHz) and filtered (3 kHz) using a Multiclamp 700B amplifier and analyzed using Clampfit 10.3 software.

Quantitative Real-Time PCR

The animals were euthanized by cervical dislocation and the brains rapidly cut into 1 mm slices using a brain matrix (Stoelting, Wood Dale, IL). The ACC/PLC and hippocampi were dissected from appropriate slices using a scalpel and total RNA was extracted using a GenElute™ Mammalian Total RNA Miniprep Kit (RTN70, Sigma, St. Louis, MO). Quality and concentrations of RNA samples were measured by Nanodrop™ 2000c (Thermo Fisher, MA). Only samples with OD260/280 readings of 1.8–2.0 were used for reverse transcription

(qScript™ cDNA supermix, Quanta Biosciences, Gaithersburg, MD). Specific primers for IL-1 β , IL-6, TNF α and β -actin were designed with Primer 2 Software (Applied Biosystems) (IL-1 β : F-5'-TTGAAGTTGACGGACCCCAA-3', R-5'-TGTTGATGTGCTGC TGC GA-3'; IL-6: F-5'-TTCCATCCAGTTGCCTTCTTG-3', R-5'-CATTCCACGATTCC CAGAG-3'; TNF α : F-5'-CACAAACCACCAAGTGGAGGA-3', R-5'-ACAAGGTACAACC CATCGGCT-3'; β -actin: F-5'-GCCCTGAGGCTCTTTTCC-3', R-5'-TGCCACAGGATTC CATA CCC-3'). Real-time PCR reactions were run on StepOne Plus™ (Applied Biosystems) using PerfeCTa SYBR® Green Supermix (Quanta Biosciences). Quantification was performed using the comparative threshold cycle (Ct) method and β -actin as standard (Schmittgen and Livak, 2008). All samples were run in triplicates.

Statistics

Statistical analyses were all based on biological replicates and performed using Prism 7.0 software (Graphpad, La Jolla, CA). Data that failed the normality test (D'Agostino & Pearson) were Box-Cox transformed or analyzed by Mann Whitney or Kruskal-Wallis followed by Dunn's test as indicated in the Figure legends. Multiple group means of normally distributed data were compared by two-way analysis of variance (ANOVA) followed by Fisher's LSD.

Outliers were identified using the boxplot function of Minitab 18 (Minitab Inc., State College, PA) and strictly excluded from statistical analyses. Nine outliers were identified and removed from nine group means. The only data set affected by this procedure was the diet effect in Fig. 4F ($F_{(1,31)} = 5.53$, $p = 0.025$), which was significant only after elimination of an outlier.

RESULTS

Chronic treatment of HFD of male mice results in glucose intolerance and neuroinflammation.

Towards establishing an obesity-induced model of MDD we exposed C57BL/6J male and female mice to a HFD starting at 5-weeks of age. Control mice were fed a matched control diet (CD) in parallel. The HFD-exposed male mice rapidly gained weight and after three weeks on HFD were significantly heavier than CD controls (Figure 1A) (for statistics of data illustrated in Figures please see Figure legends). By 18 weeks of exposure, the HFD-exposed male mice were almost twice the weight of controls. Obesity of HFD-exposed mice was paralleled by development of glucose intolerance as evidenced by the increased area under curve of the glucose tolerance test (Figure 1B,C) and the increased blood glucose level measured 8 h after food deprivation (Figure 1D). Quantitation of brain mRNAs encoding proinflammatory cytokines (TNF α , IL-6 and IL-1 β) by real-time PCR revealed an overall increase in inflammation in both hippocampus ($F_{(1,49)} = 17.36$, $p < 0.001$, ANOVA) and ACC/PLC ($F_{(1,62)} = 4.296$, $p < 0.05$) of HFD-exposed animals. Posthoc tests for individual cytokines revealed significantly increased expression of TNF α and IL-6 mRNAs in the hippocampus (Figure 1E), as well as TNF α mRNA in ACC/PLC (Figure 1F) ($p < 0.05$, Fisher's LSD, for all three effects). In ACC/PLC, neuroinflammation of HFD-exposed mice was further corroborated by an increased percentage of the area covered by immunostaining

for the microglia marker Iba-1 (Figure 1H), an established index of microglia activation (Wohleb et al., 2011). However, no such effect was evident in the hippocampus (Figure 1G). Attempts to confirm changes in cytokine expression by ELISA were not successful because the proteins were expressed below reliable quantitation (not shown).

HFD exposure of female mice led to weight gain similar to HFD exposure of males (Figure 2A) but with only marginally impaired glucose tolerance and unaltered fasting glucose (Figure 2B–D). Similarly, there was at best a trend for an increase of proinflammatory cytokines in ACC/PLC (ANOVA, diet effect, $F_{(1, 38)} = 3.554$, $p = 0.07$) but not in hippocampus ($F_{(1, 28)} = 1.454$, $p = 0.24$) (Figure 2E, F). Therefore, our further analyses were limited to male mice (see also Discussion).

HFD-exposed mice exhibit an anxious-depressive-like phenotype and cognitive impairment that is largely resistant to chronic desipramine and fluoxetine treatment.

To assess the behavioral consequences of chronic HFD-exposure, we subjected 18-week-HFD and CD-exposed mice to tests of locomotion, followed by a battery of tests with predictive validity for anxiolytic and antidepressant drug effects, as well as working memory. In addition, to assess whether HFD-induced behavioral changes can be reversed by antidepressant drug treatments, we treated separate groups of HFD-exposed mice with either the norepinephrine-selective reuptake inhibitor desipramine (40 mg/kg/day) or the selective serotonin reuptake inhibitor fluoxetine (18 mg/kg/day) for at least three weeks, using drug dosages, application routes and treatment durations previously established as effective in reversing anxious-depressive-like phenotypes in mice (David et al., 2009; Shen et al., 2010). In the OFT, HFD-exposed mice showed a significant reduction in the distance traveled compared to CD controls, albeit without drug effects (Figure 3B). This phenotype prompted us to limit further behavioral assessments to measures that are relatively insensitive to changes in locomotion. In the Novelty Suppressed Feeding Test (NSFT), HFD-exposed mice exhibited an increased latency to feed, indicative of heightened anxiety, and this phenotype was significantly ameliorated by both desipramine and fluoxetine treatment (Figure 3C). Caloric intake in a Home Cage Feeding (HCF) test was unaffected by HFD exposure and drug treatments (Figure 3D), confirming that the behavioral changes in the NSFT reflected differences in emotional reactivity rather than appetite. In the Sucrose Splash Test (SST), HFD-exposed animals showed a robust reduction in the time spent grooming (Figure 3E). Grooming behavior in this test is known to be reduced by chronic stress exposure and reversed by antidepressant drug treatment, indicating that it is relevant to anxiety and depression (Nollet et al., 2013). However, the grooming behavior of HFD-exposed mice was insensitive to desipramine and fluoxetine treatment, suggesting resistance to both types of drug treatments. In the Female Urine Sniffing Test (FUST), the total time spent sniffing a female urine-soaked cotton tip was significantly reduced in HFD-exposed mice compared to CD-exposed mice, yet the behavior was unaffected by desipramine and fluoxetine treatment (Figure 3F). Behavior in the FUST is associated with elevated dopamine release in the nucleus accumbens, suggesting that this test assesses pleasure seeking (Mallesman et al., 2010) and that HFD-exposed mice exhibit an antidepressant drug-resistant, anhedonia-like phenotype. Lastly, in a Y-maze test, HFD-exposed mice showed a significant reduction in the percent spontaneous alternations (Figure 3G), indicating a defect in spatial working

memory. Both HFD and CD animals showed alternations of arm visits in this test at a rate significantly above chance level (22.2%, Wolf et al., 2016). However, the cognitive deficit of HFD-exposed mice was again insensitive to desipramine and fluoxetine treatment (Figure 3G). Collectively, the data suggest that HFD treatment of mice results in emotional and cognitive deficits that are largely resistant to norepinephrine and serotonin reuptake inhibitors representative of currently used antidepressants. Anxiolytic-like effects in the NSFT were a notable exception and confirmed that the drugs were dosed appropriately. However, anxiety-like behavior in this test is effectively reversed by classical acutely effective anxiolytics such as diazepam (Bodnoff et al., 1988) that are ineffective as antidepressants, suggesting that responsiveness in this test alone is insufficient to predict antidepressant efficacy.

HFD-exposure-induced behavioral changes are largely resistant to ketamine.

Ketamine is rapidly evolving into a treatment of choice for otherwise treatment-resistant patients and is effective in reversing anxious-depressive-like behavior in stress-based rodent models of emotional disorders. Therefore, we next examined whether ketamine had antidepressant effects in HFD-exposed mice. The anxious-depressive-like phenotype of these mice was again confirmed in the NSFT, SST, FUST and Y-maze (Figure 4C–F), along with reduced locomotion in the OFT (Figure 4B). However, ketamine failed to reverse the behavioral defects of HFD-exposed mice in all of these tests. To verify that the dosage (6 mg/kg) and time-point of testing (8 h post drug injection) were within the effective range of ketamine, we added a Forced Swim Test (FST) (Figure 4G) as an additional test with predictive validity for antidepressant drug effects. HFD mice showed increased immobility in this test, which may indicate a depressive-like phenotype or could simply be due to the increased buoyancy or non-emotional defects in locomotion of obese mice. However, an acute dose of ketamine reduced the time spent immobile of HFD-exposed mice, without affecting control animals, which is consistent with an antidepressant effect. Ketamine did not affect the distance traveled of CD- and HFD-exposed mice in the OFT (Figure 4B), indicating that increased mobility in the FST was not simply due to increased locomotion. Thus, HFD-exposed mice are resistant to ketamine in most tests, with the notable exception of the FST.

Chronic exposure to HFD leads to increased neural excitability and I_h currents in the PLC.

