

Reconsolidation of fresh, remote, and extinguished fear memory in medaka: old fears don't die

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Abstract

Long-term fear memory in the medaka fish (*Oryzias latipes*) regains transient sensitivity to a consolidation blocker immediately after memory reactivation in retrieval ('reconsolidation'). Here we show that reconsolidation occurs in fresh long-term memories but not in remote memories, and that the apparent amnesia induced by blockade of reconsolidation can be reinstated by an unpaired reinforcer, a procedure that has no effect on amnesia induced by blockade of consolidation. Extinction memory also undergoes post-reactivation reconsolidation, the blockade of which exposes the previously acquired fear. Hence in medaka, the process manifested in reconsolidation seems itself to consolidate; moreover, even when the post-reactivation application of the consolidation blocker is still able to disrupt the memory, the conditioned fear does not seem to go away permanently.

Introduction

Memory consolidation is the process by which the long-term memory of an item stabilizes and becomes resistant to certain sources of interference, such as distracting stimuli, electric shock or metabolic inhibitors (McGaugh, 2000; Dudai, 2004). The question of whether for any item in long-term memory consolidation occurs just once, or whether memories reconsolidate each time anew after retrieval, has recently been revitalized (Sara, 2000; Nader *et al.*, 2000; Dudai, 2004). Ample evidence indicates that indeed, after activation, items in long-term memory may regain transient sensitivity to consolidation blockers (Misanin *et al.*, 1968; Nader *et al.*, 2000; Sara, 2000; Taubenfeld *et al.*, 2001; Kida *et al.*, 2002; Eisenberg *et al.*, 2003). It is still unclear, however, whether this 'reconsolidation' recapitulates the original post-acquisition consolidation (Dudai, 2004). For example, is the amnesia induced by reconsolidation blockade, manifestation of a memory storage deficit or, alternatively, retrieval or performance deficits? In addition to being of remarkable theoretical importance, this question, and the stability of retrieved memory traces in general, has potential practical implications. It is particularly relevant to attempts to understand and develop treatments for certain pathologies that involve persistent activation of undesired memories, such as post-traumatic stress disorder (PTSD; Marks, 1987; Bouton & Swartzentruber, 1991; O'Shea, 2001).

In approaching the nature of consolidation, extinction and reconsolidation in the vertebrate brain, the use of relatively simple systems may prove advantageous. In this respect, teleost fish offer a convenient choice. They have a relatively simple brain, with undeveloped neocortex, and hence are expected to display primitive modes of vertebrate learning, while lacking some of the complexities contributed to the control of even elementary learned

behaviours of higher vertebrates by the frontal brain (Quirk *et al.*, 2000; Myers & Davis, 2002). The medaka fish (*Oryzias latipes*) is particularly noteworthy, as in addition to being relatively easily bred in large numbers, it is highly suitable for neurodevelopmental and neurogenetic analysis (Ishikawa, 2000; Wittbrodt *et al.*, 2002).

Elemental fear conditioning in medaka regains transient sensitivity to a consolidation blocker immediately after its retrieval, i.e. it 'reconsolidates' (Eisenberg *et al.*, 2003). Here we report that this reconsolidation occurs in fresh long-term memories, but not in old memories. Even when the reactivated long-term memory is fresh and prone to reconsolidation, the apparent amnesia induced by blockade of reconsolidation can be reinstated by an unpaired reinforcer, a procedure that has no effect on amnesia induced by blockade of consolidation. We also show that blockade of reconsolidation after apparent complete long-term extinction of the fear memory fully reinstates that memory. The overall conclusion is therefore that in medaka, the process manifested in reconsolidation seems itself to consolidate; but even when reconsolidation still occurs, conditioned fear ultimately survives post-reactivation application of a consolidation blocker, although its expression is disrupted.

Experimental procedures

Animals

Medaka fish (*Oryzias latipes*), inbred strain Cab (kernel colony kindly provided by J. Wittbrodt, EMBL, Germany), were bred and housed in a system of glass aquaria with common capacity of 500 L (Muller & Pflieger, Rockenhausen, Germany). Fish were kept in aerated filtered water at 26 ± 1 °C, 14 : 10 h light–dark cycle and fed three times per day. Young adult fish (5–6 months old, 36 ± 2.2 mm long) were used. All the experimental manipulations were approved by the Animal Maintenance and Experimentation Committee of the Weizmann Institute of Science.

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The experimental set-up

A semi-transparent Plexiglas box (10 × 10 × 10 cm, outer dimensions), filled with water to a depth of 3.5 cm, was divided into four compartments by transparent Plexiglas walls, in order to allow monitoring of four fish simultaneously. Light was used as the conditioned stimulus (CS) and electric shock as the unconditioned stimulus (US). The CS was delivered by two 24-V, 2-W lamps positioned beneath the floor of the box. The US, 0.5 s, 2 V AC, 50 Hz, was delivered through stainless-steel mesh electrodes positioned on each side of the box. The onset and duration of CS and US were computer controlled. Subjects were recorded from above using a Philips ToUcam Pro web-camera, to allow off-line analysis of fish movements.

