ing on work from isolated transfected cells is just not good enough if one wishes to really understand disease processes and their implications and treatment possibilities. Focusing more specifically on migraine aura, the new work provides a very satisfactory way to understand why a patient with such a mutation is more susceptible to triggering their aura. At once the work provides reinforcement of the importance of the mutations and a plausible way to think about how this change renders the patient susceptible to aura, as well as a target mechanism for the development of anti-aura strategies. The importance of the latter should not be underestimated. A patient with familial hemiplegic migraine has untreatable, unpredictable attacks of weakness that can last for days; the effects can be devastating and very difficult to manage. With recent data suggesting that patients with aura are at risk for some degree of brain change on MRI (Kruit et al., 2004), the new information here acts as a beacon for the development of new therapeutic approaches at a time when the consequences of migraine aura are beginning to be questioned. From the patients' and clinicians' viewpoint, this is very timely basic biology.

Migraine is not, however, solved. The work in hand (van den Maagdenberg et al., 2004) is extremely important in terms of aura, but much is needed to understand how the mutations described thus far in familial hemiplegic migraine may translate into migraine without aura. A particular issue in the literature at the moment is the extent to which understanding aura can inform the description of the pain mechanisms or indeed how aura, or mechanisms of cortical spreading depression, relate to the initiation of the attack (Goadsby, 2001). The clinical evidence is that an interesting set of symptoms, known as premonitory symptoms, precede migraine in many patients (Giffin et al., 2003). There is no evidence that they involve aura mechanisms as we understand them from brain imaging. Moreover, it has been postulated that the pain of migraine is in some way related to aura (Moskowitz et al., 2004). This seems problematic in the face of the facts that most patients do not experience aura, there are patients with aura and no pain, and some patients have aura well into the attack or at the end. It seems more likely, as has been considered for many years, that aura is a part, albeit an important part, of a more complex brain disorder (Goadsby, 2003), the genesis of which is likely to be in subcortical structures (Matharu et al., 2004; Weiller et al., 1995). Whatever the ultimate nature of the underlying problem, integrating molecular genetics with in vivo physiology and pharmacology must be the way forward to understand many of the disorders our research efforts target. This new work (van den Maagdenberg et al., 2004) is a very good step in that direction.

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Are Fear Memories Made and Maintained by the Same NMDA Receptor-Dependent Mechanisms?

A recent finding indicates that inducible knockout of the NR1 NMDA receptor subunit promotes the loss of fear memories formed months earlier. One view is that posttraining NMDA receptor activation protects modified synapses from "synaptic drift." An alternative view is that NMDA receptors help maintain appropriate connectivity in memory-encoding networks.

That nothing in the brain lasts as long as a memory poses a fundamental problem in memory research. How do seemingly stable memories persist in an ever-changing brain? A second question of fundamental importance concerns learning. Specifically, what are the initial triggers of learning-related brain change? Over the last several decades, significant advances have been made on both fronts, and both are relevant to the target article by Cui et al. (2004) in this issue of *Neuron*.

What, then, are the neural triggers for learning? Undoubtedly there are many, but for fear conditioning, NMDA receptors play a critical role. NMDA receptor antagonists disrupt fear learning when given prior to training but do not consistently disrupt performance when given prior to testing (e.g., Kim et al., 1991; Miserendino et al., 1990). These dissociations suggest that effects on learning are not attributable to impaired con-

ditioned stimulus (CS) processing or to general anxiolytic effects because these effects would also disrupt performance. For reasons discussed elsewhere (e.g., Gewirtz and Davis, 1997; Kim et al., 1991), it has also been difficult to attribute effects on learning to impaired sensory processing of the unconditioned stimulus (US; e.g., footshock). Instead, many believe that NMDA receptor activation during CS-US pairings directly triggers the neural changes upon which fear memories depend. Although many issues remain, the critical contribution of NMDA receptors to fear learning is generally recognized.

An answer to the question "How do memories persist in an ever-changing brain?" has been somewhat more elusive. Many learning-related cellular changes have been identified, and many if not most of these may contribute to short-term retention. Covalent modifications of synaptic proteins, for example, might mediate the short-term increases or decreases of synaptic efficacy that presumably mediate short-term retention. It is not clear, however, how these changes would also mediate long-term retention. Kinases, for example, might phosphorylate synaptic proteins, but how would these phosphorylated proteins be protected from routine or stimulated phosphatase activity or, for that matter, from protein turnover? Various creative schemes have been proposed (cf. Lisman et al., 2002) but remain largely conjectural.

Early findings that protein synthesis inhibitors disrupted long- but not short-term retention, coupled with other observations of experience-dependent structural changes, suggested another possibility—namely, that long-term retention might be mediated by more stable changes of neuronal architecture. It has become increasingly clear, however, that synapses and other structural elements are anything but stable. On a time scale measured in hours and even minutes, synaptic populations fluctuate dramatically in both number and morphology in response to neural activity, circulating hormones, and presumably many other factors (e.g., Gould et al., 1990; Kirov and Harris, 1999). Moreover, the life span of the protein building blocks of these structural elements is almost certainly less than the life span of the memories that they are purported to maintain. Again, the questions arises: "How do long-lasting memories persist?"

Cui et al. (2004) consider the possibility that the same processes that trigger neural modifications during training also participate actively in memory maintenance and do so for as long as the memory persists. They evaluate this possibility using transgenic mice in which expression of the NR1 subunit—a necessary component of NMDA receptors—can be turned off by doxycycline, fed to the animals either in their drinking water or food supply. Five days of doxycycline treatment completely eliminates NR1 protein in the cortex, hippocampus, and striatum, but within 2 months of doxycycline withdrawal, NR1 levels return to normal.

