Enhancement of synaptic plasticity by NYX-2925: Sleep cycle EEG studies in rats

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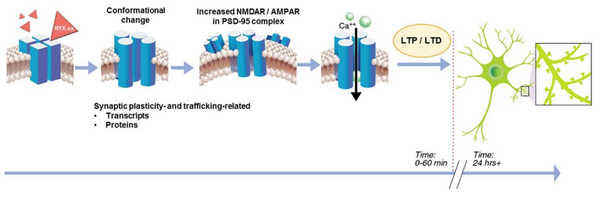

INTRODUCTION

Aptinyx has developed a novel class of small-molecule N-Methyl-D-Aspartate receptor (NMDAr) modulators with broad applicability across neurologic and psychiatric disorders. NYX-2925 is orally bioavailable and currently entering a Phase II trial for neuropathic pain, a condition in which sleep disruption is a core symptom. NYX-2925 facilitates synaptic plasticity, as measured by enhancement of long-term potentiation (LTP) both *in vitro* and *ex vivo* 1-7 days post-dosing (1-10 mg/kg, PO). NYX-2925 also enhances medial prefrontal cortex (MPFC)-dependent novel object recognition and positive emotional learning.

The present studies examine the effects of NYX-2925 on activity-dependent long-term synaptic plasticity *in vivo* using 24-hr EEG recording from the MPFC in the presence or absence of sleep deprivation. Sleep deprivation has been shown to suppress plasticity and decrease pain thresholds. NYX-2925 significantly facilitates non-REM (NREM) during the sleep period, and this effect persisted for 3 days following a single dose. Sleep bout duration and NREM-to-REM transition times were increased without adversely affecting total REM sleep, suggesting better overall sleep quality. Delta power during wake was decreased, suggesting less drowsiness. In contrast, the NMDAR antagonist ketamine acutely suppressed REM and NREM sleep and increased delta power during wake. Ketamine also increased gamma power during wake, indicative of its dissociative effect.

CONCLUSIONS

1) NYX-2925 is unique in its ability to facilitate NREM sleep during the sleep cycle without altering REM sleep or inducing daytime grogginess.
2) NYX-2925 facilitates synaptic plasticity *in vivo,* as measured by increased NREM sleep during the light cycle and facilitates learning and memory after sleep deprivation.
3) Facilitation of NREM sleep may be useful as a biomarker for drug effects.

FINANCIAL DISCLOSURES

JSB, KL, EMC, NGH, ALG, TMM, MAK, RAK, JRM are employees of Aptinyx Inc. employees.

PKS, MHV, and FWT are consultants for Aptinyx Inc.

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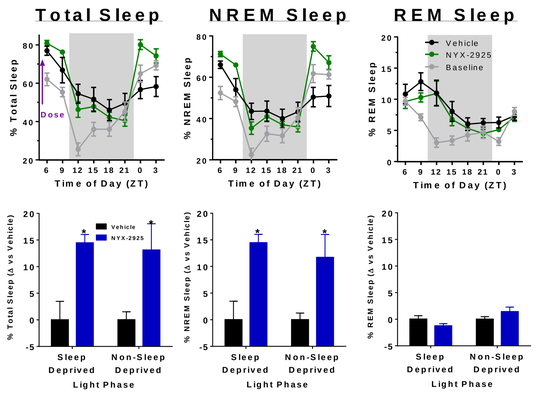
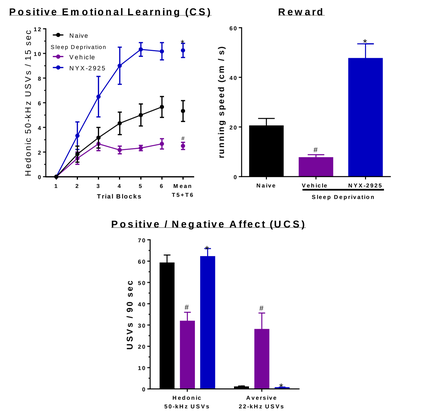
(1) NYX-2925 increases NREM sleep during the light phase in both sleep-deprived and non-deprived rats



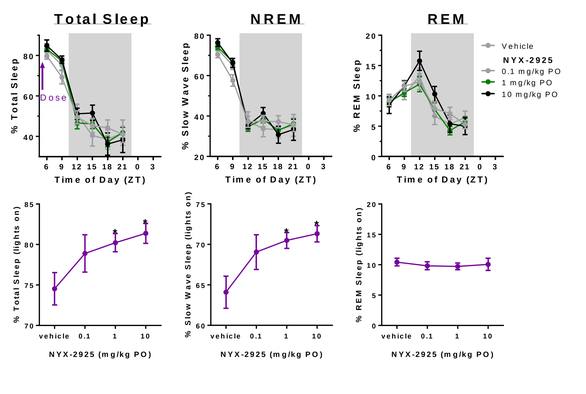
Adult male Sprague Dawley rats were dosed with NYX-2925 (1 mg/kg PO) or saline vehicle at ZT5, and cortical EEG and EMG were recorded continuously via skull screws and neck muscle EMG wires. EEG was captured via a tethered system (Pinnacle, USA), and sleep wake states were scored with combination of manual scoring and machine learning (Gao, Turek, Vitaterna (2016); Journal of neuroscience methods, 264, 33-39). **Top panels** - the effect of 6-hr sleep deprivation (Pinnacle, USA) from ZT0-6 on total sleep, NREM sleep and REM sleep. **Bottom panels** – comparison of NYX-2925 effects of sleep during the lights on period with or without pre- exposure to sleep deprivation.
* P < .05 vehicle vs drug ANOVA.