Behavioural protocols

Conditioning and extinction

Fish were adapted to the experimental box for 3 days, 30 min daily. In between they were kept in 500-mL aquaria, the water in which was changed daily with water from the home tank. On the first experimental day, fish were subjected to fear conditioning in a delay conditioning protocol. In each CS–US pairing they received the CS for 5.5 s, the last 0.5 s of which overlapping with the US. Intertrial interval (ITI) within sessions of ten trials was 120 s. The subjects received two such sessions with a 30-min intersession interval (ISNI), during which the water in the experimental box was changed. Pseudoconditioned fish received an equal number of CS and US exposures in a quasirandom sequence. Memory was tested for retention on days 4, 9 or 15 (depending on the experimental objectives). The test session consisted of ten CS presentations in the absence of the US. Further extinction training, when applicable, consisted of ten daily trials. The number of such extinction sessions varied as detailed in the Results section below.

Analysis of behaviour

The conditioned response (CR) was experience-dependent modification in locomotion. This was analysed on the basis of activity recorded at a rate of 20 frames/s, in time slots of 5 s before and 5 s after CS onset. A computer program registered two parameters for each frame: coordinates of the central pixel of the fish image in the x - y plane of the frame, and angular position, defined as the angle of the fish longitudinal axis relatively to the x -axis of the frame. Two parameters were then used to quantify the CR: (i) activity, defined as multiplication of the length of the subject's trajectory path and the sum of all turns over time, and (ii) variance of angular locomotion, calculated as the standard deviation of angular positions. A subject was defined as responding to the CS if its activity differed four-fold or its variance of angular locomotion differed two-fold in the 5-s period preceding the CS onset compared with that 5 s after CS onset. The probability of fear response, calculated as the number of responses/number of trials within a session, was plotted as a function of training.

Drug treatment

The Na⁺-channel blocker 3-aminobenzoic acid ethyl ester (tricaine, MS222, Sigma, A-5040) was used at a concentration of 0.01% (w/v) in the water. Subjects were placed in the treated water for 20 min. The immobilizing effect of the drug was detectable within 1 min, whereas swimming and feeding behaviour returned to normal after drug washout within *c.* 10 min. Unless otherwise indicated, to block consolidation, subjects were treated immediately after the second conditioning session (day 1), whereas to block reconsolidation,

MS222 was applied immediately after a single non-reinforced retrieval trial on day 2.

Memory recovery protocols

These were applied after one of the following types of memory manipulations: (i) extinction, induced by two daily extinction sessions; (ii) disruption of consolidation by MS222 treatment after acquisition; and (iii) disruption of reconsolidation by application of MS222 after retrieval. Three types of memory recovery protocols were tested: spontaneous recovery, renewal and reinstatement.

In the spontaneous recovery protocol (see Fig. 2a), all fish were subjected to two conditioning sessions on day 1. The experimental groups were presented with a single retrieval trial on day 2 and tested on days 4 and 11. Experimental groups were either treated with MS222 immediately after the second conditioning session (Con) or after a single retrieval (Recon), or were left untreated (No MS222). The control group (SR, Fig. 2e) was subjected to two daily extinction sessions (days 4 and 5) and tested on day 12.

In the renewal protocol (Fig. 2b), two contexts were used. Context A included mild vibrations generated in the aquarium by an R-301 air pump (Rena OEM Sales, Charlotte, NC, USA). In context B, the aquarium was kept stable. The R+Recon group was conditioned in context A on day 1, subjected to a single retrieval trial followed by MS222 treatment in context B (day 2) and tested on day 4 in context A. The R+Con group was conditioned in context B and then immediately treated by MS222 (day 1); this group was tested in context A on day 4. The Recon group was conditioned, subjected to a retrieval trial and tested in context B. In order to demonstrate renewal of extinguished fear memory, two additional groups were conditioned on day 1 (ABA group in context A, BBA group in context B, Fig. 2e), extinguished on two subsequent days (both groups in context B) and tested on day 4 (both groups in context A).

The reinstatement procedure (Fig. 2c) involved administration of 2 × US [interstimulus interval (ISI) = 120 s], in the absence of the CS, 24 h before the test, which was performed on day 4. In the Con group, MS222 was applied immediately after conditioning on day 1, whereas in the two Recon groups fish were treated immediately after a single retrieval trial either 4 h (Recon 4hr group, Fig. 2d) or 24 h after conditioning (Recon 24hr group, Fig. 2d). Fish subjected earlier to extinction training (Reinst group, Fig. 2e) were subjected to 2 × US on day 6 and tested 1 day later.

