NASA-Ames Contract NCC2-862 Final Report
Project title: Development of sensory receptors in skeletal muscle.
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Background.

Rationale. Life on Earth evolved with gravity as a natural, pervasive, physical condition. Muscle receptors provide sensory information to the central nervous system about the length and tension status of most skeletal muscles in the bodies of amniote animals. That is particularly the case for muscles of the limbs and trunk, which counter the effects of gravity. These sensory receptors are susceptible to permanent structural change if their innervation is perturbed, either directly or indirectly, during late prenatal or early postnatal development. Consequently, it is important to know if exposure to a near-zero gravity condition during the second part of prenatal development affects the proximate causation processes (as opposed to 'ultimate causation') responsible for the formation of skeletal muscle receptors.

Experimental protocol. The offspring of pregnant female, Sprague-Dawley rats (*Rattus norvegicus*) were studied. There were four groups of pregnant rats. The Flight or experimental dams were housed in Animal Enclosure Modules (AEMs) and, being aboard the space shuttle *Atlantis* during STS-66, from days 9-20 of their gestation (G9-20, where G0=day of conception) they were in a microgravity condition. STS-66 landed at Dryden Flight Research Facility in California. There were several groups of ground control rats. They were time-pregnant animals delayed by one day relative to the Flight rats and were either housed in AEMs or in a Vivarium at the Kennedy Space Center in Florida. None of these ground control groups were exposed to the sounds and vibrations of a simulated space shuttle launch and landing. There were two sub-groupings of the Vivarium ground control rats. Approximately half of them had a laparotomy done on G7 to confirm pregnancy, and a partial hysterectomy done at G20 to obtain fetuses. This group was designated 'Vivarium A Control.' These procedures had been done to all of the Flight and the Synchronous Control rats as well. The remainder of the Vivarium controls (i.e. 'B') had no surgery at all.

Objectives. There were two major goals for my project. One was to examine the hindlimb walking pattern of offspring from the Flight dams as compared with offspring of the ground control groups from initiation of walking up to two months thereafter. This initial goal was subsequently modified so that additional developmental measures were taken (e.g. body weight, eye opening) as the progeny developed, and the study period was lengthened to eighty days. Also videotapes taken shortly after the pregnant Flight dams returned to Earth were scored for locomotor activity and compared to those for the Synchronous control dams at the same stage of pregnancy.

The second goal was to examine skeletal muscle. Selected hindlimb skeletal muscles were to be identified, weighed, and examined for the presence and integrity of muscle receptors, (both muscle spindles and tendon organs), at the level of the light and electron microscope. Muscles were examined from rats that were at fetal (G20), newborn (postnatal day 1 or P1, where P1 = day of birth), and young adult (~P100) stages. At the present time data from only the last group of rats (i.e. P100) has been completely examined.

Results of specific projects.

Achievement of early postnatal developmental milestones.

This work involved studying offspring from Flight, Synchronous Control, Vivarium Control (groups A and B) dams. The measures taken were: a) body weight (from P1-P80), b) when walking began and how it progressed, c) when eye opening occurred, and d) whether offspring themselves were able to produce viable progeny. These offspring were sacrificed at ~P100 and their muscles were studied (see below).

The findings on rate of attainment of developmental landmarks and related data on the motor activity and birthing by the dams have been published (Wong and DeSantis 1997. Integrat. Physiol. Behav. Sci. 32:322-342). Details of the results can be found in that paper, a copy of which is included with this report. The results may be summarized as follows. Only two things that we measured showed indications of a significant measureable difference between Flight animals and ground controls, either Synchronous or Vivarium. First, the activity of Flight dams was markedly diminished in the period immediately after the space flight. Second, Flight dams and their offspring had a diminished record for producing progeny that survived past the initial 48 postnatal hours. For all other developmental observations that we made, there were no statistically significant differences between Flight rats and those from all control groups. That was particularly true when the measured parameter was one for which rats are sexually dimorphic (e.g. body weight) and the gender differences among the treatment groups being compared were factored out.

