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ORIGINAL INVESTIGATION

A novel touchscreen-automated paired-associate learning (PAL) task sensitive to pharmacological manipulation of the hippocampus: a translational rodent model of cognitive impairments in neurodegenerative disease

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Abstract
Rationale Paired-associate learning (PAL), as part of the Cambridge Neuropsychological Test Automated Battery, is able to predict who from an at-risk population will develop Alzheimer's disease. Schizophrenic patients are also impaired on this same task. An automated rodent model of PAL would be extremely beneficial in further research into Alzheimer's disease and schizophrenia.
Objective The objective of this study was to develop a PAL task using touchscreen-equipped operant boxes and test its sensitivity to manipulations of the hippocampus, a brain region of interest in both Alzheimer's disease and schizophrenia.
Materials and methods Previous work has shown that spatial and non-spatial memory can be tested in touchscreen-equipped operant boxes. Using this same apparatus, rats were trained on two variants of a PAL task differing only in the nature of the S− (the unrewarded stimuli, a combination of image and location upon the screen). Rats underwent cannulation of the dorsal hippocampus, and after recovery were tested under the influence of intra-hippocampally administered glutamatergic and cholinergic antagonists while performing the PAL task.
Results Impairments were seen after the administration of glutamatergic antagonists, but not cholinergic antagonists, in one of the two versions of PAL.
Conclusions De-activation of the hippocampus caused impairments in a PAL task. The selective nature of this effect (only one of the two tasks was impaired), suggests the effect is specific to cognition and cannot be attributed to gross impairments (changes in visual learning). The pattern of results suggests that rodent PAL may be suitable as a translational model of PAL in humans.

Keywords Lidocaine · MK-801 · CNQX · Scopolamine · Mecamylamine · Schizophrenia · Alzheimer's disease · Direct administration

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Introduction

Alzheimer's disease (AD) is difficult to diagnose in its earliest stages (Nestor et al. 2004). Recent studies have shown that a test of object-in-place paired-associated learning (PAL), as part of the Cambridge Neuropsychological Test Automated Battery (CANTAB), is very effective at distinguishing patients in the earliest stages of AD from those suffering cognitive impairment caused by different etiologies, even more so than other clinical tests (Blackwell et al. 2004;

Sahakian et al. 1988; Swainson et al. 2001). Moreover, performance in PAL is neurochemically dissociable from delayed-matching-to-sample paradigms (Robbins et al. 1997). Thus, PAL performance is dependent upon cognitive functions other than just working memory, functions that may be particularly susceptible to AD pathologies. PAL's sensitivity however, is not limited to AD. Performance on this task has been shown to be impaired in first-episode psychotics as well as established schizophrenic patients and chronic drug abusers (Barnett et al. 2005; Wood et al. 2002; Ersche et al. 2006). This wide-ranging sensitivity may make it an ideal tool to study cognitive decline associated with multiple cognitive disorders. As such, a translational model of PAL could advance the search for novel therapies to treat numerous neurodegenerative disorders.

In humans, as a component of CANTAB, patients are shown a series of "boxes" on a computer screen. Under each box is hidden an object and, one at a time, these boxes are turned over to reveal their contents (sample phase). Once all the objects have been displayed, a previously seen sample object is presented in the centre of the screen. The participant must then match this object with the location where it was previously displayed, demonstrating an association between an object and location (Sahakian et al. 1988). In light of the requirement to associate an object with a spatial location, it is not surprising that in humans PAL has been found to be dependent upon the medial temporal lobe (Owen et al. 1995). Based on an evolving body of literature in the rat and monkey, it might be expected that the hippocampus and entorhinal cortex may contribute to the spatial component (Burgess 2008; Gaffan 1977; O'Keefe et al. 1975; Owen and Butler 1981; Parron et al. 2004), whereas the perirhinal and perhaps entorhinal cortex would contribute to the object component of the task (Meunier et al. 1993; Murray et al. 2007; Saksida et al. 2006; Winters et al. 2004). It is likely that this dependency on multiple brain regions, all of which show pathologies very early in the progression of AD, makes PAL so predictive of who will eventually develop the disorder. As previously mentioned, PAL performance is also impaired in patients with schizophrenia; however, it is unclear whether this is because of dysfunction within the medial temporal lobe, frontal cortices, or a combination of both regions.

An analogue of the human PAL CANTAB task also exists for the monkey as part of the nonhuman primate CANTAB. This task has been proposed as a pre-clinical model of AD because of its similarity to the human task and is also sensitive to manipulations of the cholinergic and glutamatergic systems (Katner et al. 2004; Taffe et al. 2002, 2004). It has also been shown to be sensitive to manipulations of the dopaminergic system (Von Huben et al. 2006), adding still further support for the use of PAL as a preclinical model of neurodegeneration. However, neither selective lesions nor localised pharmacology have been performed in primates trained on this task, making it impossible to more than speculate on the specific brain regions involved. Although the primate version of PAL is without a doubt a powerful tool, a rodent model of the impairments seen in AD and schizophrenia on PAL would still be advantageous because of the increase in utility associated with testing in the rodent (e.g. throughput, cost, ease of surgical manipulations).

Previous work has shown that rodents can successfully perform visual discriminations in a touchscreen-equipped operant box (Bussey et al. 2008; Janisiewicz and Baxter 2003; Morton et al. 2006; Bussey et al. 1998, 1994; Brigman et al. 2005, 2008). More recently, a test of hippocampal-dependent spatial cognition has been developed that can be run in an identical apparatus (Talpos et al. 2008), demonstrating that both the visual and spatial domains can be tested within the touchscreen environment. These findings provide the groundwork for the development of a touchscreen-based object-in-place PAL task. If successful, such a task could reduce many of the confounding variables associated with non-automated paradigms currently in use (e.g. timing variability, observer bias, stress). Thus, the goal of the present study was to develop an automated object-in-place PAL task using operant boxes equipped with touch screens in an effort to build a better translational model of the CANTAB PAL task that is highly sensitive to cognitive deficits in neurodegenerative disorders. In addition, we tested whether the task was sensitive to intra-hippocampal manipulations that can be used to model disease; specifically, selective manipulations of the cholinergic and glutamatergic systems within the dorsal hippocampus.

