Meta-analysis of the Efficacy of Different Training Strategies in Animal Models of Ischemic Stroke

Anjie Schmidt, MD*; Jürgen Wellmann, PhD*; Matthias Schilling, MD; Jan-Kolja Strecker, PhD; Clemens Sommer, MD; Wolf-Rüdiger Schäbitz, MD; Kai Diederich, PhD; Jens Minnerup, MD

Background and Purpose—Although several studies have shown beneficial effects of training in animal stroke models, the most effective training strategy and the optimal time to initiate training have not been identified. The present meta-analysis was performed to compare the efficacy of different training strategies and to determine the optimal time window for training in animal stroke models.

Methods—We searched the literature for studies analyzing the efficacy of training in animal models of ischemic stroke. Training was categorized into forced physical training, voluntary physical training, constraint-induced movement therapy, and skilled reaching training. Two reviewers independently extracted data on study quality, infarct size, and neurological outcome. Data were pooled by means of a meta-analysis.

Results—Thirty-five studies with >880 animals were included. A meta-analysis of all treatments showed that training reduced the infarct volume by 14% (95% confidence interval, 2%–25%) and improved the cognitive function by 33% (95% confidence interval, 8%–50%), the neuroscore by 13.4% (95% confidence interval, 1.5%–25.3%), and the running function by 6.6% (95% confidence interval, 1.4%–11.9%). Forced physical training reduced the infarct volume and enhanced the running function most effectively, whereas skilled reaching training improved the limb function most effectively. A meta-regression illustrated that training was particularly efficacious when initiated between 1 and 5 days after stroke onset.

Conclusions—Our meta-analysis confirms that training reduces the infarct volume and improves the functional recovery in animal stroke models. Forced physical training and skilled reaching training were identified as particularly effective training strategies. The efficacy of training is time dependent. (Stroke. 2014;45:00-00.)

Key Words: exercise • meta-analysis • stroke

Several studies have shown that training reduces the infarct volume and improves the functional recovery in animal models of stroke, whereas others have reported negative results. Common training strategies include forced physical training, voluntary physical training, constraint-induced movement therapy (CIMT), and skilled reaching training. Despite successes in many experimental studies, there is still controversy about the concept of forced physical training, which mostly involves treadmill running. It is criticized that the stressful component of forced paradigms may elevate the serum levels of glucocorticoid hormones and thereby exacerbate ischemic injury. Voluntary physical training, which mostly involves optional wheel running, produces less stress. However, studies comparing the effectiveness of voluntary physical training and forced physical training are scarce and not conclusive. The concept of CIMT involves a restraint of the unaffected upper limb to enhance the use of the affected limb in daily activities. In animal studies, restraint is usually achieved using a plaster cast. Skilled reaching training includes retrieval tasks with an exclusive use of the paretic limb (e.g., repeated reaching for food pellets).

Beyond the different training strategies, the optimal time window to initiate training is a matter of debate. It is assumed that training augments endogenous repair mechanisms following stroke. Because these repair mechanisms are pronounced within the first 2 weeks after stroke, training is likely to be most effective when started within this phase. The latter is supported by studies showing that the efficacy of rehabilitative treatment decreases with time after stroke onset. Other studies, by contrast, have suggested that training early after stroke exacerbates ischemic injury.
Overall, several studies suggest that training might be beneficial in animal models of stroke, but the ideal strategy has not been defined to date. Characterizing the most effective training strategy is not only relevant for the guidance of future clinical studies but may also help to elucidate further mechanisms of training-induced recovery in experimental stroke. Furthermore, it may improve the quality of experimental stroke studies which use training as a control treatment of known efficacy. The present meta-analysis was performed to compare the efficacy of different training strategies with respect to infarct size and functional outcome and to determine the optimal time window for training.

Materials and Methods

Retrieving the Literature

We searched the database PubMed from the beginning through November 30, 2011, with the keywords constraint-induced movement therapy or forced arm use or forced limb use or forced disuse or exclusive use or motor skill training or skilled reaching training or task-specific training or coordination training or treadmill training or forced exercise or voluntary exercise or running or rehabilitative training or physical exercise or physical therapy or movement therapy and stroke or cerebral ischemia. The bibliographies of relevant articles were cross-checked for further articles.