I conclude from this part of the project that fetal development in microgravity during the latter part of gestation does no preclude the subsequent achievement of developmental milestones by rats subsequent to their birth on Earth. There is at least a transient effect on the locomotor activity of dams when they return to Earth gravity. There is also evidence for impaired perinatal well-being and survival of offspring (and their own progeny as well) of Flight dams after microgravity exposure during their pregnancy.

Findings for hindlimb skeletal muscles.

Muscle weights. After perfusion fixation of anesthetized young adult (~P100) rats, selected flexor and extensor muscles from each hindlimb of Flight (n=10; 7♀, 3♂), Synchronous Control (n=10; 5♀, 5♂) and Vivarium Control (n=10; 5♀, 5♂) were removed and weighed to the nearest 0.01gm. The average values (±standard deviation) are given in Table 1. The extensor digitorum longus and tibialis anterior muscles are flexors at the ankle joint, whereas the soleus, plantaris and gastrocnemius muscles are

extensors at that same joint. It can be seen from Table 1 that for any given muscle, there was consistently more of a difference in weight when comparing between the sexes (i.e. horizontal comparisons) than when comparing among treatments for a given gender (i.e. vertical comparisons)

Microscopic examination of skeletal muscles. We examined by microscopy muscle spindles from the soleus and extensor digitorum longus muscles of ~P100 rats from the Flight and Synchronous Control groups. As soon as we had examined a few histological sections of muscle from a rat in the former group, it was apparent that at least some muscle spindles (and tendon organs) were present in their hindlimb muscles. Then the task was one of learning how consistently the muscle receptors in the Flight or experimental rats were organized, both intrinsically and within the overall muscle, relative to ground controls. We then did studies by light microscopy to assess the following four things:

i. average number of muscle spindles per muscle,
ii. distribution of muscle spindles along the length of the muscle,
iii. the number of intrafusal muscle fibers present in the muscle spindles, and
iv. the size of those intrafusal muscle fibers.

By electron microsocopy we addressed the following two aspects:

v. the morphological integrity of intrafusal muscle fibers, and
vi. the presence of sensory and motor terminals ending on intrafusal muscle fibers.

Soleus muscles from female, Flight (n=4) and Synchronous and Vivarium controls (n=2 and 4, respectively) rats when they had developed to adulthood were cut serially in cross-section (20μm thickness), mounted onto glass slides and stained by the VanGiesen method. Figure 1 shows the the average number of spindles per soleus muscle was not different between the Synchronous Control (X=17.8 ±0.5) and the Flight (X=18.8 ±0.5) rats. Nor was there any obvious difference in the distribution of those muscle spindles along the length of the soleus muscles (Figure 2). For both sets of muscles there were no spindles present at the very ends of a muscle and most of the spindles were located between the nerve entry (i.e. the regions designated as '0μm' in order to normalize values among the muscles studied) and the insertion of the muscle.

Two other soleus muscles from adult, female rats – one a Flight animal, the other a Synchronous Control – were embedded in epoxy resin, skip-serially cross-sectioned (1-2μm thickness) and stained on the slide with Stevenel's Blue dye in order to count the number and measure the size of intrafusal muscle fibers in muscle spindles. Fifeteen spindles were identified in each muscle. Figure 3 shows that for the muscles from both Flight and Synchronous Control rats 80% of the spindles had four intrafusal fibers and the remaining 20% of spindles contained three fibers. Figure 4 shows the size distribution for the intrafusal muscle fibers comprising muscle spindles from those two muscles. The spindles in the Flight rat's soleus had a slightly larger average (± standard deviation) diameter (12.32 ±2.08μm) and range (4-24μm) of intrafusal fiber size than did those of the control (11.45 ±2.36μm; 4-20μm). Those means were not significantly different (Students' T-test, $p<0.05$).

When muscle spindles in the soleus muscle from young adult (~P100) rats of both Flight and Synchronous Controls groups were examined by transmission electron microscopy, the typical components of mammalian muscle spindles were observed.

