SLEEPJ, 2019, 1–8

doi: 10.1093/sleep/zsz135
Advance Access Publication Date: 1 August 2019
Original Article

ORIGINAL ARTICLE

NMDAR activation regulates the daily rhythms of sleep and mood

Jeffrey S. Burgdorf[1,2,*], Martha H. Vitaterna[3], Christopher J. Olker[3], Eun Joo Song[3], Edward P. Christian[1], Laurits Sørensen[4], Fred W. Turek[3], Torsten M. Madsen[1], M. Amin Khan[1], Roger A. Kroes[1,2] and Joseph R. Moskal[1,2]

[1]Aptinyx Inc., Evanston, IL, [2]Falk Center for Molecular Therapeutics, Department of Biomedical Engineering, Northwestern University, Evanston, IL, [3]Department of Neurobiology, Northwestern University, Evanston, IL and [4]Lund Sorensen Life Sciences, Aarhus, Denmark

*Corresponding author. Jeffrey Burgdorf, Aptinyx Inc., 1801 Maple Ave, Suite 4300, Evanston, IL 60201. Email: jeffreyburgdorf@aptinyx.com.

Abstract

Study Objectives: The present studies examine the effects of NMDAR activation by NYX-2925 diurnal rhythmicity of both sleep and wake as well as emotion.

Methods: Twenty-four-hour sleep EEG recordings were obtained in sleep-deprived and non-sleep-deprived rats. In addition, the day–night cycle of both activity and mood was measured using home cage ultrasonic-vocalization recordings.

Results: NYX-2925 significantly facilitated non-REM (NREM) sleep during the lights-on (sleep) period, and this effect persisted for 3 days following a single dose in sleep-deprived rats. Sleep-bout duration and REM latencies were increased without affecting total REM sleep, suggesting better sleep quality. In addition, delta power during wake was decreased, suggesting less drowsiness. NYX-2925 also rescued learning and memory deficits induced by sleep deprivation, measured using an NMDAR-dependent learning task. Additionally, NYX-2925 increased positive affect and decreased negative affect, primarily by facilitating the transitions from sleep to rough-and-tumble play and back to sleep. In contrast to NYX-2925, the NMDAR antagonist ketamine acutely (1–4 hours post-dosing) suppressed REM and non-REM sleep, increased delta power during wake, and blunted the amplitude of the sleep-wake activity rhythm.

Discussion: These data suggest that NYX-2925 could enhance behavioral plasticity via improved sleep quality as well as vigilance during wake. As such, the facilitation of sleep by NYX-2925 has the potential to both reduce symptom burden on neurological and psychiatric disorders as well as serve as a biomarker for drug effects through restoration of sleep architecture.

Statement of Significance

This manuscript details the development of a novel method for measuring the diurnal rhythm of emotion in rats using a novel NMDAR modulator, NYX-2925, that facilitates synaptic plasticity and is in Phase II trials for neuropathic pain disorders in which sleep disturbances are a core symptom. NYX-2925 produces a robust and long-lasting facilitation of the diurnal rhythm of affect and enhances measures of NREM sleep during the light phase. A parallel clinical trial with NYX-2925 in sleep is currently being conducted.

Key words: sleep; EEG; ultrasonic vocalizations; NMDA receptors; sleep deprivation; ketamine

Submitted: 29 December, 2018; **Revised:** 29 April, 2019

Introduction

NMDA receptor (NMDAR) activity is critical for diurnal rhythmicity, sleep, and memory consolidation. The NMDAR antagonist MK-801 completely eliminates both REM and NREM sleep for approximately the first 2 hours after systemic administration [1]. Interestingly, rebound increases in both REM and NREM sleep are seen approximately 2–12 hours after MK-801 administration [1]. MK-801 also disrupts consolidation of learning and memory across a wide range of tasks [2], whereas the NMDAR glycine site partial agonist d-cycloserine facilitates learning and memory consolidation [3, 4]. Recently, it has been shown that sleep drive is controlled in part via phosphorylation of the NR2B subtype of the NDMAR receptor [5]. Similarly, sleep deprivation decreases hippocampal cell surface expression of the obligatory NMDAR subunit GRIN1, and reduces NMDAR current and disrupts NMDAR-dependent LTP and LTD [6]. In comparison, pharmacological manipulations of other neurotransmitter systems (e.g., GABA, 5-HT, histamine, melatonin) have more moderate effects on both sleep-EEG and memory consolidation [7, 8]. Thus, facilitation of NMDAR activity likely enhances memory consolidation via facilitation of REM and NREM sleep, whereas inhibiting NMDAR activity has opposite effects.

Sleep is causally linked to the retention of recently learned memories. Sleep deprivation disrupts a wide variety of learning and memory tasks including contextual fear extinction [9], spatial learning in both the Morris water maze and radial arm maze [10], motor learning consolidation [11, 12], and declarative memory [13]. At the cellular and molecular levels, sleep induces dendritic spine pruning and reduces the expression of cell surface AMPA receptors [14, 15]. In this way, sleep promotes mature dendritic spine formation and consolidation via an NMDAR-dependent process.

NYX-2925 is a novel, orally available, small-molecule NMDAR modulator and is distinct from known NMDAR agonists or antagonists such as D-cycloserine, ketamine, MK-801, kynurenic acid, or ifenprodil [16]. NYX-2925 robustly facilitates NMDAR current and NMDAR-dependent long-term potentiation (LTP) in brain slices, and NMDAR-dependent learning and memory in vivo by activating the NMDAR [16]. NYX-2925 is in development as a therapy for chronic pain and is currently under evaluation in two phase 2 clinical studies, one in subjects with painful diabetic peripheral neuropathy and the other in subjects with fibromyalgia (ClinicalTrials.gov identifiers: NCT03219320; NCT03249103). Both indications being studied are chronic pain conditions in which sleep disruption is often a core symptom reported by those affected [17, 18].