Selection of Studies and Data Extraction

We included studies analyzing the effects of training on the neurological outcome and the infarct volume in animal models of ischemic stroke. Treatment arms involving comedication were not included so that training was always the only treatment variable. Hemorrhagic stroke models and models with electrotylic brain lesions (ie, focal brain lesions induced by removing a piece of skull and delivering a current through an electrode lowered beneath the dura) were excluded. Furthermore, studies were excluded when training started before the induction of ischemia. Training was categorized into 4 groups: (1) forced physical training, which consisted of physical endurance exercise, mostly on a treadmill; (2) voluntary physical training (ie, animals had free access to running wheels); (3) CIMT with a restraint of the unaffected upper limb achieved by a plaster cast, thus enhancing the use of the affected limb in daily activities; and (4) skilled reaching training, which involved repeated reaching for food pellets with an exclusive use of the affected limb. We extracted data on training strategy, study quality, infant size, neurological outcome, and the number of animals in training and control groups. Because a meta-analysis requires a reasonable number of studies, neurobehavioral tests were divided into 4 clinically relevant groups: (1) neuroscore as gross neurological deficit score; (2) latency in the Morris water maze test for cognitive function; (3) rota rod, rotating pole, beam balance, foot-fault, and ladder rung walking tests for running function; and (4) staircase, limb placement, cylinder, and adhesive tape removal tests for limb function. With respect to previous studies, the initiation of exercise was categorized into <1 day, 1 to 5 days, and >5 days after stroke onset. The early time window was chosen because an initiation of exercise within 1 day after ischemia was reported to have negative effects on sensorimotor function. However, rehabilitation in experimental models is most pronounced within the first 5 to 14 days after stroke, and it has been shown that the efficacy of exercise declines with time from stroke onset. When neurobehavorial deficits were assessed at different times, only the last point in time was included. When data were presented only graphically, values were read off the graphics using Adobe Acrobat X Pro (Adobe Systems; San Jose, CA).

Quality Assessment

The methodological quality of the included studies was evaluated according to a previously published 11-item quality scale, modified as follows: (1) the intensity–response relationship; (2) randomization of the experiment; (3) optimal time window to initiate training; (4) monitoring of physiological parameters (temperature, blood pressure, blood glucose level); (5) blinded outcome assessment; (6) assessment of ≥2 outcome parameters; (7) outcome assessment in the acute phase (days 1–7); (8) outcome assessment in the chronic phase (beyond day 7); (9) appropriate animal model (aged, diabetic, hypertensive); (10) compliance with animal welfare regulations; and (11) statement of potential conflict of interests. The studies were thus classified into 3 quality categories (category I, 8–11 items; category II, 4–7 items; and category III, 0–3 items).

Statistical Analysis

We first performed a meta-analysis to assess both the efficacy of training in general, and the efficacy of each training strategy in particular. The meta-analysis was performed using a random-effects model. The results of included trials were analyzed separately for each of the end points: infarct volume, cognitive function, neuroscore, running function, and limb function. Each trial consisted of 1 control group and ≥1 treatment groups. We abstracted mean values and their SEs for the different outcomes for each treatment group and each control group. Some studies did not provide these statistics directly. When results were presented as minimum, median, and maximum, we obtained the mean and the SD using a previously published approximation. When the results were given as quartiles, we took the median as a substitute for the mean and approximated the SD and quartiles of a normal distribution. The SE was computed by dividing the SD by the square root of the number of animals per group. For infarct volume and cognitive function (latency in the Morris water maze test), we quantified the effect of treatment by the ratios of the outcomes of treatment groups and corresponding control groups. For this purpose, we applied a meta-analysis to the logarithms of the mean outcome values and approximated the corresponding SEs by means of the delta method. For neuroscore, running function, and limb function, we extracted minimum and maximum values and standardized the outcome values to a scale ranging from 0 to 100. The effect of treatment was quantified by the differences in the outcomes of treatment groups and corresponding control groups. For all outcomes, the effects of treatments are expressed as percentages and 95% confidence intervals (CIs). To analyze whether exercise, in general, is beneficial in experimental stroke, results from all treatment groups were averaged according to the DerSimonian and Laird random-effects approach. To compare the efficacy of different training strategies, we averaged the results of trials on forced exercise, voluntary exercise, and limb training for each of the end points: infarct volume, running function, and limb function. The results of our meta-analysis are presented as forest plots. Within the forest plots, studies are arranged according to their quality scores. In addition, we performed a meta-regression to compare the effectiveness of training when started <1 day, 1 to 5 days, and >5 days after stroke onset. For this purpose, the mean outcomes were analyzed as dependent variable in a linear mixed model with a random intercept for each trial and a random treatment effect. Treatment was also entered as fixed variable (either as binary variable or by 4 binary variables indicating forced exercise, voluntary exercise, and limb training), together with a categorical variable for start of treatment. All random variables involved in the model are assumed to follow normal distributions, whereby the random treatment effect and the random intercepts were allowed to be correlated. Apart from that, we analyzed the relationship between treatment frequency and treatment efficacy, and we analyzed the relationship between running distance and treatment efficacy (see online-only Data Supplement Methods).

