Establishment of a reversal learning assay in rats to investigate the effects of novel compounds on executive function

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Introduction

Cognitive ability declines with age, ranging from normal age-related decline (Craik and Bialystok, 2006), to mild cognitive impairment (Gauthier et al., 2006), and to dementias like Alzheimer's disease (Perry and Hodges, 1999). One domain which is particularly affected is executive function (Lien et al., 2008). Attentional set-shifting and reversal learning tasks have been widely used to quantify executive dysfunction in older humans (Robbins et al., 1998) and rats (Schoenbaum et al., 2006). Here, we describe the establishment of an operant conditioning task to assess reversal learning in rats. We investigated a series of pharmacological interventions, including drugs affecting cholinergic and serotonergic transmission, previously shown to have pro-cognitive effects in animal models and humans.

Methods and Analysis

Six week old male Sprague-Dawley rats (Envigo, UK) were food restricted to 85% of their free-feeding bodyweight and then trained to earn a 45mg sugar pellet reward in an operant non-match to sample task (Figure 1 A). Once animals were trained to a criteria of above 90% correct on the non-match response (11 sessions) the reward contingency was switched to match to sample. Following this, rats received daily drug administration (Figure 1 B) 30 minutes prior to the task for SB-399885 and 15 minutes prior to the task for nicotine. The reversal learning phase of the task continued until rats again achieved criteria (90% correct response).

Analysis of performance of the task compared the % correct response of a trial session (90 trials) between groups which occurred once daily (Figure 2 & 3 A). After the experiment was complete (13 sessions) the total number of errors made across all trials was also analysed (Figure 2 & 3 B). Following on from the statistical between groups analysis described above further examination of the reversal sessions was conducted (Figure 4). Each daily session of 90 trials was broken down into 18 bins of 5 trials, each individual animals performance was examined (Figure 4A). The average performance of each group on each day in bins of 5 trials is shown (Figure 4B).

