early vertebrates? Or is it a more pervasive phenomenon? In general, the answer is that stem-ward slippage is widespread: all fossil animals with a high proportion of missing information tend to fall out near the base of an evolutionary tree through the lack of morphological features (such as structures in the head, in the case of chordates) to ally them with more evolved groups. And the resulting tree may be biased unless the decay sequence is random relative to the tree's branching order — that is, the order in which characters evolved. As well as prompting caution in interpreting soft-bodied fossils, Sansom and colleagues' research[1] may turn out to be important in identifying a way to assign confidence limits to the placement of these extinct forms in the tree of life. ■

Derek E. G. Briggs is in the Department of Geology and Geophysics, and the Yale Peabody Museum of Natural History, Yale University, New Haven, Connecticut 06520, USA.
e-mail: derek.briggs@yale.edu

1. Sansom, R. S., Gabbott, S. E. & Purnell, M. A. *Nature* **463,** 797–800 (2010).
2. Hou, X.-G. *et al. The Cambrian Fossils of Chengjiang, China: The Flowering of Early Animal Life* (Blackwell, 2004).
3. Caron, J.-B. & Rudkin, D. (eds) *A Burgess Shale Primer: History, Geology, and Research Highlights* (Burgess Shale Consortium, 2009).
4. Briggs, D. E. G. *Annu. Rev. Earth Planet. Sci.* **31,** 275–301 (2003).
5. Gabbott, S. E., Hou, X., Norry, M. J. & Siveter, D. J. *Geology* **32,** 901–904 (2004).
6. Donoghue, P. C. J. & Purnell, M. A. *BioEssays* **31,** 178–189 (2009).
7. Schäfer, W. *Ecology and Palaeoecology of Marine Environments* (Chicago Univ. Press, 1972).

NEUROSCIENCE

Lack of inhibition leads to abuse

Arthur C. Riegel and Peter W. Kalivas

Chronic drug use can lead to addiction, which is initiated by specific brain circuits. The mystery of how one class of drugs, the benzodiazepines, affects activity in this circuitry has finally been solved.

Common illnesses such as anxiety disorders, insomnia and even muscle spasms are treated with benzodiazepine drugs, of which diazepam (Valium) is perhaps the best known. But both conventional benzodiazepines and newer benzodiazepine-like compounds (such as zolpidem) are addictive. This limits the therapeutic potential of an otherwise safe class of drugs that has broad clinical applications.

Most addictive substances activate the same brain circuitry: the dopaminergic system, which is also stimulated by natural rewards, such as food and sex. Benzodiazepines stimulate this circuitry, but the underlying mechanism was unknown, prompting speculation that benzodiazepine addiction involves systems distinct from those involved in dependence on other addictive drugs.

In this issue (page 769), Tan *et al.*[1] report that the missing link connecting benzodiazepines to the dopaminergic circuitry is a select group of $GABA_A$ receptors (γ-aminobutyric acid type A receptors) that reside on neurons known as inhibitory interneurons. These interneurons coordinate the output of dopaminergic neurons in the ventral tegmental area (VTA) of the brain. $GABA_A$ receptors are assembled from five subunits, of which there are several types; the authors find that, in inhibitory interneurons, benzodiazepines increase the stimulation of $GABA_A$ receptors that contain α1 subunits. This reduces activity in the interneurons and thereby increases excitability in VTA dopaminergic cells. Through a series of ingenious experiments, Tan *et al.* demonstrate that this mechanism contributes to benzodiazepine addiction.

In general, the urge to self-administer an addictive drug is initiated in the VTA by the 'strengthening' of excitatory glutamatergic synapses — neural junctions at which glutamate is the primary neurotransmitter — to dopaminergic neurons. Such strengthening increases the chance that the synapse will release glutamate and is caused by the recruitment of new AMPA receptors (a class of glutamate receptor) to glutamatergic synapses on dopaminergic neurons. In agreement with an earlier study[2], Tan *et al.*[1] found that benzodiazepines also cause this effect in mice, regardless of whether the drugs are injected systemically (whereupon they can interact with $GABA_A$ receptors throughout the animal's body) or directly infused into the VTA.

The authors obtained similar results with zolpidem, which binds predominantly to $GABA_A$ receptors that contain α1 subunits. But they detected no such strengthening in wild-type mice in which a benzodiazepine was co-administered with a compound that blocks the benzodiazepine binding site on $GABA_A$ receptors (a benzodiazepine antagonist), or in mice that were genetically engineered so that their α1 subunits were unable to bind benzodiazepines. Taken together, these results showed that the cellular changes that occur in dopaminergic neurons after a single exposure to benzodiazepines require functional α1 subunits in $GABA_A$ receptors.

Tan *et al.* then mapped the location of α1-containing $GABA_A$ receptors within the VTA's microcircuitry, and found that the highest density was not on dopaminergic cells, but on the inhibitory interneurons — the same class of cells targeted by opioid drugs such as morphine[3]. In fact, much of morphine's addictive potential is attributed to the inhibition of these interneurons in the VTA.

