failed to fully normalize the data in that they have just counted the number of positive cells, be they Fluoro-Jade C or caspase-3 positive, in each field of view. This method does not take into account the number of cells present in the section or the size of the section. One method is based on the generation of a "wandering mean." To generate these data, the following procedure should be undertaken. Count the number of events (caspase-3 positive or Fluoro-Jade C positive cells) and the total number of relevant cells in the first microscopic field. This will give the first apoptosis score (A1 based on N1 cells). In the second field, the process is repeated and running scores recorded to give a running mean (A2 based on N2 cells). This process is repeated to give multiple running averages (A3, N3 . . . An, Nn). If these are plotted, the mean will be seen to wander and eventually oscillate about a mean value, and as N increases, this will become less. This procedure can then define experimentally the number of events to be assessed to produce a given quality of data.[7]

Intrathecal ketamine may have a much narrower intrathecal therapeutic index compared with that of morphine. However, local anesthetic agents have been shown to have detrimental effects on neuronal apoptosis,[8] and the right balance between exposing vulnerable children to potential harmful general or regional anesthetics is yet to be established. Until then, we need to pay attention to the primary cause of morbidity and mortality in children: hypoxia.[9]

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References

1. Walker SM, Westin BD, Deumens R, Grafe M, Yaksh TL: Effects of intrathecal ketamine in the neonatal rat: Evaluation of apoptosis and long-term functional outcome. Anesthesiology 2010; 113:147–59
2. Drasner K: Anesthetic effects on the developing nervous system: If you aren't concerned, you haven't been paying attention. Anesthesiology 2010; 113:10–2
3. Westin BD, Walker SM, Deumens R, Grafe M, Yaksh TL: Validation of a preclinical spinal safety model: Effects of intrathecal morphine in the neonatal rat. Anesthesiology 2010; 113:183–99
4. Taylor SE, Dorris RL: Modification of local anesthetic toxicity by vasoconstrictors. Anesth Prog 1989; 36:79–87
5. Bruce DL, Capan L: Antidepressants do not increase the lethality of ketamine in mice. Br J Anaesth 1983; 55:457–9
6. Marhofer P, Krenn CG, Plöchl W, Wallner T, Glaser C, Koinig H, Fleischmann E, Höchtl A, Semsroth M: S(+)-ketamine for caudal block in paediatric anaesthesia. Br J Anaesth 2000; 84:341–5
7. Save V, Hall PA, Coates PJ: Detecting and quantifying apoptosis in tissue sections. Methods Mol Biol 2004; 282:67–84
8. Werdehausen R, Fazeli S, Braun S, Hermanns H, Essmann F, Hollmann MW, Bauer I, Stevens MF: Apoptosis induction by different local anaesthetics in a neuroblastoma cell line. Br J Anaesth 2009; 103:711–8
9. Bhananker SM, Ramamoorthy C, Geiduschek JM, Posner KL, Domino KB, Haberkern CM, Campos JS, Morray JP: Anesthesia-related cardiac arrest in children: Update from the Pediatric Perioperative Cardiac Arrest Registry. Anesth Analg 2007; 105:344–50

(Accepted for publication November 10, 2010.)

In Reply:

We thank Drs. Engelhardt, Blaylock, and Weiss for their comments on our article,[1] and we are happy to clarify the issues of ketamine dosing. Intrathecal ketamine (30 mg/kg) produced irreversible sedation and respiratory depression in P3 pups, and excitation and convulsions in P21 animals after emergence from anesthesia that necessitated termination. As a result, the same degree of dose escalation was not possible with ketamine as with morphine. Rather than indicating that "sublethal doses" of ketamine are associated with apoptosis, this emphasizes the narrower therapeutic window between analgesia and dose-limiting side effects with ketamine.

