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Retrograde Enhancement of Episodic Learning by a Post Learning Stimulus

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Evidence suggests encoding of recent episodic experiences may be enhanced by a subsequent salient event. We tested this hypothesis by giving rats a 3-min unsupervised experience with four odors and measured retention after different delays. Animals recognized that a novel element had been introduced to the odor set at 24, but not 48 hours. However, when odor sampling was followed within 5-min by salient light flashes or bedding odor, the memory lasted a full 2 days. These results describe a retroactive influence of salience to promote storage of episodic information and introduce a unique model for studying underlying plasticity mechanisms.

Episodic memory involves encoding of diverse forms of data -- including the identity and location of serial cues and the order in which they were encountered ('what', 'where', and 'when') --- into narratives of different lengths (Tulving 1984). It also incorporates data about the context in which event sequences were constructed and actions taken during them (the actor is part of the memory). Acquisition occurs routinely as part of daily life without practice or explicit rewards, features that distinguish episodic memory from trial and error learning (Tulving 1972). Given their ubiquity, it is not surprising that only a fraction of daily episodic experiences is transferred into long-term memory. Experimental studies have identified elements that are likely to be remembered (McGaugh 2000; Kentros et al. 2004; Sarter et al. 2009) but factors determining the likelihood of an entire sequence being retained are poorly understood. There are however suggestions that very salient stimuli markedly strengthen the encoding of immediately preceding episodic experience. For example, people commonly report highly detailed descriptions of what they were doing in the minutes prior to first learning of a traumatic event (e.g., otherwise unremarkable events occurring when they learned of the 9/11 terrorist attacks or the Kennedy assassination). Laboratory tests have questioned the accuracy of such 'flashbulb memory' (Bohannon 1988; Schmolck et al. 2000; Talarico and Rubin 2003), but the general idea accords with a large literature indicating that stressful events can retroactively enhance the strength of earlier encoding (Cahill et al. 2003; Beckner et al. 2006; Diamond et al. 2007; Preuss and Wolf 2009; Dunsmoor et al. 2015). There is also evidence that novelty or a second learning experience can similarly act back through time to enable storage of material that otherwise would

have been forgotten (Li et al. 2003; Moncada and Viola 2007; Wang et al. 2010; Takeuchi et al. 2016; van Dongen et al. 2016).

The discovery that induction of robust LTP in one set of contacts strengthens weak LTP in another set provided a plausible neurobiological substrate for the observed crosstalk between learning sessions. The effect, which is operative over extended delays between the two LTP events, is thought to reflect the production of plasticity-related proteins by the strong LTP and their use by the weak case synapses ('synaptic tagging and capture') (Frey and Morris 1997; Redondo and Morris 2011).

The present studies tested if salient cues produce retrograde enhancement of encoding in a rodent version of episodic memory. Recent work showed that mice and rats learn identities, locations, and temporal order ('what', 'where', and 'when') during a first-time encounter with a collection of intrinsically interesting cues (Cox et al. 2019). We used a protocol of this type to ask if an unrelated but prominent signal occurring after the brief sampling session prolongs memory for items encountered during the session. Rats were given a single exposure to four novel odors without prior training or explicit rewards. These conditions were intended to mimic the routine unsupervised sampling of multiple commonplace cues that characterizes episodic learning in humans. A retention trial, occurring one or two days after the initial sampling period, assessed the time the rodents sampled a novel, replacement odor relative to three previously encountered stimuli (**Fig 1A**). Given that rats have a strong disposition towards novelty, this test provided an estimate of how well they remembered the unchanged odors. After either a short or longer delay following initial odor sampling, the rats were exposed to one of two very different salient experiences. We thereby tested our predictions that i) a brief, salient environmental event will prolong the retention of an episodic memory if delivered shortly after initial learning, but not after a several minute delay, and ii) this retrograde enhancement effect would not be dependent on the specific qualities of the salient event.