Results

Study Inclusion and Study Characteristics

Our search of the literature identified 35 articles with 50 comparisons and >880 animals, which met the inclusion
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Meta-analysis of Training in Animal Stroke Models

Outcome was assessed in 539 animals for infarct size, 91 animals for cognitive function, 226 animals for neuroscore, 325 animals for running function, and 387 animals for limb function. The efficacy of forced physical training was investigated in 23 studies with 443 animals, the efficacy of voluntary physical training was assessed in 8 studies with 181 animals, the efficacy of CIMT was analyzed in 8 studies with 136 animals, and the efficacy of skilled reaching training was assessed in 8 studies with >81 animals. Two studies investigated a combination of skilled reaching training and forced physical training, and 1 study investigated a combination of skilled reaching training and voluntary physical training. In 7 comparisons, training was initiated <1 day after stroke onset; in 37 comparisons, training began 1 to 5 days after stroke onset; and in 6 comparisons, training started >5 days after stroke onset. Two studies used mice, 1 study used squirrel monkeys, 3 studies used spontaneously hypertensive rats, 1 study used aged rats, and all other studies used adult rats. The median quality score of included studies was 6 (range, 3–9).

### Overall Efficacy of Training and Comparisons of the Efficacy of Different Training Strategies

A meta-analysis of all treatments showed that training reduced the infarct volume by 14% (95% CI, 2%–25%; Figure 1). Forced physical training reduced the infarct volume by 30% (95% CI, 17%–42%; Figure 1), whereas CIMT

#### Table 1: Effect of Different Training Strategies on Infarct Volume

<table>
<thead>
<tr>
<th>Trial Description</th>
<th>Ratio (95% CI)</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voluntary + skilled training</td>
<td>0.93 (0.72–1.20)</td>
<td>53.3</td>
</tr>
<tr>
<td>Forc e physical training</td>
<td>0.69 (0.37–1.27)</td>
<td>4.6</td>
</tr>
<tr>
<td>CIMT</td>
<td>0.59 (0.37–0.92)</td>
<td>8.4</td>
</tr>
<tr>
<td>Skilled reaching training</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puglighman 2007</td>
<td>0.62 (0.28–1.40)</td>
<td>53.3</td>
</tr>
<tr>
<td>Keiser 2008</td>
<td>1.33 (0.58–2.70)</td>
<td>4.6</td>
</tr>
<tr>
<td>Maldonado 2008</td>
<td>0.85 (0.36–1.94)</td>
<td>8.4</td>
</tr>
<tr>
<td>Meta-Analysis, I²: 0%</td>
<td></td>
<td>8.4</td>
</tr>
<tr>
<td>Voluntary physical training</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marin 2003</td>
<td>1.04 (0.95–1.14)</td>
<td>78.6</td>
</tr>
<tr>
<td>Malsuda 2011</td>
<td>0.90 (0.83–1.01)</td>
<td>3.3</td>
</tr>
<tr>
<td>Maldonado 2006</td>
<td>1.18 (1.01–1.39)</td>
<td>3.3</td>
</tr>
<tr>
<td>Meta-Analysis, I²: 0%</td>
<td></td>
<td>3.3</td>
</tr>
</tbody>
</table>

