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David Reiss, Ondine Walter, Lucie Bourgoïn, Brigitte Kieffer, Abdel-Moultalib Ouagazzal. New automated procedure to assess context recognition memory in mice. *Psychopharmacology*, 2014, 231 (22), pp.4337-4347. 10.1007/s00213-014-3577-3 . hal-01384062

HAL Id: hal-01384062

<https://hal.science/hal-01384062>

Submitted on 10 Mar 2023

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New automated procedure to assess context recognition memory in mice

David Reiss, Ondine Walter, Lucie Bourgoïn,
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Abstract

Rationale and objectives Recognition memory is an important aspect of human declarative memory and is one of the routine memory abilities altered in patients with amnesic syndrome and Alzheimer's disease. In rodents, recognition memory has been most widely assessed using the novel object preference paradigm, which exploits the spontaneous preference that animals display for novel objects. Here, we used nose-poke units instead of objects to design a simple automated method for assessing context recognition memory in mice. **Methods** In the acquisition trial, mice are exposed for the first time to an operant chamber with one blinking nose-poke unit. In the choice session, a novel nonblinking nose-poke unit is inserted into an empty spatial location and the number of nose

poking dedicated to each set of nose-poke unit is used as an index of recognition memory.

Results We report that recognition performance varies as a function of the length of the acquisition period and the retention delay and is sensitive to conventional amnesic treatments. By manipulating the features of the operant chamber during a brief retrieval episode (3-min long), we further demonstrate that reconsolidation of the original contextual memory depends on the magnitude and the type of environmental changes introduced into the familiar spatial environment.

Conclusions These results show that the nose-poke recognition task provides a rapid and reliable way for assessing context recognition memory in mice and offers new possibilities for the deciphering of the brain mechanisms governing the reconsolidation process.

Keywords Recognition memory · Nose-poke units · Spatial context · Consolidation · Reconsolidation · Mice

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Introduction

Recognition memory is the ability to judge that a currently present object, person, place, or event has previously been encountered or experienced. Recognition memory is an important aspect of human declarative memory and is one of the routine memory abilities altered in patients with amnesic syndrome and Alzheimer's disease (Hildebrandt et al. 2013; Peters et al. 2013; Squire et al. 2007). One of the most common tasks for assessing recognition memory in rodents is the novel object preference (NOP) paradigm, which resembles the visual paired comparison (VPC) task given to human subjects (Ennaceur 2010). Unlike other recognition memory tasks, delayed matching to sample and delayed nonmatching to sample that involve an initial phase of rule learning, the NOP paradigm capitalizes on the animal's innate preference

for novelty. The standard procedure consists of prehabitation to spatial context alone followed by an acquisition session during which rats or mice are familiarized with two identical objects. In the testing trial, a novel object is presented together with one of the previously encountered sample objects and recognition memory is reflected by a greater exploration of the novel object than the familiar one. Variants of the procedure have also been developed to assess spatial-, temporal-, and episodic-like memory (Balderas et al. 2008; Barker and Warburton 2011; Dere et al. 2007; Dix and Aggleton 1999; Eacott and Norman 2004; Wilson et al. 2013a). For instance, the object-in-context procedure has been used to assess a form of associative recognition memory that is considered as an analog of human episodic memory (Balderas et al. 2008; Langston and Wood 2010; Wilson et al. 2013b). The procedure consists of two successive acquisition trials in which the animals are exposed to two different pairs of identical objects located within two distinct contexts. In the testing trial, both types of objects are presented in one of these familiar contexts. Normal rats or mice tend to explore more the objects presented in an incongruent familiar context indicating that they have remembered the previously encountered object-context association. Since its introduction, the NOP task has rapidly gained popularity as a recognition memory test for rodents. The relative simplicity of this paradigm has allowed for widespread use across disciplines to evaluate the cognitive alterations associated with aging, genetic manipulations, and pharmacological interventions in rodents (Aggleton et al. 2012; Antunes and Biala 2012; Bertaina-Anglade et al. 2006; Dere et al. 2007; Ennaceur 2010; Lyon et al. 2012; Winters et al. 2010). However, the manual scoring of the test is both time and labor intensive, which limits its utilization for high-throughput behavioral phenotyping and pharmacological screening. To overcome these limitations, automated versions of the task have been successfully developed by several groups using video-tracking systems (Benice and Raber 2008; Chambon et al. 2011; Rutten et al. 2008).

In the present study, we introduce a new automated method for assessing associative recognition memory that adopts the basic concept behind the NOP and VPC paradigms. The procedure is conducted in an operant chamber and involves discrimination of novel from familiar nose-poke units (NPU) that are distinguishable by their visual features and spatial location. During the acquisition session, mice are familiarized with the spatial context in the presence of blinking NPU, and during the choice session, a novel nonblinking NPU is inserted into an empty spatial location. Recognition memory is assessed by comparing the amount of exploration (number of nose poking) dedicated to each set of NPU. A series of control studies were conducted to establish that mice reliably discriminate novel from familiar NPU. We first examined whether discrimination between novel and familiar NPU varies as a function of the length of the acquisition period

and the retention delay. The effects of amnesic drugs on recognition memory were also assessed using systemic administration of scopolamine, an antagonist of the muscarinic cholinergic receptors, and MK-801, an antagonist of the glutamatergic NMDA receptors.

