Temporal binding function of dorsal CA1 is critical for declarative memory formation

Azza Sellami[a,b,1], Alice Shaam Al Abed[a,b,1], Laurent Brayda-Bruno[a,b,1], Nicole Etchamendy[a,b,1], Stéphane Valério[a,b,1], Marie Oulé[a,b], Laura Pantaléon[a,b], Valérie Lamothe[a,b], Mylène Potier[a,b], Katy Bernard[c], Maritza Jabourian[c], Cyril Herry[a,b], Nicole Mons[b,d], Pier-Vincenzo Piazza[a,b], Howard Eichenbaum[e,2], and Aline Marighetto[a,b,3]

[a]Neurocentre Magendie, Physiopathologie de la Plasticité Neuronale, U1215, INSERM, F-33000 Bordeaux, France; [b]Université de Bordeaux, F-33000 Bordeaux, France; [c]Institut de Recherche Internationale Servier, 92150 Suresnes, France; [d]Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, UMR 5287, CNRS, F-33600 Pessac, France; and [e]Center for Memory and Brain, Boston University, Boston, MA 02215

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Temporal binding, the process that enables association between discontiguous stimuli in memory, and relational organization, a process that enables the flexibility of declarative memories, are both hippocampus-dependent and decline in aging. However, how these two processes are related in supporting declarative memory formation and how they are compromised in age-related memory loss remain hypothetical. We here identify a causal link between these two features of declarative memory: Temporal binding is a necessary condition for the relational organization of discontiguous events. We demonstrate that the formation of a relational memory is limited by the capability of temporal binding, which depends on dorsal (d)CA1 activity over time intervals and diminishes in aging. Conversely, relational representation is successful even in aged individuals when the demand on temporal binding is minimized, showing that relational/declarative memory per se is not impaired in aging. Thus, bridging temporal intervals by dCA1 activity is a critical foundation of relational representation, and a deterioration of this mechanism is responsible for the age-associated memory impairment.

aging | relational memory | optogenetics | trace conditioning | radial maze

Our ability to form declarative memories depends on the hippocampus, and this capacity degrades with age (1). To identify the critical determinants of this age-associated memory loss, we explored the relationships between two fundamental functions of the hippocampus known to be age-sensitive.

First, the hippocampus plays an essential role in forming a "relational organization" that links independently acquired memories via common elements and consequently supports cardinal flexibility of declarative memory, exemplified in the capability to make inferences from memory (2) or to compare separately acquired information to guide a choice decision in a novel situation (3, 4). This capacity is compromised in aging (5–8). Second, as suggested three decades ago (9), the hippocampus supports "temporal binding," by which discrete stimuli can be associated in memory despite their temporal separation. A critical role for the hippocampus in temporal binding was demonstrated in "trace" conditioning tasks, where a brief temporal gap separates the conditioned stimulus (CS) and unconditioned stimulus (US) presentations (10–13). This temporal binding function is disrupted in aging (14).Thus, relational organization and temporal binding are well-known functions of the hippocampus and are sensitive to aging, but potential links between these functions in declarative memory and its age-related decline remain hypothetical. Here, we test the hypothesis that the bridging of temporal gaps sustained by the hippocampus is a necessary condition for relational organization of memories (15, 16).

To unveil the link between hippocampal function in temporal binding and relational organization and their critical role in aging, we combined behavioral, cellular imaging, and spatially targeted interventional approaches following a two-step strategy. First, we used a trace conditioning procedure to identify the limit of temporal binding capability in young and old mice, and demonstrated with optogenetic tools that temporal binding relies on dorsal (d)CA1 activity over temporal gaps. Then, we demonstrated that dCA1-dependent temporal binding is necessary for the development of a relational organization of memories, and that loss of this activity plays a critical role in the aging-associated decline in relational memory. To examine the development of relational organization, we used a two-phase radial-maze task in mice and its virtual analog in humans. Cumulative evidence dissociates the performance when the hippocampus is compromised between the two phases of our task. Young mice with hippocampal lesions or inactivation (3, 4), like old mice (5–7), normally acquire reward associations of individual arms presented successively in the initial phase of our task but fail in choosing the rewarded arm when subsequently challenged to choose between a pair of the same arms in the test phase. Similarly, in a second version of our radial-maze task and its virtual analog for humans, aged mice (6), like aged humans (17), can learn individual pairs of arms (with one arm rewarded and one arm not rewarded in each pair) but fail in choosing the rewarded arm when presented within a recombined pair of the same arms in the test phase. To interpret the dissociation, we propose that a relational organization of associations among individual arm experiences made during the initial learning phase is needed for flexible memory expression as assessed in the test phase. In contrast, the learning of adaptive responses to individual arms or pairs would rely on simple stimulus–reward or stimulus–response associations acquired by repetition in the initial phase. By manipulating the temporal separation between individual arm experiences in the initial phase, we here confirmed our relational interpretation and found that the formation of a relational representation necessary for flexible memory expression is restricted by the limits of dCA1-dependent temporal binding.

