Instantie voor Dierenwelzijn Utrecht

	Dossier: AVD1150020173846	
		Aanwezig
1	NTS	X
2	Aanvraagformulier	X
3	Projectvoorstel	X
4	Bijlage beschrijving dierproeven	X4
5	DEC-advies	X
6	Ontvangstbevestiging	X
	Evt. Vragen CCD aan aanvrager	X
	Evt. antwoorden aanvrager	X
7	Beschikking en vergunning	X
8	Beoordeling achteraf	X
9		
10		

Centrale Commissie Dierproeven

Niet-technische samenvatting 20173846

1 Algemene gegevens

1.1	Titel van het project	Werkingsmechanismen van hersenstimulatie therapieën na een experimentele hersenberoerte.
1.2	Looptijd van het project	5 jaar
1.3	Trefwoorden (maximaal 5)	MRI, optische imaging, hersen stimulatie, beroerte

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

- ☐ Fundamenteel onderzoek
- X Translationeel of toegepast onderzoek
- ☐ Wettelijk vereist onderzoek of routinematige productie
- ☐ Onderzoek ter bescherming van het milieu in het belang van de gezondheid
- ☐ Onderzoek gericht op het behoud van de diersoort
- ☐ Hoger onderwijs of opleiding
- ☐ Forensisch onderzoek
- ☐ Instandhouding van kolonies van genetisch gemodificeerde dieren, niet gebruikt in andere dierproeven

3 Projectbeschrijving

3.1 Beschrijf de doelstellingen van het project (bv de wetenschappelijke vraagstelling of het wetenschappelijk en/of maatschappelijke belang)

Hersenberoerte is een voornaame oorzaak van mortaliteit en invaliditeit in de moderne samenleving. Momenteel zijn er effectieve acute behandelingen beschikbaar, maar deze zijn alleen kort na de beroerte toepasbaar. Daardoor wordt slechts een beperkt aantal patiënten behandeld. Dit benadrukt de noodzaak voor alternatieve therapieën om patiënten ook in een later stadium te behandelen.

Hersenstimulatie kan een verbetering in functioneel herstel na beroerte veroorzaken. Veelbelovende technieken zijn transcraniële magnetische stimulatie (TMS), transcraniële direct current stimulatie (tDCS) en farmacologische manipulatie. Met deze technieken worden hersencellen

		tijdelijk meer of minder actief door middel van een magnetische veld (TMS), elektrische stroom (tDCS) of door farmacologische manipulatie van het brein. Het doel van deze studie is om vast te stellen welke methoden van hersenstimulatie het meest effectief zijn om herstel na een beroerte te bevorderen, en om inzichten te krijgen in werkingsmechanismen van deze stimulatie technieken.
3.2	Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?	In dit project zullen nieuwe inzichten verkregen worden in de effectiviteit en werkingsmechanismen van hersenstimulatie therapieën, tDCS en TMS en farmacologische manipulatie om herstel na een beroerte bevorderen. Deze inzichten zullen bijdrage aan verbeterd inzicht in het effect van hersenstimulatie therapie, en uiteindelijk tot verbeterde protocollen voor hersenstimulatie therapie in de mens.
3.3	Welke diersoorten en geschatte aantallen zullen worden gebruikt?	We zullen maximaal 2805 ratten gebruiken.
3.4	Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?	De dieren met een beroerte kunnen tot 20% van hun lichaamsgewicht verliezen in de eerste dagen na de beroerte en kunnen tijdelijk deels verlamd en minder bewegelijk zijn als gevolg van de beroerte.
3.5	Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?	82,9 % van dieren ervaart ernstig ongerief, 10,7% ervaart matig ongerief en 6,4% ervaart mild ongerief.
3.6	Wat is de bestemming van de dieren na afloop?	De dieren worden na afloop gedood zodat we het hersenweefsel kunnen onderzoeken.

4 Drie V's

4.1	**Vervanging** Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.	Dit onderzoek is alleen mogelijk in een levend dier. Zonder dieren met een beroerte is het niet mogelijk om onder gecontroleerde en reproduceerbare omstandigheden de bevorderende effecten van (langdurige) hersenstimulatie therapieën op het gedrag en functie van dieren met een beroerte in kaart te brengen.
4.2	**Vermindering** Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.	De beroerte wordt aangebracht door ervaren, getrainde onderzoekers. Dit zorgt voor minder uitval en variatie, waardoor minder dieren nodig zijn. Het hergebruik van dieren voor gedragstesten, hersenstimulatie therapieën, operatie training en MRI-methode optimalisatie zorgt ervoor dat er minder

		dieren nodig zijn.
4.3	**Verfijning** Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diermodel(len) de meest verfijnde zijn, gelet op de doelstellingen van het project.	In onze experimenten maken we gebruik van dieren die een beroerte ondergaan. De meeste literatuur over hersenstimulatie therapieën in dieren betreft experimenten in ratten of muizen. Omdat ons laboratorium veel ervaring heeft met beroerte modellen en MRI in ratten, maken we in dit onderzoek gebruik van ratten.
	Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de proefdieren zo beperkt mogelijk te houden.	Het welzijn van de dieren wordt in de eerste dagen na beroerte dagelijks gemonitord. In de eerste 24 uur na beroerte krijgen de dieren pijnstilling, en worden de kooien deels verwarmd. De dieren krijgen vloeibaar voedsel, standaard voer op de bodem van de kooien, en extra vocht om gewichtsverlies tot het minimale te beperken. Indien een dier meer dan 20% van het lichaamsgewicht na een beroerte verliest en (2) aanhoudend verminderd beweeglijk is, en ook niet op prikkels reageert,-zal het dier gedood worden.

5 In te vullen door de CCD

Publicatie datum	24 januari 2018
Beoordeling achteraf	Ja
Andere opmerkingen	Nee

Centrale Commissie Dierproeven

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.centralecommissiedierproeven.nl. of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

1 Gegevens aanvrager

1.1 Heeft u een deelnemernummer van de NVWA?
Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.

☒ Ja > *Vul uw deelnemernummer in* 11500

☐ Nee > *U kunt geen aanvraag doen*

1.2 Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.

Naam instelling of organisatie	UMC Utrecht
Naam van de portefeuillehouder of diens gemachtigde	[redacted]
KvK-nummer	30244197

1.3 Vul de gegevens van het postadres in.
Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.

Straat en huisnummer	Instantie voor Dierenwelzijn Utrecht
Postbus	12007
Postcode en plaats	3501AA Utrecht
IBAN	NL27INGB0000425267
Tenaamstelling van het rekeningnummer	Universiteit Utrecht

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted] ☒ Dhr. ☐ Mw.
Functie	[redacted]
Afdeling	Biomedical MR Imaging and Spectroscopy Group, Center for Image Sciences, UMC Utrecht
Telefoonnummer	[redacted]
E-mailadres	[redacted]

1.5 *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted] ☐ Dhr. ☒ Mw.
Functie	Postdoctoraal onderzoeker
Afdeling	Biomedical MR Imaging and Spectroscopy Group, Center for Image Sciences, UMC Utrecht
Telefoonnummer	[redacted]
E-mailadres	[redacted]

1.6	*(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.	(Titel) Naam en voorletters	☐ Dhr. ☐ Mw.
		Functie	
		Afdeling	
		Telefoonnummer	
		E-mailadres	
1.7	Is er voor deze projectaanvraag een gemachtigde?	☐ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*	
		☐ Nee	

2 Over uw aanvraag

2.1	Wat voor aanvraag doet u?	☒ Nieuwe aanvraag > Ga verder met vraag 3
		☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn
		Vul uw vergunde projectnummer in en ga verder met vraag 2.2
		☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn
		Vul uw vergunde projectnummer in en ga verder met vraag 2.3
2.2	Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?	☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier
		☐ Nee > Ga verder met vraag 3
2.3	Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?	☐ Nee > Ga verder met vraag 3
		☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	1 - 1 - 2018
		Einddatum	1 - 1 - 2023
3.2	Wat is de titel van het project?	Mechanisms of action of therapeutic brain stimulation therapies after experimental stroke	
3.3	Wat is de titel van de niet-technische samenvatting?	Werkingsmechanismen van hersenstimulatie therapieën na een experimentele hersenberoerte	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	DEC Utrecht
		Postadres	Postbus 85500 3508 GA Utrecht
		E-mailadres	dec-utrecht@umcutrecht.nl

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1684 Lege

☐ Wijziging € Lege

4.2 Op welke wijze wilt u dit bedrag aan de CCD voldoen.
Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.

☐ Via een eenmalige incasso

☒ Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☐

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.7). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel F van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

Naam [redacted]

Functie

Plaats Utrecht

Datum 26-10-2017

Handtekening [redacted]

[redacted]

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	11500
1.2	Provide the name of the licenced establishment.	UMCU
1.3	Provide the title of the project.	Mechanisms of action of therapeutic brain stimulation therapies after experimental stroke.

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

- ☐ Basic research
- X Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Stroke – i.e. a sudden loss of blood flow to the brain – is a disease with a high socioeconomic impact and is the leading cause of adult disability in several countries, including the Netherlands. There are three different types of stroke: ischemic stroke, hemorrhagic stroke and transient ischemic attack, of which

ischemic stroke is most prevalent. Many surviving stroke patients are left with moderate to severe functional deficits and long-term dependence on rehabilitation services. Currently, two effective therapies for acute ischemic stroke are available to restore blood flow to the brain: thrombolysis and thrombectomy. However, 85-95% of acute stroke patients do not receive these treatments due to their narrow treatment time-windows of 3-6 hours and 6-7 hours, respectively (Saver et al., 2016; Wardlaw et al., 2014). Furthermore, even after acute thrombolysis or thrombectomy, patients can experience long-term functional disabilities. This stresses the need for therapeutic strategies which are beneficial and safe to treat stroke patients at later stages.