The authors incorrectly stated that "no data on analgesic action" were provided for 0.1–0.3 mg/kg intrathecal ketamine. Figure 1B clearly presents dose-response data for antihyperalgesic effects of intrathecal ketamine in both P3 and P21 pups.[1] Because of ketamine's mode of action, increases in baseline sensory threshold (*i.e.*, antinociceptive effects) are not seen. The increased primary afferent input after injury (*i.e.*, carrageenan-induced hind paw inflammation) results in activation of *N*-methyl D-aspartate-mediated sensitization in the spinal cord, and dose-dependent reversal of hyperalgesia by ketamine can now be demonstrated. This pattern of response is discussed and referenced under "Dose-dependent effects" in our manuscript. Significant reversal of hyperalgesia was seen 30 min after intrathecal ketamine 3 mg/kg in P3 rats and 15 mg/kg in P21 rats. As we had shown that ketamine produced apoptosis within this analgesic dose range in P3 pups, repeating the same experiments with subtherapeutic doses would represent unnecessary use of animals and resources.

Engelhardt *et al.* refer to doses that "are the comparative and relevant equivalents commonly employed for caudal anesthesia." We are surprised that the authors expect there to be a direct correlation between the doses uesd in different species, at different ages, and by different routes. Again, these issues were covered in our discussion.[1] Our rationale for describing results in terms of a therapeutic index was to provide a ratio of toxic to functional doses that could facilitate comparison of different drugs at different developmental

Supported by funding from the National Institutes of Health NIH-DA15353 and NIH NS16541, Bethesda, Maryland (to Dr. Yaksh); the Association of Paediatric Anaesthetists of Great Britain and Ireland, London, United Kingdom (to Dr. Walker); and the County Council of Ostergotland, Ostergotland, Sweden, and the Swedish Society for Anaesthesiology and Intensive Care, Stockholm, Sweden (to Dr. Westin). Noldus Information Technology, Wageningen, The Netherlands, provided a loan of a CatWalk® system.

The above Letter was sent to the author of the associated Editorial View, who declined to respond to the Letter.—James C. Eisenach, M.D., Editor-in-Chief.

stages. Similarly, studies evaluating apoptosis following systemic ketamine have related doses to functionally significant general anesthetic effects in different species at specific developmental ages (*i.e.*, infusion of ketamine at doses producing surgical anesthesia in primates[2] or the ED_{50} for loss of righting reflex in rodents[3]).

Our evaluation of apoptosis was confirmed using multiple methodologies. The low standard errors and statistically significant differences comparing ketamine but not morphine with saline control groups, in the setting of evaluation of appropriately randomly coded sections, support the validity of our method of quantification. Although we agree that the size or dermatomal level may have influenced the total number of cells, the whole lumbosacral spinal cord section and not selected fields of view were examined, and for each animal, counts were performed in at least four randomly selected sections for each different evaluation (activated caspase-3 immunohistochemistry, FluoroJade C, and hematoxylin and eosin staining). The "wandering mean" method of counting would not be appropriate because of the uneven distribution of positive cells (as stated in the article, more apoptotic cells were identified in the dorsal rather than ventral horn; fig. 4[1]). Consistent with previous reports, the level of baseline neuronal apoptosis was higher in the spinal cord in early postnatal life, and consistent reductions in apoptotic counts with increasing age at time points from P4 to P22 were found in naïve and saline control groups using all our methodologies. Results from activated-caspase 3 immunohistochemistry correlated with those obtained by counting apoptotic profiles in hematoxylin and eosin-stained sections,[4] and although not reported in the manuscript, double staining with activated-caspase 3 and the neuronal marker NeuN was confirmed in preliminary experiments. Numbers of FluoroJade C-positive cells were consistent with but higher than those with activated-caspase 3, as Flouro-Jade C staining is a more sensitive method and will capture dead cells at most stages of cell death, dying by any cell death pathway. Importantly, the authors do not acknowledge that single-dose intrathecal ketamine at P3 also produced long-term functional changes in mechanical sensory threshold and paw placement during gait, or that data from adult models have shown toxicity following intrathecal ketamine, providing further evidence for caution with neuraxial administration of ketamine.