To begin to search for neural correlates of obesity and inflammation-induced changes in behavior of HFD mice, we used slice electrophysiology to assess possible changes in intrinsic properties of neurons. We focused on pyramidal cells in Layer II/III of the PLC as a key brain region implicated in MDD (Drevets et al., 2008) that also plays a role in our model, as evidenced by increased expression of TNF α and activation of microglia (Figure 1F, H), and defects in prefrontal cortex-dependent working memory (Figure 3G, 4F). The resting membrane potential (RMP) (CD, -60.68 ± 3.28 mV, HFD, -59.66 ± 3.33 mV), rheobase at RMP (CD, 75.73 ± 11.08 pA, HFD, 80.24 ± 20.70 pA) and membrane potential at first spike (CD, -45.05 ± 1.63 mV, HFD, -49.50 ± 2.80 mV) were all unaltered in HFD- vs. CD-exposed mice ($p > 0.05$, $n = 10$ – 17 cells for all three experiments, t -tests). However, we observed significantly increased voltage step-induced I_h currents in HFD- vs CD-exposed mice (Figure 5). I_h currents were also increased as a function of voltage

independent of diet. Increased I_h currents are indicative of a pathophysiological adaptation to increased excitatory drive of neurons (Friedman et al., 2014; Wanat et al., 2008). Indeed, the number of action potentials evoked by current injection was significantly increased in pyramidal cells of HFD vs. CD mice, as detailed further below (Figure 7B).

Retigabine reverses anxiety- and depression-related behavioral deficits of HFD-exposed mice, along with normalizing neural excitability and I_h currents.

Neuronal hyperexcitability and elevated I_h currents recorded from pyramidal cells of HFD-exposed mice were reminiscent of corresponding changes in intrinsic properties of dopaminergic neurons in a chronic social defeat-induced model of anhedonia that could be reversed by subchronic treatment with the $K^+(M)$ -channel opener retigabine (Friedman et al., 2014). Therefore, we adopted the protocol of Friedman et al to test whether the detrimental effects of HFD-exposure could be reversed by retigabine, using daily i.p. injection of retigabine or vehicle (1% DMSO in 0.9% saline) for at least eight days (Figure 6A). A first cohort of mice was subjected to behavioral tests 1h after the last drug injection, using one test per day (Figure 6B–G). Consistent with previous tests, HFD-exposed mice tested in an OFT showed hypo-locomotion compared to CD mice, without drug effects (Figure 6B). Importantly, retigabine significantly ameliorated the HFD-induced phenotypes in the NSFT, SST, and Y-maze, although it failed to normalize behavior in the FUST (Figure 6C–G). Caloric consumption assessed in the home cage was again unaffected (Figure 6D).

To further assess the time course and mechanism of retigabine action, we repeated the behavioral analyses of retigabine effects in yet another cohort of mice, but this time analyzed the behavior 12 h after the last drug injection (partial drug off condition). In addition, we added a group of CD-exposed mice that was subject to retigabine treatment as an additional control (Figure 6H–M). Similar to mice assessed at 1 h post drug injection, retigabine-treatment assessed 12 h post injection significantly but more modestly ameliorated the behavioral consequences of HFD-exposure in the NSFT (Figure 6I) and Y-maze (Figure 6M) and totally failed to have an effect in the SST (Figure 6K). and FUST (Figure 6L). In this cohort of mice HFD-exposure uniquely increased caloric consumption in the HCF test (Figure 6J). A correspondingly increased drive to eat would reduce the latency to feed and thus cannot explain the increased latency to feed observed in HFD- vs CD-exposed mice. Importantly, when some of the same animals were reassessed in the SST six weeks later and again subjected to daily retigabine for 8 days and tested 1 h post drug treatment, retigabine again enhanced grooming behavior in this test (Figure 6N). Thus, retigabine was most effective at the 1h timepoint when the drug was on board and started to fade at the 12 h timepoint, when most of the drug had been eliminated ($t_{1/2}$ retigabine in rodents is 2–3½ h (GlaxoSmithKline Australia Pty Ltd, 2013)). The drug effects were selectively observed in HFD-exposed mice and absent in animals maintained on the CD, indicating that retigabine acted specifically to reverse the pathological condition. The consistent failure to reverse behavior in the FUST by all four drug treatments (Figures 3L, 4E, 6F,L) may suggest that this phenotype reflects HFD-induced defects in olfaction rather than anhedonia, as previously reported (Jakupovic et al., 2008).

Retigabine normalizes neural excitability and I_h currents of chronic HFD-exposed mice.

In search of a cellular correlate for the behavioral actions of retigabine, we measured the effects of retigabine on neural excitability and I_h currents. In PLC slices of HFD-exposed mice, bath-applied retigabine effectively reduced the number of current-evoked action potentials recorded from layer II/III pyramidal cells (Figure 7A). By contrast, in slices isolated 24 h after the last of eight daily retigabine injections (drug-off conditions) retigabine had increased rather than reduced the number of current-evoked action potentials (Figure 7B). Thus, retigabine must be on board to affect neuronal excitability. Curiously, under these same conditions the I_h currents remained normalized to levels recorded from CD-exposed mice (Figure 7C). Thus, retigabine acutely reduces the excitability of pyramidal cells of HFD-exposed mice, which leads to lasting normalization of I_h currents, consistent with the idea that elevated I_h currents report a history of chronically elevated neuronal excitability. The data suggest that retigabine alleviates inflammation-induced behavioral alterations by normalizing neuronal excitability independent of its effects on I_h currents.

DISCUSSION

We have shown here that chronic HFD exposure-induced emotional behavior and cognitive alterations of male mice is largely resistant to treatment with the norepinephrine and serotonin selective uptake inhibitors desipramine and fluoxetine, as well as ketamine. We confirmed that HFD-induced behavioral alterations are associated with hyperglycemia, glucose intolerance, and multiple signs of neuroinflammation and then showed that this phenotype involves overtly increased intrinsic excitability and elevated I_h currents of pyramidal cells in the PLC. Moreover, we showed that I_h currents and neuronal hyperexcitability in this model are reversible and the behavioral changes ameliorated by subchronic treatment with the Kv7/KCNQ-channel opener retigabine, an anticonvulsant. Thus, reducing neuronal hyperexcitability is associated with normalization of HFD-induced and inflammation-mediated depressive-like behavioral alterations.

A notable limitation of our study is that HFD-exposed female mice failed to show significant neuroinflammation which precluded testing for links between inflammation and emotional behavior in the female sex. Lower susceptibility to HFD-exposure of female vs. male mice has been reported by others and may reflect sex-specific differences in fat metabolism (Zore et al., 2018) and a lower susceptibility to metabolic syndrome (Pettersson et al., 2012), or inhibitory effects of female-specific sex hormones on neuroinflammation (Au et al., 2016; Clevenger et al., 2018). Curiously, neuroinflammation has also been reported to be more common in male than female depressed patients (Maes et al., 1997), despite MDD being overall much more common in the female gender. Notably, numerous sex differences have also been described for the peripheral and central immune systems, suggesting that sex specific vulnerabilities to mood disorders may be due to differences in the immune system (Rainville and Hodes, 2019).

A second limitation of our study is that the link from neuroinflammation to altered neurophysiology and treatment-resistant behavioral changes is correlational rather than causative because the focus of our study was on a pharmacological and physiological description of this brain state and not on the mechanistic sequelae involved in reaching that

state. However, microglia and inflammation are known to act as necessary mediators of HFD-induced neuroinflammation and behavioral changes (Cope et al., 2018). Furthermore, a causal link between HFD-induced inflammation and altered neurophysiology is consistent with findings by others. For example, the increased intrinsic excitability of pyramidal cells in PLC of HFD-exposed mice can be replicated *in vitro* by acute treatment of neuron-glia co-cultures with lipopolysaccharides, which act by stimulating the release of pro-inflammatory cytokines from microglia and astrocytes (Gao et al., 2014). In addition, TNF α , which was drastically increased in both ACC/PLC and hippocampus of HFD-exposed mice (Figure 1), is known to increase the synaptic excitation-inhibition ratio of cultured neurons, which is consistent with increased excitability (Pribiag and Stellwagen, 2013b; Stellwagen et al., 2005) (see also below). Our data add to the evidence that obesity and neuroinflammation contribute to treatment resistance of MDD and that treatment resistance may be due to vulnerability factors distinct from stress. Nevertheless, neuroinflammation is also a consequence of stress and known to causally contribute to depressive-like phenotypes in stress-based models of depression (reviewed in Franklin et al., 2018; Menard et al., 2017; Weber et al., 2017; Yirmiya et al., 2015). Thus, treatment resistance of MDD may depend on the source, magnitude, chronicity and anatomical distribution of neuroinflammation, rather than simply any type of neuroinflammation.

The SSRI fluoxetine can attenuate chronic stress-induced behavioral effects of animals kept on a standard diet but fails to do so in mice maintained on a HFD (Isingrini et al., 2010). HFD-exposed mice are also resistant to the SSRI escitalopram (Zemdegs et al., 2016). Our data extend these studies to the norepinephrine selective uptake inhibitor desipramine and suggest that both classes of conventional antidepressants are ineffective in reversing the detrimental neural circuit effects of chronic HFD. Notably, while both drugs were overall ineffective, they nevertheless showed anxiolytic-like activity in the NSFT, demonstrating that they were dosed appropriately. We have previously shown anxiolytic but not antidepressant effects of fluoxetine in GABA receptor mutant mouse model of MDD (Shen et al., 2010), suggesting similar neural circuit changes in these mutants and HFD-exposed mice. In addition to conventional antidepressants, we show here that HFD-exposed mice are largely resistant to ketamine, as ketamine reversed HFD-induced behavioral changes in just one of five tests. Emerging evidence indicates that ketamine's efficacy is limited by inflammation in patients as well (Kiraly et al., 2017). Moreover, ketamine is ineffective in reversing cognitive defects associated with MDD, both in patients (Murrough et al., 2013b) and in the HFD mouse model presented here, although ketamine given prophylactically can prevent acute inflammation-induced behavioral consequences (Walker et al., 2013).

Our findings are reminiscent of studies using HFD-exposed rats that showed a behavioral phenotype comparable to the one reported here that was, however, reversible by ketamine (Dutheil et al., 2016). Importantly, in contrast to the fiber-free HFD used here with mice, the rat study relied on a HFD rich in fiber and failed to induce obesity, which may explain why the rats developed only marginal glucose intolerance and inflammation that was limited to hippocampus and absent in prefrontal cortex (Dutheil et al., 2016). By contrast, the C57BL/6J strain of mice used here is uniquely sensitive to HFD-induced obesity and glucose intolerance (Nicholson et al., 2010). Thus, apparent discrepancies between different rodent models may reflect differences between diets used, species differences in fat

metabolism, and hence the severity and neuroanatomical distribution of inflammation. Moreover, the ketamine dose that showed antidepressant effects in rats amounted to twice the dose normally used to reverse stress-induced behavioral phenotypes of rats (Liu et al., 2015), suggesting that neuroinflammation reduced the responsiveness to ketamine not only in mice but also in rats.

