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Voluntary exercise delays heart failure onset in rats with pulmonary artery hypertension

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Abstract

Increased physical activity is recommended for the general population and to patients of many diseases because of its health benefits but can be contraindicated if it is thought a risk for serious cardiovascular events. One such condition is pulmonary artery hypertension (PAH). PAH and right ventricular failure was induced in rats by a single injection of monocrotaline (MCT). MCT rats with voluntary access to a running wheel ran on average 2km per day. The time for half the animals to develop heart failure signs (median survival time) was 28 days (exercise failure (EF) group), significantly longer than sedentary animals (sedentary failure (SF) group), 23 days). The contractility of single failing myocytes in response to increasing demand (stimulation frequency) was significantly impaired compared with both sedentary control (SC) and exercising control (EC) myocytes. However, myocytes from exercising MCT rats, tested at 23 days (EM group) showed responses intermediate to the control (SC, EC) and failing (SF, EF) groups. We conclude that voluntary exercise is beneficial to rats with heart failure induced by PAH and this is evidence to support the consideration of appropriate exercise regimes for potentially vulnerable groups.

New and Noteworthy statement

In rats that developed pulmonary artery hypertension (PAH), voluntary wheel running exercise delayed both progression to right heart failure and deterioration of the myocytes' response to increased demand. Our observations suggest that appropriate exercise regimes may be useful in the treatment of PAH.

Introduction

Regular physical activity (exercise) is known to have multiple beneficial health effects and is recommended to the general population and many patients including those with heart disease (10). However conditions exist where exercise is contraindicated due to the possibility of provoking serious cardio-pulmonary events. Pulmonary artery hypertension (PAH) results in right ventricular (RV) failure and the impaired ability of the RV to increase stroke volume during exercise is given as a reason to limit physical activity in PAH patients (5; 9). Although exercise has been shown to benefit PAH patients (3; 16) this has not yet been incorporated into treatment guidelines (15).

An animal model study of PAH found treadmill running exercise was beneficial to rats with stable PAH but detrimental to those with progressive PAH, decreasing survival time (11). The interpretation of these data could be that sufferers of severe PAH will not benefit from exercise. However, there are multiple types of exercise modes and regimes used in rodents with respective strengths and weaknesses (17; 20; 24). The aim of our study was to test whether an alternative exercise regime might prove beneficial in rats destined to develop RV failure induced by PAH and thus give an alternative view of the potential role of exercise in PAH.

Methods

Male Wistar rats (200g) had either free access to a running wheel that logged rotations and thus daily running distance (designated as exercise animals, E) or no access to wheels (designated as sedentary animals, S) see (18; 22). Animals were introduced to the running wheels 2 days before treatment with monocrotaline (MCT) or saline.

Animals received either a single intraperitoneal injection of 60 mg/kg MCT to induce RV failure (F) or an equivalent volume of saline as control (C) see (1; 2). Rats were killed upon showing signs of heart failure (e.g. weight loss, dyspnea, piloerection) or on equivalent days for C animals. In addition to these 4 groups (SC, EC, SF, EF) a fifth group was given access to running wheels, injected with MCT but taken at the median end point day (± 1 day) of SF animals, for temporal comparison. This group was designated exercise + MCT (EM). The median survival time for SF

and EF groups represented the time after MCT treatment when more than 50% of the group reached the heart failure end point (see Fig. 1).

Following killing, the heart and lungs were excised, blotted dry and weighed. The heart was then attached to a Langendorff retrograde perfusion system and single RV myocytes isolated as previously described (1; 2; 14). Each group had N = 6 animals. Experiments were conducted in accord with Health Research Extension Act (public law 99-158, 1985 "Animals in Research"), UK Home Office regulations and local ethical approval.

Myocytes were placed in a bath on the stage of an inverted microscope and superfused with a Tyrode's solution containing in mM: 137 NaCl, 5.4 KCl, 0.33 NaH_2PO_4, 0.5 $MgCl_2$, 5 HEPES, 5.6 glucose 1.8 $CaCl_2$, pH 7.4 with 5N NaOH. Individual myocytes were selected for study if they had a clear, regular striated (sarcomere) pattern, did not spontaneously contract in the absence of external stimulation and responded to 1Hz stimulation with a single twitch. Myocytes were stimulated to contract and demand progressively increased by increasing stimulation frequency from 1 to 7 Hz. Cells were field stimulated at the required frequency by external Pt electrodes and the resultant cell shortening measured via analysis of a video image of the cell using an Ionoptix camera and software (Ionoptix, Milton, MA, USA). Cell shortening was expressed as % of resting cell length. All experiments were performed at 37˚ C.