Significance

Our ability to form declarative memories depends on the hippocampus, and this capacity degrades with age. To identify the critical determinants of this age-associated memory loss, we explored the relationships between two functions of the hippocampus known to be age-sensitive, temporal binding and relational organization. We found that (*i*) temporal binding relies on the activity of the dorsal (d)CA1 subfield across temporal gaps between events; and (*ii*) loss in this dCA1-dependent function, through ensuing disruption of relational organization, is the primary cause of declarative memory loss occurring in aging.

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[1]A.S., A.S.A., L.B.-B., N.E., and S.V. contributed equally to this work.

[2]Deceased July 21, 2017.

[3]To whom correspondence should be addressed. Email: aline.marighetto@inserm.fr.

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Results

Activity of dCA1 Cells Across Temporal Gaps: A Necessary Condition for Temporal Binding and a Sufficient Condition for Reversing the Aging-Related Loss of Temporal Binding Capacity. All experiments were conducted in accordance with European Directive 2010-63-EU and with approval from the Bordeaux University Animal Care and Use Committee CCEA50 (agreement number A33-063-098; authorization N°5012035A-N°1377). Young and old mice were trained in a trace fear conditioning procedure (Fig. 1*A* and *SI Methods*). In young mice, retention of the CS–US association is limited to less than 60-s trace intervals and requires activation of dCA1 neurons during acquisition (Fig. 1 *B* and *C*). Acquisition of tone conditioning was comparable among all trace conditions (as was the retention of context conditioning; Fig. S1*A*) but was retained only with trace intervals of less than 60 s. Thus, 24-h retention of the tone–shock association was similar in magnitude among groups trained with 0-, 5-, or 20-s trace intervals but was diminished in mice trained with a 40-s trace interval and null in mice trained with a 60-s trace (Fig. 1*B*, tone test). Correspondingly, conditioning with a trace interval of less than 60 s induced a specific activation of dCA1 neurons. In dCA1 (but not in other areas studied), postconditioning Fos level was higher in mice trained with a 20-s trace, compared with those trained with either a 0-s or a 60-s trace (Fig. 1*B*, Fos for dCA1 and Fig. S1*B*, other structures). Thus, dCA1 activation is associated with the combination of a demand for and success in temporal binding. Conversely, optogenetic inhibition of dCA1 neurons during the trace interval blocks otherwise successful retention of the CS–US association. In freely moving mice expressing the inhibitory ArchT in dCA1 cells and chronically implanted with optic fibers in this hippocampal subfield, transitory inhibition of dCA1 neuronal activity was performed during the conditioning using a 20-s trace interval. ArchT mice with light on in each trace interval that normally acquired the 20-s trace tone conditioning (Fig. S1*C*, *Left*) were significantly impaired in the 24-h retention test of the tone–shock association, compared with both GFP controls and ArchT mice that were submitted to conditioning with light on during equivalent periods outside the trace interval (Fig. 1*C*, tone test). In contrast, tone conditioning acquired under the 0-s trace condition was not affected by light on in the 20-s tone-CS period immediately preceding the shock (Fig. S1*C*). Also, retention of context conditioning was remarkably unaffected under all inactivation conditions (Fig. 1*C*, context test). Finally, additional experiments showed that inhibition of the dCA2/CA3 subfield failed to produce any impairment (Fig. S1*D*), hence confirming that dCA1 was the critical area involved in the impairment of temporal binding, even though marginal contribution of extra CA1 cells cannot be ruled out. Thus, dCA1 neuronal activity during the trace interval is a necessary condition for bridging the temporal gap and enabling CS and US events to be bound together in memory. In contrast, dCA1 neuronal activity does not seem to be critically needed for linking the environmental cues into a (relational) memory representation of the context. Since environmental cues are temporally contiguous, the present dissociation between trace and contextual memories indicates a selective role of dCA1 cells in temporal binding in memory.

Activation of dCA1 cells during the trace interval ameliorates the age-related impairment in the retention of the CS–US association (Fig. 1 *D* and *E*). Comparison of young and old normal mice trained with different trace intervals revealed an age-related reduction in temporal binding capacity. Both young and aged mice acquired the conditioning task successfully, and displayed significant retention of context conditioning (Fig. 1*D*, context test and Fig. S1*E*). By contrast (Fig. 1*D*, tone test), while the old mice trained with 0- or 5-s trace intervals exhibited significant 24-h retention of the tone–shock association, those trained with a 20-s trace interval exhibited no retention of the CS–US association. Thus, neither the ability to acquire an association across time nor the ability to form durable associative memories (like contextual memory) in general is altered in aged mice. Aging results in a selective deficit in long-term retention of an association between events separated by 20 s, corresponding to a reduction in the capacity for temporal binding compared with young mice. We next examined whether this selective impairment was due to a reduction in dCA1 neuronal activity bridging the trace interval. We thus tested whether optogenetic activation of dCA1 neurons during the trace interval could reverse the age-related impairment. In old mice expressing the activating channel rhodopsin 2 (ChR2) in dCA1 neurons, we compared the effects of activation "in trace" and "out of trace" and no activation of dCA1 (5 Hz, 40 s, three times) performed during conditioning using a 40-s trace, the longest trace interval associated with successful retention in young mice (Fig. 1*B*). The group trained with in-trace dCA1 activation only exhibited significant retention of the tone–shock association (Fig. 1*E*). Thus, maintaining dCA1 but not dCA2/CA3 (Fig. S1*F*) neuronal activity during the trace interval between the tone and the shock is sufficient to restore retention of the association between these events in old animals. Taken together, these findings reveal that dCA1 neuronal activity bridging the trace interval between the CS and the US is a necessary condition for subsequent storage of the CS–US association. In young mice, dCA1 activity bridges temporal gaps of up to 40 s, enabling the separate CS and US events to be bound in memory. This temporal binding capacity by dCA1 cells is compromised in aged mice, resulting in an inability to form associative memories of events separated by more than a few seconds.

