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Age-related declines in delayed non-match-to-sample performance (DNMS) are reversed by the novel 5HT6 receptor antagonist SB742457

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ABSTRACT

Alterations in synaptic plasticity and neurocognitive function with age have been well documented in the literature. These changes are accompanied by modifications of neurotransmitter systems in the central nervous system (CNS). The serotonergic system in particular plays an important role in attention, alertness and cognition. Disturbances in serotonergic function have been implicated in differing neurological and neuropsychiatric disorders including depression, psychosis aggression and dementia. The serotonin receptor subtype 5HT6 is distributed within CNS regions relevant to learning and memory, including the striatum, cortex and hippocampus. We examined here the effects of acute and chronic administration of the 5HT6 receptor antagonist SB742457 on performance in a delayed non-matching-to-sample task (DNMS), which was used to identify neurocognitive differences between middle-aged (MA, 13 months) and young adult (YG, 3 months) rats. We found that MA rats have significantly lower performance in the DNMS task compared to YG rats. Acute administration of SB742457 (3 mg/kg/po) significantly improved performance of the MA rats. Chronic administration of SB742457 (3 mg/kg) reversed the age-related deficit of the MA to match their performance to that of YG rats. Furthermore, these improvements were observed for 1 week post-SB742457 treatment cessation. The acute and chronic effects of this treatment suggest that there is both an immediate effect on neurotransmitter action and potentially a longer-term modification of synaptic plasticity. Together these data indicate a role for modulation of the serotonergic system in the development of cognition-enhancing agents.

1. Introduction

Age-related decline in cognitive function may result from numerous and widespread changes in the brain. Neurocognitive deficits related to aging also involve concomitant alterations of neurotransmitter systems (Stemmelin et al., 2000) that might be prevented or slowed by pharmacological intervention (Frolich and Forstl, 2011; Burchinsky, 1984). Delayed-response tasks have proven reliable at identifying age-related deficits in humans (Li Hegner et al., 2010), non-human primates (Bartus et al., 1978) and rodents (Dunnett et al., 1988). We focus here on the use of the delayed non-match-to-sample (DNMS) task, where the rule requires flexibly modulating behavior through time, where the animal must choose the reverse to the previous occasion (see Methods). The DNMS task was chosen over the delayed match-to-sample (DMS) task as DNMS does not inhibit the animal's natural alternation tendency (Deacon and Rawlins, 2006). Furthermore, the DNMS task is sensitive to subtle changes in hippocampal neurophysiological dynamics that may not be visible in behavior until later in life (Hok et al., 2012), specifically, between young adult (YG) and middle-aged (MA) populations. Lesions of the hippocampus, prefrontal cortex and/or subiculum all impair performance on the DNMS task (Porter et al., 2000; Hampson et al., 1999a). Insult to any of these regions can cause deficits in performance (Dunnett et al., 1988). Furthermore, performance in the DNMS task has previously been reported to be supported by the interplay of the hippocampus and subiculum (Hampson et al., 1999b, 2003). The DNMS task is functionally partitioned between neurons within subiculum and hippocampus to uniquely identify trial-specific information accounting for both spatial and temporal constraints on performance within and between trials (Deadwyler and

Abbreviations: YG, young adult; MA, middle-aged; DNMS, delayed non-matching-to-sample; DMS, delayed match-to-sample; CNS, central nervous system; AD, Alzheimer's disease.

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Hampson, 2004). At a systems level, oscillatory activity at the population level (specifically at theta frequency) between prefrontal cortex and hippocampus is highly correlated with correct trial performance (Hyman et al., 2010).

Alterations in the serotonergic system have been observed with age and with age-related impairments in spatial memory (Stemmelin et al., 2000). The 5HT6 serotonergic receptor is found almost exclusively in the CNS, with the highest concentrations found in the hippocampal formation, frontal cortex, striatum and olfactory tubercle (Gerard et al., 1996; Hamon et al., 1999). Reduced density of 5HT6 receptors were found in both the frontal and temporal cortex from post-mortem AD patients and correlated with aggression and the Present Behavioral Examination in these same patients (Garcia-Alloza et al., 2004). Critically, the 5HT6 receptor is particularly distributed in structures supporting memory formation and hence, performance in a delayed non-matching-to-sample (DNMS) task. The serotonergic system also modulates multiple neurotransmitter pathways, including central cholinergic neurotransmission (Bentley et al., 1999; Riemer et al., 2003) which has been extensively implicated in age-related cognitive decline. Modifications of the serotonergic system may affect other neurotransmitter systems, resulting in beneficial effects on cognition (Shirazi-Southall et al., 2002). Direct intra-cerebroventricular administration of antisense oligonucleotides in rats induced receptor knock-down and improved spatial learning and memory in the water maze (Bentley et al., 1997; Woolley et al., 2001), identifying a role for 5HT6 receptor knock-down in learning and memory. However, another study demonstrated increased 5HT6 expression, through targeted gene delivery, had no effect on spatial learning in the water maze, but decreased ability of rats to learn a reward-based instrumental learning operant task, as compared to sham surgery or GFP (green fluorescent protein)-expressing control rats. This 5HT6 receptor-associated deficit was reversed by administration of a 5HT6 antagonist, SB-258585 (Mitchell et al., 2007). Other studies have used pharmacological interventions: the select 5HT6 antagonist, SB742457, reverses scopolamine-induced deficits in object recognition and in a passive avoidance (Chuang et al., 2006). Other 5HT6 antagonists including Ro-04-6790 and SB271046 (King et al., 2004) and SB399885 (Hirst et al., 2006) reverse age-related deficits in spatial and recognition memory tasks. SB271046 improves spatial recognition memory in adult mice and reverses age-related consolidation deficits of episodic-like memory in aged mice (Da Silva Costa et al., 2009; Da Silva Costa-Aze et al., 2011). High doses of SB271046 reverses scopolamine-induced deficits in working memory and those of acquisition and retrieval of aversive learning; whereas scopolamine-induced deficits in episodic-like memory were only partially counteracted by SB271046 (Da Silva Costa-Aze et al., 2012). In a separate study sub-chronic treatment with SB271046 improved acquisition in the water maze in rats, while acute treatment only improved retention but SB271046 (acute or sub-chronic) treatment failed to reversed scopolamine-induced learning deficits (Marcos et al., 2008). The selective 5-HT6 receptor antagonist Ro4368554 restores memory performance in cholinergic and serotonergic models of memory deficiency in the rat (Lieben et al., 2005). GSK-742457 ameliorated scopolamine-induced deficits in object recognition task and high doses GSK-742457 also reduced scopolamine-induced deficits in the object location task in 3-months-old male Wistar rats (de Bruin et al., 2011). SB742457 is currently in phase II clinical trials and has proven to be generally safe and well tolerated and may be a potential treatment of Alzheimer's disease (Maher-Edwards et al., 2010). The DNMS task was utilized to induced delay deficits in normal rats and naturally occurring deficits in aged rats of short-term working memory, to provide confirmation of cognitive enhancement with SB742457. The current study aimed to examine the cognitive enhancing potential of the 5HT6 antagonist, SB742457, on delay induced cognitive deficits in normal rats and naturally occurring deficits in ageing rats. Together with its presumed role in cognitive enhancement (Upton et al., 2008), these data have lead us to test the hypothesis that the 5HT6 receptor antagonist SB742457 may modulate normal age-related deficits in short-term spatial-temporal memory as measured in the DNMS task in middle-aged animals.