Statistics

Unless otherwise indicated, comparisons were made between the test and the last conditioning session. Statistical significance was calculated using one- or two-way ANOVA, one-way repeated measures ANOVA or Student's *t*-test. Newman–Keuls test was used for *post hoc* analysis. All data were subjected to arcsin $\sqrt{\pi}$ transformation before statistical analysis.

Results

Reconsolidation occurs both in reinforced and non-reinforced reactivation trials

The Na⁺ channel blocker MS222 (Wang *et al.*, 1994; EMEA, 1999) is an effective consolidation and reconsolidation blocker in medaka (Eisenberg *et al.*, 2003). It blocks long-term memory when applied to the bath immediately after training but not afterwards, but has no detectable lingering effects on performance and short-term memory

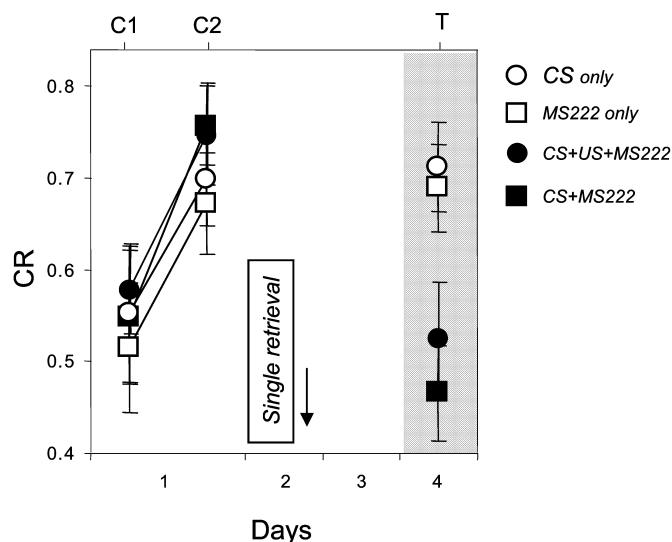


FIG. 1. Blockade of reconsolidation of fear memory by MS222. In this as well as in the following figures, graphs represent the probability of fear response as a function of training. Fish were subjected to two conditioning sessions (C1 and C2) on day 1 and tested on day 4 (T). Groups that were treated with MS222 immediately after memory reactivation in a single retrieval trial (CS+MS222, $n = 11$, closed squares) or immediately after a single conditioning trial (CS+US+MS222, $n = 8$, closed circles) on day 2 (arrow) demonstrated impaired fear memory on day 4. Control groups were either subjected to memory reactivation in the absence of MS222 treatment (CS only, $n = 10$, open circles) or received MS222 treatment in the absence of memory reactivation (MS222 only, $n = 10$, open squares). All values in this and in the following figures are means for ten trials \pm SEM.

after washout. To investigate reconsolidation, fish were subjected to fear conditioning as detailed above (two sessions of ten trials each, ITI 120 s, ISNI 30 min; Fig. 1). Twenty-four hours later, the fish were presented with a single CS reactivation (retrieval) session either in the absence (CS+MS222) or in the presence of the US (CS+US+MS222). These reactivation procedures, which do not induce experimental extinction, are expected to render the fear conditioning trace transiently susceptible to disruption by the consolidation blocker (Eisenberg *et al.*, 2003; see also Pedreira & Maldonado, 2003; Suzuki *et al.*, 2004, in other systems). Indeed, MS222 blocked reconsolidation under both reactivation conditions, as verified by one-way ANOVA of the differences in performance between test and the last conditioning session of the experimental groups (Fig. 1). Controls included fish that were either untreated (CS only) or treated with MS222 in the absence of memory reactivation (MS222 only; $F_{3,38} = 7.71$, $P < 0.001$). Newman–Keuls *post hoc* test revealed significant differences between each of the experimental groups and each of the controls ($P < 0.05$). Unless otherwise indicated, in the experiments described below, the single-trial non-reinforced, non-extinguishing reactivation procedure was used.

The amnesia caused by blockade of reconsolidation does not reverse spontaneously

To determine whether the amnesia induced by reconsolidation blockade was reversible, we applied three methods that are used in the analysis of latent memory in experimental extinction. These methods are: (i) measure of spontaneous recovery, which is the recovery of the original memory in the absence of explicit retraining; (ii) renewal, the reappearance of the original memory in a context

different from that in which retrieval was practised; and (iii) reinstatement, the reversal of amnesia by presentation of an unpaired reinforcer (reviewed in Dudai, 2002).