Those structures included: inner and outer capsular cells, intrafusal muscle fibers, and sensory and motor nerve terminals. Ultrastructural features of those cells appeared qualitatively normal when examining receptors from Flight and Synchronous Control rats.

Based on these finding for the rat, I conclude that exposure to microgravity during the latter half of gestation does not have any obvious detrimental effect either on the mass of hindlimb skeletal muscle that develops or on the structural integrity of muscle receptors that are present in those muscles by the time the rat has grown to sexual maturity. It appears that proximate causation mechanims responsible for skeletal muscle development are not permanently influenced by microgravity exposure during the latter half of gestation in this mammal.

Publications and presentations resulting from this study (as of 29/II/00).

Refereed journal articles.

Wong, A.M. and M. DeSantis. 1997. Rat gestation during space flight: outcomes for dams and their offspring born after return to Earth. *Integrative Physiological and Behavioral Science* 32:322-342.

DeSantis, M., E. Eldred, C. Helmick and A.Wong. Muscle receptor development in rats after gestation in near-zero gravity. (manuscript in preparation)

Published abstracts.

Parkman, K., A. Wong and M. DeSantis. 1995. Locomotor behavior of rats after exposure to the conditions of space flight. *Journal of the Idaho Academy of Science* 31:34-35.

Wong, A., K. Parkman and M. DeSantis. 1995. Gender, more than space flight during part of gestations, influences postnatal weight gain in rats. *American Society for Gravitational and Space Biology* 9:10.

DeSantis, M., A. Wong and K. Parkman. 1995. Two motor horizons – walking and eye opening – are unaffected by prenatal exposure to near-zero gravity. *American Society for Gravitational and Space Biology* 9:96.

DeSantis, M., C. Helmick and A. Wong. 1996. Proprioceptors in skeletal muscle of rats that developed *in vivo* aboard the space shuttle *Atlantis*. *Journal of the Idaho Academy of Science* 32:13.

Invited presentations.

DeSantis, M. Encapsulated muscle receptors: searching for answers in sundry places. Dept. of Biological Sciences, University of Idaho. September 1996. Moscow, Idaho, USA.

DeSantis, M. Encapsulated sensory receptors in skeletal muscles: lessons from the dromedary camel and space rats. Dept. of Anatomy, Faculty of Dentistry, Chulalongkorn University. October 1996. Bangkok, THAILAND.

DeSantis, M., E. Eldred, C. Helmick and A. Wong. Quantitative observations on the structure of selected proprioceptive components in adult rats that underwent half of their fetal development in space. 12th Man in Space Symposium. June 1997. Washington, District of Columbia, USA.

DeSantis, M. Muscle receptors as a model system for studying specificty of nerve connections. Dept. of Anatomy, Faculty of Medicine, Kuwait University. June 1999. Kuwait City, KUWAIT.

DeSantis, M. Camel eyes, (r)a(t)stronauts and cat legs. Neuroscience Program, Washington State University. January 2000. Pullman, Washington. USA.

Signature: _____ date: 29/II/00

Table 1. Weight of hindlimb skeletal muscle after various treatments. Values in grams are the mean ± standard deviation for female (♀) and male (♂) young adult (~P100) rats.

	Flexor Muscles				**Extensor Muscles**					
Treatment	Ext. Digit. Long.		Tibialis Anterior		Soleus		Plantaris		Gastrocnemius	
Group	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Flight	0.11 ±0.02	0.21 ±0.02	0.52 ±0.05	0.93 ±0.09	0.14 ±0.03	0.25 ±0.02	0.31 ±0.03	0.51 ±0.02	1.57 ±0.08	2.59 ±0.08
Synchronous Control	0.13 ±0.02	0.19 ±0.03	0.56 ±0.08	0.93 ±0.09	0.15 ±0.02	0.25 ±0.04	0.36 ±0.04	0.50 ±0.04	1.76 ±0.24	2.55 ±0.18
Vivarium Control	0.12 ±0.03	0.20 ±0.04	0.54 ±0.08	0.84 ±0.20	0.15 ±0.04	0.24 ±0.07	0.33 ±0.07	0.54 ±0.08	1.62 ±0.23	2.53 ±0.23