The overtly increased intrinsic excitability of pyramidal cells of HFD-exposed mice is reminiscent of hyperactive dopaminergic neurons in the ventral tegmental area of socially defeated mice (Krishnan et al., 2007). However, to the best of our knowledge no such hyperexcitability has ever been reported in the PLC and/or pyramidal cells of chronic stress-based animal models. The external cues leading to altered excitability remain poorly understood but, among others, may involve cytokine-induced alterations in the function of the amino acid transmitters, GABA and glutamate. For example, TNF α , which is prominently increased in hippocampus and ACC/PLC of HFD-exposed mice (Figure 1), is known to potentiate glutamatergic synaptic transmission and to downregulate GABAergic synaptic transmission (Pribiag and Stellwagen, 2013b; Stellwagen et al., 2005). Modest genetically induced defects in GABAergic inhibition alone are sufficient to induce behavioral, cognitive, neuroendocrine, cellular, synaptic and pharmacological sequelae expected of an animal model of depression (Crestani et al., 1999; Earnheart et al., 2007; Ren et al., 2016; Shen et al., 2012; Shen et al., 2010). TNF α may also lead to increased Ca²⁺ entry into dendrites through Ca²⁺ permeable AMPA receptors (Stellwagen et al., 2005). In addition, inflammation has been reported to increase cytoplasmic [Ca²⁺] from intracellular stores, which then increases intrinsic excitability by inhibiting Kv7/KCNQ type K⁺ channels (Tzour et al., 2017), the targets of retigabine. Elevated Ca²⁺ concentrations in dendrites are also implicated in the etiopathology of stress-based models of depression, but in this case, the effects appear to be mediated by V-gated Ca²⁺ channels (VGCCs) (Dao et al., 2010; Green et al., 2010) and NMDA receptors (Deutschenbaur et al., 2016; Skolnick et al., 1996). Indeed, recent evidence suggests that fluoxetine acts by antagonizing the function of VGCCs, rather than the serotonin transporter (Normann et al., 2018), while ketamine has been proposed to reduce dendritic [Ca²⁺] by reducing spontaneous activity of NMDA receptors (Nosyreva et al., 2013; Ren et al., 2016). Distinct sources, magnitudes and neuroanatomical distributions of elevated [Ca²⁺] in dendrites or neural somata may explain the different sensitivity of inflammation- vs. genetic vs. chronic stress-induced depressive-like brain states to different classes of antidepressants.

The HFD-induced neuronal hyperexcitability and the corresponding emotional and cognitive behavioral alterations were reversible by subchronic treatment with retigabine, an anticonvulsant that reduces the intrinsic excitability of neurons by increasing the activity of Kv7/KCNQ channels (Tatulian et al., 2001; Wickenden et al., 2000). Retigabine (Trobal®) remains FDA approved with a primary indication as anticonvulsant although its production has been discontinued in 2017 due to unwanted side effects. We found that retigabine treatment of slices *in vitro* led to rapid normalization of excitability. Both the drug effects on behavior and excitability were fading or disappearing 12 and 24 h after the end of drug treatment, respectively. The plasma half-life in rodents has previously been estimated to 2–3½ hours (GlaxoSmithKline Australia Pty Ltd, 2013), indicating that these represent partial and complete drug-off conditions. By contrast, I_h currents remained suppressed when

assessed 24 h after retigabine, which is consistent with the notion that elevated I_h currents report a history of chronically elevated excitability; they remain suppressed beyond the end retigabine treatment even as hyperexcitability reemerges.

Future studies will need to address the minimal and optimal time course of retigabine treatment for optimal therapeutic effects. In addition, it will be interesting to test whether effective retigabine treatment involved a reduction in markers of inflammation, although we consider this unlikely given the rapid reemergence of hyperexcitability after the end of retigabine treatment. Further dissection of different sources of neuroinflammation and their effects on neural function and specific neural circuits should help understand the difference between treatment-resistant and treatment-responsive depressive disorders.

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REFERENCES

- Al-Hakeim HK, Al-Rammahi DA, Al-Dujaili AH, 2015 IL-6, IL-18, sIL-2R, and TNFalpha proinflammatory markers in depression and schizophrenia patients who are free of overt inflammation. *J Affect Disord* 182, 106–114. doi: 10.1016/j.jad.2015.04.044 [PubMed: 25985379]
- Alcocer-Gomez E, Ulecia-Moron C, Marin-Aguilar F, Rybkina T, Casas-Barquero N, Ruiz-Cabello J, Ryffel B, Apetoh L, Ghiringhelli F, Bullon P, Sanchez-Alcazar JA, Carrion AM, Cordero MD, 2016 Stress-Induced Depressive Behaviors Require a Functional NLRP3 Inflammasome. *Mol Neurobiol* 53, 4874–4882. doi: 10.1007/s12035-015-9408-7 [PubMed: 26362308]
- Almeida-Suhett CP, Graham A, Chen Y, Deuster P, 2017 Behavioral changes in male mice fed a high-fat diet are associated with IL-1beta expression in specific brain regions. *Physiol Behav* 169, 130–140. doi: 10.1016/j.physbeh.2016.11.016 [PubMed: 27876639]
- Arnold SE, Lucki I, Brookshire BR, Carlson GC, Browne CA, Kazi H, Bang S, Choi BR, Chen Y, McMullen MF, Kim SF, 2014 High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice. *Neurobiol Dis* 67, 79–87. doi: 10.1016/j.nbd.2014.03.011 [PubMed: 24686304]
- Au A, Feher A, McPhee L, Jessa A, Oh S, Einstein G, 2016 Estrogens, inflammation and cognition. *Front Neuroendocrinol* 40, 87–100. doi: 10.1016/j.yfrne.2016.01.002 [PubMed: 26774208]
- Barnes J, Mondelli V, Pariante CM, 2017 Genetic Contributions of Inflammation to Depression. *Neuropsychopharmacology* 42, 81–98. doi: 10.1038/npp.2016.169 [PubMed: 27555379]
- Bessa JM, Ferreira D, Melo I, Marques F, Cerqueira JJ, Palha JA, Almeida OF, Sousa N, 2009 The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. *Mol Psychiatry* 14, 764–773, 739. doi: 10.1038/mp.2008.119 [PubMed: 18982002]
- Bodnoff SR, Suranyi-Cadotte B, Aitken DH, Quirion R, Meaney MJ, 1988 The effects of chronic antidepressant treatment in an animal model of anxiety. *Psychopharmacology (Berl)* 95, 298–302. doi: 10.1007/BF00181937 [PubMed: 3137614]

- Bonin RP, Zurek AA, Yu J, Bayliss DA, Orser BA, 2013 Hyperpolarization-activated current (In) is reduced in hippocampal neurons from Gabra5^{-/-} mice. *PLoS One* 8, e58679. doi: 10.1371/journal.pone.0058679 [PubMed: 23516534]
- Brachman RA, McGowan JC, Perusini JN, Lim SC, Pham TH, Faye C, Gardier AM, Mendez-David I, David DJ, Hen R, Denny CA, 2016 Ketamine as a Prophylactic Against Stress-Induced Depressive-like Behavior. *Biol Psychiatry* 79, 776–786. doi: 10.1016/j.biopsych.2015.04.022 [PubMed: 26037911]
- Buckman LB, Hasty AH, Flaherty DK, Buckman CT, Thompson MM, Matlock BK, Weller K, Ellacott KL, 2014 Obesity induced by a high-fat diet is associated with increased immune cell entry into the central nervous system. *Brain Behav Immun* 35, 33–42. doi: 10.1016/j.bbi.2013.06.007 [PubMed: 23831150]
- Cao JL, Covington HE 3rd, Friedman AK, Wilkinson MB, Walsh JJ, Cooper DC, Nestler EJ, Han MH, 2010 Mesolimbic dopamine neurons in the brain reward circuit mediate susceptibility to social defeat and antidepressant action. *J Neurosci* 30, 16453–16458. doi: 10.1523/JNEUROSCI.3177-10.2010 [PubMed: 21147984]
- Checkley S, 1996 The neuroendocrinology of depression and chronic stress. *Br Med Bull* 52, 597–617. doi: 10.1093/oxfordjournals.bmb.a011570 [PubMed: 8949260]
- Clevenger AC, Kim H, Salcedo E, Yonchek JC, Rodgers KM, Orfila JE, Dietz RM, Quillinan N, Traystman RJ, Herson PS, 2018 Endogenous Sex Steroids Dampen Neuroinflammation and Improve Outcome of Traumatic Brain Injury in Mice. *J Mol Neurosci* 64, 410–420. doi: 10.1007/s12031-018-1038-x [PubMed: 29450697]
- Colasanti A, Guo Q, Giannetti P, Wall MB, Newbould RD, Bishop C, Onega M, Nicholas R, Ciccarelli O, Muraro PA, Malik O, Owen DR, Young AH, Gunn RN, Piccini P, Matthews PM, Rabiner EA, 2016 Hippocampal Neuroinflammation, Functional Connectivity, and Depressive Symptoms in Multiple Sclerosis. *Biol Psychiatry* 80, 62–72. doi: 10.1016/j.biopsych.2015.11.022 [PubMed: 26809249]
- Colpo GD, Leboyer M, Dantzer R, Trivedi MH, Teixeira AL, 2018 Immune-based strategies for mood disorders: facts and challenges. *Expert Rev Neurother* 18, 139–152. doi: 10.1080/14737175.2018.1407242 [PubMed: 29179585]
- Conversano C, Carmassi C, Carlini M, Casu G, Gremigni P, Dell'Osso L, 2015 Interferon alpha Therapy in Patients with Chronic Hepatitis C Infection: Quality of Life and Depression. *Hematol Rep* 7, 5632. doi: 10.4081/hr.2015.5632 [PubMed: 25852845]
- Cope EC, LaMarca EA, Monari PK, Olson LB, Martinez S, Zych AD, Katchur NJ, Gould E, 2018 Microglia Play an Active Role in Obesity-Associated Cognitive Decline. *J Neurosci* 38, 8889–8904. doi: 10.1523/JNEUROSCI.0789-18.2018 [PubMed: 30201764]
- Crestani F, Lorez M, Baer K, Essrich C, Benke D, Laurent JP, Belzung C, Fritschy JM, Lüscher B, Möhler H, 1999 Decreased GABAA-receptor clustering results in enhanced anxiety and a bias for threat cues. *Nat Neurosci* 2, 833–839. doi: 10.1038/12207 [PubMed: 10461223]
- Crowley NA, Bloodgood DW, Hardaway JA, Kendra AM, McCall JG, Al-Hasani R, McCall NM, Yu W, Schools ZL, Krashes MJ, Lowell BB, Whistler JL, Bruchas MR, Kash TL, 2016 Dynorphin Controls the Gain of an Amygdalar Anxiety Circuit. *Cell Rep* 14, 2774–2783. doi: 10.1016/j.celrep.2016.02.069 [PubMed: 26997280]
- Dao DT, Mahon PB, Cai X, Kovacsics CE, Blackwell RA, Arad M, Shi J, Zandi PP, O'Donnell P, Bipolar Genome Study C., Knowles JA, Weissman MM, Coryell W, Scheftner WA, Lawson WB, Levinson DF, Thompson SM, Potash JB, Gould TD, 2010 Mood disorder susceptibility gene CACNA1C modifies mood-related behaviors in mice and interacts with sex to influence behavior in mice and diagnosis in humans. *Biol Psychiatry* 68, 801–810. doi: 10.1016/j.biopsych.2010.06.019 [PubMed: 20723887]
- David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, Drew M, Craig DA, Guiard BP, Guilloux JP, Artymyshyn RP, Gardier AM, Gerald C, Antonijevic IA, Leonardo ED, Hen R, 2009 Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron* 62, 479–493. doi: 10.1016/j.neuron.2009.04.017 [PubMed: 19477151]
- Delgado y Palacios R., Campo A, Henningsen K, Verhoye M, Poot D, Dijkstra J, Van Audekerke J, Benveniste H, Sijbers J, Wiborg O, Van der Linden A, 2011 Magnetic resonance imaging and spectroscopy reveal differential hippocampal changes in anhedonic and resilient subtypes of the