To demonstrate another important potential use of the nose-poke recognition task, we studied the reconsolidation phenomenon. Compelling evidence now indicates that well-established memories can return to a labile state when retrieved and again need to be restabilized in order to persist (Finnie and Nader 2012; Sara 2000). One hypothesized function of the destabilization-restabilization (or reconsolidation) process is to mediate the updating of a memory to maintain its predictive relevance (Finnie and Nader 2012; Kroes and Fernandez 2012; Lee 2009). The destabilization of neural trace is thought to enable incorporation of new relevant information present during retrieval into preexisting memory representation, but this hypothesis is not unanimously accepted. While some studies have demonstrated that memory reconsolidation occurs only under retrieval circumstances that favor novel information encoding (Jones et al. 2012; Morris et al. 2006; Pedreira et al. 2004; Rossato et al. 2007; Winters et al. 2009, 2011), others reported that the association of new information to retrieved memory requires a consolidation-like mechanism (Alberini 2011; Suarez et al. 2010; Tronel et al. 2005). More recently, new computational and theoretical models have been proposed to explain how in hippocampal-dependent tasks the availability of novel information during recall may trigger memory updating (reconsolidation process) or new learning (consolidation process) as a function of the degree of similarity/dissimilarity that exists between the event present at memory recall and the previously memorized experience (Besnard et al. 2012; Osan et al. 2011). Here, we manipulated the components of the operant chamber during a brief reactivation trial interposed between the acquisition and choice sessions to explore whether the engagement of memory reconsolidation depends on the magnitude and/or the type of the transformation introduced into the familiar spatial context.

Materials and methods

Subjects

Eight-week-old C57BL/6 N (BL6N) and C57/BL6J (BL6J) male were purchased from the Charles River Laboratory (France). Mice were housed four per cage and maintained on a 12:12 h light/dark cycle with free access to food and water and allowed to acclimatize to housing conditions until testing, at the age of 10 to 13 weeks. All experimental procedures were conducted with the approval of the local ethics committee (CREMEAS) based on adherence to European Union

guidelines (European Community Guidelines on the Care and Use of Laboratory Animals 86/609/EEC).

Drugs

Scopolamine hydrobromide (Sigma, France) and MK-801 (Sigma, St Quentin Fallavier, France) were dissolved in physiological saline (0.9 % NaCl). Drugs were injected at a volume of 10 ml/kg either subcutaneously (scopolamine) or intraperitoneally (MK-801). A 30-min pretreatment time was used in all experiments. The dose of scopolamine and MK-801 was selected based on our previous studies (Goeldner et al. 2008, 2009; Reiss et al. 2012).

Apparatus

Testing was carried out in four five-choice operant chambers (Coulbourn Instruments, Allentown, USA) dimly lit with a permanent house light. The front of the operant chamber was curved and composed of five bays filled with metal wall panels interchangeable with nose-poke modules (Model H21-10 M). Each nose-poke hole is equipped with a controlled yellow LED cue light at the end and infrared photo beam across the opening that detects the number of nose pokes. The back wall was composed of a single bay fitted with metal panels, and the plexiglass side walls were completely covered by cardboard with distinguishable geometrical motifs. The metal stainless-steel rod floor (the grid shock floor provided by the manufacturer) was covered by a grey vinyl-coated paper that was used as the standard flooring throughout the study. An infrared activity monitor (Model H24-61MC) placed on the ceiling was used for measuring the animal locomotor activity.

Experimental procedures

The standard nose-poke recognition protocol comprised an acquisition session followed by a 10-min choice session. The acquisition session consisted of the familiarization with the spatial context in the presence of a blinking nose-poke unit (NPU: two adjacent nose-poke modules spaced 4 cm apart and turned on with a blinking cue light) presented either in the right or the left corner of the front wall. The spatial location of NPU was counterbalanced between mice for each condition or pharmacological treatment. In the choice session, the familiar NPU was presented in the same corner as in the acquisition, and a novel nonblinking NPU (turned on with constant cue light) was introduced in the opposite corner (8 cm apart from familiar NPU). The present experimental design was adopted based on a series of preliminary experiments showing that the visual features of the familiar NPU do not impact novelty discrimination (supplementary Fig. 1A). The number of nose pokes made in each set of NPU was monitored during 10 min.

The recognition index (RI) was expressed by the ratio ($100 \times$ total number of exploration of novel NPU) / (total number of exploration of all NPU). An RI of 50 % corresponds to a chance level whereas a higher RI reflects a good recognition.

The reactivation protocol comprised a trial of 3-min duration interposed between the acquisition and the choice sessions. A 1-day intertrial delay was used in all experiments. During reactivation, familiar NPU and chamber floor were either manipulated separately or conjointly. The manipulation of familiar NPU consists of removing the entire modules and replacing it by metal wall panels. Manipulation of chamber floor consists of removing the grey vinyl-coated paper and keeping the metal stainless-steel grids as new flooring. For all experiments, prior to reactivation, mice were assigned into testing groups that had both an equivalent number of nose pokes and levels of locomotor activity during acquisition session.

Statistical analysis

All data are expressed as mean group value \pm standard error of the mean (SEM) and analyzed using Student's *t* test, one-way, or two-way ANOVA as appropriate. When relevant, data were submitted to post hoc Fisher's protected least significant difference (PLSD) test analysis. One-sample Student's *t* test was used to compare recognition index values to chance level (50 %). The criterion for statistical significance was $p < 0.05$.