Loss of function after stroke is related to focal injury at the ischemic site as well as global changes in connected brain areas. For instance, interhemispheric communication is altered after stroke, disrupting the balanced feedback between hemispheres necessary for coordinated movements. As a consequence of the infarct, the unaffected hemisphere tries to compensate for the loss of activity in the affected hemisphere. This results in increased activity in the unaffected hemisphere which excessively inhibits, and decrease activity in intact areas of the affected hemisphere via the transcallosal fibers that pass through the corpus callosum (alternatively known as transcallosal inhibition) (Fig. 1) (Fregni and Pascual-Leone, 2007; Murase et al., 2004). It is thought that this imbalance in communication between the two hemispheres hinders patients' potential for functional recovery leading to suboptimal motor outcomes after stroke (Bütefisch et al., 2003; Silasi and Murphy, 2014). Recently, proof-of-principle studies have demonstrated the potential of brain stimulation paradigms to elicit significant behavioral and functional improvement in recovering stroke patients. Two non-invasive and safe ways to facilitate or suppress brain activity to promote functional recovery are transcranial magnetic stimulation (TMS) and transcranial direct-current stimulation (tDCS).

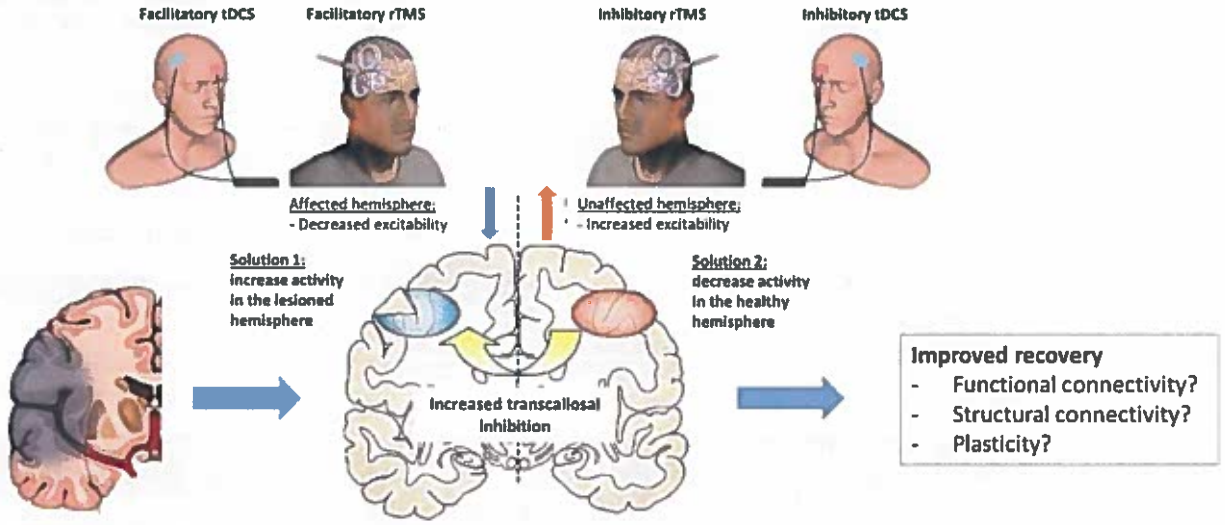

Figure 1: Using rTMS or tDCS to alter interhemispheric communication after stroke. Facilitatory rTMS or tDCS can potentially increase cortical excitability/activity in the affected hemisphere, whereas inhibitory rTMS or tDCS can decrease cortical excitability/activity in the unaffected hemisphere.

TMS induces current in the cerebral cortex with a coil that generates a magnetic field (Hallett, 2007), whereas with tDCS, weak electrical currents are transcranially delivered through two scalp electrodes (Nitsche et al., 2003). Most previous studies applied single stimulation sessions, but longer lasting effects on motor function can be achieved by multiple stimulation sessions, e.g. repetitive TMS, in acute/sub-acute stroke patients (Chang et al., 2010; Khedr et al., 2010).

Depending on the type and duration of the stimulation protocol, rTMS and tDCS can be used to counterbalance the abovementioned hemispheric imbalance by either facilitating or suppressing the underlying cortical activity in the affected or unaffected hemisphere, respectively. These stimulation techniques potentially have lasting effects beyond the stimulation period, which may promote mechanisms of synaptic plasticity and functional recovery (Gersner et al., 2011; Podda et al., 2016). The effects of rTMS and tDCS are not restricted to the target region of stimulation, but also include distantly connected cortical areas, allowing modulation of large-scale neural networks (Liew et al., 2014).

Although TMS and tDCS methods are promising approaches to promote stroke recovery, they lack stimulation focality, and the limited understanding of their mechanisms of action severely complicates

the rational design of treatment protocols (Bestmann et al., 2015; Tang et al., 2015). An alternative, recently developed technique, with high stimulation specificity and known mechanisms of action is [redacted] manipulation [redacted]. [redacted] manipulation allows activation or deactivation of [redacted] pathways that express [redacted] after systemic administration of an [redacted] Hereby, [redacted] technology can yield [redacted] control over [redacted] brain circuits. Although [redacted] has become a widely applied tool in basic neuroscience research, its application as a therapeutic brain stimulation method is basically unexplored. Despite several initial translational barriers that [redacted] manipulation has already overcome, it has yet to reach the clinic [redacted]. This is mainly due to inherent difficulties associated with [redacted] and drug delivery in human patients. Pilot trials in humans are hampered by the lack of approved [redacted] necessary for the activation of [redacted] and its potential toxicity effects [redacted]

At present, stroke treatment using brain stimulation techniques is promising, but it has several uncertainties, such as the unknown optimal treatment time-window after stroke, unclear working mechanisms of established methods such as TMS and tDCS, or the lack of information on the potential of novel methods such as [redacted] brain manipulation in patients. This emphasizes the need for preclinical studies in animal models, in which various aspects of brain stimulation techniques can be studied systematically under controlled and reproducible conditions.

We hypothesize that the above mentioned brain stimulation techniques can influence post-stroke outcome by lastingly modifying the organization of large-scale brain networks by altering structural and functional neuronal connections, as well as brain [redacted] Therefore, the primary aim of this project is to elucidate the working mechanisms of TMS, tDCS and [redacted] manipulation therapies that effectively improve functional recovery after stroke.

We will use different translational neuroimaging modalities, such as magnetic resonance imaging (MRI), [redacted] and optica[redacted] imaging [redacted] which enable longitudinal and *in vivo* assessment of functional, structural and [redacted] parameters. With these modalities we will particularly focus on imaging the following aspects:

- **MRI:** Brain perfusion, neuro-vasculature, functional network connectivity, structural connectivity, drug-induced brain activation, infarct size and location.
- [redacted] Brain [redacted] baseline levels of [redacted] and active [redacted]
- [redacted] Neuronal activation.
- [redacted] Cortical activity and functional connectivity.

References:

[redacted]

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[redacted]
Saver, J.L., Goyal, M., van der Lugt, A., Menon, B.K., Majoie, C.B.L.M., Dippel, D.W., Campbell, B.C., Nogueira, R.G., Demchuk, A.M., Tomasello, A., Cardona, P., Devlin, T.G., Frei, D.F., du Mesnil de Rochemont, R., Berkhemer, O.A., Jovin, T.G., Siddiqui, A.H., van Zwam, W.H., Davis, S.M., Castaño, C., Sapkota, B.L., Fransen, P.S., Molina, C., van Oostenbrugge, R.J., Chamorro, Á., Lingsma, H., Silver, F.L., Donnan, G.A., Shuaib, A., Brown, S., Stouch, B., Mitchell, P.J., Davalos, A., Roos, Y.B.W.E.M., Hill, M.D., Castano, C., Sapkota, B.L., Fransen, P.S., Molina, C., van Oostenbrugge, R.J., Chamorro, A., Lingsma, H., Silver, F.L., Donnan, G.A., Shuaib, A., Brown, S., Stouch, B., Mitchell, P.J., Davalos, A., Roos, Y.B.W.E.M., Hill, M.D., Collaborators, H., de Rochemont, R.D., Berkhemer, O.A., Jovin, T.G., Siddiqui, A.H., van Zwam, W.H., Davis, S.M., Castao, C., Sapkota, B.L., Fransen, P.S., Molina, C., van Oostenbrugge, R.J., Chamorro, A., Lingsma, H., Silver, F.L., Donnan, G.A., Shuaib, A., Brown, S., Stouch, B., Mitchell, P.J., Davalos, A., Roos, B.W.E.M., Hill, M.D., Collaborators, H., 2016. Time to Treatment With Endovascular Thrombectomy and Outcomes From Ischemic Stroke: A Meta-analysis. Ja 316, 1279–1288. doi:10.1001/jama.2016.13647
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Tang, A., Thickbroom, G., Rodger, J., 2015. Repetitive Transcranial Magnetic Stimulation of the Brain: Mechanisms from Animal and Experimental Models. Neurosci. 23, 82–94.
Wardlaw, J.M., Murray, V., Berge, E., del Zoppo, G.J., 2014. Thrombolysis for acute ischaemic stroke. Cochrane Database Syst. Rev. 7, CD000213. doi:10.1002/14651858.CD000213.pub3

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

The overall aim of this project is to establish effective plasticity-enhancing brain stimulation therapies that improve functional recovery after stroke, and to elucidate the working mechanisms of these brain stimulation techniques (rTMS, tDCS, [redacted] manipulation), which can be guided by imaging-based monitoring.

The main objectives of this project are:

1. To establish effective brain stimulation (rTMS, tDCS, [redacted] manipulation [redacted] protocols that improve restoration of sensorimotor function after experimental stroke (MRI, [redacted] and behavior).
2. To elucidate the underlying working mechanisms of plasticity-enhancing brain stimulation techniques (rTMS, tDCS, [redacted] manipulation [redacted]) that promote functional recovery after experimental stroke (behavior).
3. To increase insights on post-stroke system level reorganization of neuronal networks, (sub)cellular level rewiring of synaptic connections, and [redacted] level rebalancing of [redacted] homeostasis (MRI, [redacted] immunohistochemistry).
4. To identify imaging-based biomarkers of neuroplasticity that predict functional outcome after stroke.