#### Figure 1

Infarct volume. The effect size is the infarct size reduction in trained animals expressed as a proportion of the infarct size in control groups. The weight represents the inverse of the variance of the ratio of outcomes in treatment group and the corresponding control group. The area of each plot is proportional to the weight. Horizontal lines indicate the 95% confidence interval (95%-CI). Subgroup analyses show the efficacy of different training strategies. Arranging forest plots by quality score, starting with the highest quality at the top, does not illustrate a relationship between study quality and efficacy. *Five days of training, **14 days of training, #young rats, 1 week of training, ##young rats, 2 weeks of training, ###aged rats, 1 week of training, ####aged rats, 2 weeks of training, ++10 days of training, +++6 weeks of training, §1 week of training, §§2 weeks of training, §§§4 weeks of training. CIMT indicates constraint-induced movement therapy.
increased the infarct volume by 18% (95% CI, 1%–18%; Figure 1). Voluntary physical training and skilled reaching training did not significantly affect the infarct volume (Figure 1). Meta-analyses of all treatments revealed that training improved the cognitive function by 33% (95% CI, 8%–50%; Figure 2) and the neuroscore by 13.4% (95% CI, 1.5%–25.3%; Figure 3). Due to the small number of studies assessing cognitive functions and neuroscore, we did not compare the efficacy of different training strategies for these outcomes. A meta-analysis of all treatments showed that training improved the running function by 6.6% (95% CI, 1.4%–11.9%; Figure 4). Forced physical training enhanced the running function by 17.8% (95% CI, 8.4%–27.3%; Figure 4), and skilled reaching improved the running function by 11.2% (95% CI, 2.5%–19.9%; Figure 4). Voluntary physical training and CIMT did not significantly affect the running function (Figure 4). A meta-analysis of all treatments illustrated that training in general did not have a significant effect on the limb function (Figure 5). Skilled reaching training in particular improved the limb function by 26.7% (95% CI, 8.3%–26.7%; Figure 5), and voluntary physical training enhanced the limb function by 13.3% (95% CI, 0.7%–25.9%; Figure 5). Forced physical training and CIMT did not significantly influence the limb function (Figure 5). Our additional analyses did not illustrate a relationship between treatment frequency and treatment efficacy. In studies on forced physical training, there was no relationship between total running distance per animal and treatment efficacy (see online-only Data Supplement Results).

Effect of the Time of Training Initiation on Efficacy of Training

Meta-regression analysis demonstrated that training reduced the infarct volume more effectively when started between days 1 and 5 after stroke onset compared with an initiation later than 5 days after stroke onset (P<0.05; Table). Neither a comparison of an initiation <1 day versus 1 to 5 days after stroke nor a comparison of an initiation <1 day versus 1 to 5 days after stroke illustrated a significant effect on the infarct volume (Table). The cognitive function was enhanced more efficaciously when training started between days 1 and 5 after stroke onset compared with a start of treatment either <1 day or >5 days after induction of ischemia (P<0.05; Table). The neuroscore was improved more efficaciously when training began between days 1 and 5 compared with an initiation <1 day after induction of ischemia (P<0.001; Table). The running function was not significantly influenced by the time to treatment initiation. The limb function was improved more effectively when training started between days 1 and 5 compared with a start of treatment at <1 day (P<0.05; Table).

Effect of the Study Quality

The median quality score of included studies was 6 (range, 3–9). Arranging forest plots by quality score did not reveal a relationship between study quality and effect of treatment (Figures 1–5).

