

GSK-3 β disrupts neural oscillatory function to inhibit learning and memory in rats

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Research

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Abstract

Background. Alterations in glycogen synthase kinase-3 β (GSK-3 β) activity have been implicated in disorders of cognitive impairment including Alzheimer's disease and schizophrenia. Another characteristic of cognitive impairment is the dysregulation of neural oscillatory activity, macroscopic electrical rhythms in brain critical to systems communication. A direct functional relationship between GSK-3 β and neural oscillations has not been elucidated.

Methods. In the present study, the impact of increasing GSK-3 β activity in prefrontal cortex (PFC) or hippocampus (HIP) on the regulation of neural oscillations in rats was investigated using an adeno-associated viral vector containing a persistently active mutant of GSK-3 β (S9A), and changes in learning and memory and tau phosphorylation assessed.

Results. Increasing GSK-3 β activity in either region had similar effects on oscillatory spectral power, enhancing theta and/or gamma oscillatory power recorded from one or both regions. Increasing PFC GSK-3 β activity additionally suppressed high gamma PFC-HIP coherence. These oscillatory changes were accompanied by deficits in recognition memory, spatial learning and/or reversal learning. Increased pathogenic tau phosphorylation was also evident in regions where GSK-3 β activity was elevated.

Conclusions. These findings indicate that increased GSK-3 β activity in PFC or HIP dysregulates neural oscillatory function in, and between, these regions. This suggests that GSK-3 β may not only play an early role in cognitive decline in Alzheimer's disease but may also play a more central role in disorders of cognitive dysfunction through the regulation of neurophysiological network function.

Introduction

Glycogen synthase kinase-3 β (GSK-3 β) is a serine/threonine kinase with over 100 biological substrates [1] that has been repeatedly shown to play a critical role in the pathology of various neuropsychiatric and neurodegenerative diseases, and in particular those that present with cognitive dysfunction [2–5]. Whereas decreased GSK-3 β activity has been implicated in autism [6–8], most often disorders of cognitive dysfunction are associated with increased activation of the kinase, the most widely studied being Alzheimer's disease (AD) [2, 9, 10].

GSK-3 β expression and/or activity levels are elevated in the hippocampus (HIP) and prefrontal cortex (PFC) [11–13], and this finding is mimicked in animal model systems used to study the disease [14, 15]. This increase in GSK-3 β activity results in hyperphosphorylation of tau protein, the formation of soluble oligomeric tau species, and the eventual deposition of neurofibrillary tangles (NFTs), a major neuropathological hallmark of AD. These soluble oligomeric species of tau are believed to play a critical role in mediating the cognitive deficiencies observed in the disorder [16, 17]. In other disorders of cognitive dysfunction, however, the presence of GSK-3 β -induced tau hyperphosphorylation in brain and the impact on learning and memory processes has received much less attention. Further, in the absence of tau pathology, there is also the question as to whether increased GSK-3 activity and cognitive decline

may be linked through some additional mechanism. Indeed, GSK-3 has been shown to play a pivotal role in synaptic plasticity [18–20] suggesting a potential critical role for the protein in the regulation of systems function.

Another functional marker of cognitive decline is the dysregulation of neural oscillatory activity. Neuronal oscillations are synchronous macroscopic electrical rhythms in the nervous system that are generated through summed neuronal population activity, and which play a critical role in brain systems communication [21, 22]. Specifically, whereas low frequency oscillations are believed to be important in long-range communication between different brain regions, high frequency activity is more restricted to short-range communication [23, 24]. Both low frequency theta oscillations, and high frequency gamma oscillations, in HIP and cortical regions have been repeatedly linked to learning and memory processes [13, 25–30]. For example, theta oscillations in the HIP have been shown to play a significant role in the encoding of new memories possibly through facilitating interactions with other brain regions such as PFC [27, 31]. In HIP, gamma oscillations have been shown to play a crucial role in the memory encoding and retrieval [32], and thus affecting working memory and attention, as well as other cognitive responses. Gamma oscillations in the cortical regions have also been linked with higher executive functions including attention, visual processing, memory and learning [30, 33]. Thus, it is not surprising that deficits in oscillatory activity in the HIP and PFC have been associated with cognitive decline both in aging [34, 35] as well as in various disease states such as schizophrenia [36–38], autism [39], and AD [40–43].

The consistent demonstration of increased GSK-3 β activity in disorders of cognitive decline, coincident with alterations in neural oscillatory function, suggests a potential coupling of the two mechanisms. Indeed, this idea is supported by our previous evidence showing that the systemic and short-term pharmacological inhibition of GSK-3 in rats altered neural oscillatory patterns within the PFC and HIP, that were associated with improved learning and memory [44]. This study sought to expand on those findings to evaluate a direct role for GSK-3 β within the PFC or HIP in the regulation of neural oscillatory activity and learning and memory processes. Using a persistently active mutant of GSK-3 β we showed that increased activity of the kinase in either region resulted in increased theta and high gamma frequency power with discrete effects in high gamma coherence, changes associated with disruptions in memory and learning. Further, pathogenic tau phosphorylation was increased in response to these manipulations.