Results

Nose-poke recognition memory as a function of the acquisition length and the retention delay

Experiment 1 To establish the amount of time required for mice to form a robust long-term (24-h delay) recognition memory, the duration of the acquisition (or familiarization) session was varied from 5 to 20 min. To ensure that the animals did not display a bias preference for one set of cues, a control group of naïve BL6N mice was directly tested in the presence of blinking and nonblinking NPU without any previous familiarization with the spatial context. Table 1 presents the absolute number of nose pokes displayed by mice during both the acquisition and testing. The control group explored equally blinking and nonblinking NPU ($p > 0.05$, Student's *t* test), demonstrating the lack of unconditioned preference for one set of these cues. Alternately, preference for the novel NPU (nonblinking one) increased as a function of the acquisition session. Mice exposed for 5 min to the context failed to distinguish the novel from the familiar NPU, whereas those exposed for longer durations, 10 or 20 min, displayed a clear preference for the novel NPU (Table 1). One-way ANOVA performed on discrimination scores (Fig. 1a) revealed a significant main effect of duration ($(F(3, 24) = 3.12, p < 0.05)$), and

Table 1 Number of visits into familiar (FNPU) and novel (NNPU) nose-poke units during the acquisition and testing sessions

	Acquisition	Testing	
	FNPU	FNPU	NNPU
Acquisition length			
Control group ($n=6$)		10.5±1.7	9.5±1.3
5 min ($n=8$)	5.8±0.8	4.6±1.0	5.1±1.0
10 min ($n=7$)	12.1±2.1	3.6±1.0	6.3±0.9*
20 min ($n=7$)	14.0±1.7	1.7±0.7	5.3±0.6*

The control group refers to mice tested directly without previous familiarization with the spatial context

* $p < 0.05$ vs FNPU, Student's t test

post hoc analysis confirmed that the 20-min group had a better recognition performance compared with the control group ($p < 0.05$, Fisher's PLSD test, Fig. 1a).

Experiment 2 We next assessed the time course of discrimination performance in BL6N mice. BL6J mice were also studied to confirm the generality of the findings. Mice were exposed for 20 min to the spatial context, and memory retention was assessed 24, 48, or 72 h later. Separate groups of mice were used for each time point. Figure 1b reveals that discrimination performance declines as a function of the retention delay. At 48-h delay, both mouse strains were able to distinguish novel from familiar NPU ($p < 0.05$ vs chance level, one-sample Student's t test), and when the retention delay was prolonged to 72-h, only BL6N mice performed above chance ($p < 0.05$, one-sample Student's t test). Two-way ANOVA revealed a significant effect of retention delay ($(F_{2,28})=4.31$, $p < 0.05$) but failed

to detect a significant effect of strain ($(F_{1,14})=2.11$, $p > 0.05$). Figure 1c shows that BL6N mice submitted to a short acquisition session (10 min) returned to chance level at 72-h delay ($p > 0.05$, one-sample Student's t test).

Relationship between nose-poke recognition performance and exploratory behavior

To clarify the connection between long-term (24-h delay) discrimination performance and exploration activity during the acquisition session, the data of BL6N mice from experiments 1 and 2 (Fig. 1a and b) were pooled together and submitted to a linear regression analysis. A significant negative correlation was found between the recognition index and the number of nose pokes ($(F_{1,14})=12.19$, $p < 0.005$, $r^2=0.50$, Fig. 2b). By contrast, a positive correlation was detected between the recognition scores and the level of locomotor activity ($(F_{1,14})=2.11$, $p < 0.05$, $r^2=0.28$, Fig. 2a), indicating that spatial exploration facilitates subsequent discrimination behavior. The level of locomotor activity was negatively correlated to the amount of nose poking but the effect failed to reach statistical significance ($(F_{1,14})=3.23$, $p=0.09$, $r^2=0.20$, Fig. 2c). This implies that discrimination performance depends on the ability of mice to form a coherent memory representation of spatial context and not only memory of NPU.

Effect of amnesic treatments on nose-poke recognition memory formation

Experiment 3 To determine whether recognition memory was susceptible to disruption by amnesic drugs, BL6N mice were treated with systemic injections of scopolamine (0.3 and 1 mg/

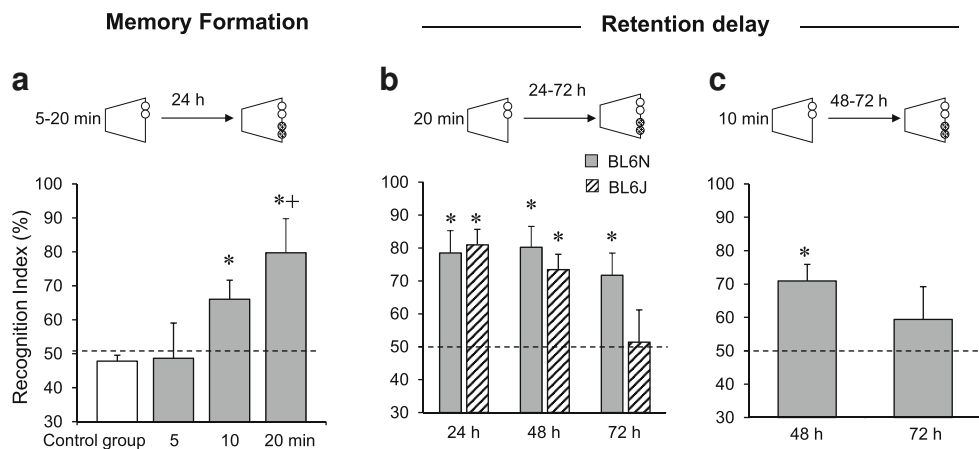


Fig. 1 Nose-poke recognition memory as function of the acquisition period and retention delay. **a** BL6N mice were familiarized 5, 10, or 20 min with spatial context in the presence of blinking nose-poke unit ($n=7-8$ per duration), and the preference for novel (nonblinking) over the familiar nose-poke units was assessed the following day during 10-min choice session. The control group ($n=6$) was tested directly in the same condition without previous familiarization with the context. **b** BL6N and