Specific research questions that we will address during this project:

1. Which brain stimulation protocols improve functional recovery after stroke? *(Objective 1)*

2. What is the optimal therapeutic time-window for improving functional recovery after stroke using brain stimulation? *(Objective 1)*
3. What are the plasticity-enhancing working mechanisms of rTMS, tDCS and [redacted] manipulation [redacted] *(Objective 2, 3)*
4. Which image-based biomarkers can be used to predict functional outcome after stroke? *(Objective 4)*

Feasibility of these objectives

To achieve the objectives described above we will employ different *in vivo* imaging modalities in rodent stroke models. Our research group has a long history of *in vivo* MRI in biomedical research, with a particular focus on neuroimaging of pathophysiology and recovery mechanisms in different rodent models of stroke (Deddens et al., 2013; Dijkhuizen and Nicolay, 2003; [redacted] van Meer et al., 2012). This includes MRI of structure, perfusion, vascularity, neuronal activation and functional connectivity in neural tissues in rats and mice. For MR [redacted] of brain [redacted] we have a long-standing collaboration with Yale University School of Medicine [redacted]

Besides MRI, our group has recently successfully implemented a number of other techniques:

- Optical [redacted] imaging [redacted] of cortical hemodynamic and neuronal activity in rodents (unpublished results). This technique has been successfully developed by our international collaborators to measure functional connectivity in mouse models [redacted].
- TMS, tDCS and [redacted] manipulation in rats. We are currently investigating the potential of rTMS to improve functional recovery in a rat model of stroke (unpublished results). TMS and tDCS have already been effectively applied in different animal models, including rodent stroke models (Notturno et al., 2014; Yoon et al., 2012, 2011; Zhang et al., 2007). Additionally, we have recently successfully developed and applied a novel approach to image [redacted] brain activation in rats [redacted]
- Behavioral test batteries and histological techniques. Over the years several behavioral tests for assessment of sensorimotor and cognitive functions in rats and mice have been implemented. In addition, we collaborate with other groups for detailed histological assessments of tissue samples.

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3.3 Relevance

What is the scientific and/or social relevance of the objectives described above?

Scientific relevance:

Current therapeutic strategies for stroke fall short and often patients still suffer from disability after intensive rehabilitation strategies. Furthermore, results from therapeutic, non-invasive brain stimulation trials in stroke patients are often contradictory due to variation in treatment protocols, small sample sizes, heterogeneity of the patients and insufficient outcome measures (Rothwell, 2016). Therefore, it is necessary to have a controlled and reproducible setting to investigate the fundamental aspects of how brain stimulation techniques, such as rTMS, tDCS and [redacted] manipulation can improve functional recovery after stroke.

This information will promote the exploitation of the therapeutic potential of brain stimulation, which can be monitored and optimized using imaging techniques. Furthermore, these results can be translated to the clinic for the development and optimization of treatment protocols that would allow for better functional recovery in stroke patients.

Societal relevance:

Stroke is the third leading cause of death in the Netherlands and a major cause of adult disability worldwide (Donnan et al., 2008; Struijs et al., 2005). The majority of stroke patients is affected by motor impairment, and despite several treatment strategies, experiences incomplete recovery, affecting independent functional activities of daily living (Bennette et al., 2014; Claflin et al., 2015; Lai et al., 2002; Langhorne et al., 2009; Ovbiagele and Nguyen-Huynh, 2011; Wilkins E, Wilson L, Wickramasinghe K, Bhatnagar P, Leal J, Luengo-Fernandez R, Burns R, Rayner M, 2017). Therefore, stroke has been identified to have a high socioeconomic impact, which will only rise due to the increasing aging population. A better understanding of how brain stimulation techniques can be used to improve stroke recovery will contribute to the ongoing search for optimal treatment strategies.

In addition, this research aims to identify MRI-based imaging markers for the prediction of functional recovery after stroke, which can be translated to clinical MRI scanners. This would provide unique possibilities for patient-specific decision making on brain stimulation therapeutics for stroke treatment.

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3.4 Research strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

Appendix 1, phase 0:

In the preparation phase of this study (Appendix 1, phase 0), we will develop, implement and validate several MR protocols for measurement of: brain [redacted] neuronal activation [redacted] functional and structural connectivity (functional MRI (fMRI) and diffusion tensor imaging (DTI)), drug-induced brain activity (pharmacological MRI (phMRI)), brain perfusion and tissue lesions. Based on our extensive experience of *in vivo* MRI within our research group (section 3.2 Purpose; Feasibility of these objectives), we would like to further improve our current MR protocols (fMRI, DTI, phMRI, perfusion & lesion imaging) as well as develop and implement new protocols [redacted] MR sequence improvement and development will first be conducted in phantom or non-living tissue samples. Consequently, further optimization, development and implementation will be done *in vivo*. Optimization of currently existing MRI protocols will be done to reduce acquisition time, increase the signal-to-noise ratio and to eliminate artifacts that might confound datasets. Together with our collaborative partners at Yale University School of Medicine, we will develop and implement protocols to measure brain [redacted] which have not yet been implemented in our lab before. In addition, we will implement, validate and optimize Optical [redacted] Imaging [redacted] for studies in rats.

By optimizing and implementing new MR protocols, we can in addition to measuring functional and structural brain changes, also get insights into changes in brain activity and [redacted] (such as alterations in [redacted] and [redacted]. In addition to MRI, the implementation of optical imaging [redacted] allows the acquisition of functional brain changes at high resolution and speed, which can be used for the validation of fMRI animal data and consequently improve translational comparisons between animal and human data.

Furthermore, we will develop and optimize three different techniques, namely: repetitive transcranial magnetic brain stimulation (rTMS), transcranial direct current stimulation (tDCS) and [redacted] manipulation [redacted] Lastly, we will evaluate and select sensitive behavioral tasks for measurement of functional motor recovery after stroke. These behavioral tests will include tests for assessment of sensorimotor function (e.g. forelimb asymmetry test, skilled reaching test, beam walk, neurological severity score, and grip strength), anxiety/locomotive activity (open field test) and cognition (e.g. Barnes maze and novel object recognition). Behavioral tests will be performed regularly to assess post-stroke recovery and efficacy of brain stimulation therapy.

Appendix 2, phase 1:

In the first phase of this study (phase 1), [redacted] manipulation [redacted] will be evaluated as a new treatment approach to improve functional recovery after stroke. During this phase we will test to what extent [redacted] targeting of [redacted] pathways within or between hemispheres can influence activity of the [redacted] network areas. Furthermore, we will determine the optimal lengths and dosages for the treatment protocol.

Appendix 3, phase 2:

In the second phase of this study (phase 2), using the optimized imaging, behavioral and brain stimulation protocols established in phases 0 and phase 1, we will longitudinally measure the effects of brain stimulation (rTMS, tDCS and [redacted] manipulation) on functional recovery after experimental stroke. In this phase we will be able to assess the therapeutic efficacy of different rTMS, tDCS and

[redacted] manipulation paradigms in two different rodent models of experimental stroke namely; photothrombotic and transient middle cerebral artery occlusion stroke models (see section 3.4.2 for a detailed description of these models). In this phase, brain stimulation therapy will be combined with multisensory stimulation therapy (enriched environment or constrained-induced movement therapy (CIMT)), since combination therapy, e.g. brain stimulation with adjunct rehabilitation treatment, has been shown to be most effective for post-stroke recovery (Takeuchi and Izumi, 2015). In addition, we will also assess the effect of timing of brain stimulation therapy by measuring effect of treatment onset in either the sub-acute or sub-chronic phase after stroke.

The therapeutic efficacy of the brain stimulation techniques will be monitored by behavioral experiments and its effects on the brain will be monitored using *in vivo* imaging techniques (MRI). Imaging will be performed before and after stroke induction, and before and after brain stimulation. Following the final imaging time point, animals will be euthanized and brain tissue will be harvested to investigate the effects of brain stimulation at the cellular and molecular level (electrophysiology on brain slices, histology and/or immunohistochemistry).

Appendix 4, phase 3:
In the third phase of this study (phase 3), the working mechanisms of the most promising rTMS, tDCS and [redacted] manipulation protocols will be investigated in the two different stroke models, by using advanced imaging protocols to measure brain activity [redacted] and brain [redacted] Imaging will be performed before and after stroke induction, and before and after brain stimulation. Following the final imaging time point, animals will be euthanized and brain tissue will be harvested to investigate the effects of brain stimulation at the cellular and molecular level (electrophysiology in brain slices, histology and/or immunohistochemistry).

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3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

The present study will investigate the effects of rTMS, tDCS and [redacted] manipulation on functional recovery in two different experimental models of ischemic stroke. Several neuroimaging techniques will be used to guide and monitor the effects of these stimulation techniques on the brain to investigate underlying working mechanisms of post-stroke plasticity enhancing stimulation therapies.

The ischemic stroke models, namely photothrombotic stroke and stroke induced by transient occlusion of the middle cerebral artery (tMCAO), have been chosen based on their low invasiveness, high reproducibility and the ability to reliably induce contralateral, functional, neurological deficits (Fluri et al., 2015; Kumar and Gupta, 2016; Schaar et al., 2010). Both stroke models will be used in appendices 1-4. Photothrombotic stroke will be induced in naïve, Sprague Dawley rats. In this model, a cortical infarct is induced by the systemic injection of a photosensitive dye (Rose-Bengal) in combination with the focal illumination of the skull (Watson et al., 1985). The illumination leads to the local activation of Rose-Bengal, which results in the disturbance of endothelial function and local thrombosis in small cortical vessels. The advantages of this model are the relatively small and reproducible infarct size, the ability to place the infarct within the desired sensorimotor subdivisions of the cortex, the minimal surgical manipulation of the animal and high survival rate (Fluri et al., 2015).

For the purpose of increasing translation of promising brain stimulation techniques to the clinic, we will also include the tMCAO stroke model, which produces a stroke pattern of damage (i.e. striatum and cortex) similar to humans (Corbett et al., 2017). Stroke will be induced in naïve Sprague Dawley rats, by transiently occluding the middle cerebral artery (tMCAO model) with an intraluminal filament (Longa et al., 1989). This model is highly reproducible, little invasive (no craniectomy), enables controllable reperfusion, and mimics human ischemic stroke (Fluri et al., 2015; Kumar and Gupta, 2016). Despite these advantages, there is a considerable (12%) incidence rate of subarachnoid hemorrhage which can

reduce the blood flow bilaterally. Therefore animals could potentially experience difficulties with eating and weight loss.

