RL-007, a novel oral neuromodulator, enhances synaptic plasticity and cognition in non-clinical models

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Abstract

Background: RL-007 is a novel small molecule neuromodulator that is currently being investigated as a procognitive therapeutic in Cognitive Impairment Associated with Schizophrenia (CIAS). RL-007 was discovered through a phenotypic screen for molecules that modulate synaptic plasticity and improve cognition. RL-007 was then interrogated across a range of in vitro, ex vivo, and in vivo models to assess its clinical potential as a procognitive neuromodulator.

Results: In hippocampal slices, RL-007 potently facilitates basal excitatory synaptic transmission and the induction of LTP, suggesting potential positive impact on learning and memory. RL-007 exhibited an inverted U-shape concentration curve on basal neurotransmission and LTP; 10–100 nM maximally enhanced while 1 nM and 1000 nM were ineffective. In cholinergic ablated hippocampal slices 100 nM of RL-007 significantly potentiated LTP , while 1000 nM remained ineffective. The enantiomer of RL-007, RL-007-03, was 100-fold less potent at potentiating LTP. Two structurally distinct $GABA_B$ antagonists, CGP-55845 (30 nM) or Saclofen (50 µM), occluded RL-007's enhancement of basal neurotransmission and LTP. In vitro, RL-007 does not directly interact with $GABA_B$ receptors, GABA transporters or a broad array of known CNS targets. In rats, the absolute oral bioavailability was 53% (males) to 88% (females), and, at equilibrium, the brain to blood ratio of unbound drug concentration is approximately 31%. 1 mg/kg diazepam (IP) significantly ($p < 0.05$) decreased mouse open field exploratory behavior while 76 mg/kg RL-007-01 (IP) was inactive, demonstrating a lack of sedation. In the Barnes task, there was a significant difference between the young and aged rats for all three home locations ($p = < 0.001$). A daily dose of 5.82 mg/kg RL-007-01 (PO) improved spatial working memory in the Barnes maze and reduced the number of errors during training at all 3 home locations as well as repeat visits to food cups during the last 3 retrievals at home location 3, when the task was most challenging. There was no statistical difference in cognitive performance between the aged rats treated with RL-007 and the young vehicle-treated rats ($p > = 0.138$).

Conclusion: RL-007 potently enhances hippocampal synaptic transmission and plasticity, independent of cholinergic afferents. Despite RL-007 hippocampal slice activity being occluded by $GABA_B$ antagonists, the molecule does not directly modulate $GABA_B$ receptors, nor does it exhibit dose limiting sedative or cognitive deficits that typically accompany GABA pharmacology. RL-007 has high oral bioavailability, readily crosses the blood brain barrier and improves the spatial working memory of aged, cognitively impaired rats. RL-007 exhibits a differentiated and well tolerated procognitive pharmacology that could be broadly beneficial in CNS disease.

Methods

Hippocampal Slice Physiology: RL-007 was evaluated for the ability to interact with basal excitatory synaptic transmission and long-term potentiation (LTP). Hippocampal slices (350 µm) were prepared from young adult male Sprague-Dawley rats and maintained in a recording chamber perfused with preheated artificial cerebrospinal fluid (aCSF) containing (in mM) NaCl 124, KCl 3, KH_2PO_4 1.25, $CaCl_2$ 3.4, $MgSO_4$ 2.5, $NaHCO_3$ 26, and D-glucose 10. Slices were continuously perfused with aCSF at a rate of 1.75-2 ml/min while the surface of the slices was exposed to warm, humidified 95%O_2 /5%CO_2 and maintained at 31 ± 1° C. Field excitatory postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum of CA1 using a single glass pipette (2-3 MΩ) filled with 2M NaCl. Stimulation pulses were delivered at two sites to the Schaffer-commissural axons passing through stratum radiatum via bipolar stimulating electrodes (twisted nichrome wires, 65uM diam) placed on either side of the recording electrode. Stimulation was administered in an alternating fashion to the two electrodes at a rate of 0.05 Hz, using constant current producing a response at 50% of maximum spike-free response. Following stable baseline recording for approximately 20 min compounds were infused into the chamber for 25 min followed by a washout period of 40 min. LTP was induced after 20 min of drug infusion to one of the two pathways via 5 brief high frequency bursts, consisting of four pulses at 100 Hz delivered at an inter-burst interval of 200ms. Infusion of the drug continued for an additional 5 min before washout began for the remaining 40 min of recording.