Statistics

Data are presented as mean ± SEM. $P < 0.05$ was considered significant. Survival was tested by a Mann-Whitney test. Running distances (on representative days 1,8,15 and 22) were compared by two-way repeated measures analysis of variance (anova), contraction-frequency relationships by two-way anova and animal weights and organ weights by one-way anova. Anovas were followed by pairwise Tukey correction tests. Proportions were tested by Chi^{-2} test. Numbers of rats, hearts and myocytes used in each experiment are given in the relevant table and figure legends.

Results

Fig. 1 shows survival for SF and EF animals. Although all animals in these groups developed heart failure signs, the median survival time for EF animals (28 days) was significantly longer than SF animals (23 days, $P < 0.05$) indicating a benefit of exercise. There was no correlation between total running distance or daily running distance prior to heart failure onset and survival time.

All animals given access to running wheels ran on them. The EF group and most EM animals ran on average, 2km per day, 22 days after injection of MCT (Fig. 2). There were no significant differences in the daily running distances of the 3 exercising groups (EC, EF, EM, $P > 0.05$). Overall, running distances were greater on days 15 and 22 ($P < 0.001$) and day 8 ($P < 0.05$) than on day 1. Mean daily distances were increased in EC and EM groups by 1-2 animals running more than others, hence the larger SEM in these groups (Fig. 2). Daily running distance in the EF group began to fall 2-3 days prior to the observation of heart failure signs.

Whole animal and organ weights showed that failing animals displayed the characteristic increase in heart weight to body weight and RV to LV weight ratios that is indicative of RV hypertrophy. MCT treated animals with voluntary access to exercise wheels, taken prior to the onset of failure (EM) had values intermediate between the control (SC, EC) and failing (SF, EF, groups) (Table 1).

When stimulated to contract, SF myocytes displayed a steep negative contraction-frequency relationship and were the only group to show statistically greater cell shortening at 1 and 3 Hz vs 7Hz ($P < 0.001$). In contrast, over the range of 1-7Hz, SC, EC and EM myocytes displayed a biphasic relationship (Fig 3A). There were no statistical differences in the magnitude of shortening at 1Hz between the 5 groups but at 3-7 Hz statistical differences were seen between control (SC,EC) and failing (SF, EF) groups. The EM group was intermediate in response to the control and failing groups (Fig 3A). All cells were able to entrain to a stimulation frequency of 1-5Hz and contract at those frequencies but some cells were not able to entrain at 7Hz and did not respond to each stimulus. The proportion of cells able to entrain at 7Hz was significantly different between the groups ($P < 0.001$) with the smallest proportions found in the 2 failing groups, SF and EF (Fig 3B).

Discussion

Male MCT treated rats voluntarily used running wheels, this is an interesting observation given lethargy can be a reported characteristic of MCT treatment. The study was performed in male rats for consistency with previous studies (1; 2). The daily running distance of 2 km/day is less than we have previously reported in female rats (18; 22) even so, this improved survival and functional characteristics of myocytes of MCT-treated animals.

The organ parameters for SF and EF animals were not different to each other indicating that although exercise delayed the onset of failure signs, the characteristics of these animals on reaching that end point were similar. In the EM group the mean values for all parameters, except LV weight, were intermediate between the C and F groups, indicating that exercise delayed the progression of organ remodelling in PAH.

An inability to respond to an increase in demand is a defining characteristic of heart failure (8). In RV failing myocytes this is manifested as a steep negative contraction-frequency relationship and a smaller proportion of cells able to respond to high frequency (7Hz) stimulation compared to the control groups. EM myocytes (i.e. MCT treated myocytes prior to the onset of failure) displayed the characteristics of control cells indicating an improvement of contractile function relative to myocytes from animals that had developed failure.

