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In Reply:

We appreciate the interest of Drs. Dilger and Steinbach in our work and are grateful for their comments on our recent publication.[1] To properly study the molecular mechanism behind nondepolarizing neuromuscular blocking agents' (NMBAs) inhibition of the nicotinic acetylcholine receptor (nAChR)-mediated signaling in the human neuromuscular junction, an isolated preparation of the junction is needed. For obvious reasons, such approach is not possible and in lieu of that various techniques are used, ranging from acute neuronal preparations to various heterogeneous cellular expression systems. All these methods have different shortcomings in terms of relevance. The neuronal preparations often have insufficient washing to do proper *in vitro* pharmacology, and the heterogeneous systems often overexpress the receptors studied. Yet, what we can do is comparative *in vitro* pharmacologic studies using similar methods to compare potency and efficacy *in vitro* using receptors from relevant species. The *Xenopus* oocyte two-electrode voltage clamp system is very well suited for such studies. It is a widely used and well-established technique yielding stable and comparable *in vitro* pharmacologic data from numerous laboratories and has been doing so for 3 decades.

We agree with Drs. Dilger and Steinbach that whole cell two-electrode voltage clamp recordings from *Xenopus* oocytes are not a system well suited for detailed kinetic studies of receptor and ligand interaction. However, we never claim this in our article.[1] What we describe is the whole cell functional pharmacology of a range of nondepolarizing NMBAs studied on the human muscle nAChR activated by acetylcholine and dimethylphenylpiperazinium. We show that acetylcholine desensitizes the receptor, whereas dimethylphenylpiperazinium does not: when using larger concentrations of ACh (10 *vs.* 1 μM), we increase receptor desensitization determined by a decrease in current activated by repeated applications of acetylcholine[1] (figs. 2C and D). At the higher concentration of agonist, a classic competitive antagonist will be less efficacious and we do not observe this. In fact, we generally observe an increased efficacy of inhibition by the nondepolarizing NMBAs[1] (table 2). A careful analysis of the inhibition curves[1] (figs. 2 and 3) shows that nondepolarizing NMBAs induce both right-shifted curves and a depression of maximum currents that are the hallmarks of competitive and noncompetitive inhibition, respectively. The noncompetitive mode of action is primarily observed at higher concentrations of acetylcholine, which also induces receptor desensitization. Further, when using the nondesensitizing antagonist dimethylphenylpiperazinium, the inhibition becomes more competitive[1] (fig. 5 and table 5).

In this context, we have to remember that the resolution of the nAChR family X-ray and electron microscopy structures are insufficient to determine the exact molecular interactions, neither with nondepolarizing NMBAs nor with acetylcholine itself.[2] The structure of an acetylcholine-binding protein is known,[3] but nondepolarizing NMBA binding to this crystal is not studied. Thus, we have to acknowledge the fact that it is still only a theoretical model framework describing receptor function by multiple open, closed, and desensitized states. The published structures of the nAChR do not have the resolution to dissect between multiple open, closed, or desensitized states and of course not the transition between these. To resolve the functional interaction and kinetics between the nAChRs and ligands, we agree with Drs. Dilger and Steinbach that one must use either binding studies or outside out isolated patch recordings. However, the former is unable to resolve the functional effect of the interaction (agonism or antagonism) and the latter suffers from

disrupted cytoskeleton, absence of normal intracellular ion concentrations, and different membrane tension compared with the whole cell.

In short, there is no single ideal *in vitro* system for replacing the human neuromuscular junction. However, we believe that by using comparative *in vitro* pharmacology one can qualitatively describe clinically relevant pharmacological interactions on the cellular level. This is possible without resolving the exact distribution between multiple desensitized states.

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Ultrasound-guided Supraclavicular Block: What Is Intraneural?

To the Editor:

We read with interest the article by Bigeleisen *et al.*[1] in the June issue of the journal. This study brings two important questions to the fore: first, can a minimum stimulating current detect intraneural needle placement; and second, can this minimum current predict whether needle placement and local anesthetic injection will cause neurologic injury? The research of Bigeleisen *et al.* is designed to deal with the former question, but in a wider context, it is concerned with the fundamental issue of avoiding nerve injury.

