Long-term survival and serial assessment of stroke damage and recovery – practical and methodological considerations

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Abstract

Impairments caused by stroke remain the main cause for adult disability. Despite a vigorous research effort, only 1 thrombolytic treatment has been approved in acute stroke (<3h). The limitations of preclinical studies and how these can be overcome have been the subject of various guidelines. However, often these guidelines focus on the acute stroke setting and omit long-term outcome measures, such as behaviour and neuroimaging. The considerations and practicalities of including the serial assessment of these approaches and their significance to establish therapeutic efficacy are discussed here.

Keywords

Stroke; middle cerebral artery occlusion; post-operative care; neurological score; behavioural battery; MRI

1. Introduction

The development of novel treatments for stroke remains a major challenge for scientists and clinicians alike. Although other neurological conditions, such as Parkinson's disease (PD), have seen the development of pharmacological compounds that at least alleviate the behavioural impairments temporarily, no such success has been achieved for ischaemic stroke, with recombinant tissue-type plasminogen activator (rtPA) remaining the only clinically-approved intervention. This is despite excellent animal models of stroke that more accurately replicate the proximal cause and pathology of the human condition compared to other disease, such as a PD. It is therefore important to understand why compounds showed promise in pre-clinical models, but failed to achieve similar success in clinical trials (Savitz 2007; Wahlgren and Ahmed 2004; Zaleska et al. 2009).

Several initiatives (Chopp et al. 2009; Feuerstein et al. 2008; Fisher 2003; Fisher et al. 2005; Fisher et al. 2009; Liu et al. 2009; Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS): bridging basic and clinical science for cellular and neurogenic factor therapy in treating stroke 2009) have compiled limitations and omissions in preclinical studies that raised concerns about the studies' reliability and validity, but also about its outcome measures and the timing of intervention. Their guidelines aim to improve the pre-clinical testing of therapeutic efficacy in stroke by failing unsuccessful interventions, while increasing the likelihood of successful compounds to succeed in the clinic. Nevertheless, the focus is often on a single short-term ex vivo outcome measure, such as histological volume

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reduction, that differs considerably from clinical outcome measures. Although this might provide an indication that a therapeutic reduces the impact of stroke, this does not necessarily translate into a behavioural benefit.

The validity of measures and their sensitivity to detect therapeutic efficacy are often poorly established. To determine if an intervention was not successful rather than outcome measures not being sensitive to the therapy, ideally within each study a validation of outcome measures using a positive control should be undertaken (Ashioti et al. 2009). Generally, there is a good correlation between ex vivo histological lesion volumes and in vivo behavioural tests or neuroimaging (Ashioti et al. 2007). Nevertheless, brain plasticity can produce recovery on neurological or behavioural tests without a concomitant improvement in lesion volume.

Merely assessing absolute lesion volume disregards the site of the lesion and omits its impact on the remaining brain. Focussing on lesion volume as a whole might average-out any regional neuroprotection. Neuroimaging is emerging though as an integrative assessment that captures the more complete anatomical impact of stroke (Liebeskind 2009). Lesion volume by MRI robustly correlates with behavioural measurements in animals (Ashioti et al. 2007; Lim et al. 2008; Virley et al. 2000) and neurological/ neuropsychological assessments in patients (Committeri et al. 2007). Additional measurements of anatomical changes in spared brain regions (i.e. where blood flow has been normal) can further elucidate the secondary impact stroke exerts on other regions, such as the thalamus, but also on the non-injured hemisphere (Modo et al. 2009). Changes in these areas can be crucial to explain behavioural improvement in stroke. Neuroimaging can hence provide a common assessment platform across species and eventually deliver validated surrogate markers of treatment efficacy (Liebeskind 2009).

To ensure that treatment is robust, experiments should not only focus on the acute short-term benefit (<48h), but also the sub-acute (2–14 days) and chronic period (>14 days). It is noteworthy that the lesion volume following 60 minutes of middle cerebral artery occlusion (MCAo) increases up to 6 weeks following insult before it stabilises (Modo et al. 2009). Hence, one would assume that as the lesion evolves the benefit from a continued treatment should become more apparent. Especially interventions impacting on secondary degeneration, such as Wallerian degeneration, will only be evident with long-term assessments (Yu et al. 2009). Therefore, serial in vivo assessments over longer time frames can be more powerful to detect therapeutic effects compared to analysis of a single acute time-point.

