Pharmacological Manipulation of a Rodent Paired Associates Learning (Pal) Paradigm, and other Tasks for Use in Disease Research

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Ethical Statement

All experiments described in this abstract have been approved by the local Ethical Committee at Janssen Pharmaceutica**.**

Introduction

Paired Associated learning (PAL) as part of the Cambridge Neurological Test Battery (CANTAB) is a task of great potential for disease research. The initial interest in this task was sparked by studies of patients with Alzheimer's disease (AD) or mild cognitive impairment (MCI), often viewed as a prodromal form of AD. AD patients were highly impaired on PAL, more importantly, so were a sub-population of MCI patients. Subsequent studies would reveal that this "impaired" sub-population of MCI patients showed a much higher conversion rate to AD than the rest of the cohort. Through the use of PAL and related tests, researchers were able to predict who would, and who would not convert to AD from an at-risk population several years earlier than with other available clinical tests (1). While the link between PAL and AD was being established, another line of research began to associate PAL with schizophrenia. Specifically patients suffering from first episode psychosis were found to be impaired in PAL. Furthermore performance on PAL was found to be more predictive of global cognitive function in schizophrenia than the extra dimensional / intra dimensional shift (ED/ID) task, often considered a gold standard for research into cognitive impairments in schizophrenia (2). As such this one task appears to have great value in researching cognitive impairments associated with AD and schizophrenia, and a pre-clinical model of this assay would be of great utility.

Although PAL exists in many forms, we are most concerned with modeling the CANTAB version. In this form PAL is a task of visuo-spatial learning. Participants are shown a series of images in distinct locations upon a computer screen (4-8 locations depending on the difficulty). One at a time, these same images are displayed in the middle of the screen and the participant is asked to touch where they were previously seen. In this way subjects are required to pair a specific object with a specific spatial location. Remembering the object or the location alone is not enough to solve the task; rather the participant must use both modalities.

The CANTAB version of PAL has been modeled in the rodent by using touch-sensitive computer monitors in an attempt to capture the visual and spatial elements of the human task. In the rodent version of the task the screen is divided into three distinct locations, each location is associated with a specific image. (Talpos et al., 2009) On each trial two images are shown, one in the correct location to earn a reward, and one in an incorrect location (see Figure 1, adapted from 4). In this way an animal can only earn a reward by making the connection between a specific object and a specific location. In order to learn this task rats or mice must be trained for many sessions. The rodent version of the task is not quickly acquired, nor can trial-unique stimuli be used as is the case in the human version of the task. However this rodent variant does require the binding of spatial and visual features in a similar way as CANTAB PAL. While this rodent PAL task has many differences from its human counterpart, it also has important similarities.

The neurobiological underpinnings of PAL in rodents are still being explored. However it has been shown that when rats have learned PAL the task is sensitive to manipulations of the hippocampus, whereas a highly similar control task is not. However the primary impetus for development of this task was specifically to aid the drug discovery process. In order to do this the pharmacological under-pinning of the task must be explored.

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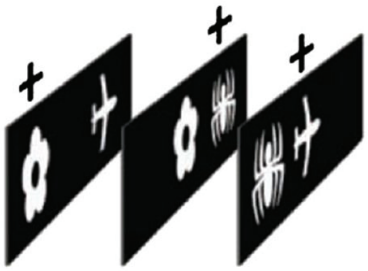

Figure 1. Scheme of the CANTAB version of PAL for rodents.

While acquisition of PAL could be used to discover potentially cognitive enhancing compounds, at the moment the task is learned far too slowly to make this practical. As such animals that have achieved a steady state will likely need to be used. However in PAL, animals routinely achieve a steady state of behavior at about 80% correct, leaving little room to detect a statistical improvement. Furthermore it is unclear if it would be possible to detect enhancement in normal animals performing at a near optimal level. To circumvent these challenges, we focus on frequently used pharmacological manipulations in an attempt to develop a model of disease and further understand the neuro-pharmacological basis of this task.

We initially focused on 4 compounds, PCP, Ketamine, amphetamine, and LSD, representing compounds used to model schizophrenia, and also known to disrupt learning, memory, or perception.

Material and Methods

Male Lister-hooded rats were used for this work. Rats were maintained on a food restricted diet keeping them at about 85% of free feed body weight.

The chambers used were modified med-associates operant boxes running commercially available software (K-Limbic, by Conclusive Solutions). Critical modification of these boxes included the addition of a "shelf"/ counter weighted flap. The purpose of this is to slightly slow the animals' response, increasing the likelihood that they will attend to the displayed stimuli. Experience dictates that this modification is extremely important for successful task acquisition. A removable mask was used to divide the chamber into distinct regions.