To test for spontaneous recovery, MS222 was applied immediately after conditioning (Fig. 2a, Con) or retrieval (Recon), as detailed above, and memory was then tested on days 4 and 11. One-way ANOVA revealed a significant difference between the Con, Recon and untreated (No MS222) groups on day 11 ($F_{2,27} = 7.88$, $P < 0.01$), indicating no spontaneous recovery in the treated groups after 7 days. In contrast, spontaneous recovery was observed in a group previously subjected to extensive extinction training (Fig. 2e, SR group; Newman–Keuls test, $P < 0.05$).

The amnesia caused by blockade of reconsolidation or reconsolidation does not reverse with change of context

To test the possibility of memory renewal with context change (Fig. 2b), we used two contexts. In context A, the conditioning aquarium was subjected to mild vibrations, as detailed above. In context B, the aquarium was kept stable. We trained and tested fish in context A, whereas a single retrieval was performed in context B (R+Recon). In the R+Con group, subjects were conditioned in context B and tested in context A. As seen in Fig. 2b, no renewal was detected after blockade of either reconsolidation or consolidation compared with fish that were trained, subjected to a single retrieval trial and tested in context B (Recon, one-way ANOVA, $F_{2,30} = 0.48$, $P = 0.6$). In contrast, renewal was observed in the groups conditioned either in context A (Fig. 2e, Renewal ABA) or in context B (Fig. 2e, Renewal BBA), subjected to extinction training in context B and tested in context A (Newman–Keul test, $P < 0.05$). Moreover, fish conditioned in context B displayed CR in the first extinction session performed in context B (ABA group, data not shown, paired t -test, $t_{11} = 4.28$, $P = 0.001$), further indicating no context-dependent failure of CR expression.

The amnesia caused by blockade of reconsolidation, but not of consolidation, can be reversed by US reinstatement

When amnesia was induced by MS222 after a single retrieval trial, 24 h after conditioning, subsequent application of US in the absence of the CS reinstated the memory tested 24 h thereafter. In contrast, a similar protocol did not rescue the memory blocked in consolidation (Fig. 2c; $t_{21} = 2.24$, $P < 0.05$). It is noteworthy, however, that no reinstatement of reconsolidation-blocked memory was observed if the reactivation + reconsolidation blockade was performed only 4 h after conditioning (Fig. 2d). Reinstatement was also observed in an extinguished memory, as expected (Fig. 2e, Reinst group; Newman–Keul test, $P < 0.05$). These results thus indicate a difference between memory blocked in consolidation and memory blocked in reconsolidation.

Blockade of reconsolidation of the fully extinguished trace restores fear conditioning

When retrieved in the absence of the US, the CS–US ('excitatory') trace competes with the CS–noUS ('inhibitory') trace for the control of behaviour (e.g. Berman *et al.*, 2003). We have recently reported that in this situation, only the trace that retains or gains control over behaviour undergoes reconsolidation (Eisenberg *et al.*, 2003). In line with this rule, we have now found that the when applied immediately after a reactivation trial of fully extinguished condi-

