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RQ-00000009, a selective 5-HT₄ receptor partial agonist, suppressed brain Amyloid-β protein levels and improved memory and cognitive performances in rodents

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Introduction

5-Hydroxytryptamine (5-HT) 4 receptor is widely expressed in the central nervous system, mainly in the hippocampus, frontal cortex and basal ganglia. 5-HT₄ receptor stimulation has been reported to increase a release of acetylcholine which stimulates memory and cognition as well as to reduce Amyloid-beta protein (Aβ) in the experimental animal studies. In clinical, a significant improvement of ADAS-cog score and computerized cognitive testing in Alzheimer's disease (AD) patients by PRX-03140, a 5-HT₄ agonist, was reported in Phase 2a study. These evidences strongly suggest that 5-HT₄ receptor selective agonist is a promising molecular target for the therapy of AD. RQ-00000009 (RQ-9) is a novel 5-HT₄ receptor partial agonist which completed Phase 1 study. In this study, the therapeutic potential of RQ-9 for AD was investigated using preclinical rodent models, and the efficacious dose for AD therapy in patients was estimated.

Conclusion

RQ-00000009 (RQ-9) ;

- is a potent and selective 5-HT₄ receptor partial agonist
- penetrated into brain effectively at brain/plasma ratios of 8.84 (rats) and 20.8 (mice)
- significantly improved memory and cognitive function in rodent models
- dose-dependently decreased brain cortex Amyloid-β protein (Aβ₁₋₄₀ and Aβ₁₋₄₂) in Tg2576 mice
- is ready for Phase 2 clinical testing for AD therapy
 - The projected efficacious dose is 1 mg once-a-day

References

- Mohler, E.G., *et al.*, Neuropharmacology 53, 563-573, 2007
- Orsetti, M., *et al.*, Learning & Memory 10, 420-426, 2003
- Terry Jr. A.V., *et al.*, Psychopharmacology 135, 407-415, 1998
- Shacham *et al.*, ICAD 2006
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Methods

In vitro human 5-HT receptor binding and functional assay:

The binding properties of RQ-9 was tested using membrane fractions prepared from cells expressing recombinant human 5-HT receptors. Commercial radioligands for each subtype were used in the binding assays (N=3). The agonism for 5-HT₄ receptor was tested by cAMP production in the cell-based system (N=3 or 4).

Pharmacokinetics and brain penetration in rats and mice:

RQ-9 was administered to male IGS rats (1 mg/kg, PO) and female C57BL/6J mice (3 mg/kg, PO). Plasma and whole brain were collected and treated for the analysis. RQ-9 concentrations in plasma and brain were analyzed using LC/MS/MS.

Spontaneous alternation task in rats:

RQ-9 (0.03, 0.1, 0.3, 1 and 3 mg/kg) or vehicle (0.5% methylcellulose containing 0.1% Tween 80) was administered orally to male IGS rats (7 week-old, N=15/group) 60 min before the test. Impairment of spontaneous alternation was induced by 0.5 mg/kg (IP) scopolamine 30 min before the test. The test session through the Y-maze was 8 min. The sequence of arm entries was recorded and alternation was defined as entry into all three arms on consecutive choices. The % alternation as an indicator of spontaneous alternation performance was calculated as (number of alternations/total number of arm entries minus 2) x 100.

Novel object recognition task in rats:

RQ-9 (0.1, 0.3 and 1 mg/kg), donepezil HCl (1 mg/kg) or vehicle was administered orally to male IGS rats (9 week-old, N=15/group) 60 min before the training and 60 min before the retention trial. The animal was allowed to explore two identical objects in a bin for 3 min in the training trial. Following a 24-h delay, animal was returned to the bin where one of the original (familiar) objects has been replaced by a novel object. The amount of time that the animal spent exploring each object was recorded using overhead camera and analyzed blindly. The objects are plastic LEGO bricks that vary in shape, color and size. The discrimination index, defined as the difference in exploration time for the objects divided by total exploration time, was calculated.

Brain contents of Amyloid-β in Tg2576 mice:

RQ-9 (0.1, 1 and 10 mg/kg BID) was administered orally to female Tg2576 mice (31 week-old, N=9 or 10/group) for 3 weeks. Three hours after the last dosing, brain tissues (cortex) were dissected and Aβ peptides were extracted with CHAPS-guanidine. Aβ peptides (Aβ₁₋₄₀ and Aβ₁₋₄₂) were quantified by ELISA.

