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RESEARCH ARTICLE



Reducing brain kynurenic acid synthesis precludes kynurenine-induced sleep disturbances

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Summary

Patients with neurocognitive disorders often battle sleep disturbances. Kynurenic acid is a tryptophan metabolite of the kynurenine pathway implicated in the pathology of these illnesses. Modest increases in kynurenic acid, an antagonist at glutamatergic and cholinergic receptors, result in cognitive impairments and sleep dysfunction. We explored the hypothesis that inhibition of the kynurenic acid synthesising enzyme, kynurenine aminotransferase II, may alleviate sleep disturbances. At the start of the light phase, adult male and female Wistar rats received systemic injections of either: (i) vehicle; (ii) kynurenine (100 mg kg⁻¹; i.p.); (iii) the kynurenine aminotransferase II inhibitor, PF-04859989 (30 mg kg⁻¹; s.c.); or (iv) PF-04859989 and kynurenine in combination. Kynurenine and kynurenic acid levels were evaluated in the plasma and brain. Separate animals were implanted with electroencephalogram and electromyogram telemetry devices to record polysomnography, and evaluate the vigilance states wake, rapid eye movement sleep and non-rapid eye movement sleep following each treatment. Kynurenine challenge increased brain kynurenic acid and resulted in reduced rapid eye movement sleep duration, non-rapid eye movement sleep delta power and sleep spindles. PF-04859989 reduced brain kynurenic acid formation when given prior to kynurenine, prevented disturbances in rapid eye movement sleep and sleep spindles, and enhanced non-rapid eye movement sleep. Our findings suggest that reducing kynurenic acid in conditions where the kynurenine pathway is activated may serve as a potential strategy for improving sleep dynamics.

KEYWORDS

α7 nicotinic acetylcholine receptor, neuropharmacology, non-rapid eye movement sleep, rapid eye movement sleep, sleep spindles, tryptophan

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1 | INTRODUCTION

Healthy sleep promotes synaptic plasticity and is necessary for the maintenance of newly formed memories (Aton et al., 2009). Cortical electroencephalography (EEG) combined with electromyography (EMG) is readily employed to distinguish wake from sleep states. Non-rapid eye movement sleep (NREMS) presents as high-amplitude cortical delta oscillations (0.5–4 Hz) from thalamocortical networks, and rapid eye movement sleep (REMS) exhibits high-amplitude frequency theta oscillations (4–8 Hz) from corticolimbic networks with muscle atonia. NREMS is largely implicated in procedural memory, and REMS is considered critical to synaptic pruning, behavioural refinement and memory consolidation (Abel et al., 2013; Boyce et al., 2017). Patients with neurocognitive disorders often experience sleep dysregulation, such as fragmented sleep, insomnia, poor quality of sleep, aberrant sleep spindles, REMS behaviour disorders and greater symptom severity following sleep disruption (Lucey et al., 2021; Pocivavsek & Rowland, 2018). Despite the integral relationship between sleep and cognition, there is currently a lack of FDA-approved therapeutic options suitable to treat both cognitive symptoms and sleep disturbances.

To investigate a novel pharmacological intervention, we presently explore the impact of inhibiting kynurenine aminotransferase II (KAT II) on sleep physiology in rodents. KAT II is the primary synthesising enzyme for brain-derived kynurenic acid (KYNA), a metabolite of the kynurenine pathway (KP) of tryptophan metabolism (Figure 1a). The essential amino acid tryptophan is pivotal to circadian rhythmicity via the regulatory role of serotonin and melatonin in sleep and arousal (Rapoport & Beisel, 1968). However, only about 5% of tryptophan is degraded through the melatonin pathway. The vast majority of tryptophan is metabolised via the KP, which generates the small neuroactive molecule KYNA that we hypothesise serves a critical role in modulating sleep (Milosavljevic et al., 2023; Pocivavsek et al., 2017; Rentschler et al., 2021).

Elevations in KYNA disrupt cholinergic and glutamatergic-dependent processes, including learning and memory, and restoring a balance in KYNA promotes cognitive function (Kozak et al., 2014; Pocivavsek et al., 2011). Clinical and physiological significance for studying elevations in KYNA stems from growing evidence that increased KYNA is found in the brains of individuals with systemic infections, neurocognitive disease and psychiatric disorders (Linderholm et al., 2012; Miller et al., 2006; Sathyasaikumar et al., 2011; Schwarcz et al., 2012). To combat cognitive dysfunction associated with these illnesses, translational studies have evaluated pharmacological approaches to reduce KYNA in the brain by inhibiting the synthesising enzyme KAT II (Kozak et al., 2014; Pellicciari et al., 2006; Pocivavsek et al., 2011; Pocivavsek & Erhardt, 2023).

Our present goal was to pharmacologically inhibit KAT II as a mechanistic strategy to: (i) reduce de novo KYNA production after acute treatment with kynurenine, the direct bioprecursor of KYNA; and (ii) overcome sleep disturbances induced by excessive KYNA (Pocivavsek et al., 2017). We selected PF-04859989, a brain-penetrable and irreversible KAT II inhibitor (Klausing et al., 2020; Kozak et al., 2014), and confirmed its effectiveness to reduce de novo

KYNA formation when administered prior to kynurenine challenge. We thereby hypothesise that KAT II inhibition with PF-04859989 will effectively attenuate dysregulated sleep–wake behaviour in response to an acute kynurenine challenge.

2 | METHODS

2.1 | Animals

Adult male (250–350 g) and female (200–300 g) Wistar rats were obtained from Charles River Laboratories. Animals were kept in a facility fully accredited by the American Association for the Accreditation of Laboratory Animal Care on a 12/12 hr light–dark cycle, where lights on corresponded to zeitgeber time (ZT) 0 and lights off to ZT 12. All protocols were approved by Institutional Animal Care and Use Committees, and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 | Chemicals

L-Kynurenine sulphate (“kynurenine”, purity 99.4%) was obtained from Sai Advantium (Hyderabad, India). PF-04859989 was obtained from WuXi AppTec (purity 95%; Shanghai, China). Other chemicals were obtained from various suppliers and were of the highest commercially available purity. For intraperitoneal (i.p.) injections, kynurenine was prepared daily by dissolving in phospho-buffered saline and adjusting the pH to 7.4 with 0.1 N NaOH. For subcutaneous (s.c.) injections, PF-04859989 was prepared daily by dissolving in ultrapure water.

2.3 | Biochemical analysis

Male and female rats were treated acutely with vehicle (water, s.c.) or PF-04859989 (30 mg kg⁻¹, s.c.) at ZT 23.5, and vehicle (saline, i.p.) or kynurenine (100 mg kg⁻¹, i.p.) at ZT 0. Animals were killed at ZT 2 by CO₂ asphyxiation. Whole trunk blood was collected in tubes containing 25 µl K3-EDTA (0.15%) as an anticoagulant, and centrifuged (1000 g, 10 min) to separate plasma. Rapidly removed brains were put on ice, and the following regions were dissected: cerebellum, brainstem, hypothalamus, basal forebrain, hippocampus, cortex and frontal cortex. All samples were promptly snap-frozen on dry ice and stored at –80°C until biochemical analyses. Kynurenine and KYNA levels were determined by high-performance liquid chromatography (HPLC; Buck et al., 2020; Wright et al., 2021). Briefly, plasma samples were diluted (1:10) in ultrapure water, and tissue samples were weighed and sonicated (1:5, w/v) in ultrapure water before being further diluted (final concentration: 1:10, w/v). One-hundred microlitres of sample, either diluted plasma or tissue homogenate, was acidified with 25 µl of 6% (plasma) or 25% (tissue) perchloric acid, centrifuged (12,000 g, 10 min), and

20 μ l of the supernatant was subjected to HPLC. Kynurenine and KYNA were isocratically eluted from a ReproSil-Pur C18 column (4 \times 100 mm; Dr Maisch GmbH, Ammerbuch, Germany) using a mobile

phase containing 50 mM sodium acetate and 5% acetonitrile (pH adjusted to 6.2 with glacial acetic acid) at a flow rate of 0.5 ml min^{-1} . Zinc acetate (500 mM) was delivered after the column at a flow rate of 0.1 ml min^{-1} .