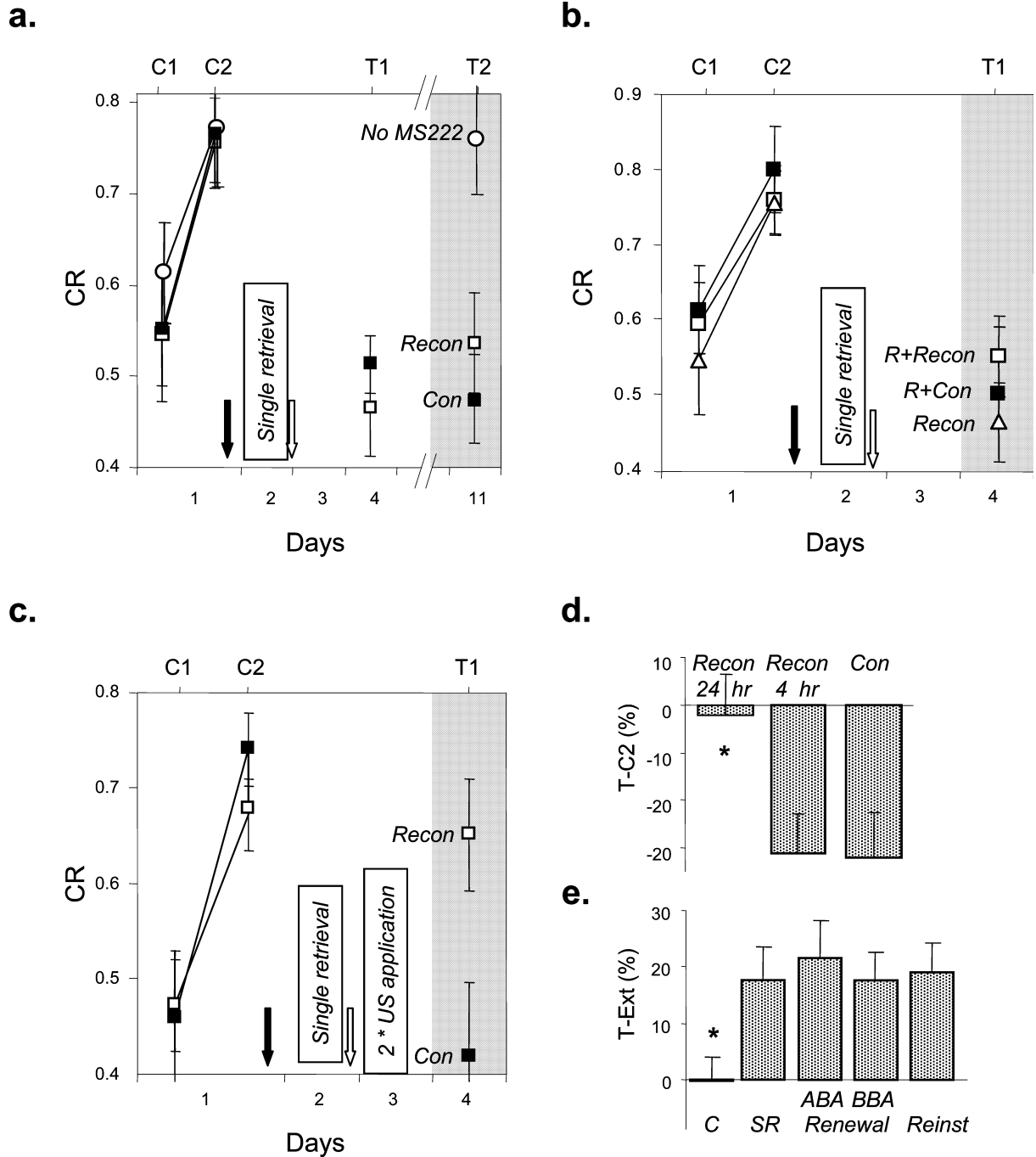


FIG. 2. Tests for spontaneous recovery (a), renewal (b) and reinstatement (c) of fear memory blocked in either consolidation or reconsolidation. (a) Fish were subjected to two conditioning sessions (C1, C2). One group (Con, $n = 8$, closed squares) was treated with MS222 immediately after C2 (black arrow). Another group (Recon, $n = 11$, open squares) was treated with MS222 immediately after a single retrieval trial on day 2 (open arrow). Memory tests were performed on day 4 (T1) and on day 11 (T2). No significant spontaneous recovery was detected in both groups (performance compared with a control receiving no MS222 treatment, No MS222, $n = 8$, open circles). (b) One group (R+Recon, $n = 12$, open squares) was conditioned on day 1 in context A (vibrating aquarium, see Experimental procedures). A single retrieval trial was performed in context B (no vibration) on day 2, immediately followed by MS222 treatment (open arrow). Memory was tested on day 4 in context A. Performance of this group was not significantly different from the group in which all stages of protocol were carried out in context B (Recon, $n = 11$, open triangles), or from the group which was trained in context B, tested in context A (R+Con, $n = 8$, closed squares) and treated with MS222 immediately after conditioning (black arrow). (c) The reinstatement procedure involved application of two USs on day 3 (ISI = 120 s). Whereas this treatment had no effect on memory blocked in consolidation (Con, $n = 11$, closed squares), it did reinstate the CR in the reconsolidation-blocked group (Recon, $n = 12$, open squares). (d) Recovery from amnesia in the reinstatement protocol depended, however, on the timing of the reactivation + MS222 treatment. Only the group in which reactivation + MS222 was performed 24 h after conditioning (Recon, 24 hr, $n = 12$) displayed reinstatement-induced memory recovery. In contrast, if MS222 was applied immediately after retrieval at 4 h (Recon, 4 hr, $n = 8$) or immediately after conditioning (Con, $n = 11$), no memory recovery was detected. Each bar represents the percentage difference between the CR probability in the test and in the last conditioning session (T1-C2, %). (e) Recovery of extinguished memory in a spontaneous recovery protocol (SR, $n = 12$), two renewal protocols (Renewal ABA and BBA) and a reinstatement protocol (Reinst, $n = 16$). A control group (C, $n = 13$) was kept untreated between conditioning, extinction training and the test (all performed in context B). Renewal groups were conditioned either in context A (ABA group, $n = 11$) or in context B (BBA group, $n = 8$), extinguished in context B and tested in context A. Each bar represents the percentage difference between the CR probability in the test and in the last extinction session (T-Ext, %). * $P < 0.05$.