2. Neurological Scoring versus Behavioural Test Battery

There is a fundamental distinction between neurological deficits and behavioural impairments. Neurological deficits are specific functions, such as the righting reflex, that are perturbed, whereas behavioural impairments are more complex readouts, such as lack of learning in the water maze. For serial and long-term measurements, one also needs to consider if there is spontaneous recovery of this task. Most tasks will eventually recover long-term, although very specific and detailed analyses will allow the continued detection of a deficit or adaptations (Alaverdashvili et al. 2008). Another essential distinction is between behavioural and functional tests. Behavioural tests are tasks where an overt response of an animal is measured, whereas functional readouts reflect measurements where brain activity is assessed (e.g. fMRI, phMRI, EEG, electrophysiology). Functional tests therefore provide the link between anatomical and behavioural measures. Although functional and behavioural measures are often linked, it is conceivable that changes in the brain's activity occur (i.e. plasticity) that do not directly translate into a behavioural benefit. For instance, the fMRI

response to somatosensory stimulation sub-acutely (1–3 days) indicates activity in the contralateral cortex. However, there is no behavioural recovery associated with this change in activity and indeed animals only recover if activity is restored to areas adjacent to those damaged (Dijkhuizen et al. 2003). Conversely, it is possible that an animal shows "recovery" on a behavioural test due to adaptation that does not necessarily reflect a measurable functional change.

Neurological deficits are often used to probe outcomes in pre-clinical stroke studies. A series of specific tests can be charted into a neurological scale to provide a read-out of overall severity (Modo et al. 2000b). For this readout to be meaningful as a general measure of severity, it is important that each deficit carries equal weight and ideally that it is either impaired (1) or normal (0). The total score hence reflects the magnitude of a general deficit. Several scales that either measure global neurological function (Table 1) or more specific stroke-related deficits are documented in the literature. Tests that are especially important and sensitive to the damage caused by stroke are best assessed separately as a graded scale. For instance, one of the most commonly used stroke-specific scales consists of the 3 point Bederson scale (Bederson et al. 1986) (Table 2). This scale is sensitive to striatal damage and measures a forelimb dysfunction that is dependent on the degree of lesioning. Importantly, this neurological deficit is visible within 45 minutes of occlusion and can be used prior to re-perfusion to surmise if a MCA occlusion was successful.

The most comprehensive approach is to use two scales to measure neurological deficits. One scale should chart the overall neurological impact of the procedure, whereas another scale should measure the degree of deficit that is relevant to stroke. Combinations of general neurological dysfunction with stroke-specific deficits have been described (Hunter et al. 2000). Often these scales, however, give different weight to different tests and therefore the overall score cannot be considered an overall measure of severity. Although neurological scales typically correlate with short-term lesion volumes (Ashioti et al. 2007), they will no longer reflect the degree of histological damage at chronic time points, as most deficits spontaneously recover between a few days to a couple of weeks post-infarction with lesion volumes increasing (Modo et al. 2000b). As such, these neurological scales are only useful in the acute to sub-acute setting. However, appropriate controls consisting of stroke-only, stroke+vehicle and/or sham-surgery need to be incorporated to provide a meaningful assessment of therapeutic effects. Recovery on these scales can at worst merely indicate better spontaneous recovery or that an intervention improves recovery from the surgical procedure itself rather than the stroke. It is therefore best to consider with great caution claims of a persisting recovery, if only neurological scales have been used as read-outs.

Neurological scales typically do not reflect the distress of animals. Neurological scales can hence be misleading in defining humane end-points. Mostly for long-term recovery experiments, it is important to ensure that animals recover fast (Figure 1) and are not persistently distressed or suffering, as this can adversely affect behavioural outcome measures. Especially in the acute setting, neurological tests can be affected if the animals are distressed/suffering. In these cases, the inclusion of a distress scale (Table 3) can be used to determine an animal's degree of suffering. Based on defined categories on these scales, it is possible to decide whether an animal should be euthanized or not. Although this type of scale can be used as an outcome measure, it does not reflect an improvement in behavioural impairments, but merely the animal's level of suffering. As such, a distress scale might be considered a complimentary measure to other more damage-specific tests.

A more robust indication of persistent impairments is achieved by applying behavioural tests (Table 4). The tests typically require the animal to perform a specific task, such as learning to swim to a platform (water maze) or to remove sticky tape from their forepaws (bilateral

asymmetry test). They mainly differ from neurological tests in their complexity and often require several functions to integrate (e.g. attention, sensorimotor detection and motor skill for the bilateral asymmetry test). Behavioural tests are often chosen based on the specific anatomical damage that is caused by a stroke. If this damage is predominantly striatal, tests that probe striatal integrity, such as amphetamine-induced rotation, are appropriate. In most cases, stroke affects a variety of anatomical regions (striatum, sensorimotor cortex, thalamus) and hence it is more appropriate to use a battery of tests that canvas damage to these regions. If one would only chose a striatal task, but have more extensive extra-striatal damage, one would potentially fail to detect an improvement on a cortical task due to neuroprotection in the cortex.