2. Materials and methods

Animal care and procedures were conducted in accordance with European Communities Council Directive (86/609/EEC) and the experimental protocols were approved by the animal experimentation committee of Trinity College Dublin.

2.1. Subjects

Young (YG; 3 months at study start, $N = 10$) and middle-aged (MA; 13 months at study start, $N = 20$) male Han Wistar rats (B&K, United Kingdom) were used in this study. We chose to look at the effects of aging on cognition; therefore we chose to use young adult rats and middle-aged rats rather than old age, therefore targeting early stages of memory deficit with age. These age groups were chosen in light of a current review on developmental stages of classification in rats (McCutcheon and Marinelli, 2009). The WHO (World Health Organization, 2007) regard middle-aged as 40+ years which proportionately corresponds to about 13 months old rat (which on average live to between 20 and 26 months of age). Average weights were 321.5 g ± 12 and 557 g ± 49, respectively, at the start of the experiment; they were maintained at 85% of free-feeding weight during training. The rats were housed in groups of two or three in a controlled environment (temperature: 20–22 °C, 12/12 h light/dark cycle) with water *ad lib* and controlled food allowance of 13 g per rat at the end of each day. The animals were initially screened for visible tumors, signs of ill-health or overt motor deficits through handling and observation in their home cage. Any animals which do not meet the above criteria would be excluded from experiment, in this case all animals were in good health and therefore included in experimental protocol. Animals were naïve to all other testing prior to inclusion in the current experiment.

2.2. Apparatus

Experiments were performed in a Plexiglas behavioral testing chamber (Med Associates; 30.5 cm L × 24.1 cm W × 21.0 cm H), with two retractable levers mounted on one wall 5.5 cm above the chamber floor (distance between levers is 11 cm), a hopper (5 cm × 5 cm) mounted between the levers 1.5 cm above the floor on the original wall where 45 mg dustless sucrose pellets were delivered (TestDiet™, 5TUT formula), and a retractable lever mounted on the center of the opposite wall 5.5 cm above the chamber floor.

2.3. Task design

DNMS was chosen as it allows us to repeatedly test each animal in the same task, meaning that each animal is its own control. We had 3 groups: YG group ($N = 10$), MA group ($N = 10$) and a DNMS stability group ($N = 10$). The DNMS task requires the animal to press the retractable lever presented on a random basis on the left or right (sample response) to initiate the trial. This initiates a delay phase randomly timed between 1 and 30 s. After the delay the animals have to press the center lever located on the opposite wall. Animals then return to where now both the left and right levers are extended (*match*/*non-match* phase). The correct response requires a press on the opposite lever pressed during the sample phase (constituting the *non-match* response), which is followed by delivery of a sucrose pellet into the hopper. An incorrect response (pressing the same lever as the one pressed in the sample phase) produces a 5 s time-out in which the overhead lights are turned off and no sucrose pellet is delivered. Trials are separated by 10 s. Overall performance on the task declines in a linear manner as the duration of the delay interval is increased from 1 to 30 s.

Animals are trained every day over c. six week period to press levers for a sucrose pellet reward; following this time the animals are started on the DNMS task where they are trained until they reach on average 85% correct criterion for 3 consecutive days. At this stage, the animals were administered drug (see below) or control daily for 7 days while completing the DNMS task. Treatment was then withdrawn and recording in the DNMS task was continued for another 7 days.

2.4. Training protocol

The rats were initially habituated to the chambers with the three levers extended. The animals were trained for 2 days to lever press for food reward on a continuous reinforcement schedule (*i.e.* pressing of any lever would result in the delivery of a sucrose pellet to the hopper). On the subsequent 2 days the levers were

programmed to retract once pressed, delivering a pellet and then extending again. This was also on a continuous reinforcement based schedule, and aimed at habituating the animals to the retraction and extension of the levers. On the 5th day, the same program was used with the exception that one specific lever could not be reinforced more than 3 consecutive times. This modification was aimed to force the animals to perform alternate lever pressing, thereby suppressing lever preferences to obtain reward.

The next phase of training involved randomized presentation of the front lever (left or right), which, once pressed, triggers the back lever extension. The reward is delivered only when the back lever is pressed. These lever combinations are repeated 60 times (30 left/center and 30 right/center) at 10 s intervals; this procedure is repeated for 2 days.

Training in the non-matching-to-sample task comprised 90 trials in a 90 min session daily. At the start of each session the house light was turned on with the levers in the retracted position. The animals were initially trained on the task contingencies with no enforced delay between the sample and the choice component (0-delay condition). At the start of each trial, one response lever was randomly selected and inserted into the chamber (the "sample"). As soon as the lever press response was registered, the lever was retracted and the rear lever on the opposite wall was extended. Once the response on the back lever was registered the two front levers were extended into the chamber together (the "choice"). A response on the non-matching-to-sample lever was designated correct, the levers were retracted, a pellet was delivered to the hopper, the house light remained on and an inter-trial interval of 10 s was initiated before the next trial began. A response on the initial sample lever resulted in an incorrect response, the levers were retracted, no pellet was delivered, the house light was extinguished and the 10 s interval was initiated before the next trial started. Rats were required to meet a criterion of 85% for 3 consecutive days on this program before introduction of the delay. In the next stage of training a randomized 1–5 s delay was introduced between the response on the sample lever and the extension of the rear lever. This phase lasted 3 days. In the final stage of training the random delay was extended to a maximum of 30 s, requiring the rat to wait for the extension of the rear lever before moving to the choice phase. Training on the DNMS task continued for 39 days, by which time a plateau in performance was reached. Continual training in the DNMS task does not alter an animal's output score of percentage correct response for up to 4 weeks once a plateau in performance has been reached following training (Fig. 1).

