United states of amnesia: rescuing memory loss from diverse conditions
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ABSTRACT
Amnesia – the loss of memory function – is often the earliest and most persistent symptom of dementia. It occurs as a consequence of a variety of diseases and injuries. These include neurodegenerative, neurological or immune disorders, drug abuse, stroke or head injuries. It has both troubled and fascinated humanity. Philosophers, scientists, physicians and anatomists have all pursued an understanding of how we learn and memorise, and why we forget. In the last few years, the development of memory engram labelling technology has greatly impacted how we can experimentally study memory and its disorders in animals. Here, we present a concise discussion of what we have learned about amnesia through the manipulation of engrams, and how we may use this knowledge to inform novel treatments of amnesia.

Introduction
Amnesia refers to a deficit of memory due to a specific cause. It is a disorder that arises as a consequence of more than 15 different types of diseases and injuries that affect the brain, such as neurodegenerative and neurological diseases, vascular disorders and traumatic lesions (Markowitsch and Staniloiu, 2012). It is often the earliest and most persistent symptom of dementia (Wells, 1979). Amnesia has a huge clinical significance – its effects on the daily life of patients who suffer from it can be enormous. As a result, there are many efforts towards developing successful treatments. Currently, therapeutic interventions are limited by the lack of understanding of how memory functions in both health and disease.

Memory is the ability to store information of past experiences in the brain. Knowledge learnt by animals alters their brain and modulates how the brain then regulates future behaviour. Understanding memory, and its mechanisms, is a central goal of modern neuroscience. In 1904, Richard Semon postulated that experiences provoke long-lasting changes in specific neurons that result in an enduring memory trace – an engram of the acquired information. Reactivation of these engram cells will result in the recall of that particular memory (Semon, 1904). To understand the mechanisms of engram formation, we have primarily relied on indirect methodological approaches, for example, by studying amnesia. The general approach is to interfere with a brain region, physiological process or gene that we hypothesize is important for memory, and then look for experimental amnesia in a given behavioural paradigm (McGaugh, 2000). Recently, we have begun to make progress in our understanding of both memory and amnesia through the development of memory engram technology.

Memory engram technology is based on the combination of transgenic, optogenetic, behavioural and electrophysiological approaches. Developed originally by Tonegawa and colleagues, the technology integrates optogenetics and immediate early gene (IEG) labelling to drive the expression of a transgene in cells that specifically respond to an experience (Boyden et al., 2005; Reijmers et al., 2007; Tonegawa et al., 2015a,b). In its first demonstration, a promoter of the IEG c-fos was used to drive the expression of channelrhodopsin (ChR2), a light-responsive ion channel, in hippocampal dentate gyrus neurons that were activated by a target contextual experience (Fig. 1). Temporal control is allowed by the tetracycline-controlled transactivator (tTA)-tetracycline response element (TRE) system, inducible by the removal of the antibiotic doxycycline so that it only labels the neurons that are responding to the controlled contextual experience. This approach demonstrated that direct activation of engram neurons for contextual memories associated with fear/threat is sufficient (Liu et al., 2012; Ramirez et al., 2013), as well as necessary (Denny et al., 2014; Tanaka et al., 2014; Trouche et al., 2016), to recall this specific episodic memory.

Amnesia
From the clinical point of view, amnesia is described as a multifaceted disorder with a frequently poor prognosis (Markowitsch and Staniloiu, 2012). Anterograde amnesia refers to the inability to acquire and retain new information, whereas retrograde amnesia affects the recall of past or recently learned memories. Recent memories are more vulnerable to amnesia than older ones, and this is known as Ribot’s law of regression (Ribot, 1881). Amnesia appears as a consequence of diverse clinical disorders, such as Alzheimer’s and Parkinson’s disease (AD and PD, respectively), depression, and head trauma, among many others. Therefore, animal models for those disorders frequently develop memory deficits (Table 1).

