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KNOCKDOWN OF ZIF268 IN THE POSTERIOR DORSOLATERAL STRIATUM DOES NOT ENDURINGLY DISRUPT A RESPONSE MEMORY OF A REWARDED T-MAZE TASK

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Abstract—Under certain conditions pavlovian memories undergo reconsolidation, whereby the reactivated memory can be disrupted by manipulations such as knockdown of *zif268*. For instrumental memories, reconsolidation disruption is less well established. Our previous, preliminary data identified that there was an increase in Zif268 in the posterior dorsolateral striatum (pDLS) after expression of an instrumental habit-like 'response' memory, but not an instrumental goal-directed 'place' memory on a T-maze task. Here, the requirement for Zif268 in the reconsolidation of a response memory was tested by knockdown of Zif268, using antisense oligodeoxynucleotide infusion into the pDLS, at memory reactivation. Zif268 knockdown reduced response memory expression 72H, but not 7d later. Western blotting revealed a non-significant increase in Zif268 in the pDLS in rats using response memories, but there was no change in Zif268 expression in the hippocampus following retrieval of a place memory. Zif268 expression increased in the basolateral amygdala after memory reactivation whether a response or place strategy was used during reactivation. We propose that Zif268 expression in the basolateral amygdala may be linked to prediction error, generated by the absence of reward at reactivation. Taken together, these results suggest a complex role for Zif268 in the maintenance of instrumental memories.

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Key words: zif268, memory, striatum, amygdala, hippocampus, T-Maze.

INTRODUCTION

Habits are an adaptive way of performing behaviors with the minimum level of cognitive effort. However *compulsive* habits, e.g. in drug addiction, are highly maladaptive. For this reason, there has been great interest in developing treatments that allow compulsive habits to be overcome once established. One such treatment would disrupt the reconsolidation of habit memories so restoring control over behavior by the values of goals (Milton and Everitt, 2012).

Reconsolidation is the process by which memories become destabilized at reactivation, and subsequently updated or strengthened (Nader et al., 2000). Reconsolidation can be disrupted by antisense oligodeoxynucleotides (ASO-ODNs) infused intra-cerebrally in key loci to knockdown the expression of the plasticity-associated gene *zif268* normally induced by memory reactivation (Lee et al., 2005). Pavlovian cue-drug memories, linking environmental stimuli to a drug high, reconsolidate (Milton et al., 2008; Sanchez et al., 2010; Theberge et al., 2010; Barak et al., 2013); but whether instrumental *habit* memories can also be specifically targeted for disruption is unclear.

Until recently, instrumental memories were thought not to reconsolidate, as protein synthesis inhibition did not produce reactivation-dependent amnesia (Hernandez and Kelley, 2004; for review Vousden and Milton, 2017). However, early studies did not take into account that instrumental behavior can be supported by either goal-directed ('action-outcome', A-O) or habitual ('stimulus-response', S-R) associations. These associations form in parallel (Dickinson, 1985) and are psychologically and neurobiologically dissociable. The A-O association is mediated by the posterior dorsomedial striatum (pDMS) while the automaticity of responding, as it becomes a S-R habit, progressively engages the anterior dorsolateral striatum (aDLS) (Haber, 2003; Belin and Everitt, 2008; Zapata et al., 2010; Murray et al., 2012) and requires an intact aDLS and posterior dorsolateral striatum (pDLS) (Packard and McGaugh, 1996; Yin et al., 2004). Although some data indicated that instrumental memories are robust because they do *not*

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Abbreviations: aDLS, anterior dorsolateral striatum; aDMS, anterior dorsomedial striatum; ANOVA, analysis of variance; A-O, 'action-outcome'; BLA, Basolateral Amygdala; IEG, Immediate Early Genes; NAc, Nucleus Accumbens; ODN, Oligodeoxynucleotides; pDLS, posterior dorsolateral striatum; pDMS, posterior dorsomedial striatum; S-R, 'stimulus-response'; Zif268, Zinc Finger Protein 225.

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undergo reconsolidation (Hernandez and Kelley, 2004), other studies have challenged this, showing that systemic NMDAR antagonism can disrupt instrumental memory reconsolidation under specific conditions (Exton-McGuinness et al., 2014).