Discussion

Efficacy of Exercise in Animal Models of Ischemic Stroke

Our meta-analysis of all treatments showed that training reduced the infarct volume by 14% and improved the cognitive function by 33%, the neuroscore by 13.4%, and the running function by 6.6%. Forced physical training reduced the infarct volume by 30% and improved the running function by 17.8% but did not improve the limb function. In contrast, skilled reaching training improved the limb function by 26.7% and enhanced the running function by 11.2% but did not significantly affect the infarct volume. Voluntary physical training improved the limb function by 13.3% but did not significantly affect the infarct volume and the running function. CIMT increased the infarct volume by 18% and did not have a significant effect on the running function and the limb function. Our meta-regression analysis illustrated that training was particularly efficacious when initiated between 1 and 5 days after stroke onset. Further analyses did not illustrate a relationship between treatment frequency and treatment efficacy. In studies on forced physical training, there was no relationship between running distance and treatment efficacy.

Figure 2. Cognitive function. The effect size is the reduction of the latency in the Morris water maze expressed as a proportion of the latency in control groups. The weight represents the inverse of the variance of the ratio of outcomes in the treatment group and the corresponding control group. The area of each plot is proportional to the weight. Horizontal lines indicate the 95% confidence interval (95%–CI). Arranging forest plots by quality score, starting with the highest quality at the top, does not illustrate a relationship between study quality and efficacy. ++Six weeks of training.
To date, this is the first meta-analysis of the efficacy of different training strategies in animal models of ischemic stroke. Although a large number of experimental stroke studies on forced physical training have shown beneficial effects, the forced paradigm has remained controversial. It has been argued that the stress involved in forced regimens might increase serum levels of glucocorticoid hormones, aggravating the ischemic damage. The latter is supported by studies showing that high concentrations of glucocorticoid hormones are associated with an increased infarct volume and an increased morbidity and mortality.23,24 To date, there are only 2 studies comparing the efficacy of voluntary physical training and forced physical training, both demonstrating superiority of voluntary physical training.6,8 Our meta-analysis does not mirror these findings but is in accordance with another study suggesting that the combination of stress and training is superior to training alone.9 These contradictory results of previous studies might be attributable to variable experimental designs of those studies. Constraint-induced movement therapy encourages use of the paretic limb and was designed to counteract the phenomenon Taub first described in a deafferented monkey as learned nonuse of the paretic limb.25 The present meta-analysis does not confirm the efficacy of CIMT in animal models of ischemic stroke. Hence, our findings are in concert with the only previous studies which demonstrated recovery-enhancing effects of task-specific training after focal lesions of the sensorimotor cortex.27,28

Beyond the training strategy, the optimal time window is another matter of dispute. Evidence from animal studies suggests a time-limited period of enhanced neuroplasticity after stroke.12,18 Neuroplasticity, which includes activity-dependent dendritic reorganization and synapse strengthening, is heightened within the first 2 weeks after stroke, when growth-promoting genes are upregulated.19 Outside this critical period of enhanced neuroplasticity, growth-inhibitory genes are gradually upregulated, and recovery slows down.18 In a previous study, rats were subjected to rehabilitative treatment starting at 5, 14, or 30 days after stroke. The results demonstrated that early treatment was most effective, whereas delayed treatment (30 days after stroke) achieved only minor behavioral improvements.13 In other studies, by contrast, exercise initiated within the first day after stroke has been associated with increased ischemic cell death.14-16 However, increased ischemic cell death does not necessarily translate into worse behavioral outcome15,20 but may also reflect a pruning effect with an early elimination of energy-compromised dysfunctional neurons because of use-dependent activation.15 Increased dendritic plasticity in the perifocal region and the expansion of motor maps might thus be better indicative of neuronal recovery than lesion size alone.

**Implications for Clinical and Experimental Studies**

Although training is routinely applied in human stroke rehabilitation, the most beneficial paradigm has not been characterized. In the Extremity Constraint Induced Therapy Evaluation (EXCITE) randomized clinical trial, CIMT achieved significant and sustained improvements in motor arm function in patients who had a stroke within the previous 3 to 9 months,12,30 but subsequent clinical studies on CIMT have yielded variable results, and a Cochrane systematic review showed no persisting benefit of CIMT.13,31-33 The efficacy of voluntary physical training, forced physical training, skilled
training, and a combination therapy of physical training and skilled training has not been compared in a clinical study to date. Similar to the experimental setting, evidence from clinical studies suggests a critical time window for human stroke rehabilitation,14–16,34,35 but the optimal time to start in-hospital rehabilitation has not been identified. The critical time window in human stroke certainly differs from that observed in animal studies because stroke recovery generally takes ≈4 weeks in animals, whereas humans continue to recover spontaneously over ≈3 months. However, both experimental and clinical studies provide evidence for a time-limited period of enhanced neuroplasticity. Altogether, our meta-analysis identifies the most effective training strategies in animal models of stroke and may thus help to design future experimental and clinical studies. Characterizing the most effective training strategies in animal stroke models is of importance because it may help to elucidate training-induced mechanisms of stroke recovery and may improve the quality of experimental stroke studies that use training as a control treatment of known efficacy.