Methods

Animals

Forty adult male Sprague Dawley rats (Charles River, Quebec, Canada) weighing approximately 350-400 grams were used. Rats were double-housed in polypropylene cages until surgery after which they were housed singly. Both the housing room and experimental room were kept on a reverse 12-hour light/dark cycle. Due to the duration of the present study, rats were food restricted, receiving 15 g of 18% chow per day to maintain weights. They were handled for 5 minutes per day for five days before the beginning of

the experiments. All the protocols were in accordance with the guidelines set out by the Animal Care Committee (ACC) at the University of Guelph.

Constructs

AAV8-hSYN1-GSK-3 β (S9A)-HA-WPRE and AAV8-hSYN1-HA-WPRE viruses were generated by Vector Biolabs (Malvern, PA). Construct size restrictions for the AAV required the use of an HA tag with GSK-3 β . The GSK-3 β S9A pcDNA3 construct was a gift from Jim Woodgett (Addgene plasmid # 14754 ; <http://n2t.net/addgene:14754> ; RRID:Addgene_14754) (Stambolic and Woodget, 1994). The constitutively active GSK-3 β (S9A) was generated by a point mutation in serine 9 converting it to adenine, thus preventing Akt-mediated phosphorylation and inhibition of GSK-3 β .

Surgery

Rats underwent stereotaxic surgery to introduce the AAV8-hSYN1-GSK-3 β (S9A)-HA-WPRE or control virus bilaterally into the prelimbic region of PFC or ventral HIP. Isoflurane was used to anesthetize the rats at 5% induction and 2.5% maintenance and body temperature maintained at 37 °C using a thermostat regulated heating pad. Animals were injected subcutaneously with 0.9 % saline (3 mL) to keep them hydrated during surgeries, 5mg/ml carprofen (0.4 mL) as well as a lidocaine/bupivacaine injection at the incision site. Injection coordinates to the PFC (AP +3.24, ML \pm 0.6, DV 3.5) or ventral HIP (AP -5.5, ML \pm 5.1, DV 7.6 & 5.6) were obtained from Paxinos and Watson (2013). AAV was infused at a rate of 0.3 μ L/min and syringes were not removed for 5 minutes post-injection to avoid backflow of the virus to the surface of the skull. Four weeks following the AAV infusion surgery rats underwent a second surgery to implant electrodes bilaterally into the PFC and HIP at the same coordinates as the AAV infusion. Custom electrode microarrays were built using pre-fabricated Delrin templates and polyimide-insulated stainless-steel wires (A-M Systems: 791600, 0.008"). All arrays used had an electrode impedance of less than 2M Ω . Local field potential (LFP) recordings were collected and placements verified at the end of the study.

Electrophysiology

Four days post electrode implantation surgery animals underwent five minutes habituation in transparent plexiglass recording chambers (45cm x 45cm x 45cm) for four days. Following that, LFP recordings were taken from freely moving animals for 30 minutes using a wireless W2100 system (MultiChannel Systems) and were recorded at a sampling frequency of 1000 Hz. The spectral power of LFP oscillations in each region, coherence between regions, and cross correlation analysis was performed using routines from the Chronux software package for MATLAB (MathWorks). Recordings were downsampled, segmented, detrended and low-pass filtered to remove high frequencies greater than 100 Hz. Continuous multitaper spectral power and coherence (tapers = [5 9]) between regions was calculated for each segment in the following frequency bands: delta (1–4 Hz), theta (>4–12 Hz), beta (>12–32 Hz), slow gamma (>32–59 Hz), and fast gamma (>61–100 Hz). Electrode placements were verified post-mortem.

Behavioural Tests

Four days after the second surgery, rat groups underwent five minutes of habituation to an open field arena every day for four days. Animals then underwent three different tests in this arena that included the Novel Object Recognition task (NOR), Object Location (OL) and Object in Place test (OiP). Discrimination ratio was used as the output variable for these tests which was calculated as the difference between the time spent exploring the novel object and familiar object divided by the sum of both times. Animals also underwent tests for spatial memory and reversal learning in the T-maze.

Novel Object Recognition

The NOR task was used to evaluate recognition memory and was conducted in a square opaque open field arena (1 m²). The NOR task was comprised of an acquisition phase and a test phase that were separated by a 2 hour delay. Rats were allowed explore two identical objects placed in two corners of the arena. During the delay time, objects were again cleaned and placed in the same position, however one of the objects was switched with a novel object. Object types were randomized, and positions counterbalanced between rats. The time spent exploring the novel object was compared to the time spent exploring the familiar object.

Object Location

To evaluate spatial memory the OL task was used and was comprised of an acquisition phase and a test phase, three minutes in length each, that were separated by 5-minute delay. In the acquisition phase rats were allowed to explore two similar objects placed in the corners of the arena. During the delay period, the two objects were cleaned with 10% ethanol. For the testing phase, one object was relocated to opposite corner in which it was originally placed. The position of the objects was counterbalanced between animals. In this test phase the time spent exploring the object in the changed position was compared to the time spent exploring the object in the original position.

Object in Place

The OiP task, used to test associative object recognition memory, requires coordinated PFC and HIP communication [45]. The task was comprised of two phases, an acquisition phase as well as a test phase separated by a 10 minute delay. During the acquisition phase animals were placed in the open field and allowed to explore four different objects for five minutes. In the test phase, two objects were switched positions and the other two remained unmoved. Rats were then allowed three minutes to investigate the objects. The time spent exploring the objects in the changed positions was compared to the time spent exploring the objects in the original positions. Object locations were counterbalanced between rats and cleaned between trials with 10% ethanol. All trials were recorded using AnyMaze software (Stoelting).