- chronic mild stress rat model. *Biol Psychiatry* 70, 449–457. doi: 10.1016/j.biopsych.2011.05.014 [PubMed: 21762877]
- Dennehy EB, Robinson RL, Stephenson JJ, Faries D, Grabner M, Palli SR, Stauffer VL, Marangell LB, 2015 Impact of non-remission of depression on costs and resource utilization: from the COMorbidities and symptoms of DEpression (CODE) study. *Curr Med Res Opin* 31, 1165–1177. doi: 10.1185/03007995.2015.1029893 [PubMed: 25879140]
- Deutschenbaur L, Beck J, Kiyhankhadiv A, Muhlhauser M, Borgwardt S, Walter M, Hasler G, Sollberger D, Lang UE, 2016 Role of calcium, glutamate and NMDA in major depression and therapeutic application. *Prog Neuropsychopharmacol Biol Psychiatry* 64, 325–333. doi: 10.1016/j.pnpbp.2015.02.015 [PubMed: 25747801]
- Dobos N, Korf J, Luiten PG, Eisel UL, 2010 Neuroinflammation in Alzheimer's disease and major depression. *Biol Psychiatry* 67, 503–504. doi: 10.1016/j.biopsych.2010.01.023 [PubMed: 20185031]
- Donahue RJ, Muschamp JW, Russo SJ, Nestler EJ, Carlezon WA Jr., 2014 Effects of striatal DeltaFosB overexpression and ketamine on social defeat stress-induced anhedonia in mice. *Biol Psychiatry* 76, 550–558. doi: 10.1016/j.biopsych.2013.12.014 [PubMed: 24495460]
- Douglas RJ, 1966 Cues for spontaneous alternation. *J Comp Physiol Psychol* 62, 171–183. doi: 10.1037/h0023668 [PubMed: 5969595]
- Drevets WC, Price JL, Furey ML, 2008 Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct Funct* 213, 93–118. doi: 10.1007/s00429-008-0189-x [PubMed: 18704495]
- Dulawa SC, Holick KA, Gundersen B, Hen R, 2004 Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology* 29, 1321–1330. doi: 10.1038/sj.npp.1300433 [PubMed: 15085085]
- Duman RS, 2014 Neurobiology of stress, depression, and rapid acting antidepressants: remodeling synaptic connections. *Depress Anxiety* 31, 291–296. doi: 10.1002/da.22227 [PubMed: 24616149]
- Dutheil S, Ota KT, Wohleb ES, Rasmussen K, Duman RS, 2016 High-Fat Diet Induced Anxiety and Anhedonia: Impact on Brain Homeostasis and Inflammation. *Neuropsychopharmacology* 41, 1874–1887. doi: 10.1038/npp.2015.357 [PubMed: 26658303]
- Earnheart JC, Schweizer C, Crestani F, Iwasato T, Itohara S, Mohler H, Luscher B, 2007 GABAergic control of adult hippocampal neurogenesis in relation to behavior indicative of trait anxiety and depression states. *J Neurosci* 27, 3845–3854. doi: 10.1523/JNEUROSCI.3609-06.2007 [PubMed: 17409249]
- Fava M, 2003 Diagnosis and definition of treatment-resistant depression. *Biol Psychiatry* 53, 649–659. doi: 10.1016/S0006-3223(03)00231-2 [PubMed: 12706951]
- Fava M, Davidson KG, 1996 Definition and epidemiology of treatment-resistant depression. *Psychiatr Clin North Am* 19, 179–200. doi: 10.1016/S0193-953X(05)70283-5 [PubMed: 8827185]
- Franklin TC, Xu C, Duman RS, 2018 Depression and sterile inflammation: Essential role of danger associated molecular patterns. *Brain Behav Immun* 72, 2–13. doi: 10.1016/j.bbi.2017.10.025 [PubMed: 29102801]
- Friedman AK, Juarez B, Ku SM, Zhang H, Calizo RC, Walsh JJ, Chaudhury D, Zhang S, Hawkins A, Dietz DM, Murrough JW, Ribadeneira M, Wong EH, Neve RL, Han MH, 2016 KCNQ channel openers reverse depressive symptoms via an active resilience mechanism. *Nat Commun* 7, 11671. doi: 10.1038/ncomms11671 [PubMed: 27216573]
- Friedman AK, Walsh JJ, Juarez B, Ku SM, Chaudhury D, Wang J, Li X, Dietz DM, Pan N, Vialou VF, Neve RL, Yue Z, Han MH, 2014 Enhancing depression mechanisms in midbrain dopamine neurons achieves homeostatic resilience. *Science* 344, 313–319. doi: 10.1126/science.1249240 [PubMed: 24744379]
- Fuchs T, Jefferson SJ, Hooper A, Yee PH, Maguire J, Luscher B, 2017 Disinhibition of somatostatin-positive GABAergic interneurons results in an anxiolytic and antidepressant-like brain state. *Mol Psychiatry* 22, 920–930. doi: 10.1038/mp.2016.188 [PubMed: 27821870]
- Furtado M, Katzman MA, 2015 Examining the role of neuroinflammation in major depression. *Psychiatry Res* 229, 27–36. doi: 10.1016/j.psychres.2015.06.009 [PubMed: 26187338]

- Gao F, Liu Z, Ren W, Jiang W, 2014 Acute lipopolysaccharide exposure facilitates epileptiform activity via enhanced excitatory synaptic transmission and neuronal excitability in vitro. *Neuropsychiatr Dis Treat* 10, 1489–1495. doi: 10.2147/NDT.S65695 [PubMed: 25170268]
- GlaxoSmithKline Australia Pty Ltd, 2013 Australian Public Assessment Report for Retigabine. In: Department of Health, T.G.A. (Ed.). Commonwealth of Australia 2013, pp. 1–61.
- Green EK, Grozeva D, Jones I, Jones L, Kirov G, Caesar S, Gordon-Smith K, Fraser C, Forty L, Russell E, Hamshere ML, Moskvina V, Nikolov I, Farmer A, McGuffin P, Wellcome Trust Case Control, C., Holmans PA, Owen MJ, O'Donovan MC, Craddock N, 2010 The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. *Mol Psychiatry* 15, 1016–1022. doi: 10.1038/mp.2009.49 [PubMed: 19621016]
- Griffin AL, 2015 Role of the thalamic nucleus reuniens in mediating interactions between the hippocampus and medial prefrontal cortex during spatial working memory. *Front Syst Neurosci* 9, 29. doi: 10.3389/fnsys.2015.00029 [PubMed: 25805977]
- Guillemot-Legris O, Muccioli GG, 2017 Obesity-Induced Neuroinflammation: Beyond the Hypothalamus. *Trends Neurosci* 40, 237–253. doi: 10.1016/j.tins.2017.02.005 [PubMed: 28318543]
- Han Y, Chen J, Zou D, Zheng P, Li Q, Wang H, Li P, Zhou X, Zhang Y, Liu Y, Xie P, 2016 Efficacy of ketamine in the rapid treatment of major depressive disorder: a meta-analysis of randomized, double-blind, placebo-controlled studies. *Neuropsychiatr Dis Treat* 12, 2859–2867. doi: 10.2147/NDT.S117146 [PubMed: 27843321]
- Hare BD, Ghosal S, Duman RS, 2017 Rapid Acting Antidepressants in Chronic Stress Models: Molecular and Cellular Mechanisms. *Chronic Stress (Thousand Oaks)* 1, 247054701769731. doi: 10.1177/2470547017697317
- Hassan AM, Mancano G, Kashofer K, Frohlich EE, Matak A, Mayerhofer R, Reichmann F, Olivares M, Neyrinck AM, Delzenne NM, Claus SP, Holzer P, 2018 High-fat diet induces depression-like behaviour in mice associated with changes in microbiome, neuropeptide Y, and brain metabolome. *Nutr Neurosci*, 1–17. doi: 10.1080/1028415X.2018.1465713
- Isingrini E, Camus V, Le Guisquet AM, Pingaud M, Devers S, Belzung C, 2010 Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: a model of fluoxetine resistance in mice. *PLoS One* 5, e10404. doi: 10.1371/journal.pone.0010404 [PubMed: 20436931]
- Jacka FN, Cherbuin N, Anstey KJ, Butterworth P, 2014 Dietary patterns and depressive symptoms over time: examining the relationships with socioeconomic position, health behaviours and cardiovascular risk. *PLoS One* 9, e87657. doi: 10.1371/journal.pone.0087657 [PubMed: 24489946]
- Jacka FN, Pasco JA, Mykletun A, Williams LJ, Hodge AM, O'Reilly SL, Nicholson GC, Kotowicz MA, Berk M, 2010 Association of Western and traditional diets with depression and anxiety in women. *Am J Psychiatry* 167, 305–311. doi: 10.1176/appi.ajp.2009.09060881 [PubMed: 20048020]
- Jakupovic J, Kang N, Baum MJ, 2008 Effect of bilateral accessory olfactory bulb lesions on volatile urinary odor discrimination and investigation as well as mating behavior in male mice. *Physiol Behav* 93, 467–473. doi: 10.1016/j.physbeh.2007.10.005 [PubMed: 17991495]
- Kappelmann N, Lewis G, Dantzer R, Jones PB, Khandaker GM, 2018 Antidepressant activity of anti-cytokine treatment: a systematic review and meta-analysis of clinical trials of chronic inflammatory conditions. *Mol Psychiatry* 23, 335–343. doi: 10.1038/mp.2016.167 [PubMed: 27752078]
- Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE, Wang PS, National Comorbidity Survey R., 2003 The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *JAMA* 289, 3095–3105. doi: 10.1001/jama.289.23.3095 [PubMed: 12813115]
- Kiraly DD, Horn SR, Van Dam NT, Costi S, Schwartz J, Kim-Schulze S, Patel M, Hodes GE, Russo SJ, Merad M, Iosifescu DV, Charney DS, Murrough JW, 2017 Altered peripheral immune profiles in treatment-resistant depression: response to ketamine and prediction of treatment outcome. *Transl Psychiatry* 7, e1065. doi: 10.1038/tp.2017.31 [PubMed: 28323284]