3. Serial in vivo evaluation of stroke impact

Apart of neurological and behavioural assessments, non-invasive neuroimaging can monitor serially the neuropathological impact of stroke (Table 5). Although neuroimaging does not provide the same anatomical detail relative to extensive histopathological assessments, it affords a serial assessment of lesion pathology that can be applied to pre-clinical, as well as clinical studies. By serially assessing the same animal/patient over time, a dramatic improvement in statistical power can be achieved (Figure 2). Merely comparing two groups using a T-test at a single time point necessitates a large group size to reach statistical significance, even with a large effect size. By using a repeated measures approach, the total number of animals needed is dramatically reduced. Therefore this results in a reduction in the number of animals/patients needed to establish therapeutic efficacy. A further refinement of this approach is to use pre-treatment scans to calculate the percentage change over time due to an experimental intervention. This calculation will lead to a reduction in the intra-animal variability and further reduce the number of animals needed to compare different groups (Figure 3). In addition to lesion volumes, other structural changes outside the area of primary infarction, such as Wallerian degeneration, can also be accounted for (i.e. atrophy or hypertrophy) on an animal-by-animal basis. Sophisticated imaging analysis methods can be further developed to, for instance, link changes in the lesion environment with impairments (Lo et al.). These approaches potentially can dramatically improve our understanding of therapeutic efficacy compared to merely assessing lesion volume histologically and therefore should result in a more likely clinical translation.

A major challenge to reconciling pre-clinical with clinical studies to date has been the administration of therapeutic agents in animals before occlusion or immediately after occlusion. This scenario is unlikely to occur in a clinical situation. However, with imaging it is now possible to investigate a therapeutic effect based on particular imaging characteristics rather than estimated time passed since occlusion. A pre-treatment scan can establish baseline pathology as in a clinical trial and monitor its progression over days. These pre-treatment scans can define inclusion and exclusion criteria for both preclinical and clinical studies. So far, in most animal studies, it has not been possible to determine very reliable inclusion or exclusion criteria based on neurological scales, as even sham surgery can produce similar transient signs of occlusion. Nevertheless, having clearly defined objective inclusion criteria will reduce variance within each group, as only animals with a clearly defined pathology will be included. Similar inclusion and exclusion criteria can in principle be applied in animal and human studies.

Apart of structural information, all non-invasive imaging techniques that are used clinically can also provide preclinical information about cerebral blood flow (Zaro-Weber et al. 2009) and in some cases penumbral tissue (Massaweh et al. 2009; Rivers et al. 2006). Being able to detect the molecular or cellular components involved in ischaemic brain damage will also increasingly afford a more targeted intervention (Rojas et al. 2007). As many of these

aspects are detectable within minutes of occlusion (Table 6), it is possible to determine therapeutic windows during which particular treatments will be effective. Although a variety of relevant and important information regarding the stroke damage can potentially be derived from neuroimaging, especially by combining various approaches, time constraints on each of these limit their application (Felberg and Naidech 2003). One of the major challenges for years to come therefore will be to develop a robust and informative imaging paradigm that will be sufficiently fast to provide relevant physiological information to conduct efficacy studies (i.e. define inclusion/exclusion criteria).

It is important here to potentially distinguish efficacy and mode-of-action studies. In efficacy studies, the main focus will be to establish if there is a benefit of an intervention and hence the aim is to merely distinguish subjects with or without a treatment. In mode-of-action studies, specific mechanisms through which an intervention is working are under investigation. These will require more detailed studies that involve a variety of imaging techniques to probe specific processes in vivo (e.g. apoptosis). However, it is likely that for mode-of-action studies more traditional ex vivo measures will remain superior to in vivo imaging studies for the coming years.