BL6J mice underwent 20-min acquisition session and tested 24, 48, or 72 h later ($n=6-8$ per interval). **c** BL6N mice were submitted to 10-min acquisition session and tested 48 ($n=6$) or 72 h ($n=8$) later. Values are mean of percent recognition index (%RI) \pm SEM. The horizontal arrows denote the passage of time. The dashed line shows a chance level of 50%. * $p < 0.05$ vs chance level, one-sample Student's t test. + $p < 0.05$ vs the control group, Fisher's PLSD test

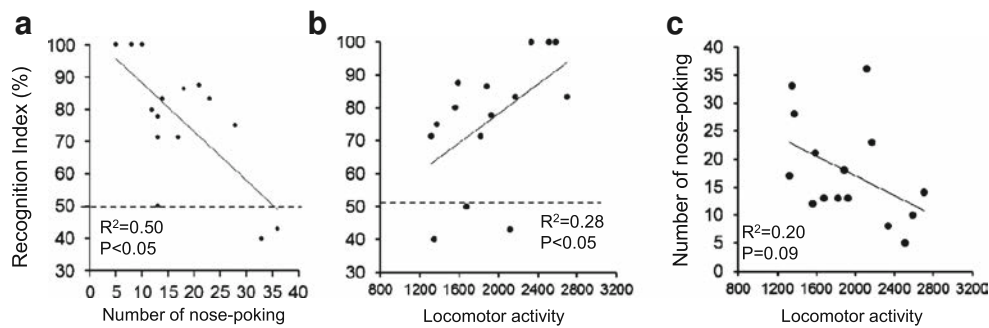


Fig. 2 Relationship between nose-poke recognition memory and level of exploratory behavior during the acquisition. **a** The set of data of BL6N mice from Fig. 1a, b were submitted to a linear regression analysis ($n=15$). Recognition performance is negatively correlated to number of nose

pokes ($p<0.05$). **b** Recognition performance is positively correlated to spatial exploration ($p<0.05$). **c** Negative relationship between spatial exploration and the amount of investigation of nose-poke units ($p>0.05$). The dashed line shows a chance level of 50 %

kg) or MK-801 (0.1 mg/kg) prior to the acquisition and tested the following day. Control groups received systemic injections of the corresponding vehicles. As expected, scopolamine produced dose-dependent memory impairment (Fig. 3a). One-way ANOVA revealed a significant effect of the treatment ($(F_{2, 21})=3.62, p<0.05$) and post hoc comparison indicated that a 1-mg/kg dose significantly reduced discrimination performance when compared with vehicle treatment ($p<0.05$, Fisher's PLSD test). A similar amnesic effect was obtained with MK-801 treatment ($p<0.05$, Student's t test, Fig. 3b). From Table 2, it can be seen that neither scopolamine nor MK-801 changed the level of NPU exploration during the acquisition session ($p>0.05$, one-way ANOVA, and Student's t test, respectively, Table 2). This shows that activation of muscarinic and NMDA receptors is required for the formation of long-term recognition memory.

Effect of spatial context transformations on recognition memory stability upon retrieval

In this series of experiments, we modified the spatial context configuration during a brief (3 min) reactivation trial interposed between the acquisition and choice sessions to examine whether destabilization of original memory depends on the magnitude and/or the type of environmental manipulation.

Experiment 4 We first examined whether removal of the familiar NPU was effective in triggering destabilization of original memory. On day 1, mice were exposed for 20 min to the standard context and on day 2, they received a scopolamine (1 mg/kg) injection prior to reactivation. Memory retention was assessed the following day (48 h post-acquisition). We hypothesized that scopolamine would return discrimination performance to chance level if the original memory trace underwent destabilization or a remodeling process. By contrast, if the memory trace remained intact, this antagonist would be ineffective. Figure 4a shows that mice treated with the vehicle injection had a good discrimination performance ($p<0.05$ vs chance level, one-sample Student's t test), while

those pretreated with scopolamine (1 mg/kg) displayed a clear-cut memory impairment ($p<0.05$ vs controls, Student's t test). To confirm these findings, we used MK-801 prior to the reactivation trial. Again, vehicle-treated mice performed significantly above the chance level ($p<0.05$, one-sample t test), whereas those pretreated with 0.1 mg/kg MK-801 were unable to discriminate novel from familiar NPU ($p>0.05$, one-sample t test, Fig. 4a). Supplementary Fig. 1B shows that the amnesic effects of scopolamine and MK-801 could be replicated using a shorter (10 min) learning session that generates a weaker recognition memory. These findings suggest that reexposure to the spatial context without the familiar NPU triggered destabilization of the original memory trace.

Experiment 5 A series of control experiments were then conducted to verify whether the engagement of a reconsolidation process requires a novelty encoding mode. We first examined whether the amnesic drugs could impair recognition memory in the absence of reactivation. On day 1, BL6N mice were exposed for 20 min to the spatial context and 24 h later, they received the scopolamine (1 mg/kg) or MK-801 (0.1 mg/kg) injection in the home cage. Memory performance was assessed the following day (48 h post-acquisition). The control groups received the corresponding vehicle treatments at the same time. Neither scopolamine nor MK-801 impaired discrimination performance (Fig. 4b), indicating that recognition memory was consolidated within 24 h and became resistant to amnesic treatments. We next examined whether memory reactivation per se was sufficient to trigger the reconsolidation process. Figure 4c shows that administration of scopolamine or MK-801 prior to reactivation in the original learning context had no effect on the subsequent expression of recognition memory ($p>0.05$, Student's t test). To confirm these findings, we used the same reactivation protocol with a 10-min learning session that generates a weak recognition memory (supplementary Fig. 1C). Again, mice treated with scopolamine performed significantly above chance and similar to the vehicle-treated group ($p>0.05$ Student's t test, supplementary Fig. 1C). Together, the findings from experiments

