Sleep aromatherapy curbs conditioned fear

John T Wixted

We know that humans are capable of learning during sleep. Research now shows that they are also capable of unlearning during sleep, and in a way that alters the neural representation of a feared stimulus: re-exposure to an odor during slow-wave sleep promotes extinction of an aversive visual association learned in that odor context.

Over the past 20 years, two intriguing lines of research on the consolidation of memory have developed more or less independently of each other. One line of research has studied the role of sleep in the consolidation of emotionally neutral memories[1,2] and the other has studied the role of consolidation in extinguishing conditioned fear[3,4] (emotionally arousing memories). In this issue, Hauner *et al.*[5] bring these two lines of research together by showing that, in humans, a conditioned fear response to a stimulus that was previously associated with mild electric shock can be extinguished during slow-wave sleep without ever having to present the feared stimulus itself. Moreover, this effect is accompanied by changes in the neural representation of the conditioned stimulus in the hippocampus and the amygdala.

A recent study using classical conditioning revealed that humans can acquire new learning during sleep[6]. Hauner *et al.*[5] took this further by investigating the 'un-conditioning' of a learned response during sleep (**Fig. 1**). While awake and in a scanner, human subjects underwent contextual fear conditioning in which two faces were paired with mild electric shock (CS+ stimuli) and two other faces were not paired with shock (CS– stimuli). Two of the faces (one CS+ and one CS–) always appeared in the presence of one odor, whereas the other two faces always appeared in the presence of a different odor. The odors were conceptualized as context stimuli. As expected, after having been paired with shock, both of the CS+ faces elicited a significantly elevated fear response (indicated by increased skin conductance) in comparison with the CS– faces. During a later nap, one of the two odors was repeatedly presented to subjects as soon as they entered slow-wave sleep. The question was whether the CS+ previously paired with shock in the presence of the re-exposed odor (termed the target CS+) would lose any of its ability to elicit a fear response and, if so, what changes in target CS+ brain activity (before versus after sleep) would be observed.

Previous work with animals[7,8] found that, after pairing a tone CS+ with shock, unreinforced presentations of the fear-conditioning context (that is, exposure to the context by itself) have the surprising effect of extinguishing the fear response previously elicited by the CS+ (as if CS+ extinction trials had also been presented). Hauner *et al.*[5] observed the same effect in humans when context re-exposure was presented during slow-wave sleep.

When the participants were later tested in the scanner while awake, the target CS+ elicited a reduced fear response, but no such effect was observed for the non-target CS+ (that is, for the CS+ that was paired with shock in the presence of the non-re-exposed odor). Hauner *et al.*[5] also found that hippocampal activity associated with the target CS+ following sleep was reduced in comparison with pre-sleep levels, whereas no changes were observed for the non-target CS+. In addition, post-sleep entorhinal activity associated with the target CS+ was negatively correlated with the duration of odorant re-exposure during slow-wave sleep (which varied across subjects) and, finally, the pattern of activity in the amygdala (assessed by multivariate pattern analysis) was selectively altered for the target CS+.

The authors interpreted these findings to mean that re-exposure to the odorant context during slow-wave sleep cued retrieval of the target CS+, perhaps accelerating the consolidation of memory for a new ('safe') version of that face in the amygdala. Theoretically, this accelerated consolidation either updated the previous memory representation of the CS+ being associated with shock (thereby recoding the target CS+ as a safe stimulus) or instead created additional safe memories of the target CS+ face—memories that would inhibit the accessibility of older (but still available) memories of the CS+ paired with shock. Either way, the accelerated consolidation of safe memories of the target CS+ theoretically accounts for the reduced hippocampal and entorhinal activity associated with post-exposure CS+ presentations, and the newly encoded version of the safe CS+ accounts for the altered pattern of activity in the amygdala.

The idea that the presentation of the conditioning context prompts the retrieval of the previously conditioned CS+ (thereby explaining why the CS+ itself becomes extinguished despite not having been re-exposed) is consistent with explanations of the context re-exposure effect dating back to when the phenomenon was first observed in rats[7]. However, a somewhat puzzling result reported by Hauner *et al.*[5] is that context re-exposure had the effect of extinguishing the previously conditioned CS+ only when the odorant context was re-exposed during sleep. When the same context re-exposure procedure was used with an awake control group, the target CS+ showed no evidence of extinction (and, not surprisingly, no pre- versus post-sleep changes in brain activity). Why would an effect that is consistently observed in awake animals only be observed in sleeping humans? The answer is not known, but one possibility may be that the awake state is not as homogenous as is sometimes thought.