4. Perspective

Many investigations into the therapeutic efficacy in stroke focus on acute histological outcome measures. Although these studies are less time-consuming, they often fail to implement a clinically-relevant experimental design. Nevertheless, these fast and reliable measures should be regarded as screening tools for potential therapeutic candidates. To establish candidates for clinical translation, serial assessments over a longer time frame, with the inclusion of both behavioural and in vivo neuropathological measures, will yield more appropriate indications of therapeutic potential. Especially, the use of neuroimaging is increasingly mandated, as it will afford the same outcome measures to be used in preclinical and clinical studies. Although these measures can improve the quality of our preclinical studies and decrease the risk of ineffective therapies being prematurely tested in patients, it does still not guarantee a successful clinical translation. Closer links between clinical and preclinical studies are required to ensure that similar approaches are used to establish therapeutic efficacy. Only if we can close the methodological gap between animal and human studies are we likely to achieve similar efficacy.

Acknowledgments

Abbreviations

CT	Computer tomography
EEG	Electroencephalography
fMRI	functional Magnetic Resonance Imaging
MCA	Middle Cerebral Artery
MRI	Magnetic Resonance Imaging
PD	Parkinson's Disease

PET	Positron Emission Tomography
phMRI	pharmacological Magnetic Resonance Imaging
rtPA	recombinant tissue-type Plasminogen Activator
SPECT	Single Photon Emission Computer Tomography