Determining whether instrumental responding is goal-directed or habitual can be achieved through outcome devaluation (Dickinson, 1985) and contingency degradation (Hammond, 1980). A related method, first employed by Tolman et al. (1946) and adapted by Packard and McGaugh (1996), uses a modified T-maze task, which produces a different behavioral outcome depending upon which association is retrieved during a probe test. Briefly, animals are trained to run to a specific rewarded location in a T-maze. Animals can retrieve the reward either by using extramaze (allocentric) cues to produce a spatial 'place' representation of the goal, or by encoding the motion (egocentric) cues required to reach the goal (e.g. 'turn left'). In a probe test, animals start opposite the original starting location. Therefore, an A-O response leads to 'place' learners correctly choosing the previously baited arm on the probe test, whereas 'response' learners employ the body turns used in training (i.e. respond incorrectly/S-R).

Inactivation studies have shown the hippocampus to be necessary for expression of the 'place' memory whereas the dorsolateral striatum supports the 'response' memory in this T-Maze task (Packard and McGaugh, 1996). Of particular interest, from a reconsolidation perspective, is the finding that instrumental training can increase striatal expression of *zif268,* and that after extensive training it remains elevated only in lateral striatal regions (Maroteaux et al., 2014). This is consistent with our preliminary data, showing that Zif268 was upregulated in the posterior (but not anterior) dorsolateral striatum (pDLS) of response learners in the T-Maze task (Milton and Everitt, 2012). As Zif268 is critical for appetitive pavlovian memory reconsolidation (Lee et al., 2006), we analyzed the expression of Zif268 after extended training in the T-Maze task and investigated whether *zif268* knockdown in the pDLS using ASO-ODNs during memory reactivation would disrupt the subsequent expression and persistence of a response memory.

EXPERIMENTAL PROCEDURES

Subjects

Subjects were 101 male Lister-Hooded rats (Charles River, Bicester, UK), weighing 250 g at the start of the experiment, that were housed in pairs in a vivarium maintained at 21 °C, on a reversed light–dark cycle (lights on at 1900 h). Water was available *ad libitum* except for during behavioral training and testing sessions, and the animals were food-restricted at 85–90% of their free-feeding weight, being fed after behavioral procedures each day. Weights were monitored thrice-weekly. All procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

Behavioral apparatus

Each animal was tested individually on a plus maze with four arms of 50 cm long and 15 cm wide, at a height of 50 cm from the floor, with raised sides of 4 cm. One arm of the plus maze, opposite to the start arm, was occluded by a white Perspex door, converting the apparatus into a T-maze. The maze was situated in a room with many external cues located around the maze, and these cues remained the same throughout training and testing of each batch of animals.

Surgery

Rats were anesthetized with intramuscular injections of a mixture of ketamine (Ketaset; Henry Schein, Dumfries, Scotland, 0.1 ml/100 g body weight) and xylazine (Rompun; Henry Schein, 0.05 ml/100 g body weight). Each rat was placed into a stereotaxic frame (David Kopf, USA) and implanted with guide cannulae (24-gauge, 11-mm; Cooper's Needleworks) targeting the pDLS, using the following co-ordinates (mm): AP −0.4 mm, ML ±4.0 mm (from bregma), DV −3.8 mm (from the skull surface). Wire stylets (Cooper's Needleworks) were inserted into the guide cannulae to maintain patency. Rats were allowed at least 7 days of recovery from surgery before behavioral procedures began.

Behavioral procedures

Behavioral procedures were adapted from those described by Packard and McGaugh (1996). Prior to training, each rat received two days of habituation to the T-maze, and to the sucrose pellet reward (Noyes 45-mg pellets, Sandown Scientific, UK). Each rat was placed in the maze for 5 min and allowed to freely explore, and following return to the home room was given 10 sucrose pellets in the home cage.

During behavioral training, rats were removed from their home cages and placed in a holding cage prior to the start of the trial. At the start of the trial each rat was placed in the 'start' arm, which was the same for each rat, and the timer started. One arm of the T-maze was baited with a single sucrose pellet; the rewarded arm was counterbalanced between rats, but remained the same throughout training for each rat. Each rat was given four trials on the maze each day, with trials separated by a 30-s intertrial interval (ITI) during which the rat was placed back into the holding cage. If the rat entered the incorrect arm during training, it was allowed to remain in the maze until the correct arm was chosen, or a predetermined 'time-out' of 120 s was reached. The experimenter remained in the room throughout testing, manually recording the latency to retrieve the pellet and the number of incorrect responses on each trial. The experimenter stood in the same position, behind the start arm, during all trials. On the last two days of training, the rats were habituated to the intracerebral infusion procedure at least once.

Following the completion of training, the rats underwent a memory reactivation session, designed as

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