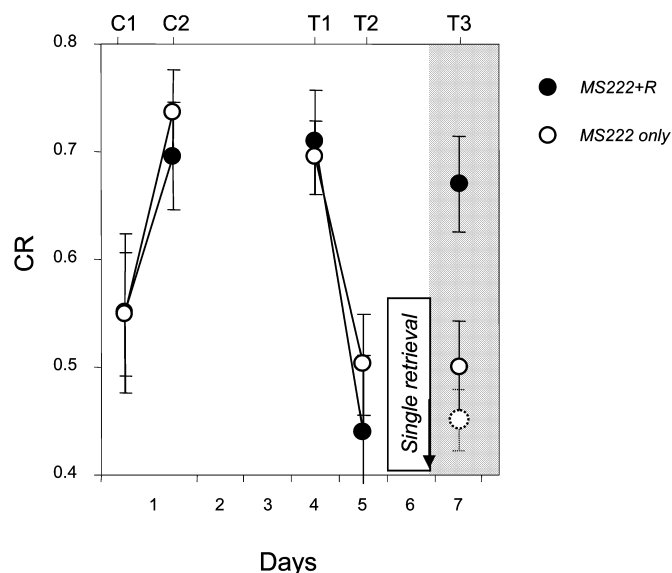


FIG. 3. The effect of reconsolidation blockade on extinguished fear memory. Fish were subjected to two conditioning trials (C1, C2) and subsequently to two extinction sessions, each composed of ten non-reinforced trials (T1, T2). One group was subjected to an additional single non-reinforced retrieval trial on day 6, and treated immediately afterwards with MS222 (MS222+R, $n = 10$, closed circles). Another group was treated with MS222 at the same time, but in the absence of memory reactivation (MS222 only, $n = 13$, open circles). Memory was then tested again, once, on day 7 (T3). Whereas the group treated with MS222 in the absence of memory reactivation displayed extinguished performance, the group treated with MS222 after memory reactivation regained its fear conditioned response. The dotted symbol represents the performance of subjects treated with MS222 after pseudoconditioning (see Experimental procedures).

tioned fear (day 6, Fig. 3), the consolidation blocker blocked the inhibitory trace and restored the excitatory trace (Student's t -test, $t_{21} = 2.53$, $P < 0.05$).

Reconsolidation occurs only in reactivated fresh memories

Groups of medaka were subjected to fear conditioning and then subjected to a single non-reinforced trial on day 2, on day 8 or on day 14 after conditioning. This memory reactivation was followed immediately by MS222 treatment. The different groups were then tested on days 4, 9 and 15, respectively, and compared with control groups in which MS222 was administered without memory reactivation (Fig. 4). Only fish tested on day 4 showed clear evidence of reconsolidation upon memory reactivation ($t_{19} = 3.34$, $P < 0.01$), whereas those tested on day 15 showed no evidence for reconsolidation of memory reactivated a day earlier; values for the group of fish tested on day 9 were between those tested on day 4 and those tested on day 15. Two-way ANOVA conducted on test performance of the six above-mentioned groups revealed a significant (reactivation) \times (conditioning–reactivation interval) interaction effect ($F_{2,62} = 3.98$, $P < 0.05$). The data thus show that the reconsolidation effect diminishes as a function of time between conditioning and memory reactivation.

A potential explanation for the decrease in reconsolidation with age might be that the remote memory extinguishes rapidly, compared with fresh memory, and therefore the extinction trace, becoming dominant after reactivation, is the trace that becomes sensitive to the consolidation blocker, rather than the original trace (Eisenberg *et al.*, 2003; see also above). This, however, was not the case (Fig. 4, inset). Comparison between the first and last five trials within the test session did not reveal a significant difference for 2-day- and 14-day-old memories ($t_{20} = 0.31$, $P = 0.76$).