### Methodological Considerations

Our meta-analysis was based on 35 studies with a median quality score of 6 of 11. Compared with previous meta-analyses and systematic reviews of experimental stroke studies, the included studies were of relatively high quality.17,36–38 In contrast to previous meta-analyses, which have reported a trend toward greater benefit in studies of lower quality,26,39,40 we found no relationship between study quality and treatment effect. Nevertheless, some methodological weaknesses must be considered. First, only 3 studies investigated the efficacy of training in animals with comorbidity. All other studies have used healthy animals, which raises the possibility that treatment effects are overestimated.41 Second, our meta-analysis contains multiple sources of heterogeneity (species, stroke model, time to initiate training, training intensity, time of assessment, and outcome measures), which raises the possibility that treatment effects within subgroups are underestimated.26 The infarct location and the initial infarct size may well have an effect on the efficacy of a treatment.
In our meta-analysis, most studies used middle cerebral artery occlusion models of stroke, and only 9 studies used models of focal cortical stroke. These 9 studies differed with respect to treatment strategy and assessed outcomes, so it is unlikely that they influenced the results in either direction. Comparing the efficacy of forced physical training and voluntary physical training, the running distance is of interest. Studies on voluntary physical training did not provide sufficient data about the running distance per animal. However, among animals subjected to forced physical training, there was no clear relationship between running distance and infarct volume or functional outcome. Therefore, it is unlikely that the unknown running distance in studies on voluntary physical training conceals a significant treatment effect. A limitation of our meta-regression analysis is that CIMT was predominantly initiated within 1 day after stroke so that CIMT has an impact on the early time window in our meta-regression. A potential weakness of meta-analyses of experimental studies in general is a high susceptibility to publication bias attributable to unpublished negative or neutral studies. However, in the present meta-analysis, many studies have reported nonsuperiority of training. Compared with many previous meta-analyses, our meta-analysis is less prone to publication bias because several studies have compared the efficacy of different training strategies, and some studies have used training as a control treatment to demonstrate superiority of another candidate stroke drug. Another strength of our meta-analysis is the reasonable number of included studies, which allows us to draw meaningful conclusions on the efficacy of different training strategies in animal models of stroke.

**Conclusions**

Our results confirm that training reduces the infarct volume and improves the functional outcome in animal models of ischemic stroke. Forced physical training and skilled reaching training were identified as the most effective training strategies. Results of a meta-regression illustrated that training was
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Disclosures

None.

References

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Supplemental material

Supplemental methods

Relationship between treatment frequency and treatment efficacy

To analyze the relationship between treatment frequency and treatment efficacy, we extracted the total number of training units from studies on forced physical training and skilled reaching training. We confined our analysis to forced physical training and skilled reaching training. CIMT was not included, because all studies on CIMT applied only one continuous training period using a plaster cast to immobilize the intact forelimb. Voluntary physical training was not included, because studies on voluntary physical training did not provide information on training frequency. We performed meta-analyses using a random-effects model as described in the methods section of the main manuscript. The results of included trials were analyzed separately for each of the end points infarct volume, running function and limb function. Due to the small number of studies assessing cognitive functions and neuroscore, we did not analyze the relationship between treatment frequency and treatment efficacy for these outcomes. The results are presented as forest plots. Within the forest plots, studies are arranged according to the treatment frequency.