To better understand commonalities between benzodiazepines and opioids, the authors[1] treated α1-mutated mice with morphine. Surprisingly, they observed that morphine caused the cellular changes

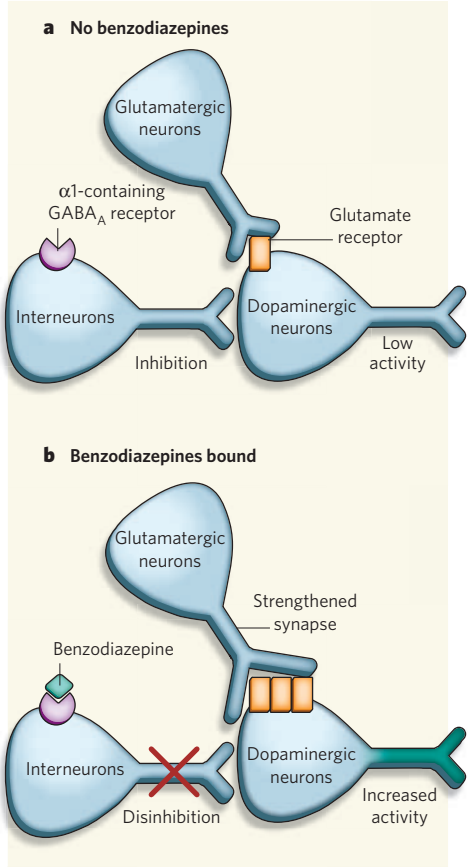

Figure 1 | Proposed mechanism of addiction of benzodiazepine drugs. Tan *et al.*[1] report that benzodiazepine drugs bind to and activate certain $GABA_A$ receptors (γ-aminobutyric acid type A receptors) — those that contain α1 subunits — on interneuron cells in the ventral tegmental area (VTA) of the brain. **a**, These interneurons ordinarily inhibit the activity of dopaminergic neurons in the VTA. Forked branches are synapses (neural junctions). **b**, Activation of the $GABA_A$ receptors by benzodiazepines reduces the amount of inhibition (a process known as disinhibition, red cross), which increases the activity of the VTA's dopaminergic neurons. This increased activity strengthens the VTA's glutamatergic synapses (neuronal junctions at which the primary neurotransmitter is glutamate) to dopaminergic neurons, making them more excitable. Such strengthening, caused by an increase in the number of glutamate receptors on the dopaminergic neuron, is the cellular response to natural rewards (such as food), but it also forms the basis of the addictive properties of drugs.

normally associated with benzodiazepines — the recruitment of new AMPA receptors to glutamatergic synapses on dopaminergic neurons. This result suggested that, despite differences in their binding sites, benzodiazepines and opioids ultimately share the same interneuron target. The authors therefore reasoned that benzodiazepines, like opioids, might increase the excitability of dopaminergic neurons by reducing interneuron-mediated inhibition, a process known as disinhibition.

Tan *et al.* tested this theory by sampling VTA dopaminergic cells from brain slices and recording the inhibitory currents mediated by $GABA_A$ receptors in these cells. In tissue from wild-type mice, they observed that a typical benzodiazepine (midazolam) reduced the inhibitory current in dopaminergic neurons, in line with the disinhibition hypothesis. Conversely, in α1-mutated mice, the inhibitory currents in dopaminergic cells increased in response to the drug, suggesting that benzodiazepines reduce the release of GABA in wild-type mice.

The authors went on to record the activity of VTA neurons *in vivo*, and noted that, in wild-type mice, benzodiazepines simultaneously activated dopaminergic neurons and inhibited GABAergic interneurons. This disinhibition effect was reversed when the authors subsequently administered a benzodiazepine antagonist to the animals. What's more, disinhibition was altogether absent in α1-mutated mice.

Finally, the researchers studied how prone their mice were to self-administering benzodiazepines. When given a choice between drinking either a sugar solution or a sugar solution laced with midazolam, wild-type mice preferred the midazolam-containing solution. The α1-mutated mice, however, showed no such preference, even though they drank as much liquid overall as the wild-type animals, and preferred sugar solution to pure water (indicating that they were capable of reward-motivated discrimination).

Taken together, Tan and colleagues' data[1] suggest that the activation of α1-containing $GABA_A$ receptors by benzodiazepines calms GABAergic interneurons, reducing their overall inhibitory output. Consequently, dopaminergic neuron firing increases in the VTA, which elevates the number of AMPA receptors in the membranes of the excited dopaminergic neurons and strengthens the excitatory synapses that favour addiction (Fig. 1). This general disinhibition mechanism is analogous to that involved in opioid drug abuse[3].

More studies are required to fully appreciate the context of the proposed mechanism[1]. For example, synaptic scaling — a homeostatic process that adjusts the strength of a neuron's excitatory synapses up or down to stabilize firing[4,5] — also modulates the number of AMPA receptors in neuron membranes, but its relevance in the VTA isn't clear and will be challenging to determine. Another consideration is that both dopaminergic and GABAergic cells receive GABAergic innervation, and the distribution of α1-containing $GABA_A$ receptors in these systems is both presynaptic and postsynaptic. This somewhat ambiguous distribution suggests that additional α1-dependent mechanisms of addiction might exist in which benzodiazepines modulate the excitability of dopaminergic cells.