After stroke, an interhemispheric imbalance between the affected and unaffected hemisphere has been suggested to prohibit functional recovery after stroke (Silasi and Murphy, 2014). The three stimulation techniques each provide a unique opportunity to restore the interhemispheric imbalance after stroke:

rTMS induces electrical current in the brain, by producing a rapidly changing magnetic field, which results in the elicitation of action potentials (Hallett, 2007). With rTMS, the interhemispheric imbalance after stroke can potentially be restored in at least two ways: 1) Facilitatory rTMS to increase cortical activity in the affected hemisphere; 2) Inhibitory rTMS to decrease cortical activity in the unaffected hemisphere.

tDCS delivers constant low intensity electrical current into the brain with two scalp electrodes (Nitsche et al., 2003), and cannot directly elicit action potentials, but modulates excitability. With tDCS, the interhemispheric imbalance after stroke can be restored in at least three ways: 1) Facilitatory tDCS to increase cortical excitability in the affected hemisphere; 2) Inhibitory tDCS to decrease cortical excitability in the unaffected hemisphere; 3) Bilateral tDCS; by applying inhibitory tDCS to the unaffected hemisphere and facilitatory tDCS to the affected hemisphere simultaneously.

[redacted] manipulation [redacted] activates a [redacted] neuronal pathway by [redacted] in [redacted] neuronal [redacted] and subsequently activating the [redacted] with the corresponding [redacted] Hereby, a [redacted] neuronal pathway can be activated or silenced, which may be applied to restore the interhemispheric imbalance after stroke. [redacted] pathways within the affected hemisphere can be activated, whereas [redacted] pathways within the non-affected hemisphere could be silenced to restore the imbalance between the hemispheric activity after stroke. In addition, the connections between the two hemispheres could be targeted, to directly manipulate the interhemispheric interaction. Stimulating the connection from [redacted] areas in the affected to the unaffected hemisphere, or inhibiting the connection from [redacted] areas in the unaffected to the affected hemisphere, may also have the potential to restore the interhemispheric imbalance after stroke.

To guide and monitor the effects of the different brain stimulation modalities and to investigate their effects on the brain, we will use different imaging modalities in our institute:

MRI [redacted] are based on detection of radio frequency signals from specific, magnetic nuclei in a large external magnetic field. MRI involves the detection of signals from water protons, which can be spatially encoded to generate images. MRI can be sensitized to different aspects of the brain, including structural and functional connectivity, drug-induced brain activity, perfusion and tissue lesions.

To measure neuronal activation with MRI [redacted] a paramagnetic ion, is used as a contrast agent [redacted] enabling detection of activated brain regions after systemic injection. Measurement of brain [redacted] is based on the detection of signals from tissue [redacted] which can provide information about the [redacted] status or active [redacted] pathways in the investigated tissue.

Optical [redacted] imaging [redacted] is a technique in which temporal changes in light reflection on the upper brain, i.e. the cortex, are detected with a light-sensitive camera. These changes in light reflection can be caused by a variety of neuronal activity-related processes, including changes in blood volume, blood flow, hemoglobin concentration, ions and water displacement around neurons, volume of neurons, neurotransmitter release etc. Thus, optical imaging [redacted] can be used to monitor (stimulation-induced) neuronal activity (changes) in the cortex.

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The present research proposal consists of the following steps:

Preparation, phase 0 (Appendix 1):

0.1 Implementation, optimization & safety of rTMS stimulation paradigms.

0.2 Implementation, optimization, feasibility & safety of tDCS stimulation paradigms.
0.3 Development, implementation and optimization of [redacted] manipulation [redacted]
0.4 Development and optimization of MR ([redacted] protocols to measure brain [redacted]
0.5 Development and optimization of MRI ([redacted] protocols to measure neuronal activation.
0.6 Setup and selection of behavioral tests for assessment of sensorimotor and cognitive function.
0.7 Training and optimization of photothrombotic and tMCAO stroke model induction in rats.
0.8 Optimization of current MRI protocols for measurement of functional and structural connectivity, drug-induced brain activity, perfusion and tissue lesions.
0.9 Optimization of current optical imaging ([redacted] protocols to measure cortical activity.

Phase 1 (Appendix 2):
1.1 Validation of [redacted] manipulation of the [redacted] network.
1.2 Optimization of duration and dose of [redacted] manipulation paradigms.

Phase 2 (Appendix 3):
2.1 Multiparametric imaging study of the efficacy of rTMS on motor recovery after stroke.
2.2 Multiparametric imaging study of the efficacy of tDCS on motor recovery after stroke.
2.3 Multiparametric imaging study of the efficacy of [redacted] manipulation on motor recovery after stroke.

Phase 3 (Appendix 4):
3.1 Gain insights into the working mechanisms of rTMS after stroke.
3.1.1 Gain insights into the brain areas activated by rTMS [redacted]
3.1.2 Gain insights into the effects of rTMS on brain [redacted]
3.1.3 Gain insights into the effects of rTMS on brain activity [redacted]

3.2 Gain insights into the working mechanisms of tDCS after stroke.
3.2.1 Gain insights into the brain areas activated by tDCS [redacted]
3.2.2 Gain insights into the effects of tDCS on brain [redacted]
3.2.3 Gain insights into the effects of tDCS on brain activity [redacted]

3.3 Gain insights into the working mechanisms of [redacted] manipulation after stroke.
3.3.1 Gain insights into the brain areas activated by [redacted] manipulation [redacted]
3.3.2 Gain insights into the effects of [redacted] manipulation on brain [redacted]
3.3.3 Gain insights into the effects of [redacted] manipulation on brain activity [redacted]

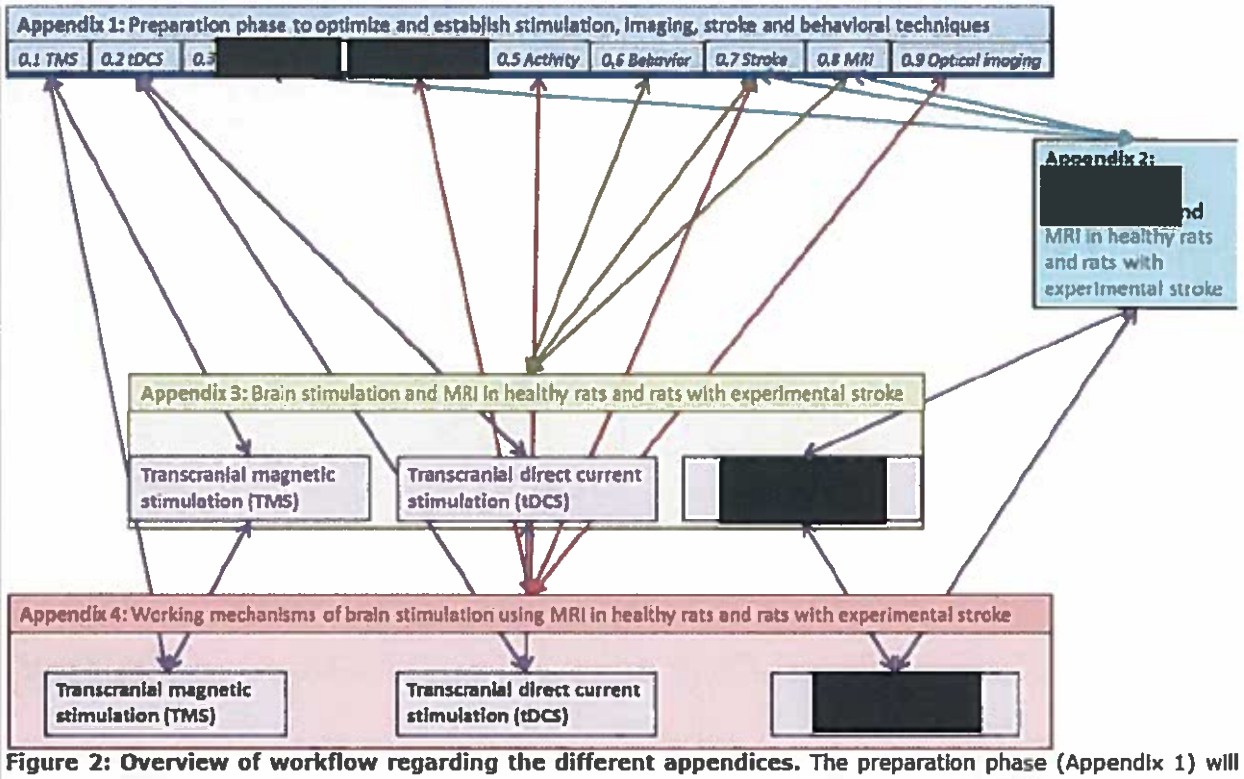


Figure 2: Overview of workflow regarding the different appendices. The preparation phase (Appendix 1) will last during the whole 5 year period. During this phase, the different techniques will be developed that are necessary for appendices 2-4. Studies using different brain stimulation techniques (TMS, tDCS and [redacted] manipulation) can be performed in parallel. The double headed arrows indicate that we can more forwards and backwards between the different parts of appendices that are connected to each other.

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

In this study, we would like to establish the efficacy of different brain stimulation techniques and paradigms on functional recovery after experimental stroke. Therefore, we would like to investigate the effects of two promising non-invasive brain stimulation techniques to improve motor recovery after stroke: rTMS (step 2.1) and tDCS (step 2.2).

When stimulating the brain with either rTMS or tDCS, the focality of the stimulation is not very high (Deng et al., 2013; Lang et al., 2005), and effects might be attributable to stimulation of remote regions. In addition, the mechanisms of action and most optimal stimulation parameters are not yet known. Alternatively, [redacted] manipulation [redacted] allows stimulation or deactivation of a [redacted] neuronal pathway. Thereby [redacted] manipulation may offer a unique stimulation therapy to improve motor recovery after stroke (step 2.3).

When we have identified the most promising stimulation protocols to improve functional recovery after experimental stroke, we will investigate their underlying working mechanisms (step 3.1, 3.2 and 3.3). We hypothesize that brain stimulation influences brain [redacted] and activity, resulting in alterations in the structure and function of neuronal connections, thereby altering functional outcome after stroke. Testing our hypothesis with these brain stimulation techniques requires spatiotemporal assessment of brain [redacted] brain activity [redacted] and structural and functional network connectivity [redacted] before and after the experimental induction of stroke, and before and after brain stimulation therapy.