Figure 1

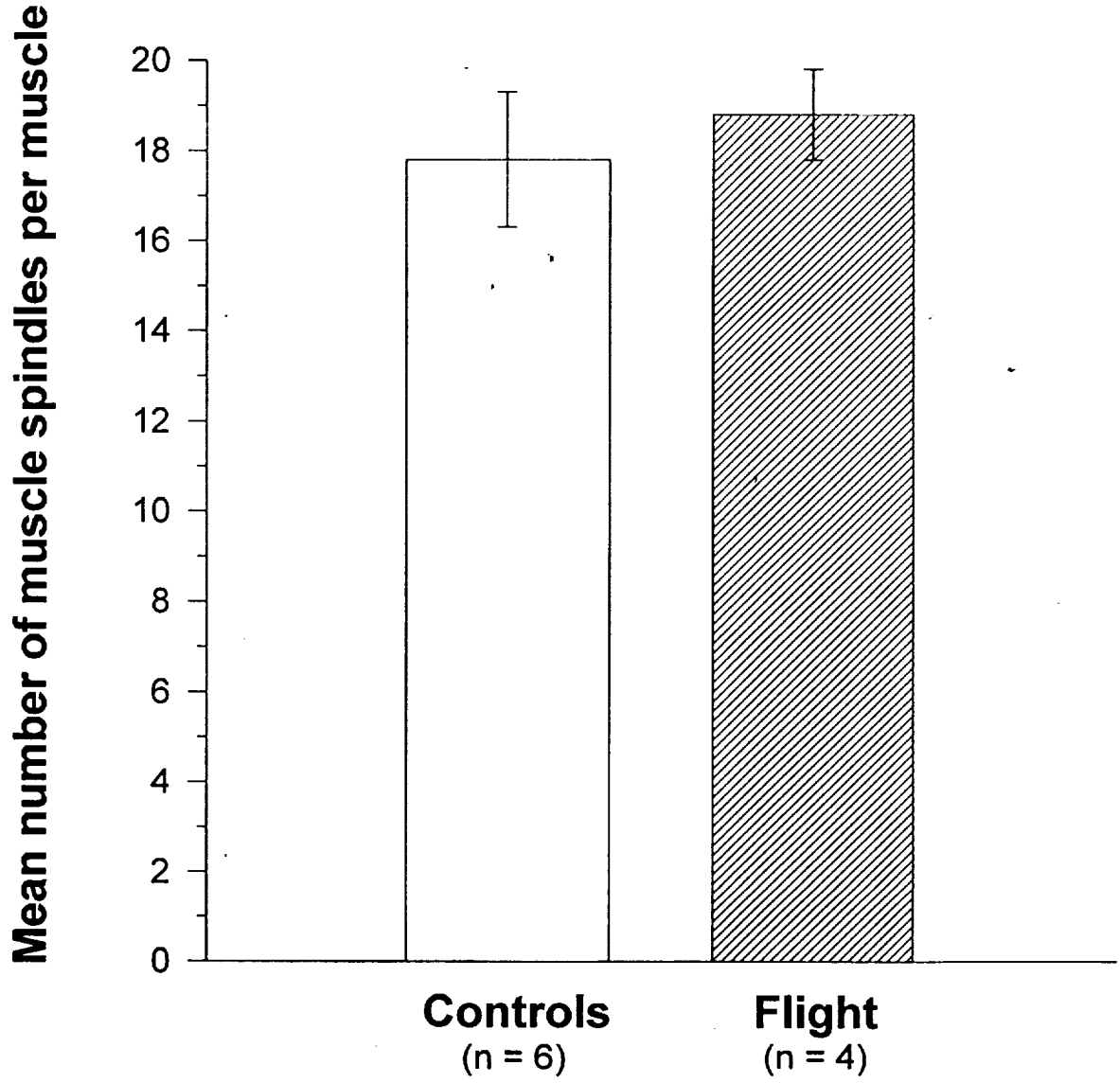

Mean number of muscle spindles per muscle
20
18
16
14
12
10
8
6
4
2
0
Controls
(n = 6)
Flight
(n = 4)