An important variable determining the effectiveness of context re-exposure in creating a safe memory of the CS+ may be the rate at which other memories are being formed at the same time that the odorant context is

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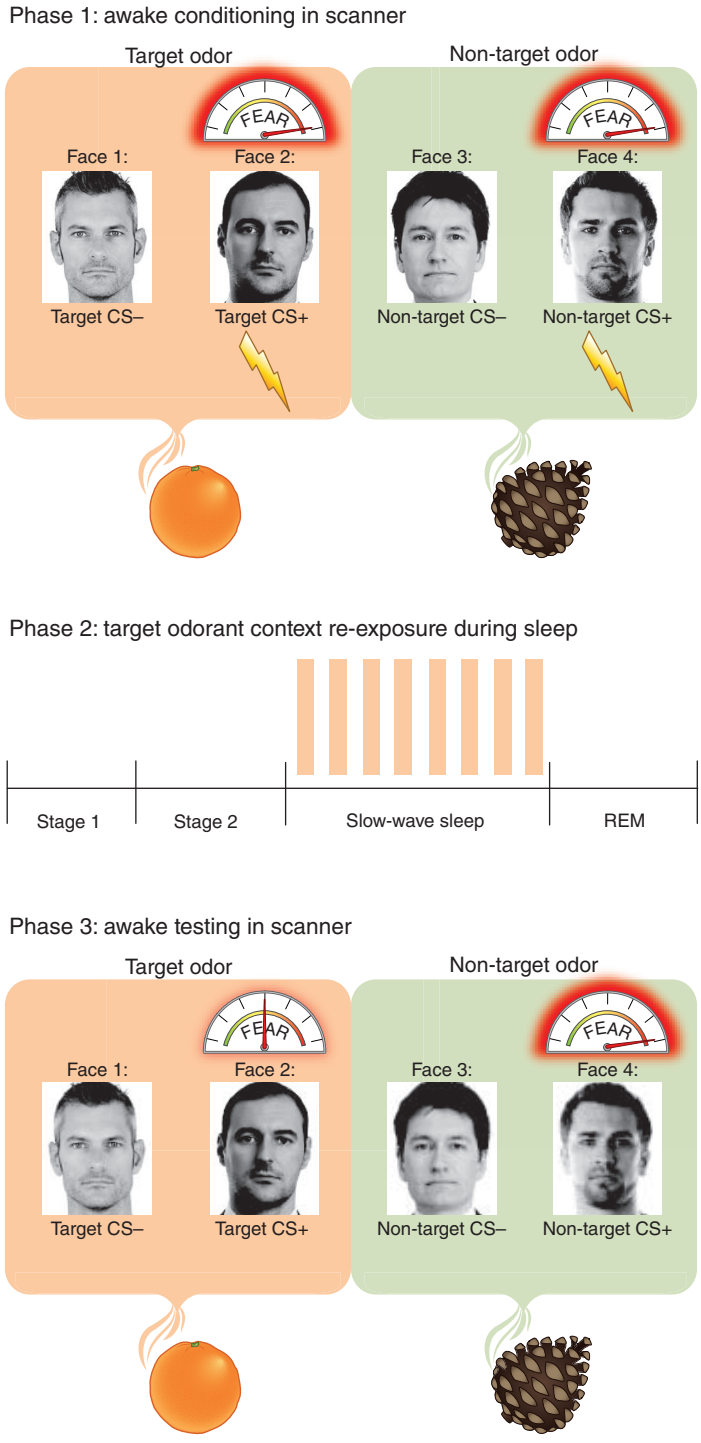

Marina Corral Spence

Figure 1 Simplified illustration of the experimental protocol. In phase 1, two faces (face 1 and face 3) were presented without being paired with shock and two other faces (face 2 and face 4) were paired with shock. Face 1 (the target CS–) and face 2 (the target CS+) were presented in the presence of one odor (the target odor, here oranges), and face 3 (the non-target CS–) and face 4 (the non-target CS+) were presented in the presence of a different odor (the non-target odor, here pine). After having been paired with shock, both face 2 and face 4 elicited fear (indicated by an elevated skin-conductance response). In phase 2, the subject napped and the target odor was re-exposed during slow-wave sleep in 30-s on-off cycles. Finally, in phase 3, the faces were presented again. Face 2 (the target CS+) then elicited less fear than face 4 (the non-target CS+). REM, rapid eye motion sleep.