The experiments described above require optimized and new MR protocols for rats on our MRI scanners for measuring [redacted] (step 0.4), neuronal activity (step 0.5) and structural and functional connectivity, drug-induced brain activity, perfusion and tissue lesions (step 0.8), and optimized protocols

on our optical imaging system (step 0.9) (see section 3.4 Research Strategy for explanation). In addition, they require the development and validation of [redacted] manipulation (step 0.3, 1.1 and 1.2), and the optimization of the non-invasive brain stimulation protocols of TMS and tDCS (step 0.1 and 0.2). In parallel, evaluation of behavioral tests for measurement of sensorimotor recovery after stroke will be performed (step 0.6). Personnel will be trained to induce experimental stroke using two different animal models (step 0.7). Animals used for the evaluation of behavioral tests can also be used for assessment of MRI protocols (step 0.4, 0.5 and 0.8), optimization of optical imaging (step 0.9) and optimization of brain stimulation techniques (step 0.1-0.3).

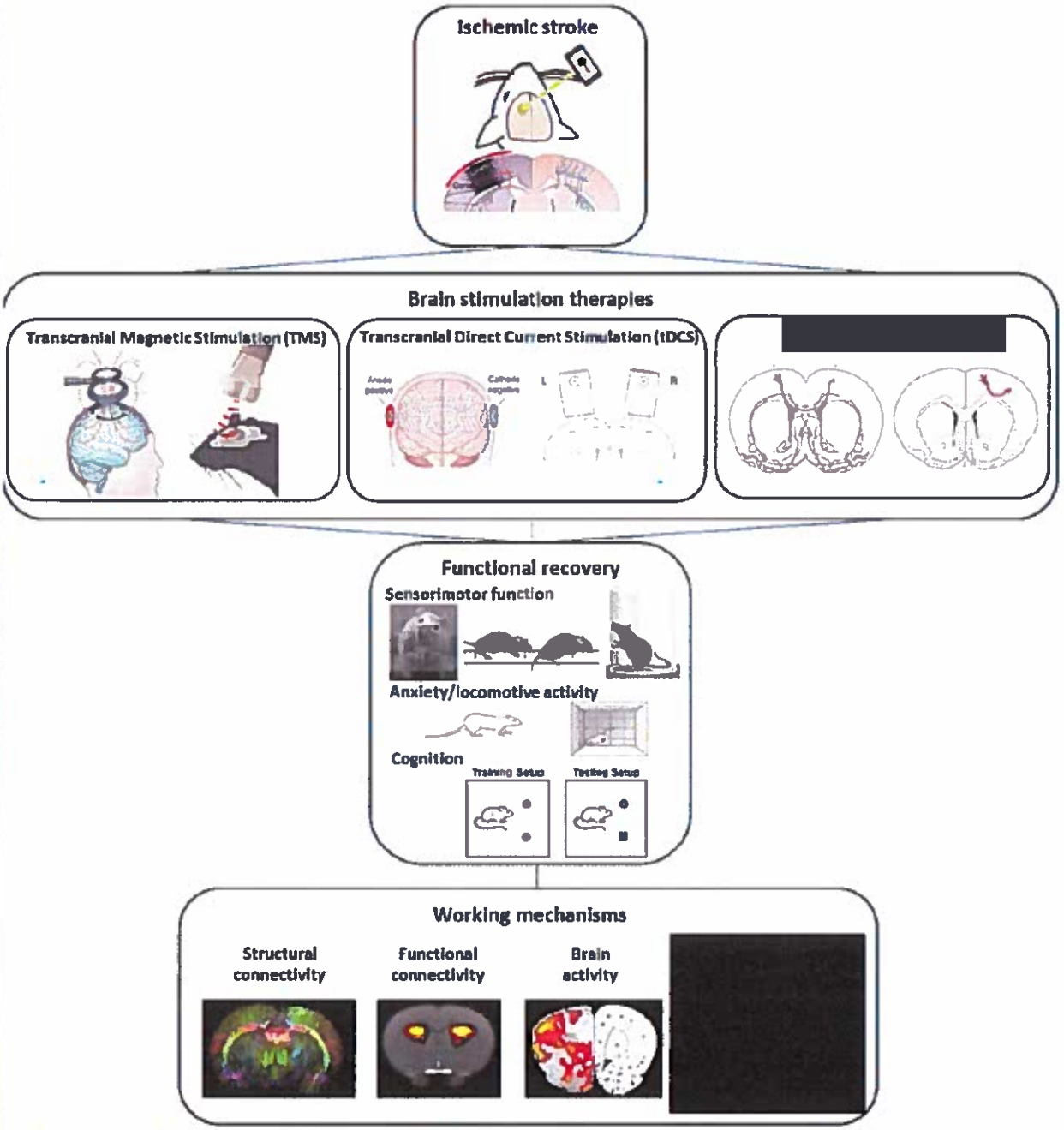


Figure 3: Coherence between the different parts of the proposal. We will investigate three different brain stimulation techniques (TMS, tDCS and [redacted] manipulation) as treatment strategies to improve functional recovery after ischemic stroke. All these brain stimulation techniques will manipulate activity within the [redacted] network. We will also investigate the working mechanism of these brain stimulation techniques, by investigating their

effects on structural connectivity, functional connectivity, brain activity and brain [redacted]

Selection points:
Time points for *in vivo* imaging of brain stimulation effects: We will select one time point before and after stroke and one time point before and several time points after stimulation. The exact time points cannot be specified yet, as the optimal stimulation protocols are not yet known.

Go/No-go criteria:
Prior to the described longitudinal studies, all necessary imaging protocols will first be tested and optimized using post-mortem rat brains. For these studies, brains can be collected from animal carcasses euthanized for other purposes. Subsequently, the imaging protocols will be further optimized in healthy and/or stroke rats.

The longitudinal studies of phase 2 will only start when corresponding preparation phases have been established:

- *2.1: Multiparametric imaging study of the efficacy of rTMS on motor recovery after stroke:*
  - Phase 0.1: Implementation, optimization & safety of TMS stimulation paradigms.
  - Phase 0.6: Setup behavioral tests for sensorimotor and cognitive function.
  - Phase 0.8: Optimization of current MRI protocols for measurement of functional and structural connectivity, drug-induced brain activity, perfusion and tissue lesions.
  - Phase 0.7: Training and optimization of photothrombotic and tMCAO stroke model in rats.
- *2.2: Multiparametric imaging study of the efficacy of tDCS on motor recovery after stroke:*
  - Phase 0.2: Implementation, optimization, feasibility & safety of tDCS stimulation paradigms.
  - Phase 0.6: Setup behavioral tests for sensorimotor and cognitive function.
  - Phase 0.8: Optimization of current MRI protocols for measurement of functional and structural connectivity, drug-induced brain activity, perfusion and tissue lesions.
  - Phase 0.7: Training and optimization of photothrombotic and tMCAO stroke model in rats.
- 2.3: *Multiparametric imaging study of the efficacy of* [redacted] *manipulation* [redacted] *on motor recovery after stroke:*
  - Phase 0.3: Development, implementation and optimization of [redacted] manipulation [redacted]
  - Phase 0.6: Setup behavioral tests for sensorimotor and cognitive function.
  - Phase 0.8: Optimization of current MRI protocols for measurement of functional and structural connectivity, [redacted] brain activity, perfusion and tissue lesions.
  - Phase 0.7: Training and optimization of photothrombotic and tMCAO stroke model in rats.
  - Phase 1.1: Validation of [redacted] manipulation of the [redacted] network.
  - Phase 1.2: Optimization of duration and dose of [redacted] manipulation paradigms.

The mechanisms of action studies of phase 3 will only start when promising stimulation protocols are identified.

References:
Deng, Z.-D., Lisanby, S.H., Peterchev, A. V., 2013. Electric field depth-focality tradeoff in transcranial magnetic stimulation : simulation comparison of 50 coil designs. Brain Stimul. 6, 1–13. doi:10.1016/j.brs.2012.02.005.

Lang, N., Siebner, H.R., Ward, N.S., Lee, L., Nitsche, M.A., Paulus, W., Rothwell, J.C., Lemon, R.N., Frackowiak, R.S., 2005. How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? Eur. J. Neurosci. 22, 495–504. doi:10.1111/j.1460-9568.2005.04233.

3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure

1	Phase 0: Preparation phase to optimize and establish stimulation, imaging, stroke and behavioral techniques.
2	Phase 1: [redacted] manipulation and MRI in healthy rats and rats with experimental stroke.
3	Phase 2: Brain stimulation and multiparametric MRI in healthy rats and rats with experimental stroke.
4	Phase 3: Working mechanisms of brain stimulation and multiparametric MRI in healthy rats and rats with experimental stroke.
5	
6	
7	
8	
9	
10	

[redacted]

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1

General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	11500
1.2	Provide the name of the licenced establishment.	UMCU
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 Type of animal procedure: Phase 0: Preparation phase to optimize and establish stimulation, imaging, stroke and behavioral techniques

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

In the preparation phase (Phase 0) (Fig. 1) we will:

0.1 Implement, optimize & test the safety of rTMS stimulation paradigms.
0.2 Implement, optimize, and test the feasibility & safety of tDCS stimulation paradigms.
0.3 Develop, implement and optimize [redacted] manipulation[redacted] within the [redacted] network.
0.4 Develop and optimize MR ([redacted] protocols to measure brain [redacted]
0.5 Develop and optimize MRI ([redacted] protocols to measure neuronal activation.
0.6 Setup and select behavioral tests for assessment of sensorimotor and cognitive function.
0.7 Train personnel to induce photothrombotic and tMCAO stroke in rats.
0.8 Optimize current MRI protocols for measurement of functional and structural connectivity, drug-induced brain activation, perfusion and measurement of lesion extent.
0.9 Optimize current optical imaging ([redacted] protocols to measure cortical activity.