Can the combination of memory engram labelling technology and disease models help us to understand the neuropathology of amnesia? In a model of drug-induced amnesia, mice administered with a protein synthesis inhibitor after a fear-inducing training session develop retrograde amnesia for that fear memory (McGaugh, 2000). Surprisingly, using the engram labelling technology, optogenetic activation of neurons in these mice elicited a context-specific fear response, indicating that the memory was still there (Fig. 1) (Ryan et al., 2015). This approach opens the possibility that the information is not completely lost in retrograde amnesia, but it is just inaccessible.

The same methodological approach was subsequently applied to models of early AD – the major neurodegenerative disease that affects memory storage (Roy et al., 2016; Perusini et al., 2017). AD is associated with the deposition of amyloid-β peptide in...
extracellular plaques and with the aggregation of the microtubule-associated protein tau in neurofibrillary tangles inside neurons (Braak and Braak, 1991). As a consequence of these aggregates, synapses are compromised, and there is selective neuronal death and a decrease in specific neurotransmitters (reviewed in Masters et al., 2015). The APP/PS1 mouse model recapitulates many of the hallmarks of human AD, including deficits in spatial, social and cognitive memory (Gong et al., 2004; Lalonde et al., 2005), but some strategies have successfully improved cognition in these models. Environmental enrichment was shown to be beneficial by stimulation of synaptic activity (Jankowsky et al., 2005; Lazarov et al., 2005; Fischer et al., 2007). Photonic stimulation of the visual cortex by chronic application of light in frequencies of 40 Hz improved contextual and fear memory, and significantly reduced amyloid-β plaque deposition (Iaccarino et al., 2016). Although these studies show that certain activities and interventions can ameliorate the deterioration of memory, they do not show whether the engram itself survives amnesia. In the APP/PS1 mouse model, short-term memories (minutes to hours) are intact, whereas long-term memories (a day or more) are compromised, indicating a consolidation deficit as a cause of the amnesia (Kilgore et al., 2010; Ryan et al., 2015). The fact that this kind of amnesia is retrograde (because the initial short-term memory is observed) indicates that the engram might still be present in the brain. Using the engram tagging approach in this model, animals with amnesia due to early-stage AD were able to remember a contextual memory through optogenetic stimulation of the labelled engram neurons. This proved that, firstly, the memory is maintained and, secondly, the cells responsible for encoding the original memory are not properly reactivated in early-stage AD models (Roy et al., 2016; Perusini et al., 2017). Furthermore, engram technology has provided insights into the role of memory loss in depression. Based on human studies, it has been hypothesized that depression may be due in part to a loss of access to positive memories (Dalgleish and Werner-Seidler, 2014). Engram technology has provided strong experimental evidence in favour of this idea. When positive or pleasurable memory engrams were labelled in the mouse hippocampus prior to the induction of depression, subsequent optogenetic stimulation of these engram cells ameliorated depressive behaviour, and chronic stimulation seemed to induce new plasticity in those cells that restored natural access to the engram and normal behaviour thereafter (Ramirez et al., 2015).

**Treatment of amnesia**

The idea that the information survives in the context of the pathology is changing the paradigm of amnesia and instigating the search for therapeutic strategies to make seemingly lost memories obtainable again, rather than simply preventing the memory loss in the first place. Such therapeutics would have wide-ranging utility, since amnesia is a common symptom of many different brain disorders (Table 1). The first objective will be to identify which kinds of retrograde (and perhaps in some cases, anterograde) amnesia are due to retrieval deficits. The subsequent step will be to find ways to restore access to those engrams in a sustainable manner. Animal studies are best placed to achieve both these initial aims before the strategies can be translated into human clinical cases. Importantly, any treatment or intervention designed to reverse amnesia, whether chronic or acute, needs to also be tested in control wild-type animals. This is crucial to account for general cognitive effects (e.g. arousal, attention, emotional response, etc.) that might affect behavioural performance independently of any improvement of memory engram function.