Positive effects of treadmill running have previously been reported when exercise was begun 2 weeks prior to MCT administration (4; 21). However this pre-training might improve resistance to the conversion of MCT into the active agent. Another MCT study showed that while exercise had beneficial effects in animals with stable PAH, in those with progressive PAH exercise had detrimental effects on cardiac hemodynamics, caused increased RV inflammation and decreased survival times (11). In that study exercise began 2 weeks after MCT treatment, once PAH had developed, exercise volume was 2 km *per week* (at 13.3 m/min; 0.5 VO_2max.) under an enforced continuous treadmill running regime with a noxious reinforcement stimulus (11).

In contrast, our study, using the same heart failure end point, found a beneficial effect of voluntary exercise (2 km *per day)* in progressive PAH. However, the studies are not direct comparisons. Though voluntary running can attain speeds of 60 m/min the running is intermittent and carried out in short bursts (6; 19). In addition, we began voluntary exercise prior to development of PAH. This design was chosen because voluntary running distances take several days to build up and the development of PAH in this model is rapid. We were therefore concerned that a later exercise starting date would give little time for any exercise benefits to accrue, this is supported by our observation that daily running distance began to fall 2-3 days prior to the observation of heart failure signs.

Exercise mode may influence study outcomes. With enforced regimes, the loss of control over locomotion and use of a reinforcement stimulus can trigger stress responses (17; 20; 24). Furthermore, continuous running is an unnatural mode of locomotion for rodents (17; 24). High intensity, treadmill running exercise does produce physiological training responses (12) but at lower intensity levels these responses may not outweigh the negative effects of stress. It is acknowledged that stress has a negative effect on heart failure prognosis (7; 13) and enforced exercise is not used with humans.

In left heart failure patients, the greatest benefits of exercise are seen with high intensity training (23). MCT-treated animals were still exercising when heart failure signs developed. This raises the possibility of experimentally increasing voluntary exercise volume and intensity using reward techniques or wheel loading to enhance the effects of exercise. This may allow exercise to be introduced to MCT rats at a later time point to better model the use of exercise as a treatment in established PAH patients.

Our observations do show that voluntary exercise slowed the development of severe PAH and RV failure, this is in accord with the wide ranging health benefits of increased physical activity in both healthy and diseased populations and suggests these extend even to severe PAH. Extrapolating

these animal data to patients, we conclude that no group should be considered beyond the beneficial effects of an *appropriate* exercise regime

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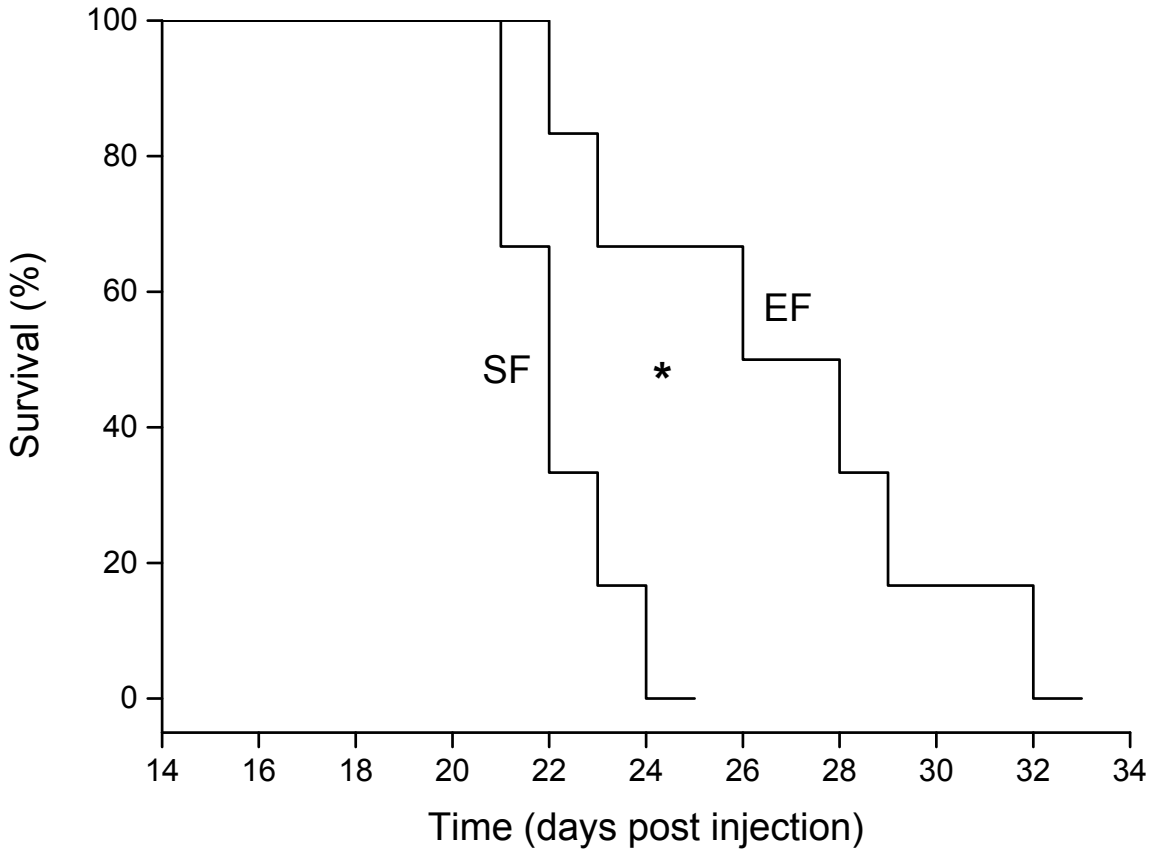