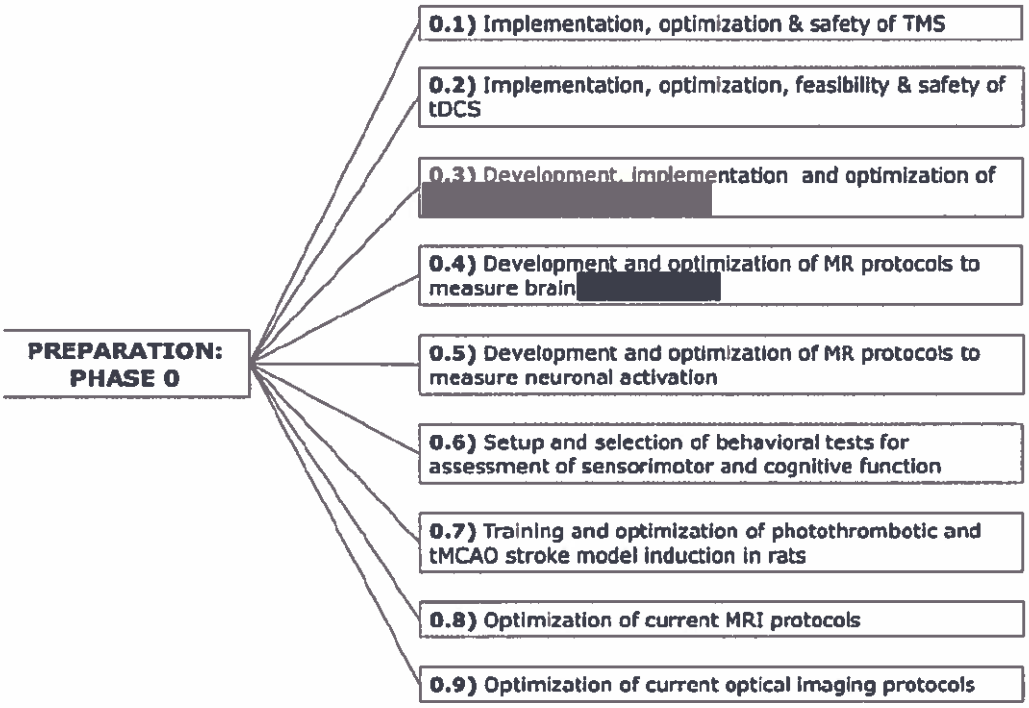


Figure 1: Overview of several procedures that will be conducted to develop, optimize and train personnel in preparation for experiments that will be conducted in appendices 2-3. For an overview of procedures related to the respective appendices, see Figure 2 in the Project Proposal.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Preparation: Phase 0

In this phase of the study, we will optimize and establish several stimulation, imaging, stroke modeling and behavioral techniques using naive Sprague Dawley rats (see diagram below). The pilot studies in the preparation phase will be performed in parallel to one another where possible.

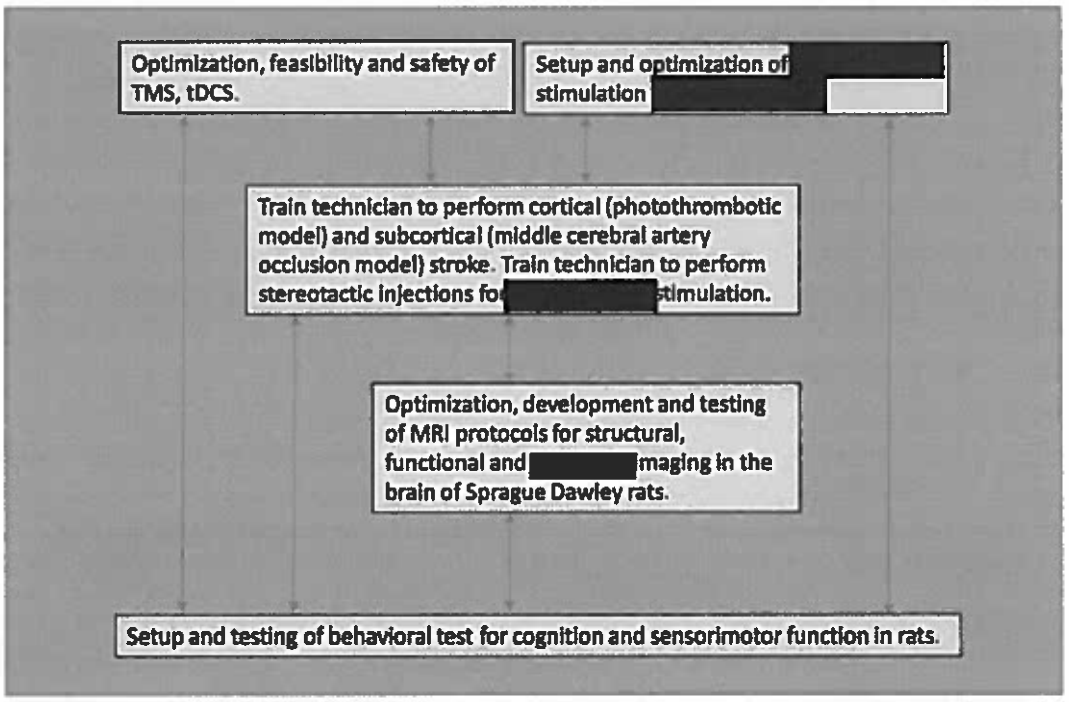


Figure 2: Overview of the different sub phases that can be performed in parallel as much as possible.

0.1-0.2) Optimization, feasibility & safety of transcranial magnetic stimulation and transcranial direct current stimulation

We will optimize and test the feasibility and safety of several rTMS and tDCS setups and stimulation protocols. These pilot experiments would particularly focus on (1) verifying setups that are ideal with regards to stimulation of the animal, anesthetized or awake stimulation, correct placement of the TMS coil or tDCS electrodes, safety of stimulation paradigms; (2) making sure that the electromyography setup is robust for measurements during stimulation and; (3) to check whether the tDCS cannula/s on the skull cause disturbances on MRI images. During these experiments, animals will either be exposed to TMS or tDCS, which will last for a maximum of 2 hours.

Transcranial magnetic stimulation (TMS)
While animals are exposed to TMS, they will be anesthetized when determining the optimal positioning of the animal for receiving target-specific stimulation. Anesthesia will either be induced through inhalation or infusion. Whilst the animals are in a fixed position, we will test the electromyography setup by inserting needle electrodes into the limbs of the animal to measure muscle responses (motor evoked potentials) induced by the stimulation, which gives an indication of cortical excitability.

Outcome parameters:
- Motor evoked potential; (1) this will give an indication of the specificity of stimulation and therefore it can indicate whether the positioning and fixation of both the coil and animal is ideal, (2) used to determine the optimal dose of stimulation.
- Histology/immunohistochemical staining; to determine if the stimulation caused any neuronal damage.

Transcranial direct current stimulation (tDCS)
To set up tDCS, animals will be anesthetized and fixed in a stereotact for the placement of the electrode cannula/s. A vertical incision will be made on the skull to expose the cranium. The periosteum will be removed, to ensure that the skull is completely dry for the proper fixation of the cannula/s. The cannula/s will be fixed on the skull using dental cement and screws. After the cement has dried, the skin will be sutured around the fixed cannula/s. This procedure will take a maximum of 3 hours. The surgery will first be practiced using material collected from available carcasses. Subsequently, the surgery will be

performed *in vivo* under anesthesia and in case the surgery is considered successful, and performed within 3 hours at maximum, rats will be allowed to recover and will be subjected to:

1) One single session of awake or anesthetized stimulation within 7-14 days after cannula fixation. When animals are stimulated in the awake condition, they will be able to move around freely in a container or perform a behavioral task while the stimulation electrodes (attached to a swivel device) are attached to the cannula/s on the skull of the animal.
2) One single *in vivo* MRI session within 7-14 days after cannula fixation, after the stimulation session. This will be done to determine whether (1) cannula/s stays fixed and intact on the skull, (2) cannula/s on the skull cause disturbances on MR images.
3) Perfusion-fixation after the MRI session, followed by histological/immunohistochemical staining to check if the stimulation protocol/s resulted in neuronal death.

Outcome parameters:

- MRI data, to check if cannula/s causes disturbances on the MR images.
- Behavior, to determine the effect of awake stimulation.
- Histology/immunohistochemical staining; to check if the stimulation caused any neuronal damage.

0.3) Setup and optimization of [redacted] stimulation [redacted]

[redacted] manipulation [redacted] will be established within the [redacted] network. The technique requires the infusion of [redacted] into two sites that are connected through [redacted] neuronal [redacted] and represent a neuronal pathway [redacted] [redacted] neuronal pathways will be targeted in healthy rats by the [redacted] approach consisting of (1) [redacted] of an [redacted] [redacted] carrying an [redacted] [redacted] [redacted] into the [redacted] network, and (2) injection of a [redacted] [redacted] into the [redacted] areas of the [redacted] neurons targeted by (1). This will result in the [redacted] [redacted] of only those neuronal projections within the [redacted] network.

[redacted] manipulation will be established and validated in 3 sub-phases:

1) Validation of inter- and intrahemispheric [redacted] sites
   During this phase, we will establish and determine the precise coordinates of the intra- and interhemispheric [redacted] sites, for the introduction of the above [redacted] into a [redacted] [redacted] In this phase, brain materials can be used from available carcasses, to practice the stereotactical [redacted] sites will be verified by infusing ink into the [redacted] sites. The brain tissue will be removed, sectioned, and examined under a microscope to verify the precision of the [redacted] site.
   Outcome parameter:
   - Histological evaluation/verification of the [redacted] sites.

2) [redacted] of neuronal [redacted]
   In this phase, it is necessary to verify how effectively the [redacted] [redacted] bind to and [redacted] a particular neuronal pathway. These [redacted] can have different coats, which may influence their efficiency in infecting a particular neuronal pathway. During this phase [redacted] with different coats may be tested and we will validate the combined use of [redacted] and [redacted] to [redacted] neural pathways within the [redacted] network. Two weeks after [redacted] of the [redacted] they are [redacted] in the targeted neuronal pathway. [redacted] of these neuronal pathways in the [redacted] network might induce changes in [redacted] activity.
   Outcome parameters:
   - Behavioral changes in [redacted] activity.
   - MRI, to detect any functional changes induced by [redacted] activation, followed by euthanasia.
   - Immunohistochemical staining for a [redacted] coupled to the [redacted] ([redacted] construct will quantify successful transfection.

