Neurobiology of Learning and Memory 104 (2013) 25–31

Contents lists available at SciVerse ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Reconsolidation and extinction of an appetitive pavlovian memory

Charlotte R. Flavell [1], Jonathan L.C. Lee *

University of Birmingham, UK

ARTICLE INFO

Article history:
Received 21 January 2013
Revised 19 April 2013
Accepted 19 April 2013
Available online 29 April 2013

Keywords:
NMDA receptor
MK-801
Instrumental
Food-seeking

ABSTRACT

When memories are retrieved, they can enter a labile state during which the memory may be modified and subsequently restabilized through the process of reconsolidation. However, this does not occur in all situations, and certain "boundary conditions" determine whether a memory will undergo reconsolidation. Naïve male lister hooded rats were trained for 5 days to press a lever in order to retrieve a food reward associated with a pavlovian light stimulus. Three days post-training, animals were injected with either MK-801 (0.1 mg kg^{-1}; i.p.) or saline vehicle, 30 min before they were placed back into the training context for a retrieval session. Lever pressing was reinforced only by the light stimulus and was restricted to either 10, 30 or 50 presentations of the light conditioned stimulus. After 48 h, animals were again returned to the boxes and light-reinforced lever-pressing activity was recorded. MK-801-treated animals in the 10CS group significantly reduced lever pressing at test, compared to saline controls. In contrast, MK-801-treated rats in the 50CS group demonstrated a significant increase. There was no effect of MK-801 in the 30CS group. Additionally, there were no effects of MK-801 in an analogous, pure instrumental, setting when the cue lights were omitted. The opposing effects of MK-801 under different parametric conditions likely reflect impairments of appetitive pavlovian memory reconsolidation and extinction, respectively. These results demonstrate a competition between reconsolidation and extinction. However, there are also conditions under which MK-801 fails to impair either process.

1. Introduction

Reconsolidation is the process proposed to occur that re-stabilizes a memory that has been reactivated through retrieval (Finnie & Nader, 2012; Lewis, Bregman, & Mahan, 1972; Nader, 2003; Nader, Schafe, & Le Doux, 2000). If this reconsolidation process is interrupted, memories are prevented from returning to a stable state resulting in long-lasting amnesia. This reactivation-dependent amnesia has been demonstrated across a number of species (e.g. Achterberg, Trezza, & Vanderschuren, 2012; Debiec, LeDoux, & Nader, 2002; Eisenberg & Dudai, 2004; Lewis et al., 1972; Nader et al., 2000; Pedreira & Maldonado, 2003; Pedreira, Perez-Cuesta, & Maldonado, 2004; Rose & Rankin, 2006), including humans (Brunet et al., 2008; Forcato et al., 2007; Hupbach, Gomez, Hardt, & Nadel, 2007; Kindt, Soeter, & Vervliet, 2009), in both appetitive (Achterberg et al., 2012; Flavell, Barber, & Lee, 2011; Lee & Everitt, 2008a; Lee & Everitt, 2008b; Lee & Everitt, 2008c) and aversive settings (Debiec et al., 2002; Nader et al., 2000). Moreover, under certain conditions it is possible to enhance (Debiec, Bush, & LeDoux, 2011; Tian et al., 2011) and even incorporate new information (Choi, Kim, & Kaang, 2010; Lee, 2010) into existing memories, leading to the suggestion that the process of destabilization and subsequent reconsolidation is a mechanism which allows the memory updating required for learning (for review see Lee, 2009).

In pavlovian conditioning settings, memory destabilization is generally achieved by re-exposure to the conditioned stimulus (CS) in the absence of the previously-associated unconditioned stimulus (US). However, the presentation of the CS alone is operationally a short extinction session and could lead to either a reconsolidation of the existing trace or the formation of a new extinction (CS-No US) memory. The conditions in which these two opposing outcomes occur are dictated by several important factors, or boundary conditions (Lee, 2009; Nader & Hardt, 2009). In particular, the balance between the strength of training and the extent of non-reinforced CS exposure appears to determine which of reconsolidation and extinction occurs in aversive pavlovian conditioning settings. When training is kept constant, several reactivation parameters have been identified as important. However, two appear to be critical; first the presentation of new information during the reactivation session (Lee & Everitt, 2008b; Morris et al., 2006; Winters, Tucci, & DaCosta-Furtado, 2009) and second, altering the duration of stimulus re-exposure, as increasing exposure to the CS during reactivation increases the likelihood of extinction rather than reconsolidation being impaired (Pedreira & Maldonado, 2003; Power, Berlau, McGaugh, & Steward, 2006; Suzuki et al.,

* Corresponding author. Address: University of Birmingham, School of Psychology, Edgbaston, Birmingham, B15 2TT, UK.
E-mail address: j.l.c.lee@bham.ac.uk (J.L.C. Lee).
[1] Current address: Psychiatric Epigenomics Laboratory, Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072, Australia.

1074-7427/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nlm.2013.04.009

2004). Varying the strength of training, while keeping reactivation parameters constant, also impacts upon whether a memory will undergo reconsolidation, with the suggestion that "stronger' memories are harder to destabilize (Eisenberg, Kobilo, Berman, & Dudai, 2003; Reichelt & Lee, 2012; Suzuki et al., 2004; Wang, de Oliveira Alvares, & Nader, 2009; Winters et al., 2009). At the present time, no studies have systematically examined the effect of manipulating the extent of CS re-exposure within an appetitive pavlovian setting.