Temporal Binding Supported by dCA1 Is Critical to the Development of a Relational Memory Organization, and Lack of Sustained dCA1 Activity During Periods of Temporal Binding Is the Cause of Age-Associated Loss of Relational Memory in Mice. To study the role of temporal binding in the formation of relational organization allowing flexible memory expression, we used a radial-maze task in which aged mice and mice with hippocampal damage or hypofunction succeed in learning the individual arms in the acquisition phase but fail on the subsequent flexibility test (4–7). This selective deficit in flexible memory expression is believed to come from an impairment of relational organization of the maze arms and reward associations in memory.

Temporal binding capacity is a limiting factor in the formation of relational/flexible memories. We here manipulated the temporal separation between the individual learning events during the acquisition phase by varying the intertrial interval (ITI) among different groups of young and old mice (Fig. 2*A*). Increasing the ITI did not interfere with initial learning, since all groups learned the individual arm–reward associations at similar rates (Fig. S2*A*), but performance in the flexibility test varied with ITI and age (Fig. 2*B*). Thus, young mice performed equally well in the flexibility probe when successive events had occurred up to 20 s apart during acquisition. With longer ITIs during learning, subsequent probe performance progressively declined and dropped to chance level when the ITI was 60 s. Aged mice performed well on the flexibility test when learning occurred with a 0- to 5-s ITI but performance dramatically dropped when learning occurred at longer ITIs (as early as 20 s). These findings show that separate arm experiences made during learning have to be related to one another to form a flexible memory, and such relational memory organization is limited by the temporal binding capability of each age. Thus, the findings demonstrate that (*i*) flexible memory expression assessed in our radial-maze task does rely on relational memory organization of individual arm experiences, just as we hypothesized, and (*ii*) temporal binding is a critical determinant of relational memory organization underlying flexible memory expression. Thus, flexibility per se is not impaired in aged mice, because test performance was normal when original learning occurred at the short ITI. This finding demonstrates that relational/declarative memory is normal in aging but that it is the reduction of temporal binding capability necessary for relating discontiguous events in memory which is responsible for the apparent degradation of relational/declarative memory occurring in aging.

Temporal binding relies on dCA1 activity during learning. Analyses of Fos activation induced by learning the individual arm associations