Figure 1: Survival, measured in days to heart failure signs, was significantly shorter in sedentary rats (SF) than those with voluntary access to running wheels (EF). $P < 0.05$, Mann-Whitney test, N=6 each group.

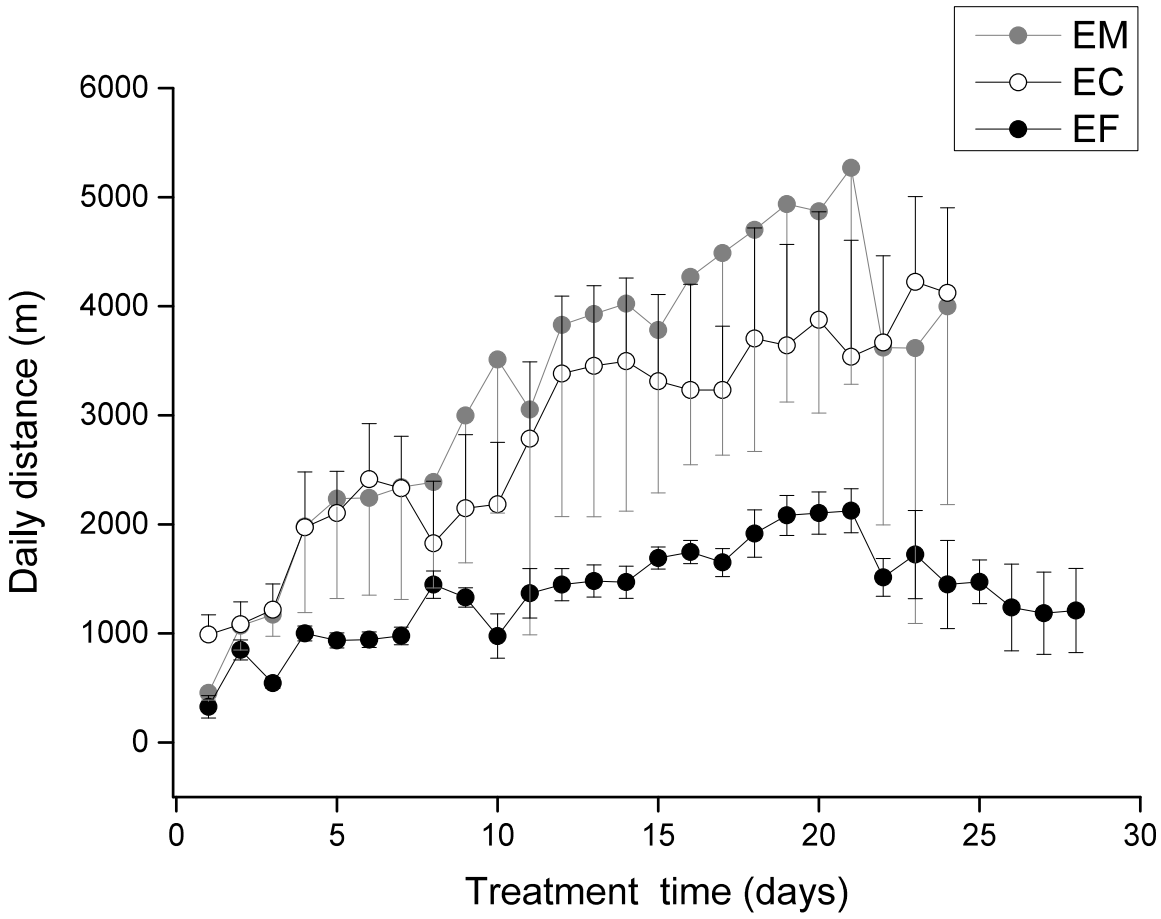

Figure 2: Mean ± SEM daily running distance in groups with voluntary access to running wheels. Control rats (EC), MCT treated rats that reached heart failure signs end point (EF), MCT-treated rats taken at the median ± 1 end point day of sedentary MCT-treated rats (EM). Animals were injected with MCT or saline on day 1, running distances were compared at days 1, 8, 15 and 22 of treatment and were significantly greater on day 8 ($P < 0.05$) and days 15 and 22 ($P < 0.001$) than day 1. There were no significant differences between the groups on days 1,8,15 or 22. Two way repeated measures anova. N = 6 in each group until animals reach designated end points from day 21 onwards.

	Sedentary Control (SC)	Exercise Control (EC)	Sedentary Fail (SF)	Exercise Fail (EF)	Exercise MCT (EM)