Reversal Learning

This test was comprised of a four-day training phase with a test of reversal learning on the fifth day. Prior to testing, animals were habituated to the T-maze for four days by raising all doors and baiting both food

wells. In the training phase, only one of the two food wells were baited, and animals received four trials daily for four days with a minimum of 10 minutes between each trial. The time required to reach the baited food well was recorded and used to calculate the cut-off time for the reversal testing phase (3 seconds). In the reversal learning phase, the bait was placed in the opposing food well and the number of trials to needed reach three consecutive successes within the cut-off time recorded.

Immunohistochemistry

Fluorescence immunohistochemistry was performed as done previously [46] on PFA-fixed free-floating brain sections (30 μ m). Free-floating sections were first washed in TBS (60.5 mM Tris, 87.6 mM NaCl pH 7.6), then blocked for 2 hours (10% goat serum, 1% BSA, 0.2% Triton-X, 1X TBS) and incubated with rabbit anti-GSK3 β , rabbit anti-HA and mouse anti-phospho tau (AT8) primary antibodies (source: Cell Signalling) for 60 hours at 4 °C. Following the primary incubation, sections were washed in TBS, blocked (5% goat serum, 0.5% BSA, 0.01% Triton-X, 1X TBS), and incubated in anti-mouse-Alexa 488 and Alexa 594 anti-rabbit secondary antibody for 2 hours. Brain slices were then washed three times in TBS and mounted on slides using Prolong Gold (Thermo Fisher Scientific). Fluorescence microscopy (Etaluma Lumascope) was used to evaluate construct expression in the PFC and HIP. Control brains that received GFP injection were sectioned, mounted on slides and visualized directly under the fluorescence microscopy.

Data Analysis

For LFP data, power and coherence curves are presented as normalized data with jackknife estimates of SEM. Data may log-transformed to better exhibit group differences as indicated. Quantification of the LFP power and coherence data at each frequency measure is reported as means \pm sem for between group comparisons, or as percent change from baseline for within group comparisons. LFP power data analysis were performed using independent samples t-test. Data analysis for the behaviours were performed using student's t-test, with the exception of the T-maze training where a repeated measure ANOVA was used, followed by student's t test for comparisons at each day of training. For the IHC data, baseline sampling was taken outside the regions of construct expression with paired t-tests used for analysis. Prior to all analyses, normality was assessed using the Shapiro-Wilk test and Levene's test for equality of variance. Computations were performed using IBM SPSS 24 software.

Results

To determine the effect of increased PFC GSK-3 β activity on neural oscillations recorded from PFC and HIP, LFP recordings from both regions were acquired in freely moving animals. Analysis of PFC spectral power showed a significant increase selectively in PFC theta power in the GSK-3 β rats compared to controls ($t(33) = 2.57$, $P = 0.014$) (Fig. 1a,b). No significant differences in PFC delta, beta, low gamma, or high gamma power were observed. In HIP, the GSK-3 β animals exhibited increased low ($t(33) = 2.78$, $P = 0.009$) and high gamma power ($t(33) = 2.22$, $P = 0.034$), with no effects in any of the other frequencies (Fig. 1c,d). Analysis of the coherence between the PFC and HIP regions revealed changes selectively within the high gamma frequency, with reduced PFC-HIP high gamma in the PFC GSK-3 β rats ($t(33) =$

2.66, $P = 0.014$) (Fig. 1e,f). There were no significant differences in PFC-HIP coherence in any of the other frequencies. Cross-correlation analysis was next performed to determine the impact of increased PFC GSK-3 β on the temporal relationship between HIP theta and PFC gamma cycles, a relationship known to be critical to normal cognitive functioning [43]. We found no significant time shifts in theta-gamma coupling, although increased PFC GSK-3 β activity enhanced the coupling strength (Fig. 1g).

The effects of increased GSK-3 β activity on cognition was next evaluated in a variety of behavioural tests for recognition memory, spatial memory and reversal learning. In the NOR task, used to assess recognition memory, animals with elevated GSK-3 β activity in the PFC exhibited deficits, spending less time exploring a novel object than control animals ($t(17) = 2.42$, $P = 0.027$) (Fig. 2a). In the OL task for spatial memory, the GSK-3 β rats also showed impaired ability to discriminate between the moved object and stationary object ($t(17) = 2.90$, $P = 0.010$) (Fig. 2b). The OiP task requires the rats to make an association between an object and the place in which it was previously encountered. In this task, there were no significant group differences (Fig. 2c). For cognitive flexibility, we evaluated the animals during a reversal learning task. Over the course of four days, all of the rats were able to learn the location of the food reward (Fig. 2d, left panel). However, when the treat was shifted to the opposite arm of the T-maze, a deficit in the GSK-3 β rats was observed, with animals requiring significantly more trials to ascertain the location of the food reward ($t(17) = 2.76$, $P = 0.013$) (Fig. 2d, right panel). Following the study the brains were removed, and immunohistochemistry performed to visualize expression of GSK-3 β , and determine whether there were any observable differences in pathogenic tau phosphorylation (Fig. 2e,f). We determined that although some animals showed a minor increase in tau phosphorylation, overall changes in tau phosphorylation were not significant (Fig. 2f, right panel).