The previous demonstrations of a relationship between reconsolidation and extinction in rodent settings have been achieved by the administration either of a protein synthesis inhibitor administered intracerebrally (Eisenberg et al., 2003) or systemically (Suzuki et al., 2004) or by the systemic injection of the NMDA receptor (NMDAR) antagonist MK-801 (Lee & Everitt, 2008a). Although there are mechanistic differences between reconsolidation and extinction at the cellular level (de la Fuente, Freudenthal, & Romano, 2011), both processes are NMDAR-dependent in appetitive pavlovian memory settings (Feltenstein & See, 2007; Holahan, Clarke, & Hines, 2010; Lee & Everitt, 2008a; Milton, Lee, Butler, Gardner, & Everitt, 2008). Therefore, in the present study, we have used systemic injections of MK-801 to determine the behavioural conditions under which the reconsolidation and extinction of an appetitive pavlovian memory occur.

2. Methods

2.1. Subjects

Experimentally-naïve male lister hooded rats ($n = 75$) weighing 220–250 g at the beginning of the experiment, were housed in groups of 4 in a holding room maintained at 21 °C on a 12 h light/dark cycle (lights on at 07:00). Once acclimatised to the holding room (48–72 h), access to food was restricted to 15 g standard rat chow, per rat, per day. Water was available ad libitum throughout, except during behavioural sessions. All procedures complied with the UK 1986 Animals (Scientific Procedures) Act, and performed under project licence PPL 40/3205. Animals were placed into one of 5 groups, based on the nature of training and the type of retrieval session they received (see Table 1).

2.2. Behavioural apparatus

All behavioural training and testing took place in eight operant chambers (MedAssociates, Vermont), each measuring 25 cm × 32 cm × 25.5 cm, housed within a sound-attenuating chamber. Two sides of the operant chambers were constructed of steel, while the ceiling, front and back walls were Perspex, the front also serving as a door. On the side walls were located several modules (retractable levers, LED stimulus lights, food magazine, and auditory stimulus generators). The auditory stimulus generators were not used during any behavioural procedure. The grid floors consisted of 19 stainless steel rods (4.8 mm diameter; 1.6 mm from centre-to-centre). The interior of each box was cleaned with a 70% ethanol solution after each subject and below the grid floor was a removable tray, which was also cleaned between animals.

Table 1
Summary of experimental groups that vary in the length and nature of both training and retrieval. The total numbers of subjects (both saline- and MK-801-administered) for each group are given.

Group	Training	Retrieval	*n*
10CS	5 days	10CS	15
30CS	5 days	30CS	14
50CS	5 days	50CS	16
10 press	5 days/no CS	10 lever presses/no CS	14
50 press	5 days/no CS	50 lever presses/no CS	16

2.3. Behavioural procedures

2.3.1. Training

Rats received 5 days of training. At the beginning of training, subjects were placed into the chambers and the start of the procedure was indicated by illumination of the house light and the presentation of two levers. In the CS groups, depressing the active lever (pseudo-randomly assigned as left or right, counterbalanced across groups) resulted in the house light being extinguished, both levers being retracted and the delivery of a sucrose pellet to the food magazine, as well as a 10-s illumination of a CS light above the active lever. In the instrumental (10 press and 50 press) groups, an active lever press resulted in the retraction of the levers and the delivery of a pellet, but the house light remained on and no CS light was presented. Ten seconds after pressing the active lever, a new trial was signalled by both levers being presented again and (in the CS groups only) the re-illumination of the house light. There were no contingent outcomes when depressing the inactive lever. This repeated until there had been 30 active lever presses or until 30 min had elapsed, whichever was soonest.

2.3.2. Retrieval

Three days after the last training session, rats were placed back into the training context, with both levers presented and the house light on. An active lever press had the same outcome as in training except that it was no longer reinforced with sucrose pellet delivery. Dependent upon the treatment group, sessions were restricted to a maximum of 15 min or until either 10, 30 or 50 active lever presses had occurred. Subjects received the non-competitive N-methyl-D-aspartic acid (NMDA) receptor antagonist (+)-MK-801 [(+)-5-methyl-10,11-dihydro-SH-dibenzo[a,d]cyclohepten-5,10-imine maleate] (0.1 mg/kg; 0.1 mg/ml) or sterile saline, intraperitoneally, 30 min before the beginning of the session. The dose and timing of the injection is the same as used in our previous study of reconsolidation and extinction in conditioned fear (Lee, Milton, & Everitt, 2006b).

2.3.3. Test

Forty-eight hours after retrieval, animals were returned to the operant chambers for a 30-min test session, in which no limit was imposed upon the possible number of active lever presses. Again, the session commenced with the extension of both levers and illumination of the house light. Active lever presses in the CS groups resulted in the illumination of the CS light above the active lever and extinction of the house light for 1 s, because brief presentations of a pavlovian CS are optimal for it to act as a conditioned reinforcer (Mackintosh, 1974), during which the levers remained inserted in the chamber.

2.4. Statistical methods

Data are presented as mean ± S.E.M. active and inactive lever presses at test, total numbers of CS-pellet pairings (or pellet deliveries) during training, and number of unreinforced CS presentations (or lever presses or nosepokes) at memory retrieval. The data from the 30-min test are presented in three 10-min bins, as responding on the active lever declined substantially during the course of the session. Data were checked for consistency and rats were excluded if they were statistical outliers (lying more than two standard deviations from the group mean) during training, memory retrieval or test. The data were analysed (PASW Statistics 18 software) using repeated measures ANOVA with factors lever