2.5. Pharmacological treatment

Subsequent to the 39 days training in the DNMS task, a 7 day baseline recording was made for each animal. Animals were orally given 0.2 ml maple syrup daily for 1 week, 24 hr prior to performance in the DNMS task. Based on a previous publication (Foley et al., 2008) 24 hr appeared to be the most appropriate interval, given the drugs pharmacokinetics in rats. Animals were administered orally the 5HT6 receptor antagonist SB742457 (GSK), 3 mg/kg suspended in 0.2 ml maple syrup. SB742457 has an oral bioavailability of 76%, has an effective dose range of 0.5–4.4 mg/kg (p.o.) in cognitive models, pK_i is 9.6 and selectivity of ≥100-fold (for review see Upton et al., 2008). Animals received the mix daily 24 hr before performance in the DNMS task for 1 week. Drug was withdrawn at this time and DNMS performance was continued for 1 additional week (see Fig. 1A).

2.6. Statistical analysis

DNMS trials were sorted by performance according to length of delay on individual trials and were grouped according to 5-s intervals (1–5, 6–10, 11–15, 16–20, 21–25, and 26–30). The data are presented graphically by correct percentage of response at 5-s delay groups. All data were statistically analyzed with SPSS, using ANOVAs and Bonferroni *post-hoc* tests. Repeated measures (RM) one- or two-way ANOVA's were used for within animal analysis and regular one- or two-way ANOVA's were used for between group analysis where indicated. For further analysis, data were divided into groups of short and long delays and analyzed by Friedman repeated measures ANOVA with Dunn's multiple comparison tests.

3. Results

The DNMS task was chosen as it allows us to repeatedly test each animal in the same task. Once optimal performance is reached following a 39 day training regime, animals can be repeatedly trained in this task without spontaneous modification of performance. The DNMS stability group demonstrates 28 days of repeated training in the DNMS task does not significantly alter performance in the task (Fig. 1A: RM ANOVA $F_{(27,243)} = 1.358$, $p = 0.1182$). There was no difference in DNMS performance from week to week within the DNMS stability group (Fig. 1B: two-way RM ANOVA: Interaction: $F_{(15,162)} = 1.774$, $^{*}p = 0.0423$; Delay: $F_{(5,162)} = 47.43$, $^{***}p < 0.0001$; Time (weeks): $F_{(1,90)} = 1.608$, $p = 0.1897$; subject matching: $F_{(18,90)} = 4.21$, $^{***}p < 0.0001$). Thus, DNMS task performance is reliable over a long period for repeated measures within the same animal and therefore allows each animal to be its own control.

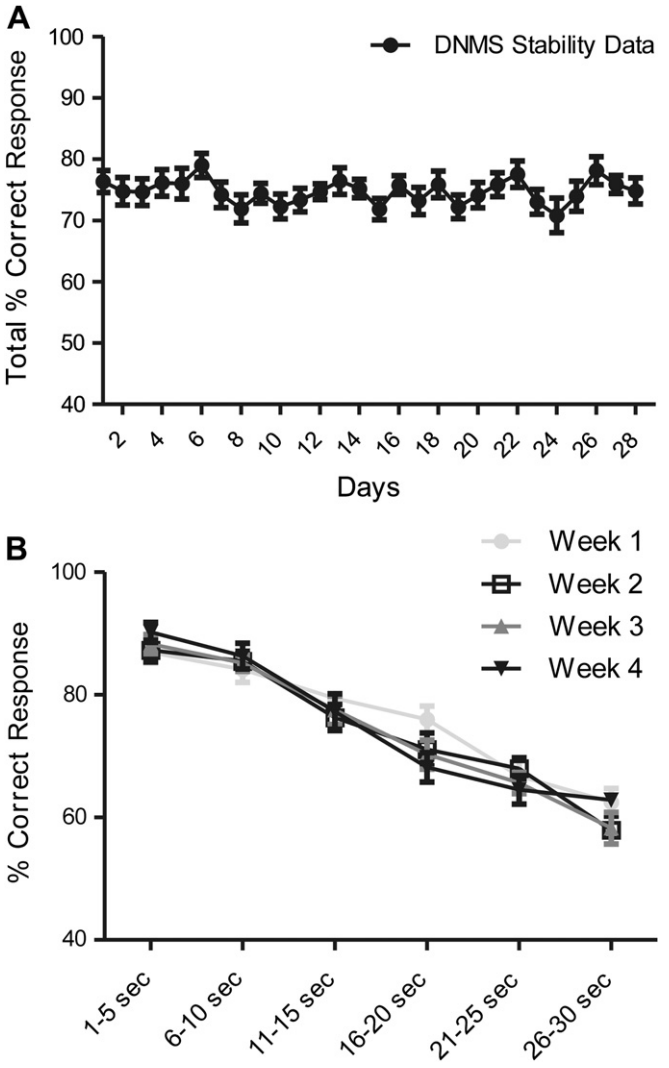

Fig. 1. Stability of DNMS performance over 28 days. A. Daily recordings of DNMS stability group "total % correct response" in recordings over 28 days. B. DNMS trials were sorted by performance according to length of delay on individual trials and were grouped according to 5-s intervals (1–5, 6–10, 11–15, 16–20, 21–25, and 26–30). Data are represented on a weekly basis.

The DNMS task was used to identify cognitive differences between YG (3 months, $n = 10$) and MA (13 months, $n = 10$) male Han Wistar rats. Following the training regime in the DNMS task, rats were assessed for 7 days to give a baseline recording. Analysis of baseline performance over 7 days showed age had a significant effect on performance of the DNMS task (Fig. 2B: two-way ANOVA: Interaction: $F_{(6,126)} = 0.6745$, $p = 0.6705$; Age: $F_{(1,126)} = 19.69$, $^{***}p < 0.0001$; Days: $F_{(6,126)} = 0.2071$, $p = 0.9740$). Statistical analysis revealed that age had a significant effect on total performance in the task and this was independent to repetition. Further analysis of individual baselines revealed no significance difference in performance in the task over baseline training (RM ANOVA's: YG: $F_{(6,54)} = 0.7203$, $p = 0.3002$; MA: $F_{(6,54)} = 0.3517$, $p = 0.9059$). MA rats were impaired in the working memory task compared to YG rats in terms of % correct responses (Fig. 2C: two-way ANOVA: Interaction: $F_{(5,108)} = 0.7203$, $p = 0.6096$, Age: $F_{(1,108)} = 28.75$,