Hippocampal Cholinergic Lesion: Ablation of the cholinergic septal afferents to hippocampus was performed in 5-week-old male Sprague-Dawley. Briefly, rats were anesthetized and a window of bone overlaying the septal pole of the hippocampus was removed. A syringe was used to displace the neocortex overlying the anterior part of the hippocampus and then ablate the fimbria/fornix anteriorly and medially. At 10-14 days post lesion, the rats were used for hippocampal slice preparation.

Pharmacokinetics (PK): RL-007 PK was evaluated after a single intravenous (2.63 mg/kg RL-007) and oral dose (5.34 mg/kg RL-007) to male and female Sprague Dawley rats at Covance Laboratories, Inc. The blood-brain barrier permeability of RL-007 was determined in male Sprague Dawley rats implanted with a microdialysis probe in the jugular vein as well as the striatum of the brain. Unbound concentrations in blood and brain matrices were measured in freely moving rats after continuous intravenous infusion (21,000 ug/kg/hr over 8 hours). Microdialysis samples were collected for 18 hours at intervals of 30 minutes. Plasma and microdialysis samples were analyzed by LC-MS/MS using a PE Sciex API 3000 mass spectrometer.

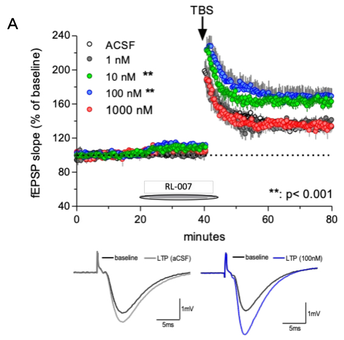
Locomotor: Locomotor behavior was assessed in an open field apparatus consisting of a square plexiglass enclosure with 16 photobeams along the base of each wall. C57BL/6 male mice were treated with RL-007 (76 mg/kg IP) and placed in the locomotor chamber and allowed to explore for 30 or 120 minutes. Diazepam (1, 1.5, or 3 mg/kg IP)) were evaluated alongside RL-007 as positive controls.

Barnes Maze: In this task, rats are trained to leave their home cage to find a food reward placed in one of several food cups throughout the middle of a circular maze. Rats are then required to return to the correct escape tunnel to their home to consume the food reward. Animals were trained to criterion (retrieving all 3 food pellets) sequentially with 3 different home cage locations, and at each home location were given 1 trial/day for a maximum of 10 days. Number of errors and repeat visits to food cups are presented as dependent variables and were analyzed using a repeated measures analysis of variance (ANOVA). Young (10 months old, n=12) and aged Fischer/Norway F1 hybrid male rats (26 months old) were dosed once daily PO with vehicle (n= 10) or 5.82 mg/kg PO RL-007-01 (n=9, aged only) 60 minutes post-training during the training period of up to 10 days.

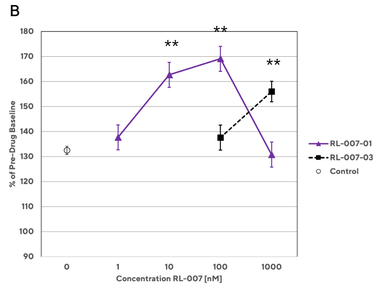
Results

RL-007 Potently Modulates Hippocampal Plasticity

Time course of Effect of RL-007 on Basal Synaptic Transmission and Long-Term Potentiation (LTP)



Hippocampal LTP Enhancement with Inverted U-Shaped Dose Response



In hippocampal slices, 10 nM RL-007 modestly enhanced Schaffer Collateral CA1 synapse neurotransmission ($p < 0.05$).

RL-007 exhibits an inverted U-shaped dose response to enhance LTP. Upon TBS stimulation, 10 and 100 nM RL-007 enhanced LTP, while 1000nM was inactive. The enhancement of LTP is sensitive to RL-007 stereochemistry.

An inverted U-shaped dose response suggests that optimal non-clinical and clinical efficacy will be observed at less than maximally tolerated exposures.

Figure 1 RL-007 enhances hippocampal Schaffer Collateral-CA1 synapses neurotransmission and long-term potentiation (LTP) of synaptic strength. **A)** Time course of effects of a 25min bath-application of RL-007-01 to hippocampal slices, which significantly facilitated synaptic transmission at Schaffer Collateral-CA1. **B)** 10 and 100nM RL-007-01 significantly enhanced the magnitude of LTP. The enantiomer of RL-007, RL-007-03 was 100X less potent, but significantly enhanced magnitude of LTP at 1000 nM concentrations. Individual t-test (two-tailed, unpaired) comparison values are shown.* indicates $p < 0.05$ and ** indicates $p < 0.01$ vs. corresponding vehicle LTP.