The effect of AAV-mediated HIP GSK-3 β activity on neural oscillatory activity recorded from the PFC and HIP was next evaluated. Increased activity of GSK-3 β in the HIP region increased theta power in the PFC in comparison to the control group ($t(27) = 3.43$, $P = 0.009$) (Fig. 3a,b). These changes were accompanied by an increase in beta power ($t(27) = 2.19$, $P = 0.037$) and high gamma power ($t(27) = 2.87$, $P = 0.008$), with no significant changes observed in the delta or low gamma bands (Fig. 3a,b). In HIP, increased GSK-3 β activity led to an increase in theta power ($t(27) = 2.82$, $P = 0.030$) and in high gamma power ($t(27) = 2.49$, $P = 0.040$) with no changes in the other frequency bands (Fig. 3c,d). No HIP GSK-3 β -induced changes in PFC-HIP coherence were evident (Fig. 3e,f). Cross-correlation analysis showed that increased HIP GSK-3 β activity had no effect on the timing of theta-gamma coupling however, in contrast to that observed when PFC GSK-3 β was increased, the coupling strength was reduced (Fig. 3g).

In tests to evaluate cognition, increased GSK-3 β activity in the HIP resulted in disruptions in recognition memory, whereby the GSK-3 β rats showed a reduced discrimination ratio in the NOR task ($t(16) = 2.52$, $P = 0.048$) (Fig. 4a). In the OL task, there were no significant group differences (Fig. 4b). However, in the OiP task, there was a significant deficit in associative recognition memory in the GSK-3 β rats ($t(16) = 3.43$, $P = 0.003$) (Fig. 4c). In the T-maze, there was a significant effect of Treatment such that increased HIP GSK-3 β activity reduced the animals ability to learn the location of the treat ($F(1, 14) = 7.86$, $P = 0.014$) (Fig. 4d, left panel). During reversal learning, when the treat was moved to the opposite arm of the maze, the GSK-

3 β rats showed a deficit, requiring significantly more trials to learn the treat location ($t(16) = 2.98$, $P = 0.009$) (Fig. 4d, right panel). Following the behavioural tests, immunohistochemistry analysis showed increased HIP GSK-3 β expression (Fig. 4e) that was concomitant with increase phosphorylation of tau protein ($t(8) = 9.0$, $P < 0.001$) (Fig. 4f).

Discussion

In the present study the impact of an AAV-induced increase in GSK-3 β activity within the PFC or HIP on neural oscillatory activity, learning and memory, and tau phosphorylation was evaluated. It was demonstrated that increasing GSK-3 β activity in either region altered neural oscillations particularly in the theta and gamma frequencies and was sufficient to induce pathogenic tau phosphorylation. These changes were associated with disruptions in recognition memory, spatial memory, and reversal learning.

Our findings showing GSK-3 β -induced increases in theta and gamma spectral power are consistent with previous clinical reports examining oscillatory dysfunction in AD and schizophrenia, two disorders of cognitive dysfunction that also present with increased expression and activity of HIP and/or cortical GSK-3 β [2–4]. For example, electroencephalogram (EEG) studies revealed that persons with AD or schizophrenia exhibit increased global theta power [47, 48] and enhanced resting state and evoked gamma power has been reported for both disorders [49–52], with increased high gamma activity in schizophrenia inversely associated with cognitive performance both in patients and their first-degree relatives [51]. Furthermore, in a recent preclinical study using a transgenic AD model overexpressing the A152T variant of human tau, increased delta and low theta frequency cortical power was shown [53], demonstrating a direct role for tau phosphorylation in the regulation of oscillatory function. HIP tau-mediated changes in oscillations have also been reported using a triple transgenic AD model [54]. Although the findings of that study showed reduced HIP theta power in this model when evaluated in mice *ex vivo*, this study used adolescent mice only, and therefore it is unknown whether this reduction in HIP theta persisted into adulthood. In a murine model of fragile X syndrome, a disorder also with elevated GSK-3 β activity and associated with significant cognitive impairments [55, 56], increased HIP theta was evident [57], and in autism, there is a higher relative EEG theta in autistic youth [58, 59], or a greater theta/beta frequency ratio [59]. Yet, it is noteworthy that although GSK-3 β has recently emerged as a potential signaling hub in autism [5], evidence indicates protein activity may actually be downregulated [6–8]. This raises the possibility that GSK-3 β may play a more homeostatic role in brain systems function, with aberrant up or downregulation in protein activity having consequent negative effects on cognitive performance. This would also suggest, however, that GSK-3 β would have additional regulatory control over oscillatory function that is independent of its actions at tau protein.

With the exception of AD, few studies have examined a role for tau hyperphosphorylation in disorders of cognitive dysfunction. A link between GSK-3 β , tau and disrupted reelin signaling in schizophrenia has been proposed [60] and, more recently, GSK-3 β -mediated tau phosphorylation was shown to underlie cognitive deficits in an animal model system of type 2 diabetes [61]. Another preclinical study showed enhanced GSK-3 β activation and tau phosphorylation mediated early-onset cognitive dysfunction after