re-exposed. Presumably, no competing memories were formed when context re-exposure occurred during slow-wave sleep (and probably not when animals were re-exposed to a familiar context in earlier studies either). In contrast, awake subjects in Hauner *et al.*[5] viewed a nature-themed documentary film during odorant context re-exposure. Memory for the documentary film was not subsequently tested, but it seems likely that, had it been tested, the subjects would have remembered much of what they had seen in the film. The encoding of these film-related memories during odorant context re-exposure undoubtedly activated hippocampal encoding and consolidation processes that could interfere with other memories formed around the same time[9]. Thus, for the awake controls, film-related memories may have competed with the consolidation of any CS+ memories that were concomitantly cued by the presentation of the odorant context (perhaps preventing consolidation of memory for a safe version of that stimulus).

In studies using rats, a distinction is often drawn between active wake versus quiet wake. In Buzsáki's two-stage model of encoding and consolidation[10], for example, active wake (such as exploration of a novel environment) is associated with encoding activity, whereas quiet wake is associated with the consolidation of recently formed memories (perhaps via neural replay, which occurs not only during slow-wave sleep, but also during quiet wake[11]). Presumably, humans watching a nature-themed documentary film are not in a state of quiet wake, but are instead in a state that is more akin to rats exploring a novel environment (that is, in a state that is more akin to active wake). If so, the encoding of extraneous memories that will inevitably occur during active wake may serve to interfere with recently formed (and, therefore, still labile) memories of the safe CS+ cued by context re-exposure—memories that might otherwise have been consolidated if a state of quiet wake had been in effect instead[12,13]. Indeed, in other kinds of learning tasks, post-learning wakeful resting has yielded effects on the consolidation of memory not unlike the effects associated with slow-wave sleep[14,15].

The potential therapeutic benefit of extinguishing a feared CS without having to present the CS itself is considerable and would be considerably greater if it were possible to achieve in the awake state. Context stimuli often consist of visual cues, such as when a PTSD patient acquires an exaggerated fear of driving in close proximity to a truck following a serious motor vehicle accident. Obviously, visual context cues cannot be conveniently presented during sleep, but the results reported by Hauner *et al.*[5] raise the possibility that context re-exposure during quiet wake may

reduce fear-related responses to a conditioned stimulus that does not actually have to be presented to the patient. Testing the Hauner *et al.*[5] context re-exposure procedure in humans who are in a state of quiet wake would seem to be a logical next step to further evaluate the potential clinical relevance of this procedure. These results may show that the extinction-related effects of procedures used during slow-wave sleep are also true of procedures used during quiet wake. Such a finding would have both clinical and theoretical relevance. Which consolidation-related effects are specific to sleep and which are instead specific to conditions in which the formation of extraneous memories is reduced to a minimum (whether the subject is awake or asleep)? The answers to theoretically intriguing questions such as these are not yet known. Whether or not such effects can be achieved during the awake state, the study by Hauner *et al.*[5] adds a new dimension to our understanding of the learning—or, to be more precise, unlearning—that can be achieved by the sleeping brain.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Rasch, B., Büchel, C., Gais, S. & Born, J. *Science* **315**, 1426–1429 (2007).
2. Diekelmann, S. & Born, J. *Nat. Rev. Neurosci.* **11**, 114–126 (2010).
3. Maren, S. *Neuron* **70**, 830–845 (2011).
4. Lattal, K.M. & Wood, M.A. *Nat. Neurosci.* **16**, 124–129 (2013).
5. Hauner, K.K., Howard, J.D., Zelano, C. & Gottfried, J.A. *Nat. Neurosci.* **16**, 1553–1555 (2013).
6. Arzi, A. *et al. Nat. Neurosci.* **15**, 1460–1465 (2012).
7. Marlin, N.A. *Learn. Motiv.* **13**, 526–541 (1982).
8. Stout, S.C. & Miller, R. *Behav. Processes* **66**, 7–16 (2004).
9. Ben-Yakov, A., Eshel, N. & Dudai, Y. *J. Exp. Psychol. Gen.* published online, doi:10.1037/a0033558 (1 July 2013).
10. Buzsáki, G. *Neurosci* **31**, 551–570 (1989).
11. Karlsson, M.P. & Frank, L.M. *Nat. Neurosci.* **12**, 913–918 (2009).
12. Wixted, J.T. *Annu. Rev. Psychol.* **55**, 235–269 (2004).
13. Mednick, S.C., Cai, D.J., Shuman, T., Anagnostaras, S. & Wixted, J.T. *Trends Neurosci.* **34**, 504–514 (2011).
14. Dewar, M., Alber, J., Butler, C., Cowan, N. & Della Sala, S. *Psychol. Sci.* **23**, 955–960 (2012).
15. Tambini, A., Ketz, N. & Davachi, L. *Neuron* **65**, 280–290 (2010).

