tutes. As well as Akt phosphorylation via the tropomyosin receptor kinase signaling pathway, p75NTR has been shown to increase phosphorylated Akt in some systems using the neurotrophin NGF.6 In figure 4C, when DIV-5 cultures were treated with control small interfering ribonucleic acid, the isoflurane treated cultures had a higher level of p75NTR than control cultures. p75NTR staining of cultures or western blot analysis of p75NTR levels would allow this hypothesis to be further investigated.

In addition to the regulation of TPA secretion, p75NTR levels are also an important determinant of isoflurane-mediated neuronal changes. In summary, there may be a two-part mechanism to the isoflurane-mediated neuronal response, an increase in p75NTR levels, and a decrease in TPA release, a threshold of which is required to obtain the isoflurane-mediated neuronal changes.

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In Reply—We thank Panni and Panni for their interest in our research article published in Anesthesiology.1 The data in that publication provided strong proof that isoflurane neurotoxicity in neonatal rodent pups and in neurons in culture (days in vitro [DIV] 5–7) is mediated at least in part by reduced tissue plasminogen activator release and increased probrain-derived neurotrophic factor signaling via the p75 neurotrophic receptors (p75NTR). This contention is supported by a reduction in tissue plasminogen activator release, increased p75NTR-mediated e-Jun N-terminal kinase activation, prevention of toxicity by Fc-TrkB (scavenges probrain-derived neurotrophic factor), and by exogenous tissue plasminogen activator and prevention of toxicity by Pep5 (a specific peptide inhibitor of p75NTR). Moreover, knockdown of p75NTR by small interfering ribonucleic acid also mitigated toxicity. Multiple lines of evidence therefore support our contention.

That said, in a comprehensive study, new questions about the possible mechanisms inevitably arise; these serve as impetus for future studies. Panni and Panni have raised several concerns. Apoptosis was evaluated by activated caspase-3 staining only, and other means of identification of apoptotic cells, such as terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling staining, were not used. The use of activated caspase-3 staining for the detection of apoptosis is well established. Nonetheless, to corroborate the activated caspase-3 data, we also used caspase-activated DNase, a highly specific marker of apoptosis, in our immunoblot studies. In those studies, caspase-activated DNase and activated caspase-3 results were similar. We have also previously used caspase-activated DNase, a highly specific marker of apoptosis, in our immunoblot studies. In those studies, caspase-activated DNase and activated caspase-3 results were similar. We have also previously used caspase-activated DNase, a highly specific marker of apoptosis, in our immunoblot studies. In those studies, caspase-activated DNase and activated caspase-3 results were similar.

We agree with Panni and Panni that levels of p75NTR expression might account for some of our findings. As indicated by them, p75NTR expression decreases with increasing age.3,4 The relatively high expression of p75NTR at postnatal days 5–7 (or DIV 5–7) would make neurons more vulnerable upon anesthetic exposure. The proposed mechanism of p75NTR expression changes based on age is interesting from a developmental standpoint, and of course would be strengthened with data revealing the expression profile of p75NTR in the developing central nervous system. The possibility that isoflurane increases p75NTR is also of interest, as indicated by Panni and Panni. While the immunoblot data are suggestive of an increase in p75NTR expression with isoflurane, we currently do not have definitive data. Unpublished data from our laboratory have indicated that isoflurane neurotoxicity is evident as early as 30 min after exposure in vitro, and this toxicity is abolished by p75NTR inhibition. This time frame is quite short and argues against the premise that isoflurane increases p75NTR expression. Nonetheless, we are in the process of defining not only age-related effects, but also the effect of isoflurane on the expression of p75NTR in our experimental models.

While total p75NTR expression levels are certainly of interest, the precise means by which p75NTR signals and its interaction with other partner proteins is just as important. p75NTR, which is a member of the tumor necrosis factor receptor family, protein expression can increase in pathologic states.5,6 However, p75NTR can interact with tropomyosin receptor kinase (Trk) to induce neurite outgrowth and cell survival through either recruitment and transportation of Trk receptors or through enhanced affinity and specificity.7–9 or it can induce neuronal apoptosis independent of Trk receptors through alternative signaling pathways.5,9 An alternative explanation to age-related reduction in receptor expression is an alteration in the coupling between the p75NTR and Trk A/B/C receptors, thus moving p75NTR more towards prosurvival signaling via downstream effectors such as Akt, Src or ERK1/2, and further away from a p75NTR-c-Jun N-terminal kinase inhibitor, TAT-Pep5. When taken in aggregate, our data clearly demonstrate the multiple facets of injury produced by isoflurane.

A second concern is that apoptosis was evaluated within a very narrow window (2 h) after exposure, and injury was not evaluated at later time points. In published studies of anesthetic neurotoxicity, apoptosis is detected early after exposure. In fact, much of apoptosis is not observed 24 h after exposure. The intention of our study was not to repeat the work previously published with respect to the time course of neuronal apoptosis, but to define the underlying molecular mechanisms of injury. With that intent, the selection of a single time point at which a substantial amount of injury is evident is entirely justified.

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Difficult Mask Ventilation and Neuromuscular Blockade

To the Editor:—We read with interest the article by Kheterpal et al. 1 regarding impossible mask ventilation. This is a very important but rare event, and this large study gives us a clear idea about its incidence and, for the first time, what the associated risk factors are.

We note that in all but 4 of the 77 cases of impossible mask ventilation, the patients had received neuromuscular blockade “in the process of induction or management of the airway,” with succinylcholine being used in 65 patients and a nondepolarizing agent in the remaining patients. However, it is not clear at what stage of airway management that the neuromuscular blocker was administered in these cases—was it before difficulty with mask ventilation being encountered or given after problems occurred to improve the situation, or was it before difficulty with mask ventilation being encountered at all? The authors do go on to discuss the problem in assessing the role of neuromuscular relaxation before intubation and the presence of a patent airway being critical to intubation. However, this leaves a large population of patients who had difﬁcult intubations, in whom the role of neuromuscular blockade cannot be assessed.

We believe it is important to note that in a large study, such as this, a large population sample would be required.

In our experience, optimum depth of anesthesia and neuromuscular blockade provide the best conditions for both mask ventilation and tracheal intubation in patients in whom an awake technique, transtracheal catheter, or awake tracheostomy are not indicated. Neuromuscular blockade given at induction and before attempts at mask ventilation is the most common practice in our institution for patients requiring tracheal intubation. In addition, we have found that using intermittent positive pressure ventilation by means of a Penlon Nuffield 200 ventilator (Penlon Ltd., Abingdon, United Kingdom) while holding a mask is beneficial for assessment of adequacy of mask ventilation and also useful for training. This approach has the advantage of allowing a two-handed mask technique for more challenging airways and continual monitoring of airway pressure from the pressure gauge on the ventilator. Monitoring airway pressure in this way provides an objective measure of the seal that is achieved with the mask and patency of the airway. Mask ventilation can then be optimized by reference to clinical signs (e.g., chest expansion), airway pressure/peak pressure, and capnography. We also encourage initial management of the awake airway without use of an oropharyngeal/Guedel airway to improve and optimize these fundamental airway skills. Mask ventilation is our core skill, and we believe subjective and objective assessment throughout training is required to maintain this art and limit airway disasters.

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Reference


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