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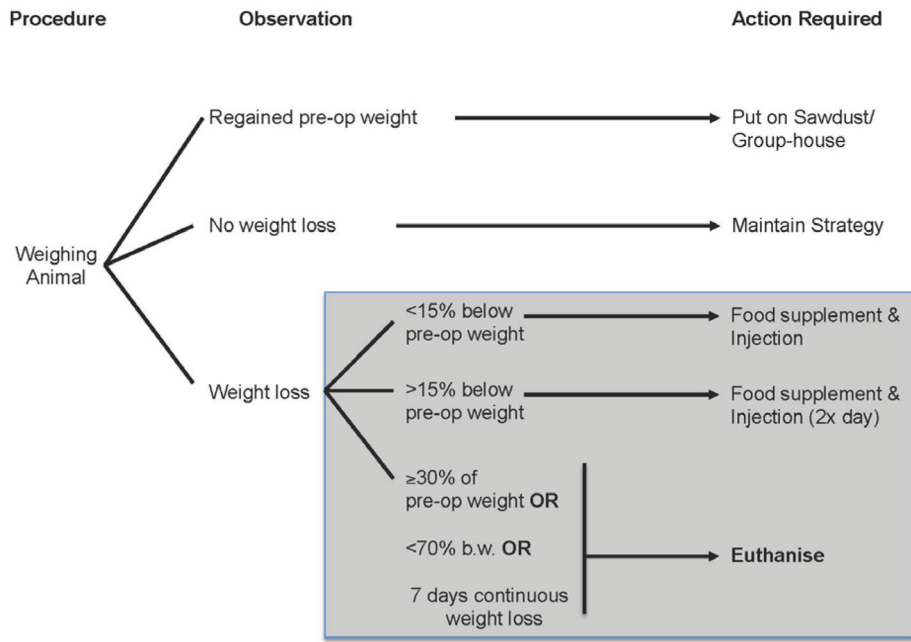
Figure 1.
Flow-chart for post-operative care strategies (Modo et al. 2000b). Animals are assessed daily after stroke. The best time for assessment is early mornings after the animals come out of their active period during which they are most likely to have consumed food. One of the most crucial features of animals' recovery from stroke is their evolution of body weight. Body weight is a good indicator of the animals' health status. If animals have re-gaining preoperative weight after surgery, they can be group-housed again. Ideally animals are kept in separate cages overnight to prevent them removing each other's sutures and to allow an assessment of food intake and defecation. If the animal maintains weight, it is best to maintain the current strategy (i.e. same level of care). In the case of animals undergoing stroke, they typically loose weight (10–15%). If they do not, this often is a good indication that they were not properly occluded or that only minor damage was caused. Different levels of weight loss require different strategies to improve the animal's recovery. Mashed food, as well as food supplements (e.g. Complan), in addition to injection with gluocsaline (sometimes mixed with Duphalyte) is known to improve post-operative recovery without affecting the evolution of the lesion per se. Typically fresh wet food is provided straight after measuring their weight in the morning and if needed as well just before the dark period. These strategies are designed to ensure the long-term survival of animals. To counteract distress/suffering of animals, additional injections of analgesics should be considered. Nevertheless, if these interfere with the evaluation of a therapeutic, experimenters should discuss alternatives with their local veterinary service.
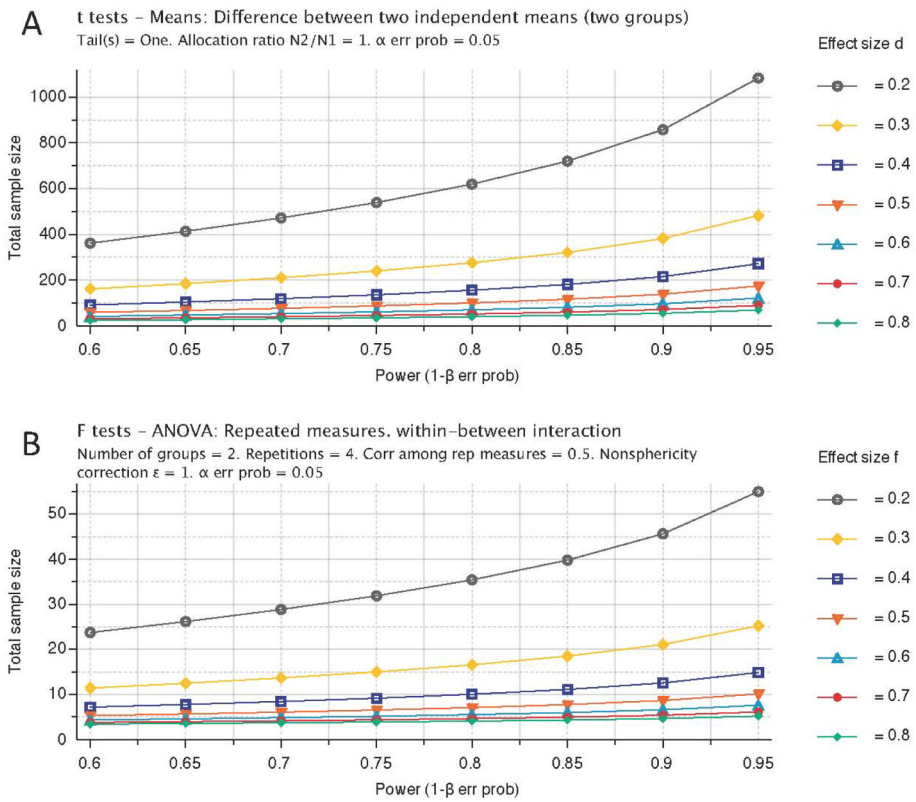
Figure 2.
Reducing animal numbers through serial assessments. A comparison of T- and F-test statistics using power calculations (generated using G*Power software 3.0.10, freely available at www.psycho.uni-duesseldorf.de/aap/projects/gpower/) indicate that an almost 20-fold reduction in sample size can be achieved with a similar effect size (0.3) by repeatedly (3×) measuring the same subjects. For instance, to compare two groups (i.e. treated versus untreated) at a single time point with an effect size of 0.5 at the 95% power level, a total sample size of 88 subjects is needed. In contrast, if there are 3 serial measurements on these subjects using a repeated measure Analysis of Variance (ANOVA), a total sample of only 12 is required. The variance used for the power calculations here is based on the Modo et al. (Modo et al. 2009) MRI lesion volume data that yielded an effect size of 0.5 at the final time point.
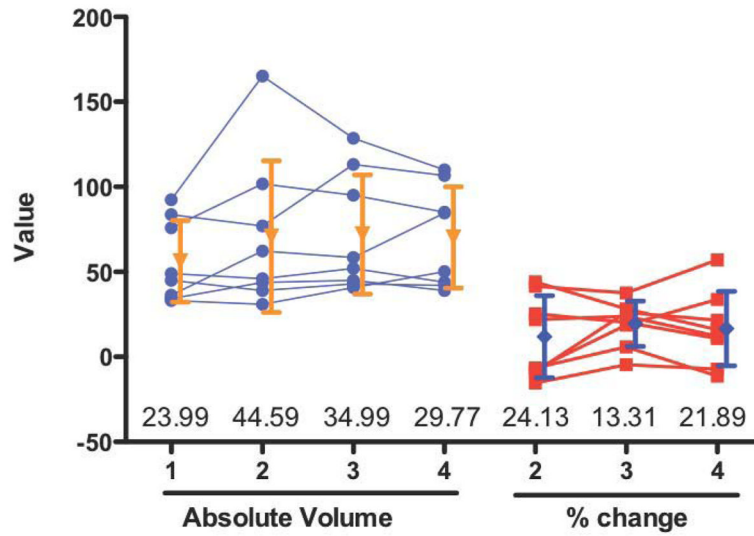
Figure 3.
Reduction of group variability through serial measurement. If baseline information can be acquired prior to animals undergoing any treatment, this data can be used to calculate the percentage (%) change of these animals at later time points. For instance, here MRI-based lesion volume data (expressed as mm^3 for absolute and % change) from Modo et al. (Modo et al. 2009) is presented for 8 animals at 4 time-points. The standard deviation for % change measures is reduced, as the baseline intra-animal variability is accounted for (values at the bottom of the graph indicate the standard deviation for each time point). The reason for this reduction in variability is that % change removes the initial distance between animals (i.e. initial intra-animal variability). Removing this intra-animal variability will increase the power of a statistical comparison (i.e. reduces the number of animals needed for each group).

