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Neurogenesis in the adult is involved in the formation of trace memories

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The vertebrate brain continues to produce new neurons throughout life^{1–12}. In the rat hippocampus, several thousand are produced each day, many of which die within weeks¹³. Associative learning can enhance their survival^{13,14}; however, until now it was unknown whether new neurons are involved in memory formation. Here we show that a substantial reduction in the number of newly generated neurons in the adult rat impairs hippocampal-dependent trace conditioning, a task in which an animal must associate stimuli that are separated in time¹⁵. A similar reduction did not affect learning when the same stimuli are not separated in time, a task that is hippocampal-independent^{16,17}. The reduction in neurogenesis did not induce death of mature hippocampal neurons or permanently alter neurophysiological properties of

the CA1 region, such as long-term potentiation. Moreover, recovery of cell production was associated with the ability to acquire trace memories. These results indicate that newly generated neurons in the adult are not only affected by the formation of a hippocampal-dependent memory¹³, but also participate in it.

We used a toxin for proliferating cells, the DNA methylating agent methylazoxymethanol acetate (MAM)^{18–20}, to diminish the number of adult-generated cells in the dentate gyrus of rats. First, we identified a dose that would deplete the new cells without impairing overall health. Adult male Sprague–Dawley rats (*n* = 18, 3 per dose) were injected subcutaneously with MAM at daily doses of 0, 3, 4, 5, 7 and 15 mg kg⁻¹ for 14 d. We chose this regime because numbers of newly generated cells increase up to 10 d after mitosis and decline thereafter²¹. Thus, 14 d of treatment should prevent their incorporation to the granule cell layer. Twelve hours after the last MAM injection, rats were injected with 5-bromodeoxyuridine (BrdU), a marker of dividing cells²², and perfused 2 h later (see Supplementary Information). Daily treatment for 14 d with 5 mg kg⁻¹ MAM decreased the number of granule cells labelled with BrdU and TUC-4, a marker of immature neurons²³, by roughly 80% (*P* < 0.05). Rats treated with 5 mg kg⁻¹ MAM continued to gain weight during treatment and were indistinguishable in general appearance from vehicle-treated rats. Liver cytology was normal. Doses lower than 5 mg kg⁻¹ had smaller effects on cell number, 7 mg kg⁻¹ was associated with weight loss, and 15 mg kg⁻¹ with weight loss and decline in overall health. Therefore, in the subsequent studies, rats were treated with 5 mg per kg per day, for 14 d.

In the first conditioning experiment, rats were fitted with headstages and electrodes to deliver periorbital stimulation to the eyelid and to record electromyographic (EMG) activity associated with eyelid closure. After 5 days of recovery, rats were injected subcutaneously with 5 mg kg⁻¹ MAM or saline daily, for 14 d. Rats received three injections of BrdU, on days 10, 12 and 14 (10, 8 and 6 d before perfusion) to label large numbers of immature cells. Rats were untreated for 2 d, then trained with delay or trace conditioning. In both tasks, a white noise conditioned stimulus predicts the occurrence of an unconditioned stimulus of periorbital eyelid stimulation. As the animal learns, it blinks in response to the conditioned

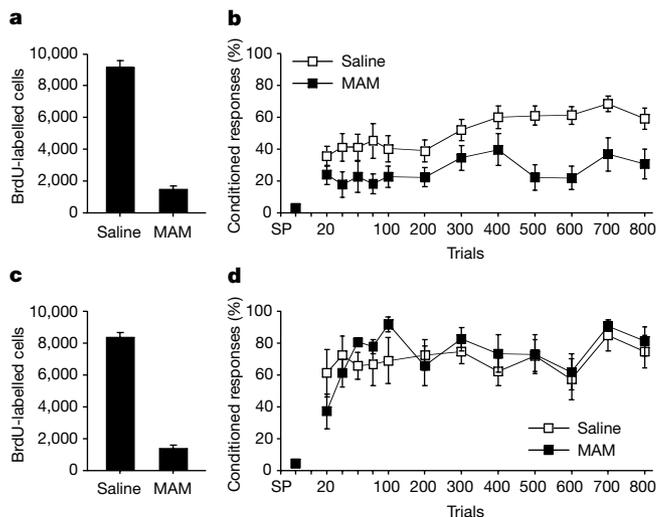


Figure 1 Adult-generated neurons are involved in trace but not in delay conditioning. **a**, Total numbers of BrdU-labelled cells in the dentate gyrus before trace conditioning. Bars represent mean ± s.e.m. **b**, Spontaneous blink rate (SP) and the percentage of conditioned responses (mean ± s.e.m.) across 800 trials of hippocampal-dependent trace conditioning in rats treated with MAM versus saline. **c**, Total numbers of BrdU-labelled cells in the dentate gyrus before delay conditioning. **d**, SP and percentage of conditioned responses across 800 trials of hippocampal-independent delay conditioning in rats treated with MAM versus saline.