However, such approaches to ameliorate or reverse amnesia need to be complemented with continuing efforts to address the
underlying cause of the disorder. This is especially the case for chronic forms of amnesia, as the memory will become inaccessible again if the problem is still present, such as in neurodegenerative disorders. Since amnesia is not the primary cause of these diseases, efforts put into finding therapies that palliate the memory deficits should be tied to therapies designed to stop the overall progress of the amnesia-causing disease in question.
What achievements in treating amnesia should be expected in the short and long term? Therapies based on optogenetic stimulation are very invasive, and this is a major obstacle to their translation to the clinic. The light required to optogenetically stimulate labelled neurons needs to be delivered through optic fibres, and gene therapy is required to make cells susceptible to light-mediated activation. This limitation might be overcome in the future by the development of the next-generation optogenetic implantable devices (Zhao and Hubin, 2017; Rudmann et al., 2018; Shoffstall et al., 2018) and by optimization of gene delivery (Dobson, 2006; Wang et al., 2017). Examples of non-invasive alternatives to target and activate engrams are transcranial direct-current stimulation (tDCS) and transcranial magnetic stimulation (TMS). tDCS applies weak electrical currents with electrodes that either hyperpolarize or depolarize the neurons to modify brain function. TMS achieves the same effect by generating a magnetic field inside a coiled wire that in turn generates an electrical field at the intracranial level. Although safer and much less invasive, the benefits of tDCS and TMS on cognitive function in AD are acute and not maintained in the long term (Freitas et al., 2011), probably because of their lack of specificity in targeting neurons. Most crucially, unlike researchers, physicians are not generally present at the time of memory engram specificity in targeting neurons. Most crucially, unlike researchers, physicians are not generally present at the time of memory engram formation in the patient’s brain, and so are not in the privileged observational position to label human engrams in clinical cases, even if safe and appropriate technology was available. However, given the progress in the past 10 years, there is every reason to be optimistic about future possibilities of overcoming this caveat.

As memory engram technology has become available as a new tool, the memory research field has advanced in its understanding of memory storage, consolidation and retrieval processes. Combining these approaches with disease models associated with amnesia will help us better understand the pathology on a neurobiological level, and this would certainly be followed by better management and therapeutic treatment of patients affected by memory loss.

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References
Ahmad, A., Ramakrishna, S., Meara, J. and Doran, M. (2010). Autoimmune limbic encephalitis: a reversible form of rapidly progressive amnesia and seizures. J. R. Coll. Physicians Edinb. 40, 123-125.

Albert, M. S., Butters, N. and Levin, J. (1979). Temporal gradients in the retrograde amnesia of patients with alcoholic Korsakoff’s Disease. Arch. Neurol. 36, 211-216.

Alegre, A., Hjoman R., de Haan, E. H. and Kahn, R. S. (1988a). Anterograde and retrograde amnesia in patients with chronic progressive multiple sclerosis. Arch. Neurol. 45, 611-619.

Beatty, W. W., Goodkin, D. E., Monson, N., Beatty, P. A. and Hertsgaard, D. (1988a). Anterograde and retrograde amnesia in patients with chronic progressive multiple sclerosis. Arch. Neurol. 45, 611-619.

Beatty, W. W., Salmon, D. P., Butters, N., Heindel, W. C. and Granholm, E. L. (1988b). Retrograde amnesia in patients with Alzheimer’s disease or Huntington’s disease. Neurobiol. Aging 9, 181-186.

Beers, D. R., Henkel, J. S., Kesner, R. P. and Stroop, W. G. (1995). Spatial recognition memory deficits without notable CNS pathology in rats following herpes simplex encephalitis. J. Neurol. Sci. 131, 119-127.

Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G. and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. Nat. Neurosci. 8, 1263-1268.

Braak, H. and Braak, E. (1991). Neuropathological staging of Alzheimer-related changes. Acta Neuropathol. 82, 239-259.

Burt, D. B., Zembar, M. J. and Niederehe, G. (1995). Depression and memory impairment: a meta-analysis of the association, its pattern, and specificity. Psychol. Bull. 117, 285-305.

Butters, N. and Cermak, L. S. (1974). Some comments on Warrington and Baddeley’s report of normal short-term memory in amnesic patients. Neuropsychologia 12, 283-285.

Cermak, L. S. and O’connor, M. (1983). The anterograde and retrograde retrieval ability of a patient with amnesia due to encephalitis. Neuropsychologia 21, 213-224.