traumatic brain injury in mice [62]. Clearly more research is required to discern a role for tau protein phosphorylation in oscillatory systems processes, however, other potential mechanisms by which GSK-3 β may influence neural oscillations should also be considered. For instance, GSK-3 β regulates the activity of voltage-gated ion channels such as sodium [63, 64], potassium [65, 66] and calcium channels [67] and irregular expression and/or function of various channels have been linked to schizophrenia [68–70], AD [71–74], as well as with aberrant gamma oscillations [71, 75, 76]. Ligand-gated ion channels, critical to neuronal plasticity and long-term potentiation, and functionally relevant to cognitive disorders including AD [77, 78] and schizophrenia [79, 80], are also regulated by GSK-3 β . GSK-3 β forms a complex with the GluA1 and GluA2 subunits of the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor in rat HIP [19], and phosphorylates accessory proteins associated with AMPA receptor mobilization and removal from the plasma membrane [81–83]. Similarly, GSK-3 β plays a critical role in N-methyl-D-aspartate (NMDA) receptor-dependent plasticity, with its activity determining whether NMDA receptor activation induces, or inhibits, long-term depression [19]. β -catenin, perhaps the most a well-known substrate of GSK-3 β , has also been implicated in both AD [84] and schizophrenia [85]. Some evidence also suggests that GSK-3 β can also inhibit brain-derived neurotrophic factor signaling [86, 87], a protein widely known to play a pivotal role in cognition [88].

Conclusions

In conclusion, our findings indicate that increased GSK-3 β activity and neural oscillatory dysfunction in disorders of cognitive decline are intimately connected. That increased GSK-3 β activity in PFC or HIP could dysregulate neural oscillatory patterns and induce learning and memory deficits suggests that this protein may not only play a more prominent role in the early cognitive deficits associated with AD, but may also have a more widespread and functionally relevant involvement in regulating systems activity in disorders of cognitive dysfunction. More research into the mechanisms by which GSK-3 β regulates oscillatory systems function, via tau-dependent and independent processes, will provide fundamental information on its role in cognition.

Declarations

Ethics Approval and Consent to Participate

All the protocols have been followed are in accordance with the guidelines set out by the Animal Care Committee (ACC) at the University of Guelph.

Consent for Publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

AA performed the experiments, analyzed the data, and assisted writing the manuscript. OW assisted performing experiments. MP designed the study, assisted with the data analysis and contributed to writing the manuscript. All authors read and approved the final manuscript.

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Not applicable.