stimulus. During delay conditioning, the conditioned stimulus and unconditioned stimulus overlap, and acquisition does not require an intact hippocampus^{16,17}. During trace conditioning, there is a temporal gap between the conditioned stimulus and the unconditioned stimulus, and the animal maintains or resurrects a memory 'trace' of the conditioned stimulus to associate it with the unconditioned stimulus. Acquisition of trace conditioning requires the hippocampus^{15,24,25}. The interstimulus interval was 750 ms for both delay and trace conditioning, and all rats were trained with 800 trials of paired stimuli, 200 trials a day for 4 d.

Treatment with MAM for 14 d reduced the number of newly generated cells in the dentate gyrus by 84% (1,487 for MAM ($n = 10$) and 9,140 for saline ($n = 12$)) ($P < 0.00001$) (Figs 1a and 2a, c). Most BrdU-labelled cells co-expressed markers TuJ1 (Fig. 2b), a marker for mature and immature neurons, and NeuN (Fig. 2d), a marker for mature neurons. MAM treatment reduced the overall number of conditioned responses during trace conditioning ($F(1,20) = 7.62$, $P = 0.01$) (Fig. 2b). Treatment did not alter spontaneous blink rate ($P > 0.05$), responses to the conditioned stimulus before training ($P > 0.05$), or responses on the first trial

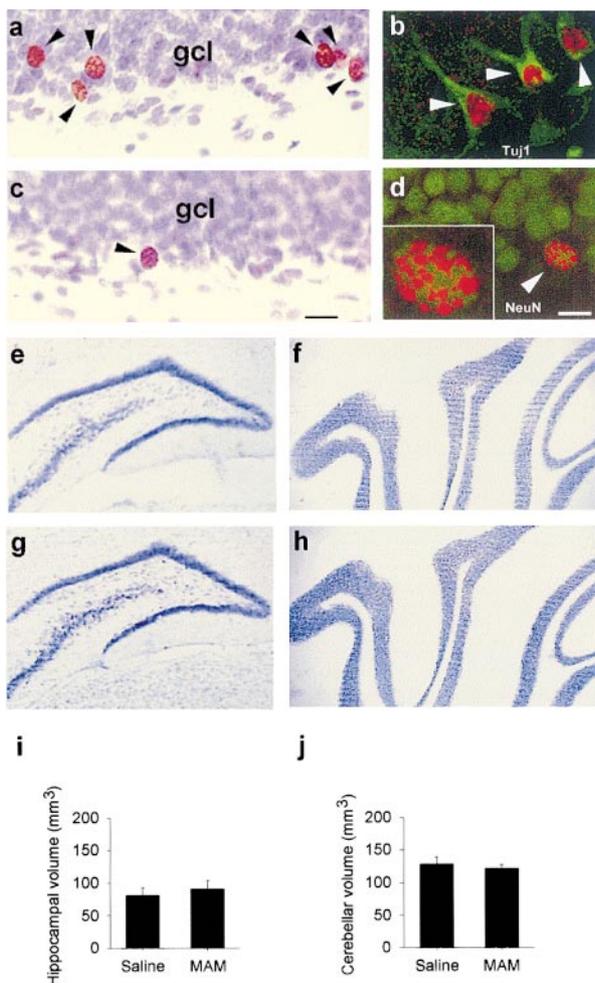


Figure 2 MAM treatment decreases number of BrdU-labelled cells in dentate gyrus and does not alter gross morphology. **a**, BrdU-labelled cells in saline-treated rat (arrowheads). gcl, granule cell layer. **b**, Cells labelled with BrdU (red, nuclei) and TuJ1 (green, cytoplasm). **c**, BrdU-labelled cells in MAM-treated rat (arrowhead). **d**, Cell labelled with BrdU (red, nucleus) and NeuN (green, nucleus). Insert depicts a cell stained with NeuN. **e**, **f**, Cresyl violet stained section of dentate gyrus (**e**) and cerebellar cortex (**f**) from a saline-treated rat. **g**, **h**, Cresyl violet stained section of dentate gyrus (**g**) and cerebellar cortex (**h**) from a MAM-treated rat. **i**, **j**, Hippocampal (**i**) and cerebellar (**j**) volume (mean \pm s.e.m.). Scale bars: **a**, **c**, 30 μ m; **b**, **d**, 10 μ m.