Having established the regional and temporal specificity of the knockout, the authors use these mice to examine the contribution of NMDA receptors to fear memory maintenance. For these experiments, mice received three presentations of tone and shock and, 10 months later, were tested for tone- and for context-elicited fear using conditioned freezing as a behavioral measure. In control and inducible knockout mice, doxycycline was administered for either 7 or 30 days, beginning 7 months after training. Thirty but not seven days of doxycycline treatment severely disrupted retention.

Even though NR1 protein was given ample time (i.e., 2 months) to recover, lingering effects secondary to NR1 depletion might have influenced performance. However, when animals were trained and tested in a different context, several days after the initial test, control and NR1 knockout mice performed comparably. Not surprisingly, given the difference in train-test intervals, freezing scores of control animals on this short-term retention test were considerably greater than freezing scores from the long-term retention test in which doxycycline treatment did disrupt retention. Thus, it is possible that the conditions of the control experiment were not optimal for detecting performance effects of prior NR1 depletion. However, performance on several other behavioral tasks (i.e., novel object recognition, rotorod, and open-field) was also comparable in the two groups.

How, then, should the results be interpreted? An obvious possibility is that the same processes that are required for memory formation are also required for memory retention. Covert learning, perhaps during offline states such as sleep, may reinforce and maintain memories and protect them from what Cui et al. (2004) refer to as "synaptic drift." As the authors point out, these covert learning episodes need not involve reactivation of the entire neuronal pattern corresponding to a given memory. Instead, it may be sufficient to reactivate, at different times, subsets of the original pattern, strengthening in piecemeal fashion various elements of the mnemonic whole. The authors point to the cortex as the likely site for long-term storage and suggest, for reasons discussed below, that cortical NR1 depletion is responsible for the observed deficits.

In previous studies, hippocampal manipulations have been shown to disrupt retention when made soon after training but not when made many weeks after training (e.g., Kim and Fanselow, 1992). These results have suggested that the hippocampus plays a time-limited role in memory consolidation. Cui et al. (2004) suggest, therefore, that the effect on retention of doxycycline treatment begun 7 months after fear conditioning cannot be attributed to hippocampal NR1 depletion because fear memories would have become independent of the hippocampus long before. However, behavioral tests after delayed lesions have typically been conducted within several days of hippocampal ablation. Because the authors find, with a long training-knockout interval, that 30 but not 7 days of doxycycline administration does disrupt retention, one wonders if negative effects of hippocampal lesions in previous studies might also have been a function of treatment duration. In other words, if lesion-to-test intervals in previous studies had been extended to 30 days or more, perhaps retention deficits would have been apparent even when hippocampal lesions had been made many weeks or even months after training.

The authors also point out that hippocampal lesions typically disrupt context- but not tone-elicited fear (e.g., Kim and Fanselow, 1992). In the present study, doxycycline disrupted both, again suggesting that hippocampal NR1 depletion could not alone account for the behavioral findings. However, the authors here used a short-

duration tone CS during training and a long-duration CS during testing. With this procedure, deficits to a tone CS have been observed following lesions restricted to the hippocampus (cf., Sanders et al., 2003).

Interestingly, Villarreal et al. (2002) have reported that daily administration of the NMDA receptor antagonist CPP prevents the normal decay of hippocampal long-term potentiation and that CPP administration on each of 5 days beginning 1 day after training prevents forgetting in a spatial learning task. Thus, the effect of post-training NMDA receptor blockade in that study was opposite in direction to that reported here. There are many differences between the two studies (e.g., the behavioral task and species used, treatment onset and duration, and the temporal dynamics of NMDA receptor disruption following repeated antagonist injections versus NR1 knockout). It will be important in future studies to determine which variables influence whether posttraining NMDA receptor manipulations enhance or impair retention.

The finding that sustained NR1 knockout long after training disrupts memory maintenance is exciting and potentially groundbreaking. However, it may be premature to conclude that the effect is specifically attributable to a failure to protect modified synapses from the synaptic drift that would otherwise undo learning-related neural change. Perhaps instead, sustained NR1 depletion leads to a more general instability of the neural network within which these changes occur. In many systems, NMDA receptors play a necessary role in the appropriate ordering and connectivity of synaptic elements—particularly, but not exclusively, during development. If NMDA receptors contribute to network maintenance in memory-encoding brain areas, then information encoded within this network might be lost following sustained NR1 depletion, not because learning-related changes in synaptic connectivity, in particular, are lost, but simply because the entire neural network within which these changes occur has become disordered. This possibility seems to have been appreciated in a previous study by Riedel et al. (1999). In that study, the AMPA/kainate receptor antagonist LY293558 was chronically infused into the hippocampus through osmotic minipumps either 1–7 or 5–12 days after water maze training (rats were tested 15 days after training). With both intervals, retention was severely impaired. The authors concluded that "without a temporal gradient, we are obliged to recognize the possibility that LY infusion, rather than affecting stabilization/consolidation, may disrupt the integrity of storage sites in the hippocampus."

The concept of "consolidation" has, in recent years, undergone considerable revision. Renewed interest in reconsolidation following reactivation (cf., Nader, 2003) has forced a reappraisal of the permanency of consolidated memories, and evidence presented by Cui et al. (2004) suggests that even consolidated memories are not static entities that persist indefinitely but are instead actively maintained by processes similar to if not qualitatively indistinguishable from those which mediate their initial acquisition. As with most groundbreaking studies, the report by Cui et al. (2004) is exciting not only for the questions it answers but also for those it raises.

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