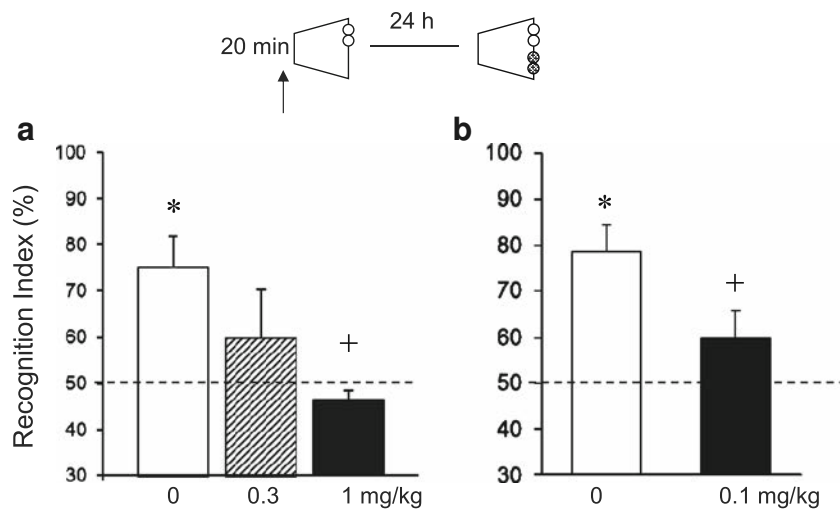


Fig. 3 Effect of amnestic treatments on formation of nose-poke recognition memory. **a** BL6N mice treated with scopolamine (0.3 or 1 mg/kg, s.c., $n=8$ and 7 , respectively) or vehicle ($n=9$) prior to the acquisition session and tested the following day. **b** Mice treated with MK-801 (0.1 mg/kg, i.p., $n=9$) or vehicle ($n=7$) prior to the acquisition and tested

the following day. Values are mean of %RI \pm SEM. The *horizontal arrows* denote the passage of time. The *vertical arrow* stands for drug injections. The *dashed line* shows a chance level of 50%. * $p<0.05$ vs chance level, one-sample Student's t test. + $p<0.05$ vs vehicle-treated mice, Fisher's PLSD test or Student's t test

4 and 5 show that novelty encoding during retrieval was necessary for the engagement of memory reconsolidation.

Experiment 6 In subsequent studies, we examined whether manipulation of the chamber flooring could promote the reconsolidation phenomenon. To this end, the smooth vinyl-coated paper used as the standard flooring was replaced by stainless-steel grid flooring (see "Materials and methods" section). Mice were submitted to 20-min familiarization in the standard context and treated the following day with scopolamine (1 mg/kg) or MK-801 (0.1 mg/kg) prior to reactivation with the new flooring. Memory retention was assessed 24 h later (48 h post-acquisition) in the original learning context. Figure 5a shows that none of the antagonists impaired discrimination performance compared with the corresponding vehicle treatments ($p>0.05$, Student's t test), suggesting that

original memory trace remained intact upon retrieval. A series of experiments were then conducted to investigate whether encoding of novel changes was mediated by a consolidation-like mechanism. We first verified whether in the presence of the new flooring mice still displayed preference for the novel NPU. To achieve this, animals were familiarized during 20 min with the standard context and tested the following day in the presence of the new flooring. As observed, trained mice behave like naïve animals ($p>0.05$ vs chance level, one-sample Student's t test, Fig. 5b). This shows that in the presence of new flooring, trained mice treated the familiar NPU as a novel cue. We then examined whether the brief reactivation with the new chamber flooring was sufficient for mice to acquire a long-term recognition memory. Figure 5b shows that control mice familiarized with the standard context and tested 48 h later in the presence of the new flooring performed at chance level ($p>0.05$, one-sample t test), replicating previous results. By contrast, those submitted to the reactivation trial had a good recognition performance compared with the control group ($p<0.05$, Student's t test Fig. 5b), indicating that the mice successfully acquired the novel context configuration. Figure 5c shows that the blockade of muscarinic or NMDA receptors prior to reactivation could prevent memory improvement ($p<0.05$, Student's t test). These findings suggest that in the presence of new flooring, encoding of novel information took place through a consolidation rather than a reconsolidation mechanism.

Table 2 Number of visits into familiar (FNPU) and novel (NNPU) nose-poke units during the acquisition and testing sessions

Drug	Acquisition	Testing	
		FNPU	NNPU
Scopolamine (mg/kg)			
0 ($n=9$)	20.9 \pm 3.9	2.1 \pm 0.6	5.3 \pm 1.1*
0.3 ($n=8$)	28.5 \pm 4.0	3.4 \pm 0.8	5.8 \pm 1.2
1 ($n=7$)	22.9 \pm 3.4	7.1 \pm 1.1	5.9 \pm 0.7
MK-801 (mg/kg)			
0 ($n=7$)	16.4 \pm 1.8	2.4 \pm 0.8	6.7 \pm 0.9*
0.1 ($n=9$)	18.7 \pm 3.0	6.4 \pm 1.1	9.2 \pm 1.1

* $p<0.05$ NNPU vs FNPU, Student's t test

Experiment 7 Finally, we used a reactivation trial in which the familiar NPU was removed in the presence of the new flooring. On day 1, mice were familiarized during 20 min in the standard context. On day 2, they were reexposed for 3 min to