3) Validating the dose of the [redacted] needed to [redacted] neuronal pathways
   In this phase, it is necessary to verify which dose of the [redacted] sufficiently [redacted] the pathway [redacted] in the [redacted] network. Two weeks after [redacted] of the [redacted] they are [redacted] in the targeted neuronal pathway. Activation of these neuronal pathways in the [redacted] network might induce changes in [redacted] activity.

Outcome parameters:

- Behavioral changes in [redacted] activity.
- MRI, to detect any functional changes induced by [redacted] activation, followed by euthanasia.
- Immunohistochemical staining for a [redacted] coupled to the [redacted] construct will quantify successful transfection.

0.4 – 0.5) Setup and optimization of MR protocols

We will develop, implement and validate two new MR protocols:

1) The first MR protocol [redacted] will allow for the detection of changes in brain [redacted] namely baseline levels of [redacted] and active [redacted] For *in vivo* [redacted] animals will be fasted over night before MR experiments [redacted] A femoral artery or tail vein will be cannulated for the monitoring of blood gasses (arterial pO_2 and pCO_2), pH and blood pressure [redacted] The femoral vein will be cannulated for the infusion of [redacted] during MR acquisition. Rats will be infused with [redacted] for 140 minutes according to a previously described protocol [redacted] MRI will be performed once per animal with a maximum duration of 5 hours scan time. Animals will be under anesthesia during MRI, and will be euthanized directly after MRI.

2) The second MR protocol [redacted] will be used for the spatial assessment of neuronal activation. [redacted] in the brain and can therefore be used as a contrast agent in [redacted] for functional imaging [redacted] As [redacted] has a high chemical similarity with [redacted] it may [redacted] and is hereby a [redacted] measure of neural activity. [redacted] before *in vivo* [redacted] will be injected systemically (iv or ip), because contrast enhancement seems to reach its equilibrium [redacted] following administration. MRI will be performed once per animal with a maximum duration of 5 hours scan time. Animals will be under anesthesia during MRI, and will be euthanized directly after MRI.

Outcome parameters:

- [redacted] concentrations, quantified from MR [redacted]
- Perfusion MRI to confirm blood supply to the brain during the infusion of [redacted]
- Signal enhancement [redacted] in the brain

<u>The first development of these MR protocols can be performed using phantoms and brain materials from available carcasses. Subsequently, to further optimize and implement these MR techniques, we need to perform these MR protocols in vivo.</u>

0.6) Setup and validation of behavioral tests for sensorimotor function

Prior to TMS, development and optimization of MRI and optical imaging protocols and after tDCS and [redacted] manipulation, the same animals will be used to setup and evaluate different behavioral tests for assessment of sensorimotor function (e.g. forelimb asymmetry test, skilled reaching test, beam walk, neurological severity score, and grip strength), anxiety/locomotive activity (open field test) and cognition (e.g. Barnes maze and novel object recognition) in our laboratory. Animals will be used for behavioral testing once per day at maximum. In addition, animals that underwent stroke surgery will perform behavioral tests to select the most sensitive tests to measure functional impairment after stroke.

0.7) Training and optimization of stroke induction

Training of personnel to induce cortical (photothrombotic model) and predominantly subcortical (transient occlusion of the middle cerebral artery (tMCAO) with an intraluminal filament) stroke. Photothrombotic stroke will be induced in naive, male, Sprague Dawley rats. In this model, a cortical infarct is induced by the systemic injection of a photosensitive dye (Rose-Bengal) in combination with the focal illumination of the skull (Watson et al. 1985). The illumination leads to the local activation of Rose-Bengal, which results in the disturbance of endothelial function and local thrombosis in small cortical vessels. The advantages of this model are the relatively small and reproducible infarct size, the ability to place the infarct within the desired sensorimotor subdivisions of the cortex, the minimal surgical manipulation of the animal and high survival rate (Fluri et al. 2015).

To increase translation of promising brain stimulation techniques to the clinic, we will also include another stroke model resulting in a predominantly subcortical stroke, which produces a pattern of damage (i.e. striatum and cortex) similar to humans (Corbett et al., 2017). Stroke will be induced in naïve Sprague Dawley rats, by the transient occlusion of the middle cerebral artery (tMCAO model) with an intraluminal filament (Longa et al. 1989). This second model is chosen based on similarities in pathology with stroke patients (caused by the focal occlusion of a large cerebral artery), high reproducibility and low invasiveness (Fluri et al. 2015; Kumar and Gupta 2016). However, there is a relatively large (12%) rate of subarachnoid hemorrhage which can reduce the blood flow bilaterally, and there could be potential difficulties in eating and weight loss.

These procedures will only be performed once per rat. The stroke induction procedures may take 3 hours at maximum. If the procedure takes longer, the procedure will be finished but the rat will be euthanized directly following surgery. In case the surgery is considered successful, and performed within 3 hours at maximum, rats will be allowed to recover and will be subjected to:

1) One behavioural test session and a single *in vivo* MRI session within 1-7 days after stroke or
2) One behavioural test session and a single optical imaging [redacted] session within 1-7 days after stroke
3) Perfusion-fixation directly after these imaging sessions within 1-7 days after stroke, followed by histological/immunohistochemical staining to identify the ischemic lesion.

Outcome parameters:

- Behavioral assessment of the efficacy and severity of the induced stroke, and selection of the most sensitive tests to measure functional impairment after stroke.
- MRI assessment of the lesion size and location.
- [redacted] assessment of cortical activity after infarction.
- Histological/immunohistochemical staining to identify the ischemic lesion.

In the unexpected case that rats show symptoms that cannot be explained by stroke, the animals will be directly euthanized or subjected to MRI, followed by euthanasia. Animals used for training of the tMCAO surgery will also be used to evaluate the sensitivity of the different behavioral tests for stroke-induced changes in sensorimotor function.

0.8-0.9) Optimization of current MRI and optical imaging [redacted] protocols

Alternatively, animals used for behavioral testing may also be used for the optimization of current MRI and [redacted] protocols. [redacted] will either be performed through an intact thinned skull or through a cranial window. [redacted] will take 1.5 hours at maximum. MRI will take 4 hours at maximum, and animals will be killed directly after the imaging session.

References:

[redacted]

Corbett D, Carmichael ST, Murphy TH, et al (2017) Enhancing the alignment of the preclinical and clinical stroke recovery research pipeline : Consensus-based core recommendations from the Stroke Recovery and Rehabilitation Roundtable translational working group. Int J Stroke 12:462–471. doi: 10.1177/1747493017711814

[redacted]

Fluri F, Schuhmann MK, Kleinschnitz C (2015) Animal models of ischemic stroke and their application in clinical research. Drug Des Devel Ther 9:3445–3454. doi: 10.2147/DDDT.S56071

Graboswki M, Brundin P, Johansson BB (1993) Paw-Reaching, Sensorimotor, and rotational behavior after brain infarction in rats. Stroke 24:889–895. doi: 10.1161/01.STR.24.6.889

[redacted]

Watson BD, Dietrich WD, Busto R, et al (1985) Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann Neurol 17:497–504. doi: 10.1002/ana.410170513

Whishaw IQ, Pellis SM, Gorny BP (1992) Skilled reaching in rats and humans: evidence for parallel development or homology. Behav Brain Res 47:59–70.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

To minimize the number of animals, brain stimulation optimization, training of personnel to perform stroke surgeries, implementation of new MRI protocols, optimization of [redacted] protocols and introducing new behavioral tests will be done within the same animals in case possible. The maximum number of animals required for implementation of new MRI protocols is based on our experience.

Table 1: Different procedures that can be performed within a single animal to minimize the number of animals.

Procedures	Aim
1. Behavior + 1x TMS + Electromyography + 1x non-recovery *in vivo* MRI	To test the setup of TMS, to train personnel to perform TMS. Subsequently, these animals could be used to practice/set up behavioral tests and to optimize new and existing MRI protocols in healthy animals.
1x tDCS + behavior + 1x non-recovery *in vivo* MRI	To practice tDCS surgery, to test the tDCS setup, to train personnel to perform tDCS and to test the feasibility of tDCS during behavioral tests. Subsequently, these animals could be used to optimize new and existing MRI protocols in healthy animals.
1x [redacted] of [redacted] + behavior + 1x non-recovery *in vivo* MRI	To test the efficacy of the [redacted] to activate [redacted] pathways, to check the behavioral effect of activation of [redacted] pathways and select appropriate behavioral tests. Subsequently, these animals could be used to optimize new and existing MRI protocols in healthy animals.
1x [redacted] of [redacted] + repetitive [redacted] of [redacted] + behavior + 1x non-recovery *in vivo* MRI	To test the optimal dose of the [redacted] to activate [redacted] pathways and to investigate its effects on behavior and the brain with MRI.
Behavior + 1x non-recovery *in vivo* [redacted]	To practice/set up behavioral tests and to optimize the optical imaging.
Behavior + 1x non-recovery *in vivo* MRI	To practice/set up behavioral tests, to optimize new and existing MRI protocols in healthy animals.
Behavior + non-recovery stroke	To practice/set up behavioral tests and to train personnel for stroke surgeries (photothrombotic and tMCAO models).
Stroke + behavior + non-recovery *in vivo* MRI	To train personnel for stroke surgeries, to select most sensitive behavioral tests to measure behavioral impairment and functional stroke recovery and to optimize new and existing MRI protocols in stroke animals.
Stroke + behavior + 1x non-recovery *in vivo* [redacted]	To train personnel for stroke surgeries, to select most appropriate behavioral tests to measure stroke recovery and to optimize optical imaging and new and existing MRI protocols in stroke animals.

It is necessary to allow animals to recover after tDCS surgery and after [redacted] [redacted] under anesthesia to test the feasibility of the stimulation setup, the possibility of applying tDCS during behavioral tests and to investigate the effects of [redacted] manipulation on behavioral measures.

