Characterization of the 5-HT6 receptor antagonist, SB-742457, on cognitive test performance in the rat.
Guy A. Higgins1,2, Leo Silieniek1, Winnie Lau1, Sandy Thevarkunnel1, Elijah Undyss1, Alan Henderson2, Nicholas Moore3 (1 CanCog Technologies, Toronto ON, 2 Dept. of Pharmacology, University of Toronto, 3 Albany Molecular Research Inc., Albany, NY)

Introduction
Although considerable effort is currently directed toward identifying treatments that modify the neurodegeneration that contributes to Alzheimer’s Disease (AD) pathology, there is also a need to identify treatments that can relieve the symptoms of this disease. One approach that has gained significant recent interest is selective 5-HT6 receptor antagonists. Indeed one 5-HT6 antagonist, SB-742457, has demonstrated clinical efficacy in mild-moderate AD patients as measured by ADAS-cog and CIBIC+ scales, with an effect size equivalent to donepezil (see Upton et al., 2008). However results published from a recent Phase II study of 5 month duration conducted in a total of 371 patients randomized to treatment showed improvements in only a subset of efficacy endpoints compared to placebo (Maher-Edwards et al, 2010). In both studies, SB-742457 was reported to be well tolerated, even at exposures predicted to attain ~97% occupancy of central 5-HT6 receptors.

In the present studies we have evaluated SB-742457 in a rodent model of cognition, the novel object recognition (NOR) task, and compared its profile to donepezil (Aricept®). Furthermore, we have conducted preliminary studies to follow up on the finding that 5-HT6 receptor antagonists may increase maximal electroshock seizure thresholds in rats (Routledge et al, 2000; Stein et al, 2002). These studies were extended to the mouse as despite significant sequence homology between the rat, mouse and human 5-HT6 receptor, the pharmacological profile of the mouse receptor appears distinct to that of the rat and human (Hirst et al, 2003). A similar species differences may exist with respect to the regional expression of the 5-HT6 receptor in mouse compared to rat and human (Hirst et al, 2003). Consequently, comparison of drug effect between these species offers a potential means to examine target based mechanism of action.

Methods
Locomotor activity: Male, CD-1 mice and SD rats were used as test subjects. Following a defined pretreatment time the animals are singly placed within test chambers where locomotor activity (defined as distance travelled) is measured by photocell interruptions. The spatial distribution of activity can also be measured. MES threshold test: Male, CD-1 mice and SD rats were used as test subjects. Following a defined pretreatment time the animals were tested for MES seizure thresholds using the ‘up and down’ method (Upton et al, 1997). In the mouse studies, sodium valproate was included as a positive control.

Cholinergic side effects: Male SD rats served as test subjects. Animals were checked for cholinomimetic signs (lacrimation, salivation, diarrhoea, tremor and hypothermia) at specific time points for up to 24 hours after drug treatment. General activity was measured at 1 hour after treatment (represented by distance travelled and rearing count).

Novel Object recognition: Male SD rats served as test subjects. Animals were habituated twice in the test chambers prior to test sessions. Each experiment was comprised of two trials, the sample trial (T1) and the choice trial (T2). Every exposure to the test chamber was preceded by a defined pretreatment. During T1 animals were singly placed within test chambers where two identical objects were presented. In the choice trial the animals were exposed to a duplicate of the object from T1 as well as a novel object. During both trials, animals were tracked digitally using video cameras and an automated tracking software (ANY-maze®). Comparing the amount of time spent with either object during T2, it is possible to assess how well rats are able to discern between the familiar (a) and the novel objects (b). Due to the rat’s spontaneous tendency to explore and interact with unfamiliar objects, this model is widely used as a measure of recognition memory, an important facet of human declarative memory (Winters et al, 2008), which is compromised in AD patients.

Results
A. Comparison between SB-742457 on locomotor activity & MES threshold in mouse vs. rat

Summary and conclusions
1. SB-742457 (3-10mg/kg oral) reduced motor activity and increased MES threshold in Sprague Dawley rats. The effect on MES threshold confirms previous studies by Routledge et al (2000); Stein et al (2002) using other structurally distinct 5-HT6 antagonists.

2. Interestingly, SB-742457 (3-10 mg/kg oral) did not reliably reduce activity or increase MES threshold in CD mice. This may reflect species differences in affinity of SB-742457 for the rat vs. murine 5-HT6 receptor, and/or regional species differences in expression pattern (Hirst et al, 2003).

3. In a 24 hour novel object recognition test, young vehicle pretreated rats failed to show a robust novel object preference.

4. SB-742457 (10mg/kg oral) pretreatment during the sample and choice test phases, resulted in a significant novel object preference. Mean plasma exposure immediately post treatment was measured at 14±315 ng/ml. In comparison, donepezil (0.3-3mg/kg i.p.) showed a modest, but inconsistent trend to produce an equivalent effect.

5. At doses from 3-10mg/kg, donepezil produced clear cholinomimetic signs which likely contributed to a decline in novel object test performance. In contrast, SB-742457 (30-60mg/kg oral) produced no cholinomimetic or other overt behavioral signs. These observations are consistent with a good tolerability reported in the clinic (Maher-Edwards et al, 2010).

6. The present studies support the hypothesis that 5-HT6 receptor antagonists have pro-cognitive effects although current clinical reports seem mixed (Upton et al, 2008; Maher-Edwards et al, 2010).