($P > 0.05$). Treatment of a second group with MAM reduced the number of newly generated cells by 84% (1,357 for MAM ($n = 5$) and 8,334 for saline ($n = 6$)) ($P < 0.00001$) (Fig. 1c), yet MAM-treated rats rapidly acquired the hippocampal-independent task of delay conditioning. Treatment with MAM did not alter the number of conditioned responses ($F(1,9) = 0.15$, $P > 0.05$) or conditioned responses across trials ($F(12,99) = 1.28$, $P > 0.05$) (Fig. 1d). Treatment with MAM did not affect spontaneous blink rate ($P > 0.05$), responses to the conditioned stimulus before ($P > 0.05$) or on the first trial of training ($P > 0.05$).

In a second conditioning experiment, we replicated the detrimental effect of MAM treatment on trace conditioning and evaluated alterations in neuronal responsiveness and long-term plasticity in the hippocampus. Groups were injected with MAM (5 mg kg⁻¹, $n = 6$) or saline ($n = 6$) for 14 d, and trained 2 d later with 800 trials of trace conditioning. MAM reduced the number of newly generated cells by 75% (2,284 for MAM and 9,264 for saline) and reduced the number of conditioned responses during trace conditioning ($F(1,10) = 8.93$, $P = 0.01$). Rats treated with saline emitted $53 \pm 8\%$ conditioned responses over 800 trials whereas those treated with MAM emitted $19 \pm 9\%$ conditioned responses. *In vivo* recordings of extracellular excitatory postsynaptic potentials (EPSPs) were conducted in area CA1 of the hippocampus in response to Schaffer collateral stimulation. MAM treatment did not affect presynaptic neurotransmitter release as measured by paired-pulse facilitation ($66 \pm 8\%$ increase for saline and $57 \pm 11\%$ for MAM-treated rats) ($P > 0.05$) or paired-pulse inhibition ($30 \pm 12\%$ decrease for saline and $35 \pm 7\%$ for MAM treated-rats) ($P > 0.05$). Treatment did not affect cell excitability as measured by changes in the input-output curve ($P > 0.05$) (Fig. 3a). Trained groups treated with MAM or saline showed long-term potentiation ($F(14, 140) = 5.46$, $P < 0.00001$) and the degree of potentiation was not different between groups ($P > 0.05$) (Fig. 3b). Because field potential measurements cannot detect a loss or silencing of mature cells, these measures reflect physiological responsiveness and cell excitability for those that remain. However, these results suggest that MAM treatment does not alter gross neurophysiological responsiveness in the hippocampus despite its detrimental affect on trace conditioning.

In a third set of experiments, we evaluated whether the detrimental effect of MAM on trace conditioning was due to general aversive effects of the drug on performance. We also identified the time period when the new cells become involved in learning. Groups of rats were treated with MAM (5 mg kg⁻¹, $n = 6$) or saline ($n = 6$) for 6 d to roughly ascertain a time when new cells were too young to participate in learning but mature cells could be affected by the drug. They were injected with BrdU on days 1, 3 and 5. Additional groups were treated as before with MAM ($n = 3$) or saline ($n = 3$) for 14 d (injected with BrdU on days 10, 12 and 14). All groups were

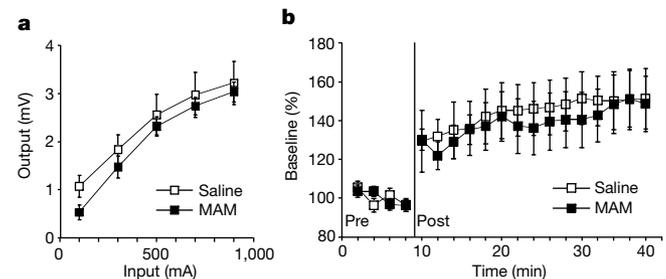


Figure 3 Reduction of adult-generated granule cells in the hippocampus does not affect neurophysiological responses in area CA1. **a**, Input-output curves for groups treated with MAM versus saline. **b**, Long-term potentiation of EPSP in area CA1 of the hippocampus in response to theta burst stimulation of Schaffer collaterals. Post-tetanic values (post) are expressed as a percentage of the pretetanus baseline (pre).