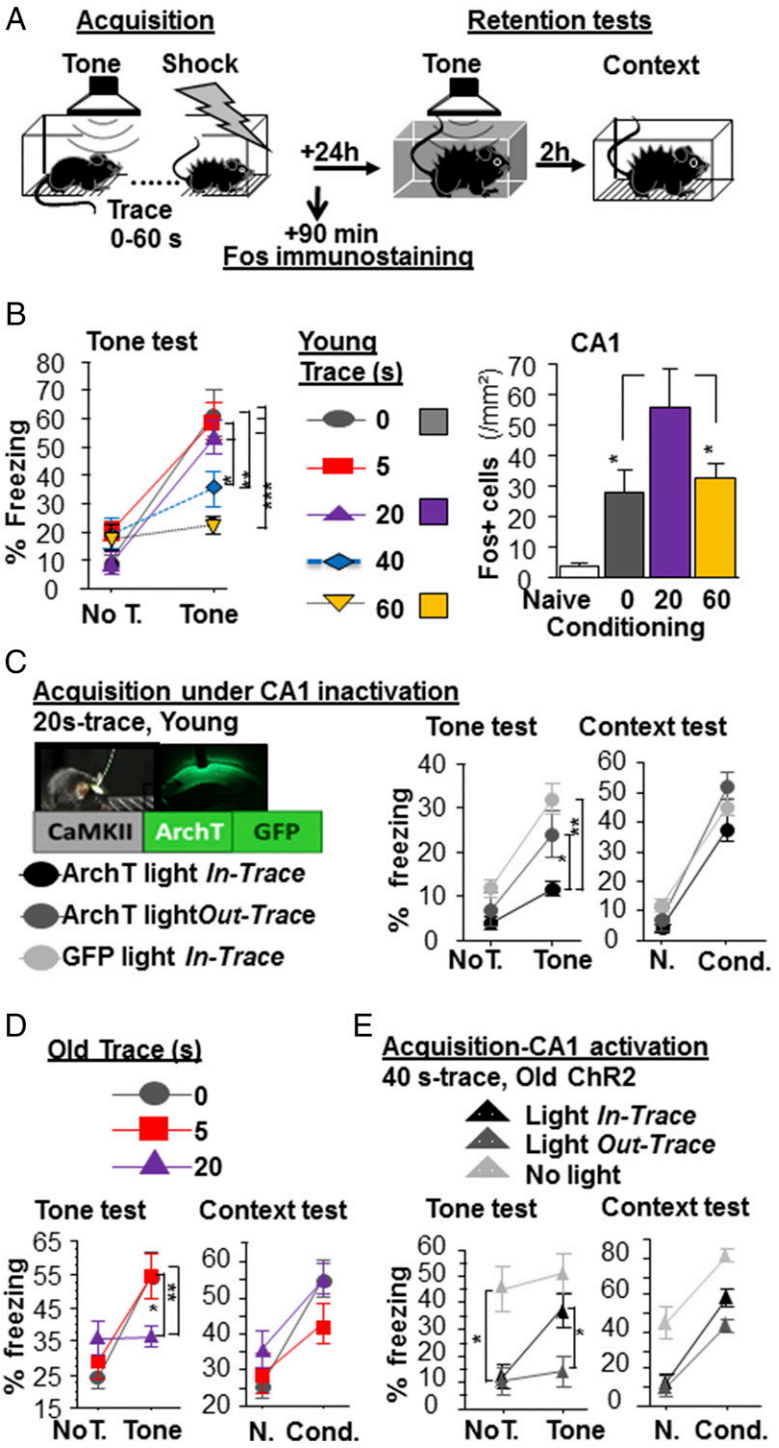

Fig. 1. In trace tone–fear conditioning, the formation of long-term memory of the trace CS–US association is limited to less than 60-s intervals between the CS and US, sustained by CA1 cell activity across the trace interval, and disrupted in aging. (*A*) Protocol: Young (3- to 4-mo-old) and aged (21- to 23-mo-old) mice were submitted to the acquisition of conditioning: three pairings of a tone (CS) and mild electric foot shock (US) with a time interval between the two (trace) of either 0, 5, 20, 40, or 60 s depending on the group. Young mice of the 0-, 20-, and 60-s groups were prepared for Fos immunostaining. The remaining mice were submitted to the "tone" and "context" retention tests the day after conditioning: % time spent freezing during 2 min of exposure to the tone (in a neutral context) and to the conditioning context, respectively, was compared with % freezing during 2 min before the tone in a neutral context (cf. *SI Methods*). (*B*) Behavior and Fos imaging in young mice. (*B*, *Left*) Tone test: The 24-h retention of the tone–shock association is dependent on the trace condition during conditioning {two-way ANOVA: significant *trace* x *tone* [repeated (rep.) measures: no tone vs. tone] interaction ($F_{4,38} = 15.457$; $P < 0.0001$); tone effect was significant for all trace conditions except for the 60-s trace [rep. measures: $P < 0.001$ for 0-, 5-, and 20-s trace, $P < 0.01$ for 40-s trace, and $P = 0.201$, not significant (ns) for 60-s trace]}, showing that successful temporal binding in long-term memory is limited to less than 60-s distant stimuli. In contrast, neither the acquisition of conditioning nor the retention of context conditioning was dependent on the trace condition (cf. Fig. S1*A*). $N = 8, 8, 8, 7$, and 12 for the 0-, 5-, 20-, 40-, and 60-s trace group, respectively. (*B*, *Right*) CA1 Fos+ cells measured after the conditioning phase are also dependent on the trace condition [one-way ANOVA: significant effect of *group* ($F_{3,46} = 10.113$; $P < 0.0001$); post hoc: $P = 0.0156$, $P < 0.0001$, and $P = 0.0031$, respectively, for 0-, 20-, and 60-s vs. naive; $P = 0.0102$ and $P = 0.0299$, respectively, for 0 and 60 s vs. 20 s; and $P = 0.6219$, ns for 0 vs. 60 s], showing that conditioning leading to maximal temporal binding (i.e., with a 20-s trace) is associated with a specific CA1 activation (cf. Fig. S1*B*). $N = 16, 11, 11$, and 12 for the naive, 0-, 20-, and 60-s trace group, respectively. (*C*) Retention effects of optogenetic inactivation of CA1 during the acquisition of 20-s trace conditioning in young mice. The 24-h retention of tone trace conditioning is altered by in-trace inactivation compared with both control conditions [significant *group* x *tone* interaction ($F_{2,52} = 6.812$; $P = 0.0024$); significant interaction for *in trace* vs. *out of trace* ($F_{1,29} = 9.622$; $P = 0.0043$) and vs. GFP ($F_{1,40} = 12.951$; $P = 0.0009$) but not for *out of trace* vs. GFP ($P = 0.4538$, ns)]. In contrast, the retention of context assessed by freezing difference between the neutral and conditioning context is similar among the groups [*group* x *context* (rep. measures: neutral vs. conditioning): $F_{2,52} = 2.278$; $P = 0.1126$, ns], and in-*trace* inhibition of CA2/CA3 instead of CA1 has no effect on tone retention (Figs. S1*D* and S4, histology). Thus, CA1 activity across the trace interval during conditioning is a necessary condition for successful temporal binding of the CS and US in memory. $n = 18$, $n = 13$, and $n = 24$ for in trace, out of trace, and GFP, respectively. (*D*) Retention of conditioning in old mice. The retention of tone conditioning is dependent on the trace, indicating that memory of the CS–US association is only retained when the temporal separation of the CS and US was less than 20 s [significant *trace* x *tone* interaction ($F_{2,31} = 5.341$; $P = 0.0102$); tone effect is significant for 0- and 5-s trace conditions (rep. measures: $P = 0.0034$ and $P = 0.0024$, respectively) but not for the 20-s trace ($P = 0.923$, ns)]. In contrast, the retention of context is largely similar among the groups (*trace* x *context* interaction: $F_{2,31} = 2.246$; $P = 0.1228$, ns), just as was the acquisition of conditioning (cf. Fig. S1*E*). Thus, temporal binding capability is limited to less than 20-s gaps in old mice, and diminished in comparison with young animals. $N = 12, 8$, and 14 for 0-, 5-, and 20-s trace group, respectively. (*E*) Retention effects of optogenetic activation of CA1 during acquisition of 40-s trace conditioning in old mice. Without affecting the retention of context conditioning, in-trace (but not out-of-trace) activation enables the retention of tone conditioning, which is normally not retained in old mice [significant *group* x *tone* interaction ($F_{2,15} = 5.17$; $P = 0.0196$); the tone effect is significant for the *in-trace* group ($P = 0.0002$) but not in the other two groups ($P = 0.114$, ns and $P = 0.335$, ns, respectively, for out of trace and no light)]. Thus, CA1 (but not CA2/CA3; cf. Fig. S1*F*) activity across temporal gaps is sufficient to restore the age-related defect of temporal binding in memory. Note that the age-related deficit is associated with overall increased levels of freezing, suggesting fear generalization that was normalized by both in-trace and out-of-trace activation. $n = 8, 5$, and 5 for the in-trace, out-of-trace, and no-light groups, respectively. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Data are presented as mean ± SEM.