(2) Sleep deprivation-induced deficits in positive emotional learning are rescued by NYX-2925



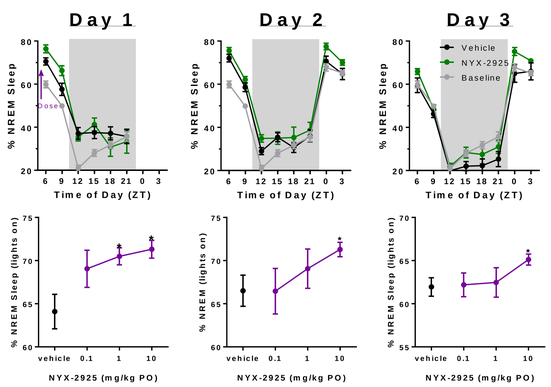
Left panel – NYX-2925 (1 mg/kg PO) increased the rates of hedonic 50-kHz USVs in response to a temporal conditioned stimulus (CS) that predicated heterospecific rough-and-tumble play in rats that had received 23 hrs of sleep deprivation as well as non-dosed / non-deprived naive controls. **Right panel** – approach latency (cm / sec) for the rats to approach the experimenter's hand in order to self-administer heterospecific rough and tumble play. **Bottom panel** – rates of hedonic 50-kHz USVs or aversive 20-kHz USVs in response to unconditional heterospecific rough-and-tumble play (UCS). * P< .05 NYX-2925 vs. vehicle, # P < .05 naive vs vehicle Fisher's PLSD post hoc test.

(3) NYX-2925 (1-10 mg/kg PO) restores NREM in sleep-deprived rats: Day 1 measurements



Adult male rats were dosed with NYX-2925 (0.1, 1, 10 mg/kg PO) or saline vehicle at ZT5, and cortical EEG and EMG were recorded continuously via skull screws and neck muscle EMG wires. EEG was captured via a tethered system (Pinnacle, USA), and sleep/wake states were scored with a combination of manual scoring and machine learning (Gao, Turek, Vitaterna (2016); *Journal of Neuroscience Methods*, 264, 33-39).
Top panels - total sleep, NREM sleep, and REM sleep on days 1-3 following sleep deprivation from ZT0-6 on days 0-1. **Bottom panels** – comparison of sleep parameters during the lights on period across doses of NYX-2925 on days 1-3.
* P < .05 vehicle vs drug Fisher's PLSD post hoc test.

(4) NYX-2925 restores NREM in sleep-deprived rats: Day 1-3 measurements



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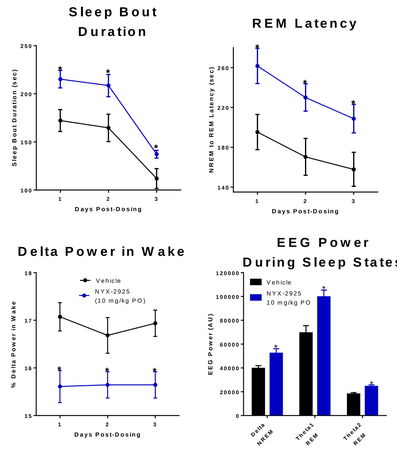
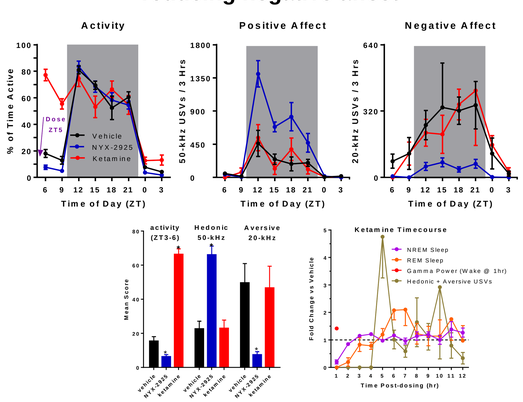
(5) NYX-2925 (10 mg/kg PO) facilitates recovery sleep and reduces grogginess days 1-3 post-dosing



Adult male rats were dosed with NYX-2925 (1 mg/kg PO) or vehicle and the following EEG parameters were quantified on days 1-3 in rats that were sleep deprived for 6 hrs on day 0 and 1 as described in panel 3. **Top left panel** - average sleep bout duration; **Top right panel** – average latency to enter REM sleep from the start of a sleep bout; **Bottom left panel** – delta power (as expressed as % of total power) that occurred during wake. **Bottom right panel** - Delta (0.5-4 Hz) EEG power during NREM and theta (theta1 = 4-8; theta2 = 8-11 Hz) power during REM.
* P < .05 vehicle vs drug Fisher's PLSD post hoc or ANOVA (bottom right).

(6) NYX-2925 increases both sleep during the light phase and positive affect, while reducing negative affect



Adult male rats were dosed with NYX-2925 (10 mg/kg PO), ketamine (10 mg/kg IV), or saline vehicle. Groups of 3 rats per chamber were continuously recorded for 24 hrs, and hedonic 50-kHz and aversive 20-kHz USVs were analyzed via sonogram (Avisoft, Germany). **Top left panel** – activity as measured by the time when sound intensity of the overall recording (sonic and ultrasonic) was above basal room noise. **Top middle, right and bottom left panels** - Rates of hedonic and aversive USVs across 24-hr recording. **Bottom right** – effect of ketamine on NREM, REM, total USVs (hedonic and aversive) for the first 12 hrs post-dosing, and gamma power during wake 1 hr post dosing .
* P < .05 vs vehicle fisher's PLSD post hoc test.