trace conditioned for 4 d, beginning 2 d after treatment. Six days of MAM treatment did not alter the number of conditioned responses ($F(1,10) = 0.75, P > 0.05$) or acquisition (an interaction between conditioned responses and trials of training) ($F(11,110) = 1.61, P > 0.05$) (Fig. 4b) despite a 78% decrease in number of BrdU-labelled cells (2,380 for MAM compared with 11,000 for saline) ($F(1,10) = 67.58, P < 0.000001$) (Fig. 4a). In contrast, 14 d of MAM treatment impaired the acquisition of trace conditioning ($F(11,44) = 10.04, P < 0.0000001$) (Fig. 4d) and reduced the numbers of new cells by 83% (1,472 for MAM compared with 9,144 for saline) ($F(1,4) = 15.11, P < 0.02$) (Fig. 4c). Thus, treatment for 6 d did not impair trace conditioning whereas treatment for 14 d did.

MAM treatment did not alter levels of motor activity in rats treated for 6 d ($P > 0.05$) or 14 d ($P > 0.05$), nor did it affect pain sensitivity (6 d groups, $P > 0.05$; 14 d groups, $P > 0.05$). MAM treatment did not alter blood levels of the stress hormone corticosterone (mean \pm s.e.m.) in those treated for 6 d ($P > 0.05$) ($124 \pm 24 \text{ ng ml}^{-1}$ for MAM and $121 \pm 13 \text{ ng ml}^{-1}$ for saline) or 14 d ($P > 0.05$) ($101 \pm 31 \text{ ng ml}^{-1}$ for MAM and $92 \pm 7 \text{ ng ml}^{-1}$ for saline). These results suggest that treatment *per se* does not alter performance, nor does it affect general activity, pain sensitivity or stress levels, as indicated by endogenous levels of glucocorticoids. Moreover, these results indicate that the new cells are about 1–2 weeks of age when they become involved in the learned response.

We conducted stereological analyses on the hippocampus and cerebellum to determine whether the MAM treatment caused structural changes other than a reduction in the number of new cells (Fig. 2). MAM treatment did not alter the volume of the hippocampal formation ($P > 0.05$) (Fig. 2e, g, i) nor the cerebellum ($P > 0.05$) (Fig. 2f, h, j). MAM treatment did not alter the volume of the granule ($P > 0.05$) or CA1 pyramidal cell layer ($P > 0.05$), nor did it reduce the number of mature granule neurons in the dentate

gyrus ($P > 0.05$). There was no evidence of degenerating cells in these regions.

We next determined whether trace conditioning would recover as neurogenesis did. Groups were treated with MAM ($n = 5$) or saline ($n = 5$) for 14 d, followed by 21 d without treatment, then trace conditioned for 4 d and perfused 24 h later. They were injected with BrdU 10, 8 and 6 d before perfusion. At the end of the 21-day recovery period, the number of BrdU-labelled cells was not different in groups treated with MAM (6,064) versus saline (7,200) ($P > 0.05$) (Fig. 4e). The ability to acquire the trace conditioned response recovered, and there was no difference between groups ($F(11,88) = 0.77, P > 0.05$) (Fig. 4f). Thus, replenishment of immature neurons after MAM treatment was associated with efficient acquisition of trace memories.

The survival of adult-generated dentate granule neurons is enhanced in response to hippocampal-dependent but not hippocampal-independent learning¹³. Specifically, survival of cells generated between one and two weeks earlier was enhanced in response to trace conditioning and spatial navigation learning, but not delay conditioning or cue-directed navigation learning. Our data indicate that these cells are not only affected by, but involved in, hippocampal-dependent learning, at least in trace conditioning. As rats treated with MAM for 6 d were not impaired, whereas those treated for 14 d were, the results are consistent with the idea that the cells become critical about 1–2 weeks after their generation. By this time, newly generated cells have become incorporated into the granule cell layer, form dendrites and extend axons into the CA3 region^{21,26}. This ability to undergo rapid structural change may be a characteristic of immature neurons that makes them ideally suited for forming associations between stimuli.

