Introduction: Skeletal muscle lacerations repaired by epimysial suturing of the cut ends and followed by immobilisation, often does not recover well [1-3]. It was proposed that in a completely lacerated skeletal muscle, the intramuscular nerve that is concomitantly cut with muscle laceration results in poor nerve recovery, and to some extent accounts for incomplete healing of skeletal muscles. While if the intramuscular nerve is preserved intact, muscle recovery improved [4,5]. We investigate the distribution of the intramuscular nerve in the medial gastrocnemius of a rat using the Sihler’s in-toto staining for myelinated nerves in skeletal muscles [6]. This paper demonstrates the differences in the intramuscular nerve distribution in lacerated skeletal muscles that have been repaired, and compares the case where the intramuscular nerve is either cut, damaged or preserved intact in a lacerated muscle, two weeks after repair.

Materials and Methods: The left medial gastrocnemius (MG) of the 12-week old Male Sprague-Dawley (SD) rat was used the lacerated skeletal muscle model. The right limb was the control where no surgery was performed (IACUC, NUS approved). In all groups, the muscles was completely divided with a surgical blade at the largest transverse diameter of the belly, just distal to the entry point of the peripheral nerve into the muscle at about 75% of the distal muscle length [4]. The muscle cut ends were surgically repaired using 4-0 Poly-propylene core sutures and a continuous epimysial suture. Sixty-four rats (mean body mass=313 ± 39g) were assigned to four groups: C-Neg, where the muscles peripheral nerve was cut and ligated; NNR, where the intramuscular nerve was not repaired; NP, where the intramuscular nerve was preserved intact but crushed at the level of the laceration, not disrupting the nerve sheath, simulating an end-to-end nerve repair. The normal contralateral limb was used as a control (Cont). All muscles were harvested at 2-weeks. Sihler’s Staining technique: The specimens were immersed in 10% unneutralised formalin (3-wks). Thereafter, the specimen was washed (30-min), and then macerated in 3% KOH (4-wks) making the muscle transparent. The macerated specimen were then washed (30-min) and decalcified (2-wks) in Sihler’s solution I [6] until they became transparent. The specimens were then washed (30-min) and soaked in Sihler’s solution II [6] for 2-wks until the finest nerve branches were visibly stained. The specimens were then washed (30-min) and immersed in 0.05% Lithium Carbonate solution (2-hr) until the nerves were noted to turn dark blue. The specimen were then washed (30-min) and decalcified (2-wks) in KOH (4-wks) making the muscle transparent or translucent with the finest nerve branches continuing beyond the lacerated site, NP more than the NR, with improved recovery in the distal region, and earlier start of repaired process at the lacerated site, compared to the NNR and CNeg, which remained necrotic.

Discussion: If the intramuscular nerve is concomitantly cut or damaged in a lacerated skeletal muscle that is repaired, then the recovery will be poor [4,5]. If the nerve is not cut and is preserved [5] or repaired [4] then the muscle recovery is much improved. This paper further demonstrates that the integrity of the nerve across the site of the laceration plays a role in the recovery of the muscle following repair.