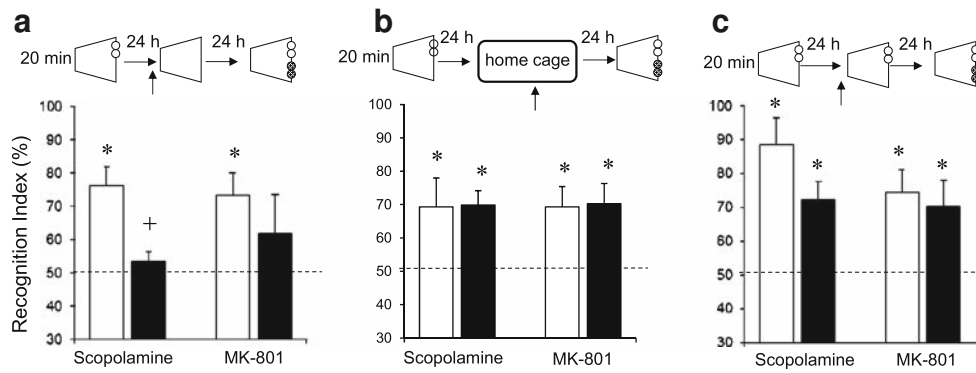


Fig. 4 Manipulation of familiar nose-poke unit alone triggers memory reconsolidation. **a** BL6N mice underwent 20-min acquisition session in the standard context, and 24 h later, they were treated with scopolamine (1 mg/kg, s.c., $n=7$, black bar), MK-801 (0.1 mg/kg, i.p., $n=10$, black bar), or corresponding vehicle (white bars, $n=10$ and 8, respectively) prior to reexposure (3 min) to the same context without the familiar nose-poke unit. Memory retention was assessed 24 h later (48 h post-acquisition) in standard context. **b** Twenty-four hours after acquisition (20 min) in the standard context, mice received scopolamine (1 mg/kg, $n=8$, black bar), MK-801 (0.1 mg/kg, i.p., $n=6$, black bar), or corresponding vehicle

injection (white bars, $n=8$ and 6, respectively) in the home cage and tested the following day (48 h post-acquisition). **c** Twenty-four hours after acquisition (20 min), mice were treated with scopolamine (1 mg/kg, $n=9$, black bar), MK-801 (1 mg/kg, $n=10$, black bar), or corresponding vehicle (white bars, $n=8$ per treatment) prior to reexposure to the original context and tested the following day (48 h post-acquisition). Values are mean of %RI \pm SEM. The horizontal arrows denote the passage of time. The vertical arrows stand for drug injections. The dashed line shows a chance level of 50%. * $p<0.05$ vs chance level, one-sample Student's t test. + $p<0.05$ vs vehicle-treated mice, Student's t test

the modified spatial context and tested the following day in the standard context. Nonreactivated mice tested directly in the original spatial context performed significantly above chance ($p<0.05$, one-sample Student's t test, Fig. 6a), while those submitted to the reactivation trial displayed a severe memory impairment ($p<0.05$, vs nonreactivated mice, Student's t test, Fig. 6a), thus revealing the retroactive interference phenomenon. To clarify the mechanisms underlying this memory impairment, we used scopolamine (1 mg/kg) prior to reactivation. Figure 6b shows that scopolamine pretreatment restored discrimination performance ($p<0.05$, Student's t test). This indicates that the retroactive interference effect was caused by the formation of a new competing memory representation through a consolidation-like mechanism.

Discussion

Previous studies have demonstrated the utility of nose-poke units (NPU) as a tool for monitoring spontaneous exploratory behavior and assessing habituation to the spatial context, a simple form of nonassociative learning (Boccia and Baratti 1999; Brodtkin 1999). Here, we demonstrate that NPU can also be employed as cues for evaluating spontaneous recognition memory in mice. We show that discrimination between the novel and the familiar NPU improved with an increasing length of the acquisition period (Fig. 1a) and declined as a function of the retention delay (Fig. 1b and c). Under our experimental conditions, a good recognition performance could be detected up to 2–3 days later depending on the duration of the acquisition and the mouse substrain. Furthermore, both scopolamine and MK-801 produced the expected

amnesic effects (Fig. 3a and b, respectively) over dose ranges used in the literature (Dodart et al. 1997; Goeldner et al. 2008, 2009; Reiss et al. 2012). Together, these findings demonstrate that nose-poke discrimination behavior provides both a reliable and valid measurement of recognition memory.

Although a nose-poke recognition task resembles the novel object preference (NOP) procedure, the experimental design contrasts in many aspects. In the latter procedure, the sample objects are presented on the floor, while here, the NPU are inserted in the wall, the boundary of the spatial context, and turned on with a blinking or nonblinking cue light. More importantly, in the conventional NOP design, animals are prehabituated to the spatial context over several sessions prior to the familiarization with the sample objects (Ennaceur 2010), while the familiarization with the NPU took place during the first exposure to the spatial context. As such, in our setting, NPUs are encoded from the beginning as part of a unitary spatial representation, while in the object recognition procedure, a robust representation of the spatial context is formed before the objects are encountered. In support of this assertion, reactivation in the absence of the NPU triggered memory reconsolidation (Fig. 4a and supplementary Fig. 1B), whereas reexposure to the familiar spatial context without the objects was consistently shown to be ineffective (Bozon et al. 2003; Rossato et al. 2007; Winters et al. 2009). Furthermore, discrimination scores were negatively correlated to the amount of NPU investigation (Fig. 2a), but positively correlated to the level of spatial exploration (Fig. 2b), which indicates that recognition performance depends primarily on the ability of mice to form a coherent configural representation of the spatial context. Finally, when mice were directly tested in the presence of new flooring, they show no preference for novel over familiar NPU (Fig. 5b) indicating that discrimination performance reflects a form of