revealed an ITI-dependent increase in dCA1 activation, similar to that observed associated with trace fear conditioning. Thus, learning-related CA1 activation was found to depend on a combination of a demand for and success in temporal binding (Fig. 2*C*). In young mice, strong demand for temporal binding when probe performance was successful (20-s ITI) resulted in

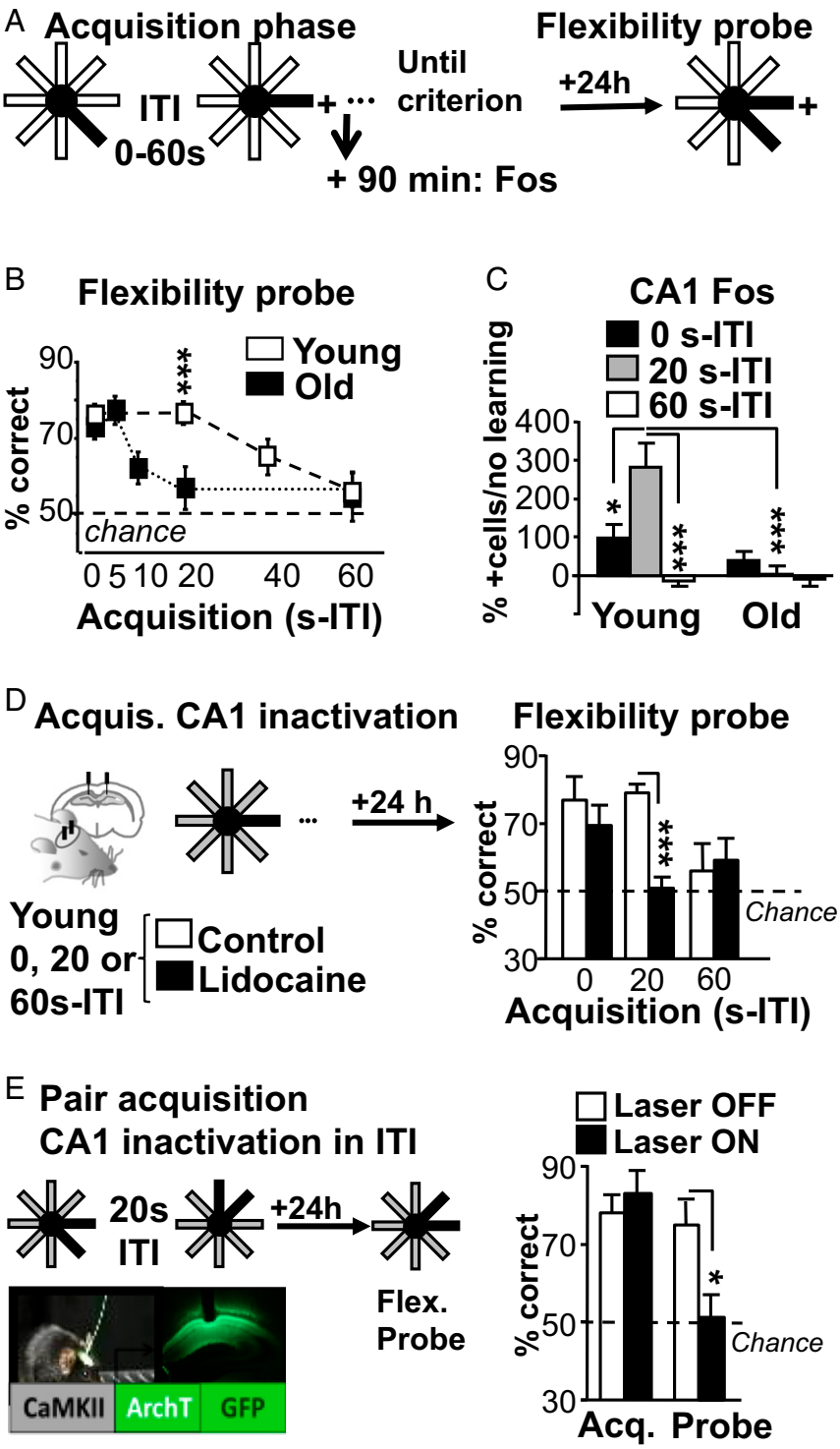

Fig. 2. Temporal binding sustained by CA1 is a critical determinant of declarative memory formation and its age-associated degradation: the radial-maze model in mice. (*A*) Protocol: In the acquisition phase, independent groups of young (3- to 4-mo-old) and aged (21- to 23-mo-old) mice learned the constant food (+)/no food (–) rewarding valence of each arm through daily sessions of 24 successive individual arm presentations, separated by an ITI of different duration among the groups. For behavioral analyses, each animal was trained until reaching the learning criterion and, 24 h after, was submitted to the flexibility probe. In this test, the reward contingencies among the arms remained unchanged but the arms were now presented by pairs to assess flexible memory expression as a model of declarative memory. For Fos analyses, groups of mice were prepared after the third training session of the acquisition phase (*SI Methods*). (*B*) Flexibility probe: Performance depends on the ITI condition under which memories were encoded, in an age-specific manner [*age* x *ITI* (0-, 20-, and 60-s ITI): $F_{2,46} = 4.975$; $P = 0.0111$; *age* effect: $P = 0.0001$, $P = 0.3967$, not significant (ns) and $P = 0.9032$, ns for 20-, 0-, and 60-s ITI, respectively]. Thus, flexible memory expression relies on the capability to relate individual arm visits across time intervals, capability limited to less than 60-s interevent separation in young mice [*ITI* (20-, 40-, and 60-s ITI) effect: $F_{2,17} = 5.045$; $P = 0.019$. Post hoc: 20 s vs. 40 and 60 s, $P < 0.05$] and to only 5-s intervals in aged mice [*ITI* (0, 5, 10, and 20 s) effect: $F_{3,26} = 5.011$; $P = 0.0071$. Post hoc: 0 s vs. 20 s, 5 s vs. 10 and 20 s: $P < 0.05$]. $n = 7$ to 10 per group. ***$P < 0.001$ vs. aged. (*C*) CA1 Fos+ cells in young and old mice: Training in the acquisition phase of the radial-maze task produces an ITI-dependent pattern of CA1 Fos activation, which resembles the one induced by trace fear conditioning in young mice, but this activation is not seen in aged mice [*age* x *ITI* (0-, 20-, and 60-s ITI): $F_{2,59} = 5.582$; $P = 0.006$; *age* effect: $P = 0.0002$, $P = 0.2381$, ns and $P = 0.885$, ns for 20-, 0-, and 60-s ITI, respectively]. $n = 7$ to 14 per group. (*D*) Transitory inactivation of CA1 through local lidocaine infusion during the acquisition phase spares the acquisition of individual arm valence, whichever the ITI condition (cf. Fig. S2*C*), but produces subsequent impairment of performance in the flexibility probe in the sole 20-s ITI condition under which temporal binding of successive learning events normally occurs [significant *lidocaine* x *ITI* interaction ($F_{2,36} = 4.36$; $P = 0.0201$); *lidocaine* effect: $P < 0.0001$, $P = 0.4546$, and $P = 0.6934$ in the 20-, 0-, and 60-s ITI condition, respectively], thus mimicking the aging effect. $n = 6$ to 8 per group. (*E*) Optogenetic inactivation of CA1 during the 20-s ITI between events in the acquisition phase also produces a subsequent impairment of performance in the flexibility probe. Here we used the second version of our radial-maze design (*SI Methods*), also used in humans (Fig. 3). The initial acquisition of separate pairs was spared by CA1 inactivation in 20-s ITI but performance was severely diminished in the "recombined" test of flexibility in the "laser on" group compared with controls [significant *group* x *acquisition probe* interaction ($F_{1,14} = 5.502$; $P = 0.034$)]. Thus, CA1 is needed to bridge a temporal gap, and this temporal binding function is crucial for relational organization sustaining the formation of flexible/declarative memory. $n = 7$ to 8 per group. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Data are presented as mean ± SEM.