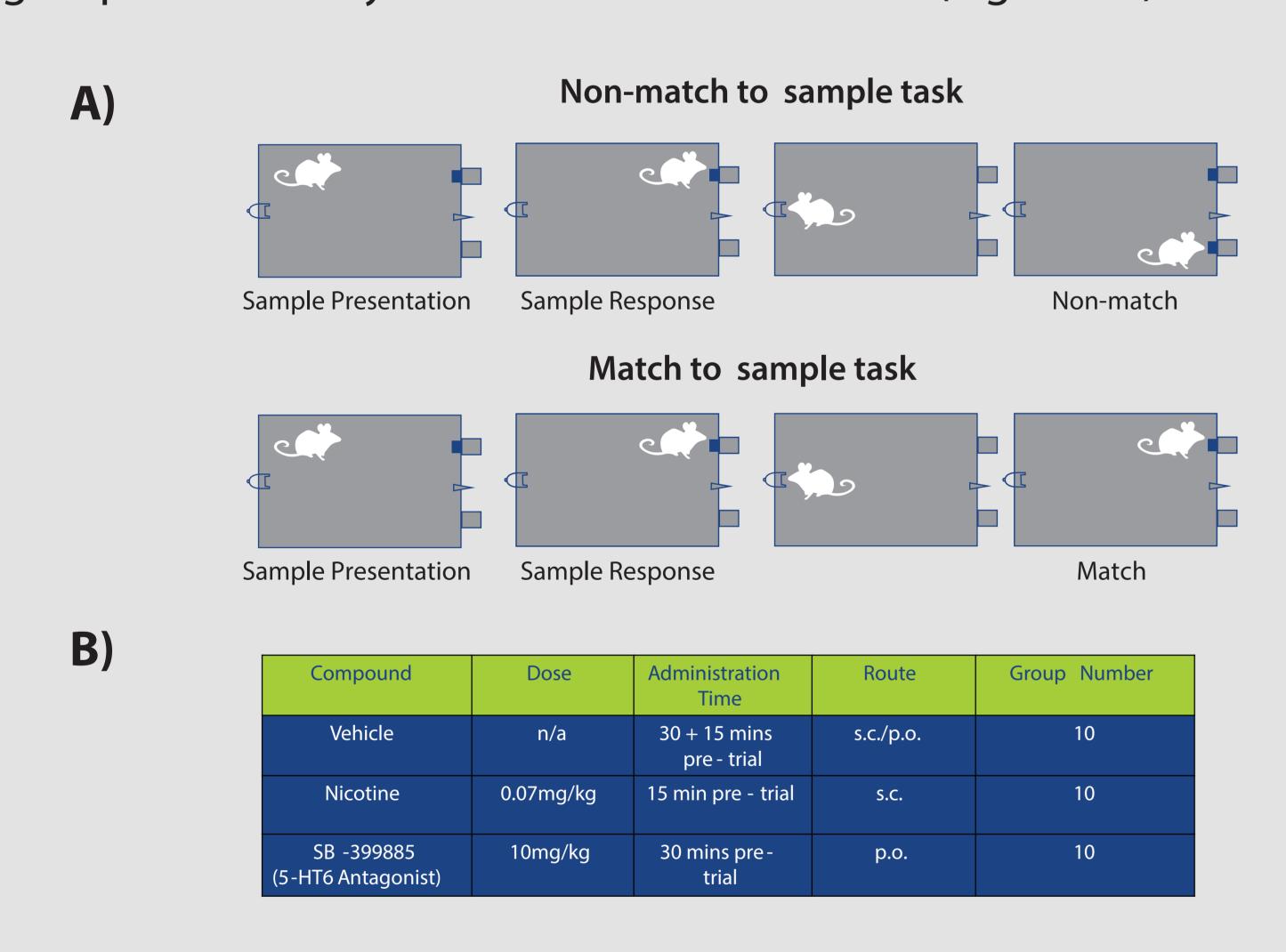


Figure 1: (A) A graphic of the non-match and match to sample tasks completed by the rats in the study. Rats were required to press a lever in the front panel of an operant box, then press a lever at the rear of the box and then was presented with two levers at the front and was required to choose the match or non-match option depending on