This study was designed to evaluate whether NMDAR activation, with a single dose of NYX-2925, enhances the amplitude of the day–night cycle of sleep and mood, and enhances learning and memory consolidation in a sleep-deprivation paradigm in rats.

Materials and Methods

Animals

Male 2- to 3-month-old Sprague-Dawley (SD) rats from Charles River (United States) were used. Rats were housed in Lucite cages with aspen-wood chip bedding, maintained on a 12:12 light:dark cycle (lights-on at 6 am), and given ad libitum access to Teklad lab chow (Envigo, United States) and tap water throughout the study. All experiments were approved by the Northwestern University Animal Care and Use Committee.

Sleep EEG studies

Rats were anesthetized using isoflurane and implanted with cortical EEG skull screws and EMG neck muscle wires (Pinnacle, USA). EEG/EMG signals were captured via a tethered system (Pinnacle, USA), and sleep/wake states were scored in 10-second epochs with a combination of manual scoring and machine learning [19] in a treatment-blinded manner.

One week after surgery, rats were placed in sleep-recording cages (35.6 cm diameter × 30.5 cm high; Pinnacle, USA) within sound-attenuating chambers that also blocked ambient outside light. After 24 hours of habituation, rats were dosed with drug or vehicle as described below at zeitgeber (ZT) 5 (11 am) and EEG/EMG was recorded for 24–72 hours post-dosing. Additional groups of rats received sleep deprivation using the Pinnacle (USA) sleep deprivation system from ZT0-6.

Rats were dosed with NYX-2925 (0.1, 1, 10 mg/kg PO; Sai Life Sciences, India) formulated in 0.5% carboxymethylcellulose/0.9% sterile saline (CMC) or CMC vehicle. A separate cohort of rats were dosed with ketamine (10 mg/kg IV) or sterile saline vehicle via the lateral-tail vein.

Sleep-deprivation learning studies

Heterospecific rough-and-tumble play was conducted as previously described [16, 20]. Animals received three consecutive days of light-touch habituation, which does not induce ultrasonic vocalizations (USVs), before testing [20]. These animals did not receive sleep-EEG surgeries or testing. Briefly, heterospecific rough-and-tumble play stimulation was administered by the experimenter's right hand. Rats received 3 minutes of play consisting of alternating 15-second blocks of play and 15 seconds of no stimulation. The experimenter was blind to the treatment condition of the animals. At the end of the 3-minute session, running speed (cm/second) for the animal to traverse a 57-centimeter arena and touch the experimenters' hand to self-administer play was measured manually with a digital stopwatch. High-frequency USVs were recorded (see below; Avisoft UltraSoundGate, Germany) during the 6 × 15-second no-stimulation blocks and analyzed by sonogram (Avisoft SASlab Pro, Germany) in a blinded manner, as described previously [18]. Rats were dosed with NYX-2925 (1 mg/kg PO; Sai Advantium, India) in CMC or CMC vehicle 1 hour before testing. Previously it has been shown that positive modulation of the NMDAR with d-cycloserine has been shown to rescue sleep deprivation-induced deficits in learning [21].

Home cage activity and USV recordings

Rats were housed three per cage by experimental condition in circular acrylic home cages (35.6 cm diameter × 30.5 cm high; Pinnacle, USA) with aspen-wood chip bedding and a Plexiglas lid with 9 × 50 cm holes and a microphone (Avisoft, Germany) suspended from the center hole. These animals did not receive sleep-EEG surgeries or testing. Rats were maintained on a 12:12

light:dark cycle (lights-on at 6 am), and given ad libitum access to lab chow and tap water throughout the study. USVs were recorded (Avisoft UltraSoundGate, Germany) in 15-minute bins for 24 hours and analyzed via sonogram (Avisoft SASlab Pro, Germany) with high (R > .90) blinded inter-rater reliability. Rats were dosed with NYX-2925 (10 mg/kg PO), CMC vehicle PO, ketamine (10 mg/kg IV), or sterile saline vehicle (IV) at ZT5, and did not previously receive EEG surgery or testing.

Frequency-modulated 50-kHz USVs and 20-kHz USVs are validated measures of positive and negative emotion in rats, respectively [22]. A wide range of hedonic stimuli (social interaction, food, drugs of abuse) increase rates of frequency-modulated 50-kHz USVs [23–26]. Aversive stimuli, on the other hand, uniformly decrease rates of frequency-modulated 50-kHz USVs and increase rates of 20-kHz USVs [23, 26, 27]. Additionally, the neural circuit of rat 50-kHz USVs and 20-kHz is essentially the same as the human positive and negative affect circuit [22, 23].

Hedonic frequency-modulated 50-kHz USVs, neutral flat 50-kHz USVs, and aversive 20-kHz USVs were classified as previously described [23]. Behavioral activation (e.g., locomotor activity, sniffing, eating, and drinking) was quantified by the total sound output from the recording microphone which captures both sonic and ultrasonic sound. Inactivity (% of total time) was defined by the amount of time in which the sound intensity was similar to levels recorded from an empty cage. Activity was defined as 100– (minus) inactivity.

Statistical analysis

Sleep, activity, and USV data were analyzed by analysis of variance (ANOVA), followed by Fisher's PLSD post hoc test (Statview, USA). The accuracy of the transition from light:dark for locomotor activity was calculated via a three parameter time-response curve (GraphPad Prism, USA), with accuracy being measured by the time to a half-maximal increase in activity relative to lights-off. The level of statistical significance was set at $p < .05$.