- Kloiber S, Ising M, Reppermund S, Horstmann S, Dose T, Majer M, Zihl J, Pfister H, Unschuld PG, Holsboer F, Lucae S, 2007 Overweight and obesity affect treatment response in major depression. *Biol Psychiatry* 62, 321–326. doi: 10.1016/j.biopsych.2006.10.001 [PubMed: 17241618]
- Kraus MR, Schafer A, Faller H, Csef H, Scheurlen M, 2003 Psychiatric symptoms in patients with chronic hepatitis C receiving interferon alfa-2b therapy. *J Clin Psychiatry* 64, 708–714. doi: 10.4088/JCP.v64n0614 [PubMed: 12823087]
- Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ, 2007 Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell* 131, 391–404. doi: 10.1016/j.cell.2007.09. [PubMed: 17956738]
- Lanquillon S, Krieg JC, Bening-Abu-Shach U, Vedder H, 2000 Cytokine production and treatment response in major depressive disorder. *Neuropsychopharmacology* 22, 370–379. doi: 10.1016/S0893-133X(99)00134-7 [PubMed: 10700656]
- Liu RJ, Aghajanian GK, 2008 Stress blunts serotonin- and hypocretin-evoked EPSCs in prefrontal cortex: role of corticosterone-mediated apical dendritic atrophy. *Proc Natl Acad Sci U S A* 105, 359–364. doi: 10.1073/pnas.0706679105 [PubMed: 18172209]
- Liu RJ, Ota KT, Duteil S, Duman RS, Aghajanian GK, 2015 Ketamine Strengthens CRF-Activated Amygdala Inputs to Basal Dendrites in mPFC Layer V Pyramidal Cells in the Prelimbic but not Infralimbic Subregion, A Key Suppressor of Stress Responses. *Neuropsychopharmacology* 40, 2066–2075. doi: 10.1038/npp.2015.70 [PubMed: 25759300]
- Lucki I, 1997 The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behavioural Pharmacology* 8, 523–532. doi: 10.1097/00008877-199711000-00010 [PubMed: 9832966]
- Lumeng CN, Saltiel AR, 2011 Inflammatory links between obesity and metabolic disease. *J Clin Invest* 121, 2111–2117. doi: 10.1172/JCI57132 [PubMed: 21633179]
- Maes M, Bosmans E, De Jongh R, Kenis G, Vandoolaeghe E, Neels H, 1997 Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. *Cytokine* 9, 853–858. doi: 10.1006/cyto.1997.0238 [PubMed: 9367546]
- Magarinos AM, Deslandes A, McEwen BS, 1999 Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress. *Eur J Pharmacol* 371, 113–122. doi: 10.1016/S0014-2999(99)00163-6 [PubMed: 10357248]
- Malkesman O, Scattoni ML, Paredes D, Tragon T, Pearson B, Shaltiel G, Chen G, Crawley JN, Manji HK, 2010 The female urine sniffing test: a novel approach for assessing reward-seeking behavior in rodents. *Biol Psychiatry* 67, 864–871. doi: 10.1016/j.biopsych.2009.10.018 [PubMed: 20034613]
- Menard C, Pfau ML, Hodes GE, Russo SJ, 2017 Immune and Neuroendocrine Mechanisms of Stress Vulnerability and Resilience. *Neuropsychopharmacology* 42, 62–80. doi: 10.1038/npp.2016.90 [PubMed: 27291462]
- Miller AH, Maletic V, Raison CL, 2009 Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* 65, 732–741. doi: 10.1016/j.biopsych.2008.11.029 [PubMed: 19150053]
- Mrazek DA, Hornberger JC, Altar CA, Degtjar I, 2014 A review of the clinical, economic, and societal burden of treatment-resistant depression: 1996–2013. *Psychiatr Serv* 65, 977–987. doi: 10.1176/appi.ps.201300059 [PubMed: 24789696]
- Murrough JW, Perez AM, Pillemer S, Stern J, Parides MK, aan het Rot M, Collins KA, Mathew SJ, Charney DS, Iosifescu DV, 2013a Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. *Biol Psychiatry* 74, 250–256. doi: 10.1016/j.biopsych.2012.06.022 [PubMed: 22840761]
- Murrough JW, Wan LB, Iacoviello B, Collins KA, Solon C, Glicksberg B, Perez AM, Mathew SJ, Charney DS, Iosifescu DV, Burdick KE, 2013b Neurocognitive effects of ketamine in treatment-resistant major depression: association with antidepressant response. *Psychopharmacology (Berl)*. doi: 10.1007/s00213-013-3255-x

- Nicholson A, Reifsnnyder PC, Malcolm RD, Lucas CA, MacGregor GR, Zhang W, Leiter EH, 2010 Diet-induced obesity in two C57BL/6 substrains with intact or mutant nicotinamide nucleotide transhydrogenase (Nnt) gene. *Obesity (Silver Spring)* 18, 1902–1905. doi: 10.1038/oby.2009.477 [PubMed: 20057372]
- Nollet M, Le Guisquet AM, Belzung C, 2013 Models of depression: unpredictable chronic mild stress in mice *Curr Protoc Pharmacol* Chapter 5, Unit 5 65 doi: 10.1002/0471141755.ph0565s61
- Normann C, Frase S, Haug V, von Wolff G, Clark K, Munzer P, Dorner A, Scholliers J, Horn M, Vo Van T, Seifert G, Serchov T, Biber K, Nissen C, Klugbauer N, Bischofberger J, 2018 Antidepressants Rescue Stress-Induced Disruption of Synaptic Plasticity via Serotonin Transporter-Independent Inhibition of L-Type Calcium Channels. *Biol Psychiatry* 84, 55–64. doi: 10.1016/j.biopsych.2017.10.008 [PubMed: 29174591]
- Nosyreva E, Szabla K, Autry AE, Ryazanov AG, Monteggia LM, Kavalali ET, 2013 Acute suppression of spontaneous neurotransmission drives synaptic potentiation. *J Neurosci* 33, 6990–7002. doi: 10.1523/JNEUROSCI.4998-12.2013 [PubMed: 23595756]
- O'Brien SM, Scully P, Fitzgerald P, Scott LV, Dinan TG, 2007 Plasma cytokine profiles in depressed patients who fail to respond to selective serotonin reuptake inhibitor therapy. *J Psychiatr Res* 41, 326–331. doi: 10.1016/j.jpsychires.2006.05.013 [PubMed: 16870211]
- Oskooilar N, Wilcox CS, Tong ML, Grosz DE, 2009 Body mass index and response to antidepressants in depressed research subjects. *J Clin Psychiatry* 70, 1609–1610. doi: 10.4088/JCP.09105226blu [PubMed: 20031110]
- Papakostas GI, Petersen T, Iosifescu DV, Burns AM, Nierenberg AA, Alpert JE, Rosenbaum JF, Fava M, 2005 Obesity among outpatients with major depressive disorder. *Int J Neuropsychopharmacol* 8, 59–63. doi: 10.1017/S1461145704004602 [PubMed: 15361263]
- Pereira-Miranda E, Costa PRF, Queiroz VAO, Pereira-Santos M, Santana MLP, 2017 Overweight and Obesity Associated with Higher Depression Prevalence in Adults: A Systematic Review and Meta-Analysis. *J Am Coll Nutr* 36, 223–233. doi: 10.1080/07315724.2016.1261053 [PubMed: 28394727]
- Pettersson US, Walden TB, Carlsson PO, Jansson L, Phillipson M, 2012 Female mice are protected against high-fat diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue. *PLoS One* 7, e46057. doi: 10.1371/journal.pone.0046057 [PubMed: 23049932]
- Pleil KE, Lowery-Gionta EG, Crowley NA, Li C, Marcinkiewicz CA, Rose JH, McCall NM, Maldonado-Devincci AM, Morrow AL, Jones SR, Kash TL, 2015 Effects of chronic ethanol exposure on neuronal function in the prefrontal cortex and extended amygdala. *Neuropharmacology* 99, 735–749. doi: 10.1016/j.neuropharm.2015.06.017 [PubMed: 26188147]
- Pribrag H, Stellwagen D, 2013a Neuroimmune Modulation of Synaptic Function, 65–94. doi: 10.1007/978-1-4614-4729-0_3
- Pribrag H, Stellwagen D, 2013b TNF-alpha downregulates inhibitory neurotransmission through protein phosphatase 1-dependent trafficking of GABA(A) receptors. *J Neurosci* 33, 15879–15893. doi: 10.1523/JNEUROSCI.0530-13.2013 [PubMed: 24089494]
- Rainville JR, Hodes GE, 2019 Inflaming sex differences in mood disorders. *Neuropsychopharmacology* 44, 184–199. doi: 10.1038/s41386-018-0124-7 [PubMed: 29955150]
- Raison CL, Capuron L, Miller AH, 2006 Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol* 27, 24–31. doi: 10.1016/j.it.2005.11.006 [PubMed: 16316783]
- Raison CL, Rutherford RE, Woolwine BJ, Shuo C, Schettler P, Drake DF, Haroon E, Miller AH, 2013 A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. *JAMA Psychiatry* 70, 31–41. doi: 10.1001/2013.jamapsychiatry.4 [PubMed: 22945416]
- Ren Z, Pribrag H, Jefferson SJ, Shorey M, Fuchs T, Stellwagen D, Luscher B, 2016 Bidirectional Homeostatic Regulation of a Depression-Related Brain State by Gamma-Aminobutyric Acidergic Deficits and Ketamine Treatment. *Biol Psychiatry* 80, 457–468. doi: 10.1016/j.biopsych.2016.02.009 [PubMed: 27062563]
- Renault PF, Hoofnagle JH, Park Y, Mullen KD, Peters M, Jones DB, Rustgi V, Jones EA, 1987 Psychiatric complications of long-term interferon alfa therapy. *Arch Intern Med* 147, 1577–1580. doi: 10.1001/archinte.1987.00370090055011 [PubMed: 3307672]