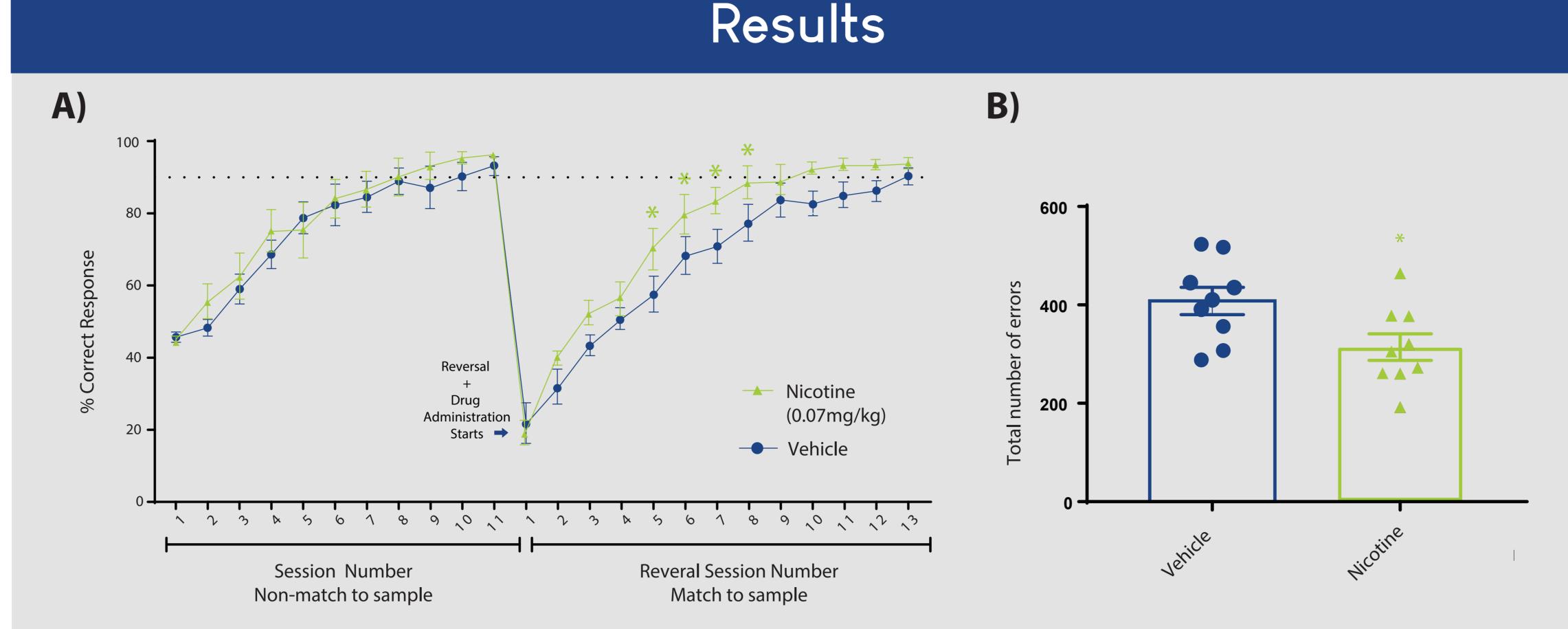


Figure 2: (A) % Correct responses between vehicle and nicotine groups in the operant reversal task. During the reversal sessions, there was main effect of nicotine compared to vehicle (P<0.05). Post-hoc analysis r evealed the nicotine group performed significantly better than the vehicle group across 4 consecutive sessions (5,6,7 & 8). (B) Total number of errors between vehicle and nicotine groups in the operant reversal task. The nicotine group performed better with significantly fewer errors made across the reversal sessions. (One animal was eliminated from the each group due to not completing the task, n=9 per group. Analysis was of the reversal session data 1-13 using a repeated measures ANOVA, Fisher's LSD post-hoc for graph A and Student's t-test for graph B)

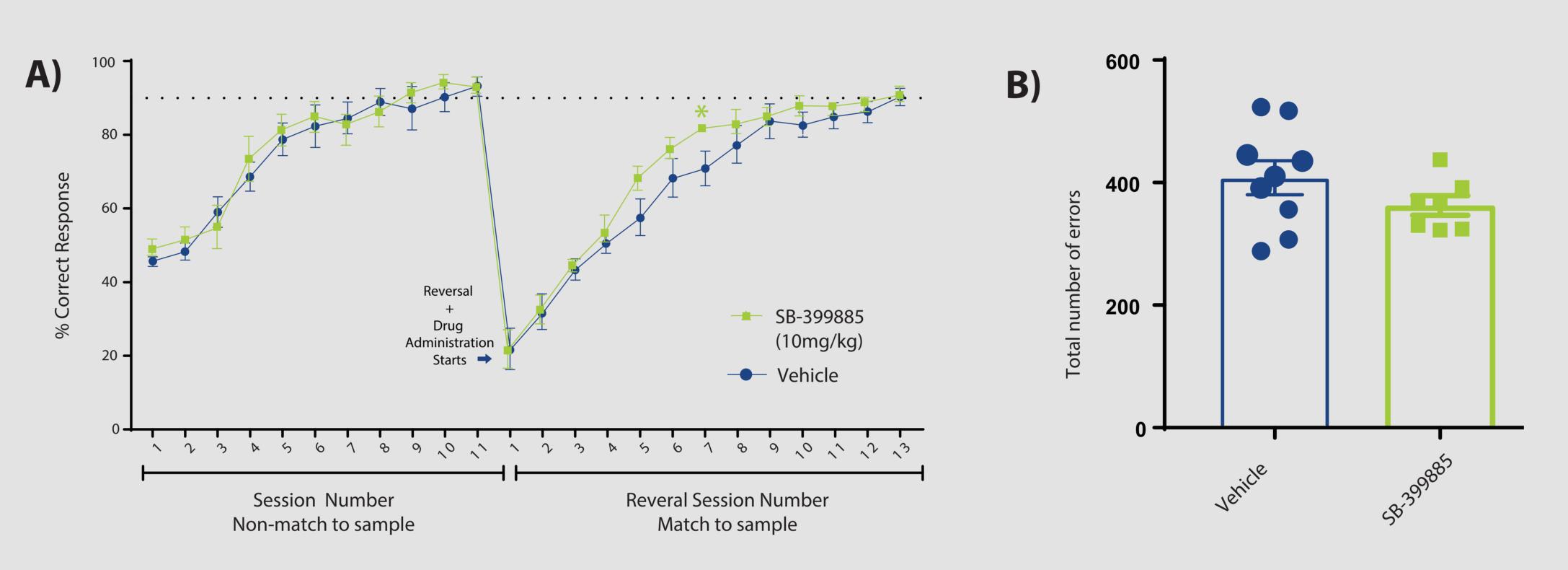


Figure 3: (A) % Correct responses between vehicle and SB-399885 groups in the operant reversal task. During the reversal sessions, there was no main effect compared to vehicle. The SB-399885 group performed significantly better than the vehicle group in session 7.

(B) Total number of errors between vehicle and SB-399885 groups in the operant reversal task There was no difference in the total number of errors made between the groups.