Results

NYX-2925 (1 mg/kg PO), as compared to vehicle, increased NREM sleep time (Figure 1, A and B; $F(1, 31) = 15.8$, $p < .05$; Fishers PLSD post hoc test NYX-2925 vs. vehicle for non-sleep-deprived rats $p < .05$ and sleep-deprived rats $p < .05$) and total sleep time ($F(1, 31) = 12.6$, $p < .05$; Fishers PLSD post hoc test NYX-2925 vs. vehicle for non-sleep-deprived rats $p < .05$ and sleep-deprived rats $p < .05$) during the sleep phase (lights-on) in both non-deprived and sleep-deprived rats without affecting REM sleep (Figure 1, D and E; $F(1, 31) = 0.01$, $p > .05$). In contrast, ketamine (10 mg/kg IV), as compared to vehicle, acutely suppressed NREM and REM sleep followed by a rebound increase in NREM (Figure 1C) and REM (Figure 1F) in non-deprived rats (NREM – Drug × Time [$F(9, 12) = 3.4$, $p < .05$]; Fishers PLSD post hoc test ketamine vs. vehicle

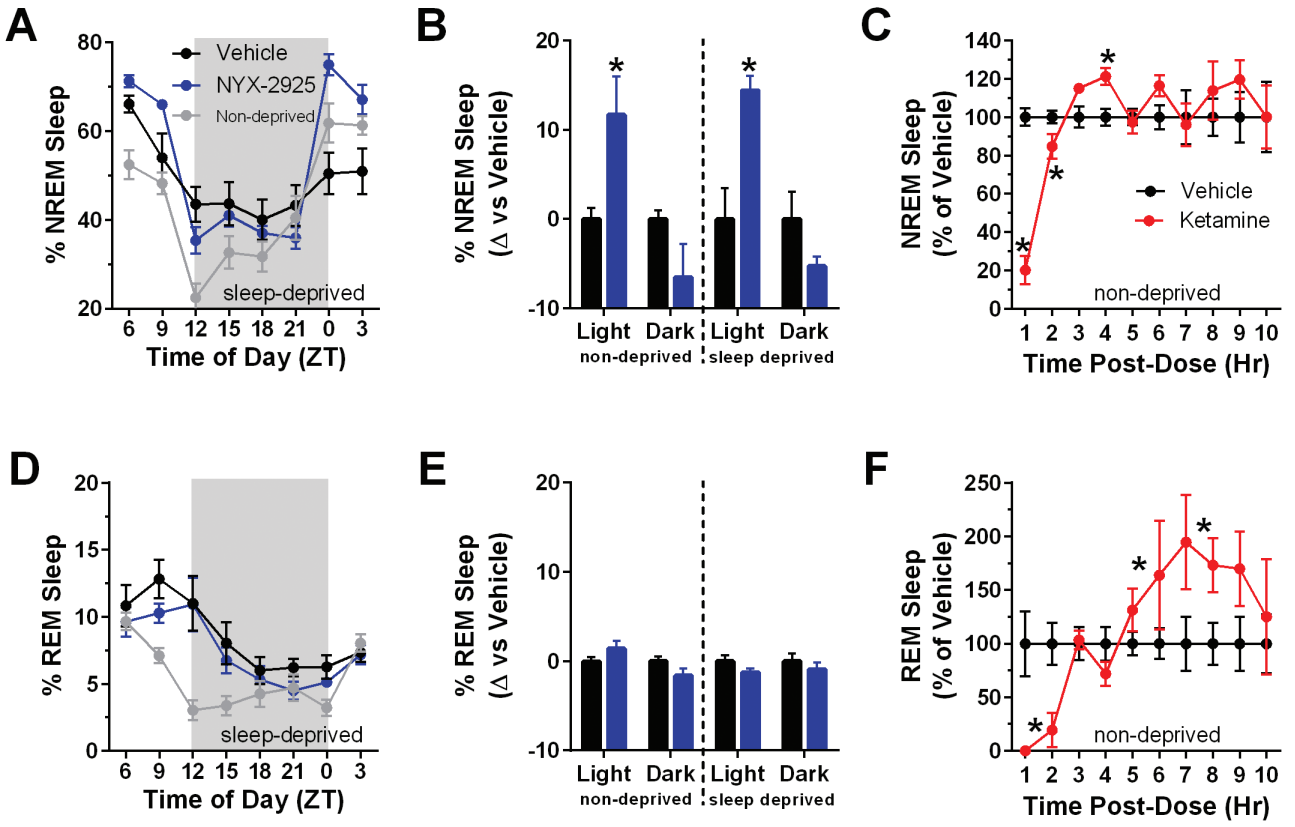

Figure 1. NYX-2925 facilitates NREM sleep during the light phase, whereas the NMDAR antagonist ketamine inhibits NREM. Non-deprived or sleep-deprived (ZT0-6) rats received either NYX-2925 (1 mg/kg PO), ketamine (10 mg/kg IV; non-deprived only) or vehicle at ZT5 and sleep EEG and EMG was recorded for 24 hours post-dosing. (A) NREM and (D) REM sleep diurnal rhythm before (baseline) and after NYX-2925 or vehicle administration. NYX-2925 increased (B) NREM sleep but did not alter (E) REM sleep during the light phase in both non-deprived and sleep-deprived rats. In contrast, ketamine acutely reduced both (C) NREM and (F) REM sleep in non-deprived rats followed by rebound increase in both sleep states. *$p < .05$ Fisher's PLSD post hoc test vs. vehicle. Data are reported as mean ± SEM. Sleep deprived (vehicle $n = 7$, NYX-2925 $n = 9$, non-deprived controls $n = 14$), non-sleep deprived (vehicle $n = 8$, NYX-2925 $n = 11$), ketamine (vehicle $n = 11$, ketamine $n = 4$).

1 and 2 hours post dose [decrease] and 4 hours post dose [increase] $p < .05$; REM – Drug × Time [$F(9, 12) = 2.8$, $p < .05$]; Fishers PLSD post hoc test ketamine vs. vehicle 1 + 2 hours post dose [decrease] and 5 + 6, 7 + 8 hours post dose [increase] $p < .05$).