Figure 2

data 2

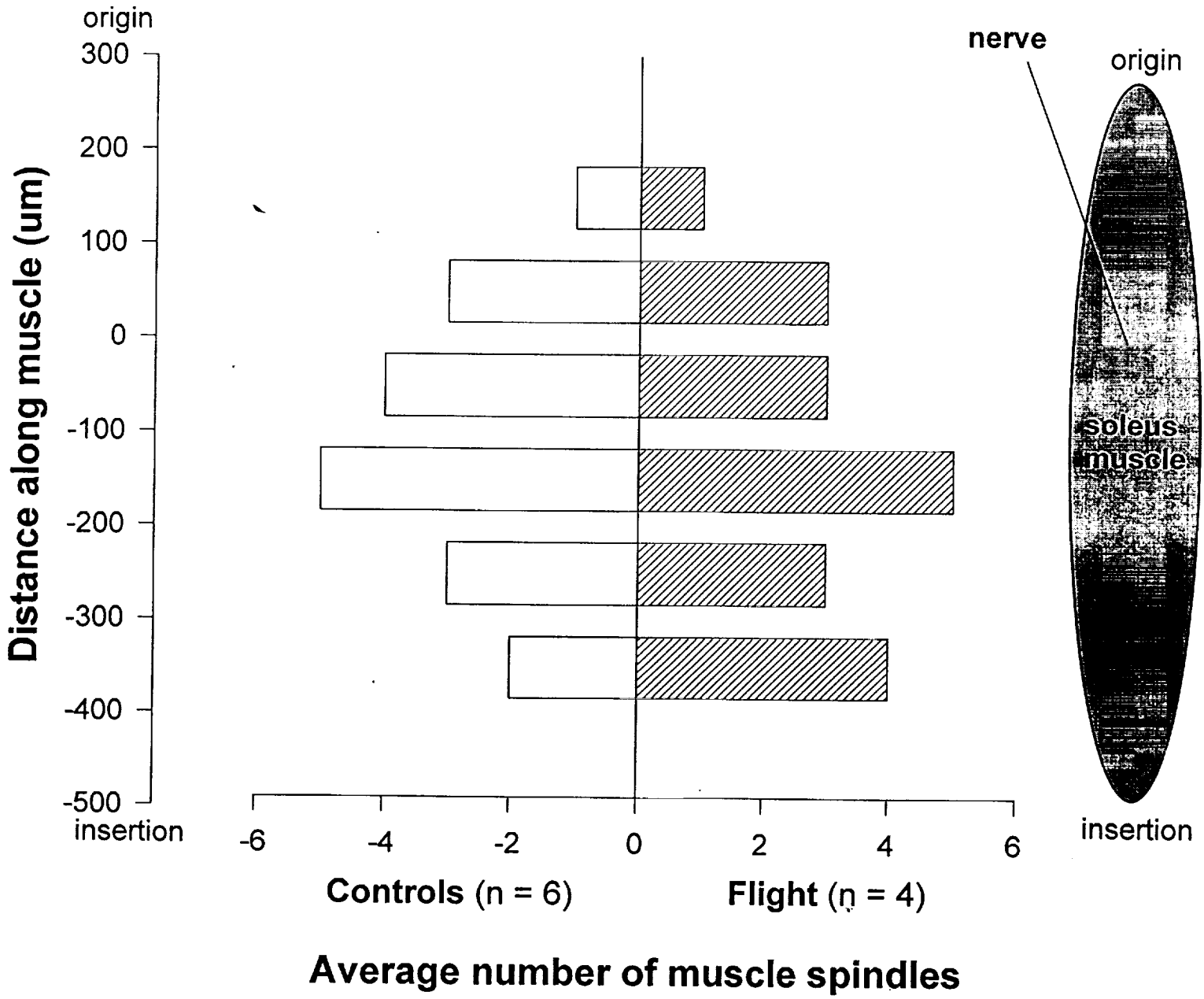
