was achieved through feeding of animals with diet containing Dox. Dox-induced reduction of JP expression led to abnormal membrane structure and compromised store-operated $Ca^{2+}$ entry in adult muscle fibers, consistent with JP's essential role in muscle development and function. This transgenic system can be applicable for inducible and reversible knockdown of different genes and in different tissues, as well as for control of transgene overexpression in an inducible and tissue-specific manner, thus providing a versatile system for eluciding the physiological gene function using viable animal models.

**3190-Pos Board B295**

**Extrusion of $Ca^{2+}$ Across the Tubular System Membrane is Dependent on Membrane Potential and the Cytoplasmic $Ca^{2+}$ in Rat Skeletal Muscle**

Joshua N. Edwards, **Bradley S. Launikonis**.

Little is known about the ability of the tubular (t-) system membrane in skeletal muscle to extrude $Ca^{2+}$ from the fibre, which is presumably due mostly to the activity of the $Ca^{2+}$-pump. Therefore we aimed to characterise t-system $Ca^{2+}$ translocation properties by changing the steady-state $[Ca^{2+}]_{cyto}$ and resting membrane potential. To do this, we imaged fluo-5N in the t-system of rat mechanically skinned extensor digitorum longus fibres bathed in a $K^+$- or $Na^+$-based internal solution on a confocal microscope. Fluo-5N was calibrated *in situ* and had a Kd of ~320 μM. Following depletion of $[Ca^{2+}]_{t\text{-}sys}$ by chronic activation of store-operated $Ca^{2+}$ entry (SOCE) in a solution containing 10 μM $Mg^{2+}$, 5 mM BAPTA and 5 mM caffeine, the fibre was exposed to an internal solution containing 1 mM $Mg^{2+}$, 1 mM EGTA and either 100, 200 or 800 nM $Ca^{2+}$. $[Ca^{2+}]_{t\text{-}sys}$ increased to hundreds of μM and mM levels in polarized and depolarized fibres, respectively, in seconds due to the different driving forces for $Ca^{2+}$ across the t-system. In some fibres, vacuolated longitudinal tubules (Edwards & Launikonis, 2008, J Physiol) were observed with a high $[Ca^{2+}]$. Under conditions of chronic SOCE activation, the transverse tubules rapidly depleted of $Ca^{2+}$ (seconds) while high $[Ca^{2+}]$ persisted in the vacuolated longitudinal tubules for $>$ 20 min (n=3). Subsequent exposure to an internal solution with 1 mM $Mg^{2+}$ and 800 nM $Ca^{2+}$ saw a rapid (seconds) increase in transverse tubular $[Ca^{2+}]$ that was trailed by a similar $Ca^{2+}$ increase in longitudinal tubules by at least 5 min.

**3191-Pos Board B296**

**Using Superfast Confocal Microscopy to Measure the $Ca^{2+}$ Release Waveform and Spread of Excitation throughout the Tubular Network in Mammalian Skeletal Muscle**

**Joshua N. Edwards**, Tanya R. Cully, Thomas R. Shannon,
Bradley S. Launikonis.

In skeletal muscle, uniform release of $Ca^{2+}$ is dependent on the rapid spread of excitation across the sarcolemma and throughout the tubular (t-) system. The t-system primarily consists of transverse, but also longitudinal tubules, interconnected throughout to form an extensive membranous network along the fibre. Both tubules can conduct action potentials, however, the rapid rising phase of the action potential-induced $Ca^{2+}$ transient and underlying release flux as well as any longitudinal spread of excitation between sarcomeres cannot be adequately measured with conventional imaging techniques. Therefore, we imaged $Ca^{2+}$ transients with Oregon Green Bapta 5N (OGB5N) at 15.5 μs $line^{-1}$ on a Zeiss 5 LIVE confocal system from electrically stimulated mechanically skinned fibres from rat. We resolved the rising phase of $Ca^{2+}$ release from the SR during field stimulation, lasting some 1.5 to 5ms. From the OGB5N fluorescence signals that displayed a very uniform release of SR $Ca^{2+}$ upon stimulation, we derived the release flux to rise to a peak of 120mM/s in about 0.6ms and continue at a rapidly decreasing rate for a further 2ms. Following field stimulation in some skinned fibres, we observed that in areas where transverse tubules failed to be excited by the initial stimulus $Ca^{2+}$ release propagated in from the adjacent regions at a rate of ~16 μm $ms^{-1}$. The areas where depolarization via the field pulse failed to depolarize transverse tubules could be up to 50 μm along the long axis of the fibre. This area was subsequently always excited by action potentials propagating inward along longitudinal tubules to initiate $Ca^{2+}$ release.