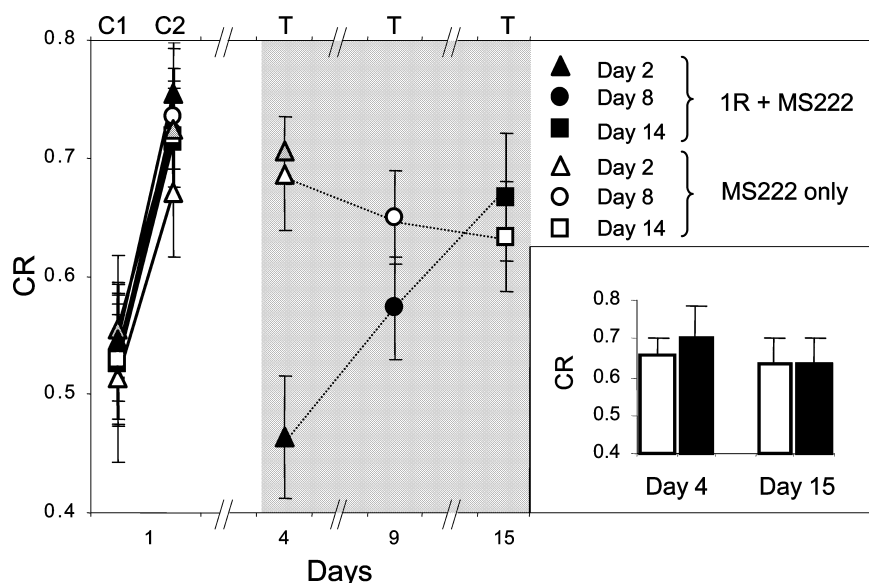


FIG. 4. The effect of the time between conditioning and reactivation on memory reconsolidation. Groups of medaka were subjected to two conditioning sessions on day 1, and tested either on day 4 (closed triangles, $n = 10$), day 9 (closed circles, $n = 12$) or day 15 (closed squares, $n = 12$). In three experimental groups (closed symbols), the test was preceded by a single non-reinforced retrieval trial, followed immediately by application of MS222 (on days 2, 8 and 14, respectively). The three other groups (open symbols, $n = 10$ – 12) were treated with MS222 only on the same days as the corresponding experimental groups, but in the absence of the preceding non-reinforced retrieval trial. The performance of a no-reactivation, no MS222 control group is represented by the grey triangle ($n = 24$). The reconsolidation effect hence diminished as the time between conditioning and memory reactivation increased. Inset: within-session extinction performance of untreated groups on days 4 and 15. For both groups there was no significant difference between the first (open bars) and the last five trials (closed bars).

Discussion

The revived interest in reconsolidation (Sara, 2000; Nader, 2003; Dudai, 2004) has stirred anew the debate concerning the nature of memory consolidation and the stability of the long-term memory trace. Ample data now indicate that reactivated items in long-term memory can regain transient sensitivity to consolidation blockers, resulting in apparent post-reactivation amnesia. However, the meaning of these data is still in dispute (Bahar *et al.*, 2004; Lee *et al.*, 2004; Salinska *et al.*, 2004; Suzuki *et al.*, 2004). Is reconsolidation recapitulation of consolidation? Is the post-reactivation amnesia a manifestation of storage, retrieval or performance deficits, and is it reversible? What do the findings tell us about the maturation and stability of long-term memory?

Our objective here was to address these questions, using the medaka fish as an experimental system. Toward that end, we have developed a fear conditioning protocol for medaka, in which a conditioned stimulus (CS, light) is paired with an unconditioned stimulus (US, shock) to yield a conditioned response (CR, altered locomotion) (Eisenberg *et al.*, 2003). Transient treatment with the sodium channel blocker MS222 immediately after acquisition, but not afterwards, blocks consolidation of long-term memory without affecting short-term memory; a similar treatment after memory reactivation blocks reconsolidation (Eisenberg *et al.*, 2003).

The effect of the consolidation blocker on the reactivated fear conditioning trace is independent of whether the reactivation is performed in non-reinforced or reinforced mode (Fig. 1), as far as this reactivation does not induce considerable extinction. Moreover, if application of MS222 after retrieval accelerated extinction, we would expect renewal of the CR in a different context, which was not the case. This supports the notion that in our system, the deficit in fear memory caused by reconsolidation blockade is unlikely to be accelerated extinction, as suggested by Fischer *et al.* (2004).

When extinction gains considerable control over behaviour, the situation changes. We have previously reported that similar to the situation in conditioned taste aversion in the rat, the trace that undergoes reconsolidation is the one that retains or gains control over behaviour immediately after the retrieval session (Eisenberg *et al.*, 2003). We have now extended this finding to show that this rule generalizes to a long-term, apparently fully extinguished trace. Hence when the overly dominant CS–noUS ('inhibitory') trace is reactivated and a consolidation blocker applied, behavioural control is reversed, and the original CS–US ('excitatory') trace regains dominance. Incidentally, this is also another piece of evidence to support the notion that experimental extinction is relearning rather than unlearning (Bouton & Swartzentruber, 1991; Rescorla, 1996; Dudai, 2002).

A fundamental question in the reconsolidation literature is whether reconsolidation blockade results in disruption of the trace, i.e. storage deficit, or merely in its suppression, i.e. retrieval or performance deficits (reviewed in Dudai, 2004). The same protocols conventionally used to demonstrate that extinction is relearning rather than unlearning could be used to analyse post-reconsolidation amnesia as well. These protocols include: (i) spontaneous recovery, the return of the original memory in the absence of explicit retraining; (ii) reinstatement, the reversal of amnesia by presentation of an unpaired reinforcer; (iii) saving, the facilitation of relearning; and (iv) renewal, the reappearance of the original memory in a context different from that in which extinction was practised. It is noteworthy, however, that the power of these protocols is inherently limited. Lack of spontaneous recovery, of unpaired US reinstatement, of saving or of context-dependent renewal cannot definitively prove the absence of

the trace. First, a negative finding is inherently inconclusive. Second, the above phenomena might be construed as summation of new experience with the residues of a damaged trace (e.g. Gold *et al.*, 1973; Bouton, 2002). Distinction between storage and retrieval/performance deficits might in the future benefit from the potential identification of specific neural signatures, and systems in which such signatures could be matched with specific learning (e.g. Hasselmo *et al.*, 2002). In the meantime, trace-seeking protocols such as used in the study of extinction are primarily useful in generating working hypotheses, and this is our intention here.