Abbreviations

AAV Adeno-associated viral vector

AD Alzheimer's disease

Akt Protein kinase-B

AMPA α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

EEG Electroencephalogram

GSK-3 β Glycogen synthase kinase-3 β

HA Hemagglutinin

HIP Hippocampus

LFP Local field potential

NMDA N-methyl-D-aspartate

NOR Novel Object Recognition

OiP Object in Place

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Figures

Fig.1

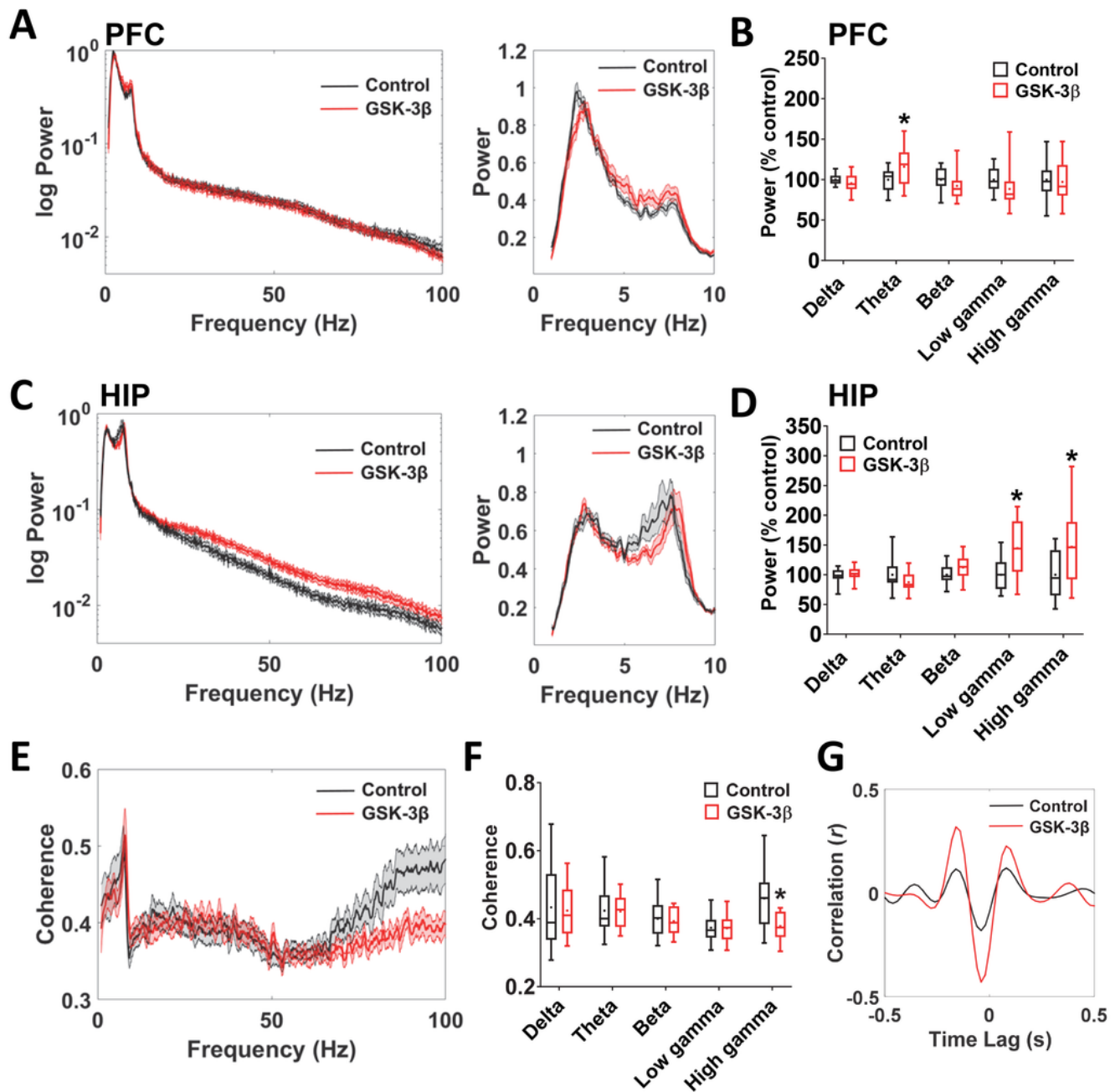


Figure 1

Effect of increased PFC GSK-3 β activity on neural oscillatory activity in rats. a Power spectrum showing changes in low and high frequency oscillatory power in PFC in response to increased PFC GSK-3 β activity. b Quantification of PFC power spectrum showing increased theta power. c Power spectrum showing changes in low and high frequency oscillatory power in HIP in response to increased PFC GSK-3 β activity. d Quantification of HIP power spectrum showing increased low and high gamma power. e, f

Increased PFC GSK-3 β suppressed PFC-HIP high gamma coherence. g Increased HIP theta-PFC gamma coupling with no effect on the temporal relationship of the two frequency bands. Power and coherence curves are presented as normalized data with jackknife estimates of SEM shown as shaded areas. N=9-10 rats, 1-2 electrodes/rat. *P<0.05, **P<0.01, student's t-test.