It has been suggested that hippocampal-dependent learning processes, such as those involved in trace conditioning, involve conscious awareness or recollection, and that the hippocampus performs such functions^{27,28}. On the basis of the relative immaturity

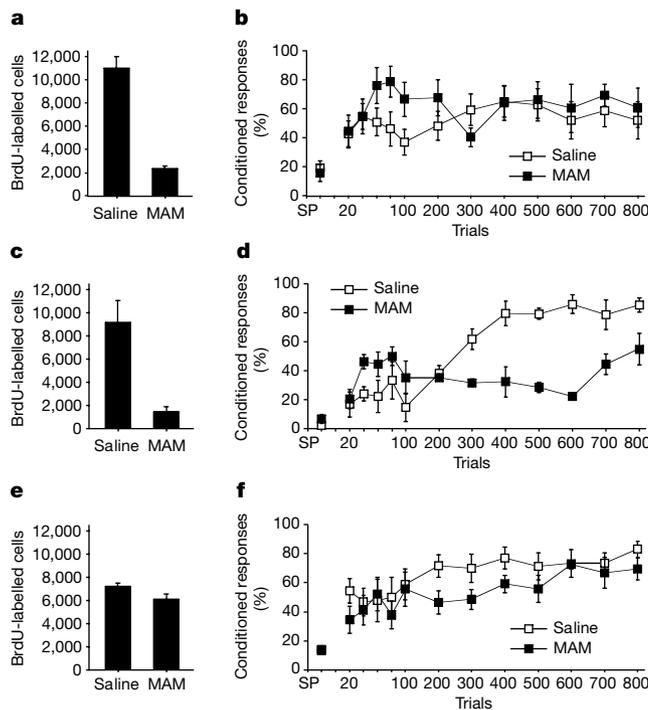


Figure 4 Trace conditioning is not affected when newly generated cells are less than one week of age or proliferation recovers. **a**, Total numbers of BrdU-labelled cells in dentate gyrus when treated with MAM or saline for 6 d. (mean \pm s.e.m.). **b**, SP and percentage of conditioned responses during trace conditioning. **c**, Total numbers of BrdU-labelled cells in dentate gyrus when treated with MAM or saline for 14 d. (mean \pm s.e.m.). **d**, SP and

percentage of conditioned responses during trace conditioning. **e**, Total numbers of BrdU-labelled cells in the dentate gyrus when treated with MAM or saline for 14 d. Training began 3 weeks later. **f**, SP and percentage of conditioned responses during trace conditioning.

of the neurons and their apparent sensitivity to both temporal and spatial associations, it could be hypothesized that the cells are used to maintain stimulus representations of newly experienced stimuli in the event that these representations are associated with subsequent events in time and space^{29,30}. Although it seems that the new cells are associated with the formation of hippocampal-dependent memories, we note that rats treated with saline learned the delay task faster than trace. Thus, newly generated neurons may not be used for learning under more lenient conditions, but become involved as task demands increase.

A reduction in the number of newly generated neurons in the dentate gyrus of the hippocampal formation impaired hippocampal-dependent, but not hippocampal-independent, forms of associative memory formation. These results suggest that immature neurons in the adult brain are involved and perhaps necessary for the acquisition of new hippocampal-dependent memories about temporal relations or the accurate timing of learned responses, such as during the acquisition of trace memories. □

Methods

Classical eyeblink conditioning

We connected headstages to four implanted electrodes: two delivered the unconditioned stimulus and two transmitted eyelid EMG, which was filtered to pass 0.3–1.0 kHz and amplified (10K). Rats were acclimated to the conditioning apparatus for 1 h, and spontaneous blink rate was recorded. Twenty-four hours later, rats were exposed to ten white noise stimuli (320 ms, 83 dB), and eyeblinks during the first 100 ms of the white noise stimulus were considered sensitized responses. We exposed rats to 200 paired trials a day for 4 d, for a total of 800 trials. They were exposed to either a delay model in which an 850 ms, 83 dB burst of white noise conditioned stimulus overlapped and co-terminated with a 100 ms, 7 mA periorbital shock unconditioned stimulus, or a trace model in which a 250 ms conditioned stimulus was separated from the unconditioned stimulus by 500 ms. Interstimulus interval was 750 ms for both of the models. The intertrial interval was 20 ± 10 ms. After training, we perfused rats and stained the brains for detection of BrdU and neuronal markers TuJ1 and NeuN.