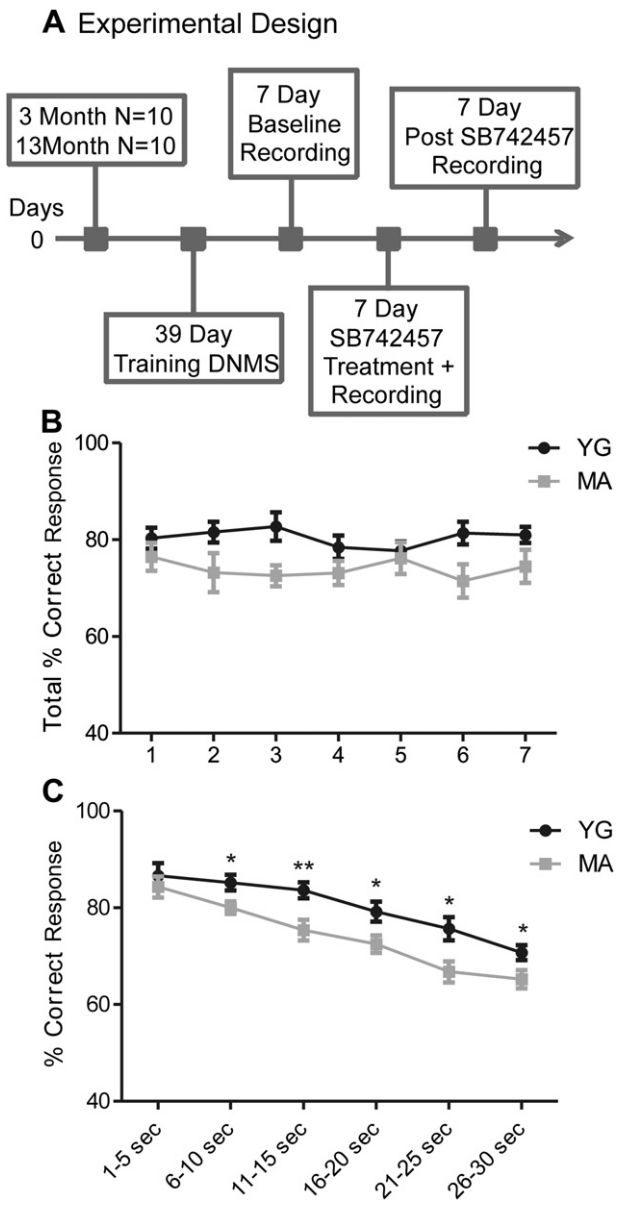

Fig. 2. Comparison of middle-aged (MA) and young rats (YG) performance in the DNMS task. A. Experimental Design for Figs 1–4. B. Daily recordings of YG and MA "total % correct response" in baseline recordings over 7 days. C. DNMS trials were sorted by performance according to length of delay on individual trials and were grouped according to 5-s intervals (1–5, 6–10, 11–15, 16–20, 21–25, and 26–30). YG Base ($n = 7$) and MA Base ($n = 7$) performance were averaged across trials, sessions and animals ($n = 10$ for both). Each point thus represents the mean (±SEM) percent of correct trials performed within each trial across delays. Values significantly different from the baseline are indicated with an asterisk (Two-way ANOVA: Bonferroni *post-hoc*, $^{*}p < 0.05$, $^{**}p < 0.01$).

$^{***}p < 0.0001$, Delay: $F_{(5,108)} = 22.95$, $^{***}p < 0.0001$). Statistical analysis revealed that age and delay had a significant effect on performance in the task and both factor were independent to each other. Further, *post-hoc* analysis revealed differences at delay intervals of 6–10 s block ($^{*}p < 0.05$), 11–15 s block ($^{**}p < 0.01$), 16–20 s block ($^{*}p < 0.05$), 21–25 s block ($^{*}p < 0.05$) and 26–30 s block ($^{*}p < 0.05$). These data suggest that the DNMS task is sensitive to age-related differences in performance on a mnemonic task.

Acute administration of SB742457 enhanced performance in the DNMS task in MA rats. Treatment with 3 mg/kg SB742457 24 hr before testing significantly improved performance in MA animals, compared to the previous day of training (i.e. Day 7 of their respective baseline; Fig. 3B: two-way RM ANOVA: Interaction: $F_{(5,90)} = 1.918$, $p = 0.0991$; Delay: $F_{(5,90)} = 12.79$, $^{***}p < 0.0001$; Acute Treatment: $F_{(1,90)} = 8.709$, $^{**}p = 0.0085$; subject matching: $F_{(18,90)} = 3.490$, $^{***}p < 0.0001$). Statistical analysis revealed that acute drug treatment had a significant effect on performance in the task and this was independent to delays. Further *post-hoc* analyses revealed significant differences at 3 time blocks (Fig. 3B and Table 1: 21–25 s ($^{*}p < 0.05$) and 26–30 s ($^{**}p < 0.01$)). No difference was found in performance of MA animals between day 6 and day 7 of baseline recordings (Data not shown: two-way RM ANOVA: Interaction: $F_{(5,90)} = 1.464$, $p = 0.2990$; Delay: $F_{(5,90)} = 13.7$, $^{***}p < 0.0001$; Daily Difference: $F_{(1,90)} = 1.981$; $p = 0.1764$; subject matching: $F_{(18,90)} = 4.978$, $^{***}p < 0.0001$). Unlike MA animals, acute treatment of SB742457 had no effect on performance in the DNMS