Normal rats were trained in one of two variants of a PAL task, with the same mnemonic demands and differing only in the nature of the S−, in touchscreen-equipped operant boxes. We used these two tasks to test the importance of the nature of the S− on PAL performance and what effect the nature of the S− might have on the sensitivity to hippocampal manipulations. Once animals had acquired the task to stable performance levels, surgery was performed to implant cannulas in the dorsal hippocampus. After recovery, rats were retrained to baseline and tested after intrahippocampal infusions of several drugs. The first of these was the fast-acting, Na+ channel blocker, lidocaine. Owing to its action at the Na+ channel, direct administration of lidocaine has an effect in many ways similar to a "temporary lesion" of the area of interest. However, the short interval of deactivation makes the likelihood of behavioural or cognitive compensation highly unlikely. In an attempt to gain further insight into the origins of any deficit seen in the task, we also investigated the glutamatergic and cholinergic systems,

both of which have been linked to paired associate learning and neurodegenerative disease (Taffe et al. 2002; Day et al. 2003; Hasselmo 2006; Robbins and Murphy 2006; Bartus et al. 1982; Farlow 2004). Specifically, to investigate the glutamatergic system, we used the non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (Wong et al. 1986) and the competitive alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/Kainate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Honoré et al. 1988). To investigate the contributions of the cholinergic system, we used the competitive non-selective muscarinic antagonist scopolamine (Frey and Howland 1992), as well as the non-competitive nicotinic receptor antagonist Mecamylamine (Martin et al. 1990). Not only are these ligands commonly used to study learning and memory, but they all have also been shown to impair performance after direct administration to the hippocampus in tests of spatial learning and memory (see "Materials and methods"). As such, they serve as an ideal starting point for the investigation of the role of the hippocampus in this PAL task.

Materials and methods

Apparatus

Med Associates operant boxes (VT USA; *h* 23 cm, *w* 30 cm, *d* 25 cm), with one end of the chamber equipped with a touch-sensitive, flat-screen, liquid crystal display computer monitor (*h* 29×*w* 24 cm viewable area, Craft Data, Chesham, UK), were used for this study. The monitors were covered with a black Perspex "mask" (*h* 38 cm× *w* 28 cm). The mask was attached to the screen leaving a 0.5-cm space between the mask and monitor and had three response windows (*h* 15 cm, *w* 6 cm) cut into it. These were 1.5 cm apart, and the outer response windows had a 4.5-cm border to the edge of the mask. A spring-hinged "shelf" (*d* 6 cm, *w* 20.5 cm) was attached at a 90° 16 cm above the grid floor. On the wall opposite the monitor was a food magazine equipped with a light, infrared beam and beam detector. Above the food magazine was a house light (3 W), and a small speaker. Each operant box was housed within a sound-attenuating chamber. The testing apparatus was controlled using IBM Netvista computers running programs written in Microsoft Visual Basic.

Subjects

Sixteen male Lister-hooded rats were used for this study (Harlan, UK; 220–240 g). They were housed four per cage and maintained on a reverse day/night light cycle. Upon arrival, rats were given 1 week to habituate prior to behavioural training. Once training began, food (standard rat chow, Purina) was restricted to maintain animals at 85–90% of normal weight (except before and after surgery; ad libitum water).

Surgery

All rats were implanted bilaterally in the dorsal hippocampus with 22-gauge indwelling guide cannulas (5 mm centre-to-centre distance). Animals were anaesthetized by intraperitoneal (i.p.) injection (60 mg/kg) of sodium pentobarbital (Sagatal; Rhône Mérieux, Essex, UK) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar set at −3.2 mm. Guide cannulas were implanted according to the following coordinates, measured relative to the skull at bregma (Paxinos and Watson 1998): anteroposterior −3.8 mm, lateral ±2.5 mm, dorsoventral −2.5 mm. The cannulas were secured to the skull using dental acrylic and four jeweller screws. Obturators, cut to sit flush with the tip of the guide cannulas and with an outer diameter of 0.36 mm, were inserted into the guides and remained there except during infusions. A screw-on dust cap kept the obturators in place. At the completion of each surgery, antibiotic powder (Acramide; Dales Pharmaceuticals, Skipton, UK) was applied. Animals were given at least 7 days to recover prior to drug testing. All experimentation was conducted in accordance with the UK animals (Scientific Procedures) Act, 1986.

Infusion procedure

Depending on the experimental phase (see Table 1. for the progression of events within this study), rats received bilateral infusions of either physiological saline (0.9% sodium chloride, pH7.0; Aquapharm, Animalcare Limited, York, UK), which was the vehicle for all drugs, lidocaine hydrochloride (69.25 or 138.5 mM; Sigma, Poole, UK), scopolamine hydrobromide trihydrate (22.8 mM; Sigma), MK-801 (10.67 mM; Sigma), CNQX (3 mM; Sigma), or mecamylamine hydrochloride (49.07 mM; Sigma) prior to the behavioural session. Bilateral infusions were conducted simultaneously using two 1 µl Hamilton syringes for the lidocaine and cholinergic phase of the experiment (10 µl syringes were used for the glutamatergic phase), which were connected to the infusion cannulas by propylene tubing. Syringes were driven by a Harvard Apparatus precision syringe pump, which delivered 1 µl (1.5 µl in the glutamatergic phase) to each hemisphere over 2 min. Different volumes were used because the largest literature-based doses were used; in the glutamatergic phase, this necessitated a greater volume. The infusion cannulas were left in place for an additional 1.5 min to allow for diffusion