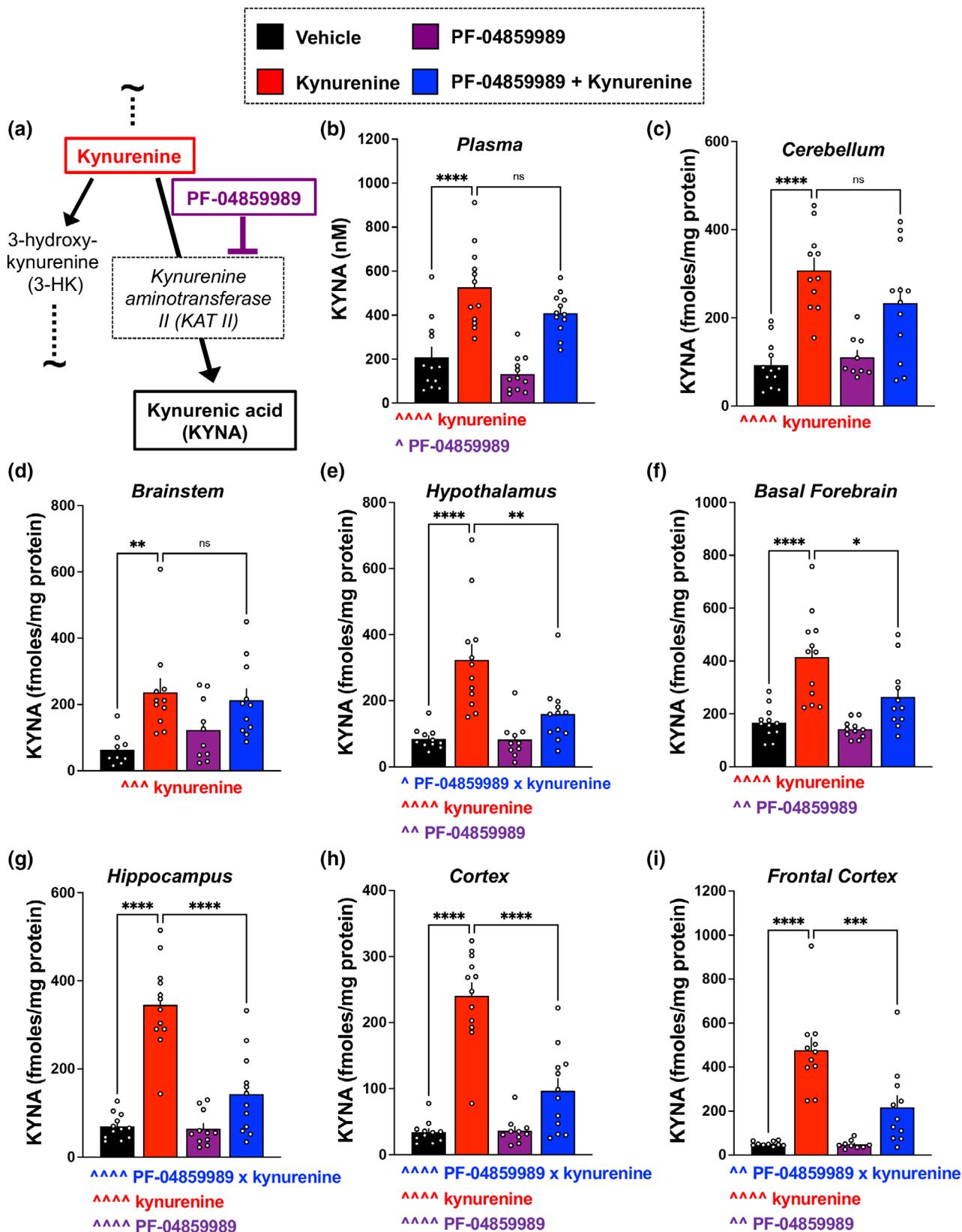


FIGURE 1 Legend on next page.

Kynurenine (excitation: 365 nm; emission: 480 nm; retention time \sim 7 min) and KYNA (excitation: 344 nm; emission: 398 nm; retention time \sim 13 min) were detected fluorometrically (Waters Alliance, 2475 fluorescence detector, Bedford, MA, USA) in the eluate. Data were integrated and analysed using Empower 3 software (Waters). The total concentration of protein in tissue samples was determined by the Lowry protein assay.

2.4 | Surgery

Under isoflurane anaesthesia (5% induction, 2%–3% maintenance), animals were placed in a stereotaxic frame (Stoelting, Wood Dale, IL, USA) for the surgical implantation of EEG/EMG telemetry transmitters (PhysiolTel HD-S02; Data Science International, St Paul, MN, USA) according to previously published protocols (Pocivavsek et al., 2017; Rentschler et al., 2021). The transmitter was inserted i.p. through a dorsal incision of the abdominal region. An incision at the midline of the head was made to anchor two EEG leads to their corresponding surgical screws (Plastics One, Roanoke, VA, USA) placed into 0.5-mm burr holes at 2.0 mm anterior/+1.5 mm lateral and 7.0 mm posterior/–1.5 mm lateral relative to bregma and secured with dental cement. Two EMG leads were inserted and sutured into the dorsal cervical neck muscle, separated by 1.0 mm. The skin on top of the head was sutured, the abdominal incision was closed with wound clips, and animals recovered for \geq 7 days prior to experimentation.

2.5 | Vaginal lavage and oestrous cycle tracking

To track the oestrous cycle, lavage of the vagina in females was taken using saline-filled transfer pipettes. Samples were viewed with a SeBa digital microscope (LAXCO, Mill Creek, WA, USA) within 30 min of sample collection. Best efforts were made to avoid drug treatments during proestrus, wherein sleep and wake parameters are vastly different from other cycle stages for female subjects (Swift et al., 2020).

2.6 | Drug treatments for sleep data acquisition

2.6.1 | Sleep study #1

Counterbalanced vehicle (saline, i.p.) or kynurenine (100 mg kg^{-1} , i.p.) injections were given 96 hr apart at ZT 0. Polysomnography data were acquired for 24 hr after each treatment.

2.6.2 | Sleep study #2

On the first day of the experiment, animals were injected with vehicle (water, s.c.) at ZT 23.5 and vehicle (saline, i.p.) at ZT 0 to obtain baseline sleep data (denoted “vehicle” in graphs). After 96 hr, animals received PF-04859989 (30 mg kg^{-1} , s.c.) at ZT 23.5, and either vehicle (saline, i.p.; denoted “PF-04859989 + vehicle” in graphs) or kynurenine (100 mg kg^{-1} , i.p.; denoted “PF-04859989 + kynurenine” in graphs) at ZT 0. The experimental schedule was counterbalanced and repeated, such that each animal received PF-04859989 + vehicle and PF-04859989 + kynurenine injections. Sleep data acquisition occurred for 24 hr after ZT 0 treatment. Treatments were spaced 96 hr apart to record sleep within the same oestrous phase for female subjects.

2.7 | Sleep data acquisition and analyses

Animals undergoing sleep recordings were left undisturbed in a designated room. EEG/EMG data and home cage activity were recorded using Ponemah 6.10 software (DSI) with a continuous sampling rate of 500 Hz (Milosavljevic et al., 2023; Pocivavsek et al., 2017; Rentschler et al., 2021). All digitised signal data were scored offline with NeuroScore 3.0 (DSI) using 10-s epochs as wake (low-amplitude, high-frequency EEG combined with high-amplitude EMG), NREMS (high-amplitude, low-frequency EEG with low-amplitude EMG) or REMS (low-amplitude, high-frequency EEG with very low-amplitude EMG). [Supplemental Information](#) includes representative hypnograms and continuous EEG and EMG recordings. Transitions among vigilance states were scored when two or more 10-s epochs of the new state were noted. Parameters assessed included total vigilance state duration, number of bouts, average bout duration, number of transitions between vigilance states, bout length distribution and spectral power frequencies during REMS and NREMS. Discrete fast Fourier transform was used to calculate the EEG power spectrum for each epoch in the delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), sigma (12–16 Hz) and beta (16–20 Hz) frequency ranges, and normalised to the total power per subject. Sleep spindles were automatically detected based on previously employed methodology (Uygun et al., 2019), and verified in NeuroScore by manual expert scoring detection (see [Supplemental Information](#)).

2.8 | Statistical analyses

Normality of data was assessed with Shapiro–Wilk test, and data were visually inspected using Q–Q plots to confirm a relative bell-shaped

FIGURE 1 Kynurenic acid (KYNA) levels in plasma and across brain regions are increased after the acute kynurenine challenge, and reversed after pretreatment with the kynurenine aminotransferase II (KAT II) inhibitor, PF-04859989. (a) Schematic representation of truncated kynurenine pathway (KP) demonstrating key metabolites and pharmacological tools used in the present study. Adult rats were peripherally injected at Zeitgeber time (ZT) 0 with kynurenine (100 mg kg^{-1}) to induce de novo KYNA formation. PF-04859989 (30 mg kg^{-1}), systemically active KAT II inhibitor, was given 30 min prior at ZT 23.5. Tissues were harvested at ZT 2. KYNA levels were evaluated in the: (b) plasma; (c) cerebellum; (d) brainstem; (e) hypothalamus; (f) basal forebrain; (g) hippocampus; (h) cortex; (i) frontal cortex. All data are mean \pm SEM. Two-way ANOVA analyses: $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.0001$; Bonferroni's post hoc test: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. $N = 9$ –12 per group.

distribution and the absence of outliers. For biochemical analyses, comparisons were made using a two-way analysis of variance (ANOVA) with kynurenine and/or PF-04859989 treatment as between-subject factors. Significant main effects were followed by Bonferroni's correction with an adjusted alpha for multiple comparisons. To evaluate sex as a biological variable in sleep architecture data (vigilance state durations, bout number, and average bout duration), we initially employed three-way repeated-measures (RM) ANOVA with treatment and ZT as within-subject factors, and sex as a between-subject factor. As no main effects of sex were determined (Supplemental Table S1), the remaining analyses were conducted with sexes combined. Durations were evaluated in 1-hr bins, followed by 6-hr bins, while bout number and average bout duration were evaluated in 6-hr bins employing two-way RM ANOVA with treatment and ZT as within-subject factors. REMS theta power spectra and NREMS delta power spectra were evaluated during the light phase by two-way RM ANOVA with frequency and treatment as within-subject factors. Spindle density, average spindle duration and spindle amplitude were evaluated by two-way RM ANOVA with treatment and ZT as within-subject factors. Power spectra during spindles were evaluated with two-way RM ANOVA with frequency and treatment as within-subject factors. Vigilance state transitions were evaluated by paired Student's *t*-test. Relative cage activity was analysed by three-way RM ANOVA with treatment and ZT as within-subject factors, and sex as a between-subject factor. Post hoc analysis was conducted with Bonferroni's correction in Sleep Study #1 and Dunnett test in Sleep Study #2, to compare drug treatments with vehicle treatment condition. Percent of vehicle calculations (REMS duration, NREMS slow-wave spectral power and spindle density) allowed comparisons between Sleep Study #1 and Sleep Study #2, thereby comparing PF-04859989 + kynurenine to kynurenine treatment alone with unpaired Student's *t*-test. All statistical analyses were performed using Prism 9.0 (GraphPad Software, La Jolla, CA, USA) and significance was defined as $p < 0.05$.

3 | RESULTS

3.1 | Systemic administration of KAT II inhibitor PF-04859989 attenuates elevation in brain KYNA levels induced by acute kynurenine challenge

We first confirmed that acute systemic kynurenine treatment at ZT 0, start of the light phase, elevates plasma kynurenine levels at ZT 2 compared with vehicle treatment (Table S2). Kynurenine-treated rats exhibited a 2.8-fold increase in plasma kynurenine levels compared with vehicle-treated rats ($p < 0.0001$). As kynurenine promptly enters the brain from the blood, we confirmed significant elevation in brain kynurenine levels in the cerebellum (6.2-fold; $p < 0.0001$), brainstem (2.3-fold; $p < 0.0001$), hypothalamus (2.5-fold; $p < 0.0001$), basal forebrain (3.9-fold; $p < 0.0001$), hippocampus (5.2-fold; $p < 0.0001$), cortex (4.1-fold; $p < 0.0001$) and frontal cortex (3.6-fold; $p < 0.0001$) compared with vehicle. Levels of kynurenine in plasma and brain remained unchanged after pretreatment with PF-04859989.