high levels of Fos activation in dCA1. However, there was less or no Fos activation in dCA1 either when there was no demand for temporal binding and probe performance was normal (0-s ITI) or when the interval between trials exceeded temporal binding capacity and probe performance failed (60-s ITI). In contrast to young animals, dCA1 neuronal activity was not recruited across the 20-s ITI in aged mice, corresponding to their poor subsequent probe test performance following training at this ITI. Instead, Fos activation in the dorsomedial striatum was greater in older mice, independent of the length of the ITI duration (Fig. S2*B*). Furthermore, neuronal activity in dCA1 is essential for temporal binding that supports subsequent flexible memory expression. First, local infusions of the anesthetic lidocaine were performed in young mice before each daily session of the initial phase but not before the flexibility-test session. We found that inactivation of dCA1 (see reduced Fos levels; Fig. S2*C*) during the entire acquisition phase

impaired flexible memory expression when animals were trained at 20-s but not 0- or 60-s ITI (Fig. 2*D*), resulting in a decrement in temporal binding capacity similar to that observed in aged mice. Second, optogenetic inhibition of CA1 during the critical 20-s ITI in the acquisition phase also resulted in a subsequent impairment in the flexibility probe (Fig. 2*E*). This result demonstrates a dependence on dCA1 information processing to bridge a temporal gap. Finally, a procholinergic drug was found to rescue probe test performance in aged mice at the dose that concomitantly recovered activation in dCA1 at the critical 20-s ITI (Fig. S2*D*).

Altogether, the present findings show that a critical engagement of dCA1 is a necessary condition for bridging a temporal gap to form a relational memory organization, and indicate that the aging-related decline in declarative memory results from a compromised dCA1 function in temporal binding.

The Age-Associated Decline in Declarative Memory Depends on Temporal Binding in Humans as Well. To test the validity of the above conclusions on the role of temporal binding in flexible/declarative memory formation for human senescence, young and aged participants selected as "cognitively normal" for their age (*SI Methods*) were submitted to the virtual analog of the radial-maze task previously used in mice (Fig. 3*A*). Experiments were approved by the following ethics committees: the CPP Aquitaine (Comité de Protection des Personnes), the CCTIRS (Comité Consultatif sur le Traitement de l'Information en Matière de Recherche dans le Domaine de la Santé), and the CNIL (Commission Nationale de l'Informatique et des Libertés). Written informed consent was obtained from all participants before any study-related procedure. Participants were divided into three intertrial interval conditions (0-, 20-, or 40-s ITI, matched for age and performance in other cognitive tests; Fig. S3*A* and Table S1). While all aged groups normally learned the initial pairs (Fig. 3*B*, *Left*), only those trained with a short ITI (0 or 20 s) could perform the flexibility test performance as well as younger adults (Fig. 3*B*, *Right*; replication Fig. S3*B* and Table S2). Thus, like in aged mice, a reduction in temporal binding causes the age-associated impairment in relational organization allowing flexible memory expression.

Discussion

The present findings in mice and humans establish a causal connection between two well-known and age-sensitive functions of the hippocampus: temporal binding and relational organization of memories. We show that (*i*) temporal binding is a necessary condition for linking experiences separated by brief time intervals into an organized representation that supports flexible memory expression, characteristic of declarative memory, and (*ii*) temporal binding critical to performance relies on dCA1 activity across temporal gaps between experiences. Our study thus validates important hypotheses established through a history of research on hippocampal function in associations across time, memory formation, and cognitive aging (9, 12, 15, 18–23).

At the psychological level, our parallel approaches in trace conditioning and radial-maze learning demonstrate that the capacity of temporal binding is a limiting factor in the formation of a relational organization associated with declarative memory. In young and aged mice, the ability to relate discontiguous experiences of individual arms into a relational memory was limited to linking experiences across the same intervals as that supporting a CS–US association in trace conditioning. Thus, in the radial-maze task, memories for individual experiences were formed through repeated exposures to each individual arm, but a relational organization was formed only when the temporal separation allowed temporal binding, a capacity measured independently in our tests on trace conditioning.

The findings identify the age-associated reduction of temporal binding capacity as the primary cause for the memory impairment. Furthermore, these results support the conclusion that the ability to form a relational organization per se is not impaired in normal aging but rather is impaired only when temporal binding between experiences is compromised. Relational organization