Relationship between running distance and treatment efficacy in studies on forced physical training

To analyze the relationship between running distance and treatment efficacy, we extracted the total running distance per animal from studies on forced physical training. Studies on voluntary physical training do not provide information about the running distance per animal, and standard deviations are not available. So these data are not useful for statistical analyses. We performed meta-analyses using a random-effects model as described in the methods section of the main manuscript. The results of included trials were analyzed separately for each of the end points infarct volume, running function and limb function. Due to the small number of studies assessing cognitive functions and neuroscore, we did not analyze the relationship between running function and treatment efficacy for these outcomes. The results are presented as forest plots. Within the forest plots, studies are arranged according to the running distance.

Supplemental results

Relationship between treatment frequency and treatment efficacy

As illustrated by supplemental figure I, there is no distinct relationship between treatment frequency and infarct volume. Supplemental figure II does not reveal a clear relationship between treatment frequency and running function, and supplemental figure III does not show a relationship between treatment frequency and limb function, either. As there was no obvious association, no specific statistical analysis was performed of the effect of the treatment frequency on the treatment efficacy.

Relationship between running distance and treatment efficacy in studies on forced physical training

As illustrated by supplemental figure IV, there is no distinct relationship between running distance and infarct volume. Supplemental figure V does not show a relationship between running distance and running function, and supplemental figure VI does not reveal an association between running distance and limb function, either. As there was no apparent
relationship, no specific statistical analysis was performed of the effect of the running distance on the treatment efficacy.

## Supplemental table

### Supplemental table I. Animal studies on training in ischemic stroke.

<table>
<thead>
<tr>
<th>First author, year of publication</th>
<th>Species</th>
<th>Stroke model (stroke focus)</th>
<th>Training category</th>
<th>Initiation of training</th>
<th>Outcome measures (n (treated / control))</th>
<th>Quality category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Araujo 2008¹</td>
<td>Gerbil</td>
<td>CCAO (10 min) (striatum)</td>
<td>Forced physical training</td>
<td>&lt; 1 d</td>
<td>Running function (12/12)</td>
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<tr>
<td>Barbay 2006²</td>
<td>Squirrel monkey</td>
<td>Cauterization of surface vessels (forelimb motorcortex)</td>
<td>Skilled reaching training</td>
<td>&gt; 5 d</td>
<td>Limb function (4/4)</td>
<td>2</td>
</tr>
<tr>
<td>Bland 2000³</td>
<td>Rat</td>
<td>ET1-induced MCAO (striatum)</td>
<td>CIMT</td>
<td>&lt; 1 d</td>
<td>Infarct volume (8/7) Cognitive function (6/6) Running function (8/8) Limb function (8/8)</td>
<td>2</td>
</tr>
<tr>
<td>Bland 2001⁴</td>
<td>Rat</td>
<td>MCAO (60 min) (striatum)</td>
<td>CIMT</td>
<td>&lt; 1 d</td>
<td>Infarct volume (10/9) Running function (10/10) Limb function (10/10)</td>
<td>2</td>
</tr>
<tr>
<td>Borlongan 2000⁵</td>
<td>Rat</td>
<td>MCAO (60 min) (striatum)</td>
<td>Forced physical training</td>
<td>1 - 5 d</td>
<td>Infarct volume (5/6)</td>
<td>1</td>
</tr>
<tr>
<td>Brown 2004⁶</td>
<td>Rat</td>
<td>Photothrombosis (hindlimb sensorimotor cortex)</td>
<td>Forced physical training</td>
<td>1 - 5 d</td>
<td>Running function (5/8)</td>
<td>2</td>
</tr>
<tr>
<td>Chang 2009⁷</td>
<td>Rat</td>
<td>MCAO (60 min) and bilat. CCAO (striatum)</td>
<td>Forced physical training</td>
<td>1 - 5 d</td>
<td>Running function (8/8)</td>
<td>2</td>
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<tr>
<td>Diederich 2012⁸</td>
<td>Rat</td>
<td>Photothrombosis (forelimb somatosensory cortex)</td>
<td>CIMT</td>
<td>1 - 5 d</td>
<td>Infarct volume (10/10) Limb function (20/20)</td>
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<tr>
<td>Ding 2004⁹</td>
<td>Rat</td>
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<td>Forced physical training</td>
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Notes: MCAO = middle cerebral artery occlusion; ER1 = endothelin-1; CIMT = constrained-induced movement therapy; SH = spontaneously hypertensive; SMA = superior mesenteric artery; CI = cerebral ischemia; S1 = somatosensory cortex; P1 = primary visual cortex; V1 = primary auditory cortex; HP = hippocampal pathology; s = 2 square millimeter; d = day; 1 = 1 square millimeter; 2 = 2 square millimeter; 3 = 3 square millimeter; 4 = 4 square millimeter; 5 = 5 square millimeter; 6 = 6 square millimeter; 7 = 7 square millimeter; 8 = 8 square millimeter; 9 = 9 square millimeter; 10 = 10 square millimeter; 11 = 11 square millimeter; 12 = 12 square millimeter; 13 = 13 square millimeter; 14 = 14 square millimeter; 15 = 15 square millimeter; 16 = 16 square millimeter; 17 = 17 square millimeter; 18 = 18 square millimeter; 19 = 19 square millimeter; 20 = 20 square millimeter; 21 = 21 square millimeter; 22 = 22 square millimeter; 23 = 23 square millimeter; 24 = 24 square millimeter; 25 = 25 square millimeter; 26 = 26 square millimeter; 27 = 27 square millimeter; 28 = 28 square millimeter; 29 = 29 square millimeter; 30 = 30 square millimeter; 31 = 31 square millimeter; 32 = 32 square millimeter; 33 = 33 square millimeter.
Supplemental figures