Upon kynurenine treatment at ZT 0, plasma KYNA increased 2.5-fold compared with vehicle-treated rats ($F_{1,43} = 73.71$, $p < 0.0001$; Figure 1b). Across brain regions (Figure 1c–i), we similarly determined that kynurenine treatment elevated KYNA content in the cerebellum (3.3-fold; $F_{1,40} = 40.95$, $p < 0.0001$), brainstem (3.7-fold; $F_{1,37} = 15.74$, $p < 0.0001$), hypothalamus (3.8-fold; $F_{1,41} = 27.20$, $p < 0.001$), basal forebrain (2.5-fold; $F_{1,42} = 32.66$, $p < 0.0001$), hippocampus (5.0-fold; $F_{1,43} = 71.33$, $p < 0.0001$), cortex (7.0-fold; $F_{1,41} = 80.26$, $p < 0.0001$) and frontal cortex (9.2-fold; $F_{1,37} = 49.44$, $p < 0.0001$). PF-04859989 significantly impacted KYNA levels noted as a main effect of treatment peripherally in plasma ($F_{1,43} = 4.848$, $p < 0.05$) and also in the hypothalamus ($F_{1,41} = 7.497$, $p < 0.01$), basal forebrain ($F_{1,42} = 7.396$, $p < 0.01$), hippocampus ($F_{1,43} = 24.23$, $p < 0.0001$), cortex ($F_{1,41} = 22.71$, $p < 0.0001$) and frontal cortex ($F_{1,37} = 9.691$, $p < 0.0001$). Pretreatment with PF-04859989 attenuated the increase in KYNA following acute kynurenine challenge, noted by a PF-04859989 \times kynurenine interaction in the hypothalamus ($F_{1,41} = 7.076$, $p < 0.05$), hippocampus ($F_{1,43} = 22.00$, $p < 0.0001$), cortex ($F_{1,41} = 23.98$, $p < 0.0001$) and frontal cortex ($F_{1,37} = 9.248$, $p < 0.01$). Post hoc analysis noted a significant reduction in KYNA when PF-04859989 was given prior to kynurenine compared with kynurenine alone in the hypothalamus ($p < 0.01$), basal forebrain ($p < 0.05$), hippocampus ($p < 0.0001$), cortex ($p < 0.0001$) and frontal cortex ($p < 0.01$). PF-04859989 pretreatment did not significantly reduce KYNA formation in the plasma, cerebellum and brainstem. In summary, Table 1 indicates the percent increase in KYNA levels from vehicle with kynurenine treatment (top panel) and the percent decrease in KYNA levels when PF-04859989 was given prior to kynurenine challenge (bottom panel) in the plasma and individual brain regions investigated.

3.2 | Kynurenine treatment reduces sleep, and alters architecture, spectral power and spindle characteristics

Sleep Study #1 was conducted in a cohort of rats used exclusively for sleep monitoring experiments. No main effects of sex were found when sex was included in the ANOVA of sleep data (Table S1). Vigilance state durations in 1-hr bins are shown separately for males and females in Figure S1. For the remaining analysis of vigilance states, we combined sexes. When analysed with 1-hr bins, REMS duration decreased significantly for kynurenine treatment compared with vehicle treatment (ZT \times treatment: $F_{23,437} = 1.635$, $p < 0.05$; treatment: $F_{1,19} = 15.14$, $p < 0.01$). The starkest REMS duration decrease occurred in the later portion of the light phase at ZT 10 ($p < 0.01$; Figure 2a). Analyses in 6-hr time bins supported the notion that REMS was disrupted with kynurenine challenge, particularly in the latter half of the light phase (ZT 6–12). REMS duration (treatment: $F_{1,19} = 15.15$, $p < 0.001$; Figure 2b) and REMS bouts (treatment: $F_{1,19} = 8.510$, $p < 0.01$; Figure 2c) were significantly reduced with kynurenine challenge. Parameters that were not significantly altered with kynurenine treatment related to REMS included the average duration of each REMS bout (Figure S2a) and spectra power in the theta range during the light phase (Figure S2b).

TABLE 1 Relative change in KYNA levels after kynurenine challenge and following PF-04859989 pretreatment

	Plasma	Cerebellum	Brainstem	Hypothalamus	Basal forebrain	Hippocampus	Cortex	Frontal cortex
% Increase from vehicle								
Kynurenine	201 ± 30****	232 ± 30****	273 ± 65**	280 ± 55****	148 ± 29****	399 ± 41****	599 ± 58****	824 ± 110****
% Reduction from kynurenine challenge								
PF-04859989 + kynurenine	34 ± 8	34 ± 17	14 ± 20	69 ± 11**	61 ± 15*	73 ± 10****	70 ± 9****	61 ± 13****

Note: Adult rats were peripherally injected at ZT 0 with kynurenine (100 mg kg⁻¹) to induce de novo KYNA formation. PF-04859989 (30 mg kg⁻¹), systemically active KAT II inhibitor, was given 30 min prior at ZT 23.5. Tissues were harvested at ZT 2. All data are mean ± SEM. N = 11–12 per group. **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001.

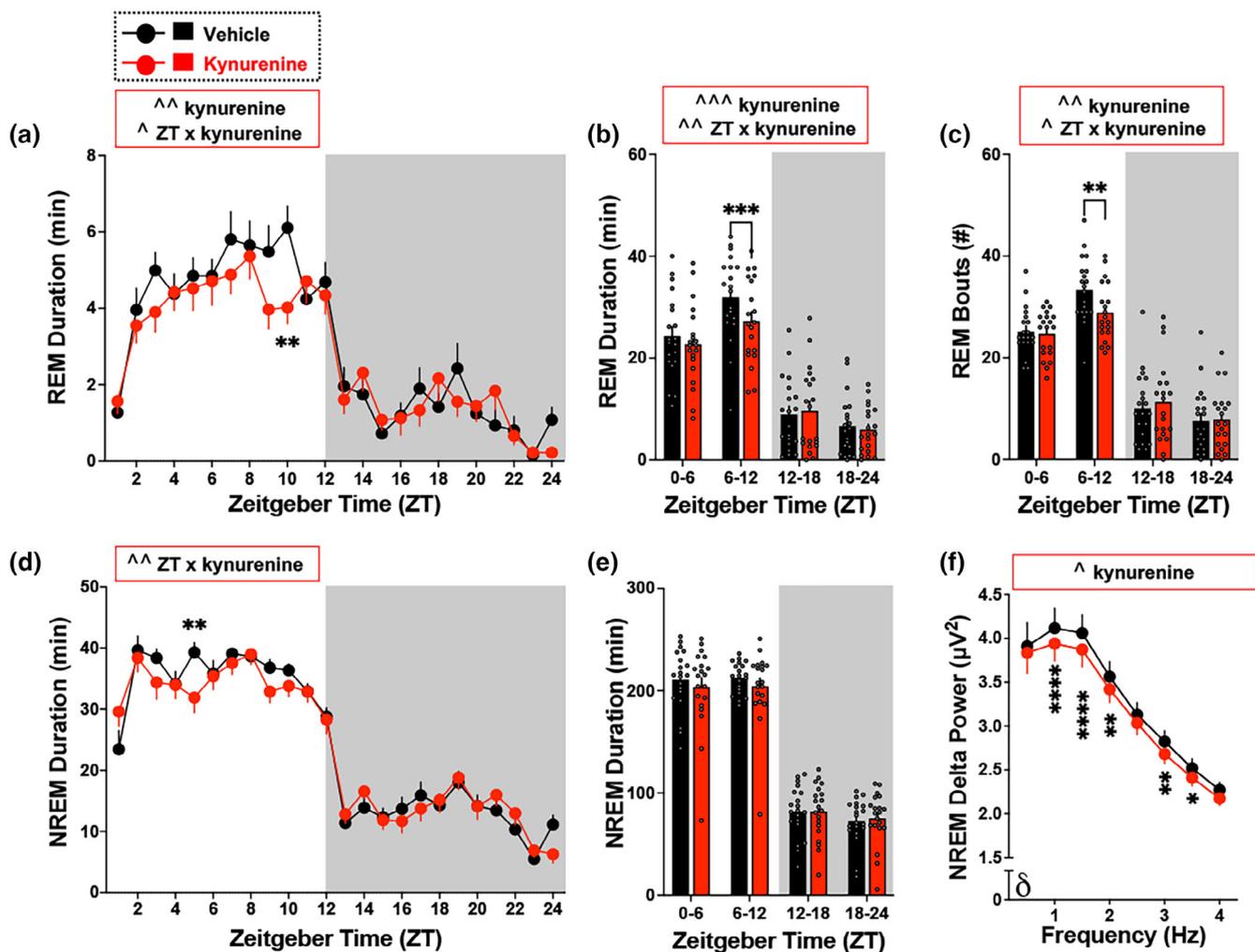


FIGURE 2 Kynurenine challenge reduces sleep and alters sleep architecture. Adult rats were treated with vehicle or kynurenine (100 mg kg⁻¹) at zeitgeber time (ZT) 0. Data are mean ± SEM, analysed by two-way repeated-measures (RM) ANOVA, with significance shown in graphs as ^*p* < 0.05, ^^*p* < 0.01, ^^^*p* < 0.001 and Bonferroni's post hoc test ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001. (a) 1-hr bins of rapid eye movement sleep (REMS) duration. ZT × treatment: $F_{23,437} = 1.635$, *p* < 0.05; treatment: $F_{1,19} = 15.14$, *p* < 0.01; ZT: $F_{23,437} = 30.82$, *p* < 0.0001. (b) 6-hr bins of REMS duration. ZT × treatment: $F_{3,57} = 4.162$, *p* < 0.01; treatment: $F_{1,19} = 15.15$, *p* < 0.001; ZT: $F_{3,57} = 99.50$, *p* < 0.0001. (c) 6-hr bins of REMS bout number. ZT × treatment: $F_{3,57} = 3.74$, *p* < 0.05; treatment: $F_{1,19} = 8.510$, *p* < 0.01; ZT: $F_{3,57} = 112.9$, *p* < 0.0001. (d) 1-hr bins of non-rapid eye movement sleep (NREMS) duration. ZT × treatment: $F_{23,437} = 2.032$, *p* < 0.01; ZT: $F_{23,437} = 69.02$, *p* < 0.0001. (e) 6-hr bins of NREMS duration. ZT: $F_{3,57} = 247.3$, *p* < 0.0001; (f) NREMS delta spectral power during the entire light phase. Treatment: $F_{1,17} = 4.615$, *p* < 0.05; frequency: $F_{7,119} = 64.15$, *p* < 0.0001. N = 20 per group (8 males, 12 females).