Pre-training: Rats were trained with a procedure very similar to that used by Talpos et al. (2009). Briefly, rats were first habituated to the chamber. This was done but putting animals in the test chambers with small amounts of a reward pellet/peanut butter mixture placed upon the "flap", "mask", and screen. This was done to encourage animals to explore the screen. Animals were left in the chambers for approximately 30mins a day until they had eaten all of the peanut butter from the screen—typically one day. Next animals were trained to associate a tone with a food reward. A trial would start with the delivery of a food pellet, the sounding of the reward tone, and the activation of the magazine light. Once the pellet was collected the reward light would go out and a 30sec delay would start. Once 30 sec had passed another pellet would be delivered in combination with activation of the reward light and tone. This continued for 60 trials or 60mins. An animal was considered trained on this stage once they managed to complete 60 trials within 60mins (typically two days). Animals would then progress to screen touch training. A session would start with the delivery of a pellet. Collecting the pellet would cause the magazine light to deactivate and the screen to illuminate. A response at any portion of the screen would start the reward sequence (de-activation of the screen, delivery of a pellet, activation of the magazine light, and a short tone). Once the pellet was collected the reward light would go out and the inter trial interval (ITI) would begin. At the end of the ITI the magazine light would again be activated, and a poke at the light would start the next trial. A session was considered complete when an animal could complete 60 trials in 45mins. An animal was considered trained when they could successfully complete a session (typically 2 or 3 days). Successful animals were advanced to the "one location" stage, where only a single region of the screen was activated/rewarded, but in all other regards this was identical to the previous stage. Typically animals only needed one day to master this

and were then progressed to the one random location. Again, this was the same as the previous stage, however now the location moved between trials. This was included to help avoid any potential side biases that may have developed during the pre-training. Most animals would complete 60 trials within 45mins on the first day, and were therefore ready to start task training.

Task Training: A trial began with activation of the reward magazine and delivery of a free pellet. Once this pellet is collected the magazine light is deactivated and two images are displayed upon the screen. A response at the correct location activates the reward sequence. Once the reward is collected the ITI begins, and at the end of the ITI the reward magazine is again illuminated. A response at the magazine will cause it to turn off, and begin the next trial. However if the incorrect stimuli is selected then the images are removed from the screen and the house light turns off (punishment period) for 10 seconds. Once 10 seconds have passed the ITI begins, and then the trial proceeds as normal except that the animal will experience the same trial until a correct response is made (correction trial). However these correction trials are not counted towards the total of trials completed. The reason for using the correction trials is to prevent the development of a side bias during the training of this long and difficult task. Animals are typically trained for 72 trials or 45mins, whichever occurs first. Training on this task is very long, requiring about 3-4 months to reach an asymptotical performance level of approximately 80% correct. Once animal behavior has stabilized animals are considered ready for compound testing.

Results

The NMDA antagonists, PCP and Ketamine, had partially distinct profiles in this test. Ketamine (up to 10 mg/kg) did not induce an impairment in accuracy. However the highest dose was associated with an increase in response latency and suppression of behavior. PCP (1.5 mg/kg) on the other hand did impair accuracy, but this effect was modest and also occurred with some side effects. A larger deficit was observed at 2.0 mg/kg, but this was also associated with a more robust side effect profile. Perhaps not surprisingly, LSD did not disrupt accuracy, although abnormalities were seen on secondary measures. Finally, amphetamine induced a substantial decrease in accuracy (0.5 mg/kg) without changes in secondary measures. While a larger, albeit non-selective impairment was also observed at 0.75 mg/kg. Of the compounds tested only amphetamine appears to possess the dynamic range and selectivity to serve as pharmacological challenge model (Figure 2). Moreover previous work has indicated these concentrations of PCP have little or no effect on performance of a touch-screen based visual discrimination, a related, but less challenging assay (Talpos et al., 2011).

Additional research has been performed with the amphetamine impairment model indicating that the effect is extremely robust (significant impairments at 0.5 mg/kg in over 15 instances). Our initial validation efforts have focused on reversing this deficit with antipsychotics. Studies indicate that clozapine, haloperidol, and risperidone are all capable of at least partially reversing this deficit; and the profiles of these compounds within this model are partially distinct.

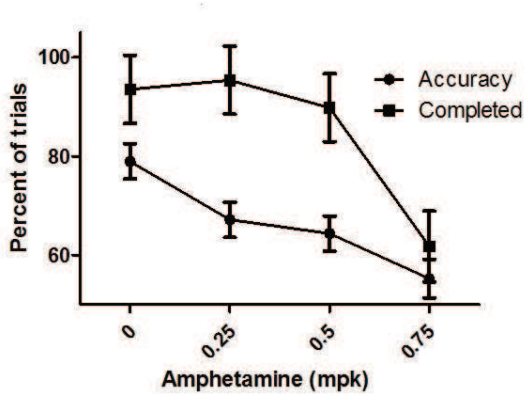

Figure 2. The effects of amphetamine on performance.

These current data indicate that PAL in the rat is exquisitely sensitive to dopaminergic hyperfunction induced by a relatively low dose of amphetamine. Moreover this deficit can be reversed by numerous antipsychotic compounds that have D2 receptor antagonism as their primary mode of action. Considering the lack of overt side effects, we do not think this is a model of hyperactivity or motoric impulsivity, although this cannot be ruled out. However additional work will be required to determine the origin of this deficit and to determine if this model of cognitive impairment can add value to the drug discovery process.

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