NYX-2925 (0.1, 1, 10 mg/kg PO) facilitated NREM sleep 24 hours post-dosing in sleep-deprived rats (Figure 2A, $F(3, 45) = 4.3$, $p < .05$, Fishers PLSD post hoc 0.1, 1, 10 vs. vehicle $p < .05$). The 10 mg/kg PO dose showed a persistent effect in continuing to facilitate NREM at 48, and 72 hours post-dosing (Figure 2B, $F(1, 21) = 6.5$, $F(1, 14)$ 5.8, $F(1, 13)$ 6.7, $p < .05$). In addition, NYX-2925 (10 mg/kg PO) increased sleep-bout duration, increased NREM to REM latency, decreased delta power in wake, and increased both delta power in NREM and theta power in REM (Figure 2C, $F(1, 14) = 10.3, 9.9, 9.4, 7.8, 13.2$, $p < .05$) as compared to vehicle across all three testing days.

Twenty-four hours of sleep deprivation resulted in a suppression of positive emotional learning (PEL), play reward as measured by running speed to self-administer play, and hedonic USVs, and an increase in aversive USVs, all of which were rescued by 1 mg/kg NYX-2925 (Figure 3A–C, $F(2, 15) = 40.5, 26.4, 17.2, 12.0$, $p < .05$; Fishers PLSD post hoc test $p < .05$ non-deprived vs. deprived-vehicle and deprived-NYX-2925 vs. deprived-vehicle separately for each dependent measure). NYX-2925 (1 mg/kg; 1 hour post-dosing) was chosen for the PEL study given a previous report showing an increase in PEL at this dose and time-point in non-deprived rats [16]. Twenty-four hours of sleep deprivation also lead to a complete elimination of REM (mean ± SEM 100 ± 0.0 reduction, within subjects $t(5) = 16.9$, $p < .05$), and a reduction in NREM (mean ± SEM 70.1 ± 2.6 reduction, within subjects $t(5) = 24.4$, $p < .05$), and total sleep time (mean ± SEM 73.7 ± 2.5 reduction, within subjects $t(5) = 29.1$ $p < .05$) as compared to non-deprived baseline values (data not shown), thus validating the 24-hour sleep-deprivation protocol.

In the 24-hour home cage USV recording study, NYX-2925 (1 mg/kg) also facilitated the amplitude of the diurnal rhythm of locomotor behavior as well as positive affect, and enhanced positive affect amplitude by suppressing wrong-time activity, whereas ketamine inhibited the amplitude of locomotor behavior by increasing wrong-time activity. As shown in Figure 4A, NYX-2925 enhanced the amplitude of the positive affect as measured by vector amplitude on activity during the dark phase ($F(2, 13) = 16.5, 21.3$, $p < .05$; Fishers PLSD post hoc test $p < .05$ NYX-2925 vs. vehicle), without altering phase angle or activity during the dark phase ($F(2, 13) = 1.3, 0.2$, $p > .05$). A clear peak in positive affect was seen across all dosing groups during the lights-off period as measured by the standard deviation of the phase angle vs. a random distribution (F test; $p < .0001$). As shown in Figure 4B, NYX-2925 suppressed negative affect across the 24-hour period measured by dark-phase and light-phase activity ($F(2, 13) = 4.2, 4.7$, $p < .05$; Fishers PLSD post hoc test $p < .05$ NYX-2925 vs. vehicle), without altering phase amplitude or angle ($F(2, 13) = 3.6, 0.2$, $p > .05$). A clear peak in negative affect was seen across all dosing groups during the lights-off period as measured by the standard deviation of the phase angle vs. a random distribution (F test [$p < .0001$]). As shown in Figure 4C, NYX-2925 enhanced, and ketamine inhibited, the amplitude of the locomotor activity rhythm as measured by the percent of total activity that occurred during the dark phase ($F(2, 13) = 13.6$, $p < .05$; Fishers PLSD post hoc test $p < .05$ NYX-2925 vs. vehicle or ketamine vs. vehicle), which was primarily due to decreased and increased activity during the light phase for NYX-2925 and ketamine, respectively ($F(2, 13) = 122.8$, $p < .05$; Fishers PLSD post hoc test $p < .05$ NYX-2925 vs. vehicle or ketamine vs. vehicle), with now change seen in activity during the dark ($F(2, 13) = 0.4$, $p > .05$). Using circular statistics, ketamine decreased the vector amplitude ($F(2, 13) = 15.4$, $p < .05$; Fishers PLSD post hoc test $p < .05$ ketamine vs. vehicle) and shifted the phase angle ($F(2, 13) = 31.1$, $p < .05$; Fishers PLSD post hoc test $p < .05$ ketamine vs. vehicle). A clear peak in locomotor behavior was seen across all dosing groups during the lights-off period as measured by the standard deviation of the phase angle vs. a random distribution as measured by an F test ($p < .0001$). The accuracy of the transition from light:dark for locomotor was facilitated by NYX-2925 as compared to vehicle ($F(1,10) = 6.1$, $p < .05$), whereas the ketamine group did not show a clear activity