Table 1
Example of a general neurological scale

Example of a general neurological scale (Modo et al. 2000b). A general neurological scale provides a neurological assessment of a variety of neurological functions. This scale can be used in different neurological conditions to chart an overall neurological deficit. However, it does not provide stroke-specific measures and some of these measures will not necessarily be affected by stroke damage, but would be affected by other types of neurological damage. For instance, the righting reflect is typically intact in animals with stroke (Modo et al. 2000b), but is often impaired in animals with global cerebral ischaemia (Modo et al. 2000a).

Neurological Test	Description	Normal	Deficit
Spontaneous Motility	The animal is put onto an empty surface in familiar surroundings (the post-operative room) and is expected to move and explore the surroundings. If the animal does not initiate this behaviour after 10 s it is considered to be impaired on spontaneous motility.	0	1
Righting Reflex	The animal is held in a supine position in the hand. The righting reflex is intact if the animal spontaneously turns and returns to its natural position. If the animal, however, fails to turn it has lost its righting reflex.	0	1
Horizontal Bar	The animal's forelimbs are placed on top of a bar (e.g. pencil), the animal is expected to grasp the bar and to hang on the bar for 3 s. The bar is placed about 30 cm above floor level. A foam pad is placed below the animal to guarantee a soft landing.	0	1
Grasping Reflex	A bar is placed under each of the limbs and the animal is expected to grasp the bar with both forepaws simultaneously.	0	1
Tilted Cage Top	The animal is placed on a tilted cage top (45°). If the animal freezes or if it moves over the edge of the top it is impaired on this task.	0	1
Placing Reaction	The animal is placed on a platform where one side of the body is near the edge. Each limb will be pulled gently in turn below the surface of the platform. The animal is impaired if it fails to re-place the limb on the platform.	0	1
Visual Placing	The animal is held around the torso and lowered slowly above the edge of the cage. If the animal reaches with the forelimbs for the edge of the cage visual placing is intact. If the animal does not reach out with its forelimbs, and does not start to move its forelimbs, it is impaired on this task.	0	1

Table 2

The Bederson Scale

The Bederson Scale (Bederson et al. 1986). This scale assesses a cardinal feature of focal ischaemia and is especially sensitive to striatal damage. Flexion of the contralateral forelimb in stroke is one of the cardinal features of a successful occlusion, but sham occlusion can also transiently produce a similar deficit. Not all animals with an occlusion exhibit circling or body twisting. Nevertheless, these features are a reasonable representation of a group deficit, even if it is not possible to determine with certitude for each animal if they indeed are occluded. It provides a best guess scenario in the absence of other more reliable measures, such as MRI. Modifications of this scale, such as the body swing test (Borlongan et al. 1995), are often used as outcome measures, although some animals will spontaneously recover over time.

Degree of deficit	Description	Score
Normal	Both forepaws reach out	0
Mild	Flexion of contralateral limb	1
Moderate	Decreased resistance to lateral push without circling	2
Severe	Decreased resistance to lateral push with circling	3

Table 3

An example of a distress scale

An example of a distress scale (Lloyd and Wolfensohn 1999). To determine the distress/suffering of an animal, a distress scale can provide a measurable state that can be used to define humane endpoints. It is important to ensure that animals are not in pain or suffering prior to conducting neurological or behavioural assessments, as these would confound stroke-related impairments. The following categories are distinguished: Normal (0–4; no action); Mild distress (5–9; monitor carefully, consider analgesics); Suffering (10–14; provide relief, observe regularly, consider euthanasia/perfusion); Severe pain or distress (15–20; immediate termination/perfusion); Refine procedure (18–20; consult with veterinary regarding procedure). For each score of 4, an additional point is added to the score.