Body weight (g)	323.17 ± 5.64	286.17 ± 7.75*	269.67 ± 10.54*	288.83 ± 12.19	271.50 ± 4.26*
Heart weight (g)	1.16 ±0.06	1.15 ± 0.04	1.39 ± 0.06	1.64 ± 0.11$^{*\#\&}$	1.26 ± 0.04
RV weight (g)	0.27 ± 0.04	0.21 ± 0.01	0.44 ± 0.05$^{*\&}$	0.45 ± 0.02$^{*\#\&}$	0.31 ± 0.03
LV weight (g)	0.58 ± 0.04	0.54 ± 0.07	0.48 ± 0.04	0.63 ± 0.05	0.52 ± 0.05
Lung weight (g)	2.32 ± 0.16	2.02 ± 0.17	2.97 ± 0.38$^{\#\&}$	2.73 ± 0.15	2.16 ± 0.22
Heart weight/Body weight (mg/g)	3.56 ± 0.14	4.03 ± 0.10	5.19 ± 0.24$^{*\&}$	5.70 ± 0.16$^{*\#\&}$	4.66 ± 0.19*
RV weight/LV weight (mg/mg)	0.46 ± 0.05	0.41 ± 0.06	0.95 ± 0.12$^{*\#\&}$	0.75 ± 0.06$^{\&}$	0.59 ± 0.06
Lung weight/Body weight (mg/g)	7.16 ± 0.44	7.13 ± 0.76	11.24 ± 1.66$^{\&}$	9.55 ± 0.68	8.02 ± 0.90

Table 1: Whole animal and organ parameters. Right ventricle (RV), left ventricle (LV). MCT treated animals with voluntary access to exercise wheels, taken prior to the onset of failure (EM) have values intermediate between the control (SC, EC) and failing (SF, EF, groups). $P < 0.05$ * vs SC; # vs EM; & vs EC. One-way anova, N= 6 in each group.