Well-handled, adult male Long-Evans rats were habituated for 5 days to an open field arena

(32.71cm x 85cm with height of 38.1cm) with two 10-minute sessions. They were then returned to the arena

on the following day, and given 3 minutes to investigate four glass cups (6.5cm diameter x 6cm height, four small holes in the lid) each containing 100µl of an odorant (odors A,B,C,D; dissolved in mineral oil; Supplementary Table 1) pipetted onto filter paper just prior to placement within the chamber. The locations of the odors were counterbalanced across rats. Animals were placed in their home cage immediately after the initial exposure session and returned to the arena 24 or 48 hours later for a 5-minute retention test, in which odor D was replaced with novel odor E (**Fig 1A**). The times spent investigating the four odors in the first (exposure) and second (test) sessions were measured by blinded offline video analysis.

The animals spent approximately equal time investigating each cue during the 3-min exposure period: they did not typically move from one odor to the next in a rapid sequence, and cue sampling was generally greatest in the first minute (**Fig 1B**). This pattern of results suggests that the rats treated the odors as a significant but not dominant component of a familiar testing environment.

Memory retention scores were calculated as the percent time exploring the new odor relative to the mean of percent sampling times for the three previously sampled cues. Rats tested 24 hours after initial exposure had a clear and statistically significant preference for the novel odor, spending 31.6 ± 2.4% of their total odor sampling time with the replacement (n=12, t_{11}=2.76, *p=0.019) (**Fig 1C**). In contrast, rats tested 48 hours after initial exposure exhibited no preference for the novel cue (n=10, t_9=1.05, p=0.323); the percent time sampling the novel cue was 21.6 ± 3.3% (**Fig 1D**), a value that was significantly less than that for the 24 hour group (n=12,10, t_{20}=2.53, *p=0.020, unpaired t-test, between-group comparison). These results indicate that the rats learned a set of odors, evident by preference for a novel cue, and the resultant memory decayed between one and two days.

The above results accord with the assumption that an episodic experience does not typically produce lasting memory traces. We next tested if the addition of a salient cue after the experience promotes stable encoding. A strobe light, positioned directly above the testing field, was activated at 15 Hz for 15 seconds immediately (within 5 seconds) following the odor exposure session, and retention was

tested 48 hours later. The intensity, duration, and distance of the strobe were the same as in a prior study (Cox et al. 2017) in which rats avoided the strobe, but nonetheless repeatedly crossed a boundary to trigger it. This

indicates that the signal may be somewhat aversive, but its novel or salient aspects can support operant behavior. Rats exposed to the strobe immediately after initial learning showed a strong preference for the novel odor 48 hours later (36.2 ± 4.1% of total sampling time; n=15, t_{14}=2.67, *p=0.018; paired t-tests for time with novel vs. familiar). Strobe-exposed rats also spent a greater portion of their sampling time with the novel odor than did no-strobe controls after 48 hours (t_{23}=2.57, *p=0.017; unpaired t-test) (**Fig 1D**).

Observations from videotapes indicated that the strobe flashes produced a strong orienting response but did not cause freezing (**Fig 2A**). We further tested for anxiety using a conventional elevated plus maze assay (Handley and Mithani 1984; Pellow et al. 1985; Dawson and Tricklebank 1995). Naïve rats were exposed to the 15 second strobe light within their home cage and immediately afterwards were placed at the center of a T-maze, 60 cm off the ground, with two open and two closed arms (arms, 50cm long and 10cm wide; 54cm high walls). During 5 minutes in the maze, both control and strobe-exposed animals spent about 75% of their time in the closed arms (Control n=6, *p=0.021; Strobe n=7, **p=0.008), in accord with prior studies (Lister 1987; Rodgers and Dalvi 1997; Horii et al. 2018) (**Fig 2B**). We conclude from these observations that positive effects of the strobe on memory were not due to induction of a fearful state, and thus were likely due simply to a strong arousal response to a salient and unexpected signal.