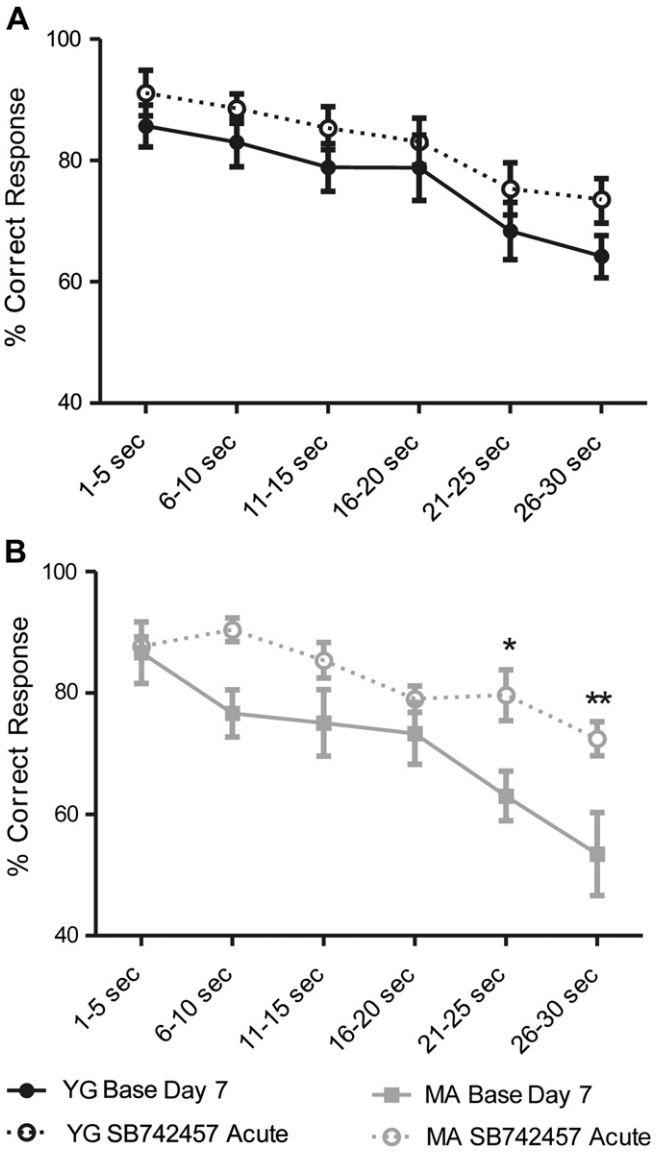

Fig. 3. Influence of acute SB742457 administration on working memory in YG and MA rats. A. YG Base Day 7 ($n = 1$ session) is compared to YG SB742457 Acute treatment ($n = 1$ session) within animal ($n = 10$). B. MA Base Day 7 ($n = 1$ session) is compared to MA SB742457 Acute ($n = 1$ session) within animal ($n = 10$). RM two-way ANOVA reveals a significant drug dependant improvement in performance of the DNMS task at 3 of the 5 s intervals in the MA group. Values significantly different from the Base Day 7 are indicated with an asterisk (Bonferroni *post-hoc*, $^{*}p < 0.05$, $^{**}p < 0.01$).

Table 1
Acute and Chronic effects of SB742457 treatment percentage correct response in the DNMS task.

	Baseline	Acute	Chronic	Post-treatment
Days	7	1	7	7
YG				
1–5 s	86.64 ± 2.59	91.12 ± 3.78	93.56 ± 1.43*	91.43 ± 0.82
6–10 s	85.18 ± 1.62	88.56 ± 2.42	89.51 ± 1.18*	89.90 ± 1.65
11–15 s	83.63 ± 1.65	85.33 ± 3.55	87.19 ± 1.84	85.73 ± 2.09
16–20 s	79.19 ± 2.07	83.09 ± 3.88	82.74 ± 1.71	83.50 ± 1.97
21–25 s	75.66 ± 2.39	75.33 ± 4.28	78.39 ± 3.31	77.42 ± 3.10
26–30 s	70.72 ± 1.55	73.57 ± 3.43	72.77 ± 3.16	73.32 ± 3.23
MA				
1–5 s	84.3 ± 2.17	87.68 ± 1.58	90.25 ± 1.81*	88.65 ± 2.08
6–10 s	80.04 ± 1.3	88.69 ± 2.27**	88.63 ± 2.45**	87.82 ± 2.35*
11–15 s	75.36 ± 2.14	85.39 ± 2.91*	85.49 ± 2.02**	84.18 ± 2.39*
16–20 s	72.48 ± 1.79	79.01 ± 2.17*	81.03 ± 2.25**	81.63 ± 2.08*
21–25 s	66.75 ± 2.19	76.35 ± 4.51*	78.46 ± 2.38**	75.21 ± 2.08*
26–30 s	65.21 ± 1.9	72.47 ± 2.80*	70.65 ± 2.84	71.15 ± 2.44

Data represent the mean ± SEM of the percentage of correct response in the DNMS task for each time interval of 5 s. Significant difference from relevant baseline is indicated by an asterisk ($^{*}p < 0.05$, $^{**}p < 0.01$, $n = 10$).

task in YG animals (Fig. 3A and Table 1, two-way RM ANOVA: Interaction: $F_{(5,90)} = 0.1343$, $p = 0.9840$; Delay: $F_{(5,90)} = 10.59$, $^{***}p < 0.0001$; Acute treatment: $F_{(1,90)} = 3.379$, $p = 0.0826$; subject matching: $F_{(18,90)} = 3.169$, $^{**}p < 0.0002$).

Chronic administration of 3 mg/kg of SB742457 daily for 1 week augmented performance in the DNMS task in the MA animals (Fig. 4B: two-way RM ANOVA: Interaction: $F_{(5,90)} = 1.128$, $p = 0.3516$; Delay: $F_{(5,90)} = 41.32$, $^{***}p < 0.0001$; Chronic treatment: $F_{(1,90)} = 14.37$, $^{**}p = 0.0013$; subject matching: $F_{(18,90)} = 5.766$, $^{***}p < 0.0001$.) with profound effects at almost all time blocks (1–5 s: $^{*}p < 0.05$; 6–10 s: $^{**}p < 0.01$; 11–15 s: $^{**}p < 0.01$; 16–20 s: $^{**}p < 0.01$; 21–25 s: $^{**}p < 0.01$). Statistical analysis revealed that chronic drug treatment had a significant effect on performance in the task and this was independent to delays. The magnitude of this effect of SB742457 was such that the performance of MA rats now matched the YG Base (two-way ANOVA: Interaction: $F_{(5,108)} = 0.1957$, $p = 0.9635$; Group Difference: $F_{(1,108)} = 3.217$, $p = 0757$; Delay: $F_{(5,108)} = 19$, $^{***}p < 0.0001$). Chronic treatment with SB742457 in YG animals appears to increase performance at shorter delays but this was not significant (two-way RM ANOVA: Interaction: $F_{(5,90)} = 0.5542$, $p = 0.7347$; Delay: $F_{(5,90)} = 36.74$, $^{***}p < 0.0001$; Chronic treatment: $F_{(1,90)} = 3.019$, $p = 0.0994$; subject matching: $F_{(18,90)} = 5.755$, $^{***}p < 0.0001$). However when data were split into shorter and longer delay blocks the increase in performance of YG following chronic treatment was significant (see below).