Engelhardt *et al.* refer to apoptotic effects of local anesthetics. The referenced study confirmed concentration-dependent apoptosis when cultured cells were exposed to local anesthetics for 24 h, and the susceptibility to apoptosis was related to the physicochemical properties of different local anesthetics.[5] Interestingly, using the same methodology, concentration- and time-dependent apoptosis after exposure to preservative-free racemic ketamine and *S*-ketamine has recently been reported.[6] The potential limitations of extrapolating *in vitro* to *in vivo* data are noted in the discussion of both studies. In particular, *in vitro* evaluation of isolated cells in culture does not allow comparison of specific age-dependent effects, particularly if mechanisms are reliant on alterations in synaptic transmission.[5,6] As noted in our discussion, prolonged exposure to morphine also produces apoptosis in cell culture, but was not seen in our *in vivo* model,[4] and further studies of age-dependent local anesthetic effects *in vivo* are warranted.

We agree with Engelhardt *et al.* that hypoxia has the potential to cause major morbidity and mortality in children; but this is a different issue and we would hope that the risk is small in children undergoing elective surgery with adequate monitoring and skilled anesthetic care. Similarly, complications after regional analgesia are fortunately rare, although the focus has been on major neurologic complications rather than on the more sensitive evaluations of sensory function. Our concern relates to neuraxial administration of drugs without adequate preclinical safety data, particularly in clinical studies of healthy children undergoing unilateral hernia repair as referenced by Englehardt *et al.*, where equivalent analgesia can be achieved with less invasive measures[7] or with neuraxial drugs with a more comprehensive safety record. As stated previously,[8] we are very aware and supportive of the important role of regional analgesia for perioperative analgesia in children. However, preclinical assessment of the relative toxicity of current and potential new neuraxial drugs, particularly in early life when the developing nervous system may be more vulnerable, has an important role in informing clinical choice. If a range of drugs have similar analgesic efficacy, it would seem prudent to choose a drug with a wide therapeutic index and documented safety record.

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References

1. Walker SM, Westin BD, Deumens R, Grafe M, Yaksh TL: Effects of Intrathecal Ketamine in the Neonatal Rat: Evaluation of Apoptosis and Long-term Functional Outcome. ANESTHESIOLOGY 2010; 113:147–59
2. Zou X, Patterson TA, Divine RL, Sadovova N, Zhang X, Hanig JP, Paule MG, Slikker W, Wang C: Prolonged exposure to ketamine increases neurodegeneration in the developing monkey brain. Int J Dev Neurosci 2009; 27:727–31
3. Young C, Jevtovic-Todorovic V, Qin YQ, Tenkova T, Wang H, Labruyere J, Olney JW: Potential of ketamine and midazolam, individually or in combination, to induce apoptotic neurodegeneration in the infant mouse brain. Br J Pharmacol 2005; 146:189–97
4. Westin BD, Walker SM, Deumens R, Grafe M, Yaksh TL: Validation of a Preclinical Spinal Safety Model: Effects of Intrathecal Morphine in the Neonatal Rat. ANESTHESIOLOGY 2010; 113:183–99
5. Werdehausen R, Fazeli S, Braun S, Hermanns H, Essmann F, Hollmann MW, Bauer I, Stevens MF: Apoptosis induction by different local anaesthetics in a neuroblastoma cell line. Br J Anaesth 2009; 103:711–8
6. Braun S, Gaza N, Werdehausen R, Hermanns H, Bauer I, Durieux ME, Hollmann MW, Stevens MF: Ketamine induces apoptosis *via* the mitochondrial pathway in human lymphocytes and neuronal cells. Br J Anaesth 2010; 105:347–54

7. Howard RF, Carter B, Curry J, Morton N, Rivett K, Rose M, Tyrrell J, Walker SM, Williams DG: Association of Paediatric Anaesthetists: Good Practice in Postoperative and Procedural Pain. Pediatric Anesthesia 2008; 18(Suppl 1):1–81
8. Walker SM, Yaksh TL: New caudal additives in children: Benefit *versus* risk? Acta Anaesthesiol Scand 2009; 53:1097–8

(Accepted for publication November 10, 2010.)

