Mapping brain neural circuitry in response to pro-cognitive therapeutics: a pharmacological MRI study in the awake rat

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Abstract

The 5-HT$_6$ receptor is a promising target for cognitive disorders, in particular for Alzheimer's disease (AD). The high-affinity and selective 5-HT$_6$ receptor antagonist idalopirdine (Lu AE58054) is currently in development for mild-moderate AD as adjunct therapy to acetylcholinesterase inhibitors (AChEIs). We studied the effects of idalopirdine alone and in combination with the AChEI donepezil on brain activity using BOLD (Blood Oxygen Level Dependent) functional magnetic resonance imaging (fMRI) in the awake rat. Idalopirdine alone had a modest effect on brain activity, with a slow onset of activity. Donepezil alone showed much greater activity with an earlier peak onset. Activity included sites of cholinergic innervation, cortical regions, areas of the septo-hippocampal system and the serotonergic raphe nucleus. When idalopirdine and donepezil were combined, there was a robust stimulation pattern with activation of 36 brain regions spread across the extended-amygdala-, striato-pallidal and septo-hippocampal networks as well as the cholinergic system. These findings indicate that, whilst idalopirdine and donepezil recruit a number of overlapping regions including one of the forebrain cholinergic nuclei, the synergistic effect of both compounds extends beyond the cholinergic system and the effects of donepezil alone towards recruitment of multiple neural circuits and neurotransmitter systems. These data provide new insight into the mechanisms via which idalopirdine might improve cognition in donepezil-treated AD patients.

Materials & Methods

Study and Imaging Protocols

Studies were performed on a Bruker BioSpec 7T / 20cm USR. Anatomical data set was collected using the RARE pulse sequence (20 slice; 1.2 mm; field of vision [FOV] 3.0 cm; 256 × 256; repetition time [TR] 2.5 sec; echo time [TE] 12.4 msec; NEX 6; 6.5 minute acquisition time). Functional images were acquired using a multi-slice half Fourier acquisition, single shot, turbo spin echo sequence. A single scanning session acquired 22 slices, 1.0 mm thick, every 6.0 seconds (TR 6.0 sec, TE 48 msec, FOV 3.0 cm, matrix size 96 x 96, NEX 1) repeated 500 times for a total time of 50 min. The in-plane pixel resolution is 312 μm^2. Each scanning session was continuous for 50 min, starting with 50 baseline image acquisitions during 5 minutes prior to treatment, then drug presentation followed by 450 image acquisitions during the following 45 minutes.

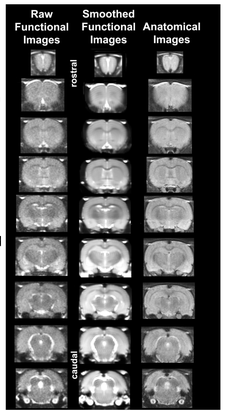
Adult male Sprague Dawley rats were divided into four groups: vehicle (n=9), idalopirdine (n=10), donepezil (n=8), idalopirdine/donepezil (n=9). All drugs were given i.v. through a tail vein catheter during the imaging session. Idalopirdine (Lu AE58054, Lundbeck) was dissolved in 5% HpBeta cyclodextrin in distilled water and evaluated at the dose of 2 mg/kg. Donepezil hydrochloride (Lundbeck) was dissolved in 5% HpBeta cyclodextrin in distilled water and evaluated at the dose of 0.3 mg/kg. The combination of idalopirdine (2 mg/kg) and donepezil (0.3 mg/kg) was also evaluated. In previous studies we have demonstrated that these doses of idalopirdine and donepezil result in clinically relevant exposure of both compounds and high 5-HT$_6$ receptor occupancy up to an hour after administration. The vehicle control was 5% HpBeta cyclodextrin in distilled water. All injections were given in a volume of 1ml/kg.

Acclimating Rats for the Imaging Protocol

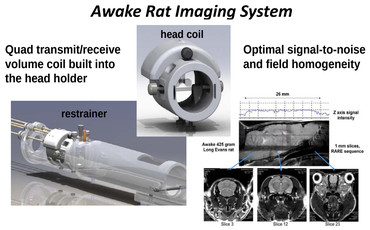
Adult, male Long Evans rats were lightly anesthetized, and placed into a copy of the restraining system used during awake imaging. When fully conscious, the animals were placed into a dark mock scanner tube with a recording of a standard MRI pulse sequence playing in the background. This procedure when repeated every other day for four days has been shown to significantly reduce plasma CORT, respiration, heart rate, and motor movements when compared to the first day of acclimation. The reduction in autonomic and somatic response measures of arousal and stress improve the signal resolution and MR image quality. (King et al. 2005)