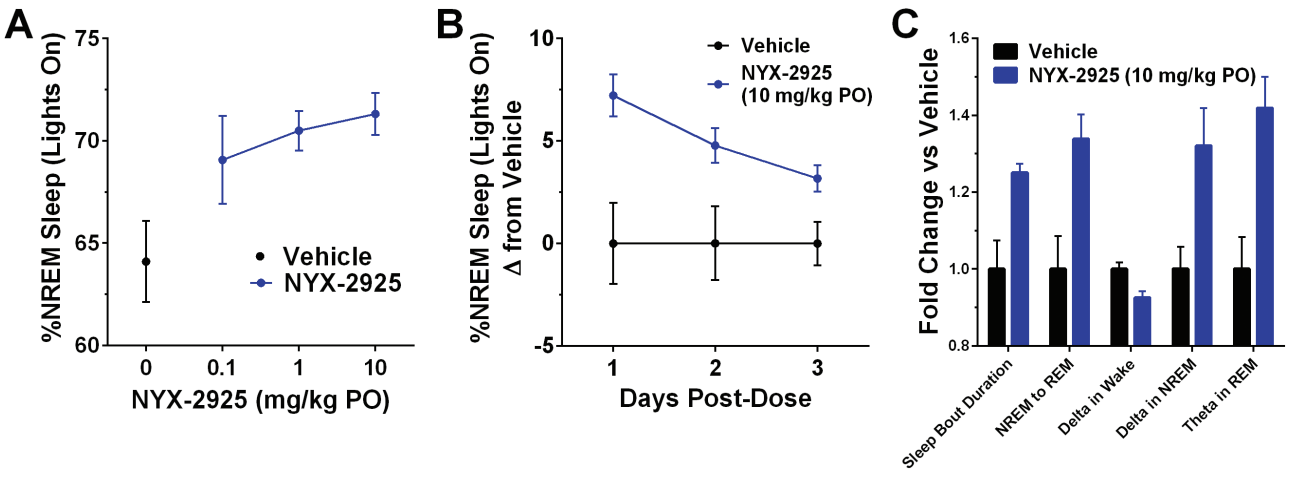

Figure 2. Dose response and time course for NYX-2925 facilitation of NREM in sleep-deprived rats. Sleep-deprived (ZT0-6; day 1–2) rats received either NYX-2925 (0.1, 1, 10 mg/kg PO) or vehicle at ZT5 and sleep EEG and EMG were recorded for 3 days post-dosing. (A) NYX-2925 (0.1, 1, 10 mg/kg PO) increased NREM sleep during the light phase as compared to vehicle during the first 24 hours after dosing, whereas (B) only the 10 mg/kg PO dose facilitated NREM across all 3 days. (C) NYX-2925 (10 mg/kg PO) across all 3 days significantly increased the duration of individual sleep bouts, the amount of time spent in NREM before transitioning to REM in a sleep bout, delta power during NREM, and theta power during REM. NYX-2925 also decreased delta power during wake. $p < .05$ Fisher's PLSD post hoc test vs. vehicle for all doses and timepoints. Data are reported as mean ± SEM. (A) Vehicle $n = 15$, NYX-2925 0.1 mg/kg $n = 9$, 1 mg/kg $n = 17$, 10 mg/kg $n = 8$; (B) vehicle $N = 15$ (d1), 8 (d2), 7 (d1), NYX-2925 $n = 8$; (C) $n = 8$ per group.

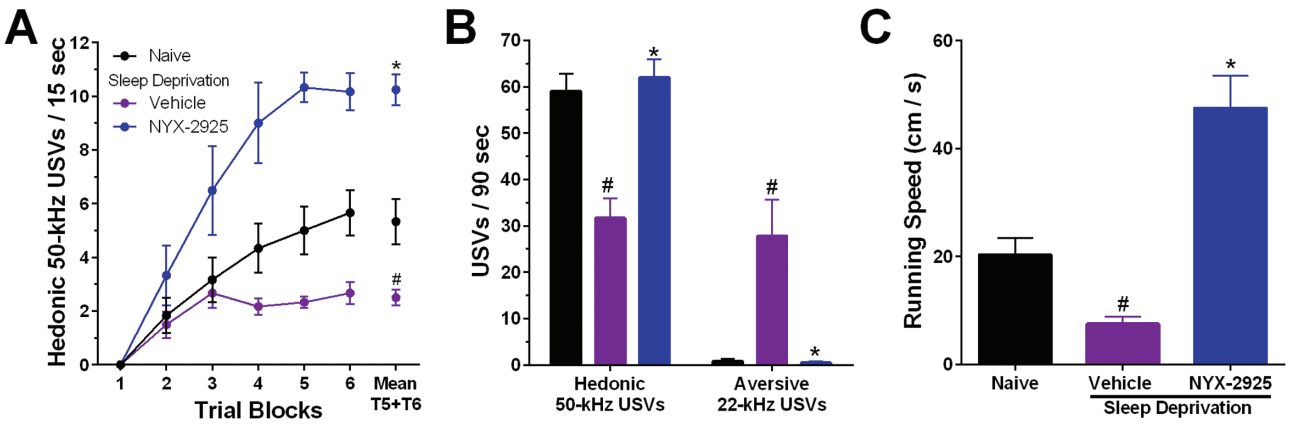

Figure 3. Sleep deprivation inhibits NMDAR-dependent positive emotional learning which is rescued with pretreatment with NYX-2925. (A) NYX-2925 (1 mg/kg PO) increased the rates of hedonic 50-kHz USVs in response to a temporal conditioned stimulus (CS) that predicated heterospecific rough-and-tumble play in rats that had received 23 hours of sleep deprivation as well as non-dosed/non-deprived naive controls. (B) Rates of hedonic 50-kHz USVs or aversive 20-kHz USVs in response to unconditioned heterospecific rough-and-tumble play. (C) Approach latency (cm/second) for the rats to approach the experimenter's hand in order to self-administer heterospecific rough and tumble play. *$p < .05$ NYX-2925 vs. vehicle, #$p < .05$ naive vs. vehicle Fisher's PLSD post hoc test. Data are reported as mean ± SEM. $N = 6$ per group.