Results

In Vitro Pharmacology

Table 1 Binding, functional and selectivity profiles

(A) Binding affinities for recombinant human 5-HT receptor subtypes				
Receptor	Cell Type	Ligand	Concentration, nM	K _i , nM (95% C.I.)
5-HT _{4d}	HEK293	[³ H]5-HT	6.35	1.4 (1.3-1.4)
		[³ H]GR113808	0.16	0.90 (0.68-1.1)
5-HT _{1A}	HeLa	[³ H]8-OH-DPAT	0.78	>4900
5-HT _{1B}	HeLa	[³ H]5-HT	4.14	>5600
5-HT _{1D}	CHO	[³ H]5-HT	2.16	>4300
5-HT _{2A}	CHO	[³ H]Ketanserin	0.39	>5800
5-HT _{2B}	CHO	[¹²⁵ I]DOI	0.20	>5000
5-HT _{3A}	HEK293	[³ H]BRL-43694	1.11	1700 (1500-2000)
5-HT ₇	CHO	[³ H]5-CT	0.67	>4800

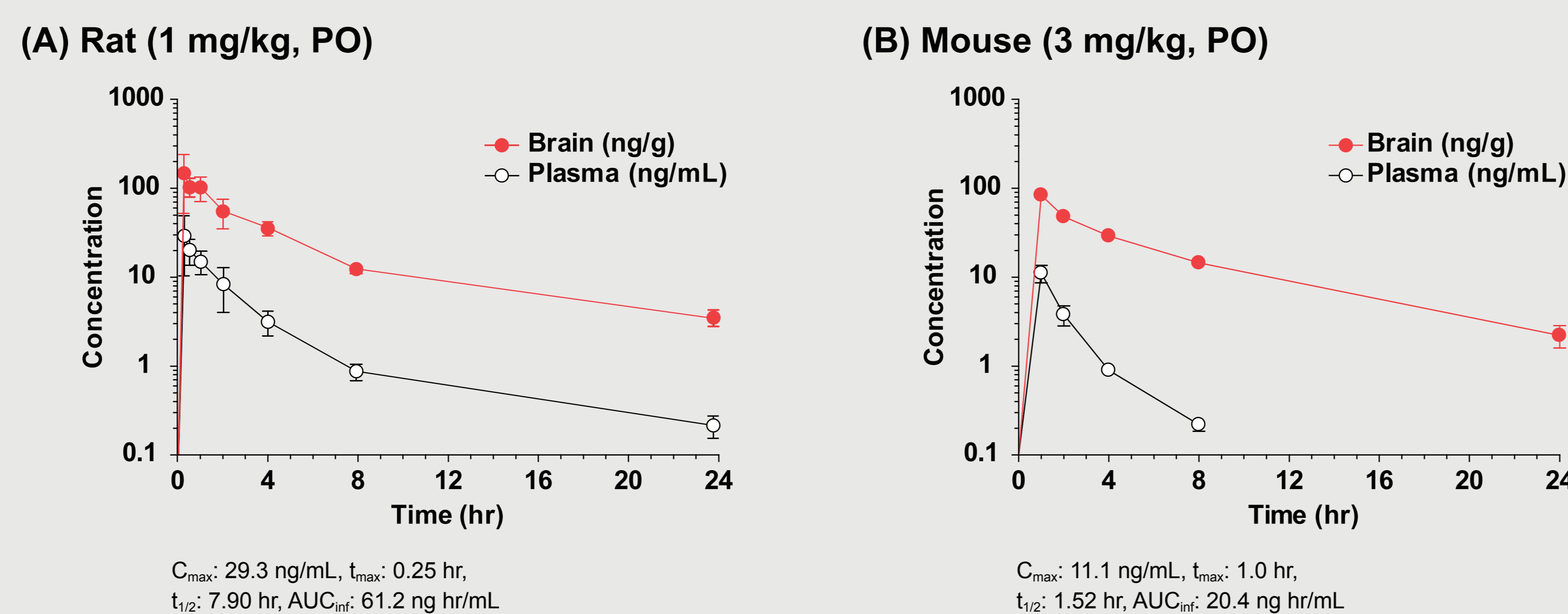
(B) Functional potency and efficacy for 5-HT ₄ receptors				
Receptor	Species	Cell Type / Tissue	EC ₅₀ , nM (95% C.I.)	E _{max} *, % (±S.E.M.)
5-HT _{4a}	Human	HEK293	0.092 (0.045-0.18)	86 ± 8.5
5-HT _{4b}	Human	HEK293	0.19 (0.11-0.32)	101 ± 4.3
5-HT _{4d}	Human	HEK293	1.3 (0.99-1.7)	70 ± 7.0
5-HT ₄	Rat	Tunica muscularis mucosae (TMM)	2.00 (1.00-3.98)	77 ± 1.0