Imaging Analysis

Functional MRI data analysis included four primary steps: 1) preprocessing, including slice timing correction, co-registration, smoothing and de-trending; 2) registration to rat brain atlas, followed by segmentation; 3) voxel-wise statistical analysis for each individual to identify voxels that experienced a signal change in relation to baseline; 4) group comparisons on the number of activated voxels per ROI and neural network. Preprocessing of functional scans was performed using in-house MATLAB® (The Mathworks, Inc, Natick MA.) software in combination with SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) batch interface. Preprocessed functional files were then exported to Medical Image Visualization and Analysis (MIVA) for registration and segmentation. The mean functional image (output from SPM) was used to register images to the rat atlas since the quality of the functional scans are structurally superior to those obtained using gradient echo (GRE) or echo planar imaging (EPI) sequences as noted above in the figure to the right.



Awake Rat Imaging System



Studies were performed with a quad transmit/receive head coil and rat restrainer developed by Animal Imaging Research, Holden, Massachusetts

Time-Dependent Changes in Activity

Donepezil

15-25 min Post Treatment

Region of Interest(ROI)	Veh	DON	P val
magnocellular preoptic nucleus	0	1	0.007
dorsal raphe	0	8	0.012
medial septum	0	3	0.02
medial orbital ctx	0	16	0.022
ventral pallidum	0	12	0.027
medial geniculate	0	22	0.028
insular ctx	14	107	0.03
superior colliculus	29	112	0.03
frontal association ctx	0	10	0.032
inferior colliculus	41	108	0.034
primary somatosensory ctx upper lip	0	41	0.041
dentate gyrus ventral	1	39	0.043
anterior olfactory nucleus	0	33	0.044
external plexiform layer	23	56	0.047
dorsal paragigantocellularis nucleus	0	3	0.047
ventral orbital ctx	0	4	0.048
entorhinal ctx	27	147	0.048
lateral geniculate	2	19	0.049
intercalated amygdaloid nucleus	0	0	0.05

Donepezil/Idalopiridine

25-35 min Post Treatment

Region of Interest(ROI)	Veh	A58/D	P val
infralimbic ctx	0	39	0.006
granular cell layer	15	81	0.006
accumbens shell	0	13	0.006
anterior olfactory nucleus	0	33	0.007
lateral preoptic area	0	8	0.008
habenula nucleus	3	18	0.009
ventral pallidum	0	5	0.009
triangular septal nucleus	0	9	0.012
medial preoptic area	0	26	0.012
3rd cerebellar lobule	4	22	0.013
lateral posterior thalamic nucleus	8	45	0.013
periaqueductal gray thalamus	9	75	0.013
accumbens core	0	2	0.013
medial dorsal thalamic nucleus	2	11	0.019
dentate gyrus ventral	4	24	0.019
prelimbic ctx	1	18	0.023
lateral septal nucleus	16	45	0.024
bed nucleus stria terminalis	2	14	0.026
diagonal band of Broca	0	7	0.026
medial pretectal area	0	0	0.028
tenia tecta ctx	8	47	0.029
posterior hypothalamic area	0	18	0.03
anterior cingulate area	4	50	0.03
secondary somatosensory ctx	4	35	0.032
frontal association ctx	0	7	0.032
reuniens nucleus	0	3	0.033
primary somatosensory ctx trunk	0	10	0.033
magnocellular preoptic nucleus	0	1	0.035
central gray	2	7	0.037
parafascicular thalamic nucleus	2	21	0.037
external plexiform layer	29	43	0.038
glomerular layer	23	92	0.038
ventromedial thalamic nucleus	0	6	0.045
premammillary nucleus	2	4	0.048
dorsomedial tegmental area	0	5	0.05
primary somatosensory ctx hindlimb	0	43	0.05

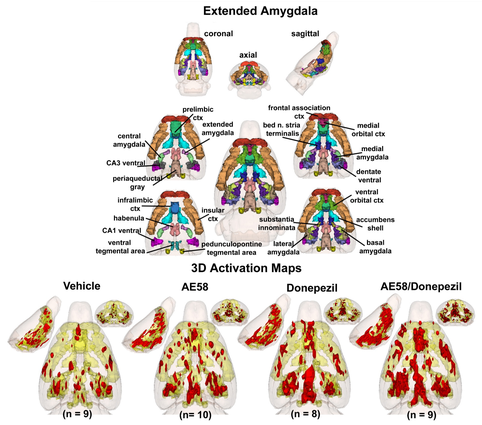
Idalopiridine (AE58)

