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The effect of training on motoneuron survival in amyotrophic lateral sclerosis: which motoneuron type is saved?

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Amyotrophic lateral sclerosis (ALS) is characterized by selective and progressive degeneration of motoneurons (MNs). Although the etiology of the disease is unknown, glutamate toxicity and reactive oxygen species toxicity have been strongly implicated in ALS pathophysiology. Training exercise has been proposed to provide a beneficial therapy during the early or late stages of ALS (Pinto et al., 1999; Drory et al., 2001); however, some studies showed deleterious effects of exercise on survival in ALS (Mahoney et al., 2004). The beneficial effects are based on the cellular adaptations induced by training exercise in the brain, spinal cord, and skeletal muscles that could counteract the oxidative stress complication in ALS. For instance, training exercise increases the capacity of antioxidant enzymes and reduces lipid peroxides in brain regions of rats (Somani and Husain, 1997; Husain and Somani, 1998). In skeletal muscle, training reduces oxidative stress following exercise (Miyazaki et al., 2001), increases the mitochondrial capacity (Holloszy et al., 1970), and increases the expression of neurotrophic factors (Gomez-Pinilla et al., 2001). The latter could be particularly beneficial in ALS because neurotrophic factors could prevent MN degeneration, preserve muscle innervation, and inhibit muscle atrophy (Acsadi et al., 2002; Manabe et al., 2002; Sun et al., 2002). Therefore, it is conceivable that training exercise could be beneficial by resisting oxidative stress and offsetting energy deficits caused by mitochondrial dysfunction in ALS. In a recent study, training exercise has been also shown to have neuroprotective effects in the spinal cord by saving MNs from degeneration, in addition to maintaining muscle fiber composition (Deforges et al., 2009). The Deforges et al. (2009) study is important for understanding the effect of training exercise in ALS because it compared two training paradigms and showed differential neuroprotective effects in the spinal cord. Here we discuss the

methodology and interpretation from the Deforges et al. (2009) study on which MN type is saved by training exercise, and try to reconcile their conclusions with the literature and more recent work.

Deforges et al. (2009) examined the effect of two training exercise paradigms (i.e., running and swimming) on MN survival and muscle fiber composition in the G93A (high expressor line) mouse model of ALS. In comparison to other ALS mouse models, this model has high level of expression of the mutant human SOD1 gene, rapid disease onset, and short life span. Training exercise was performed through 30 min daily running or swimming sessions that started at postnatal day 70 (P70) and lasted until P115 or death. Mice survival, MN count, and muscle fiber composition were assessed at P115 in the fast twitch muscles tibialis and plantaris and the slow twitch soleus muscle of the G93A mouse groups (i.e., running and swimming ALS groups), and were compared to normal, non-transgenic (control group), and untrained G93A transgenic mice (sedentary ALS group). MN count was based on soma cross-sectional area in which MNs were classified into three types: small (soma size $<300 \mu\text{m}^2$), medium (soma size $300\text{--}700 \mu\text{m}^2$), and large (soma size $>700 \mu\text{m}^2$) soma MN types. Muscle fiber composition was examined using immunofluorescence in which muscle fibers were incubated with antibodies raised against myosin heavy chains (MyHC) for slow (type I) and fast (types II, IIa, and IIb) fibers. The percentage of fast fiber type IIx was determined as the difference between the number of type II fibers and the sum of type IIa and IIb fibers [$\text{IIx} = \text{II} - (\text{IIb} + \text{IIa})$]. The results showed that in the sedentary ALS group the disease caused MN loss in the ventral horn of 49% relative to control with a reduction in the proportion of medium and large MN types and increase in the proportion of small MN type. In fast twitch muscles, the disease caused a significant transition in muscle fiber types from fast-to-slow (type II

to type I) and within the type II fiber type (from type IIb/IIx to type IIa). The slow twitch soleus muscle, on the other hand, experienced an increase in the proportion of type IIx muscle fibers. The G93A transgenic mice in the running ALS group did not have significant difference in symptom onset or survival time and had comparable loss of MNs (45%) to the sedentary group. In contrast, the G93A transgenic mice in the swimming ALS group survived longer and had delayed symptom onset relative to the sedentary ALS group. They also had higher MN total number and higher proportion of medium-size MNs relative to the sedentary ALS group, and maintained muscle fiber composition in all muscles similar to the control group. The authors concluded that swimming preferentially activated large MNs, innervating fast muscle fibers of type II), and protected them.