Table 1 A time line of events during the study

Week	Stage
1–2	Habituation and pre-training
3–10	task acquisition
11	Surgery and recovery
12	Re-baselining
13	mock infusion
	Testing
13	Lidocaine against vehicle
14	Washout
15	MK-801 and CNQX against vehicle
16	Washout
17	mecamylamine and scopolamine against vehicle
18	washout
19	MK-801 and CNQX against vehicle (repeat)
20	Histology

of the infusate. For all drug conditions except lidocaine and its control, behavioural testing began 15 min after the end of the infusion. Testing began immediately after drug infusion for rats during the lidocaine phase, as previous research suggests that lidocaine becomes active about 1 min after administration and is only effective for a further 10–15 min (Boeijinga et al. 1993; Floresco et al. 1996; Seamans and Phillips 1994). Within each phase, treatments were administered in a counter-balanced fashion. Animals were run continually during testing, but between treatment days, they were tested in a drug-free state. These data were used to check for carry-over effects of drug administration and to allow for drug clearance. At least 3 days were allowed for washout between each phase of drug testing. All doses used were taken from studies in which they had previously been shown to impair memory when administered into the hippocampus (Kim and Levin 1996a, b; Marti Barros et al. 2004; Ohno et al. 1993; Robinson and Mao 1997; Vianna et al. 2000). In order to minimise damage resulting from the infusion procedure, we avoided volumes and doses higher than those found in the literature.

Histology

Upon completion of behavioural testing, animals were anaesthetized with Dolethal (2 ml, i.p.) and transcardially perfused with 100 ml of phosphate-buffered saline (PBS), followed by 250 ml of 4% paraformaldehyde. Brains were removed and further fixed in paraformaldehyde at 4°C for 24 h. Prior to cutting, samples were soaked in 20% sucrose and PBS for 24 h. Sixty-micrometre sections were cut using a cryostat, and every fifth section was mounted on a gelatin-coated glass slide and stained with Cresyl Violet. Sections were then examined under a light microscope to determine the location of cannula implantation (Fig. 2).

Training

Initially, animals were placed in the operant chambers for 20 min to habituate to the environment with food pellets placed throughout the chamber (on the mask, screen, and pellet receptacle; 45 mg formula "P", Noyes). Animals were then trained to associate a tone with a reward pellet for one session (100 trials). This was accomplished by delivering a 0.5 s tone, followed by a food pellet, every 30 s. White squares were presented in all three of the response windows. No response resulted in the tone and a one-pellet reward after 30 s. However, if an animal touched the monitor, it was rewarded with three food pellets, and the next trial was initiated. Next, rats were required to touch any area of the monitor to earn a reward. The screen remained active until a response occurred. Once a response occurred, a tone sounded, a food pellet was delivered, and the touchscreen was deactivated. The next trial began 5 s after the pellet was collected. This was repeated until subjects could successfully complete 100 trials within 30 min (typically three sessions). Last, to avoid a development of a response bias, one of the three response locations would be randomly illuminated, and the rat was required to poke at this location to earn a reward. Pokes at other locations had no programmed consequences. This was repeated until each rat could complete 100 trials in 1 h.

When animals were placed on the PAL tasks, a trial began when the rat nose poked at the illuminated food receptacle. This deactivated the reward light and displayed the S+ and S− upon the screen. A response at the S+ would trigger a tone, delivery of a reward pellet, illumination of the receptacle, activation of the house light and cause the screen to go blank. The collection of the reward pellet caused the deactivation of the food receptacle light, while also beginning the inter-trial interval (ITI) (10 s). After 10 s, the food receptacle was illuminated, and a poke to it initiated the next trial. If the subject responded at the S−, a 10-s time out occurred, stimuli were removed from the screen, and the house light was deactivated for 10 s. After 10 s, the pellet receptacle was illuminated, and a poke to it triggered a correction trial. Correction trials were not counted toward the total trials completed, and the S+ and S− were displayed as in the previous trial. Before commencement of the full task, subjects were tested on an unpunished version where responses at the S− were ignored. This was done until the rat could complete 20 trials in 60 min, which took three to five sessions.

Two versions of the PAL task were used in this study, Same PAL (sPAL) and Different PAL (dPAL; the names refer to the nature of the S−, in sPAL the S− is the same stimulus as the S+ but in a different location, whereas in dPAL, it is a different stimulus).

As displayed in Fig. 1, in sPAL two duplicates of one of three possible stimuli were displayed on every trial. One "object" (O) was displayed in the correct response location (L), where a response to it always resulted in a reward (S+ , for example L1O1). The other "object" was displayed in a location where it was not rewarded (S−, for example L2O1, or L3O1). Accordingly, the following six trial types were possible, S+=L1O1, S−=L2O1 or L3O1; S+=L2O2, S−=L1O2 or S−=L3O2; S+=L3O3, S−=L1O3 or L2O3. dPAL was identical to sPAL, except that two different stimuli were displayed on every trial. The following six trial types were possible, S+=L1O1, S−=L2O3 or L3O2; S+=L2O2, S−=L1O3 or S−=L3O1; S+=L3O3, S−=L1O2 or L2O1. In both instances, rats were tested for a session lasting 1 h or 72 trials, whichever occurred first.

Results

Throughout analysis, repeated measure analyses of variance (ANOVAs) were used where percent correct, reaction time (time from the stimuli being displayed upon the screen, to the screen being poked) or magazine latency (time from the reward tone to poke at the reward magazine) served as the dependent variable and drug treatment as the repeated measure (except during analysis of acquisition data). Reaction time and magazine latency were both Log transformed prior to statistical analysis. Animals completing fewer than 75% of trials or failing to reach 65% correct under a saline test condition were removed from that stage of the study. Discounting the excluded animals, all rats consistently completed 72, or very near to 72 trials. No effect of drug was ever seen on trials completed, and therefore, these data are not presented. Moreover, the data from any animal for which it was unclear if the cannula tip was within the hippocampus was removed. The two animals that were removed from this study based upon histological results would have also failed inclusion criteria for accuracy and trials completed in most instances. The final sample size varied slightly because of individual variability in response to the administration process but was typically dPAL n=7, sPAL n=6. All statistics were generated with Statistica 6.1 (StatSoft, Tulsa, USA).