(Two animald was eliminated from the SB-399885 group and one from the nicotine group due to not completing the task. Analysis was of the reversal session data 1-13 using a repeated measures ANOVA, Fisher's LSD post-hoc for graph A and Student's t-test for graph B)

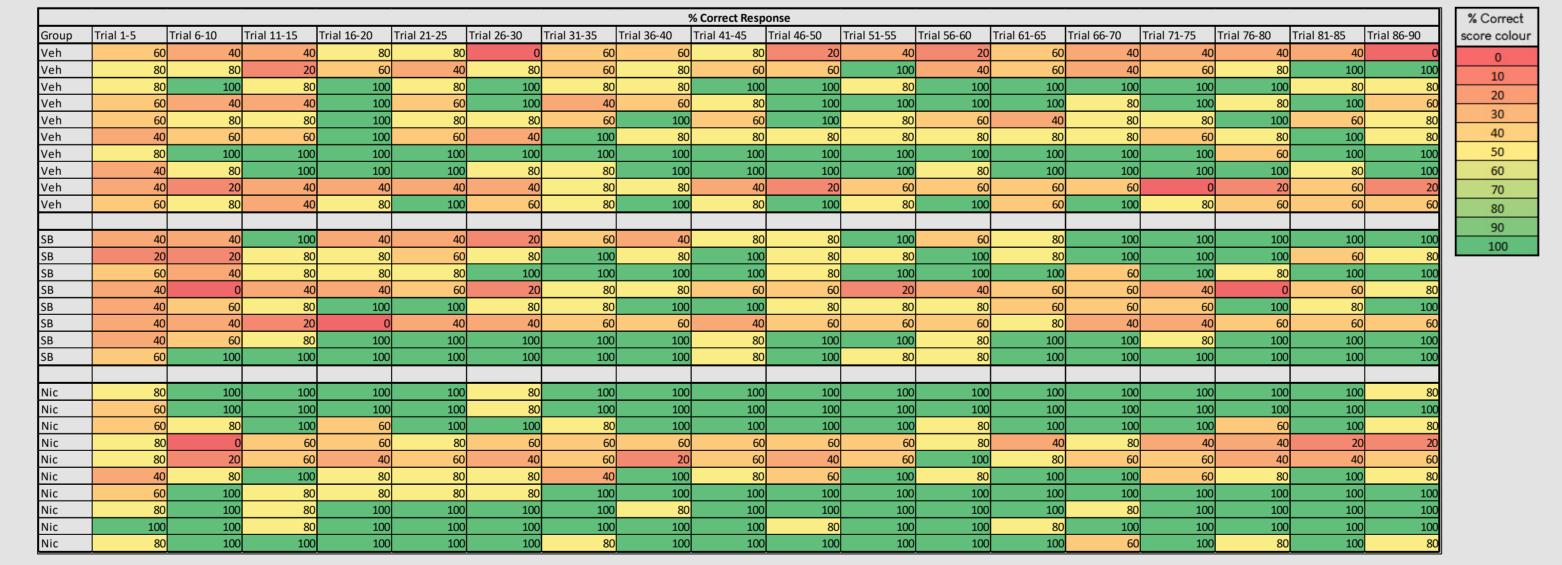
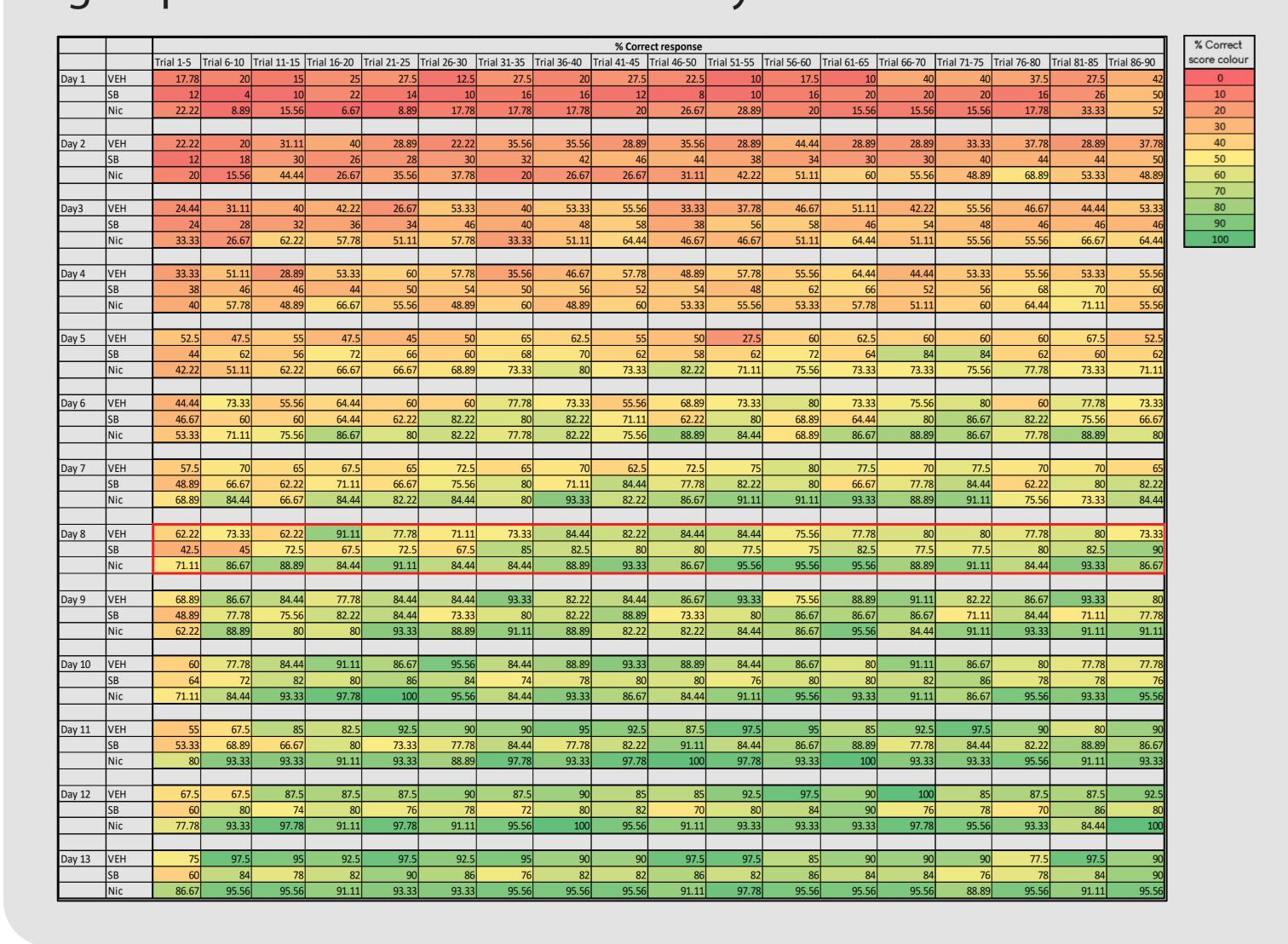