- Rice F, Harold G, Thapar A, 2002 The genetic aetiology of childhood depression: a review. *J Child Psychol Psychiatry* 43, 65–79. doi: 10.1111/1469-7610.00004 [PubMed: 11848337]
- Rizvi SJ, Grima E, Tan M, Rotzinger S, Lin P, McIntyre RS, Kennedy SH, 2014 Treatment-resistant depression in primary care across Canada. *Can J Psychiatry* 59, 349–357. doi: 10.1177/070674371405900702 [PubMed: 25007419]
- Roos E, Mariosa D, Ingre C, Lundholm C, Wirdefeldt K, Roos PM, Fang F, 2016 Depression in amyotrophic lateral sclerosis. *Neurology* 86, 2271–2277. doi: 10.1212/WNL.0000000000002671 [PubMed: 27164661]
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R, 2003 Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301, 805–809. doi: 10.1126/science.1083328 [PubMed: 12907793]
- Sarnyai Z, Sibille EL, Pavlides C, Fenster RJ, McEwen BS, Toth M, 2000 Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin(1A) receptors. *Proc Natl Acad Sci U S A* 97, 14731–14736. doi: 10.1073/pnas.97.26.14731 [PubMed: 11121072]
- Schachter J, Martel J, Lin CS, Chang CJ, Wu TR, Lu CC, Ko YF, Lai HC, Ojcius DM, Young JD, 2018 Effects of obesity on depression: A role for inflammation and the gut microbiota. *Brain Behav Immun* 69, 1–8. doi: 10.1016/j.bbi.2017.08.026 [PubMed: 28888668]
- Schmittgen TD, Livak KJ, 2008 Analyzing real-time PCR data by the comparative CT method. *Nature Protocols* 3, 1101–1108. doi: 10.1038/nprot.2008.73 [PubMed: 18546601]
- Setiawan E, Attwells S, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Xu C, Sharma S, Kish S, Houle S, Meyer JH, 2018 Association of translocator protein total distribution volume with duration of untreated major depressive disorder: a cross-sectional study. *Lancet Psychiatry* 5, 339–347. doi: 10.1016/S2215-0366(18)30048-8 [PubMed: 29496589]
- Shelton RC, Claiborne J, Sidoryk-Wegrzynowicz M, Reddy R, Aschner M, Lewis DA, Mirnics K, 2011 Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. *Mol Psychiatry* 16, 751–762. doi: 10.1038/mp.2010.52 [PubMed: 20479761]
- Shelton RC, Falola M, Li L, Zajecka J, Fava M, Papakostas GI, 2015 The pro-inflammatory profile of depressed patients is (partly) related to obesity. *J Psychiatr Res* 70, 91–97. doi: 10.1016/j.jpsychires.2015.09.001 [PubMed: 26424427]
- Shen Q, Fuchs T, Sahir N, Luscher B, 2012 GABAergic control of critical developmental periods for anxiety- and depression-related behavior in mice. *PLoS One* 7, e47441. doi: 10.1371/journal.pone.0047441 [PubMed: 23071808]
- Shen Q, Lal R, Luellen BA, Earnheart JC, Andrews AM, Luscher B, 2010 gamma-Aminobutyric acid-type A receptor deficits cause hypothalamic-pituitary-adrenal axis hyperactivity and antidepressant drug sensitivity reminiscent of melancholic forms of depression. *Biol Psychiatry* 68, 512–520. doi: 10.1016/j.biopsych.2010.04.024 [PubMed: 20579975]
- Singh JB, Fedgchin M, Daly EJ, De Boer P, Cooper K, Lim P, Pinter C, Murrrough JW, Sanacora G, Shelton RC, Kurian B, Winokur A, Fava M, Manji H, Drevets WC, Van Nueten L, 2016 A Double-Blind, Randomized, Placebo-Controlled, Dose-Frequency Study of Intravenous Ketamine in Patients With Treatment-Resistant Depression. *Am J Psychiatry* 173, 816–826. doi: 10.1176/appi.ajp.2016.16010037 [PubMed: 27056608]
- Skolnick P, Laver RT, Popik P, Nowak G, Paul IA, Trullas R, 1996 Adaptation of N-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: implications for the pharmacotherapy of depression. *Pharmacopsychiatry* 29, 23–26. doi: 10.1055/s-2007-979537 [PubMed: 8852530]
- Stellwagen D, Beattie EC, Seo JY, Malenka RC, 2005 Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor- α . *J Neurosci* 25, 3219–3228. doi: 10.1523/JNEUROSCI.4486-04.2005 [PubMed: 15788779]
- Suranyi-Cadotte BE, Bodnoff SR, Welner SA, 1990 Antidepressant-anxiolytic interactions: involvement of the benzodiazepine-GABA and serotonin systems. *Prog Neuropsychopharmacol Biol Psychiatry* 14, 633–654. doi: 10.1016/0278-5846(90)90035-F

- Surget A, Tanti A, Leonardo ED, Laugeray A, Rainer Q, Touma C, Palme R, Griebel G, Ibarguen-Vargas Y, Hen R, Belzung C, 2011 Antidepressants recruit new neurons to improve stress response regulation. *Mol Psychiatry* 16, 1177–1188. doi: 10.1038/mp.2011.48 [PubMed: 21537331]
- Tan A, Costi S, Morris LS, Van Dam NT, Kautz M, Whitton AE, Friedman AK, Collins KA, Ahle G, Chadha N, Do B, Pizzagalli DA, Iosifescu DV, Nestler EJ, Han MH, Murrough JW, 2018 Effects of the KCNQ channel opener ezogabine on functional connectivity of the ventral striatum and clinical symptoms in patients with major depressive disorder. *Mol Psychiatry*. doi: 10.1038/s41380-018-0283-2
- Tatulian L, Delmas P, Abogadie FC, Brown DA, 2001 Activation of expressed KCNQ potassium currents and native neuronal M-type potassium currents by the anti-convulsant drug retigabine. *J Neurosci* 21, 5535–5545. doi: 10.1523/JNEUROSCI.21-15-05535.2001 [PubMed: 11466425]
- Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT, Fischer JD, Matsen ME, Wisse BE, Morton GJ, Horvath TL, Baskin DG, Tschop MH, Schwartz MW, 2012 Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest* 122, 153–162. doi: 10.1172/JCI59660 [PubMed: 22201683]
- Thomas L, Kessler D, Campbell J, Morrison J, Peters TJ, Williams C, Lewis G, Wiles N, 2013 Prevalence of treatment-resistant depression in primary care: cross-sectional data. *Br J Gen Pract* 63, e852–858. doi: 10.3399/bjgp.13X675430 [PubMed: 24351501]
- Tzour A, Leibovich H, Barkai O, Biala Y, Lev S, Yaari Y, Binshtok AM, 2017 KV 7/M channels as targets for lipopolysaccharide-induced inflammatory neuronal hyperexcitability. *J Physiol* 595, 713–738. doi: 10.1113/JP272547 [PubMed: 27506492]
- Valdearcos M, Douglass JD, Robblee MM, Dorfman MD, Stifler DR, Bennett ML, Gerritse I, Fasnacht R, Barres BA, Thaler JP, Koliwad SK, 2017 Microglial Inflammatory Signaling Orchestrates the Hypothalamic Immune Response to Dietary Excess and Mediates Obesity Susceptibility. *Cell Metab* 26, 185–197.e183. doi: 10.1016/j.cmet.2017.05.015 [PubMed: 28683286]
- Viktorin A, Meltzer-Brody S, Kuja-Halkola R, Sullivan PF, Landen M, Lichtenstein P, Magnusson PK, 2016 Heritability of Perinatal Depression and Genetic Overlap With Nonperinatal Depression. *Am J Psychiatry* 173, 158–165. doi: 10.1176/appi.ajp.2015.15010085 [PubMed: 26337037]
- Walker AK, Budac DP, Bisulco S, Lee AW, Smith RA, Beenders B, Kelley KW, Dantzer R, 2013 NMDA receptor blockade by ketamine abrogates lipopolysaccharide-induced depressive-like behavior in C57BL/6J mice. *Neuropsychopharmacology* 38, 1609–1616. doi: 10.1038/npp.2013.71 [PubMed: 23511700]
- Wan LB, Levitch CF, Perez AM, Brallier JW, Iosifescu DV, Chang LC, Foulkes A, Mathew SJ, Charney DS, Murrough JW, 2015 Ketamine safety and tolerability in clinical trials for treatment-resistant depression. *J Clin Psychiatry* 76, 247–252. doi: 10.4088/JCP.13m08852 [PubMed: 25271445]
- Wanat MJ, Hopf FW, Stuber GD, Phillips PE, Bonci A, 2008 Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of Ih. *J Physiol* 586, 2157–2170. doi: 10.1113/jphysiol.2007.150078 [PubMed: 18308824]
- Wang R, Munoz EE, Zhu S, McGrath BC, Cavener DR, 2014 Perk gene dosage regulates glucose homeostasis by modulating pancreatic beta-cell functions. *PLoS One* 9, e99684. doi: 10.1371/journal.pone.0099684 [PubMed: 24915520]
- Weber MD, Godbout JP, Sheridan JF, 2017 Repeated Social Defeat, Neuroinflammation, and Behavior: Monocytes Carry the Signal. *Neuropsychopharmacology* 42, 46–61. doi: 10.1038/npp.2016.102 [PubMed: 27319971]
- Wickenden AD, Yu W, Zou A, Jegla T, Wagoner PK, 2000 Retigabine, a novel anti-convulsant, enhances activation of KCNQ2/Q3 potassium channels. *Mol Pharmacol* 58, 591–600. doi: 10.1124/mol.58.3.591 [PubMed: 10953053]
- Willner P, Belzung C, 2015 Treatment-resistant depression: are animal models of depression fit for purpose? *Psychopharmacology (Berl)* 232, 3473–3495. doi: 10.1007/s00213-015-4034-7 [PubMed: 26289353]