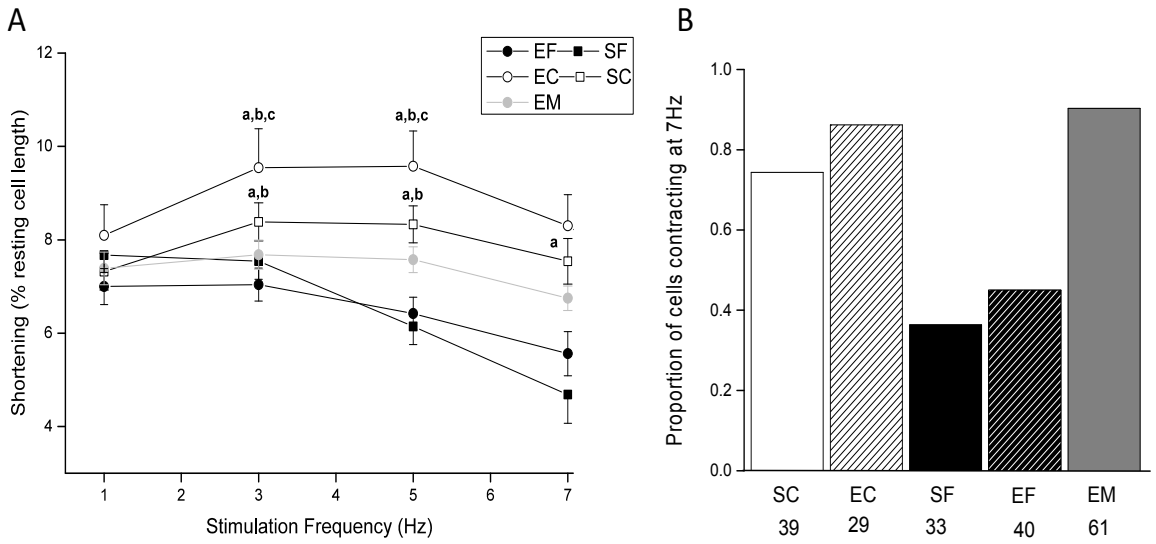

Figure 3. A. Cell shortening expressed as a fraction of resting cell length in response to stimulation frequencies between 1 and 7 Hz. Data from myocytes isolated from sedentary (SC), exercise control (EC), sedentary failing (SF), exercise failing (EF) and exercise + MCT (EM) rats. The SF and EF group shows a steep negative relationship while SC, EC and EM groups show biphasic relationships, indicating a better response to increased demand. a vs SF; b vs EF; c vs EM, P between < 0.001 and < 0.05 two-way anova. **B**. The proportion of cells in each group that entrained to a stimulation frequency of 7 Hz. The proportions were smallest in the failing groups (SF, EF), $P < 0.001$ Chi^2 test. N = 6 hearts in each group, Myocyte numbers at 1-5 Hz for each group are given in panel B.