Kynurenine treatment induced changes to NREMS duration that varied across 1-hr bin analysis (treatment \times ZT interaction: $F_{23,437} = 2.032$, $p < 0.01$). NREMS duration decreased significantly at ZT 5 ($p < 0.01$) with kynurenine treatment compared with vehicle (Figure 2d). Analyses in 6-hr time bins revealed no significant changes in NREMS duration (Figure 2e), NREMS bout number (Figure S2c) and average duration of NREMS bouts (Figure S2d). Delta (0.5–4 Hz) spectral power was highest compared with theta, alpha, sigma and beta during NREMS (light phase: $F_{38,722} = 414.2$, $p < 0.0001$; data not shown). During the entire light phase, kynurenine treatment significantly reduced NREMS delta spectral power (treatment: $F_{1,17} = 4.615$, $p < 0.05$; post hoc 1 Hz: $p < 0.001$; 1.5 Hz: $p < 0.0001$; 2 Hz: $p < 0.01$; 3 Hz: $p < 0.01$; 3.5 Hz: $p < 0.05$; Figure 2f). Power spectra across 1-hr bin analysis is shown in Figure S2e.

The NREMS sleep spindles were detected during the light phase. Kynurenine treatment modified spindle density across 4-hr bin analysis (treatment \times ZT interaction: $F_{2,16} = 7.648$, $p < 0.01$), with reduced

spindle density from ZT 0 to ZT4 ($p < 0.001$; Figure 3a). Average duration of each spindle (Figure 3b), peak to peak amplitude (Figure 3c) and the frequency distribution of spindle power (Figure 3d) remained unchanged when comparing kynurenine and vehicle treatments.

3.3 | Kynurenine challenge alters wake architecture

To understand how the observed changes in REMS and NREMS impacted arousal, wake state architecture and general home cage activity were evaluated. Analysis of wake durations in 1-hr bins revealed a kynurenine \times ZT interaction ($F_{123,437} = 2.078$, $p < 0.01$) such that kynurenine initially reduced wake duration within the first hour ($p < 0.01$; Figure S3a). When wake parameters were evaluated in 6-hr bins, we did not find any significant changes in wake duration

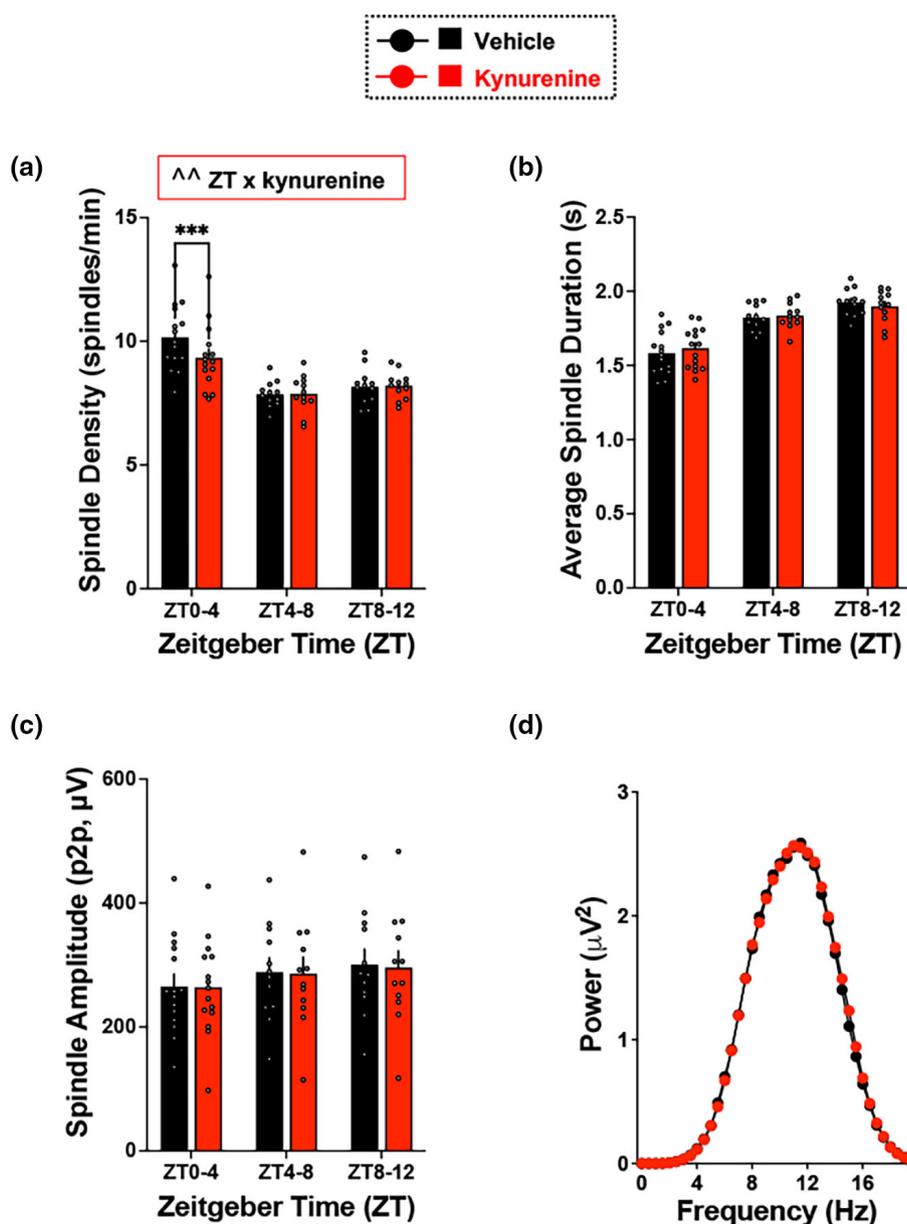


FIGURE 3 Kynurenine challenge reduces non-rapid eye movement sleep (NREMS) spindle density. Adult rats were treated with vehicle or kynurenine (100 mg kg^{-1}) at zeitgeber time (ZT) 0. Sleep spindles were evaluated during the light phase. Data are mean \pm SEM, analysed by two-way repeated-measures (RM) ANOVA. (a) NREMS spindle density (spindles per min NREMS). Treatment \times ZT interaction: $F_{2,16} = 7.648$, $p < 0.01$; ZT: $F_{2,28} = 23.43$, $p < 0.0001$. (b) Average spindle duration. ZT: $F_{2,28} = 133.0$, $p < 0.0001$. (c) Spindle peak to peak (p2p) amplitude. ZT: $F_{2,28} = 4.023$, $p < 0.05$. (d) NREMS spindle power. Frequency: $F_{39,741} = 185.0$, $p < 0.0001$. $N = 20$ per group (8 males, 12 females).

(Figure S3b), number of wake bouts (Figure S3c) or average duration of each wake bout (Figure S3d). Of note, sex significantly impacted home cage activity (three-way ANOVA, $F_{1,347} = 5.118$, $p < 0.05$), along with time of day ($F_{22,396} = 25.40$, $p < 0.0001$), yet kynurenine treatment did not change home cage activity across time (Figure S3e).

3.4 | Vigilance state transitions are impacted by kynurenine challenge

The stability of maintaining a wake or sleep episode upon kynurenine challenge was assessed by examining the number of transitions into and out of wake, NREMS sleep, and REMS sleep by light or dark phase (Table S3). Kynurenine challenge reduced REMS to NREMS ($p < 0.0001$) and NREMS to REMS ($p < 0.01$) transitions during the light phase. In the subsequent dark phase, kynurenine treatment resulted in enhanced wake to NREMS ($p < 0.05$) and REMS to wake

($p < 0.05$) transitions. Analysis of bout length distribution for the light phase showed that kynurenine treatment significantly decreased short NREMS bouts (< 0.5 min) and REMS bouts (0.5–1 min; Figure S4).

3.5 | KAT II inhibitor PF-04859989 attenuates changes in sleep architecture and spindle dynamics induced by acute kynurenine challenge

Pretreatment with PF-04859989 resulted in reduced de novo KYNA synthesis in the brain with systemic kynurenine challenge (Figure 1), thus we next explored if PF-04859989 would readily reverse the consequences of kynurenine challenge on sleep architecture. Sleep Study #2 was conducted in a new cohort of adult rats ($N = 18$), and each subject received the following treatments: (i) two vehicle injections (ZT 23.5 s.c., ZT 0 i.p.); (ii) PF-04859989 at ZT 23.5 + vehicle at ZT 0; (iii) PF-04859989 at ZT 23.5 + kynurenine at ZT 0. Our analysis focused on vigilance state parameters that were altered with