- Willner P, Scheel-Kruger J, Belzung C, 2014 Resistance to antidepressant drugs: the case for a more predisposition-based and less hippocampocentric research paradigm. *Behav Pharmacol* 25, 352–371. doi: 10.1097/FBP.0000000000000066 [PubMed: 25083567]
- Wohleb ES, Franklin T, Iwata M, Duman RS, 2016 Integrating neuroimmune systems in the neurobiology of depression. *Nat Rev Neurosci* 17, 497–511. doi: 10.1038/nrn.2016.69 [PubMed: 27277867]
- Wohleb ES, Hanke ML, Corona AW, Powell ND, Stiner LM, Bailey MT, Nelson RJ, Godbout JP, Sheridan JF, 2011 beta-Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat. *J Neurosci* 31, 6277–6288. doi: 10.1523/JNEUROSCI.0450-11.2011 [PubMed: 21525267]
- Wolf A, Bauer B, Abner EL, Ashkenazy-Frolinger T, Hartz AM, 2016 A Comprehensive Behavioral Test Battery to Assess Learning and Memory in 129S6/Tg2576 Mice. *PLoS One* 11, e0147733. doi: 10.1371/journal.pone.0147733 [PubMed: 26808326]
- World Health Organization, 2017 Depression and Other Common Mental Disorders: Global Health Estimates. http://www.who.int/mental_health/management/depression/prevalence_global_health_estimates/en/.
- Xia M, Liu T, Bai W, Zheng X, Tian X, 2019 Information transmission in HPCPFC network for spatial working memory in rat. *Behav Brain Res* 356, 170–178. doi: 10.1016/j.bbr.2018.08.024 [PubMed: 30170031]
- Yalcin I, Aksu F, Bodard S, Chalon S, Belzung C, 2007 Antidepressant-like effect of tramadol in the unpredictable chronic mild stress procedure: possible involvement of the noradrenergic system. *Behav Pharmacol* 18, 623–631. doi: 10.1097/FBP.0b013e3282eff109 [PubMed: 17912046]
- Yirmiya R, Rimmerman N, Reshef R, 2015 Depression as a microglial disease. *Trends Neurosci* 38, 637–658. doi: 10.1016/j.tins.2015.08.001 [PubMed: 26442697]
- Yoshimura R, Hori H, Ikenouchi-Sugita A, Umene-Nakano W, Ueda N, Nakamura J, 2009 Higher plasma interleukin-6 (IL-6) level is associated with SSRI- or SNRI-refractory depression. *Prog Neuropsychopharmacol Biol Psychiatry* 33, 722–726. doi: 10.1016/j.pnpbp.2009.03.020 [PubMed: 19332097]
- Zemdegs J, Quesseveur G, Jarriault D, Penicaud L, Fioramonti X, Guiard BP, 2016 High-fat diet-induced metabolic disorders impairs 5-HT function and anxiety-like behavior in mice. *Br J Pharmacol* 173, 2095–2110. doi: 10.1111/bph.13343 [PubMed: 26472268]
- Zore T, Palafox M, Reue K, 2018 Sex differences in obesity, lipid metabolism, and inflammation-A role for the sex chromosomes? *Mol Metab* 15, 35–44. doi: 10.1016/j.molmet.2018.04.003 [PubMed: 29706320]

Highlights:

Chronic exposure of mice to a high fat diet results in neuroinflammation in hippocampus and medial prefrontal cortex

High fat diet induced neuroinflammation results in overt hyperexcitability of L2/3 pyramidal cells in the prelimbic cortex

Chronic neuroinflammation results in a depressive state that resists treatment by conventional antidepressants and ketamine

High fat diet induced neurophysiological and behavioral changes are reversible by the Kv7 channel opener retigabine

Figure 1. HFD feeding induces weight gain, abnormal glucose metabolism and neuroinflammation in male mice.

A) Treatment with HFD resulted in an accelerated increase in body weight illustrated by main effects for diet ($F_{(1, 16)} = 412.3$, $p < 0.001$) and time ($F_{(18, 288)} = 77.41$, $p < 0.001$) and a significant diet $\times$ time interaction [$F_{(18, 288)} = 21.36$, $p < 0.001$, 2-way Repeated Measurement (RM) ANOVA]. A significant weight gain was evident starting at three weeks of HFD ($p = 0.014$; $n = 9$, Fisher's LSD). **B)** Glucose tolerance tests after 18 weeks of HFD revealed significant main effects for diet ($F_{(1, 15)} = 28.97$, $p < 0.001$) and time post glucose injection ($F_{(6, 90)} = 82.38$, $p < 0.001$) and interaction ($F_{(6, 90)} = 6.95$, $p < 0.001$, 2-way RM ANOVA). HFD-exposed mice showed increased blood glucose levels from 40 to 160 min post glucose injection ($p < 0.001$, $n = 9$, Fisher's LSD). **(C)** Area under curve of results in **(B)** ($p < 0.001$, t -test). **(D)** Blood glucose levels of 18-week HFD-exposed mice assessed after 8 h of fasting were increased ($p < 0.01$, t -test). **E, F)** Quantitation of TNF α , IL-6 and IL-1 β mRNA levels by qRT-PCR revealed proinflammatory effects of 18-week HFD-treatment in both hippocampus **(E)** (Diet: $F_{(1, 49)} = 17.36$, $p < 0.001$) and ACC/PLC **(F)** (Diet: $F_{(1, 62)} = 4.30$, $p = 0.042$). Posthoc tests revealed increased expression of TNF α and IL-6 in hippocampus **(E)** (TNF α , $p = 0.002$, $n = 9-11$; IL-6, $p = 0.007$, $n = 6-9$) and of TNF α in ACC/PLC **(F)** ($p = 0.025$, $n = 13-14$; Fisher's LSD). **G, H)** Representative micrographs and quantitation of the % area immunostained for Iba-1. The % area positive for Iba-1 was increased in ACC/PLC of 18-week HFD-exposed vs. control dietmice ($p = 0.004$, $n = 7$) but

unaltered in hippocampus ($p = 0.57$, $n = 7-8$; t -tests). Scale bar, 100 μm ; error bars, SEMs; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, for all Figures.

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Figure 2. HFD feeding-induced weight gain of female mice results in marginally altered glucose metabolism and insignificant trends for neuroinflammation.

A) Treatment with HFD for 18 weeks resulted in a significant increase in body weight ($p < 0.01$, $n = 6$; Mann-Whitney). **B)** Glucose tolerance test after 18 weeks of HFD. A two-way repeated measurement ANOVA for glucose level revealed significant main effects for time post glucose injection ($F_{(6, 60)} = 30.98$, $p < 0.001$) and interaction of time $\times$ diet exposure ($F_{(6, 60)} = 2.577$, $p < 0.05$). HFD-treated mice showed increased blood glucose levels at 80 and 120 min post glucose injection ($p < 0.05$, $n = 6$; Fisher's LSD). **C)** Area under the curve of results in (B) (p , *ns*). **D)** Blood glucose levels of 18-week HFD-treated mice assessed after 8 h of fasting trended higher ($p < 0.1$, $n = 6$, Mann-Whitney). **E, F)** Quantitation of TNF α , IL-6, Interferon- γ (IFN- γ) and IL-1 β mRNA by qRT-PCR revealed an overall trend for increased expression of proinflammatory cytokine mRNAs in ACC/PLC of HFD exposed mice (E) ($F_{(1, 38)} = 3.554$, $p = 0.07$) that was absent in hippocampus (F) ($F_{(1, 28)} = 1.454$, $p = 0.24$). Error bars, SEMs; * $p < 0.05$, ** $p < 0.01$.

Figure 3. HFD exposure-induced behavioral alterations are largely resistant to desipramine and fluoxetine, along with increased neural excitability and I_h currents.

A) Experimental design: Four separate groups of mice ($n = 14-15$) were fed a CD or HFD starting at 5 weeks of age, followed by treatment with desipramine (DES) or fluoxetine (FLX) in drinking water (or water alone) starting in the 19th week, and behavioral testing (three tests per week) in the 21st week of special diet exposure. **B)** In the OFT, the total distance traveled was reduced in HFD vs CD-exposed animals ($F_{(3, 55)} = 22.22$, $p < 0.001$, ANOVA, Fisher's LSD), without drug effects in HFD-exposed animals (DES, $p = 0.95$; FLX, $p = 0.25$; Fisher's LSD). **C)** In the NSFT, the latency to feed was increased by HFD exposure ($p < 0.001$, Kruskal-Wallis, uncorrected Dunn's tests) and this effect was ameliorated by antidepressant drug treatment (DES, $p < 0.001$; FLX, $p < 0.05$; uncorrected Dunn's tests). **D)** Caloric consumption of food deprived animals tested in the home cage was unaffected ($F_{(3, 56)} = 0.05$, $p = 0.98$, ANOVA). **E)** In the SST, HFD-induced reductions in the duration spent grooming were unaffected by drug treatments (DES, $p = 0.90$; FLX, $p = 0.11$; Fisher's LSD). **F)** In the FUST, the HFD-induced reduction in the duration spent sniffing was unaffected by the drug treatments ($F_{(3, 55)} = 11.08$, $p < 0.001$, ANOVA, DES induced significant reduced time spent sniffing, $p = 0.008$; FLX, $p = 0.27$; Fisher's LSD). **G)** In the Y-Maze, the HFD-induced reduction of Spontaneous Alternations was unaffected by drug treatment ($F_{(3, 56)} = 5.88$, $p = 0.0015$, ANOVA, DES, $p = 0.66$; FLX, $p = 0.92$; Fisher's

LSD). The level of alternations by chance in this test is indicated by a dashed horizontal line. Error bars, SEMs; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 4. Chronic HFD induced anxious-depressive-like behavior is resistant to ketamine.