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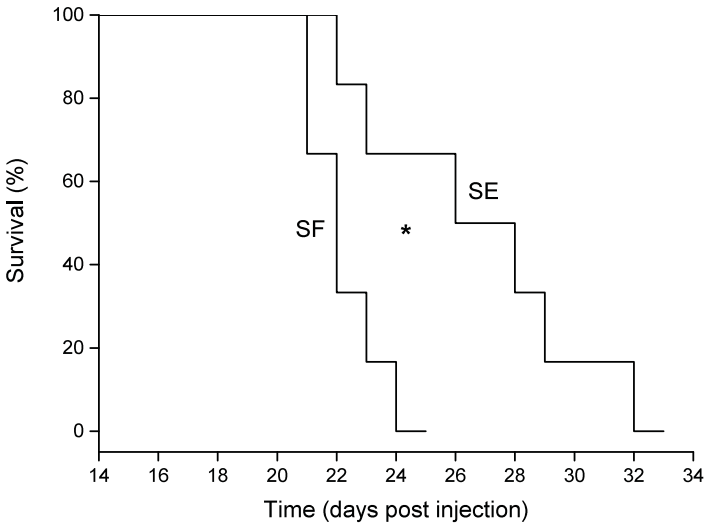
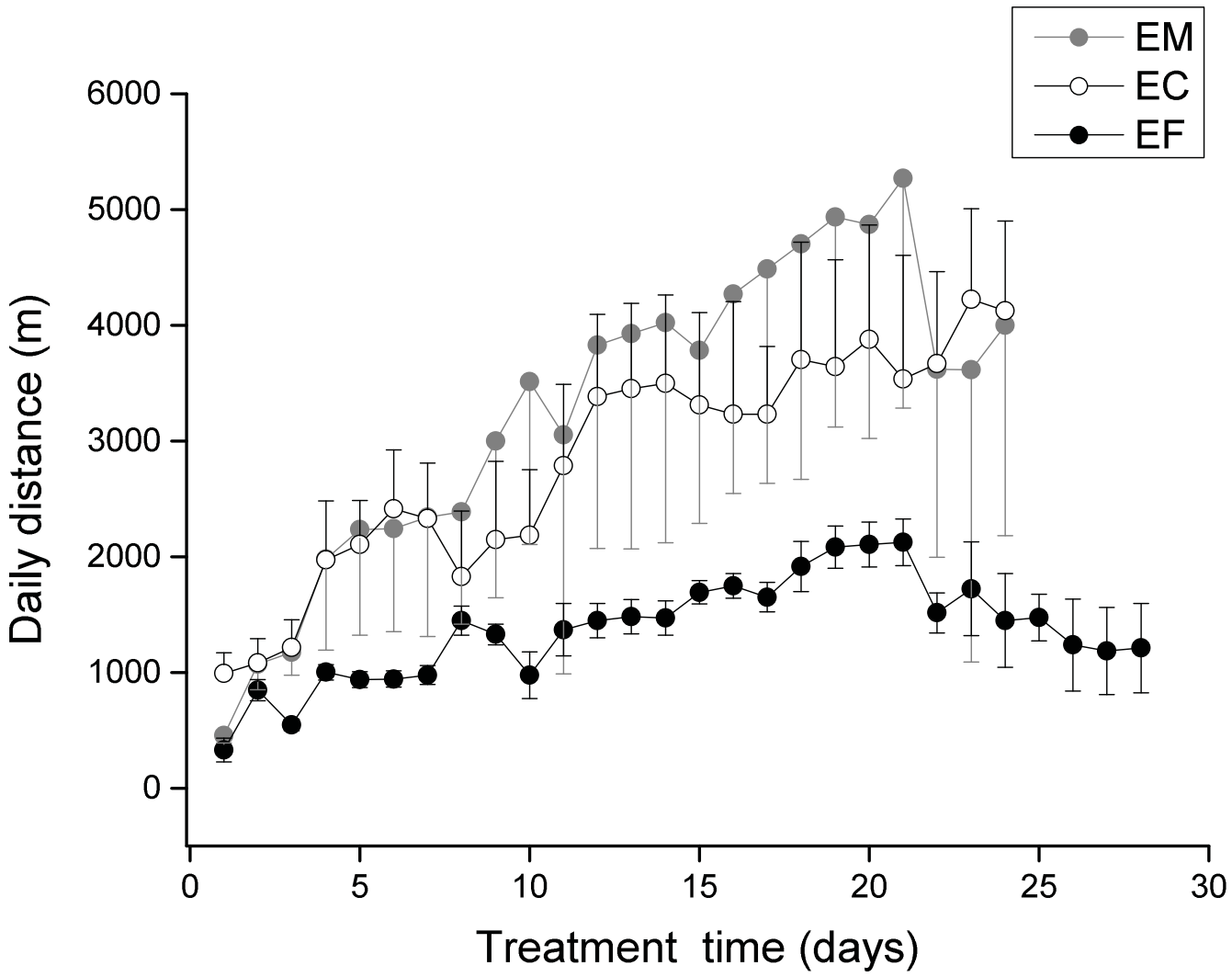

100
80
60
40
20
0
Survival (%)
SE
SF
*
14
16
18
20
22
24
26
28
30
32
34
Time (days post injection)