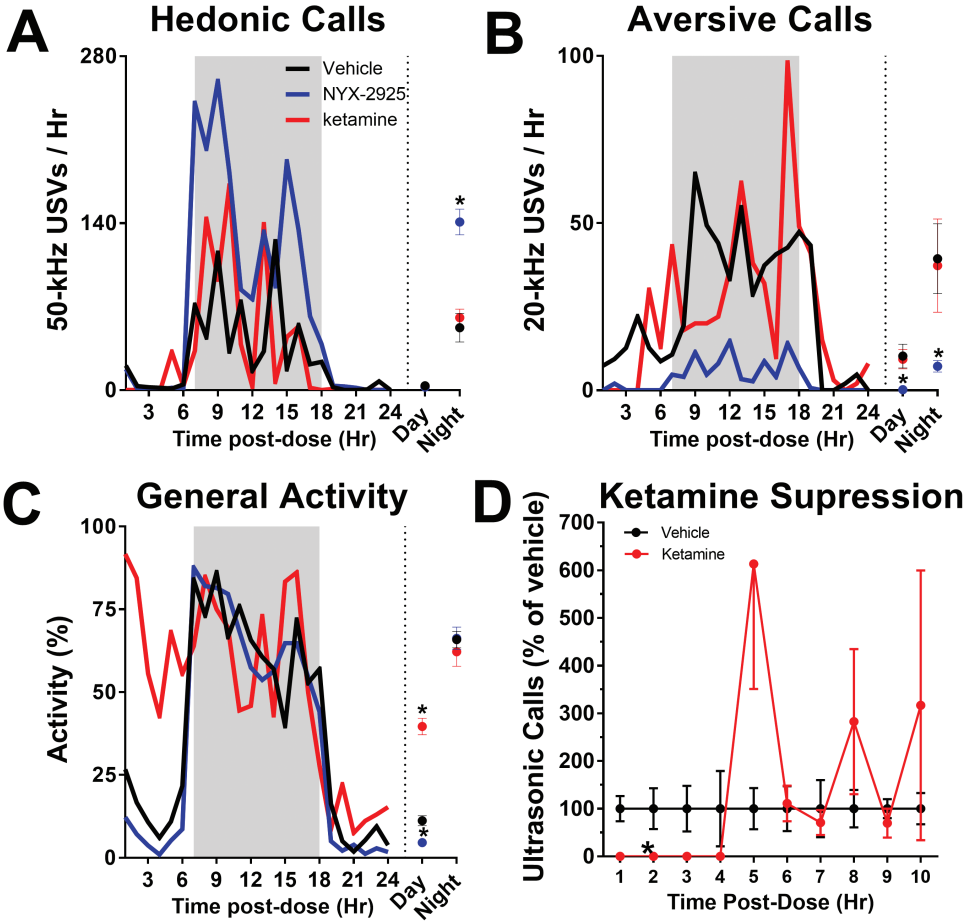

Figure 4. NYX-2925 facilitates the amplitude and phase transition timing of diurnal rhythm of both activity and emotional expression whereas ketamine does the opposite. Non-deprived rats received either NYX-2925 (10 mg/kg PO), ketamine (10 mg/kg IV) or vehicle at ZT5 and both sound levels (activity) and USVs (hedonic and aversive calls) were recorded in sound-attenuated chambers housing three rats per cage. (A–C) Diurnal rhythm of locomotor activity as well as positive and negative affect. (D) Acutely, ketamine suppressed hedonic and aversive USVs for the first 4 hours post-dosing. *$p < .05$ ANOVA NYX-2925 vs. vehicle. Data are reported as mean ± SEM. Vehicle $n = 6$, NYX-2925 $n = 6$, ketamine $n = 4$.

transition (i.e., they did not adequately fit the three-phase model). The mean ± SEM absolute error (in min) vs. lights-on time values were: vehicle 34.0 ± 5.6; NYX-2925 15.9 ± 4.7; ketamine 128.3 ± 41.5. Fragmentation was indexed as the percent of the time in which phase inappropriate behavior was exhibited, namely locomotor behavior and/or ultrasonic calling during the lights-on

period, and lack of locomotor behavior and/or ultrasonic calling during the lights-off period. NYX-2925 decreased and ketamine increased fragmentation using this measure ($F(2,13) = 19.0$, $p < .05$; Fishers PLSD post hoc test $p < .05$ for each pairwise comparison); the mean ± SEM% fragmentation values were: vehicle 34.0 ± 5.8; NYX-2925 14.6 ± 3.5; ketamine 59.4 ± 4.6. As shown in Figure 4D, ketamine eliminated rates of positive affect and total affect for the first 4 hours post-dosing ($F(2, 13) = 7.9, 6.4$, $p < .05$; Fishers PLSD post hoc test $p < .05$ ketamine vs. vehicle) without affecting negative affect ($F(2, 13) = 2.5$, $p > .05$).

Discussion

The data presented here demonstrate that NMDAR activation enhances the amplitude of the day–night cycle of NREM sleep and emotion. The transition from sleep and non-affect to wake and positive affect occurs at lights-off. The quality of this transition, namely accuracy of the transition time, the amplitude of the change, and the lack of fragmentation in the activity cycle requires NMDAR activation. Positive emotional expression also follows the day–night cycle. Negative affect appears to occur when out-of-phase activity is exhibited. NMDAR activation improves sleep and mood by facilitating these behaviors at the appropriate times of day. The ability of the lights-off stimulus to trigger a rapid and long-lasting increase in activity and positive affect appears to be a naturalistic form of NMDAR-dependent behavioral plasticity. The long-lasting effects of NYX-2925 are likely due to enhancement of structural plasticity, as evidenced by trafficking of NMDA and AMPA receptors into the synapse, as well as mature dendritic spine formation [16].

The homeostatic regulation of sleep and affective states during wake appear to be regulated by a form of NMDAR-dependent behavioral plasticity. In rats, lights-off is a strong signal for the induction of locomotor activity and pro-social behavior resulting in hedonic 50-kHz calls (Figure 4). The temporal precision (phase angle) as well as the amplitude and the fidelity of the response (fragmentation) is facilitated by NMDAR activation and inhibited by NMDAR antagonism (Figure 4). Thus, NMDAR activation appears to alter primarily the activity transitions from lights-on/off as depicted by the model in Figure 5.

Ketamine may produce its antidepressant effects through an acute suppression of emotion followed by a rebound increase in positive emotion and sleep. In the studies reported here, ketamine produces an immediate and robust suppression of positive and negative affect followed by a rebound increase in positive affect (Figure 4), which is consistent with the acute antidepressant effect of ketamine [28]. In addition, the rebound increase in NREM and REM after ketamine treatment are consistent with the rat and human literature on ketamine and sleep [29, 30], and may contribute to the long-lasting therapeutic effects of the drug [29].

