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In Reply:—It was an honor to read the response of Dr. Moore to my editorial about defining professionalism within anesthesiology. Dr. Moore's comments are presented from the perspective of the President of the American Society of Anesthesiologists (ASA), highlighting several important issues not emphasized in my manuscript because of space constraints of the editorial format. The Lifeline Campaign is an important initiative to improve the public image of the anesthesiologists, and many of the elements professionalism defined in the editorial will be essential to the success of the Lifeline Campaign. Defining professionalism as a competency with standards to measure achievement has implications beyond residency. Initial board certification is now time-limited, and those with non-time–limited certificates are voluntarily recertifying. Professionalism undoubtedly will be a core competency. Just as assessment drives behavior and learning during training (medical school, residency), there is every reason to hope that the same effect will continue into practice. The leadership role of the ASA has been sustained. The efforts to counteract the plague of substance abuse and the efforts to define ethical issues within anesthesiology are evidence of decades of focused, funded efforts to advance these elements of professionalism, among others. The action of the 2008 House of Delegates to advance wellness among anesthesiologists was the most recent contribution to this important effort. The absence of direct praise for these efforts in my editorial is another casualty of word count.

For years, I have arbitrarily presented anesthesiology professionalism in four parts. The obligation to participate in the larger world of state and national issues, and the interface between anesthesiology and society as a whole was embedded in one of these elements ("within anesthesiology"). Again to be concise in my editorial, involvement did not get the emphasis or urgency it deserves. Without space constraints, our residency presentations expand this element of professionalism. As program director of a large anesthesiology residency, I have been fortunate to be supported by chairs that have supported active roles in our state society, and the ASA. Our resident participation in the ASA Political Action Committee has been substantial and a direct result of exposure to the issues as a result of participation (or "involvement").

Given the urgency of the issues, "involvement" will become the fifth element of professionalism when I discuss this subject in the future. Dr. Moore's concerns for the future of the specialty are undoubtedly a daily issue for the President of the ASA. The *Men's Health* article[1]; the movie "Awareness;" the sustained efforts by nurses to encroach on the practices of anesthesia, pain medicine and procedural sedation; and the media attention to deaths related to substance abuse set the stage. Pending healthcare reform will provide the script, which has not been written as of now. Whether the play is a drama (patient safety), comedy (with the physician anesthesiologist for humor) or tragedy (with the trivialization of anesthesia as the practice of medicine) will be determined in the near future.

Dr. Moore's call to "involvement" should be taken seriously by all physicians who are making a living providing, teaching, or studying anesthesiology, including perioperative medicine, critical care, and pain medicine. Medical students should observe involvement as an essential element of anesthesiology during their first experiences. Residencies should provide faculty and peer mentorship for participation in state and national societies and governmental affairs. Faculty should demonstrate the value of ASA Political Action Committee participation for residents by their own participation. Academic departments that sponsor residency programs should encourage involvement with time, financial support, and senior faculty mentorship. Departments should sponsor medical students from their sites to attend our national meeting. Residents should be encouraged to join the ASA, state societies, and participate in resident components of state and the national society, and to seek appointment on state and national committees. All of this good work by those in training should be based on imitation and mentorship of faculty, who must be the best possible role models. If the "rank and file" anesthesiologist does not meet this challenge, the future of anesthesiology professionalism may be less important, if the role of the profession in our healthcare system diminishes.

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Role of p75 Neurotrophin Receptor in Isoflurane-mediated Neuronal Changes

To the Editor:—We read with interest the article by Head *et al.*, investigating the role of p75 neurotrophin receptor ($p75^{NTR}$) in isoflurane-mediated neuronal changes.[1] The central premise presented is that isoflurane neurotoxicity results mainly from the prevention of activity-dependent release of tissue plasminogen activator (tPA). tPA converts plasminogen to plasmin, which then cleaves probrain-derived neurotrophic factor to mature brain-derived neurotrophic factor.[2] In the absence of tPA, excess probrain-derived neurotrophic factor levels are thought to activate a $p75^{NTR}$-mediated synaptic reduction and increase cleaved caspase 3 levels. The increased cleaved caspase 3 is assumed to indicate increased apoptosis; however, no other evidence is presented of this, such as terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling staining at a 24-/48-h time point.[3]

There is no investigation as to why this isoflurane-mediated toxicity is only seen in early neuronal cultures and early postnatal ages. We would like to suggest that this may be because of altered $p75^{NTR}$ expression levels. There is an inverse relationship to $p75^{NTR}$ levels in the hippocampus with age.[4,5] The absence of isoflurane effects at postnatal Day 21 may in part be a result of lower or redistributed $p75^{NTR}$ levels. The apoptotic pathway *via* $p75^{NTR}$ could also require additional coreceptors/cofactors that are themselves developmentally regulated.

The cell culture experiments are confounded by the fact that mixed neonatal cortex and hippocampal cultures are used. This would mean that there is inherent variation in neurite morphologies. No plating density is given, but we assume that 5 days *in vitro* (DIV) cultures have low numbers of cell-cell contacts, whereas by DIV-14 or 21, a neurite network has been established.

In addition to changing $p75^{NTR}$ levels with neuronal development and culture times, we suggest that isoflurane treatment may also alter the level of $p75^{NTR}$. In figure 2D, isoflurane treatment of DIV-5 cultures in the presence of tPA seems to increase levels of phosphorylated Akt, as compared with control tPA treated DIV-5 cul-

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-