Other findings indicate that benzodiazepine abuse is not always attributable to α1-containing $GABA_A$ receptors. For example, it is unclear why the overall incidence of zolpidem addiction is low relative to that of less-selective benzodiazepines (those that, unlike zolpidem, show no binding preference for α1 GABA subtypes), opioids or other drugs of abuse[6]. It is also unclear why primates will self-administer[7] a benzodiazepine known as L-838 417, which binds to, but does not activate, α1-containing $GABA_A$ receptors and (as Tan *et al.*[1] show) does not change AMPA-receptor distribution in dopaminergic neurons.

Nevertheless, Tan and colleagues' discovery[1] is a landmark for the field — these authors are the first to identify a molecular mechanism contributing to benzodiazepine abuse. Given that the α1 subunits of $GABA_A$ receptors are not responsible for the therapeutic effects of benzodiazepines[8], the work highlights an exciting possibility: if benzodiazepines can be designed that lack affinity for this subunit, then the addictive properties of these versatile and useful drugs might be reduced. ■

Arthur C. Riegel and Peter W. Kalivas are in the Department of Neurosciences, Medical University of South Carolina, Charleston, South Carolina 29425, USA.
e-mails: riegel@musc.edu; kalivasp@musc.edu

1. Tan, K. R. *et al. Nature* **463,** 769–774 (2010).
2. Heikkinen, A. E., Möykkynen, T. P. & Korpi, E. R. *Neuropsychopharmacology* **34,** 290–298 (2009).
3. Johnson, S. W. & North, R. A. *J. Neurosci.* **12,** 483–488 (1992).
4. Nelson, S. B. & Turrigiano, G. G. *Neuron* **60,** 477–482 (2008).
5. Turrigiano, G. G. *Cell* **135,** 422–435 (2008).
6. Hajak, G., Müller, W. E., Wittchen, H. U., Pittrow, D. & Kirch, W. *Addiction* **98,** 1371–1378 (2003).
7. Rowlett, J. K., Platt, D. M., Lelas, S., Atack, J. R. & Dawson, G. R. *Proc. Natl Acad. Sci. USA* **102,** 915–920 (2005).
8. McKernan, R. M. *et al. Nature Neurosci.* **3,** 587–592 (2000).

PARKINSON'S DISEASE

Mitochondrial damage control

Asa Abeliovich

Defects in mitochondria are implicated in Parkinson's disease. Study of a quality-control pathway involving the proteins PINK1 and Parkin provides further clues about the mechanism involved.

The pursuit of a unifying mechanism for Parkinson's disease has been refuelled by the identification of genetic mutations that underlie inherited variants of the disorder[1]. For instance, mutants of a particular enzyme — the mitochondrial PTEN-induced kinase-1 (PINK1) — cause a rare, early-onset form of Parkinson's[2], directly implicating altered mitochondrial regulation in the disease process. Furthermore, mice with mutations in PINK1 display reduced mitochondrial function[3]. Mitochondria are double-membrane-bound organelles that produce energy in the form of ATP, but in the course of this process they can accumulate toxic by-products as well. They are thus crucial to a cell's well-being — as is their disposal when they malfunction.

Writing in *PLoS Biology*, Narendra *et al.*[4] now describe a specific role for PINK1 in mitochondrial quality control and disposal. The authors observe that PINK1 accumulates within minutes at mitochondria that have lost the electric-potential gradient that spans their inner membrane, as is seen on exposure to mitochondrial poisons or in ageing. Such poisons have been linked epidemiologically to Parkinson's disease[5]. Whereas normal cells that are exposed to these depolarizing toxins eventually dispose of the damaged mitochondria, Narendra *et al.* find that cells deficient in PINK1 (or with disease-associated mutant forms of PINK1) fail to do so.

As the authors point out, reactive oxygen species or other damaging agents may leak from damaged mitochondria. This suggests a mechanism for the loss of midbrain neurons that produce dopamine neurotransmitter — a defining feature of Parkinson's disease. The accumulation of defective mitochondria might in part explain other features associated with mutation in the *PINK1* gene, such as altered mitochondrial morphology[6] and reduced ATP production. In contrast to PINK1 deficiency, overexpression of the normal protein promotes mitochondrial loss, further implicating PINK1 in the disposal process.

These findings shed light on a study from the same group[7] that focused on *parkin*, a second gene that is mutated in an inherited form of Parkinson's disease. The Parkin protein occurs mainly in the cell cytoplasm, but re-localizes to mitochondria that have been treated with depolarizing agents. Cells that are deficient in Parkin, or that have disease-associated Parkin mutations, fail to rid themselves of defective mitochondria; by contrast, Parkin overexpression induces excessive mitochondrial disposal, reminiscent of