In rats, the daily rhythm of positive affect appears to be maximal during the active lights-off period (Figure 4A) and is driven by an NMDAR-dependent process. Descriptively, there appears to be early and late activity cycle peaks in positive affect, especially in the NYX-2925 group, which may help explain conflicting reports in the human literature showing either the strongest peak in positive affect occurring in the morning or in the night, before bed [31, 32]. This may translate to an ability to coordinate pro-social play behavior right after the start of

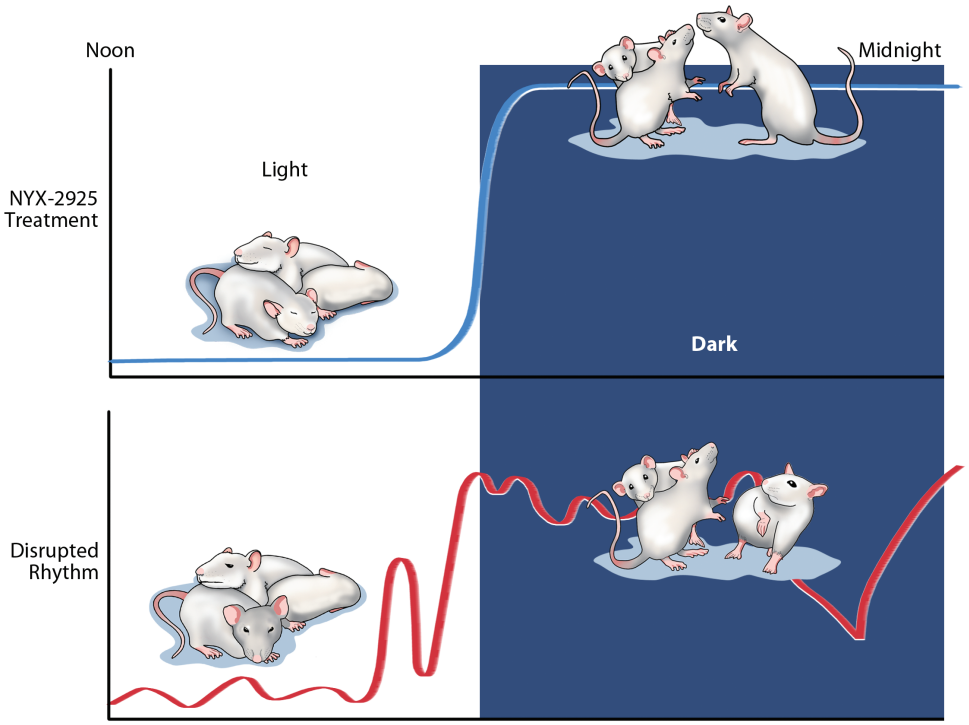

Figure 5. Sleep-affect model. (top panel) NYX-2925 treatment synchronized day–night activity patterns results in limited wake/affect during the day and robust positive affect during the night. (bottom panel) NMDAR antagonists or sleep deprivation disrupts the rhythm. Desynchronized activity patterns lead to increased wake and negative affect during the day and mixed affect during the night. The synchronization of activity and affect appear to be a form of NMDAR-dependent behavioral plasticity which is regulated by the lights-on and lights-off stimuli.

the wake cycle and to complete pro-social behavior right before the end of the wake cycle, which is likely NMDAR-dependent. Future studies should further examine the potential biphasic function of positive affect. Interestingly, error in this diurnal pattern leads to increased negative affect, especially during wake activity in the dark phase. This observation mirrors the increased negative affect seen in humans following sleep deprivation [33]. NMDAR activation facilitates the synchronization of these two bouts of play with lights-on and lights-off, respectively (Figure 4A).

Negative affect appears to represent an error or termination signal during 24-hour home cage recordings. Positive affect is associated with the coordination of pro-social play behavior whereas negative affect occurs when these behaviors are not coordinated between individual rats. In particular, negative affect is evident during arousal that occurs during sleep in the light phase, and during the transition from play to sleep during lights-on. It is hypothesized that NMDAR activation reduces negative affect and facilitates the transition from inactivity to activity during lights-off and activity to inactivity during lights-on with negative affect being an index of error in these states.

The facilitation of sleep and the daily rhythm of mood by NYX-2925 may serve as a biomarker for brain NMDAR activation and contribute to its therapeutic effects. Disrupted sleep is a common symptom of chronic pain, thus improving sleep quality may prove to be beneficial when treating these disorders. This hypothesis is supported by the finding that sleep deprivation has been shown to increase pain sensitivity [34]. Patients with chronic and/or neuropathic pain show a marked reduction in sleep quality; this is especially evident in increased sleep fragmentation and feeling “unrefreshed” in the morning, and these sleep disturbances are positively correlated to pain [17, 18]. Interestingly pregabalin, one of the most widely used therapies in chronic pain, both reduces pain scores and improves sleep quality in post-herpetic neuralgia patients [35] but comes with substantial side effects and a burdensome dosing regimen. Facilitation of NMDAR activity with NYX-2925 has the potential to address the symptoms of chronic pain while also treating the associated deficits in sleep, which are essential for restorative homeostatic plasticity in centrally mediated pain circuits.

Funding

J.R.M. was supported by National Institutes of Health (NIH) grant NS100173. J.S.B. was supported by NIH grants MH094835 and CA199928.

Conflict of interest statement. J.S.B., T.M.M., M.A.K., R.A.K., and J.R.M. are full-time employees of Aptinyx. L.C. is an Atpyinx consultant. E.P.C. was an Aptinyx consultant.