Enhanced Hippocampal Plasticity Requires $GABA_B$ Receptors and Is Independent of Cholinergic Input

RL-007 Hippocampal LTP Effects Are Occluded By GABA Receptor Antagonists

Ablation of Cholinergic Afferents Does Not Affect RL-007 Mediated LTP Enhancement

Endogenous $GABA^B$ receptor tone is required for RL-007 plasticity, as evidenced by occlusion of RL-007 LTP when co-infused with $GABA_B$ antagonists.

In contrast, endogenous cholinergic tone is not required for RL-007 plasticity, as hippocampal LTP was enhanced when cholinergic afferents were ablated.

RL-007 plasticity mechanism is through a unique, novel target. Although RL-007 activity is occluded by $GABA_B$ antagonists. 10 uM RL-007 did not exhibit any significant activity in a broad (> 400 targets) radioligand displacement or enzyme inhibition screen) that included $GABA_B$ receptors, GABA transporters, and GABA enzymes.

Figure 2 Pharmacology of RL-007. **A)** In *ex vivo* hippocampal brain slices, 100 nM RL-007, 30nM CGP-55845 or 50 uM Saclofen, two structurally unrelated selective $GABA_B$ v were bath perfused or co-perfused for 25 minutes. Alone, CGP-55845 and Saclofen did not significantly alter basal synaptic transmission or LTP. Individual t-test comparison values are shown for effects on LTP (two- tailed, unpaired). * indicates $P < 0.05$ and ** indicates $P < 0.01$* vs pre-drug and aCSF control LTP, respectively. **B)** Depletion of hippocampal acetylcholine by cholinergic septo-hippocampal lesioning did not alter 100nM RL-007 potentiation of hippocampal slice LTP. Loss of cholinergic afferents was confirmed by loss of physostigmine-induced increase in basal neurotransmission compared to non-lesioned slices (data not shown). Individual t-test (paired, two-tailed comparisons) values are shown. * indicates $P < 0.05$ and ** indicates $P < 0.01$ vs. corresponding within-slice vehicle LTP.

Oral RL-007 Is Bioavailable And Lacks Acute High Dose CNS Adverse Effects

RL-007 is Orally Bioavailable

2.0 mg/kg Intravenous				
	Male		Female	
Parameter	Mean	SD	Mean	SD
t1/2 (hr)*	0.54	0.04	0.82	0.04
C0 (ng/mL)	2200	150	1960	330
Vss (mL/kg/hr)	1180	160	1270	30
AUC0-last (ng hr/mL)*	1720	200	3210	190

4.0 mg/kg Oral Solution				
	Male		Female	
Parameter	Mean	SD	Mean	SD
t 1/2 (hr)*	0.62	0.03	0.86	0.05
Cmax (ng/mL)*	1340	150	3050	210
Tmax (hr)	0.438	0.12	0.25	0
AUC0-last (ng hr/mL)*	1830	160	5750	390
F (%)*	53	4.2	87.6	5.4

	Blood	Brain
Css (ng/ml)	11500	3720
Css Ratio Br/Bl (%)		31 ± 11

RL-007 Lacks Sedative or Locomotor Effects

In vivo, RL-007 is highly orally bioavailable and readily crosses into the brain. At 4mg/kg, RL-007 reached plasma Cmax concentrations (~5uM) and exceed drug concentrations that stimulate hippocampal LTP ex vivo.

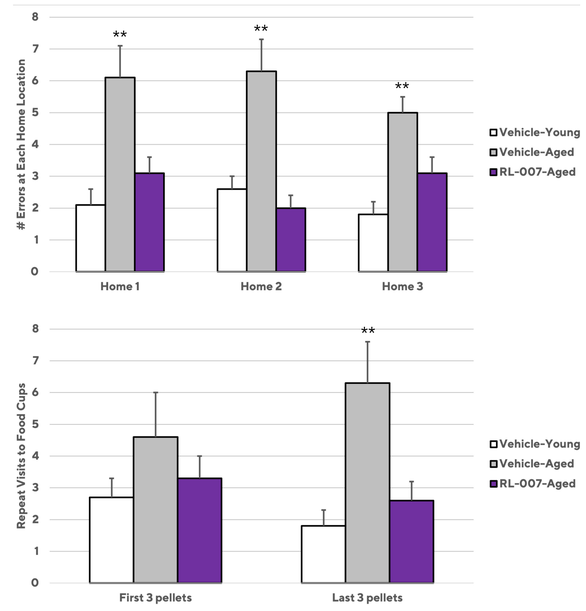
Diazepam, a $GABA_A$ benzodiazepine, induced significant locomotor and sedative effects. In contrast, treatment with a high dose of RL-007 (76 mg/kg) failed to induce sedative or locomotor effects.