Histology

Examination of cannula tracks revealed distinct marks within the hippocampus in seven out of eight animals in each experimental group. In two animals, one from each group, it was not clear if the cannula tip had entered the hippocampus. It appeared that the placement may have been too dorsal. Interestingly, both of these subjects failed to reach criteria for inclusion into the study based upon a low number of trials completed under the saline condition of the drug phase. As these animals were not included in the statistical analysis, they also were not included in the

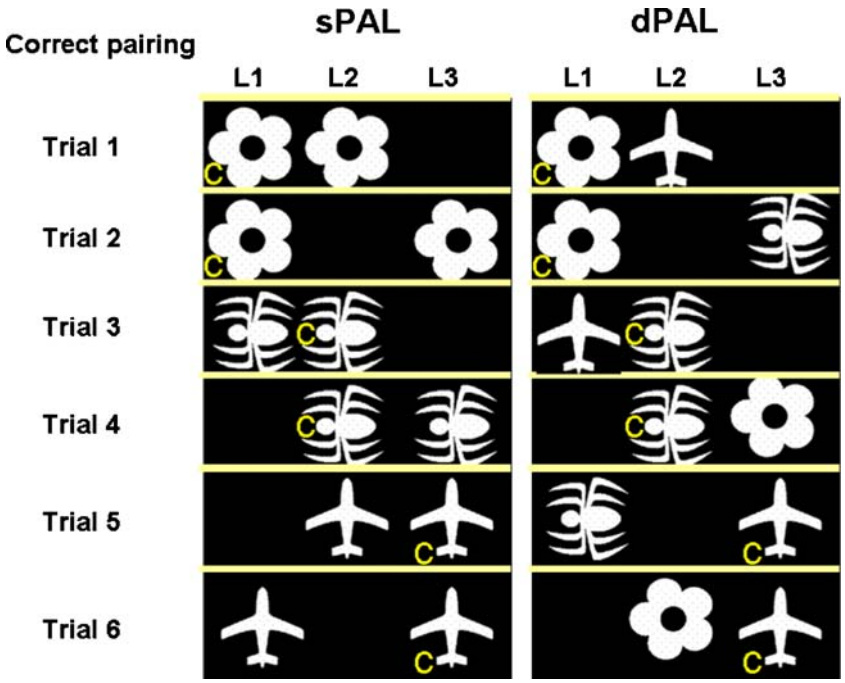

Fig. 1 An illustration of the six different trial types and the correct location object pairing in sPAL and dPAL. The S+ is signified by the *C*

depiction of cannula placements. As depicted in Fig. 2, the remaining cannulas were all placed in the dorsal hippocampus with only small variation in medial/lateral or anterior/posterior locations. Too much variation existed in the dorsal/ventral placement to comment about subregion involvement in animals performing this task (e.g. CA3 vs. dentate gyrus). However, the dorsal hippocampus had been successfully cannulated in all remaining animals.

Task acquisition

A two-way repeated measures ANOVA, with blocks of five sessions serving as the repeated measure and task serving as the independent variable, indicated main effects of block ($F(8,104)=63.9$, $P<0.0001$) and task ($F(1,13)=6.90$, $P=0.021$; Fig. 3) suggesting that dPAL was more difficult than sPAL. No significant interaction between block and task was seen ($F(8,104)=1.883$, $P=0.070$).

Lidocaine

Owing to the short-lived effect of lidocaine, only responses occurring within the first 10 min of testing were considered. Lidocaine was tested twice at two separate doses, each with a vehicle control. The first dose had no effect in either task (20 mg/ml; data not shown). When tested at a higher concentration (40 mg/ml), no effect of lidocaine on accuracy was seen in sPAL ($F(1,5)=1.72$, $P=0.24$). However, a significant effect of intra-hippocampal lidocaine was seen in dPAL ($F(1,6)=10.95$, $P=0.016$), where the drug group made significantly more errors than the vehicle control (Fig. 4). As lidocaine caused a significant decrease in accuracy in dPAL, one-tailed ANOVAs were used in subsequent analyses of specific drug effects. P values have been adjusted accordingly. Lidocaine had no effect on reaction time in sPAL ($F(1,5)=0.168$, $P=0.69$). However, it did cause a significant decrease in reaction time in dPAL ($F(1,6)=9.74$, $P=0.20$, see Table 2). No effect of lidocaine was seen on magazine latency in sPAL ($F(1,5)=0.792$, $P=0.414$), or dPAL ($F(1,6)=0.0002$, $P=0.98$).

Mecamylamine

No effect of intra-hippocampal infusions of mecamylamine on accuracy was seen in sPAL ($F(1,6)=0.45$, $P=0.52$), or dPAL ($F(1,6)=0.185$, $P=0.68$; see Fig. 5). Moreover, mecamylamine had no effect on reaction time (sPAL $F(1,6)=0.19$, $P=0.672$; dPAL $F(1,6)=0.42$, $P=0.53$) or magazine latency (sPAL $F(1,6)=0.0074$, $P=0.93$; dPAL $F(1,6)=0.053$, $P=0.825$).

Scopolamine

No effect of intra-hippocampal infusions of scopolamine was seen on accuracy in sPAL ($F(1,6)=0.15$, $P=0.71$) or dPAL ($F(1, 6)=0.05$, $P=0.41$; see Fig. 6). Scopolamine caused a significant lengthening of reaction time in both tasks (sPAL $F(1,6)=16.24$, $P=0.0068$; dPAL $F(1,6)=9.78$, $P=0.02$). The effects of scopolamine on magazine latency approached significance in sPAL ($F(1,6)=4.76$, $P=0.071$), while no such tendencies were seen in dPAL ($F(1,6)=1.80$, $P=0.22$).