EM
EC
EF
Daily distance (m)
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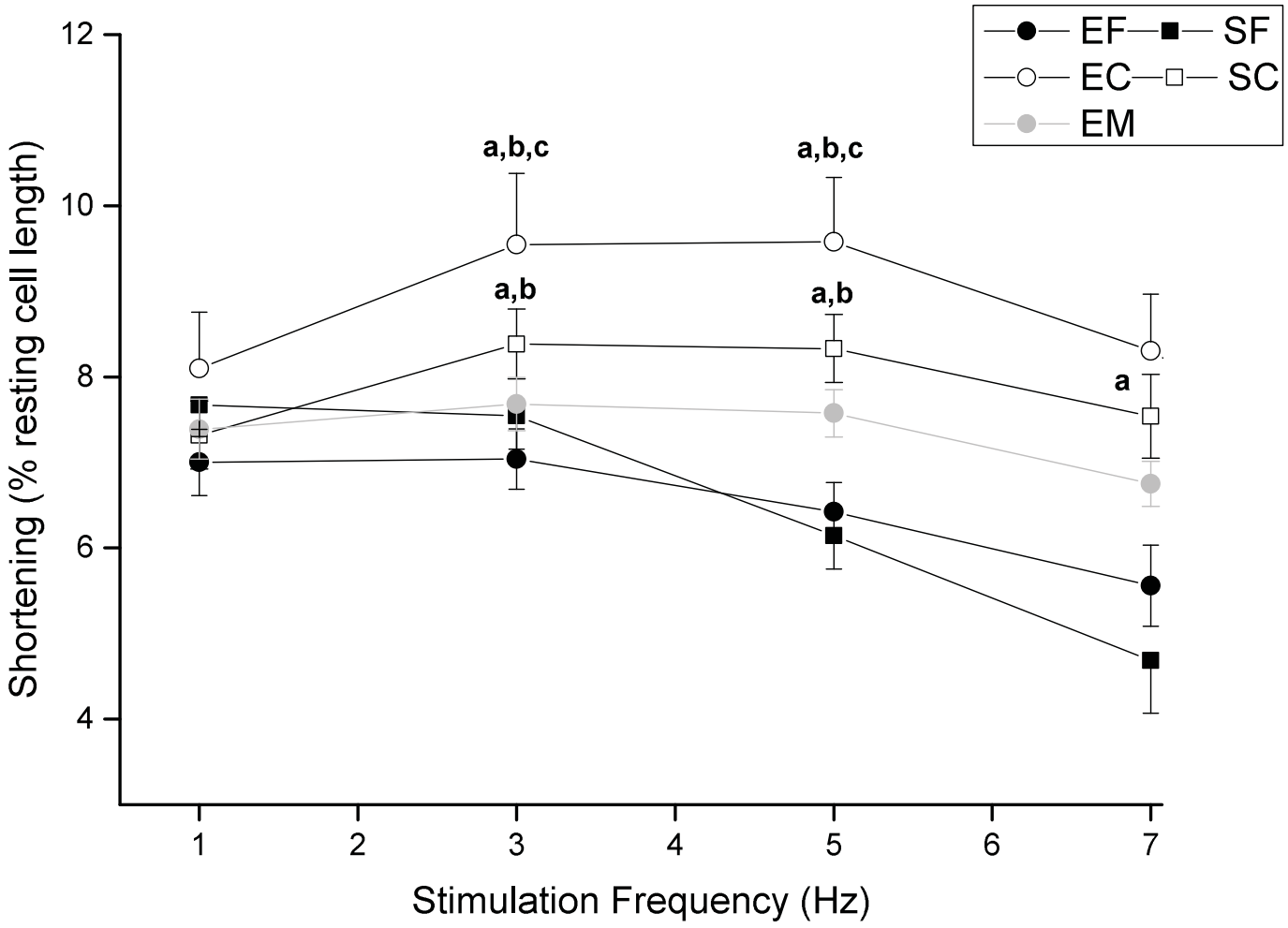
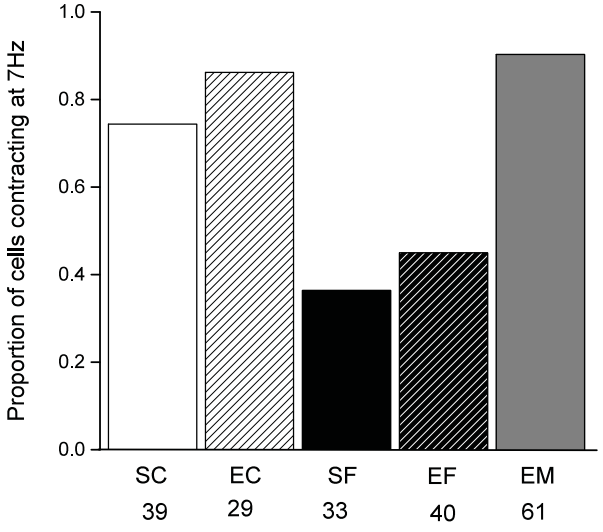
5000
4000
3000
2000
1000
0
0
5
10
15
20
25
30
Treatment time (days)


EF
SF
EC
SC
EM
Shortening (% resting cell length)
12
10
8
6
4
1
2
3
4
5
6
7
Stimulation Frequency (Hz)
a,b,c
a,b,c
a,b
a,b
a


Proportion of cells contracting at 7Hz
1.0
0.8
0.6
0.4
0.2
0.0
SC
EC
SF
EF
EM
39
29
33
40
61

	Sedentary Control (SC)	Exercise Control (EC)	Sedentary Fail (SF)	Exercise Fail (EF)	Exercise MCT (EM)
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Lung weight (g)	2.32 ± 0.16	2.02 ± 0.17	2.97 ± 0.38#&	2.73 ± 0.15	2.16 ± 0.22
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