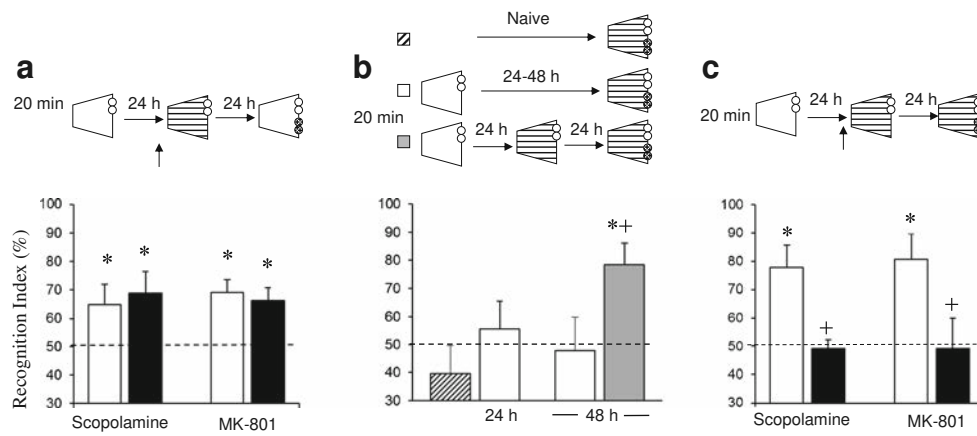


Fig. 5 Manipulation of chamber flooring alone did not trigger memory reconsolidation. **a** Twenty-four hours after acquisition (20 min) in the standard context, mice were treated with scopolamine (1 mg/kg, $n=12$, black bar), MK-801 (1 mg/kg, $n=7$, black bar), or corresponding vehicle (white bars, $n=11$ and 7, respectively) prior to reactivation with new flooring. Testing was carried the following day (48 h post-acquisition) in the standard context. **b** Reactivation with new flooring promotes memory changes. Dashed bar, naïve mice tested directly in the modified context. White bars, mice familiarized with standard context and tested 24 ($n=6$) or 48 h ($n=10$) later in the modified context. Grey bar, mice reactivated with

the new floor and tested the following day in the modified context ($n=6$). **c** Mice submitted to a 20-min learning session in the standard context and treated with scopolamine (1 mg/kg, black bar, $n=7$), MK-801 (0.1 mg/kg, black bar, $n=7$), or corresponding vehicles (white bars, $n=8$ and 6, respectively) prior to reactivation in the modified context. Testing was carried 24 h later (48 h post-acquisition) in the same modified context. Values are mean of %RI \pm SEM. The horizontal arrows denote the passage of time. The vertical arrows stand for drug injections. The dashed line shows a chance level of 50%. * $p<0.05$ vs chance level, one-sample Student's t test. + $p<0.05$ vs nonreactivated mice or vehicle-treated group, Student's t test

associative recognition memory (the association of the familiar NPU with the old chamber configuration). All together, these observations suggest that the nose-poke recognition task assesses contextual recognition memory and not only recognition memory for individual item.

As mentioned earlier, a challenge to the memory-updating hypothesis of reconsolidation comes from a set of studies showing that novel information encoding during retrieval does not systematically engage destabilization of reactivated memory (Alberini 2011; Pedreira and Maldonado 2003; Suarez

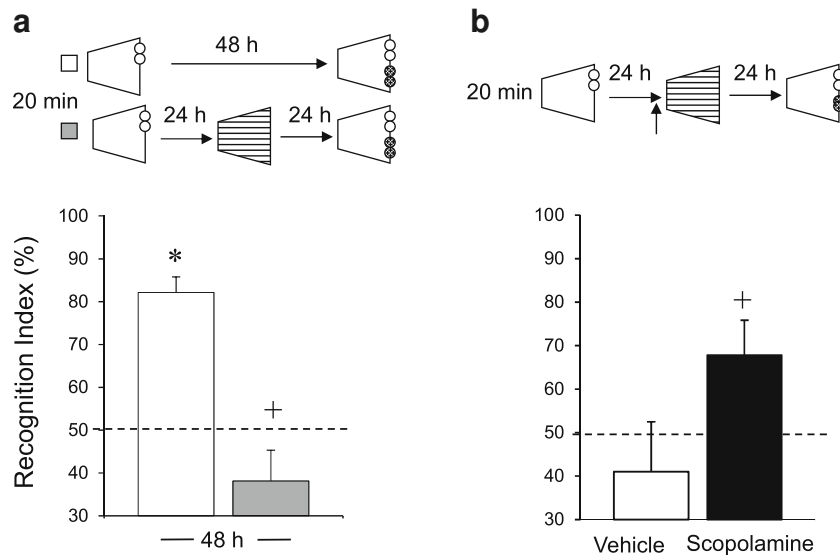


Fig. 6 Conjoint manipulation of nose-poke unit and chamber flooring promotes formation of new competing memory. **a** Removal of nose-poke unit in the presence of new flooring during reactivation trial impairs subsequent recall of old memory. White ($n=6$) and grey ($n=7$) bars represent nonreactivated and reactivated mice, respectively. **b** Reactivation in the modified context engages formation of new competing

memory trace. The horizontal arrows denote the passage of time. The vertical arrows stand for the injection of vehicle (white bar, $n=8$) or scopolamine (1 mg/kg, black bar, $n=9$). Values are mean of %RI \pm SEM. The dashed line shows a chance level of 50%. * $p<0.05$ vs chance level, one-sample Student's t test. + $p<0.05$ vs nonreactivated mice or vehicle-treated group, Student's t test