MK-801

No main effect was seen of intra-hippocampal infusions of MK-801 on accuracy in sPAL ($F(1,5)=2.15$, $P=0.20$). However, a significant effect was seen in dPAL ($F(1,6)=7.04$, $P=0.019$), indicating MK-801 caused an increase in errors (see Fig. 7). MK-801 had no significant effect on reaction in sPAL ($F(1,5)=0.853$, $P=0.39$), although it did approach a significant decrease in dPAL ($F(1,6)=4.56$, $P=3.18$). Interestingly, MK-801 caused an increase in magazine latency in sPAL ($F(1,5)=12.21$, $P=0.011$),

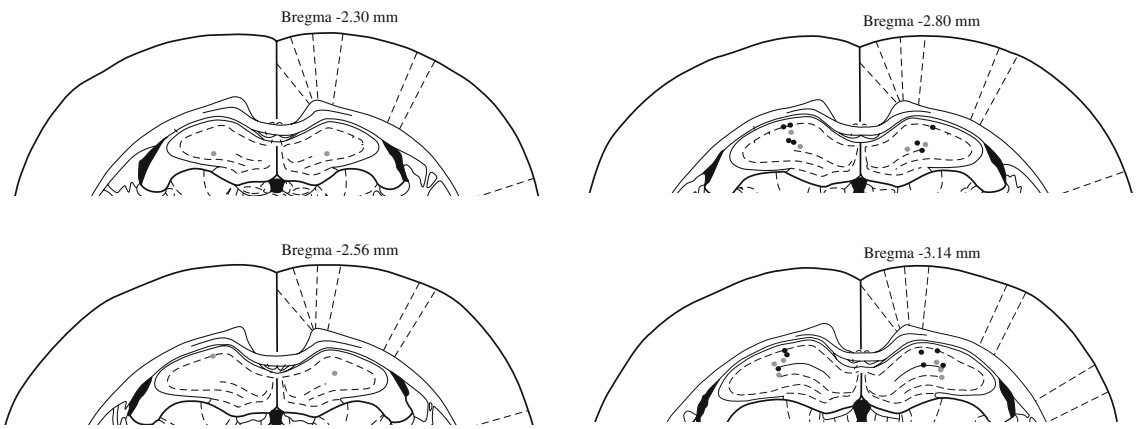

Fig. 2 A reconstruction of the location of cannula injection sites. *Black* indicates the location of cannula used in sPAL, and *gray* indicates the location of those used in dPAL

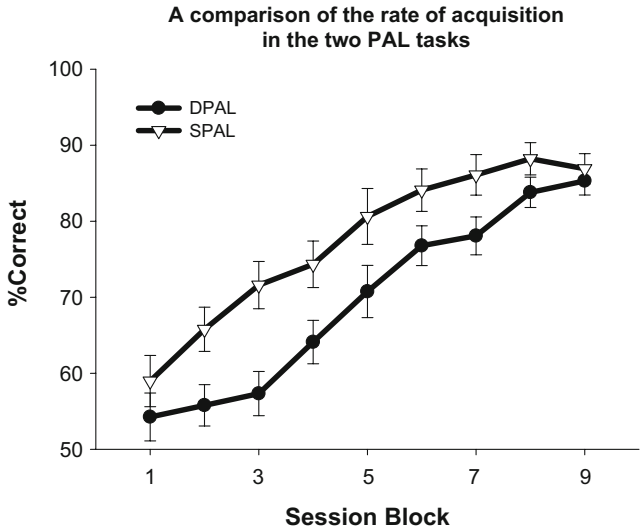

Fig. 3 A comparison of the learning curves seen in sPAL and dPAL. Each block represents five testing sessions. A main effect of task was seen. Data are expressed as means±SEM

whereas no significant effect was seen in dPAL ($F(1,6)=0.044$, $P=0.83$).

CNQX

No effect of intra-hippocampal infusions of CNQX on accuracy was seen ($F(1, 5)=0.965$, $P=0.34$) in sPAL. However, a significant effect was seen in dPAL ($F(1,6)=4.346$, $P=0.041$), indicating that CNQX caused an increase in errors (see Fig. 8). No effect of CNQX was seen on reaction time in either task (sPAL $F(1,5)=0.57$, $P=0.48$; dPAL $F(1,6)=0.46$, $P=0.52$). Similarly, no effect of CNQX was seen in magazine latency in either task (sPAL $F(1,5)=0.06$, $P=0.8$; dPAL $F(1,6)=0.17$, $P=0.68$).

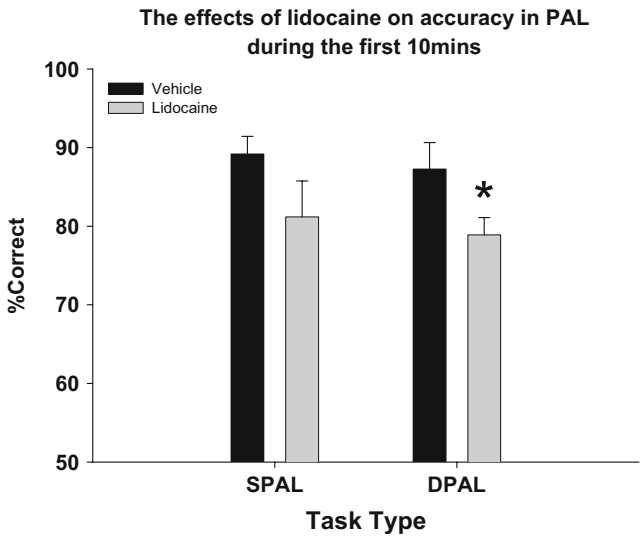

Fig. 4 The effect of intra-hippocampal lidocaine on accuracy in sPAL and dPAL. Data are expressed as means±SEM. *$P>0.05$