Previous studies have shown that 5HT6 receptor antagonists can produce long term effects on synaptic plasticity (see Discussion) and it was therefore decided to assess potential effects of SB742457 on DNMS performance after cessation of drug administration. Chronic administration of SB742457 resulted in sustained increasing performance in DNMS for at least 1 week post-treatment in MA animals. Analyses of DNMS performance 1 week post-treatment with SB742457 revealed a sustained and significant effect of the drug on DNMS task performance (Fig. 5B: two-way RM ANOVA: Interaction: $F_{(5,90)} = 0.7432$, $p = 0.5931$; Delay: $F_{(5,90)} = 42.79$, $^{***}p < 0.0001$; Post-treatment: $F_{(1,90)} = 11.37$, $^{**}p = 0.0034$; subject matching: $F_{(18,90)} = 6.061$, $^{**}p < 0.0001$). This statistical analysis reveals chronic drug treatment had a sustained significant effect on performance in the task for 1 week follow drug withdrawal, and this was independent to delays. *Post-hoc* analyses revealed significant difference at 4 time blocks between MA Base and MA Post SB742457 (6–10 s: $^{*}p < 0.05$; 11–15 s: $^{*}p < 0.05$; 16–20 s: $^{*}p < 0.05$; 21–25 s: $^{*}p < 0.05$) represented in Fig. 5B and Table 1. No differences were observed between MA SB742457 Chronic and MA Post SB742457. For the YG group the performance appears to remain elevated but this is not significant (Fig. 5A, two-way RM ANOVA: Interaction: $F_{(5,90)} = 0.3734$, $p = 0.8657$; Delay: $F_{(5,90)} = 34.11$, $^{***}p < 0.0001$; post-treatment: $F_{(1,90)} = 2.220$, $p = 0.1535$; subject matching: $F_{(18,90)} = 6.077$, $^{***}p < 0.0001$).

In further analysis we split the time blocks into two groups of shorter (1–15 s) and longer (16–30 s) delays to assess the overall effects of SB742457 on shorter and longer delays (Fig. 6). Administration of 3 mg/kg SB742457 daily for 1 week augmented performance in the DNMS task at shorter delays in the YG animals (Friedman statistic = 8.867, $^{*}p = 0.012$) but had no effect at longer delays (Friedman statistic = 2.467, $p = 0.29$). SB742457 administration also improves YG performance in the DNMS task at shorter delays. Similar analysis of the MA animals revealed treatment of SB742457 significantly improved DNMS performance at shorter

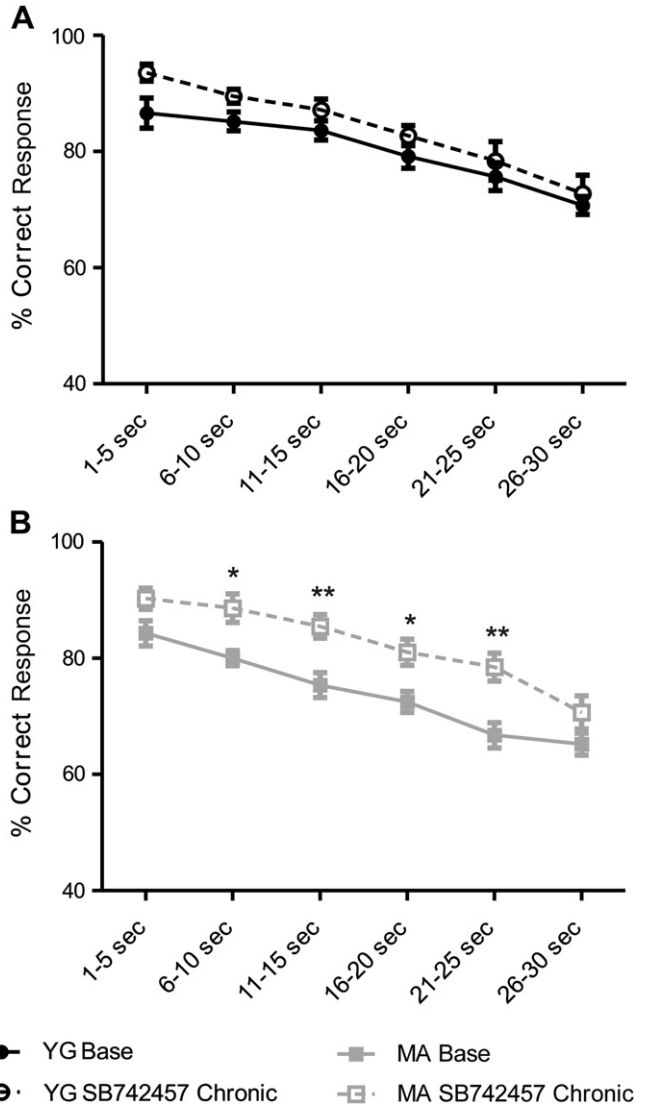

Fig. 4. Influence of chronic SB742457 administration on working memory in YG and MA rats. A. YG Base ($n = 7$ sessions) is compared to YG SB742457 Chronic ($n = 7$ sessions) within animal ($n = 10$). B. MA Base ($n = 7$ sessions) is compared to MA SB742457 Chronic ($n = 7$ session) within animal ($n = 10$). RM two-way ANOVA reveals a significant drug dependant improvement in performance of the DNMS task. Values significantly different from the Base are indicated with an asterisk (Bonferroni *post-hoc*, $^{*}p < 0.05$, $^{**}p < 0.01$).