**3192-Pos Board B297**

**Differential Recording of Voltage Changes at the Surface and Transverse Tubular System Membranes of Mammalian Skeletal Muscle Fibers using Di-8-Anepps and Global and TIRFM**

**Joana C. Capote**, Marino DiFranco, Julio L. Vergara.

Aiming to investigate the distribution of ClC-1 and $K_{IR}$ channels at the sarcolemma and transverse tubular system (TTS) membranes of mammalian skeletal muscle fibers, we used global and total internal reflection fluorescence microscopy (TIRFM) to monitor voltage changes in these compartments, respectively. Enzymatically-dissociated fibers from murine *FDB* and *interosseus* muscles were stained with the potentiometric dye di-8-ANEPPS, and voltage-clamped with a two-microelectrode system. Ion substitutions were used to isolate and characterize the specific ClC-1 ($I_{Cl}$) and $K_{IR}$ ($I_{KIR}$) currents: 70 mM internal $[Cl^-]$ and 120 mM external $[K^+]$, respectively. Also, 9-ACA and TEA were used to, respectively, block these currents. Global di-8-ANEPPS signals report, early after the onset of large hyperpolarizing pulses, $I_{Cl}$-dependent attenuations with respect to those recorded in the presence of 9-ACA. Peak attenuation levels of ~35% were observed for $I_{Cl}$ of ~900 μA/$cm^2$. Large attenuations were similarly observed in global signals recorded the presence of large $I_{KIR}$'s with respect to those in TEA. In contrast, TIRFM di-8-ANEPPS signals demonstrate only minor current-dependent attenuations ($<$10%) under conditions in which global signals evidenced much larger attenuations. Overall, our results demonstrate that voltage changes at the TTS membranes display prominent current-dependent attenuations while the sarcolemma is largely under voltage-clamp control. A radial cable model of the TTS, including equations for each current pathway and luminal accumulation/depletion of ions, was used to quantitatively predict the ionic currents and to assess their effects on average TTS voltage changes. Comparative analysis of global optical data with model predictions of voltage changes in the TTS suggests that both ClC-1 and $K_{IR}$ channels are equally distributed in both membrane compartments. Supported by NIH grants AR047664, AR041802, and AR054816.

**3193-Pos Board B298**

**Fatigue-Induced Kinetic Changes in Tetanic $Ca^{2+}$ Transients in Enzymatically Dissociated Mouse Fibers**

**Juan C. Calderón**, Pura Bolaños, Carlo Caputo.

We used enzymatically dissociated FDB and *Soleus* fibers loaded with the fast $Ca^{2+}$ dye Magfluo-4 AM (Calderón et al. 2010), to test whether repetitive stimulation induces progressive changes in the kinetics of $Ca^{2+}$ release and reuptake in a fiber type-dependent fashion. Control experiments applying repetitive stimulation to FDB fibers loaded with Fluo-3 AM demonstrated that the preparation reproduced the main previously published findings on $Ca^{2+}$ transients during fatigue, namely the tetanic $Ca^{2+}$ transient amplitude decrease and the basal $Ca^{2+}$ increase (Westerblad & Allen, 1991). When loaded with Magfluo-4 AM, a subgroup of MT-II fibers (fMT-II) showed significant reductions, of 28,1 and 29% (n=7), of the amplitude and the amplitude/rise time relationship of the first peak of the tetanic transient, respectively, after 40 tetani. A subgroup of more resistant MT-II fibers (rMT-II) showed reductions of 21,6 and 28,8% (n=6) only after 60 tetani. MT-I fibers (n=5) showed significant changes in both variables only after 80 tetani. fMT-II fibers also showed an increase of 33,6% in the half width value of the first peak. MT-I fibres did not show kinetic changes in the decay phase of the tetanic transients, while significant changes of 29,3 and 13,3% in the first time constant of decay ($t_1$) values were seen after only 20 tetani in fMT-II and rMT-II fibers, respectively. These changes were reversed after an average recovery period of 10 min. Further experiments ruled out the possibility that the differences in the kinetic changes of $Ca^{2+}$ release between fibers MT-I and MT-II could be related to the inactivation of $Ca^{2+}$ release mechanism. In conclusion, we show that a fatiguing protocol induces important kinetic alterations of both $Ca^{2+}$ release and reuptake, which are larger in fibers MT-II than in fibers MT-I (FONACIT G-2001000637).

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**Role of C-Term Tail of DHPR β1A in the DHPR/RyR1 Interaction**

Feng Wei, Kim Truong, Paul D. Allen, Isaac N. Pessah, **Claudio F. Perez**.

Although the mechanism by which the DHPR β1a subunit supports EC-coupling is still debatable it is apparent that C-terminal domain of β1a (β-Ct) is an intricate component of the interaction between the DHPR complex and RyR1. To characterize the molecular components of β-Ct involved in DHPR/RyR1 signaling we tested the effect of progressive truncation of β1a subunit on EC-coupling and RyR1 activity. To do this cDNA constructs carrying truncations of either the 52 (β-52), 38 (β-38) or 14 (β-14) most C-terminal amino acid residues of β1a were expressed in β-null myotubes and then tested for their ability to restore depolarization-induced $Ca^{2+}$ release in Fluo-4 loaded cells. Whereas β-null myotubes expressing constructs β-52 and β-38 were unresponsive to $K^+$ depolarization the cells expressing β-14 displayed EC-coupling that was indistinguishable from that of cells expressing wt-β1a ($K^+EC_{50}$: β1a = 23mM, β-14 =21mM). Thus, these results identify a segment of β-Ct of up to 24 amino acids that appears to be critical for the functional interaction between DHPR and RyR1 during EC-coupling. To test for specific interactions between the β-Ct and RyR1 we then studied the effect of purified β subunits on RyR1 activity. Using $^3$H-ryanodine