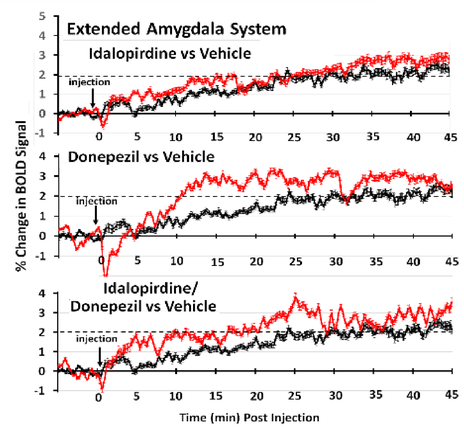
35-45 min Post Treatment

Region of Interest(ROI)	Veh	AE58	P val
ventral pallidum	0	8	0.006
accumbens shell	0	10	0.008
diagonal band of Broca	0	7	0.008
medial preoptic area	1	8	0.013
infralimbic ctx	0	14	0.015
substantia nigra reticularis	6	17	0.033
periaqueductal gray thalamus	24	47	0.037
magnocellular preoptic nucleus	0	1	0.042

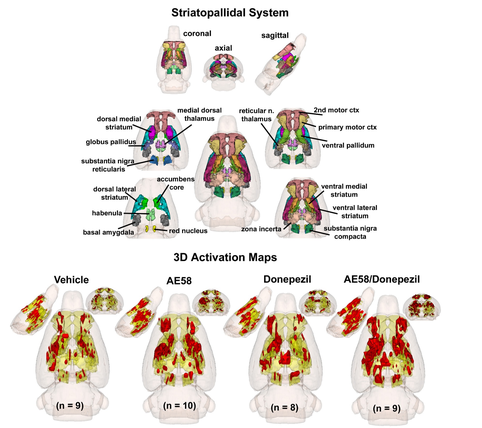
Main Results

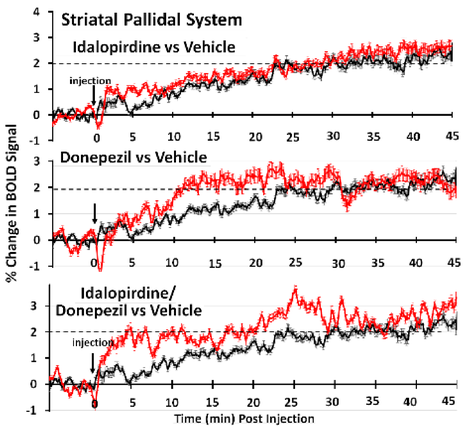
Brain Activity in the Extended Amygdala Integrated Neural Circuit



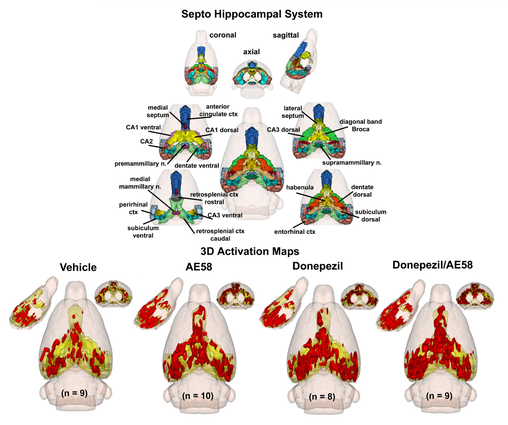


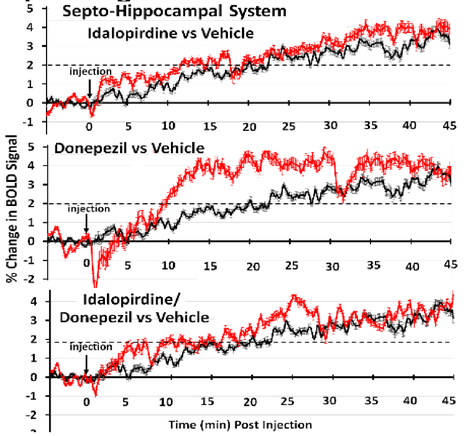
Brain Activity in the Striato-Pallidal Integrated Neural Circuit





Brain Activity in the Septo-Hippocampal Integrated Neural Circuits





Summary

In summary, the current data indicate that, whilst idalopirdine and donepezil recruit a discrete number of overlapping brain regions including one of the forebrain cholinergic nuclei, the synergistic effect of combining treatment extends beyond the effects of donepezil alone and the cholinergic system, towards recruitment of multiple neural circuits and neurotransmitter systems. Indeed, the combination treatment recruits a constellation of integrated neural circuits associated with cognition, emotion and motivation as well as extroceptive (olfaction) and introceptive cues (brainstem). These may collectively contribute to enhancing cognition, by enriching learning and memory processes with motivational salience and the context of extro- and introception. These data provide new insight into how idalopirdine may extend and complement the benefits of donepezil observed in patients with AD.