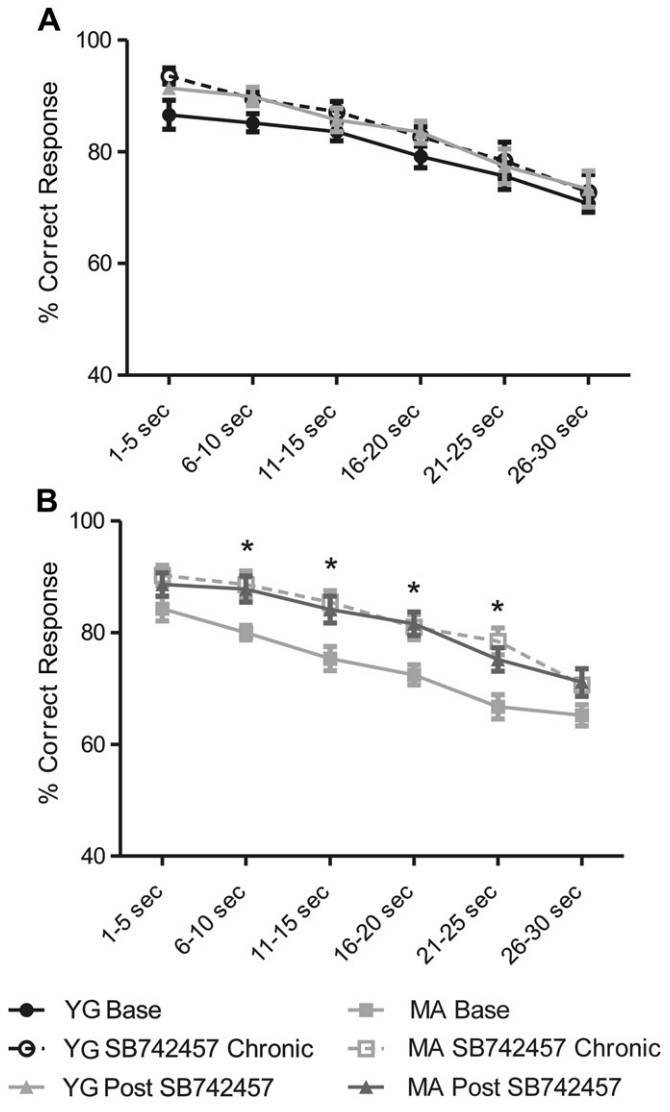

Fig. 5. Effect of chronic SB742457 administration, one week post-treatment on performance of the DNMS task in YG and MA rats. A. YG Base ($n = 7$ sessions) is compared to YG SB742457 Chronic ($n = 7$ sessions) and YG Post SB742457 ($n = 7$ sessions) within animal ($n = 10$). B. MA Base ($n = 7$ sessions) is compared to MA SB742457 Chronic ($n = 7$ session) and MA Post SB742457 ($n = 7$ sessions) within animal ($n = 10$). RM two-way ANOVA reveals a significant post-drug treatment sustained improvement in performance of the DNMS task compared to Base. Values significantly different from the Base are indicated with an asterisk (Bonferroni *post-hoc*, $^{*}p < 0.05$).

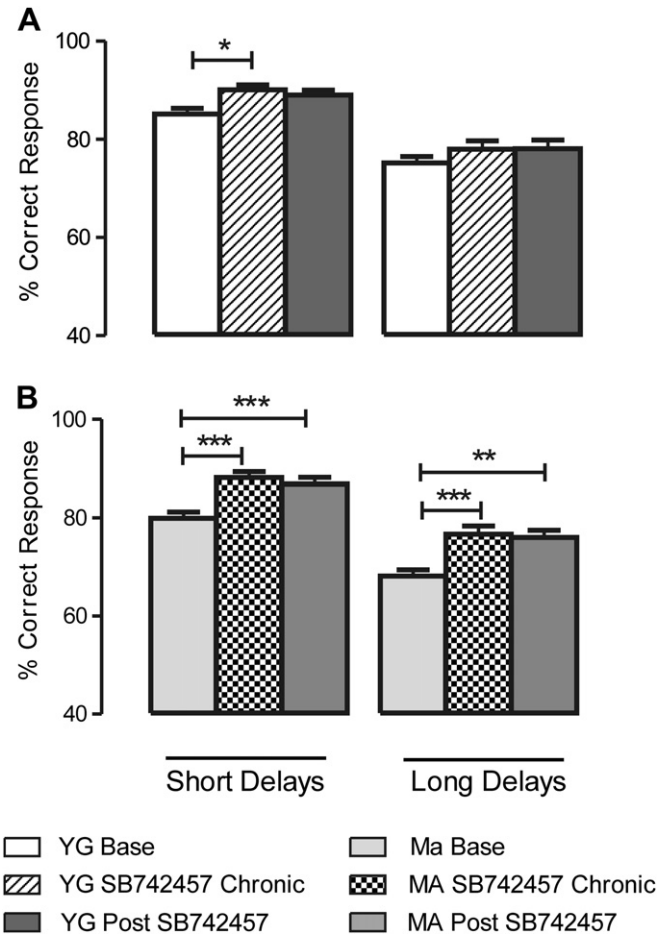

Fig. 6. Administration of SB742457 improves both short and long term memory in the rat at both short and long delays in the DNMS task. Histograms represent % correct response at short delays (1–15 s) and long delays (16–30 s). A. There is a significant increase in performance by YG SB742457 Chronic compared to YG Base at short delays in the DNMS task when analyzed by Friedman's repeated measures ANOVA (Friedman statistic = 8.867). No differences were observed at long delays for the YG animals (Friedman statistic = 2.467). B. Analysis by Friedman's repeated measures ANOVA revealed a significant increase in performance between MA Base and MA SB742457 Chronic at short delays (Friedman statistic = 22.84). Analysis of MA groups at long delays revealed significant increase in performance between MA base and MA SB742457 Chronic, and between MA Base and MA Post SB742457 (Friedman statistic = 23.41). Values significantly different from the Base are indicated with an asterisk (Dunn's post-hoc analysis, $^{**}p < 0.01$, $^{***}p < 0.0001$).

delays (Friedman statistic = 22.84, $^{***}p < 0.0001$) and longer delays (Friedman statistic = 23.41, $^{***}p < 0.0001$). Dunn's multiple comparison test confirmed between group significant difference ($p < 0.05$).

4. Discussion

In this study we report three major findings. First, we showed that the DNMS task can be used to identify subtle changes in cognition and performance that occur in early aging. The DNMS task identifies a deficit in short-term working memory in middle-aged (MA) rats compared to young adult (YG) rats. Secondly, this deficit in short-term working memory is relieved by both acute and chronic treatment with the 5HT6 receptor antagonist, SB742457. Finally, the deficit in memory continues to be alleviated for at least one week post-treatment with SB742457. Importantly, both groups were able to learn the task, with both groups performing well above chance, even at the longest delays.

We hypothesise that this reduction in cognitive performance is due to subtle changes within the neurotransmitter systems and modifications in the structural plasticity of the brain that are becoming more apparent in older populations. These changes may begin at an earlier phase of life, but are not always evident until older age. The effects of early-term aging on cognition are not always identifiable using common behavioral assays. Old Wistar rats (24 months) show a deficit in spatial memory in the water maze compared to young (3 months) controls, whereas MA (12 months) do not show a deficit compared to young animals (Long et al., 2009). Behavioral tasks may not always be sensitive enough to detect subtle differences in cognitive performance between YG and MA. The present study shows that both YG and MA rats were able to learn the DNMS task, although the MA subjects showed performance impairments compared to the YG controls, corroborating observations made by Wisco et al. (2008) and Herndon et al.