Next, we tested if temporal contiguity is required for the light flashes to retroactively enhance encoding. At the conclusion of the 3-minute exposure session, the odors were removed from the testing chamber and the fifteen-second light flashes were delivered after a delay of either 1-3 (**Fig 3A**) or 5-10 (**Fig 3B**) minutes. Retention was tested 48 hours later. The shorter (1-3 min) delay group again had a clear preference for the new odor (n=14, 32.5 ± 2.7% of total sampling time; t_{13}=2.77, *p=0.016, paired t-test). The effect of the strobe was less evident with the longer, 5-10 min delay: rats spent 28.7 ± 2.2% of their sampling time with the novel cue, a value that was not statistically different from time with familiar cues (n=13, t_{12}=1.72, p=0.112, paired t-test) (**Fig 3B**). In all, the positive effect of the strobe is reproducible but tends to weaken when the delay between it and cue sampling increases.

Finally, we tested if a more naturalistic, emotionally arousing cue reproduces the results obtained with the strobe light. Inclusion of emotional content is one factor that appears to strengthen encoding of

episodic memory in humans (Cahill and McGaugh 1995; Tang et al. 2016). Accordingly, an odor cup containing standard bedding and nesting material from a female rat cage, after 5 days of use, was placed in the testing arena immediately after the male rats sampled the four initial odors (A-D) for 3 minutes. A control group was exposed to a cup containing clean, unused cage bedding by the same schedule. In both cases, the initial four odors were removed prior to the introduction of the bedding cup. Rats were allowed to actively sample the bedding odors for 15 seconds with a maximum of 45 seconds in the chamber. Then, 48 hours later the animals were tested for retention of initial odor cues as above.

Rats that were exposed to the salient female bedding retained the odor memory 48 hours later, spending 34.1 ± 4.0% of their sampling time investigating the novel odor (n=14, t_{13}=3.62, **p=0.003, paired t-test, time with novel vs. familiar cues). This was not the case for rats presented with clean bedding after initial odor exposure (26.2 ± 1.4% time with novel odor; n=20, t_{19}=0.85, p=0.407). The time sampling the novel odor in the 48-hour retention trial was also significantly greater for the female bedding group than the clean bedding group (t_{32}=2.98, **p=0.006, unpaired t-test) (**Fig 3C**).

The above results constitute evidence that prominent cues occurring after an episodic learning event can prolong the memory of that event in rodents. They thus provide experimental support for popular accounts of otherwise mundane experiences seemingly burned into stable memory when followed by dramatic, unexpected information (Brown and Kulik 1977; Conway et al. 1994; McGaugh 2013). Our data also suggest that the retroactive influence operates over a brief time period, information that could be useful for pinpointing the underlying plasticity mechanisms.

Post-trial facilitation of operant learning via a diverse array of pharmacological treatments and brain manipulations is a well-established phenomenon (Cahill and McGaugh 1996; McGaugh et al. 1996; Okuda et al. 2004) that in some cases reportedly involves epinephrine acting on the amygdala (LaLumiere et al. 2003). But the type of memory studied in the earlier experiments is quite different than the encoding of multiple cues during a brief period of unsupervised sampling. Moreover, available evidence suggests that in the operant situations the post-training manipulations are effective over a longer time frame than

that described here for the strengthening of episodic information. Relatedly, a sizable body of work points to

stress as a potent modulator of learning and memory, whether it occurs before or after training (Diamond et al. 1996; Diamond et al. 1999; Cahill et al. 2003; Woodson et al. 2003; Beckner et al. 2006; Diamond et al. 2007; Preuss and Wolf 2009; Cadle and Zoladz 2015; Dunsmoor et al. 2015). While stress effects are generally reported to be negative, there are clear examples of memory facilitation and theoretical studies suggest underlying variables that are potentially relevant to episodic paradigms. However, it should be noted that the secondary stimuli used in our experiments did not generate signs of fear or anxiety as might be expected if they were stressful. It will in any case be of interest to test if manipulations found to effect retrograde enhancement in earlier work, have positive effects in the paradigms used in the present studies.

