tures. As well as Akt phosphorylation *via* the tropomyosin receptor kinase signaling pathway, $p75^{NTR}$ 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 $p75^{NTR}$ than control cultures. $p75^{NTR}$ staining of cultures or western blot analysis of $p75^{NTR}$ levels would allow this hypothesis to be further investigated.

In addition to the regulation of tPA secretion, $p75^{NTR}$ 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 $p75^{NTR}$ 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 neutrotrophic factor signaling *via* the p75 neurotrophic receptors ($p75^{NTR}$). This contention is supported by a reduction in tissue plasminogen activator release, increased $p75^{NTR}$-mediated c-Jun N-terminal kinase activation, prevention of toxicity by Fc-TrkB (scavenges probrain-derived neutrotrophic factor), and by exogenous tissue plasminogen activator and prevention of toxicity by Pep5 (a specific peptide inhibitor of $p75^{NTR}$). Moreover, knockdown of $p75^{NTR}$ 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 immunofluorescence and the results with this technique are identical to those used from activated caspase-3 staining.[2] Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling staining, by contrast, is not specific for apoptosis as deoxyribonucleic acid methylation is observed in cells undergoing necrosis. While additional methods of apoptosis detection would provide some incremental information, the relative value of this information, in so far as the support or refutation of the primary hypothesis is concerned, would be at best limited. We are therefore comfortable with the use of activated caspase-3 and caspase-activated DNase for detection of apoptosis. We also wish to point out that apoptosis was not the only endpoint of the study. Additional immunofluorescence with drebrin staining and electron microscopic analysis revealed the damage at a cytoskeletal/morphologic level. The morphologic alterations induced by anesthesia on developing neurons included $p75^{NTR}$-mediated loss in dendritic spines (as indicated by drebrin loss) and morphologic loss of intact synapses, both of which were attenuated by the intracellular $p75^{NTR}$ 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.

We agree with Panni and Panni that levels of $p75^{NTR}$ expression might account for some of our findings. As indicated by them, $p75^{NTR}$ expression decreases with increasing age.[3,4] The relatively high expression of $p75^{NTR}$ at postnatal days 5–7 (or DIV 5-7) would make neurons more vulnerable upon anesthetic exposure. The proposed mechanism of $p75^{NTR}$ expression changes based on age is interesting from a developmental standpoint, and of course would be strengthened with data revealing the expression profile of $p75^{NTR}$ in the developing central nervous system. The possibility that isoflurane increases $p75^{NTR}$ is also of interest, as indicated by Panni and Panni. While the immunoblot data are suggestive of an increase in $p75^{NTR}$ 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 $p75^{NTR}$ inhibition. This time frame is quite short and argues against the premise that isoflurane increases $p75^{NTR}$ expression. Nonetheless, we are in the process of defining not only age-related effects, but also the effect of isoflurane on the expression of $p75^{NTR}$ in our experimental models.

While total $p75^{NTR}$ expression levels are certainly of interest, the precise means by which $p75^{NTR}$ signals and its interaction with other partner proteins is just as important. $p75^{NTR}$, which is a member of the tumor necrosis factor receptor family, protein expression can increase in pathologic states.[5] However, $p75^{NTR}$ 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,[6–8] 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 $p75^{NTR}$ and Trk A/B/C receptors, thus moving $p75^{NTR}$ more towards prosurvival signaling *via* downstream effectors such as Akt, Src or ERK1/2, and further away from a $p75^{NTR}$– c-Jun N-terminal kinase-

apoptotic pathway. Use of immunoprecipitation experiments at different developmental time points after receptor agonism may explain whether this is an alteration in receptor signaling or changes in receptor expression with age. What does appear to be known is that $p75^{NTR}$ expression and signaling is not only temporally but also spatially dependent on some unknown intracellular mechanism. Studies to characterize $p75^{NTR}$ expression and its coupling with known partners (*e.g.*, Trk) at varying ages are currently underway in our laboratory. The expectation is that these studies will provide more detail about the mechanisms by which isoflurane injures developing neurons.

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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, and did ventilation indeed improve? Furthermore, only 19 patients (25%) proved difficult to intubate, which suggests that there was opportunity for improving the conditions for mask ventilation. Kheterpal *et al.* do go on to discuss the problem in assessing the role of muscle relaxants in mask ventilation difficulties, but the documentation for each case did not include an assessment of mask ventilation before and after neuromuscular blockade. It would be interesting to note if there is a difference in the incidence of impossible mask ventilation with or without neuromuscular blockade being given at induction (before attempts at mask ventilation). This may be an area for further investigation, although as with this study, 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 technique 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 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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In Reply:—We thank Drs. Myatt and Patel for their interest in our data and comments. We agree that detailed, controlled data collection regarding the ease or difficulty of mask ventilation before and after administration of neuromuscular blockade would be of great interest. Unfortunately, as our original manuscript mentioned,[1] collecting these data using a large observational dataset is difficult. Aggregation of a 50,000-patient dataset has necessary limitations. Although observational data are exceptional for establishing the real-world effectiveness