Dalgleish, T. and Werner-Seidler, A. (2014). Disruptions in autobiographical memory processing in depression and the emergence of memory therapeutics. Trends Cogn. Sci. 18, 596-604.

Davis, H. P. and Squire, L. R. (1984). Protein synthesis and memory: a review. Psychol. Bull. 96, 518-559.

De Renzi, E. and Luccelli, F. (1993). Dense retrograde amnesia, intact learning capability and abnormal forgetting rate: a consolidation deficit? Cortex. Masson Italia Periodici S.r.l., 29, 449-466.

Denny, C. A., Khreiba, M. A., Alba, E. L., Tanaka, K. F., Brachman, R. A., Laughman, K. B., Torn, K. M., Glosnycz, A. and Hen, R. (2014). Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. Neuron 83, 189-201.

Dobson, J. (2006). Gene therapy progress and prospects: magnetic nanoparticle-based gene delivery. Gene Ther. 13, 283-287.

Engstrom, O., Hortobagyi, T., Pidsley, R., Troakes, C., Bernstein, H. G., Kreutz, M. R., Mill, J., Nikolic, M. and Giese, K. P. (2011). Schizophrenia is associated with dysregulation of a Cd68 activator that regulates synaptic protein expression and cognition. Brain 134, 2408-2421.

Fischer, A., Sananbenesi, F., Wang, X., Dobbm, M. and Tsai, L.-H. (2007). Recovery of learning and memory is associated with chomatrin remodelling. Nature 447, 178-182.

Freitas, C., Mondragón-Llorca, H. and Pascual-Leone, A. (2011). Noninvasive brain stimulation in Alzheimer’s disease: systematic review and perspectives for the future. Exp. Gerontol. 46, 611-627.

Goldberg, E., Antin, S., Biber, R., Gerstman, L., Hughes, J. and Mattis, S. (1981). Retrograde amnesia: possible role of mesencephalic reticular activation in long-term memory. Science 213, 1392-1394.

Gong, B., Vitolo, O. V., Trinchese, F., Liu, S., Shelsangi, M. and Arancio, O. (2004). Persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model after rolipram treatment. J. Clin. Investig. 114, 1624-1634.

Iaccarino, H. F., Singer, A. C., Martorell, A. J., Rudenko, A., Gao, F., Gillingham, T. Z., Mathys, H., Seo, J., Kritskiy, O., Abdurroob, F. et al. (2016). Gamma frequency entrainment attenuates amyloid load and modifies microglia. Nature 540, 230-235.

Jankowsky, J. L., Melnikova, T., Fadale, D.J., Xu, G.M., Haggarty, S. J., Sweatt, J. D. and Werner-Seidler, A. (2014). Disruptions in autobiographical memory processing in depression and the emergence of memory therapeutics. Trends Cogn. Sci. 18, 596-604.

Kapur, N. (1993). Transient epileptic amnesia—a clinical update and a reformulation. J. Neurol. Neurosurg. Psychiatry 56, 1184-1190.

Kapur, N. (1999). Syndromes of retrograde amnesia: a conceptual and empirical synthesis. Psychol. Bull. 125, 800-825.

Kapur, N., Mcclellan, D. L. and Burrows, E. H. (1992). Focal retrograde amnesia following bilateral temporal lobe pathology. A neuropsychological and magnetic resonance study. Brain J. Neurol. 115, 73-85.

Kesner, R. P., Dixon, D. A., Pickett, D. and Berman, R. F. (1975). Experimental animal model of transient global amnesia: role of the hippocampus. Neuropsychologia 13, 465-480.

Kim, D. Y., Hao, J., Liu, R., Turner, G., Shi, F.-D. and Rho, J. M. (2012). Inflammation-mediated memory dysfunction and effects of a ketogenic diet in a murine model of multiple sclerosis. PLoS ONE 7, e35476.

Kinoshita, K., Muroi, Y., Unno, T. and Ishii, T. (2017). Rolipram improves facilitation of contextual fear extinction in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced mouse model of Parkinson’s disease. J. Pharmacol. Sci. 134, 55-58.