A) Mice were subjected to behavioral testing starting in the 19th week of special diet exposure, with ketamine (6 mg/kg, i.p.) or vehicle (0.9% saline) given 8 h before each test and performing one test per week. **B)** In the OFT, ketamine did not affect the distance traveled (Diet: $F_{(1,40)} = 5.70$, $p = 0.022$; Drug: $F_{(1,40)} = 0.66$, $p = 0.42$; Interaction: $F_{(1,40)} = 1.49$, $p = 0.23$; ANOVA). **C)** In the NSFT, ketamine failed to ameliorate the anxious phenotype (Diet: $F_{(1,39)} = 111.6$, $p < 0.001$; Drug: $F_{(1,39)} = 0.61$, $p = 0.44$; Interaction: $F_{(1,39)} = 0.02$, $p = 0.88$; ANOVA). **D)** In the SST, ketamine failed to reverse the HFD induced reduction in grooming duration (Diet: $F_{(1,35)} = 32.59$, $p < 0.001$; Drug: $F_{(1,35)} = 0.02$, $p = 0.89$; Interaction: $F_{(1,35)} = 1.17$, $p = 0.29$; ANOVA). **E)** In the FUST, ketamine failed to reverse the anhedonia-like reductions in the duration spent urine sniffing (Diet: $F_{(1,25)} = 4.42$, $p = 0.046$; Drug: $F_{(1,25)} = 0.13$, $p = 0.72$; Interaction: $F_{(1,25)} = 0.001$, $p = 0.97$; ANOVA). **F)** In the Y-Maze, ketamine had no effect on % Alternations (Diet: $F_{(1,31)} = 5.53$, $p = 0.025$; Drug: $F_{(1,31)} = 0.51$, $p = 0.48$; Interaction: $F_{(1,31)} = 2.64$, $p = 0.11$; ANOVA). **G)** In the FST, there was a HFD exposure-induced increase in the BOX-COX transformed time spent immobile that was reversed by ketamine ($F_{(1,37)} = 6.17$, $p < 0.018$ for diet, ANOVA and $p = 0.046$ drug effects, Fisher's LSD). Error bars, SEMs; * $p < 0.05$, *** $p < 0.001$.

Figure 5. Chronic HFD exposure results in increased I_h currents.

Recordings of I_h currents from PLC L2/3 pyramidal cells with representative current traces on the left and summary data on the right. The I_h currents increased with increasing hyperpolarizing membrane potentials ($F_{(5, 95)} = 5.12$, $p < 0.001$) and showed a significant diet $\times$ voltage interaction ($F_{(5, 95)} = 2.77$, $p = 0.022$, 2-way RM ANOVA). Posthoc tests showed greater I_h currents in HFD vs. CD-exposed animals at -120 mV ($p = 0.003$, $n = 9$ – 12 cells from at least four animals per condition, Fisher's LSD). Error bars, SEMs; * $p < 0.05$, ** $p < 0.01$.

Figure 6. Retigabine reverses chronic HFD induced anxious-depressive-like behavior at different time points.

A) Mice exposed to HFD starting from 5-weeks of age were subjected to a daily injection of retigabine or vehicle, starting at the end of the 18th week of diet exposure. CD-exposed mice were vehicle injected and analyzed in parallel. Behavioral testing was initiated on the 8th day of drug treatment, 1 h (B–G, N) or 12 h (H–M) after drug injection, with one behavioral test every 2–3 days. **B)** In the OFT, retigabine (Ret) had no effect on distance traveled ($F_{(2,30)} = 9.75$, $p < 0.001$, ANOVA, $p = 0.87$, Fisher's LSD). **C)** In the NSFT, HFD exposure increased the latency to feed ($F_{(2,31)} = 11.09$, $p < 0.001$, ANOVA), and retigabine treatment partially reversed this effect ($p = 0.040$, Fisher's LSD). **D)** Caloric intake in HCF tests was unaffected by diet and retigabine ($F_{(2,32)} = 0.81$, $p = 0.45$, ANOVA). **E)** In the SST, retigabine partially reversed the HFD-exposure-induced reduction in grooming duration ($F_{(2,31)} = 44.27$, $p < 0.001$, ANOVA; CD-Veh vs HFD-Veh, $p < 0.001$; HFD-Veh vs HFD-Ret, $p < 0.001$, Fisher's LSD). **F)** In the FUST, neither diet nor retigabine had significant effects on grooming

duration ($F_{(2,31)} = 2.17$, $p = 0.13$, ANOVA). **G**) In the Y-Maze, retigabine reversed HFD-exposure-induced reductions in % alternations ($F_{(2,32)} = 3.82$, $p = 0.03$ for diet and $p = 0.049$ for drug effects, ANOVA, Fisher's LSD). **H**) In the OFT, retigabine had no effect on distance traveled, independent of diet (Diet, $F_{(1,44)} = 22.59$, $p < 0.001$; Drug, $F_{(1,44)} = 3.81$, $p = 0.06$; Interaction, $F_{(1,44)} = 0.007$, $p = 0.94$, ANOVA). **I**) In the NSFT, the HFD increased the latency to feed (Diet, $F_{(1,44)} = 147.5$, $p < 0.001$, ANOVA), and retigabine partially reversed this effect (Drug, $F_{(1,44)} = 0.19$, $p = 0.67$; Interaction, $F_{(1,44)} = 5.86$, $p = 0.020$, ANOVA, $p = 0.0499$, Fisher's LSD), without effect on CD mice ($p = 0.17$, Fisher's LSD). **J**) In the HCF test, caloric intake was increased in HFD-exposed animals but was unaffected by retigabine (Diet, $F_{(1,44)} = 22.59$, $p < 0.001$; Drug, $F_{(1,44)} = 0.15$, $p = 0.70$; Interaction, $F_{(1,44)} = 3.64$, $p = 0.063$, ANOVA). **K**) In the SST, the grooming duration of HFD-exposed animals was reduced but unaffected by retigabine (Diet, $F_{(1,44)} = 39.34$, $p < 0.001$; Drug, $F_{(1,44)} = 0.045$, $p = 0.83$; Interaction, $F_{(1,44)} = 1.31$, $p = 0.26$, ANOVA). **L**) In the FUST, the duration spent sniffing was reduced in HFD-exposed mice and unaffected by retigabine (Diet, $F_{(1,44)} = 30.49$, $p < 0.001$; Drug, $F_{(1,44)} = 1.34$, $p = 0.25$; Interaction, $F_{(1,44)} = 0.048$, $p = 0.83$, ANOVA). **M**) In the Y-maze, retigabine reversed the HFD-induced reduction in % alternations but had no effect in CD mice (Diet, $F_{(1,43)} = 6.30$, $p = 0.016$; Drug, $F_{(1,43)} = 3.55$, $p = 0.067$; Interaction, $F_{(1,43)} = 3.92$, $p = 0.054$, ANOVA; CD-Veh vs HFD-Veh, $p = 0.003$; CD-Ret vs HFD-Veh, $p = 0.004$; HFD-Veh vs HFD-Ret, $p = 0.0098$, Fisher's LSD). **N**) Some of the mice tested in (H–M) were taken off retigabine or vehicle for 6 weeks and then tested again in the SST [untreated controls (Unt)]. The same mice were then subjected to daily retigabine injections for 8 days and retested 1 h after the last injection. Retigabine partially reversed HFD-induced reductions in grooming duration without effects in CD mice (Diet, $F_{(1,25)} = 38.58$, $p < 0.001$; Drug, $F_{(1,25)} = 7.24$, $p = 0.013$; Interaction, $F_{(1,25)} = 0.29$, $p = 0.59$, two-way RM ANOVA; CD-Veh vs CD-Ret, $p = 0.13$; HFD-Veh vs HFD-Ret, $p = 0.034$, Fisher's LSD). Error bars, SEMs; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 7. Retigabine reduces I_h currents and normalizes neural excitability of HFD-exposed animals to control levels.

A) Current injection data from layer II/III PLC pyramidal cells of HFD-exposed mice before and after bath application of 100 μ M retigabine, with representative traces shown on the left and summary data on the right. We observed significant main effects of current injected ($F_{(20,160)} = 23.07$, $p < 0.001$) and drug ($F_{(20,160)} = 23.07$, $p < 0.001$) and an interaction of drug and current ($F_{(1,8)} = 39.8$, $p < 0.001$, 2-way RM ANOVA). Retigabine reduced the number of action potentials in response to currents of 20 pA to 200 pA (20 pA, $p = 0.037$; 30 pA: $p = 0.01$; 40 pA, $p = 0.006$; 50 pA, $p = 0.001$; 60 pA, $p = 0.002$; 70–200 pA: $p < 0.001$, $n = 5$ cells per condition, Fisher's LSD). **B)** Representative traces and current injection data from layer II/III PLC pyramidal cells from vehicle injected CD- or HFD-exposed mice, in comparison to retigabine treated HFD mice, using slices isolated 24 h after the last of eight daily doses of retigabine. A two-way RM ANOVA revealed a main effect of current injected ($F_{(1.544,44.78)} = 69.63$, $p < 0.001$), treatment ($F_{(2,29)} = 3.79$, $p = 0.035$) and an interaction of

current and treatment ($F_{(40,580)} = 5.16$, $p < 0.001$). HFD exposure (vs. CD) increased the number of action potentials in response to currents of 180 pA to 200 pA (180 pA, $p = 0.035$; 190 pA, $p = 0.047$; 200 pA, $p = 0.042$, Fisher's LSD). Retigabine increased the number of action potentials in response to currents of 60 pA to 120 pA (60 pA–80 pA, $p < 0.01$; 90 pA, $p = 0.011$; 100 pA, $p = 0.022$; 110 pA, $p < 0.01$; 120 pA, $p = 0.027$; Fisher's LSD, $n = 6$ –14 cells from at least four animals per condition). C) Representative traces and summary data of I_h currents recorded from layer II/III PLC pyramidal cells from behaviorally naïve CD- or HFD-exposed mice, using slices isolated 24 h after the last of eight daily doses of retigabine or vehicle. A two-way RM ANOVA revealed a main effect of V step ($F_{(5,125)} = 6.75$, $p < 0.001$) and an interaction of diet and voltage ($F_{(10,125)} = 2.73$, $p = 0.005$, 2-way RM ANOVA). HFD exposure (vs. CD) increased I_h currents in response to V-steps of -110 mV to -120 mV (-110 mV, $p = 0.026$; -120 mV, $p = 0.002$, $n = 7$ –12 cells from at least four animals per condition). Retigabine (vs vehicle) reduced I_h currents of HFD-exposed mice in response to V steps of -100 mV to -120 mV (-100 mV, $p = 0.03$; -110 mV, $p = 0.002$; -120 mV, $p = 0.001$; $n = 9$ –12 cells from at least four animals per condition, Fisher's LSD). Error bars, SEMs; *, $p < 0.05$, **, $p < 0.01$.