References

1. Campbell IG, *et al*. NREM delta stimulation following MK-801 is a response of sleep systems. *J Neurophysiol*. 1996;**76**(6):3714–3720.
2. van der Staay FJ, *et al*. Effects of the cognition impairer MK-801 on learning and memory in mice and rats. *Behav Brain Res*. 2011;**220**(1):215–229.
3. Ledgerwood L, *et al*. Effects of D-cycloserine on extinction of conditioned freezing. *Behav Neurosci*. 2003;**117**(2):341–349.
4. Monahan JB, *et al*. D-cycloserine, a positive modulator of the N-methyl-D-aspartate receptor, enhances performance of learning tasks in rats. *Pharmacol Biochem Behav*. 1989;**34**(3):649–653.
5. Wang Z, *et al*. Quantitative phosphoproteomic analysis of the molecular substrates of sleep need. *Nature*. 2018;**558**(7710):435–439.
6. Chen C, *et al*. Altered NMDA receptor trafficking contributes to sleep deprivation-induced hippocampal synaptic and cognitive impairments. *Biochem Biophys Res Commun*. 2006;**340**(2):435–440.
7. Idzikowski C. The pharmacology of human sleep, a work in progress? *Curr Opin Pharmacol*. 2014;**14**:90–96.
8. Izquierdo I, *et al*. Behavioural pharmacology and its contribution to the molecular basis of memory consolidation. *Behav Pharmacol*. 2000;**11**(7-8):517–534.
9. Melo I, *et al*. Sleep supports cued fear extinction memory consolidation independent of circadian phase. *Neurobiol Learn Mem*. 2016;**132**:9–17.
10. Smith C. Sleep states and memory processes. *Behav Brain Res*. 1995;**69**(1-2):137–145.
11. Yang G, *et al*. Sleep promotes branch-specific formation of dendritic spines after learning. *Science*. 2014;**344**(6188):1173–1178.
12. Walker MP, *et al*. Practice with sleep makes perfect: sleep-dependent motor skill learning. *Neuron*. 2002;**35**(1):205–211.
13. Stickgold R, *et al*. Sleep, learning, and dreams: off-line memory reprocessing. *Science*. 2001;**294**(5544):1052–1057.
14. Vyazovskiy VV, *et al*. Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nat Neurosci*. 2008;**11**(2):200–208.
15. Maret S, *et al*. Sleep and waking modulate spine turnover in the adolescent mouse cortex. *Nat Neurosci*. 2011;**14**(11):1418–1420.
16. Khan MA, *et al*. NYX-2925 Is a Novel NMDA receptor-specific spirocyclic-β-lactam that modulates synaptic plasticity processes associated with learning and memory. *Int J Neuropsychopharmacol*. 2018;**21**(3):242–254.
17. McCracken LM, *et al*. Disrupted sleep patterns and daily functioning in patients with chronic pain. *Pain Res Manag*. 2002;**7**(2):75–79.
18. Foley D, *et al*. Sleep disturbances and chronic disease in older adults: results of the 2003 National Sleep Foundation Sleep in America Survey. *J Psychosom Res*. 2004;**56**(5):497–502. doi:10.1016/j.jpsychores.2004.02.010
19. Gao V, *et al*. Multiple classifier systems for automatic sleep scoring in mice. *J Neurosci Methods*. 2016;**264**:33–39.
20. Ghoreishi-Haack N, *et al*. NYX-2925 Is a Novel N-methyl-d-aspartate receptor modulator that induces rapid and long-lasting analgesia in rat models of neuropathic pain. *J Pharmacol Exp Ther*. 2018;**366**(3):485–497.
21. Silvestri AJ, *et al*. Effects of REM deprivation and an NMDA agonist on the extinction of conditioned fear. *Physiol Behav*. 2008;**93**(1-2):274–281.
22. Burgdorf J, *et al*. Frequency-modulated 50 kHz ultrasonic vocalizations: a tool for uncovering the molecular substrates of positive affect. *Neurosci Biobehav Rev*. 2011;**35**(9):1831–1836.
23. Burgdorf J, *et al*. Ultrasonic vocalizations of rats (Rattus norvegicus) during mating, play, and aggression: behavioral concomitants, relationship to

reward, and self-administration of playback. *J Comp Psychol*. 2008;**122**(4):357–367.
24. Burgdorf J, *et al*. Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats. *Behav Neurosci*. 2001;**115**(4):940–944.
25. Panksepp J, *et al*. 50-kHz chirping (laughter?) in response to conditioned and unconditioned tickle-induced reward in rats: effects of social housing and genetic variables. *Behav Brain Res*. 2000;**115**(1):25–38.
26. Burgdorf J, *et al*. Anticipation of rewarding electrical brain stimulation evokes ultrasonic vocalization in rats. *Behav Neurosci*. 2000;**114**(2):320–327.
27. Burgdorf J, *et al*. Evaluation of rat ultrasonic vocalizations as predictors of the conditioned aversive effects of drugs. *Psychopharmacology (Berl)*. 2001;**155**(1):35–42.
28. Zarate CA Jr, *et al*. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry*. 2006;**63**(8):856–864.
29. Duncan WC, *et al*. Concomitant BDNF and sleep slow wave changes indicate ketamine-induced plasticity in major depressive disorder. *Int J Neuropsychopharmacol*. 2013;**16**(2):301–311.
30. Feinberg I, *et al*. Stimulation of NREM delta EEG by ketamine administration during waking: demonstration of dose dependence. *Neuropsychopharmacology*. 1995;**12**(1):89–90.
31. Stone AA, *et al*. A population approach to the study of emotion: diurnal rhythms of a working day examined with the Day Reconstruction Method. *Emotion*. 2006;**6**(1):139–149.
32. Boivin DB, *et al*. Complex interaction of the sleep-wake cycle and circadian phase modulates mood in healthy subjects. *Arch Gen Psychiatry*. 1997;**54**(2):145–152.
33. Scott JP, *et al*. Effects of sleep deprivation and exercise on cognitive, motor performance and mood. *Physiol Behav*. 2006;**87**(2):396–408.
34. Roehrs T, *et al*. Sleep loss and REM sleep loss are hyperalgesic. *Sleep*. 2006;**29**(2):145–151.
35. Sabatowski R, *et al*.; 1008-045 Study Group. Pregabalin reduces pain and improves sleep and mood disturbances in patients with post-herpetic neuralgia: results of a randomised, placebo-controlled clinical trial. *Pain*. 2004;**109**(1-2):26–35.