Discussion

Using touchscreen-equipped operant boxes, we have developed and validated an automated PAL task for rodents. Normal rats were first trained on one of two variants of the PAL task. In sPAL, the S+ and S− were visually the same, but only the S+ was in the rewarded location. This is in contrast to dPAL, in which the S− was a different stimulus from the S+. Once the task was acquired, rats were tested under the influence of several drugs delivered directly to the dorsal hippocampus. The hippocampus was chosen because dysfunction in this structure is thought to be one of the leading contributors to memory deficits in AD (Braak and Braak 1997; Nestor et al. 2004), and changes to hippocampal function are also seen in schizophrenia (Boyer et al. 2007). These disease-related changes and the fact that human studies strongly suggest a role for the hippocampus in paired-associate learning make manipulations of this structure an ideal starting point for the validation of the rodent PAL task.

In sPAL, the S+ and S− were the same object, differing only in their location on the screen, whereas in dPAL, the S− was a different image, one that would be correct if displayed in another location. Interestingly, dPAL was more difficult to learn than sPAL, although there was no significant difference between these two tasks in terms of final levels of performance. This finding suggests that, although the two tasks are highly similar, they may be dependent upon partially dissociable cognitive processes and neural substrates. Indeed, because in sPAL the S+ and S− shapes are identical, the task may be solved most readily via a conditional rule (e.g. "if object A is presented, then choose location 1"). Consistent with this interpretation, lesions of the hippocampus or fornix can leave conditional learning in the touchscreen unaffected (Bussey et al. 2000; Talpos 2006). A further dissociation between sPAL and dPAL was indicated by the differential effects of MK-801 on magazine and response latencies in these two versions of the task.

After acquiring the task to an asymptotic performance level, animals underwent hippocampal cannulation. Once recovered, animals were again tested in PAL but, in this instance, under the influence of intra-hippocampal infusions of several drugs including lidocaine, scopolamine, mecamylamine, MK-801, and CNQX. None of these drugs caused significant impairments in sPAL, but impairments were seen in dPAL with lidocaine, MK-801, and CNQX. These data indicate that dPAL is more sensitive to manipulations of the dorsal hippocampus than sPAL. Furthermore, the finding that these agents had effects even

Table 2 A summary of logged (msec) mean latency data. *P<0.05, *** P<0.001

Task	Vehicle	Lidocaine	Vehicle	Mecamylamine	Vehicle	Scopolamine	Vehicle	CNQX	Vehicle	MK-801
Magazine latency										
sPAL	2.91	2.94	2.88	2.89	2.9	2.71	2.91	2.9	2.94	2.82*
dPAL	2.93	2.93	2.88	2.88	2.91	2.74	2.91	2.89	2.9	2.91
Reaction time										
sPAL	3.25	3.24	3.33	3.27	3.3	3.75***	3.3	3.27	3.32	3.28
dPAL	3.32	3.2*	3.21	3.25	3.24	4.26*	3.24	3.22	3.24	3.18

after dPAL had been well learned is consistent with a recent functional magnetic resonance imaging study in humans showing that the hippocampus is activated during both acquisition and retention in the CANTAB PAL task (de Rover and Pironti 2008). The specific effects of the various agents tested are discussed below.

Lidocaine

Lidocaine is a non-selective, fast-acting Na+ channel blocker, which can inhibit depolarisation and neuronal firing. This effect is short-lasting and can temporarily deactivate the area to which it is administered, in this case the dorsal hippocampus. Previous work has shown that when administered to the dorsal hippocampus, lidocaine causes impairments in several spatial tasks (Broadbent et al. 2006; Chang and Gold 2003; Farr et al. 2000). In the present study, lidocaine caused impairment in dPAL, but not in sPAL, at doses previously shown to be behaviourally effective within the hippocampus. It is possible that an effect of lidocaine would have been observed in sPAL with a larger sample size, as the magnitude of the decrease caused by lidocaine was similar in both sPAL and dPAL conditions. However, subsequent experiments showed that sPAL, unlike dPAL, was not sensitive to more pharmacologically selective manipulations of the hippocampus (discussed below). The overall pattern of results therefore suggests that dPAL is more sensitive to hippocampal disruption than sPAL.

Cholinergic function and PAL

Administration into the dorsal hippocampus of the nicotinic receptor antagonist mecamylamine or the muscarinic receptor antagonist scopolamine at doses known to be behaviourally effective (Kim and Levin 1996a, b; Ohno et al. 1993; Robinson and Mao 1997) failed to induce deficits in either PAL task. To date, no study has been published presenting data on the effects of cholinergic ligands in an appetitive rodent PAL paradigm, but blockade of the nicotinic and muscarinic receptors has been studied in monkeys performing a version of PAL. PAL, when tested in monkeys as part of CANTAB, consists of four progressively more difficult stages. As these stages progress, more objects and locations are introduced into the problem sets, thus increasing task difficulty. In monkeys, both mecamylamine and

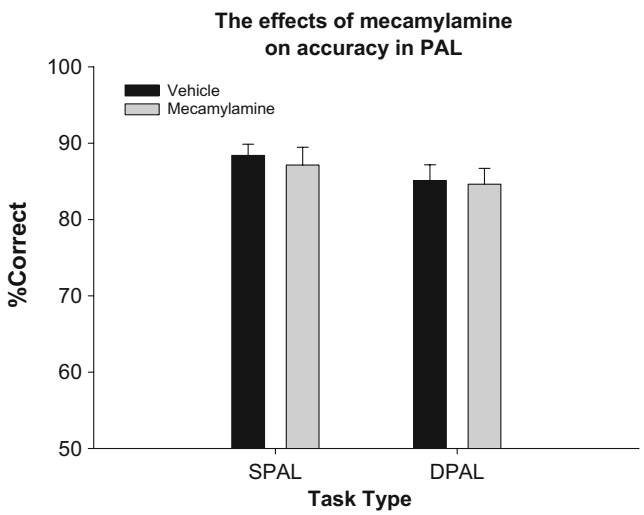

Fig. 5 The effect of intra-hippocampal mecamylamine on accuracy in sPAL and dPAL. Data are expressed as means±SEM