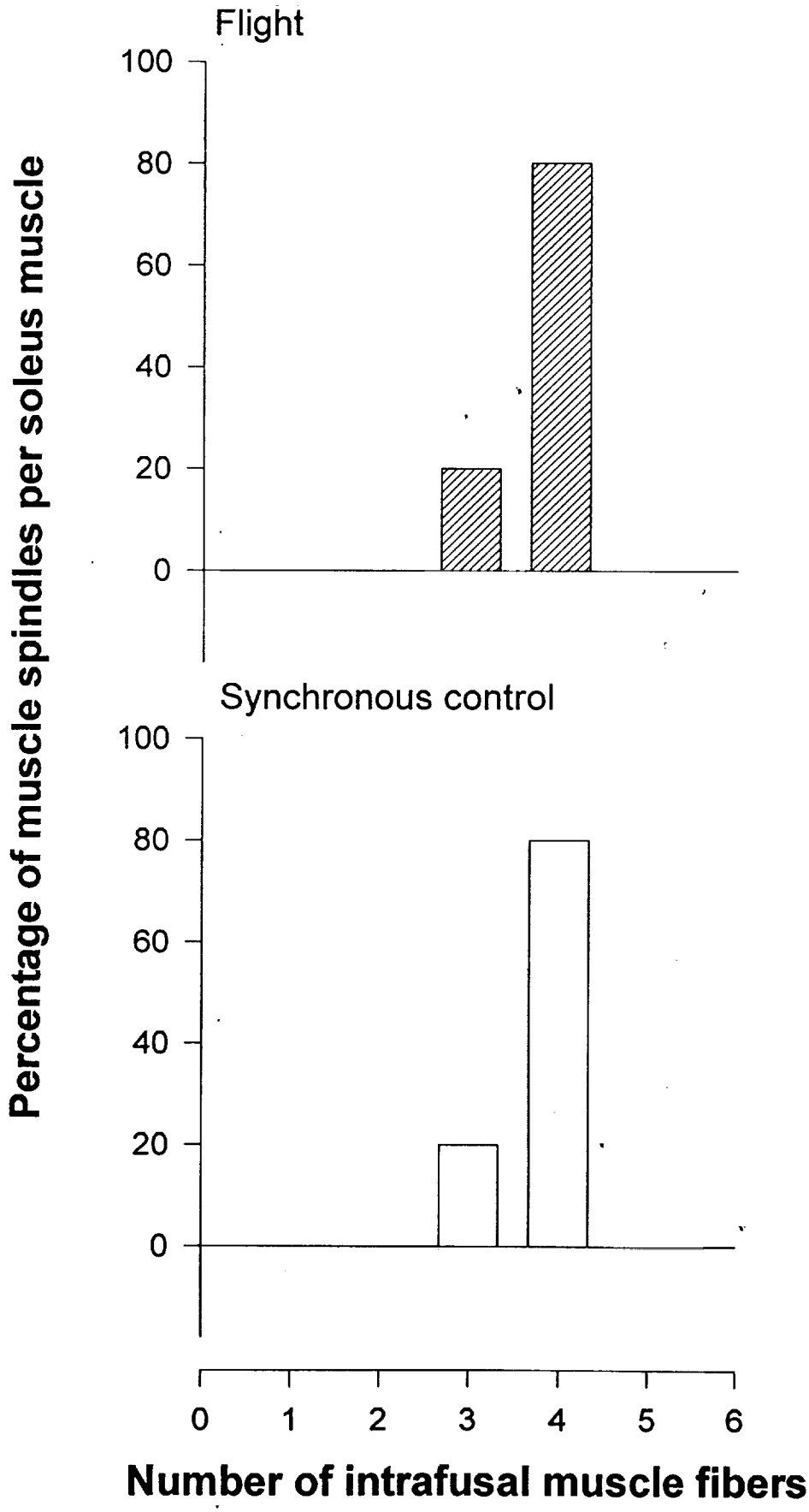