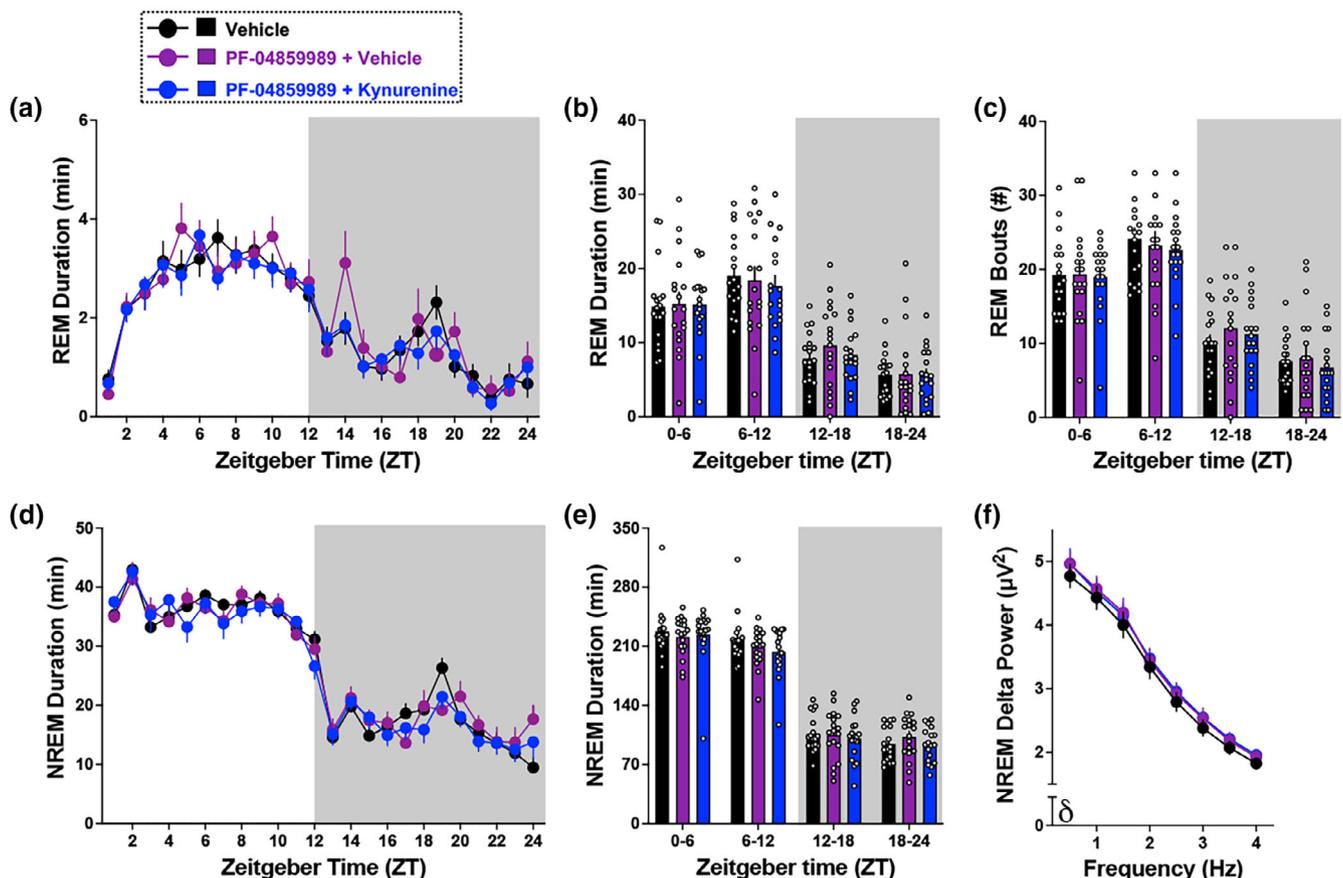


FIGURE 4 PF-04859989 treatment protects non-rapid eye movement sleep (NREMS) architecture. Adult rats were treated with vehicle, PF-04859989 (30 mg kg^{-1}) or PF-04859989 + kynurenine (100 mg kg^{-1}) at the start of the light phase. Data are mean \pm SEM, analysed by two-way repeated-measures (RM) ANOVA. (a) 1-hr bins of rapid eye movement sleep (REMS) duration. ZT: $F_{23,391} = 23.98$, $p < 0.0001$. (b) 6-hr bins of REMS duration. ZT: $F_{3,51} = 62.44$, $p < 0.0001$. (c) 6-hr bins of REMS bout number. ZT: $F_{3,51} = 76.02$, $p < 0.0001$. (d) 1-hr bins of NREMS duration. ZT: $F_{23,391} = 86.51$, $p < 0.0001$. (e) 6-hr bins of NREMS duration. ZT: $F_{3,51} = 304.4$, $p < 0.0001$. (f) NREMS delta spectral power during the entire light phase. Frequency: $F_{7,91} = 135.0$, $p < 0.0001$. $N = 18$ per group (9 males, 9 females).

kynurenine challenge, as reported above. Thus, focusing initially on REMS, we found that PF-04859989 pretreatment prevented kynurenine-induced REMS deficits, thereby stabilising REMS duration (1-hr bins: Figure 4a; 6-hr bins: Figure 4b), REMS bout number (Figure 4c), average duration of each REMS bout (Figure S5a) and theta spectral power during REMS (Figure S5b) to vehicle treatment levels. PF-04859989 pretreatment did not influence NREMS duration (1-hr bins: Figure 4d; 6-hr bins: Figure 4e), NREMS bout number (Figure S5c) and average duration of each NREMS bout (Figure S5d). Of note, PF-04859989 treatment, with or without kynurenine, did not alter NREMS delta power, in 1-hr bin (Figure S5e) or entire light phase (Figure 4f) analysis.

Evaluation of spindle dynamics revealed no significant differences in NREMS spindle density between treatments in Sleep Study #2 (Figure 5a), suggesting that PF-04859989 attenuated the reduction in NREMS spindle density elicited by kynurenine (comparison to Figure 3a). Average spindle duration and peak-to-peak amplitude were not impacted by PF-04859989 treatment (Figure 5b,c). Spectral

power within the 10–15-Hz sigma range was also not impacted by PF-04859989 treatment (Figure 5d).

3.6 | PF-04859989 prevents kynurenine-induced changes in wake behaviour

We found no significant differences in wake duration between Sleep Study #2 treatment conditions when evaluating data in 1-hr bins (Figure S6a) or 6-hr bins (Figure S6b). Wake bout number (Figure S6c) and average duration of each wake bout (Figure S6d) were also not altered by PF-04859989 treatment. As in Sleep Study #1 home cage activity data, we similarly evaluated activity separately by sex in Sleep Study #2. Home cage activity was significantly impacted by an interaction between ZT and treatment in males, with increased activity at ZT 1 (PF-04859989 + vehicle: $p < 0.001$; PF-04859989 + kynurenine: $p < 0.001$), and reduced activity at ZT 4 (PF-04859989 + kynurenine: $p < 0.05$) and ZT 24 (PF-04859989 + vehicle: $p < 0.001$; PF-04859989 + kynurenine:

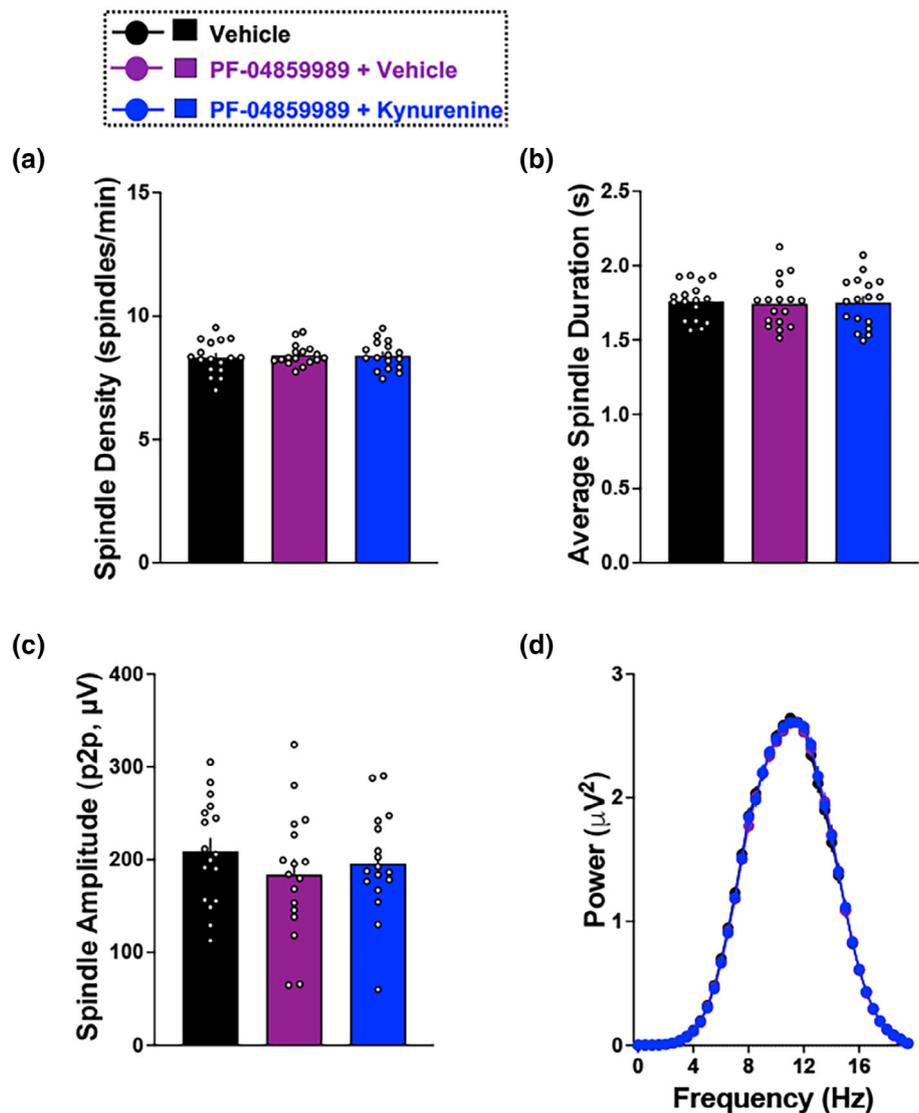


FIGURE 5 Pretreatment with PF-04859989 prior to kynurenine challenge restores non-rapid eye movement sleep (NREMS) spindle dynamics. Adult rats were treated with vehicle, PF-04859989 (30 mg kg⁻¹) or PF-04859989 + kynurenine (100 mg kg⁻¹) at the start of the light phase. Sleep spindles were evaluated from ZT 0 to ZT 4. Data are mean ± SEM, analysed by repeated-measures (RM) ANOVA. (a) NREMS spindle density (spindles per min NREMS). $F_{2,32} = 0.2635$, $p = 0.7700$. (b) Average spindle duration. $F_{2,32} = 0.1742$, $p = 0.8409$. (c) Spindle peak to peak (p2p) amplitude. $F_{2,32} = 1.953$, $p = 0.1583$. (d) NREMS spindle power. Frequency: $F_{39,663} = 840.3$, $p < 0.0001$. $N = 18$ per group (9 males, 9 females).

$p < 0.01$; Figure S6e). PF-04859989 treatment with or without kynurenine did not change home cage activity in female subjects.

3.7 | Vigilance state transitions are not impacted by PF-04859989 treatment

PF-04859989 successfully rescued kynurenine-induced changes in sleep transitions (Table S4), as in Sleep Study #2 no significant differences in transitions were noted between treatment conditions. Bout length distribution analysis in the light phase showed no impact of PF-04859989 treatment (Figure S7).

3.8 | PF-04859989 treatment attenuates sleep disruptions induced by kynurenine challenge

Vigilance state durations, sleep spindle density and NREMS slow-wave spectral power were normalised to a percent change from vehicle treatment to compare results from Sleep Study #1 with Sleep Study #2. Kynurenine challenge resulted in a 11% decrease in REMS duration, and this impact was significantly attenuated with PF-04859989 + kynurenine treatment ($p < 0.0001$; Figure 6a). PF-04859989 given prior to kynurenine challenge significantly attenuated the reduction in slow-wave (0.5–2 Hz) spectral power during NREMS with kynurenine challenge alone (Figure 6b). Lastly, spindle density was reduced by 12%

with kynurenine challenge and PF-04859989 + kynurenine treatment substantially rescued this phenotype ($p < 0.01$; Figure 6c). Taken together, we demonstrate the ability of KAT II inhibitor PF-04859989 to reverse kynurenine-induced sleep spindle and REMS abnormalities (Figure 6).