Supplemental figure I. Relationship between treatment frequency and infarct volume in studies on forced physical training and skilled reaching training. The effect size is the infarct size reduction in trained animals expressed as a proportion of the infarct size in control groups. Horizontal lines indicate the 95% CI. Arranging forest plots by treatment frequency, starting with the highest number of training units at the top, does not illustrate a relation between treatment frequency and efficacy.
Supplemental figure II. Relationship between treatment frequency and running function in studies on forced physical training and skilled reaching training. Outcome values of included studies were standardized to a scale ranging from 0 to 100. The effect size is the difference of the outcomes of treatment group and corresponding control group. Horizontal lines indicate the 95% CI. Arranging forest plots by treatment frequency, starting with the highest number of training units at the top, does not demonstrate a clear relation between treatment frequency and efficacy.
Supplemental figure III. Relationship between treatment frequency and limb function in studies on forced physical training and skilled reaching training. Outcome values of included studies were standardized to a scale ranging from 0 to 100. The effect size is the difference of the outcomes of treatment group and corresponding control group. Horizontal lines indicate the 95% CI. Arranging forest plots by treatment frequency, starting with the highest number of training units at the top, does not show a relation between treatment frequency and efficacy.
Supplemental figure IV. Relationship between total running distance per animal and infarct volume in studies on forced physical training. The effect size is the infarct size reduction in trained animals expressed as a proportion of the infarct size in control groups. Horizontal lines indicate the 95% CI. Arranging forest plots by running distance, starting with the highest running distance per animal at the top, does not illustrate a relation between total running distance per animal and treatment efficacy.
Supplemental figure V. Relationship between total running distance per animal and running function in studies on forced physical training. Outcome values of included studies were standardized to a scale ranging from 0 to 100. The effect size is the difference of the outcomes of treatment group and corresponding control group. Horizontal lines indicate the 95% CI. Arranging forest plots by running distance, starting with the highest running distance per animal at the top, does not illustrate a relation between total running distance per animal and treatment efficacy.
Supplemental figure VI. Relationship between total running distance per animal and limb function in studies on forced physical training. Outcome values of included studies were standardized to a scale ranging from 0 to 100. The effect size is the difference of the outcomes of treatment group and corresponding control group. Horizontal lines indicate the 95% CI. Arranging forest plots by running distance, starting with the highest running distance per animal at the top, does not illustrate a relation between total running distance per animal and treatment efficacy.

References


31. Wurm F, Keiner S, Kunze A, Witte OW, Redecker C. Effects of skilled forelimb training on hippocampal neurogenesis and spatial learning after focal cortical infarcts in the