Figure 3

data 3



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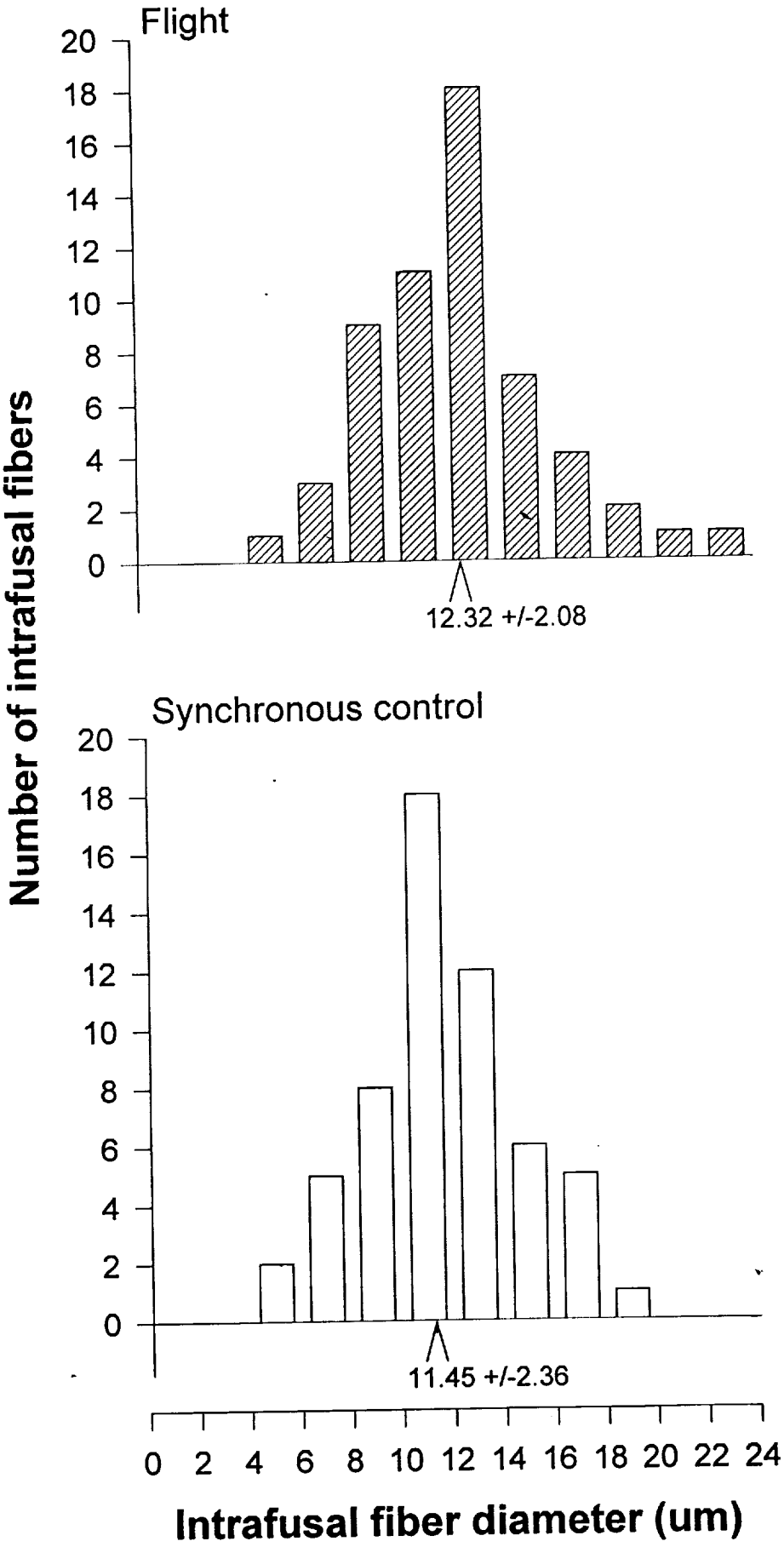
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