(1997) in non-human primates. Interestingly, the particular profile of memory impairment observed in our study might reveal which specific components are at risk during the early onset of the aging process. It has been demonstrated recently that both the hippocampus and subiculum show striking neuronal correlations with respect to the different phases of the DNMS task (Hampson et al., 1993, 2004). It appears (Deadwyler and Hampson, 2004) that the encoding of trial-specific information is functionally partitioned between those two structures: the subiculum encoding the relevant information mainly during short delays (1–15 s), while the hippocampus proper (CA1) is required during the longer delays (16–30 s). It seems that the impairment affecting the MA rats is not exclusively a hippocampal or a subicular one, but may result from an alteration of the interaction between those two structures.

The second main result of our study focused on the putative neurotransmitter systems that are at risk during the early age-related neuronal alterations. Age has consequences for neurotransmitter systems which affect cognitive function; these are changes which are detectable at MA and continue to progress into old age (Wang et al., 1995). Here, we report acute administration of the 5HT6 receptor antagonist SB742457 improves the MA animal's performance in the DNMS task. SB742457 administration appears to enhance short-term working memory in MA rats. Acute administration of SB742457 has an immediate effect on MA animals, significantly improving their performance in the DNMS task. Similarly, King and colleagues demonstrate an acute effect of 5HT6 antagonists Ro-04-6790 (10 mg/kg i.p.) and SB271046 (10 mg/kg i.p.) on performance in an object recognition task (King et al., 2004). Acute treatment of SB271046 (10 mg/kg) improved retention and not acquisition of the Morris water maze and did not reverse scopolamine-induced learning deficits (Marcos et al., 2008). It should be noted that SB742457 is also an antagonist of 5HT2a receptors, although with less affinity (Upton et al., 2008). Selective antagonism of 5HT2a receptors has been shown to enhance performance in a DMS task in adult rhesus monkeys (Terry et al., 2005).

Chronic treatment with the antagonist, SB742457 (3 mg/kg), significantly enhanced DNMS performance in MA rats compared to their relevant baseline after one week, improving performance in the DNMS task to match that of YG rats. Previously, oral dosing of SB742457 (1.5 mg/kg) has been shown to reverse spatial task deficits in aged rats in the Morris water maze while improving task retention (Chuang et al., 2006). While GSK-742457 (3 mg/kg) ameliorated scopolamine-induced deficits in object recognition task and 10 mg/kg GSK-742457 also reduced scopolamine-induced deficits in the object location task in 3-months-old male Wistar rats (de Bruin et al., 2011). Similarly, chronic administration of SB399885 (10 mg/kg P.O.) for 7 days, fully reversed an age-dependent deficit in 22 months old rats in the Morris water maze compared to age matched controls (Hirst et al., 2006). Other studies also identified the potential of another 5HT6 antagonist, SB271046 (10–20 mg/kg p.o. and 1–6 mg/kg i.p.) in reversal of age-related deficits in spatial memory (Callahan et al., 2004; Foley et al., 2008). These studies all demonstrate improvements in both acquisition and retention, suggesting that in ageing rats this task is sensitive to the cognitive enhancing properties of 5HT6 receptor antagonists. Here we provide evidence that the DNMS task is also sensitive to the cognitive enhancing potential of SB742547.

In YG animals we found that SB742547 increased performance in the DNMS task at short delays only. Studies investigating the effects of Ro-04-6790 and SB271046 (10 mg/kg I.P.) on acquisition, consolidation and retention in the object recognition task in young adult rats found enhanced consolidation of recognition memory (King et al., 2004). The current study indicates that SB742457 has a more profound cognitive enhancing action in MA animals than YG; Foley et al. (2008) also found that SB271046 had a more substantial effect on aged animals than young in the water maze. This is in contrast to previous studies which did not find a cognitive-enhancing action of that 5HT6 receptor antagonist on spatial task acquisition in mature animals (Rogers and Hagan, 2001; Woolley et al., 2001). In a similar operant task aimed at auto-shaping and attention shifting in the rat, it was found that both acute and chronic administration of 5HT6 receptor antagonists SB-357134 (1–30 mg/kg/po) and SB399885 (1–30 mg/kg/po) improved performance in young rats (Perez-Garcia and Meneses, 2005). 5HT6 antagonists also have the ability to reverse pharmacological induced deficits in behavioral paradigms. SB742547 (0.5–4.5 mg/kg) has previously been shown to reverse scopolamine-induced deficits in the object recognition paradigm and in a passive avoidance paradigm (Chuang et al., 2006). SB-357134 (1–30 mg/kg/po) and SB399885 (1–30 mg/kg/po) reversed memory deficits produced by scopolamine or dizocilpine (MK-801) in an auto-shaping operant task (Perez-Garcia and Meneses, 2005). One of the proposed mechanisms to explain those results states that the blockade of the 5HT6 receptor leaves more acetylcholine and glutamate available at the synapse, aiding synaptic function. This hypothesis is further supported by King and colleagues' data revealing that pre-treatment with the NMDA receptor antagonist MK-801 prevented the cognitive-enhancing effects of Ro-04-6790 on delay-induced deficits in an object recognition task (King et al., 2004). We found that the pro-cognitive effects of SB742457 were sustained for up to one week post-drug administration; 5HT6 antagonists may therefore have long lasting effects on neuronal and synaptic plasticity (see also Foley et al., 2008) after drug withdrawal. It is possible that with continued overtraining in the DNMS task MA animals would eventually reach a similar performance level to that of the YG, although here administration of SB742457 has an immediate effect on their performance. Treatment with SB742457 had equal effect on performance at both short and long delays in MA suggesting the drug is aiding encoding in both the subiculum and hippocampus and thereby increasing performance in the DNMS task. 5HT6 receptor blockade appears to compensate for the lower level deficits seen in MA animals. However, we cannot be certain that it will have beneficial effects when pathology is present, such as β-amyloid in AD. We note however, that SB742457 is currently in phase II clinical trials in subjects with mild to moderate AD (Maher-Edwards et al., 2010).

In conclusion, we provide in this study a behavioral model of age-related cognitive decline in MA animals. Using the DNMS task we identify a short-term working memory deficit in middle-aged rats. Our findings with the DNMS task expand on previous findings demonstrating that 5HT6 antagonism may be beneficial in early stages of cognitive decline facilitating short-term working memory in middle-aged rats. These actions of 5HT6 receptor antagonists may therefore have significant implications in terms of the future development of neurocognitive agents.

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