4 | DISCUSSION

Altered tryptophan metabolism, including KP dysregulation, impacts sleep and arousal in normal and pathological conditions (Milosavljevic et al., 2023; Mukherjee et al., 2018; Pocivavsek et al., 2017; Rentschler et al., 2021; Yamashita, 2020). Using telemetric recordings for the acquisition of EEG, EMG and activity data in freely moving animals, we obtained ethologically relevant polysomnography recordings to evaluate sleep–wake behaviour with acute elevation of KYNA (kynurenine challenge) or inhibition of newly produced KYNA in the brain (PF-04859989 + kynurenine). Building upon previous work, which evaluated changes in sleep with a dose–response of kynurenine challenge in male rats (Pocivavsek et al., 2017), we presently expanded our understanding of the impact on sleep–wake behaviours by inhibiting KAT II, thereby reducing de novo KYNA formation, with a brain-penetrable and irreversible compound, PF-04859989, in male and female subjects.

Acute kynurenine challenge notably reduced the total duration of REMS and the architecture of sleep, denoted as reduced REMS bouts, time bin distribution of sleep bouts, and transitions from NREMS to

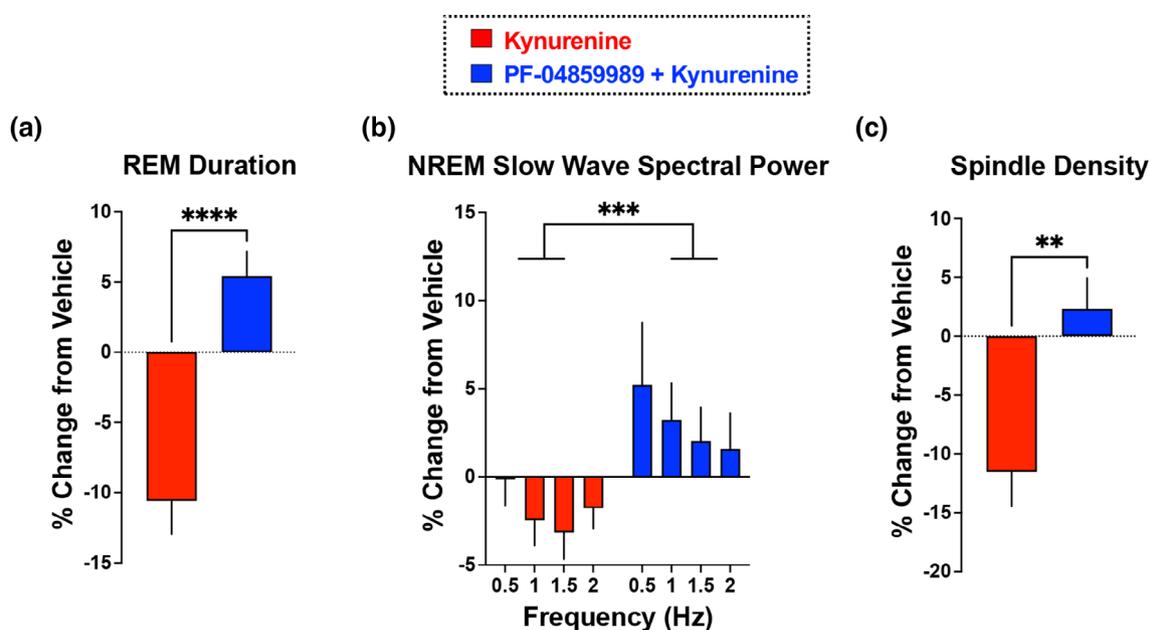


FIGURE 6 Pretreatment with PF-04859989 attenuates consequences of kynurenine challenge on rapid eye movement sleep (REMS) and non-rapid eye movement sleep (NREMS) parameters. In Sleep Study #1, adult rats were treated with vehicle or kynurenine (100 mg kg^{-1}) at zeitgeber time (ZT) 0. In Sleep Study #2, adult rats were treated with vehicle (ZT 23.5 and ZT 0), PF-04859989 (30 mg kg^{-1} ; ZT 23.5) + vehicle (ZT 0) or PF-04859989 (30 mg kg^{-1} ; ZT 23.5) + kynurenine (100 mg kg^{-1} ; ZT 0). To compare results from the two sleep studies, data were evaluated as percent change from vehicle. (a) REMS duration (ZT 0–12); Student's t -test, $t_{36} = 5.344$, **** $p < 0.0001$. (b) NREMS slow-wave spectral power (ZT 0–12), two-way ANOVA, $F_{1,144} = 12.31$, *** $p < 0.001$. (c) Spindle density (ZT 0–4); Student's t -test, $t_{36} = 3.495$, ** $p < 0.01$. All data are mean \pm SEM. Kynurenine: $N = 20$; PF-04859989 + kynurenine: $N = 18$.

REMS and vice versa. Changes in sleep–wake architecture induced by kynurenine challenge were prevented with prophylactic PF-04859989 treatment, and we observe that the reduced KYNA levels across various brain regions contributed to the attenuated sleep behaviour phenotypes. Namely, PF-04859989 effectively reduced KYNA levels in the hypothalamus, basal forebrain, hippocampus, cortex and frontal cortex, suggesting that the majority of KYNA synthesis in these regions is KAT II dependent. While we saw slight trends towards reduced KYNA levels in the cerebellum and brainstem with PF-04859989 treatment prior to kynurenine challenge, the effects were not significant thereby endorsing evidence of alternative means to regulate KYNA formation within these brain regions (Blanco Ayala et al., 2021). At the transcriptome level, KAT II mRNA encoded by *Aadat* are found prominently in cerebral regions such as the hippocampus, and much less in cerebellum and brainstem (Song et al., 2018). In the case of the cerebellum, KYNA formation may be more dependent on non-enzymatic formation with a role for reactive oxygen species, and a peripheral KAT II inhibitor may be less effective (Blanco Ayala et al., 2021).

The basal forebrain, a region wherein KYNA was both elevated with kynurenine challenge and reduced with PF-04859989 pre-treatment, is richly implicated in modulation of sleep and arousal behaviour (Lelkes et al., 2018; Szymusiak et al., 2000). A large amount of acetylcholine is released from the basal forebrain during REMS (Jing et al., 2020), and we postulate that KYNA-mediated modulation of cholinergic neurotransmission may impede these dynamics (Zmarowski et al., 2009). Within the extracellular milieu, KYNA is present within low micromolar concentrations and optimally positioned to inhibit receptor targets including $\alpha 7$ nACh receptors and NMDA receptors (Albuquerque & Schwarcz, 2013; Konradsson-Geuken et al., 2010). The stark decrease in REMS with acute KYNA elevation is presumably related to the inhibition of these targets. The fact that systemic application of PF-04859989 downregulated KYNA production and reversed the consequences of KYNA elevation on sleep behaviour provides compelling evidence for the role of KYNA, rather than the alternate arm of the KP that produces the metabolites 3-hydroxykynurenine, 3-hydroxyanthranilic acid and quinolinic acid (Schwarcz et al., 2012), in modulating changes in sleep physiology. Ongoing work with mouse models genetically modified to eliminate key enzymes of the KP will provide further support for the implicated role of KYNA in sleep behaviour (Erhardt et al., 2017).

Key sleep disruptions bring further attention to the concept that KYNA serves as a molecular intersection between sleep and cognitive function (Pocivavsek et al., 2011; Pocivavsek et al., 2017). Reduced NREMS duration, NREMS delta spectral power and disrupted transitions between NREMS and REMS with elevated KYNA may influence long-term memory consolidation (Tartar et al., 2006). Temporally, NREMS changes occurred during the early light phase, and REMS duration and architecture, which impact synaptic plasticity and memory (Boyce et al., 2016; Tartar et al., 2006), were subsequently impacted in the late light phase. Sleep spindles, oscillations produced by the thalamic reticular nucleus, support memory consolidation (Fernandez & Luthi, 2020) and are speculated to predict onset of REMS (Bandarabadi et al., 2020). Reduced spindle density is a hallmark endophenotype of sleep dysfunction in patients with psychotic disorders and considered a

predictor for cognitive dysfunction (D'Agostino et al., 2018; Ferrarelli & Tononi, 2017; Manoach et al., 2014). Preclinical models relevant to the study of neuropsychiatric illnesses have noted reduced sleep spindle density in rodents (Aguilar et al., 2020; Thankachan et al., 2019). Importantly, we show for the first time that a reduction of brain-derived KYNA, achieved with prophylactic PF-04859989 treatment, effectively corrected for kynurenine-induced spindle density deficiencies and REMS disturbances.

It is noteworthy that PF-04859989 precluded the reduced NREMS delta power induced by kynurenine treatment alone, suggesting that reducing KYNA accumulation improves the quality of slow-wave sleep (Lundahl et al., 2012). NREMS quality is directly correlated with improved neuropsychological functioning and remains a physiological point of therapeutic interest for cognitive impairment associated with insomnia, neuropsychiatric disorders and aging (Wilckens et al., 2018). It is plausible that KYNA modulates NREMS by influencing γ -aminobutyric acid (GABA) neurotransmission, as evidenced in microdialysis studies wherein elevated KYNA associates with reduced local extracellular GABA levels in rats (Beggiato et al., 2014; Wright et al., 2021). GABA receptor agonists are commonly prescribed for insomnia and NREMS disorders (Lankford et al., 2008; Walsh et al., 2011), yet these drugs, including benzodiazepines or zolpidem, carry a risk of drug dependence and side-effects including prolonged sleepiness, delirium, aggressivity and complex behaviours such as sleepwalking (Earl & Van Tyle, 2020). Our findings place new attention on KAT II inhibitors to modulate sleep quality, improve daytime functioning and arousal. Complementary experiments wherein we consider the use of KAT II inhibitors at different times of day and apply chronobiology to therapeutic approaches may have significant translational value to the treatment of sleep disturbances (Milosavljevic et al., 2023). Lastly, despite a number of disruptions in sleep parameters, we did not find major changes in arousal phenotypes with increased KYNA. However, enhanced home cage activity with PF-04859989 immediately after treatment warrants further investigation into its short-term efficacy in attentional tasks, as KAT II inhibitors have been shown to acutely enhance cognition (Klausing et al., 2020; Pocivavsek et al., 2011). Lastly, unanswered questions about microarousals and local sleep events could conceivably be addressed with technical advancements, including in automatic approaches to sleep scoring in future studies (Rayan et al., 2022; Vyazovskiy et al., 2011).