Intraneural needle placement does not inevitably lead to nerve injury,[2,3] in the etiology of which the perineurium may be a more critical barrier than the epineurium.[4,5] Nerve fascicles may escape direct injury from subepineural needles because of the tough perineurium that surrounds them and the considerable amount of compliant connective tissue within the epineurium.[6,7] Nevertheless, the possibility of causing nerve injury by direct trauma with a needle, or by toxic or ischemic effects of injection of local anesthetic, has made the avoidance of intraneural injection a basic rule of regional anesthesia.[1,4] However, the anatomical site and the methodology chosen by Bigeleisen *et al.* have led us to question whether the authors achieved their primary objective of comparing intraneural and extraneural minimum stimulating currents and to address our own technique of ultrasound-guided supraclavicular block.

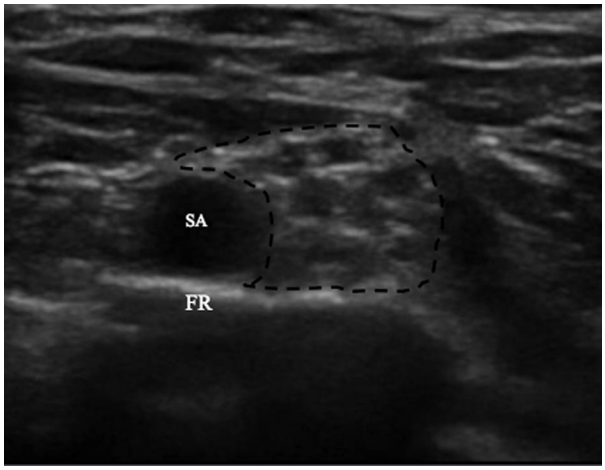

Fig. 1. Ultrasound image of brachial plexus before supraclavicular block. *Dashed line* = approximate area of brachial plexus lateral to artery. FR = first rib; SA = subclavian artery.

Our question rests on the definition of intraneural needle placement at the level of the supraclavicular brachial plexus. Bigeleisen *et al.* describe the outer border of the entire plexus as the epineurium, breach of which defines intraneural. The brachial plexus is a network of nerves—if each of these is considered to have its own epineurium, then the definitions in this study do not hold true. However, the area of enquiry is a compact segment of a plexus, ultrasound images of which rarely resolve into distinct trunks or divisions (fig. 1) and where separate trunks and divisions may not be visually distinct on cadaver cross sections.[1,7]

In our practice of ultrasound-guided supraclavicular block, we intentionally breach the layer that Bigeleisen *et al.* describe as epineurium.[8,9] This is observed in real time and often felt by the operator as a loss of resistance or "pop." We attempt to avoid injecting into what we believe to be nerves (seen as predominantly hypoechoic circular structures lateral to the subclavian artery; fig. 1) by observing both the needle tip as it advances and the spread of local anesthetic (fig. 2), which will likely cause the nerves to move but should not

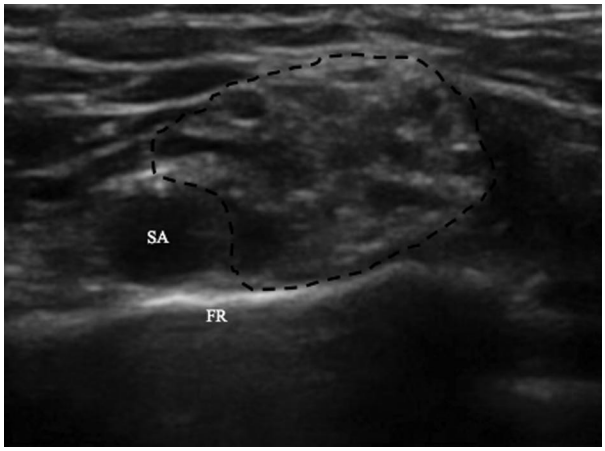

Fig. 2. Appearance after injection of 30 ml local anesthetic. *Dashed line* = approximate area of brachial plexus lateral to artery. FR = first rib; SA = subclavian artery.