*: % of maximal 5-HT response

Pharmacokinetics & Brain Penetration

Figure 1 Brain penetration in rat and mouse

The brain to plasma ratios in rats and mice on the basis of AUC₀₋₂₄ were 8.84 and 20.8, respectively. Data are expressed as the mean ± SD (N=3).



In Vivo Pharmacology

Figure 2 Spontaneous alternation task in rats

RQ-9 at 0.3 and 1 mg/kg significantly restored the scopolamine (SCOP)-induced impairment of spontaneous alternation performance. No significant change of total arm entry among groups was observed. Data are expressed as the mean ± S.E.M. (N=15/group).

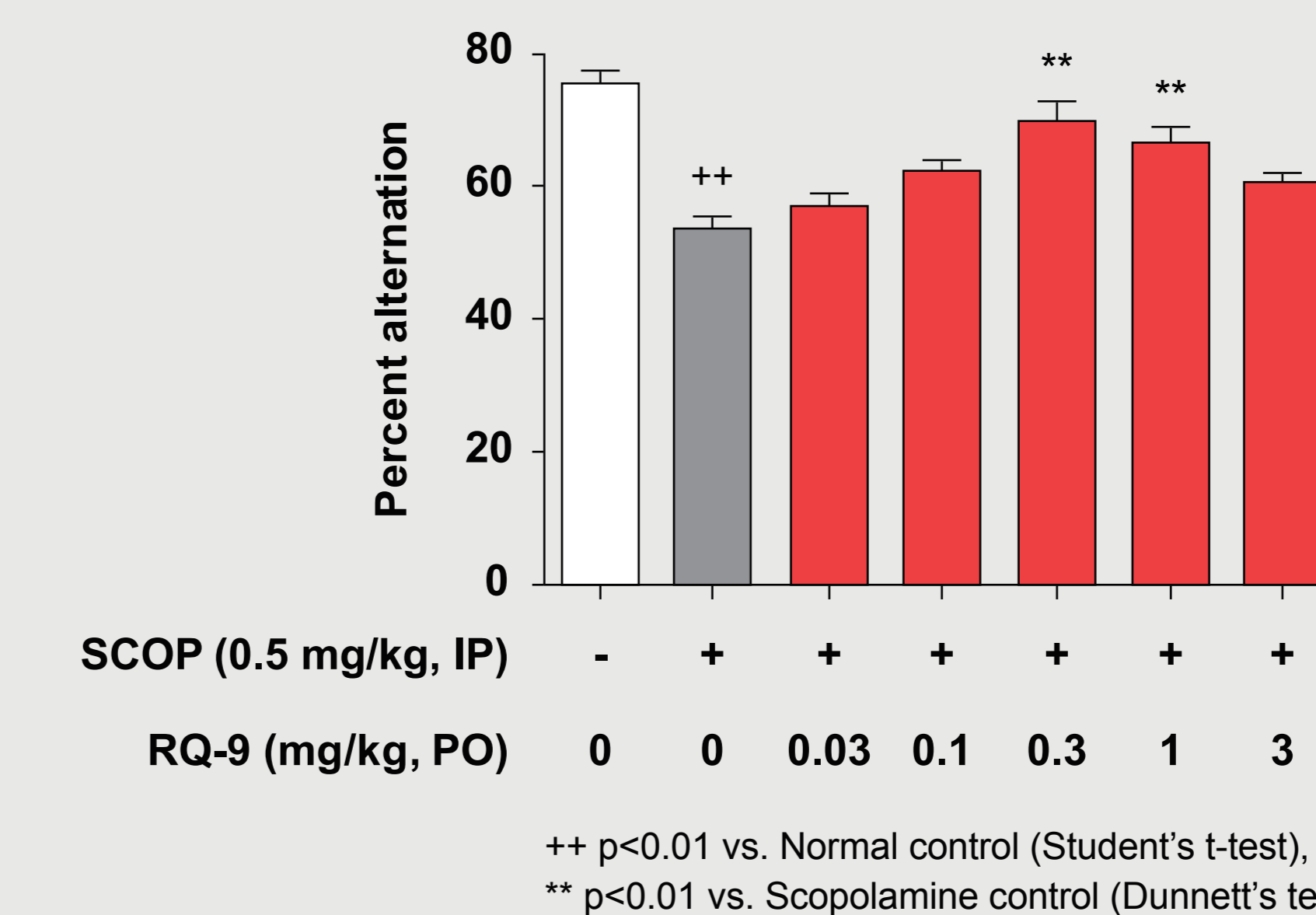


Figure 3 Novel object recognition task in rats

RQ-9 at 0.1, 0.3 and 1 mg/kg significantly increased the discrimination index (A) and the time spent exploring the novel object compared with the familiar object (B). The efficacy was comparable to that of 1 mg/kg donepezil (DPZ). No significant difference in total time spent exploring both objects was observed. Data are expressed as the mean ± S.E.M. (N=15/group).