Kopelman, M. D. (1992). Storage, forgetting, and retrieval in the anterograde and retrograde amnesia of the Alzheimer’s type. Memory functioning in dementia, pp. 45-71.

Kuroda, T., Futamura, A., Sugimoto, A., Midorikawa, A., Honma, M. and Kawamura, M. (2015). Autobiographical age awareness disturbance syndrome in autoimmune limbic encephalitis: two case reports. BMC Neuro. 15, 238.
Lalonde, R., Kim, H. D., Maxwell, J. A. and Fukuchi, K. (2005). Exploratory activity and spatial learning in 12-month-old APP695SWE/PS1A9 mice with amyloid plaques. *Neurosci. Lett.* 390, 87-92.

Lazarov, O., Robinson, J., Tang, Y.-P., Hairston, I. S., Korede-Mirnica, Z. Lee, V. M.-Y., Hersh, L. B., Saposky, R. M., Mirnics, K. and Sisodia, S. S. (2005). Environmental enrichment reduces Aβ levels and amyloid deposition in transgenic mice. *Cell* 120, 701-713.

Lister, R. G. (1985). The amnesic action of benzodiazepines in man. *Neurosci. Biobehav. Rev.* 9, 87-94.

Liu, X., Ramirez, S., Pang, P. T., Puryear, C. B., Govindarajan, A., Deisseroth, K. and Tonegawa, S. (2012). Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature* 484, 381-385.

Markowitsch, H. J. and Staniloiu, A. (2012). Amnesic disorders. *Lancet* 380, 1429-1440.

Masters, C. L., Bateman, R., Blennow, K., Rowe, C. C., Sperling, R. A. and Cummings, J. L. (2015). Alzheimer’s disease. *Nat. Rev. Dis. Primers.* 1, 1-18.

Mcgaugh, J. L. (2000). Memory—a century of consolidation. *Science (New York, N.Y.)* 287, 248-251.

Michel, P., Beaud, V., Eskandari, A., Maeder, P., Demonet, J. F. and Eskiglou, E. (2017). Ischemic amnesia. *Stroke* 48, 2270-2273.

Misanin, J. R., Miller, R. R. and Lewis, D. J. (1968). Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. *Science* 160, 554-555.

Pang, T. Y. C., Stam, N. C., Nithianantharajah, J., Howard, M. L. and Hannan, A. J. (2006). Differential effects of voluntary physical exercise on behavioral and brain-derived neurotrophic factor expression deficits in huntington’s disease transgenic mice. *Neuroscience* 141, 569-584.

Perusini, J. N., Cajigas, S. A., Cohensedgh, O., Lim, S. C., Pavlova, I. P., Domokonec, Z. R. and Denny, C. A. (2017). Optogenetic stimulation of dentate gyrus engrams restores memory in Alzheimer’s disease mice. *Hippocampus* 27, 1110-1122.

Ramirez, S., Liu, X., Lin, P.-A., Suh, J., Pignatelli, M., Redondo, R. L., Ryan, T. J. and Tonegawa, S. (2013). Creating a false memory in the hippocampus. *Science* 341, 367-370.

Ramirez, S., Liu, X., Macdonald, C. J., Moffa, A., Zhou, J., Redondo, R. L. and Tonegawa, S. (2015). Activating positive memory engrams suppresses depression-like behaviour. *Nature* 522, 335-339.

Rau, V., Decola, J. P. and Fanselow, M. S. (2005). Stress-induced enhancement of fear learning: An animal model of posttraumatic stress disorder. *Neurosci. Biobehav. Rev.* 2007, 1207-1223.

Reijmers, L. G., Perkins, B. L., Matsuo, N. and Mayford, M. (2007). Localization of a stable neural correlate of associative memory. *Science* 317, 1230-1233.

Ribot, T. A. (1881). Diseases of memory—an essay in the positive psychology, the international scientific series, Vol. XLI. *Diseases of memory: An Essay in the Positive Psychology, The International Scientific Series, Volume XLI*, p. 236.

Roy, D. S., Arons, A., Mitchell, T. I., Pignatelli, M., Ryan, T. Á. and Tonegawa, S. (2016). Memory retrieval by activating engram cells in mouse models of early Alzheimer’s disease. *Nature* 531, 508-512.