Best-laid schemes for interneuron origin of mice and men

Zoltán Molnár & Simon J B Butt

Two studies emphasize similarities in the developmental origin of cortical interneurons across mammals. They suggest that most interneurons in humans and macaques have a subcortical origin.

The complex function of the mammalian neocortical circuitry depends on the balance between diverse subpopulations of excitatory pyramidal projection neurons and inhibitory GABAergic interneurons. Understanding how these cell types sculpt behavior has become increasingly reliant on genetic technologies to a priori identify cell types with distinct morphological and electrophysiological properties—aspects that in murine models can be largely predicted by embryonic origin. In primates, including humans, evidence has suggested there is a substantial difference from rodents in the location from which cortical interneurons originate. In this issue of *Nature Neuroscience*, Hansen *et al.*[1] and Ma *et al.*[2] extensively characterize developmental gene expression patterns in the ganglionic eminences. These patterns indicate that the vast majority of interneurons in human and macaque monkey have a subcortical origin.

It is generally accepted that most GABAergic interneurons in the mouse are generated in ventral embryonic forebrain and reach the cortex—the site of pyramidal projection neuron neurogenesis—through tangential migration. Furthermore it would appear that interneuron precursors do not proliferate further after reaching the cortical plate. Estimates are that, in the dorsal telencephalon of rodents, less than 5% of GABAergic interneurons are generated locally. It has become evident that there are several distinct sources in the ventral telencephalon for interneurons. Initial *in vitro* and *in vivo* cell tracing and cell transplantation experiments in rodents have suggested that the medial ganglionic eminence (MGE) is the primary source of cortical interneurons. However, it was clear from these studies that other sources provide cells, and subsequent genetic fate mapping experiments identified the caudal ganglionic eminence (CGE) as a major contributor to interneuron diversity.

How such complex processes could be scaled up in the developing brain of primates has sparked debate, not least because of evidence that some, if not the majority, of cortical interneurons may originate from the dorsal, pallial ventricular zone (VZ). Hansen *et al.*[1] and Ma *et al.*[2] revisited this question and analyzed the organization and expression pattern of the human and monkey fetal ganglionic eminences and their contributions to the origin of cortical interneurons. By doing so, they draw parallels between the anatomical and genetic organization of the ganglionic eminences in the mouse and the human. These studies provide evidence for the production of neuronal precursors from the medial, lateral and caudal ganglionic eminences with genetic profiles similar to those that we would expect in the mouse.

Although the basic organization of cortical microcircuits is conserved across mammals, cell numbers and gene expression patterns in adult and developing human and monkey cortex are very different from those in the mouse. Since the identification of distinct morphological subtypes of interneuron by Ramón y Cajal, it has often been argued that the variety and diversity of these cells increases in higher mammals[3]. These differences can also be extended to the embryonic origin of interneurons, with monkey and human telencephalic germinal zones possessing more elaborate cytoarchitectonic distinctions and some striking differences in embryonic and adult gene expression patterns[4,5].

The first indication that the dorsal VZ or subventricular zone (SVZ) might produce interneuron subtypes came from a combination of immunohistochemistry and retroviral labeling of human fetal organotypic slice preparations[6]. As many as 65% of GABAergic neurons in some human neocortical areas expressed the DLX1, DLX2 and MASH1 (also known as ASCL1) transcription factors (associated with interneuron development in rodents) and originated from MASH1-expressing progenitors of the VZ-SVZ of the dorsal forebrain. This view

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