et al. 2010). A number of explanations have been promulgated over the past years to reconcile the disparate evidence (Finnie and Nader 2012; Lee 2009; McKenzie and Eichenbaum 2011; Nadel et al. 2012; Pedreira et al. 2004; Rodriguez-Ortiz and Bermudez-Rattoni 2007). More recently, Besnard et al. (2012) have proposed a theoretical model that explains why in hippocampal-dependent tasks the availability of novel information during recall may lead to memory updating or new learning (see also (Osan et al. 2011)). They posit that a high degree of similarity will trigger the reconsolidation process that mediates the updating of old-memory representation, while a low degree of similarity will engage the consolidation process that supports formation of new-memory representation. The present study provides empirical evidence in support of this prediction by showing that the engagement of reconsolidation depends on the magnitude of context changes introduced during retrieval. Specifically, a substantial transformation of the spatial context, such as removal of the NPU and replacement of the chamber flooring, led to the formation of a new competing memory (Fig. 6a), as indicated by the protective effect of scopolamine (Fig. 6b). By contrast, a minor context transformation, the removal of the NPU, triggered memory reconsolidation (Fig. 4a and supplementary Fig. 1B). Indeed, the fact that reexposure to the standard context without the familiar NPU rendered recognition memory susceptible to the amnesic treatments suggests that the original memory trace underwent a destabilization process. As a consequence, the blockade of muscarinic or NMDA receptors prior to the reactivation prevented not only the encoding of the novel information but also the restabilization of the original memory trace, thereby resulting in amnesia. It might be argued that these antagonists may have simply speeded up memory loss upon reactivation. However, the set of studies conducted with the manipulation of chamber floor either alone (Fig. 5a) or conjointly with the NPU (Fig. 6b) argues against this possibility. It should be also stressed that neither scopolamine nor MK-801 impaired recognition memory when administered in the home cage (Fig. 4b) or prior to reactivation in the presence of the familiar NPU (Fig. 4c and supplementary Fig. 1C) suggesting that the engagement of reconsolidation only occurs when the retrieved memory needs to be updated with new relevant information in the environment. Overall, our findings corroborate previous studies showing that a dual encoding retrieval state is necessary to trigger destabilization of the memory trace and provide new behavioral evidence supporting the memory-updating hypothesis of reconsolidation.

An interesting finding was that the engagement of memory reconsolidation was also dependent on the type of context changes introduced during retrieval. Unlike removal of the NPU (Fig. 4a and supplementary Fig. 1B), replacement of the chamber flooring did not trigger destabilization of the original memory (Fig. 5a). These results may be explained by the fact

that mice have a greater contact with the chamber floor and that the sensory experiences (e.g., visual, tactile, proprioceptive, etc.) elicited by the new (stainless-steel grids) and the old (smooth vinyl-coated paper) floorings are radically distinct. As such, upon reexposure to the chamber, the novel sensory experience is encoded as a distinct episodic memory from older one. Consistent with this idea, when familiarized mice were directly tested in the presence of new flooring, they behave like naïve animals and engaged in active sampling of all sets of NPU (Fig. 5b), more likely to form a new memory representation. This result extends those reported in the fear conditioning paradigm showing that mice treated the conditioning context as a novel environment when the floor texture was modified, a phenomenon illustrating a form of behavioral pattern separation (McHugh et al. 2007). Further evidence that animals use sensory information supplied by the floor to discriminate between similar environments comes from electrophysiological studies in rats showing that modifying the floor color alone resulted in activation of completely different assemblies of hippocampal place cells (global remapping or pattern separation process) like changing the entire recording chamber, thus reflecting the creation of a new hippocampal representation or spatial map for the modified environment (Jeffery 2007; Jeffery and Anderson 2003). Interestingly, the brief re-activation episode with the new flooring was sufficient for the familiarized mice to acquire a long-term recognition memory (Fig. 5b and c), while a longer duration (>5 min) was necessary for naïve mice (Fig. 1a). Further studies are required to clarify whether the learning improvement displayed by reactivated mice reflects formation of new independent memory or a form of memory updating (or integrative encoding), which consists of linking together novel and retrieved context information by a consolidation-like mechanism (Alberini 2011).

In conclusion, the above findings demonstrate that our new automated method using the NPU permits a rapid and reliable way for assessing recognition memory in rodents. Even though the procedure described here can be used in its current version for characterizing the effects of various pharmacological and genetic manipulations on recognition memory, further optimization of the procedure might be necessary depending upon the experimental conditions (e.g., mouse strains, types of operant chambers, etc) and the questions addressed. One shortcoming of the current experimental design lies in the fact that animals can only be tested once, but this may be overcome by implementing few modifications to make it suitable for repeated testing, for instance, by shortening the initial acquisition session (e.g., 10 min) to prevent an over-habituation of the animals to the chamber and by performing the testing in the presence of a novel NPU configuration (e.g., blinking or nonblinking NPU displaced to a novel spatial location or a third NPU unit introduced in an empty location). For routine screening of new drugs and behavioral

phenotyping of new mouse lines, counterbalancing for the identity of the NPU as well the spatial location is also recommended to control for a potential nonspecific changes in novelty discrimination. Overall, the development of the nose-poke recognition task should provide a valuable complement to existing rodent learning paradigms by offering new possibilities for assessing contextual memory and deciphering the neural and genetic mechanisms underpinning the reconsolidation process.

Acknowledgments This work was supported by grants from the Centre National de la Recherche Scientifique (CNRS), the Institut National de la Santé et de la Recherche Médicale (INSERM), the Université de Strasbourg (UDS). The authors thank Dr. Steve Brooks for the English corrections.

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