In a rat single dose GLP CNS safety functional observation study, RL-007 did not induce acute neurobehavioral effects at doses up to 1527 mg/kg PO.

RL-007 is well tolerated and not dose limited by CNS side effects commonly associated with GABA modulators.

Figure 3 PK and Tolerability of RL-007. **A)** Single dose intravenous, oral PK, and brain microdialysis (males only) of RL-007 was assessed in Sprague Dawley rats . **B)** In male, C57B/6 mice, RL-007 did not influence spontaneous locomotor activity at 100 mg/kg, IP. There was a significant overall effect of treatment on locomotor activity [$F(2,29) = 20.7$; $p < 0.001$]. Bonferroni post-hoc comparisons indicated that mice given diazepam (any dose) were significantly less active than vehicle animals ($p < 0.001$ for diazepam comparison)* indicates $p < 0.05$ and ** indicates $p < 0.01$ vs. vehicle.

RL-007 Reverses Rat Age-Related Spatial Learning and Memory Deficits



Compared to young and RL-007 treated aged animals, vehicle-treated aged rats exhibited significant memory and learning deficits in homing and foraging.

Once daily oral administration of RL-007 improved learning and memory performance of aged rats

Figure 4 RL-007 reverses rat age-related cognitive deficits in the Barnes Maze. **A)** Aged, vehicle-treated rats made significantly more homing/foraging errors compared to young vehicle-treated rats at all three home locations. There was a significant effect of treatment on number of errors at all three home locations: Home 1 [$F(2,30) = 8.9$, $p<0.001$]; Home 2 [$F(2, 30) = 11.4$, $p<0.001$]; Home 3 [$F(2,30) = 13.2$, $p<0.001$]. Post-hoc Bonferroni comparisons indicated that these differences were a result of the significant difference between young vehicle-treated rats and aged vehicle-treated rats ($p\leq 0.001$ at all three home locations). By contrast, RL-007 treated rats were not significantly different than young control rats with respect to number of errors or repeat visits to food cups ($p>0.05$ for all post-hoc comparisons). **B)** RL-007 decreased repeat visits to food cups in aged rats. There was a significant effect of treatment on repeat visits to food cups for the last three retrievals at home location 3 (the most challenging task) [$F(2,30) =7.6$, $p=0.002$]. Post-hoc Bonferroni comparisons indicated that aged vehicle-treated rats performed significantly worse than young vehicle and aged RL-007-treated rats ($p=0.003$ and $p=0.021$ respectively). By contrast, RL-007-treated aged rats were not statistically different on any retrieval measure compared to young vehicle-treated rats ($p>0.05$ for all comparisons).

Conclusions & Impact

- RL-007 is well tolerated, engages a novel mechanism that specifically and potently promotes ex vivo hippocampal plasticity, in vivo cognition and has the potential to be broadly beneficial in CNS diseases.
- Ex vivo, RL-007 exhibits a stereo-specific pharmacology: an inverted-U shaped dose response in hippocampal slices and its enantiomer is 100X less potent.
- Although RL-007 mediated LTP enhancement is blocked by $GABA_B$ receptor antagonists, the molecule does not induce cognitive deficits or exhibit dose limiting sedative effects commonly associated with GABA modulators.
- In vitro, RL-007 does not directly modulate the $GABA_B$ receptors, GABA transporters or a broad array of known CNS targets. Additionally, RL-007's hippocampal plasticity mechanism is independent of the cholinergic system, which has been exhaustively investigated as a pro-cognitive pathway.
- RL-007 has high oral bioavailability, readily crosses into the brain, and reverses spatial memory deficits in aged rats.
- RL-007 is currently being clinically investigated for treatment of cognitive impairment associated with schizophrenia (CIAS).

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Disclosures: Pando MP, Walker GA and Donello JE are employees of Recognify Life Sciences.

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