Maximum EMG response that occurred during a 250 ms pre-stimulus baseline was added to four times its standard deviation, and responses that exceeded that value and had a width greater than 3 ms were considered eyeblinks. Those that occurred within 500 ms of unconditioned stimulus onset were considered conditioned responses. For data analysis we used analysis of variance (ANOVA) with repeated measures and planned comparisons.

Activity, pain sensitivity and stress hormones

For motor activity, horizontal and vertical motions were recorded during 30 min of free movement in a 30 cm × 30 cm chamber. For pain analysis, rats were gently restrained and tails were exposed to a beam of light of increasing intensity. Latency to withdraw was measured four times and averaged. We collected, centrifuged and stored the trunk blood for radio-immunoassay of corticosterone (Coat-a-Count).

Neurophysiological recording procedures

Within 5 d of training, subgroups were anaesthetized and placed in a stereotaxic instrument. A concentric bipolar stimulating electrode was placed 3.5 mm posterior and 3.5 mm lateral to Bregma, and a recording electrode was positioned 3.5 mm posterior and 2.7 mm lateral to Bregma. Responses were amplified 1K, filtered (1 Hz–1 kHz) and collected using BrainWave software. Input–output curves were generated by manipulating current stimulation between V_{min} and V_{max} , averaging six responses at five intensities. Maximum response was reduced to half and data from paired-pulse facilitation (40 ms interstimulus interval) and inhibition (20 ms interstimulus interval) were collected. Baseline was established as 1/2 V_{max} and a tetanus of ten, 40 ms 100 Hz bursts at 5 Hz was delivered. Responses were collected every 30 s, averaging every four, for 30 min, and are presented as a percentage of pretetanus baseline. We perfused rats and later stained the cells for detection of BrdU and neuronal markers, TuJ1 and NeuN.

Histology

Brains were cut on an oscillating tissue slicer (40- μ m thick sections) throughout the dentate gyrus and cerebellum. We stained tissue for BrdU using peroxidase and fluorescence techniques. For peroxidase, tissue was heated at 75 °C for 1 h, followed by 2 M HCl for 1 h. After rinsing in PBS, sections were incubated overnight at 4 °C in mouse monoclonal anti-BrdU (1:250; Novocastra). We reacted the sections using a standard Vector ABC kit. For immunofluorescence, sections were treated with 2 M HCl for 30 min. After rinsing in TBS, tissue was incubated in rat monoclonal anti-BrdU (1:250; Accurate) and then in mouse monoclonal anti-NeuN (1:200; Chemicon), mouse monoclonal anti-TuJ1 (1:500, a gift from A. Frankfurter), or rabbit polyclonal anti-TUC-4 (antibody 25, TOAD/ulip/crmp-4, formerly TOAD-64; 1:10,000, a gift from S. Hockfield). Sections were reacted with biotinylated anti-rat followed by streptavidin-Alexa 568 and anti-mouse or anti-rabbit Alexa 488.

We coded slides before quantitative analysis. Stereological analyses of total number of BrdU-labelled cells in the dentate gyrus were conducted using a modified version of the optical fractionator method on every twelfth section of peroxidase stained tissue. For each section, the number of BrdU-labelled cells was determined in the dentate gyrus, excluding those in the outermost focal plane to avoid counting cell caps. Resulting numbers were thinned and multiplied by tissue thickness (40 μ m) and number of intervening sections ($n = 12$). Double labelling of BrdU and NeuN or BrdU and TuJ1 was assessed with a fluorescence microscope (Olympus BX-60) and a Zeiss confocal laser scanning microscope (510 LSM; lasers HENE 543 NM and ARGON 458/488 NM). Estimates of the total number of mature granule cells were carried out using an optical fractionator method (Stereoinvestigator, MicroBrightfield). Volume estimates of the cerebellum and hippocampus were determined using Cavalieri's principle, with area measurements obtained from every twelfth section using Stereoinvestigator.

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Supplementary information is available on Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from the London editorial office of Nature.