Figure 4: (A)Breakdown of reversal session on day 8 of testing. Data shows each individual animals % correct responses for every 5 trials in the task. (Session 8 was chosen to illustrate this method of analysis as it is the one session where both treatment groups performed significantly better than the vehicle group) **(B)** Average performance of each treatment group by day split into bins of 5 trials in the reversal task. Data shows % correct response for each group at each trial bin for each day.



Results

A main effect of nicotine (P<0.05) was observed across the 13 reversal sessions with the nicotine group performing significantly better compared to vehicle. Post-hoc analysis demonstrated that a significant effect of nicotine was observed during sessions 5,6,7 and 8 (P<0.05) (Figure 2A). Analysis of the total number of errors made throughout the reversal sessions also revealed a significant effect (P<0.05) with the nicotine group having lower number of errors compared to vehicle (Figure 2B).

There was no main effect observed for the SB-399885 group compared to vehicle however post-hoc testing demonstrated a significant difference (P<0.05) between the SB-399885 group and the vehicle group during session 8 with the SB-399885 group performing significantly better (Figure 3A). Examination of the total number of errors showed there was no difference between the two groups (Figure 3B).

Further investigation was conducted by dividing each session into bins of 5 trials (Figure 4A) this has allowed for a more comprehensive and informative view of the data. The breakdown of individual sessions allows for detailed examination of how each animal is performing at any given time. This can be applied to examine the performance of a particular animal that is for example, an outlier or to give an improved account of the time course of the efficacy of a compound. As well as analysing each individual animal by day it is also possible to average performances of each treatment group by day in these trial bins (Figure 4B) This gives a clearer picture of the effects of the treatments throughout testing to potentially assess peak efficacy within and between sessions.

In the present study pro-cognitive effects have been observed both using nicotine (acetylcholine receptor agonist) and SB-399885 (5-HT6 antagonist) compared to control. A breakdown of test sessions into smaller trial bins has yielded a better temporal resolution of the data to assess changes within as well as between testing sessions.

Conclusion

These data demonstrate the successful establishment of an operant reversal learning task in rats. The task is sensitive to the pharmacological interventions used, both cholinergic and serotonergic and therefore can be used to investigate the potential pro-cognitive effects of drugs under development for treatment of cognitive dysfunction associated with psychiatric and neurodegenerative disease.

Future studies (delayed due to the COVID-19 pandemic) in aged animals and disease models will explore the possibility of employing the task to assess disease specific deficits in executive functioning and screen potential pharmacological interventions.

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