5 | CONCLUSIONS

In summary, our findings indicate an important association between KYNA, the KAT II enzyme, and sleep–wake behaviour. Our data suggest that pharmacological agents that inhibit KAT II activity in the brain may attenuate sleep disturbances. KYNA is considered a modulator of cognitive and sleep mechanisms, thereby KYNA may causally disrupt the relationship between sleep and cognition in pathological conditions. Sleep dysfunction and cognitive impairments are associated with a variety of neurocognitive disorders (Brzecka et al., 2018; Manoach et al., 2014), and similarly elevated KYNA levels are found in

the brains of patients with these conditions (Linderholm et al., 2012; Miller et al., 2006; Sathyaikumar et al., 2011). As such, regulating KYNA levels with KAT II inhibition should be carefully considered in the development of novel therapeutic strategies to treat sleep and cognitive disturbances (Pocivavsek & Erhardt, 2023), and these strategies may provide value to improving the quality of life in patients afflicted with neurocognitive disorders and associated sleep disruptions.

AUTHOR CONTRIBUTIONS

Katherine M. Rentschler: Writing – original draft; writing – review and editing; visualization; investigation; conceptualization; formal analysis; methodology. **Snezana Milosavljevic:** Investigation; writing – review and editing; visualization; formal analysis; methodology. **Annalisa M. Baratta:** Investigation; writing – review and editing; methodology; formal analysis; visualization. **Courtney J. Wright:** Investigation; writing – review and editing; visualization; methodology; formal analysis. **Maria V. Pirolli:** Investigation; methodology; visualization; writing – review and editing; formal analysis. **Zachary Tentor:** Investigation; writing – review and editing; methodology. **Homayoun Valafar:** Writing – review and editing; supervision; resources; formal analysis. **Christian O'Reilly:** Methodology; validation; writing – review and editing; investigation; formal analysis; resources. **Ana Pocivavsek:** Conceptualization; investigation; funding acquisition; writing – original draft; visualization; writing – review and editing; formal analysis; project administration; supervision; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All protocols were approved by Institutional Animal Care and Use Committees, and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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REFERENCES

- Abel, T., Havekes, R., Saletin, J. M., & Walker, M. P. (2013). Sleep, plasticity and memory from molecules to whole-brain networks. *Current Biology*, 23(17), R774–R788. <https://doi.org/10.1016/j.cub.2013.07.025>
- Aguilar, D. D., Strecker, R. E., Basheer, R., & McNally, J. M. (2020). Alterations in sleep, sleep spindle, and EEG power in mGluR5 knock-out mice. *Journal of Neurophysiology*, 123(1), 22–33. <https://doi.org/10.1152/jn.00532.2019>
- Albuquerque, E. X., & Schwarcz, R. (2013). Kynurenic acid as an antagonist of alpha7 nicotinic acetylcholine receptors in the brain: Facts and challenges. *Biochemical Pharmacology*, 85(8), 1027–1032. <https://doi.org/10.1016/j.bcp.2012.12.014>
- Aton, S. J., Seibt, J., Dumoulin, M., Jha, S. K., Steinmetz, N., Coleman, T., Naidoo, N., & Frank, M. G. (2009). Mechanisms of sleep-dependent consolidation of cortical plasticity. *Neuron*, 61(3), 454–466. <https://doi.org/10.1016/j.neuron.2009.01.007>
- Bandarabadi, M., Herrera, C. G., Gent, T. C., Bassetti, C., Schindler, K., & Adamantidis, A. R. (2020). A role for spindles in the onset of rapid eye movement sleep. *Nature Communications*, 11(1), 5247. <https://doi.org/10.1038/s41467-020-19076-2>
- Beggiato, S., Tanganelli, S., Fuxe, K., Antonelli, T., Schwarcz, R., & Ferraro, L. (2014). Endogenous kynurenic acid regulates extracellular GABA levels in the rat prefrontal cortex. *Neuropharmacology*, 82, 11–18. <https://doi.org/10.1016/j.neuropharm.2014.02.019>
- Blanco Ayala, T. B., Ramírez Ortega, D., Ovalle Rodríguez, P., Pineda, B., Pérez de la Cruz, G., González Esquivel, D., Schwarcz, R., Sathyaikumar, K. V., Jiménez Anguiano, A., & Pérez de la Cruz, V. (2021). Subchronic N-acetylcysteine treatment decreases brain Kynurenic acid levels and improves cognitive performance in mice. *Antioxidants*, 10(2), 147. <https://doi.org/10.3390/antiox10020147>
- Boyce, R., Glasgow, S. D., Williams, S., & Adamantidis, A. (2016). Causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation. *Science*, 352(6287), 812–816. <https://doi.org/10.1126/science.aad5252>
- Boyce, R., Williams, S., & Adamantidis, A. (2017). REM sleep and memory. *Current Opinion in Neurobiology*, 44, 167–177. <https://doi.org/10.1016/j.conb.2017.05.001>
- Brzecka, A., Leszek, J., Ashraf, G. M., Ejma, M., Ávila-Rodríguez, M. F., Yarla, N. S., Tarasov, V. V., Chubarev, V. N., Samsonova, A. N., Barreto, G. E., & Aliev, G. (2018). Sleep disorders associated with Alzheimer's disease: A perspective. *Frontiers in Neuroscience*, 12, 330. <https://doi.org/10.3389/fnins.2018.00330>
- Buck, S. A., Baratta, A. M., & Pocivavsek, A. (2020). Exposure to elevated embryonic kynurenic acid in rats: Sex-dependent learning and memory impairments in adult offspring. *Neurobiology of Learning and Memory*, 30(174), 107282. <https://doi.org/10.1016/j.nlm.2020.107282>
- D'Agostino, A., Castelnovo, A., Cavallotti, S., Casetta, C., Marcattili, M., Gambini, O., Canevini, M., Tononi, G., Riedner, B., Ferrarelli, F., & Sarasso, S. (2018). Sleep endophenotypes of schizophrenia: Slow waves and sleep spindles in unaffected first-degree relatives. *NPJ Schizophrenia*, 4(1), 2. <https://doi.org/10.1038/s41537-018-0045-9>
- Earl, D. C., & Van Tyle, K. M. (2020). New pharmacologic agents for insomnia and hypersomnia. *Current Opinion in Pulmonary Medicine*, 26(6), 629–633. <https://doi.org/10.1097/MCP.0000000000000722>
- Erhardt, S., Pocivavsek, A., Repici, M., Liu, X. C., Imbeault, S., Maddison, D. C., Thomas, M. A. R., Smalley, J. L., Larsson, M. K., Muchowski, P. J., Giorgini, F., & Schwarcz, R. (2017). Adaptive and behavioral changes in kynurenic acid 3-monooxygenase knockout mice: Relevance to psychotic disorders. *Biological Psychiatry*, 82(10), 756–765. <https://doi.org/10.1016/j.biopsych.2016.12.011>
- Fernandez, L. M. J., & Luthi, A. (2020). Sleep spindles: Mechanisms and functions. *Physiological Reviews*, 100(2), 805–868. <https://doi.org/10.1152/physrev.00042.2018>
- Ferrarelli, F., & Tononi, G. (2017). Reduced sleep spindle activity point to a TRN-MD thalamus-PFC circuit dysfunction in schizophrenia. *Schizophrenia Research*, 180, 36–43. <https://doi.org/10.1016/j.schres.2016.05.023>
- Jing, M., Li, Y., Zeng, J., Huang, P., Skirzewski, M., Kljatic, O., Peng, W., Qian, T., Tan, K., Zou, J., Trinh, S., Wu, R., Zhang, S., Pan, S.,