On their methodology, Deforges et al. (2009) measured the number of surviving MNs by counting MN cell bodies in the ventral horn of spinal cord. Although this provides anatomical evidence, this technique does not provide information on the functional state of MNs (i.e., whether surviving MNs produce muscle contractions). This is exceptionally important in ALS because MNs retract from the neuromuscular junction before the MN cell body dies (Fischer et al., 2004). Second, Deforges et al. (2009) classified MNs based on their morphological properties (e.g., soma size). This method could be misleading in ALS because WT and mutant MNs exhibit differences in morphology and cell size (Amendola and Durand, 2008) and, for all mice, the separation between MN types based on soma size is problematic because there is a continuum of sizes (McHanwell and Biscoe, 1981). To avoid these problems, functional motor unit electrophysiology, in which the number of surviving MNs is estimated from isometric whole muscle and single motor unit twitch forces (Gordon et al., 2010), could be used together with MN cell body

count to measure the number of functional surviving MNs. Although conducting functional motor unit electrophysiology would add complexity to the experiment, it would also allow for distinguishing between motor unit types (fast vs. slow) accurately based on their contractile properties.

Regarding their interpretation, the authors in Deforges et al. (2009) concluded from their soma size measurements that swimming rescued large MNs and saved them from degeneration. However, in their MN classification the authors did not consider gamma-MNs, which have the smallest soma size in the ventral horn of the spinal cord. With that in mind, the results of the Deforges et al. (2009) could be reinterpreted in light of published literature on the soma size of various MN types in adult WT mice. Accordingly, MNs that were saved by swimming exercise (whose soma size is between 300 and 700 μm^2) actually correspond to small alpha-MNs (whose soma size ranges between 450 and 580 μm^2 ; McHanwell and Biscoe, 1981; Ishihara et al., 2001), which innervate slow muscle fibers. Also, MNs that were reduced in proportion by swimming exercise (whose soma size is $<300 \mu\text{m}^2$) actually correspond to gamma-MNs (whose soma size ranges between 200 and 250 μm^2 ; McHanwell and Biscoe, 1981; Ishihara et al., 2001), which are spared in the disease (Sobue et al., 1981). Moreover, the proportion of large alpha-MNs (whose soma size is $>700 \mu\text{m}^2$), which innervate fast muscle fibers, did not change with swimming, indicating that “true” large MNs were not protected. In sum, swimming exercise actually increased the proportion of small alpha-MNs, which innervate slow muscle fibers, even if large MNs were preferentially activated as claimed by the authors. This effect could be due to MN conversion from the large to small type by the effect of the training exercise. This reinterpretation is also consistent with a previous study in which swimming training in normal (i.e., non-transgenic) mice was shown to induce a fast-to-slow fiber conversion (Clément et al., 2008). In light of this reinterpretation, the Deforges et al. (2009) comes in agreement with Gordon et al. (2010) in which increased neuromuscular activity in G93A transgenic mice saved fast motor units by converting them to slow

motor units, which are innervated by the small alpha-MNs. This conversion was supported by both electrophysiological recordings and histochemical staining techniques.

In Deforges et al. (2009), a fundamental concept the authors claimed is that swimming exercise preferentially recruits the larger, faster motor units in contrast to the running exercise that recruits the smaller, slower motor units. Certainly, recruitment is orderly from small to large motor units although the relative recruitment of large motor units may differ with the exercise. In other words, swimming exercise must have involved activation of both small and large MNs, whereas running exercise involved mainly activation of small MNs, with maybe some activation of large MNs. With that in mind, the beneficial effect of swimming, but not running, exercise on MN survival in ALS could be explained in the context of their electrical properties. In the neonatal phase, large mutant MNs are vulnerable and selectively exhibit 30% reduction in their input resistance relative to WT (Bories et al., 2007), which makes them harder to recruit. Therefore, exercise of sufficient intensity to activate large MNs, such as swimming exercise as in the Deforges et al. (2009) study or functional overload of spared motor units as in the Gordon et al. (2010) study, could then be essential to both recruit and convert them to slow MNs. Also, specific types of synaptic inputs to MNs are lost in ALS [e.g., cholinergic synapses on MNs are lost in ALS as shown by Nagao et al. (1998)] and it is possible that training exercise rescues vulnerable MNs in part by strengthening remaining inputs to MNs. These are open questions that warrant further investigations.

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