Presenting Data *versus* Predictions as Basic Scientific Information: Target-controlled Infusions *versus* Microgram per Kilogram per Minutes

To the Editor:

In the August issue of ANESTHESIOLOGY, I found two articles of particular interest regarding propofol administration.[1,2] I was simply confounded by the fact that target-controlled infusion (TCI) "predicted" concentrations have become the basic jargon for scientific papers. In both articles, I never found any TRUE raw data disclosing the actual dose of drug administered to these patients. In addition, the index of anesthesia in the article by Rigouzzo *et al.*[2] was the bispectral index value (another proprietary, *i.e.*, undisclosed program). In particular, the article by Rigouzzo *et al.*[2] demonstrated that true differences exist between the multiple studied TCI models (and probably all others as well). I was confounded for several reasons: (1) TCI is not currently used in the United States and probably will remain withheld from clinical use by the Food and Drug Administration; (2) there apparently are multiple TCI devices with unknown (to any U.S. clinician) validity and deviations in ability/accuracy; and (3) TCI values are *predictions* and not measured values in any individual study; (4) multiple variables influence *actual* plasma concentrations in any given patient or patient group; and (5) finally, the actual TCI infusion rates change over time. Our journal (ANESTHESIOLOGY) is a publication of the American Society of Anesthesiologists, where practice remains relevant in terms of microgram per kilogram per minute during propofol infusion. It would seem appropriate to require, at a bare minimum, presentation of this pertinent information to the readership (at least alongside TCI values) for several reasons: (1) microgram per kilogram per minute is the American "frame of reference"; (2) microgram per kilogram per minute is REAL and not proposed/extrapolated scientific information; and (3) TCI devices should/must disclose the instantaneous infusion rate during the relevant study periods.

Although I understand the practicability of "indexing anesthetic depth" to some form of electroencephalogram monitor for studies for total intravenous anesthesia anesthetics, I would hope the *Journal* would also require end-tidal gas concentration disclosure for any inhaled agent mentioned in a manuscript. As a clinical scientist, it is essential to *know* what is *actually* being administered to correlate to truly dependent variables such as bispectral index or TCI, especially because no single electroencephalogram monitor or TCI program has been accepted as the standard for scientific studies or even clinical use in the United States. I concluded that I simply came away from both articles without meaningful clinical information—clinical information being why I read this journal. I personally suspect Bandschapp *et al.*[1] found "analgesic properties of propofol" simply because pain is the *conscious* perception of noxious stimulation, and impairment of consciousness resulted in these findings (with probably 60 microgram per kilogram per minute of propofol infusing). Perhaps ANESTHESIOLOGY might lead the world's journals to take on such a basic standard of presenting facts (infusion rates) instead of predictions (TCI/bispectral index) as basic science and in the interests of our readership.

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References

1. Bandschapp O, Filitz J, Ihmsen H, Berset A, Urwyler A, Koppert W, Ruppen W: Analgesic and antihyperalgesic properties of propofol in a human pain model. ANESTHESIOLOGY 2010; 113: 421–8
2. Rigouzzo A, Servin F, Constant I: Pharmacokinetic-pharmacodynamic modeling of propofol in children. ANESTHESIOLOGY 2010; 113:343–52

(Accepted for publication November 24, 2010.)

In Reply:

We have read with great interest and also some concern the letter of Dr. Kempen regarding our manuscript.[1] To begin with the last point, the problem of discriminating analgesia from other effect-like sedation is discussed in our article. This is a typical problem when studying pain and has been discussed in a recent article on ketamine, where the euphoric effect of the drug interacted with its analgesic effect.[2]

With regard to Dr. Kempen's main criticism, he is completely right that dose is basic information that should be given in the article. Therefore, we are glad to have the opportunity to supply this information *ex post*: the total dosage was 4.4 mg/kg in 45 min with a maintenance infusion rate of approximately 90 $\mu g \cdot kg^{-1} \cdot min^{-1}$. However, we do not agree with Dr. Kempen that the predicted/targeted concentrations are not "real." Of course, these concentrations are predicted values that will differ from measured concentrations. However, if one looks at these concentrations as "targets," the view changes a bit: for the user, the target concentration set at the target-controlled infusion system is as "real" as the infusion rate set at a normal infusion pump. If one uses a defined system, that means a commercially available target-controlled infusion system with a defined pharmacokinetic parameter set, the information that a defined concentration was targeted is as definite as the information that a defined infusion rate was chosen. This means that any