Work on the substrates for memory-related LTP also suggests potential mechanisms for retroactive enhancement of memory for items in an episode. Recently induced potentiation gradually stabilizes over several minutes with much of this consolidation occurring in less than 5 minutes (Arai et al. 1990; Lynch et al. 2007; Babayan et al. 2012). The pertinent molecular mechanisms involve a complex set of signaling cascades that reorganize the subsynaptic actin cytoskeleton and then anchor the network in its new configuration (Kramar et al. 2006; Rex et al. 2009; Rex et al. 2010; Lynch et al. 2013). Experimental results suggest that odor learning induces synapse specific LTP (Roman et al. 1987; Wang et al. 2018). Possibly, then, LTP–like changes produced by odor sampling can be enhanced by the later arrival of salient environmental cues. A report suggesting that novelty enhances subthreshold LTP but only if delivered within 5 min of induction is of interest in this regard (Li et al. 2003). This argument can be made more specific by linking it to the synaptic tagging hypothesis mentioned earlier. The secondary stimuli in this case would mobilize memory-related proteins that find their way to synapses activated by the odors and thereby promote consolidation of what would otherwise be rapidly decaying LTP.

An alternative explanation involves the possibility that memories are held in a buffer system prior to transfer into long-term storage (Brady et al. 2016). One version of this argument posits that reverberating networks underlie the transient encoding, as in postulate 2 of Hebb's influential theory of

memory (Hebb 1949). Recent studies found that field CA3 of hippocampus, via its singular associational system of feedback connections, maintains self-sustained activity for minutes after a brief input, and that blocking

this effect disrupts encoding of temporal order (Cox et al. 2019). Possibly, then, a salient event arriving after an episodic experience interacts with a still active trace left by the experience and thereby facilitates the transition to stable memory. The effective period for retroactive enhancement would then correspond to the time over which cue-induced reverberating brain activity is maintained.

Finally, we note that the present description of a novel retroactive faciliatory mechanism is potentially related to a long standing and poorly understood issue in episodic memory research. Such encoding occurs continuously as part of everyday life, typically without advance knowledge as to what in the vast amount of material being surveyed might be significant on a later occasion. The issue is what should be retained or lost? Or as William James famously put it: 'The stream of thought flows on; but most of its segments fall into the bottomless abyss of oblivion' (James 1890). James goes on to ask if we can explain why some memories barely survive the instant of their passage while others are preserved for remarkable periods. Part of the answer may be that the insertion of a notable event into the stream of experience serves to shift preceding material into long term memory.

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Figure 1. A novel environmental event retroactively enhances unsupervised encoding of olfactory cues. A. Rats were exposed (session 1) to 4 odors (A-D) for 3 min and tested (session 2) 24 or 48 hours later with one of the cues replaced with a novel odor (E, orange). A 15 second strobe light was flashed on the arena immediately after session 1 in one group. **B.** (Left) Rats did not have preferences for the different odors used in the initial exposure period. (Right) On average, ~50% of the time spent investigating the cues occurred in the first minute of session 1. **C.** During the 24 hr retention test, rats preferably sampled novel odor 'E' (n=12, *p=0.019, within group comparison for seconds sampling E vs. mean time sampling cues A-C). **D**. When tested 48 hr after session 1, control (no strobe) rats did not distinguish between novel and familiar odors (n=10, $p > 0.05$) whereas rats given the strobe flashes did preferentially explore novel odor E (n=15, *p=0.018, within group comparison for sampling times; *p = 0.017, between group comparison for % time sampling the novel cue). The sampling time for cue E recorded for the 24 hr control (no strobe) group was statistically greater than for the 48 hr control group (*p=0.020, not shown in figure).