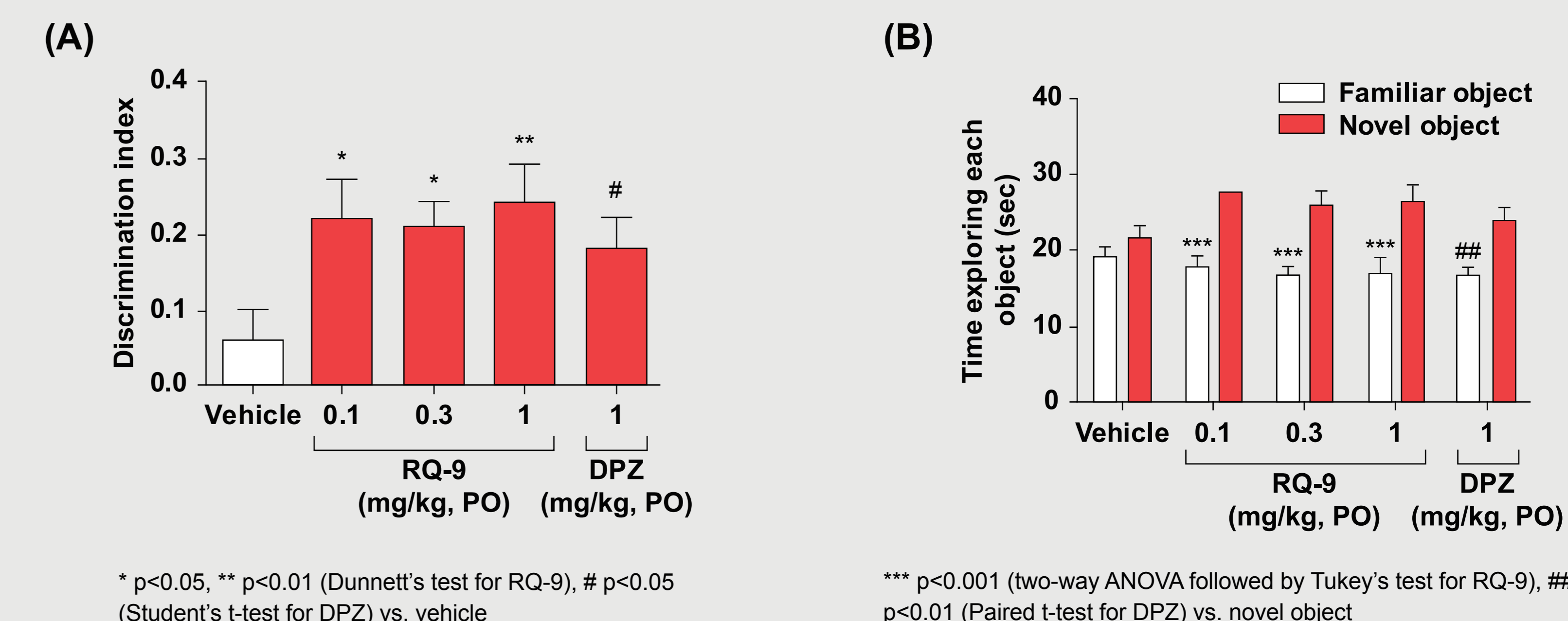
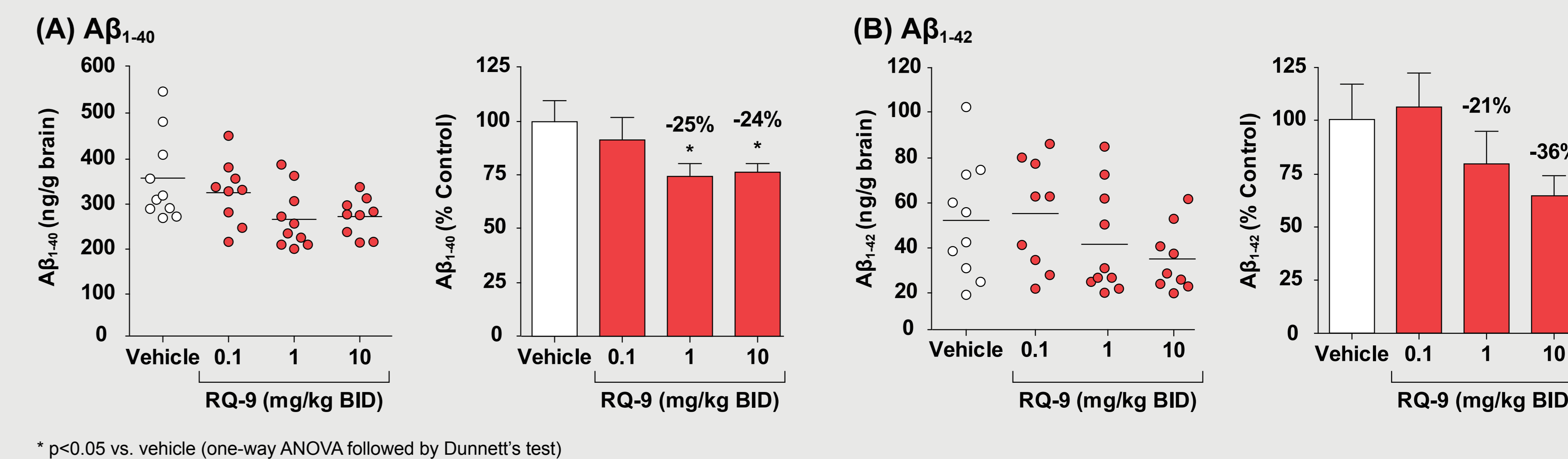


Figure 4 Brain Amyloid-β level in Tg2576 mice

Three-week treatment of RQ-9 at 1 and 10 mg/kg significantly reduced brain cortex Aβ₁₋₄₀ content (A), and also dose-dependently decreased brain cortex Aβ₁₋₄₂ content (B). Data are expressed as the mean ± S.E.M. (N=9-10/group).



Human Efficacious Dose Prediction for AD Therapy

Table 2 Human efficacious dose prediction for AD therapy

A human efficacious dose of RQ-9 as a CNS drug was calculated to be ca. 1 mg once-a-day. The minimum effective doses of RQ-9 in the rat novel object recognition test and the spontaneous alternation study were 0.1 and 0.3 mg/kg, PO, respectively. In a separate experiment, the mean plasma C_{max} at 0.1 and 0.3 mg/kg, PO, in rats were 1.7 and 5.0 ng/mL, respectively. A dose of RQ-9 which achieves a brain concentration of these C_{max} values were calculated using *in vitro* functional potencies and protein binding in human and rats, brain/plasma distribution ratio in rats and plasma PK profiles in Phase 1 clinical study.

	Novel Object Recognition	Spontaneous Alternation
Rat MED (mg/kg, PO)	0.1	0.3
Rat plasma C _{max} at MED (ng/mL)	1.7	5.0
Predicted human efficacious dose (mg QD)	0.87	1.3