We found no evidence for spontaneous recovery of the reconsolidation-blocked trace over 10 days, nor did we find evidence for renewal of the blocked conditioned behaviour in a different context. We did, however, succeed in completely reinstating the blocked long-term trace by unpaired US reinstatement. This could be taken, the above reservations notwithstanding, to favour the hypothesis that reconsolidation blockade in medaka culminates in a retrieval or performance deficit. This finding is also in line with earlier interpretations that recall deficits induced by post-reactivation blockade in the rat (Mactutus *et al.*, 1979) and in the chick (Anokhin *et al.*, 2002) reflect expression rather than storage deficits, and differs from the report on the apparent irreversibility of the post-activation amnesia in reconsolidation blockade of fear conditioning in the rat (Debiec *et al.*, 2002).

Two caveats are, however, appropriate. First, reinstatement was ineffective if reconsolidation blockade was performed on a 4-h memory – a point in time at which the memory is already insensitive to the consolidation blocker in the absence of memory reactivation (Eisenberg *et al.*, 2003). This might imply that with time the reactivated trace becomes less sensitive to the consolidation blocker (see below), in which case the larger trace residues might add to the new US experience to restore the CR. Second, the preference of retrieval or performance deficits interpretations over storage deficits interpretations of reconsolidation blockade does not imply that reconsolidation and consolidation differ profoundly. For example, in the reinstatement experiments, the similarity of the data regarding reconsolidation blockade of 4-h memory and those of consolidation blockade could be construed as indicating that reconsolidation and consolidation differ quantitatively, not qualitatively.

Indeed, the zeitgeist prefers storage deficits explanations for post-consolidation-blockade amnesia, but whether this explanation is conclusive is still a matter of debate (Millin *et al.*, 2001; de Hoz *et al.*, 2004; Dudai & Eisenberg, 2004; Dudai, 2004). Elsewhere, we have argued that interpretation of the reconsolidation data should preferably diverge from the chronic storage/retrieval/performance debate (Dudai & Eisenberg, 2004).

A major finding of our study is that old fears in medaka become resistant over time to post-reactivation interference. Litvin & Anokhin (2000) have noted a temporal gradient of susceptibility to protein synthesis inhibition of reactivated passive avoidance in the young chick within 48 h post-training. Milekic & Alberini (2002) have demonstrated a temporal gradient of sensitivity to protein synthesis inhibition over 1–2 weeks after reactivation of long-term memory in the hippocampal-dependent inhibitory avoidance task in the rat. The age-dependent gradient detected in our study spans over 1 week, a time course that resembles that of systems consolidation, which is characteristic of hippocampal-dependent declarative memories (McClelland *et al.*, 1995; Dudai, 2004). We have not yet identified the brain circuits that subserve fear conditioning in medaka. It has been suggested that the medial and lateral telencephalic pallia maintain functional similarity with the mammalian hippocampus and amygdala (Rodriguez *et al.*, 2002; Portavella *et al.*, 2004). It is not implausible

therefore that systems consolidation does occur in teleosts. But even if this were the case, it is contextual fear conditioning, rather than elemental fear conditioning as used in our study, that is expected to be hippocampal-dependent. It is therefore possible that even in hippocampus-independent memory systems, the time window of consolidation closes much later than thus far assumed (see also Litvin & Anokhin, 2000), and that this lingering consolidation is reflected in progressively reduced sensitivity to post-reactivation application of consolidation blockers (Dudai & Eisenberg, 2004). This interpretation is indifferent to the issue of whether consolidation itself is a process that establishes trace stability, retrievability or both (Dudai & Eisenberg, 2004; Dudai, 2004). It might be possible, for example, that a major role of consolidation in the first place is to establish retrieval links; that blockade of consolidation prevents the formation of all or the majority of these links, rendering the trace practically irretrievable and thus generating an apparent storage-deficit phenotype; and that only reactivated links are prone to interference in reconsolidation, resulting in a retrieval-deficit phenotype (Dudai, 2004).

Resolution of the debate on the nature of consolidation and reconsolidation will require a better understanding of the neuronal encoding of distinct internal representations in identified brain circuits. In the meantime, the medaka data suggest that even a relatively simple trace is unstable for much longer than assumed so far, and further, that old fear memory might be immune to erasure.

Abbreviations

CR, conditioned response; CS, conditioned stimulus; ISI, interstimulus interval; ISNI, intersession interval; ITI, intertrial interval; US, unconditioned stimulus.

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