Figure 2. The strobe light did not disrupt exploration or produce anxiogenic effects. A. Rats maintained a consistent level of exploration while sampling odors during session 1: There was no evidence for freezing during the strobe flashes or during the following 15 seconds (No strobe N=7, Strobe=7; $p >0.05$ at 15 sec strobe mark). **B.** Rats performed equivalently on an elevated plus maze whether they received strobe light or not. Both groups remained within the closed arm for the majority of the trial and for nearly identical amounts of time (control/no strobe=6, *p=0.021; strobe n=7, **p=0.008).

Figure 3. Retroactive enhancement is delay dependent and not specific to visual cues. **A**. Rats that were exposed to a strobe light 1-3 min after the initial 3-minute session with four odors spent a greater percent time (seconds) sampling novel odor E than the mean for the three previously sampled cues (A-C) during a delayed (48 hrs.) retention trial (n=14, *p=0.016, paired t-test for time with novel vs. familiar cues).
B. Preference for novel odor E was not significant when the delay between the initial odor exposure session and the strobe was increased to 5-10 min (n=13, p=0.112). However, when comparing the 1-3 min and 5- 10 min groups, the amount of time spent with novel odor E was not significantly different (n=14,13; t_{24}=1.078; p=0.291). **C.** Rats exposed to female rat bedding (instead of a strobe light) after odor cue sampling in session 1, had excellent retention scores 48 hours later (time/seconds investigating the novel vs. the previously sampled odors: n=14, **p=0.003, within group t-test). Rats exposed to control bedding after session one did not prefer the novel cue E in the delayed retention trial (n=20, p=0.407, paired t-test for sampling times). The percent time spent with novel odor E was greater for the female bedding group than the control bedding group (**p=0.006, unpaired test).

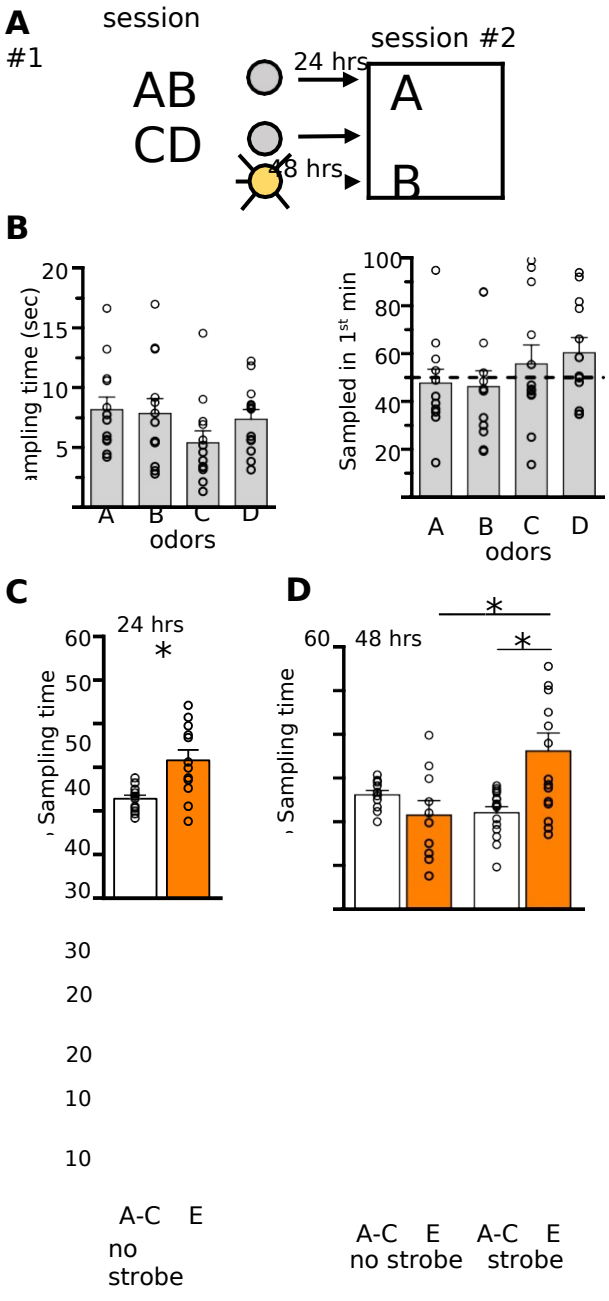

Figure 1. A novel environmental event retroactively enhances unsupervised encoding of olfactory cues