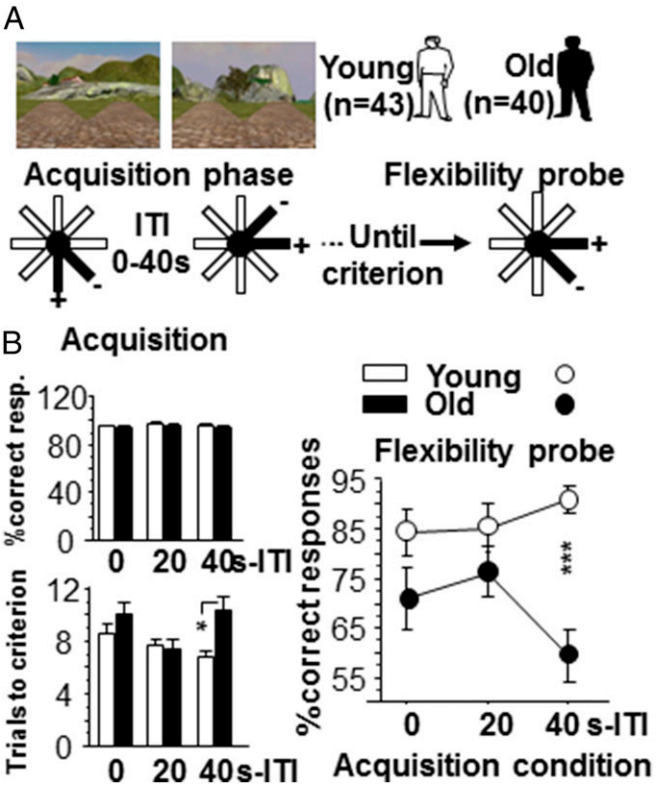

Fig. 3. Age-associated decline in flexible/declarative memory depends on temporal binding in humans: the virtual radial-maze task. (*A*) Protocol: In the acquisition phase, young (18- to 25-y-old; $n = 43$) and aged (59- to 75-y-old; $n = 40$) participants learned the constant rewarding (+, virtual coin)/no rewarding (–) valence of each arm through successive presentations of invariant pairs (1 arm+, 1 arm–), separated by an ITI of 0, 20, or 40 s, depending on the group, until reaching the learning criterion. In the flexibility probe, the reward contingencies among the arms remained unchanged, but the arms were rearranged into novel pairings to assess flexible memory expression. Participants were matched among the ITI conditions according to their age and performance in other cognitive tests (*SI Methods*, Fig. S3*A*, and Table S1). (*B*) Results: In acquisition, aged participants required more training to reach the learning criterion but eventually learned the task as well as the young participants, whichever the ITI condition (*Left Top*: *final performance age*, *ITI* and *age* x *ITI*: all $P > 0.14$, not significant (ns); *Left Bottom*: *trials to criterion age* effect: $F_{1,77} = 6.02$, $P = 0.0164$; *ITI* and *age* x *ITI*: $P = 0.0676$, ns and $P = 0.0729$, ns). In contrast, in the flexibility probe, there was an age-related impairment dependent on the ITI condition under which the task was acquired [*Right*: *flexibility age* x *ITI* ($F_{2,77} = 3.473$, $P = 0.036$); significant *ITI* effect in aged groups ($F_{2,37} = 3.828$, $P = 0.0308$; post hoc 40 s vs. 0 and 20 s, $P < 0.05$) but not in young ($P = 0.74$, ns); significant *age* effect for 40- ($P < 0.0001$) but not 0- or 20-s ITI (all $P > 0.057$, ns)]. Thus, the age-related loss of flexibility is due to a reduction of temporal binding capability. $n = 10$ to 18 per group. ***$P < 0.001$. Data are presented as mean ± SEM.

for spatial memory was intact in aged mice and humans as long as the demand on temporal binding was minimized by the temporal proximity of learning experiences. These findings challenge the commonly held view that aging produces impairments in declarative (1, 24) and spatial learning and memory (25, 26), and suggest the possibility that these aging-sensitive declarative tasks include a demand for binding memories across time.

The present findings not only show that temporal binding is a critical determinant of declarative memory formation and its age-related decline but also provide a potential basis for reconciling two conflicting theories of hippocampal function, spatial mapping and declarative memory. Commonly, these two functions have been respectively studied in animals and in humans, making their comparison difficult. However, a common feature of spatial mapping and declarative memory is that they both allow flexible expression of memories in modified testing situations. Hence, the present

observations suggest that temporal binding plays a critical role in both spatial mapping and declarative memory by supporting the ability to form relational memory organizations.

What might be the underlying mechanisms of temporal binding in dCA1? The peculiarity of temporally distant stimuli is that they cannot be associated in memory through Hebbian mechanisms of synaptic plasticity, which are known to sustain the formation of long-lasting associations among co-occurring stimuli (27). Hebbian plasticity is triggered by the coactivation of neuronal assemblies encoding each stimulus during learning. To overcome the limitation of Hebbian plasticity for linking events separated in time, the presence of time cells in the CA1 subfield (20) provides a potential mechanism by which distinct events separated in time may be bound together in memory (20, 22, 23, 28). Namely, time cells were found to fire at successive moments during an empty temporal interval between key events, so that collectively the population of cells filled in the temporal gap and bridged the time intervals between the events. Such time-cell sequences in CA1 during temporal intervals would induce Hebbian plasticity among successively activated cells, and ultimately could thereby sustain the formation of an association between temporally separated events. This hypothesis implies that CA1 cell activity across time intervals between events must be a necessary condition for encoding a long-lasting associative memory of these events, just as shown by the present studies using optogenetic manipulations. Thus, our findings are compatible with the time-cell hypothesis even though they do not directly identify "time cells" as the underlying mechanism. Indeed, it is surprising that maintaining a randomly selected subset of cells by artificial activation was sufficient to restore the age-related memory deficit. However, it is possible that artificial stimulation of a few cells in dCA1 might lead to concomitant activation of large assemblies through recurrent collateral activation, and thereby induce Hebbian plasticity in synaptic contacts within the assemblies and with the cells activated by the event occurring just before and after the interval. Thereby, optogenetic stimulation of a few dCA1 cells could enable an association to be made across the interval in aged mice lacking (spontaneous) activation of time cells that would naturally enable the temporal binding process in young adult animals. Whatever the case might be, our findings are consistent with observations on the age-related loss in the excitability of CA1 cells (18, 29, 30) as a potential source of the reduction in temporal binding capacity.

In conclusion, our study identifies the bridging of temporal intervals by dCA1 activity as a critical determinant of relational organization that sustains characteristic flexibility of declarative memory expression, and shows that a deterioration of the temporal binding mechanism is the primary cause for age-associated declarative memory impairment.

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