- Hires, S. A., Xu, M., Li, H., Saksida, L. M., Prado, V. F., ... Li, Y. (2020). An optimized acetylcholine sensor for monitoring in vivo cholinergic activity. *Nature Methods*, 17(11), 1139–1146. <https://doi.org/10.1038/s41592-020-0953-2>
- Klausing, A. D., Fukuwatari, T., Bucci, D. J., & Schwarcz, R. (2020). Stress-induced impairment in fear discrimination is causally related to increased kynurenic acid formation in the prefrontal cortex. *Psychopharmacology*, 237(7), 1931–1941. <https://doi.org/10.1007/s00213-020-05507-x>
- Konradsson-Geuken, A., Wu, H. Q., Gash, C. R., Alexander, K. S., Campbell, A., Sozeri, Y., Pellicciari, R., Schwarcz, R., & Bruno, J. P. (2010). Cortical kynurenic acid bi-directionally modulates prefrontal glutamate levels as assessed by microdialysis and rapid electrochemistry. *Neuroscience*, 169(4), 1848–1859. <https://doi.org/10.1016/j.neuroscience.2010.05.052>
- Kozak, R., Campbell, B. M., Strick, C. A., Horner, W., Hoffmann, W. E., Kiss, T., Chapin, D. S., McGinnis, D., Abbott, A. L., Roberts, B. M., Fonseca, K., Guanowsky, V., Young, D. A., Seymour, P. A., Dounay, A., Hajos, M., Williams, G. V., & Castner, S. A. (2014). Reduction of brain kynurenic acid improves cognitive function. *The Journal of Neuroscience*, 34(32), 10592–10602. <https://doi.org/10.1523/JNEUROSCI.1107-14.2014>
- Lankford, D. A., Corser, B. C., Zheng, Y. P., Li, Z., Snively, D. B., Lines, C. R., & Deacon, S. (2008). Effect of gaboxadol on sleep in adult and elderly patients with primary insomnia: Results from two randomized, placebo-controlled, 30-night polysomnography studies. *Sleep*, 31(10), 1359–1370. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/18853933>.
- Lelkes, Z., Abdurakhmanova, S., & Porkka-Heiskanen, T. (2018). Cholinergic basal forebrain structures are not essential for mediation of the arousing action of glutamate. *Journal of Sleep Research*, 27(4), e12605. <https://doi.org/10.1111/jsr.12605>
- Linderholm, K. R., Skogh, E., Olsson, S. K., Dahl, M. L., Holtze, M., Engberg, G., Samuelsson, M., & Erhardt, S. (2012). Increased levels of kynurenic acid and kynurenic acid in the CSF of patients with schizophrenia. *Schizophrenia Bulletin*, 38(3), 426–432. <https://doi.org/10.1093/schbul/sbq086>
- Lucey, B. P., Wisch, J., Boerwinkle, A. H., Landsness, E. C., Toedebusch, C. D., McLeland, J. S., Butt, O. H., Hassenstab, J., Morris, J. C., Ances, B. M., & Holtzman, D. M. (2021). Sleep and longitudinal cognitive performance in preclinical and early symptomatic Alzheimer's disease. *Brain*, 144(9), 2852–2862. <https://doi.org/10.1093/brain/awab272>
- Lundahl, J., Deacon, S., Maurice, D., & Staner, L. (2012). EEG spectral power density profiles during NREM sleep for gaboxadol and zolpidem in patients with primary insomnia. *Journal of Psychopharmacology*, 26(8), 1081–1087. <https://doi.org/10.1177/0269881111424457>
- Manoach, D. S., Demanuele, C., Wamsley, E. J., Vangel, M., Montrose, D. M., Miewald, J., Kupfer, D., Buysse, D., Stickgold, R., & Keshavan, M. S. (2014). Sleep spindle deficits in antipsychotic-naïve early course schizophrenia and in non-psychotic first-degree relatives. *Frontiers in Human Neuroscience*, 8, 762. <https://doi.org/10.3389/fnhum.2014.00762>
- Miller, C. L., Llenos, I. C., Dulay, J. R., & Weis, S. (2006). Upregulation of the initiating step of the kynurenic acid pathway in postmortem anterior cingulate cortex from individuals with schizophrenia and bipolar disorder. *Brain Research*, 1073-1074, 25–37. <https://doi.org/10.1016/j.brainres.2005.12.056>
- Milosavljevic, S., Smith, A. K., Wright, C. J., Valafar, H., & Pociavsek, A. (2023). Kynurenic acid aminotransferase II inhibition promotes sleep and rescues impairments induced by neurodevelopmental insult. *Translational Psychiatry*, 13(1), 106. <https://doi.org/10.1038/s41398-023-02399-1>
- Mukherjee, D., Krishnamurthy, V. B., Millett, C. E., Reider, A., Can, A., Groer, M., Fuchs, D., Postolache, T. T., & Saunders, E. F. H. (2018). Total sleep time and kynurenic acid metabolism associated with mood symptom severity in bipolar disorder. *Bipolar Disorders*, 20(1), 27–34. <https://doi.org/10.1111/bdi.12529>
- Pellicciari, R., Rizzo, R. C., Costantino, G., Marinozzi, M., Amori, L., Guidetti, P., Wu, H. Q., & Schwarcz, R. (2006). Modulators of the kynurenic acid pathway of tryptophan metabolism: Synthesis and preliminary biological evaluation of (S)-4-(ethylsulfonyl)benzoylalanine, a potent and selective kynurenic acid aminotransferase II (KAT II) inhibitor. *ChemMedChem*, 1(5), 528–531. <https://doi.org/10.1002/cmdc.200500095>
- Pocivavsek, A., Baratta, A. M., Mong, J. A., & Viechweg, S. S. (2017). Acute kynurenic acid challenge disrupts sleep-wake architecture and impairs contextual memory in adult rats. *Sleep*, 40(11), zsx141. <https://doi.org/10.1093/sleep/zsx141>
- Pocivavsek, A., & Erhardt, S. (2023). Kynurenic acid: Translational perspectives of a therapeutically targetable gliotransmitter. *Neuropsychopharmacology*. In press. <https://doi.org/10.1038/s41386-023-01681-6>
- Pocivavsek, A., & Rowland, L. M. (2018). Basic neuroscience illuminates causal relationship between sleep and memory: Translating to schizophrenia. *Schizophrenia Bulletin*, 44(1), 7–14. <https://doi.org/10.1093/schbul/sbx151>
- Pocivavsek, A., Wu, H. Q., Potter, M. C., Elmer, G. I., Pellicciari, R., & Schwarcz, R. (2011). Fluctuations in endogenous kynurenic acid control hippocampal glutamate and memory. *Neuropsychopharmacology*, 36(11), 2357–2367. <https://doi.org/10.1038/npp.2011.127>
- Rapoport, M. I., & Beisel, W. R. (1968). Circadian periodicity of tryptophan metabolism. *The Journal of Clinical Investigation*, 47(4), 934–939. <https://doi.org/10.1172/JCI105785>
- Rayan, A., Agarwal, A., Samanta, A., Severijnen, E., van der Meij, J., & Genzel, L. (2022). Sleep scoring in rodents: Criteria, automatic approaches and outstanding issues. *The European Journal of Neuroscience*. Online ahead of print. <https://doi.org/10.1111/ejn.15884>
- Rentschler, K. M., Baratta, A. M., Ditty, A. L., Wagner, N. T. J., Wright, C. J., Milosavljevic, S., Mong, J. A., & Pociavsek, A. (2021). Prenatal kynurenic acid elevation elicits sex-dependent changes in sleep and arousal during adulthood: Implications for psychotic disorders. *Schizophrenia Bulletin*, 47(5), 1320–1330. <https://doi.org/10.1093/schbul/sbab029>
- Sathyasaikumar, K. V., Stachowski, E. K., Wonodi, I., Roberts, R. C., Rassoulpour, A., McMahon, R. P., & Schwarcz, R. (2011). Impaired kynurenic acid pathway metabolism in the prefrontal cortex of individuals with schizophrenia. *Schizophrenia Bulletin*, 37(6), 1147–1156. <https://doi.org/10.1093/schbul/sbq112>
- Schwarcz, R., Bruno, J. P., Muchowski, P. J., & Wu, H. Q. (2012). Kynurenic acid in the mammalian brain: When physiology meets pathology. *Nature Reviews Neuroscience*, 13(7), 465–477. <https://doi.org/10.1038/nrn3257>
- Song, C., Clark, S. M., Vaughn, C. N., Nicholson, J. D., Murphy, K. J., Mou, T. M., Schwarcz, R., Hoffman, G. E., & Tonelli, L. H. (2018). Quantitative analysis of kynurenic acid aminotransferase II in the adult rat brain reveals high expression in proliferative zones and corpus callosum. *Neuroscience*, 369, 1–14. <https://doi.org/10.1016/j.neuroscience.2017.11.001>
- Swift, K. M., Keus, K., Echeverria, C. G., Cabrera, Y., Jimenez, J., Holloway, J., & Poe, G. R. (2020). Sex differences within sleep in gonadally intact rats. *Sleep*, 43(5), zsz289. <https://doi.org/10.1093/sleep/zsz289>
- Szymusiak, R., Alam, N., & McGinty, D. (2000). Discharge patterns of neurons in cholinergic regions of the basal forebrain during waking and sleep. *Behavioural Brain Research*, 115(2), 171–182. [https://doi.org/10.1016/s0166-4328\(00\)00257-6](https://doi.org/10.1016/s0166-4328(00)00257-6)
- Tartar, J. L., Ward, C. P., McKenna, J. T., Thakkar, M., Arrigoni, E., McCarley, R. W., Brown, R. E., & Strecker, R. E. (2006). Hippocampal synaptic plasticity and spatial learning are impaired in a rat model of sleep fragmentation. *The European Journal of Neuroscience*, 23(10), 2739–2748. <https://doi.org/10.1111/j.1460-9568.2006.04808.x>
- Thankachan, S., Katsuki, F., McKenna, J. T., Yang, C., Shukla, C., Deisseroth, K., Uygun, D. S., Strecker, R. E., Brown, R. E.,

- McNally, J. M., & Basheer, R. (2019). Thalamic reticular nucleus Parvalbumin neurons regulate sleep spindles and electrophysiological aspects of schizophrenia in mice. *Scientific Reports*, 9(1), 3607. <https://doi.org/10.1038/s41598-019-40398-9>
- Uygun, D. S., Katsuki, F., Bolortuya, Y., Aguilar, D. D., McKenna, J. T., Thankachan, S., McCarley, R. W., Basheer, R., Brown, R. E., Strecker, R. E., & McNally, J. M. (2019). Validation of an automated sleep spindle detection method for mouse electroencephalography. *Sleep*, 42(2), zsy218. <https://doi.org/10.1093/sleep/zsy218>
- Vyazovskiy, V. V., Olcese, U., Hanlon, E. C., Nir, Y., Cirelli, C., & Tononi, G. (2011). Local sleep in awake rats. *Nature*, 472(7344), 443–447. <https://doi.org/10.1038/nature10009>
- Walsh, J. K., Coulouvrat, C., Hajak, G., Lakoma, M. D., Petukhova, M., Roth, T., Sampson, N. A., Shahly, V., Shillington, A., Stephenson, J. J., & Kessler, R. C. (2011). Nighttime insomnia symptoms and perceived health in the America insomnia survey (AIS). *Sleep*, 34(8), 997–1011. <https://doi.org/10.5665/SLEEP.1150>
- Wilckens, K. A., Ferrarelli, F., Walker, M. P., & Buysse, D. J. (2018). Slow-wave activity enhancement to improve cognition. *Trends in Neurosciences*, 41(7), 470–482. <https://doi.org/10.1016/j.tins.2018.03.003>
- Wright, C. J., Rentschler, K. M., Wagner, N. T. J., Lewis, A. M., Beggiano, S., & Pocivavsek, A. (2021). Time of day-dependent alterations in hippocampal Kynurenic acid, glutamate, and GABA in adult rats exposed to elevated Kynurenic acid during neurodevelopment. *Frontiers in Psychiatry*, 12, 734984. <https://doi.org/10.3389/fpsy.2021.734984>
- Yamashita, M. (2020). Potential role of neuroactive tryptophan metabolites in central fatigue: Establishment of the fatigue circuit. *Int J Tryptophan Res*, 13, 1178646920936279. <https://doi.org/10.1177/1178646920936279>
- Zmarowski, A., Wu, H. Q., Brooks, J. M., Potter, M. C., Pellicciari, R., Schwarcz, R., & Bruno, J. P. (2009). Astrocyte-derived kynurenic acid modulates basal and evoked cortical acetylcholine release. *The European Journal of Neuroscience*, 29(3), 529–538. <https://doi.org/10.1111/j.1460-9568.2008.06594.x>

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