	Description	Score
Clinical Signs	Normal	0
	General lack of grooming, ocular/nasal discharge	1
	Coat staring	2
	Pinched features, ridge lines	4
Body Weight	Normal – gaining weight	0
	B.W. < 5% reduction	1
	B.W. 5–15% reduction	2
	B.W. > 15% reduction	4
Disease score	Normal	0
	Loss of forelimb grip	0.5
	Forelimb paralysis	1
	Incomplete hind limb paralysis	2
	Forepaw and hindlimb paralysis	3
	Loss of righting reflex	4
Provoked behavior	Normal	0
	Minor depression or exaggerated response	1
	Moderate change/isolated	2
	Very still/lethargic	4

Table 4

Overview of behavioural tasks affected by stroke damage

Overview of behavioural tasks affected by stroke damage. Behavioural tests are designed to uncover damage inflicted by stroke to specific brain regions, but can also uncover damage to areas undergoing secondary damage due to, for instance, Wallerian degeneration. Anatomical areas in *italics* reflect sites of secondary damage, whereas other regions are directly affected by middle cerebral artery occlusion (MCAo). Degree of damage to these areas is, nevertheless, dependent on the duration and type of occlusion. Most of these tests can be used repeatedly and are fairly resistant to learning effects. A serial assessment on these tasks dramatically improves the statistical power to detect treatments effects. However, behavioural tasks with an asterix are difficult to adapt for serial measurement. For long-term assessments, it is also important to establish if a given behavioural test is resistant to spontaneous recovery. Often with time, brain plasticity and behavioural adaptations allow animals to perform at the level of controls. It is therefore crucial to include appropriate lesion-only controls that determine the persistence of a lesion effect. Sham-surgery controls are also needed for this.

Impairment	Damage	Test	References
Motor	Striatum	Rotameter	(Borlongan et al. 1998; Grabowski et al. 1993; Janowski et al. 2008; Mattsson et al. 1997; Modo et al. 2002; Modo et al. 2003)
	Striatum & Motor Cortex	Running Wheel/Rotarod	(Bouet et al. 2007; Gertz et al. 2006; Ishrat et al. 2009; Janowski et al. 2008; Kadam et al. 2009; Willing et al. 2002)
		Staircase Test	(Bouet et al. 2007; Freret et al. 2006; Grabowski et al. 1993; Grabowski et al. 1995; Machado et al. 2009)
		Footfault Test	(Badin et al. 2009; Modo et al. 2002; Modo et al. 2003)
		Forelimb Placing/Cylinder Test	(Borlongan et al. 1998; Freret et al. 2006; Hayase et al. 2009; Kadam et al. 2009; McGill et al. 2005; Tennant and Jones 2009)
		Ladder Rung Test	(Tennant and Jones 2009)
		Grip Strength Meter	(Ishrat et al. 2009)
		Suspension Test	(Borlongan et al. 1998; Brown et al. 2003; Mattsson et al. 1997)
		Rotating Pole	(Risedal et al. 1999; Zou et al. 2006)
		Beam Walk Test	(Brown et al. 2003; McGill et al. 2005; Michalski et al. 2009)
		von-Frey Hairs/Weight Bearing	(Lim et al. 2008)
		Gait Analysis	(Wang et al. 2008)
		Chimney Test	(Bouet et al. 2007)
	Motor Cortex	Skilled Forelimb	(Alaverdashvili and Whishaw 2008; Allred et al. 2008; Bax et al. 2008; Knieling et al. 2009; Tennant and Jones 2009)
Sensory	Sensorimotor Cortex	Bilateral Asymmetry Test	(Ashioti et al. 2009; Ashioti et al. 2007; Bouet et al. 2007; Freret et al. 2009; Freret et al. 2006; Holmberg et al. 2009; Modo et al. 2009; Modo et al. 2002; Modo et al. 2003; Roulston et al. 2008; Tennant and Jones 2009)
		Whisker Test	(De Ryck et al. 1992; Hurwitz et al. 1990; Pazos et al. 1995; Woodlee et al. 2005)
Cognition	Striatum/Frontal/ Cortex/ Hippocampus	Water Maze	(Borlongan et al. 2005; Hayase et al. 2009; Modo et al. 2002; Modo et al. 2003; Soderstrom et al. 2009)
		Working Memory	(Kadam et al. 2009)

Impairment	Damage	Test	References
Emotion	Amygdala	Passive Avoidance*	(Borlongan et al. 2005; Bouet et al. 2007; Gupta et al. 2002; Haelewyn et al. 2007; Romanova et al. 2006; Willing et al. 2002)
		T-maze	(Hurwitz et al. 1991)
		Open Field	(Babu and Ramanathan 2009; Kadam et al. 2009; Lyden et al. 1997)
		Elevated Plus Maze	(Gupta et al. 2002)
		Corner Test	(Bouet et al. 2007; Michalski et al. 2009)

Table 5

Overview of non-invasive translational imaging techniques to visualise stroke deficits

Overview of non-invasive translational imaging techniques to visualise stroke deficits. Shaded imaging techniques involve exposure to radioactivity and serial measurements are often difficult to achieve. MRI is gradually emerging as a core assessment technique. However, PET and SPECT are far superior to MRI in assessing specific cellular and molecular aspects of stroke damage (e.g. microglia, apoptosis).

