Circadian rhythms deeply affect our lives: our general energy level and ability to perform exacting work are profoundly affected by the time of day, to the point where the impact of irregular work hours on ability to safely deliver anaesthesia or perform surgery are a matter of concern. Circadian changes in hormone levels drive fluctuations in functioning of our bodily systems, and chronobiology, the study of such rhythms, shows that almost every organ system is affected. It is no surprise, therefore, that both the pharmacokinetics and pharmacodynamics of drugs can be influenced by the time of their administration. More of a surprise is that such chronopharmacology has received relatively little attention until recently. A timely review of these issues as pertaining to anaesthesia shows that many of the drugs we use in our practice are influenced by the body’s circadian rhythms.

In this issue of the BJA, Sato and colleagues report an investigation into the mechanism behind the time-dependent effect of ketamine. Loss of righting reflex (LORR) in mice was used as an endpoint for hypnotic effect of the drug after administration at different times of day. The experiment was repeated in mice lacking the ε1 (also known as NR2A) N-methyl-d-aspartate (NMDA) glutamate receptor. The authors found that whereas the wild-type mice showed circadian variation in ketamine-induced LORR, the ε1 knock-out animals did not. The authors therefore suggest a role for the ε1 NMDA receptor subunit in the circadian effect of ketamine.

This study uses an elegant, powerful technique to investigate gene function, and yields a result that is of great interest. It demonstrates why knockout—i.e. the selective functional removal of one gene and its product—has become such a common approach to identifying the function of genes. However, a number of confounders exist in such studies, and they should be carefully considered before definitive conclusions are drawn. The study by Sato demonstrates some of these issues.

In knockout studies, it is always possible that the lack of a certain receptor subtype may be compensated for (and possibly in an abnormal manner) by upregulation of other subtypes. The NMDA receptor is constructed from five subunits, with poorly defined stoichiometry, and it is not known how the expression of those other subtypes was affected by the lack of the ε1 subunit. Three other ε subtypes exist, and any of these may have taken the place of ε1, leading to a receptor composition that may behave neither as a native NMDA receptor, nor as one simply lacking ε1. Worse, some of the remaining subunits are subject to circadian variation in expression. In addition, whereas the reason the investigators chose to study NMDA receptors is clear (they are the main target for ketamine), the reasons for selecting ε1 rather than any other of the ε subtypes are not clear. It would have been important, therefore, for the authors also to study knockouts of these related subtypes.

It is important in such studies to differentiate between the pharmacokinetic and pharmacodynamic effects of a knockout. Sato and colleagues established that, in wild-type animals, circadian ketamine sensitivity did not result from changes in the bioavailability of ketamine: serum concentrations of ketamine were not affected by the time of day. However, although a serum level was obtained at one time point in knockout mice, it was not formally determined that pharmacokinetics were unaffected by the ε1 knockout.

Most difficult, however, can be the interpretation of an observed effect of a knockout. The systems under consideration in such studies are very complex, and the ‘obvious’ interpretation of an observed effect is not always the only one. In the paper under consideration, Sato and colleagues conclude that ‘the NMDA receptor ε1 subtype is involved in the anaesthetic effect of ketamine, and plays a major role in the induction of the dosing time-dependency’. One should be very careful in drawing such a conclusion, and I do not believe either statement has been conclusively shown by this paper. Other explanations are possible. For example, the NMDA receptor may be a part of the righting reflex pathway, and knockout of the ε1 subunit may therefore result in loss of the circadian rhythm of this reflex, independent of the drug used to elicit it. This hypothesis can be tested fairly easily: does LORR induced by a compound known not to act through the NMDA receptor (such as a barbiturate or benzodiazepine) exhibit a circadian rhythm? And is such a circadian rhythm abolished by ε1 subunit knockout? If so, it is unlikely that the interaction between ketamine and the NMDA receptor is responsible for the circadian effects observed in this study. And one would certainly not conclude from such a result that the ε1...
subunit was responsible for the anaesthetic effect of the barbiturate! Such experiments are required before any conclusive statements can be made about the role of ε1 in the action of ketamine, and it would have been very helpful if the authors had investigated any effect, or lack thereof, of barbiturates or benzodiazepines on the circadian LORR. (It is some comfort to know that the knockout of ε1 did not result in elimination of all circadian rhythms, as circadian changes in water intake were similar in both groups.)

Molecular genetics has provided us with some extraordinarily powerful tools for investigating the molecular actions of our drugs. Sato and colleagues demonstrate how such tools can be used to address important pharmacological questions in anaesthesia. At the same time, they also show us that appropriate use of such techniques requires extensive control experiments to assure validity of the results, and that it is possible to draw unwarranted conclusions in the absence of such controls. Powerful tools, in short, require very careful handling.

M. E. Durieux
Department of Anesthesiology
University of Virginia

PO Box 800710
Charlottesville
VA 22908–0710
USA
E-mail: durieux@virginia.edu

References

2 Chassard D, Bruguerolle B. Chronobiology and anesthesia. Anesthesiology 2004; 100: 413–27
4 Michaelis EK. Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. Prog Neurobiol 1998; 54: 369–415
5 Ishida N, Matsui M, Mitsui Y, Mishina M. Circadian expression of NMDA receptor mRNAs, epsilon 3 and zeta 1, in the suprachiasmatic nucleus of rat brain. Neurosci Lett 1994; 166: 211–15

DOI: 10.1093/bja/aeh165