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Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5

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Cocaine enhances dopamine-mediated neurotransmission by blocking dopamine re-uptake at axon terminals. Most dopamine-containing nerve terminals innervate medium spiny neurons in the striatum of the brain. Cocaine addiction is thought to stem, in part, from neural adaptations that act to maintain equilibrium by countering the effects of repeated drug administration^{1,2}. Chronic exposure to cocaine upregulates several transcription factors that alter gene expression and which could mediate such compensatory neural and behavioural changes^{1–4}. One such transcription factor is Δ FosB, a protein that persists in striatum long after the end of cocaine exposure^{3,5}. Here we identify *cyclin-dependent kinase 5* (*Cdk5*) as a downstream target gene of Δ FosB by use of DNA array analysis of striatal material from inducible transgenic mice. Overexpression of Δ FosB, or chronic cocaine administration, raised levels of *Cdk5* messenger RNA, protein, and activity in the striatum. Moreover, injection of *Cdk5* inhibitors into the striatum potentiated behavioural effects of repeated cocaine administration. Our results suggest that changes in *Cdk5* levels mediated by Δ FosB, and resulting alterations in signalling involving D1 dopamine receptors, contribute to adaptive changes in the brain related to cocaine addiction.

Transgenic mice displaying inducible and targeted expression of Δ FosB in the nucleus accumbens and caudatoputamen—together, the striatum of the brain—were engineered⁶. Analysis of complementary DNA expression array profiles from mice overexpressing Δ FosB indicated that the neuronal protein kinase *Cdk5* was a downstream target gene for Δ FosB in these brain regions (Fig. 1a). This effect was confirmed by quantitative *in situ* hybridization analyses of coronal brain sections from mice that did (H₂O) or did not (Dox) overexpress Δ FosB (Fig. 1b, left). Increased expres-

sion of *Cdk5* mRNA in response to Δ FosB accumulation was evident in both the caudatoputamen ($160.0 \pm 14.2\%$ of control, $P < 0.05$) and the nucleus accumbens ($152.6 \pm 12.9\%$, $P < 0.05$). Adult rats injected with cocaine for 8 days showed elevated levels of *Cdk5* mRNA in the caudatoputamen ($151.8 \pm 11.8\%$, $P < 0.05$) and nucleus accumbens ($150.5 \pm 10.0\%$, $P < 0.05$) in comparison to animals injected with saline (Fig. 1b, left). An increase was also observed in the level of mRNA encoding the neuron-specific *Cdk5*-activating cofactor, p35, in response to Δ FosB induction or chronic cocaine administration in both the caudatoputamen ($121.8 \pm 5.7\%$ and $129.8 \pm 7.5\%$, respectively, $n = 6$, $P < 0.05$) and the nucleus accumbens ($119.8 \pm 5.2\%$ and $126.3 \pm 7.4\%$, respectively, $n = 6$, $P < 0.05$) (Fig. 1b, right). Increased levels of *Cdk5* protein were observed in striatal tissue dissected from transgenic Δ FosB-expressing mice ($130 \pm 10\%$, $P < 0.05$) and from rats exposed chronically to cocaine ($180 \pm 20\%$, $P < 0.05$) compared to control animals (Fig. 1c, left and middle panels). Increased striatal p35 protein levels also occurred in response to chronic cocaine ($147.3 \pm 11\%$, $n = 12$, $P < 0.05$) (Fig. 1c, right panel). These results indicated that *Cdk5*, together with its activating cofactor p35, are downstream targets of chronic cocaine exposure, and raised the possibility that this protein kinase is involved in the behavioural effects of cocaine.

A characteristic behavioural effect of cocaine is potentiation of locomotor activity. The effects of daily intra-accumbens infusions of a potent *Cdk5* inhibitor, roscovitine, on cocaine-induced locomotor activity were examined over a 60-min period daily for 5 days. Administration of cocaine resulted in a marked and progressive increase in locomotor activity over this 5-day period, indicating the development of locomotor sensitization, during the initial 10–20 min period following injection (data not shown). Roscovitine infusions did not significantly affect locomotor responses to initial cocaine administration. However, roscovitine markedly potentiated the locomotor effects of repeated cocaine exposures. This was evident as an augmentation of cocaine's effects over successive days of injections (Fig. 2a). By day 4, significant differences were observed between the saline/cocaine and roscovitine/cocaine groups. By day 5, mean cocaine-induced activity rates for roscovitine-infused animals were almost double those measured for vehicle-infused animals. This effect of roscovitine was most evident 40–60 min after cocaine administration (Fig. 2b).