Fig.2

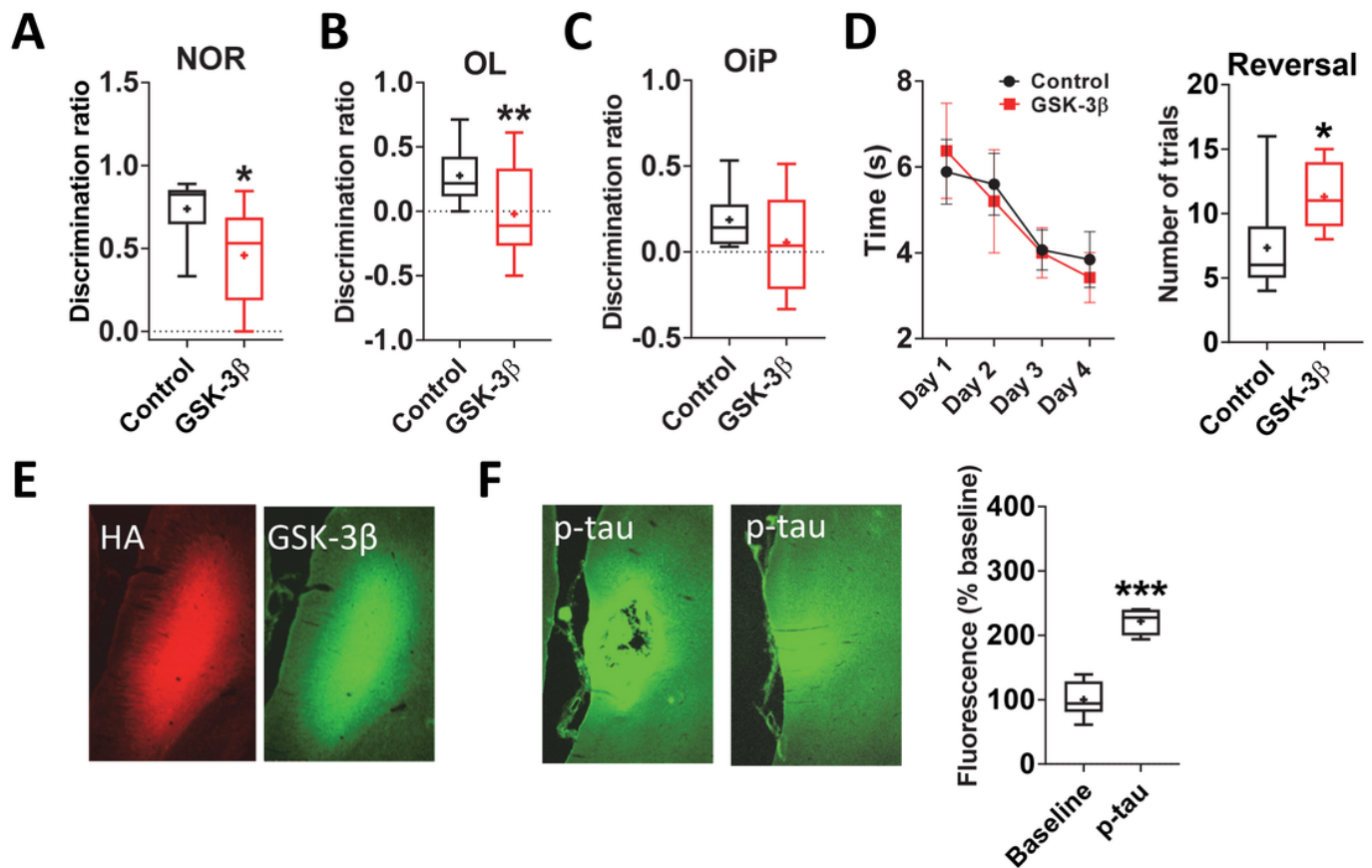


Figure 2

Increased GSK-3 β activity in PFC induces deficits in learning and memory. a, b Elevated GSK-3 β activation in PFC induced deficits in object recognition in the NOR test, but not in associative recognition memory when tested in the OiP. c Deficits in spatial memory in the OL test were also evident. d In the T-maze, both groups learned the location of the treat (left panel), with PFC GSK-3 β rats requiring more trials to learn the new location of the treat in a reversal learning test (right panel). *P<0.05, **P<0.01, student's t-test. e Representative immunohistochemistry images showing AAV-induced expression of the HA tag concomitant with increased expression of GSK-3 β in the PFC. f Representative images and quantification

showing tau hyperphosphorylation in response to increased GSK-3 β . N=9 rats/group, ***P<0.001, paired t-test.

Fig.3

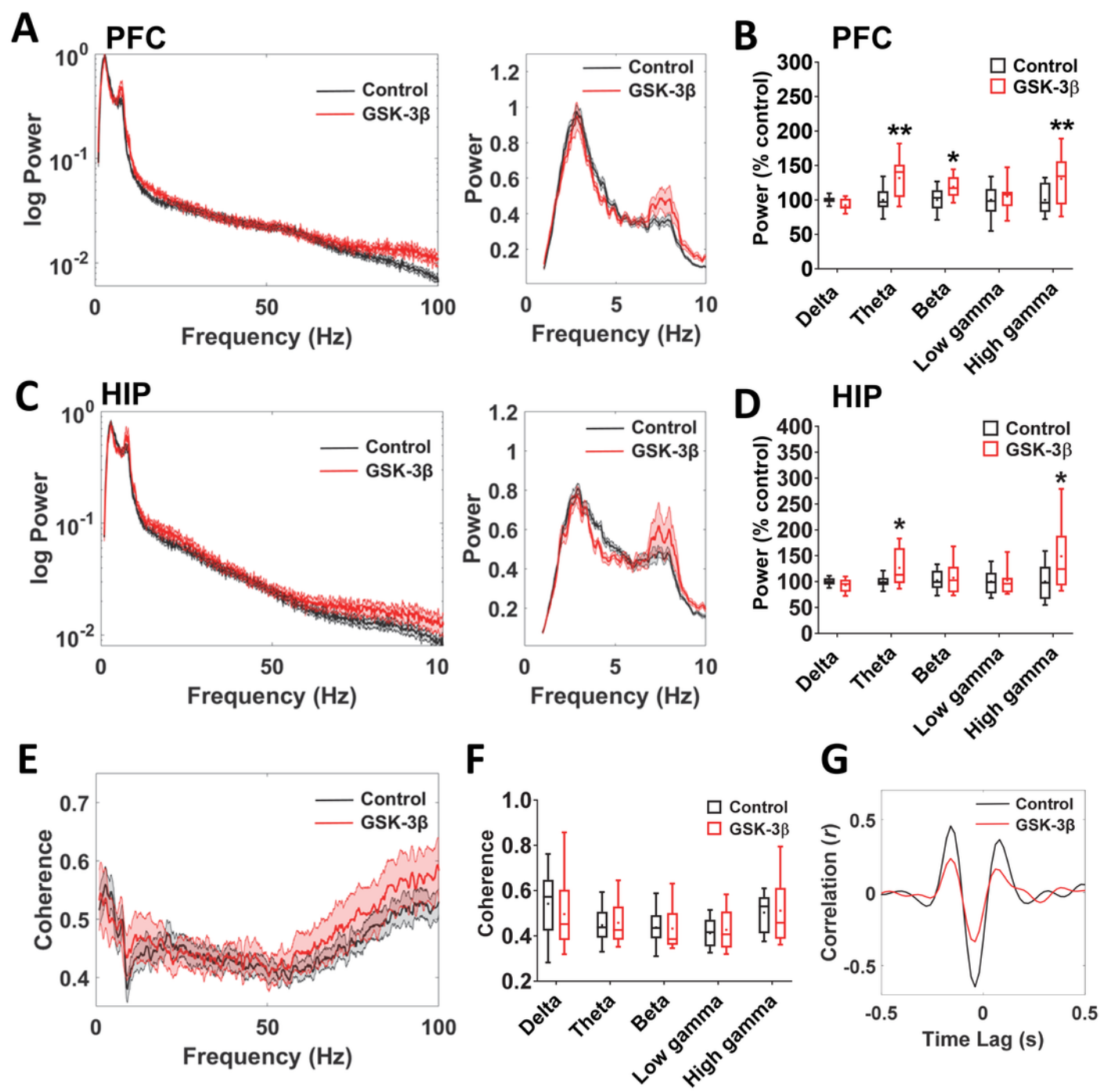


Figure 3

Effect of increased HIP GSK-3 β activity on neural oscillatory activity in rats. a Power spectrum showing changes in low and high frequency oscillatory power in PFC in response to increased HIP GSK-3 β activity. b Quantification of PFC power spectrum showing increased theta power, beta power, and high gamma

power. c Power spectrum from HIP displaying alterations in low and high frequency oscillatory power. d Quantification of HIP power spectrum with increased theta power and high gamma power shown. e, f There were no group differences in PFC-HIP coherence. g There was a reduction in HIP theta-PFC gamma coupling with no effect on the temporal relationship of the two frequency bands. Power and coherence curves are presented as normalized data with jackknife estimates of SEM shown as shaded areas. N=8-10 rats, 1-2 electrodes/rat. * $P<0.05$, ** $P<0.01$, student's t-test.