Technique	Scan Type	Pathology	References
Magnetic Resonance Imaging (MRI)	T2	Ischaemic Lesion	(Berger et al. 1998; Bihel et al. 2009; Siemonsen et al. 2009)
		Haemorrhage	(Chen et al. 2008; Tsubokawa et al. 2007)
	Diffusion	Ischaemic Lesion	(Sood et al. 2007; Srivastava et al. 2008; Taheri et al. 2009)
	Perfusion	Blood Flow	(Ebinger et al. 2009; Ma et al. 2009; Poppe et al. 2009; Rivers et al. 2006; Zaro-Weber et al. 2009)
	Diffusion Tensor	White Matter Damage	(Bihel et al. 2009; Lindberg et al. 2009; Yu et al. 2009)
	Functional	Loss of Activity	(Carvalho et al. 2008; Geisler et al. 2006; Murata et al. 2006)
	Spectroscopy	Metabolite Changes ($\downarrow$NAA)	(Kang et al. 2009; Lei et al. 2009; Munoz Maniega et al. 2008)
	Angiogram	Vessel Occlusion	(Khan et al. 2009)
	Electron Paramagentic Resonance Imaging	Brain Oxygen	(Shen et al. 2009)
Computer Tomography (CT)	Structural	Ischaemic Lesion	(Andersen et al. 2009)
		Haemorrhage	(Andersen et al. 2009)
	Angiogram	Vessel Occlusion	(Delgado Almandoz et al. 2009; Khan et al. 2009; Maas et al. 2009; Nikolova et al. 2009; Puetz et al. 2009)
	Perfusion	Blood Flow	(Blau et al. 2000)
Positron Emission Tomography (PET)	^{15}O	Blood Flow	(Zaro-Weber et al. 2009)
	^{11}C-PK11195	Microglia	(Rojas et al. 2007; Schroeter et al. 2009)
	^{18}F-fluorodeoxyglucose	Metabolism	(Martin et al. 2009; Schroeter et al. 2009)
	^{18}F-fluoroacetate	Glial Metabolism	(Marik et al. 2009)
	^{18}F-Flumazenil	Penumbra	(Massaweh et al. 2009)
	^{18}F-5-fluoropentyl-2-methyl- malonic acid	Apoptosis	(Reshef et al. 1998)
Single Photon Emission Tomography (SPECT)	^{99m}Tc-HMPAO	Blood Flow	(Kaakinen et al. 2006; Umemura et al. 2000)
Transcranial Ultrasound (TUS)	^{99m}Tc-ethylcysteinate dimer	Blood Flow	(Murakami et al. 2008) (Holzer et al. 2009; Khan et al. 2009; Nikolova et al. 2009; Schreiber et al. 2009)

Table 6

Overview of the detection of pathological changes caused by stroke on MRI scans

Overview of the detection of pathological changes caused by stroke on MRI scans (based on Sen (Ebinger et al. 2009)). Within minutes of stroke onset, it is possible to detect pathological changes in the area of stroke due to a loss of blood flow and changes in the extracellular matrix that are reflected on perfusion and diffusion scans, respectively. A mismatch between perfusion (area larger than final lesion volume) and diffusion scans has been suggested to reflect penumbral tissue (Ma et al. 2009). Gradually further physiological changes occur that eventually can result in a complete tissue loss. However, at these different stages various imaging protocols and hallmarks exist to use imaging to reach a differential diagnosis and potentially select an appropriate intervention.

Time	Sequence	Characteristic	Aetiology
2–3 min	Diffusion	↓ADC	↓ proton motion
	Perfusion	↓CBF, CBV, MTT	↓ CBF
0–2 h	T2	Absent flow	Occlusion
	T1	Arterial enhancement	Slow flow
2–4 h	T2	Sulcal effacement	Edema
	T1	Slightly hyperintense	Incomplete infarction
8h	T2	Hyperintense	Edema
16–24 h	T1	Hypointense	Edema
>5 days	T2	Hyperintense	Tissue loss