Rudmann, L., Alt, M. T., Ashouri Vajari, D. and Stieglitz, T. (2018). Integrated optoelectronic microprobes. *Curr. Opin. Neurobiol.* 50, 72-82.

Ryan, T. J., Roy, D. S., Pignatelli, M., Arons, A. and Tonegawa, S. (2015). Engram cells retain memory under retrograde amnesia. *Science* 348, 1007-1013.

Sackheim, H. A., Prudic, J., Devanand, D. P., Nobler, M. S., Lisanby, S. H., Peyser, S., Fitzsimons, L., Moody, B. J. and Clark, J. (2000). A prospective, randomized, double-blind comparison of bilateral and right unilateral electroconvulsive therapy at different stimulus intensities. *Arch. Gen. Psychiatry* 57, 425.

Savage, L. M., Hall, J. M. and Resende, L. S. (2012). Translational rodent models of korsakoff syndrome reveal the critical neuropeptidyl substrates of memory dysfunction and recovery. *Neuropsychol. Rev.* 22, 195-209.

Schuckit, M. A. (2009). Alcohol-use disorders. *Lancet* 373, 492-501.

Semon, R. (1904). Die mneme [The mneme]. Edited by W. Engelmann. Leipzig.

Shoffstall, A. J., Srinivasan, S., Willis, M., Stiller, A. M., Ecker, M., Voit, W. E., Pancrazio, J. J. and Capadora, J. R. (2018). A mosquito inspired strategy to implant microprobes into the brain. *Sci. Rep.* 8, 122.

Staniloiu, A., Markowitsch, H. J. and Kordon, A. (2018). Psychological causes of autobiographical amnesia: a study of 28 cases. *Neuropsychologia* 110, 134-147.

Sullivan, E. V. and Fama, R. (2012). Wernicke’s encephalopathy and Korsakoff’s syndrome revisited. *Neuropsychol. Rev.* 22, 69-71.

Tanaka, K. Z., Pevzner, A., Hamidi, A. B., Nakazawa, Y., Graham, J., Witgen, B. J. (2014). Cortical representations are reinstated by the hippocampus during memory retrieval. *Neuron* 84, 347-354.

Tonegawa, S., Liu, X., Ramirez, S. and Redondo, R. (2015a). Memory engram cells have come of age. *Neuron* 87, 918-931.

Tonegawa, S., Pignatelli, M., Roy, D. S. and Ryan, T. J. (2015b). Memory engram storage and retrieval. *Curr. Opin. Neurobiol.* 35, 101-109.

Troupe, S., Perestenko, P. V., Van De Ven, G. M., Bratley, C. T., McNamara, C. G., Campo-Urriza, N., Black, S. L., Reijmers, L. G. and Dupret, D. (2016). Recoding a cocaine-place memory engram to a neutral engram in the hippocampus. *Nat. Neurosci.* 19, 554-557.

Van Der Kolk, B. A. (1994). The body keeps the score: memory and the evolving psychobiology of posttraumatic stress. *Harv. Rev. Psychiatry.* 1, 253-265.

Viana, M. B., Tomaz, C. and Graeff, F. G. (1994). The elevated Tmaze: A new animal model of anxiety and memory. *Pharmacol. 49*, 549-554.

Wang, S., Kugelman, T., Buch, A., Herman, M., Han, Y., Karakatsani, M. E., Hussain, S. A., Duff, K. and Konofagou, E. E. (2017). Non-invasive, focused ultrasound-facilitated gene delivery for optogenetics. *Sci. Rep.* 7, 39955.

Warrington, E. K. and McCarthy, R. A. (1988). The fractionation of retrograde amnesia. *Brain Cogn.* 7, 184-200.

Wells, C. E. (1979). Diagnosis of dementia. *Psychosomatics* 20, 517-523.

Zhao, H. and Hubin, (2017). Recent progress of development of optogenetic implantable neural probes. *Int. J. Mol. Sci.* 18, 1751.

Zola-Morgan, S. (1996). Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. *Neurocase* 2, 259aw-25298.