Fig.4

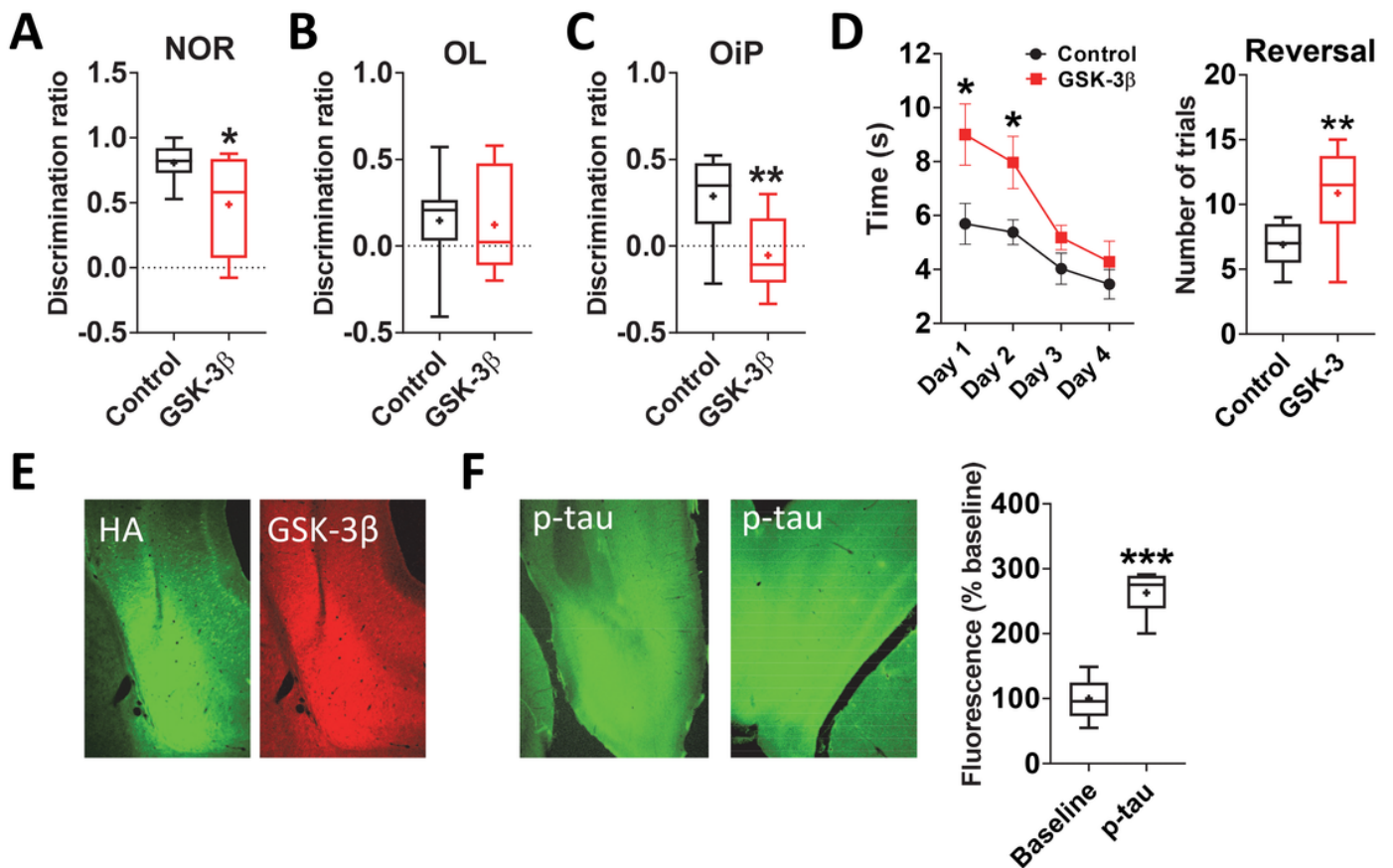


Figure 4

Increased HIP GSK-3 β activity induces deficits in learning and memory. a, b Increased HIP GSK-3 β activity induced deficits in object recognition and associative recognition in the OiP test. c No effects were observed in spatial memory in the OL test. d In the T-maze, increased HIP GSK-3 β activity inhibited spatial learning, taking longer to learn the location of the treat on days 1 and 2 of training ($F(1, 14)=7.86$, $P=0.014$, repeated measures ANOVA) (left panel). HIP GSK-3 β rats showed also exhibit a deficit in reversal learning, requiring more trials to learn the treat location (right panel). e Representative immunohistochemistry images showing AAV-induced expression of the HA tag concomitant with increased expression of GSK-3 β in the HIP. f Representative images and quantification showing tau hyperphosphorylation in response to increased GSK-3 β N=8 rats/group. *** $P<0.001$, paired t-test.