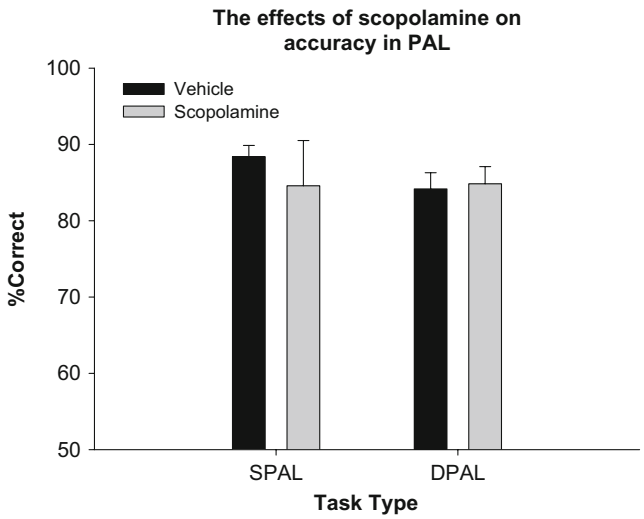

Fig. 6 The effect of intra-hippocampal scopolamine on accuracy in sPAL and dPAL. Data are expressed as means±SEM

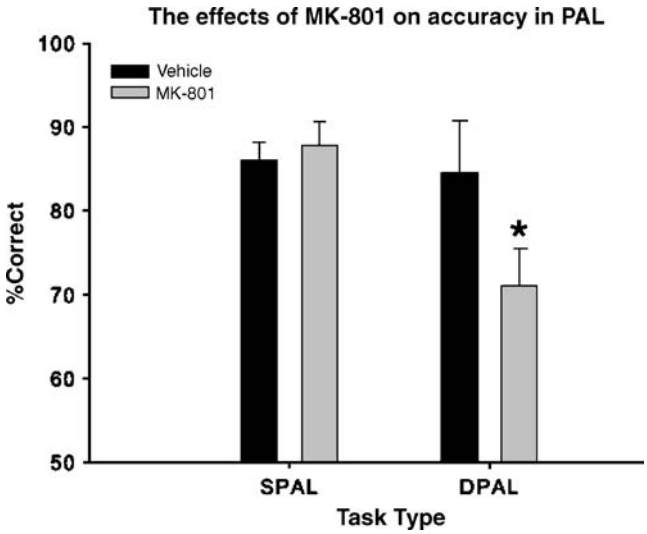

Fig. 7 The effect of intra-hippocampal MK-801 on accuracy in sPAL and dPAL. Data are expressed as means±SEM. *$P>0.05$

scopolamine have been shown to induce deficits in performance, especially in the more difficult stages of PAL (Katner et al. 2004; Taffe et al. 1999, 2002, 2004). Furthermore, administration of systemic scopolamine impairs acquisition of a the dPAL task in mice (Saksida et al. 2008). One possible explanation for the discrepancy between the results from these systemic studies and the lack of effect of intra-hippocampal scopolamine in the present study is that the impairment following systemic scopolamine administration may not be mediated by the hippocampus. Thus, impairments following systemic scopolamine could be the consequence of a dysfunction in attention or other processes mediated by extra-hippocampal structures. Consistent with the idea that intra-hippocampal cholinergic function is not necessary for the memory component of PAL is the

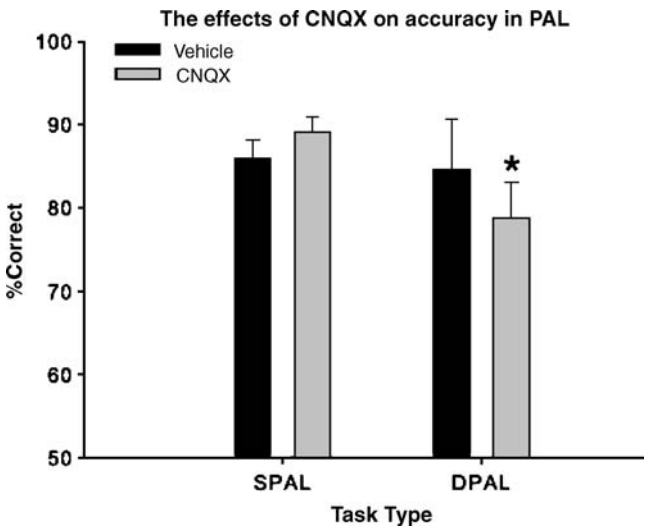

Fig. 8 The effect of intra-hippocampal CNQX on accuracy in sPAL and dPAL. Data are expressed as means±SEM. *$P>0.05$

observation that cholinergic integrity of the hippocampus is not necessary for many tests of spatial cognition (Parent and Baxter 2004). Furthermore, several authors have argued that cholinergic function is most important for memory acquisition processes, but not necessarily retention (Hasselmo 2006; Hasselmo and McGaughy 2004; Power et al. 2003), and scopolamine was administered in the present study long after the task had been well-learned. It is also worth noting that scopolamine has only marginal effects on the performance of humans tested on the CANTAB version of PAL (Robbins et al., 1997). Although ideally the lack of effect of scopolamine would need to be confirmed by testing higher doses, the use of higher doses would be problematic due to non-specific effects that were already being observed at the dose used in the present study (see Table 1).