Repeated intra-accumbens infusions of a less selective *Cdk5* inhibitor, olomoucine, also potentiated cocaine's locomotor effects. This action was similar to that produced by roscovitine, except that a marked behavioural effect was already observed as early as day 3 (Fig. 2c). In response to cocaine treatment on days 4 and 5, olomoucine-treated animals exhibited stereotypy, which denotes enhanced sensitization and is known to compete with increases in locomotor activity¹⁰. In contrast, intra-accumbens infusions of the inactive congener, iso-olomoucine, failed to enhance either locomotor (Fig. 2c) or stereotypic responses to cocaine. These behavioural findings indicate that cocaine-induced increases in *Cdk5* levels may serve a homeostatic function to dampen responses to subsequent drug exposure.

One way in which *Cdk5* could regulate the psychomotor effects of chronic cocaine is through regulation of dopamine signalling. *Cdk5* phosphorylates a key molecule involved in striatal dopamine signalling, DARPP-32—dopamine and cyclic AMP-regulated phosphoprotein, M_r 32K—at threonine-75 (Thr75)⁷. Immunocytochemistry studies revealed that DARPP-32 and *Cdk5* are colocalized: all medium spiny neurons in the nucleus accumbens that contained DARPP-32 also contained *Cdk5* (Fig. 3a). Furthermore, dendrites in the neuropil were typically double-labelled and sometimes contained puncta that were immunoreactive to both proteins. Increased levels of phospho-Thr75 DARPP-32 were observed in striatal tissue dissected from transgenic mice overexpressing Δ FosB (Fig. 3b). In rats subjected to chronic cocaine

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correction

Neurogenesis in the adult is involved in the formation of trace memories

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The original Fig. 2e–j is in error because it was based on incorrectly sorted brain tissue. A corrected figure is shown below and demonstrates that the anti-mitotic agent MAM does not affect the gross morphology or the volume of either the hippocampus or the cerebellum. The revised figure is based on a new set of tissue obtained from brains in experiment 3 that had been exposed to MAM ($n = 3$) or saline ($n = 3$) for 14 days and processed as described. Sections from each of these brains are presented. This error (and its correction) does not alter the main findings we reported, namely that treatment with MAM depletes newly generated neurons in the hippocampus and impedes learning of trace eyeblink conditioning in the adult rat. □

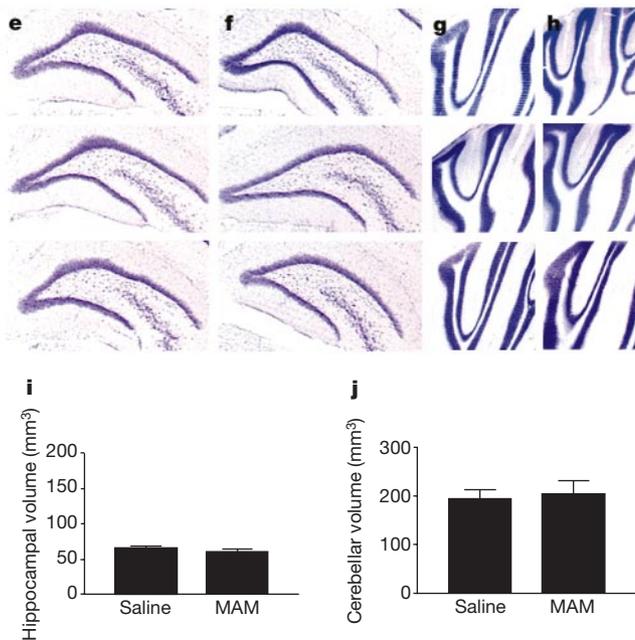


Figure 2e–j MAM treatment does not alter the gross morphology of the hippocampus or cerebellum. **e, f**, Cresyl violet stained sections of the dentate gyrus from three saline-treated (**e**) and three MAM-treated (**f**) adult rats. **g, h**, Cresyl violet stained sections of the cerebellar cortex from three saline-treated (**g**) and three MAM-treated (**h**) adult rats. **i, j**, Hippocampal (**i**) and cerebellar (**j**) volume (mean + s.e.m.).