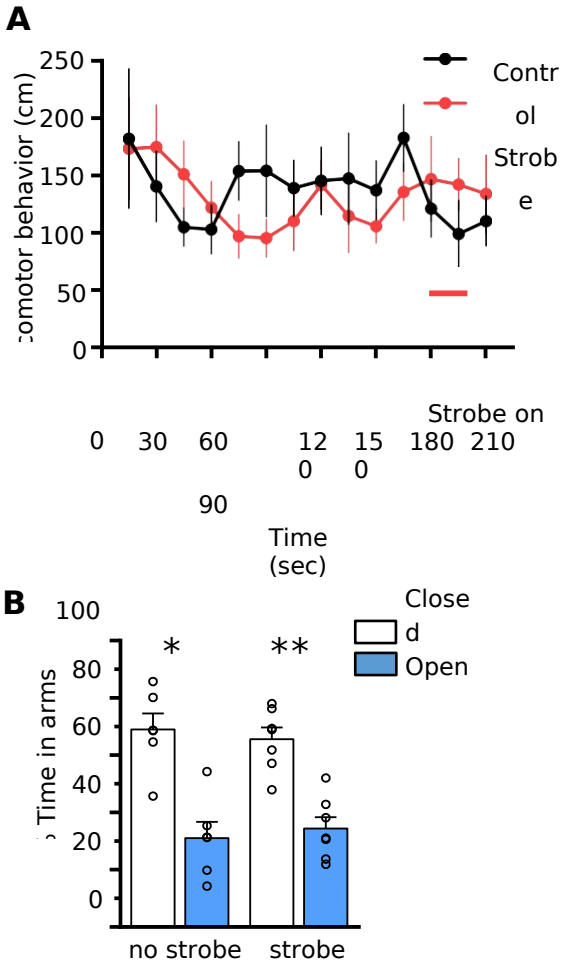

Figure 2. The strobe light did not disrupt exploration or produce anxiogenic effects

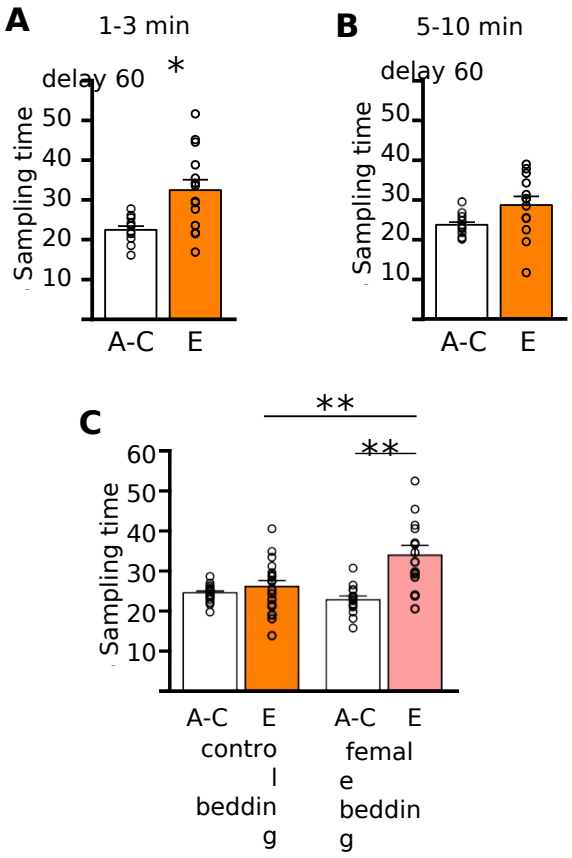

Figure 3. Retroactive enhancement is delay dependent and not specific to visual cues