Glutamatergic function and PAL

CNQX, an AMPA receptor antagonist, and MK-801, an NMDA receptor antagonist, disrupted performance in dPAL but not sPAL. CNQX has been previously found to impair PAL acquisition and recall in a rapidly acquired paired-associate learning task (Day et al. 2003), and our results agree with this finding. This result is not surprising as AMPA receptor activation is thought to be necessary for learning as well as continued expression of a memory. However, in the study by Day et al. (2003), the NMDA receptor antagonist AP5 impaired only acquisition and not retention, a result that seems to conflict with the effect of MK-801 on well-learned associations reported in the present study. One difference between dPAL and the task used by Day et al. (2003) is that during a choice trial in dPAL, not only does spatial and non-spatial information need to be recalled, but a conflict must be resolved between two familiar shapes, the S+ and S−, both of which can be correct depending on the location in which they are presented. It is conceivable that the requirement to resolve this conflict increases the need to re-encode the representations into memory for comparison with stored representations. Consistent with this interpretation is the observation that MK-801 had no effect on sPAL, in which this type of conflict resolution is not required—however, this lack of effect may simply reflect the fact that hippocampal activity in general seems to be less important for the performance of sPAL.

Direct administration of substances to the brain area of interest has many advantages. Chief amongst these is the ability to study the effects of agents in the region of interest in near isolation from the rest of the brain. This consideration was of particular importance in the present study, in which we wished to be certain that our effects were due the action of agents in an area of importance to neuropsychiatric diseases: the hippocampus. The

intracerebral infusion technique is limited, however, with respect to the number of infusions one can make before potential tissue damage becomes a consideration. In this instance, the use of intracerebral injections precluded the performance of dose response curves *and* testing the involvement of multiple neurotransmitter systems. This leaves the question, would higher doses have resulted in larger cognitive impairments (lidocaine, CNQX, mk-801) or impairments where they had not been previously seen after lower doses were administered (scopolamine and mecamylamine)? Such a possibility cannot be ruled out. Yet, all of the doses used in this series of experiments were taken from previous studies in which the same dose had been shown to impair memory when administered into the hippocampus (Kim and Levin 1996; Marti Barros et al. 2004; Ohno et al. 1993; Robinson and Mao 1997; Vianna et al. 2000). Furthermore, the dose of scopolamine used in the present study was already producing nonspecific effects, precluding the use of higher doses. The greatest unknown remains mecamylamine where no behavioural effects were seen, yet the dose used had previously been shown to be behaviourally active. We are inclined to think that greater doses of mecamylamine would not have caused cognitive effects.

The goal of this study was to focus on basic neurotransmitter involvement rather than to establish the relationship between dose and effect size. Notwithstanding, the addition of a dose response curve could have been particularly revealing in the case of glutamatergic manipulations as the effects seen, though reliable, are modest. It is unclear if this is because only partial inactivation of the glutamatergic system was achieved or that other areas of the brain are able to contribute to dPAL in the absence of the hippocampal input. Unfortunately, this was beyond the scope of this study, but it does not detract from the fact that dPAL is impaired by manipulations of the hippocampus, when a stringent control task is not.

The PAL task tested in the present study has obvious similarities to the PAL task used in the human CANTAB battery, but there are also differences. For example, in the human version of the task, stimulus–location pairings are presented and then re-presented one at a time along with a choice of several locations; the subject must choose with which location the stimulus had previously been paired. The sensitivity of the human PAL task to neurodegenerative disorders such as AD is likely due to the need for simultaneous activation of both object and spatial representations. This dual activation likely recruits brain regions such as the hippocampus, perirhinal and entorhinal cortex, which are the areas of the brain first to show pathology in AD (Braak and Braak 1997). PAL may be dependent upon each of these regions, and it could be this dual dependency that makes PAL so predictive of conversion from mild cognitive impairment to AD. However, the neuronal substrates of PAL are still not completely understood and likely not limited to structures within the medial temporal lobe. Other regions, such as the frontal cortices, have been shown to contribute to PAL performance (Owen et al. 1995). Accordingly, it is too early to determine if the impairments seen in first episode psychotic patients are the result of frontal cortex or hippocampal dysfunction (Barnett et al. 2005), or even an interaction between the two. Similarly, the changes in performance in PAL after chronic drug abuse could be the result of long-lasting changes to either of these systems or changes in global 5-HT function which has also been shown to influence performance on PAL (Ersche et al. 2006; Park et al. 1994). dPAL is by no means the first paired-associate learning task that has been developed for the rodent. In addition to the task used by Day et al. (2003) described above, object-in-place, odour-in-place, flavour-in-place, and odour-with-object paradigms have all been developed for the rat (Bunsey and Eichenbaum 1993; Day et al. 2003; Gilbert and Kesner 2002, 2003). Results between these paradigms vary, but deficits are not generally seen following lesions of the hippocampus unless one of the two associates is spatial in nature. For example, no impairment was seen in odour and object pairing tasks, while pairings of object and location or odour and location were both impaired by lesions of the hippocampus (Gilbert and Kesner 2002). If one of the associates is within the spatial domain, then lesions of the hippocampus cause impairments during task acquisition, but not always in retention (Gilbert and Kesner 2004; Tse et al. 2006, 2007). Such tasks can be rapidly learned and the data highly informative; however, the touchscreen version of PAL will be a particularly attractive alternative for those researchers interested in mimicking as closely as possible the test setting of the human version of the task. Moreover, dPAL can be added to the growing battery of automated rodent touchscreen tasks used for high-throughput assessment of cognitive functions relating to human disorders (Brigman et al. 2005; Bussey et al. 1997a, b; Morton et al. 2006; Talpos et al. 2008).

The aim of the present study was to develop a new PAL task for the rodent, test whether manipulations of the hippocampus affected this new task, and not a very similar control task, and finally to begin to understand what neurotransmitter systems might be important for task performance. These goals were achieved. In the future, we hope to expand upon the relative contributions of different neurotransmitter systems and brain regions in PAL, a current focus of ongoing research.

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