In addition, it is necessary to allow animals to recover after the stroke surgery, to select the most sensitive behavioral tests to measure functional impairment after stroke, and to optimize (optical) imaging techniques in stroke animals.

The first developmental phases of different techniques (tDCS surgery, [redacted] manipulation, development of new MRI protocols) will be performed using phantoms or brain materials of available carcasses. In addition, animals will be euthanized after the imaging sessions and the brains will be collected to enable post-mortem experiments.

Training of the two stroke models (photothrombotic and tMCAO model) leads to refinement of the procedure, and will reduce animal loss to a minimum (photothrombotic model: ~0%, tMCAO model: < 25%).

Behavioral testing does not require extra animals, as these animals will always be used for optimizing brain stimulation, implementation of the new MRI protocols, [redacted] and/or training of the stroke models.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Preparation phase: maximum 325 rats.

Adult male and female Sprague Dawley (Sprague Dawley/CRL:CD(SD) rats from Charles River will be used in this part of the study. The rat's cerebral anatomy and blood supply are well described, and our lab has extensive experience using these animals in stroke models.

Emerging data have suggested that stroke incidence, mortality and severity may be sex dependent (Ahnstedt et al. 2016). For example, fluctuating levels of estrogen have been linked to the extent of brain damage after experimental stroke (Carswell et al. 2000), and young female animals demonstrate less ischemic damage than young males (Manwani et al. 2013). Biological sex may also influence how patients/animals respond to stroke treatments, since the mostly applied treatment for stroke (thrombolysis) has been suggested to be more effective in women than men (Kent et al. 2005).

Most of the experimental work has been done in male animals, despite the higher clinical burden of female stroke patients. To increase translational possibilities, and because of the above mentioned sex differences, we willl include both males and females but perform separate analyses for both sexes.

The number of rats needed for this preparation phase are based on our own experience or on suggestions from experts in the field.

Personnel will be trained during the full 5 years of the proposal to perform the photothromtobic or tMCAO stroke induction. Based on our experience, the tMCAO procedure is more difficult to learn than the photothrombotic stroke procedure. Therefore, only 2 people will learn the tMCAO technique during the 5 years, while 5 people will be trained to induce a photothrombotic stroke during the 5 year period. The number of animals needed to learn a reproducible stroke induction with the tMCAO procedure is based on experience and will be 50 per person, whereas for the photothrombotic stroke procedure it is 10. This leads to a total number of 150 rats needed to train personnel for the induction of a photothrombotic or tMCAO stroke.

For the animals that will undergo a non-recovery stroke surgery, surplus animals will be used whenever possible. Animals that will recover after the stroke surgery will also be used to select/evaluate sensitive behavioral tests and MRI and [redacted] protocols, which will be 20 rats per imaging technique, leading to a total of 40 rats.

For the optimization of current TMS protocols, three new people will be trained during the 5 years. Based on our experience, 10 rats per person are needed to reliably perform TMS in rats, leading to 30 rats.
For the optimization of tDCS, also three new people will be trained. Since this technique also requires a surgery, 15 rats per person are needed to realiably perform tDCS in rats, leading to 45 rats.

The rats used to optimize TMS and tDCS protocols can also be used to optimize current MRI protocols and to setup new behavioral tasks and train personnel to perform behavioral tests. In addition, we will use a total of 20 rats to optimize the optical imaging protocols ([redacted] and 20 rats to setup and develop new MRI protocols.

Lastly, for the set up of [redacted] manipulation, we need a total of 60 rats. The setup of [redacted] maniuplation requires three steps, of which the first step can be performed on brain material from available carcasses. For the second part, to test the efficacy of the [redacted] to [redacted]/[redacted] a [redacted] pathway in the [redacted] network, we need 3 rats per manipulation paradigm. Since we will use 4 different paradigms, this leads to 12 rats. For the last part, to determine the dose-response curve of the [redacted] [redacted] we need 12 rats per manipulation paradigm, which leads to 48 rats.

This adds up to a maximum total of 325 rats. The age of the animals used in the preparation phase will be matched to the age of the animals that will be used in the following studies (see appendices 1-3).

References

Ahnstedt H, Mccullough LD, Cipolla MJ (2016) The Importance of Considering Sex Differences in Translational Stroke Research. Transl Stroke Res 7:261–273. doi: 10.1007/s12975-016-0450-1

Carswell HVO, Dominiczak AF, Macrae IM (2000) Estrogen status affects sensitivity to focal cerebral ischemia in stroke-prone spontaneously hypertensive rats. Am J Physiol Hear Circ Physiol 278:290–294.

Kent DM, Price LL, Ringleb P, et al (2005) Sex-Based Differences in Response to Recombinant Tissue Plasminogen Activator in Acute Ischemic Stroke A Pooled Analysis of Randomized Clinical Trials. Stroke 36:62–65. doi: 10.1161/01.STR.0000150515.15576.29

Liu F, Yuan R, Benashski SE, McCullough LD (2009) Changes in experimental stroke outcome across the lifespan. J Cereb Blood Flow Metab 29:792–802. doi: 10.1038/jcbfm.2009.5

Manwani B, Liu F, Scranton V, et al (2013) Differential effects of aging and sex on stroke induced inflammation across the lifespan. Exp Neurol 249:1–25. doi: 10.1016/j.expneurol.2013.08.011

C. Re-use

Will the animals be re-used?

X No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☐ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:

Animals are required to set up and test the effects of brain stimulation therapies and imaging modalities *in vivo*, as the complex interaction between different cells, growth factors etc. of the brain can never be mimicked completely *in vitro*. In addition, an intact animal is needed to study the stimulation effects on behavioral and clinical relevant parameters.

Where possible, all imaging protocols will first be tested using phantom or post-mortem tissue samples, and we will use available carcasses of animals were possible.

Reduction:

Information gathered from pilot studies executed in this preparation phase will be used to define optimal stimulation protocols, behavioral tests, stroke induction methods, MRI and optical imaging protocols, leading to a reduction of the total number of animals to be used in the present study.
Animals used for TMS, tDCS and behavioral testing will always be re-used for the implementation of new MRI protocols, optimization of the [redacted] protocols and/or training of the induction of stroke models. This leads to a reduction in the animals to be used.
There is a lot of experience with animal models of stroke within our lab. This allows optimal training of the stroke surgery, resulting in skilled personnel. This leads to less animal loss and less variation in the outcome parameters.
MRI is the main imaging method of choice because it allows the verification of our results from animal studies to clinical outcomes. In our laboratory we have a long history of neuro-MRI in rodents, leading to a reduction in the number of animals used.

Refinement:
All imaging experiments, surgeries and and stimulation therapies (where necessary) will be performed under anaesthesia. During these procedures, animal physiology (temperature, heart rate, respiration rate, oxygenation and temperature) will be continuously monitored and kept within physiological range.
In case physiological parameters during surgery or imaging indicate that the animals are experiencing unexpected discomfort, animals will be euthanized.

Where possible, animals will be housed socially, with at least two animals per cage. Only in exceptional circumstances animals will be housed individually, i.e. when a cage mate dies during an experiment, or when animals have an epicranial device (tDCS setup) fixed to the skull and a suitable cap cannot be designed to protect the structure from gnawing damage. If animals were to be housed individually, it would be for a maximum of 5 weeks.

Moreover, in case animals reach the humane endpoints described in this proposal, animals will be euthanized.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

All animals will have the appropriate anaesthesia and analgesics (pre-operatively and post-operatively) in case of brain stimulation, stroke induction and imaging. All animals will be carefully monitored for any adverse effects. During brain stimulation, [redacted] and MRI, animals will be under anaesthesia, and the physiology parameters of the animal (temperature, heart rate, respiration rate) will be continuously monitored and kept within physiological range. Furthermore, animals will be supplemented with subcutaneous saline before each imaging session, to prevent dehydration during imaging.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

X No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

X Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Animals will be monitored closely after surgical procedures. While the animal is deeply anesthetized, lidocaine will be applied on the skin before an incision is made for the stereotactic injection surgery. Lidocaine will also be applied on the skin before induction of stroke and at suture wounds upon closure. Analgesia will be administered 2x 24h after stereotactic injection and stroke induction surgery.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Due to the photothrombotic and tMCAO procedures, animals may expose unusual behavior such as failure to groom, a hunched posture, and/or inappetence. Moreover, animals may experience partial paralysis, explaining behavioral deficits and reduced ability to eat. Animals are expected to lose weight, up to 10% of pre-stroke body weight within 7 days after stroke, based on previous experiments of post-stroke rats compared to sham-controlled animals. Beyond the time window of 7 days post stroke, animals are expected to gain weight.

Explain why these effects may emerge.

The expected effects are a direct consequence of stroke surgery.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Liquid food and/or food pellets will be placed inside the cage. Furthermore, animal cages will be placed on a heating mattress. In case of exacerbated weight loss, Ringer Lactate will be subcutaneously administered.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

In case stroke surgery takes longer than 3 hours, or if one of the blood vessels at the surgical site ruptures, the animal will directly be killed.

In the event that any animal shows a progressive weight loss of over 20%, compared to pre-surgery weights, the animal will be killed.

In addition we will use an animal motility score to idenfity the humane endpoint for animals suffering from stroke. The following motility scores will be assessed in their home cage:

(0) Normal exploratory behavior;
(1) Slightly reduced exploratory behavior;
(2) Moving limbs without proceeding;
(3) Moving only to stimuli;
(4) Unresponsive to stimuli, with normal muscle tone;
(5) Severely decreased tone, premortal signs.

Animals with a motility score of 5 will be killed. Animals with a motility score of 4 will be observed 2 times daily. In case no progression is observed within 2 days, the animal will be killed.

Indicate the likely incidence.

In post-stroke studies, this is a rare occurrence in fewer than 5% of the entire study population.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

PREPARATION PHASE			
	Stroke	**Discomfort**	**Estimated % of animals**
Behavior + 1x TMS + Electromyography + 1x non-recovery *in vivo* MRI	**no**	**mild**	**9**
1x tDCS + behavior + 1x non-recovery *in vivo* MRI	**no**	**moderate**	**14**
1x [redacted] of [redacted] + behavior + 1x non-recovery *in vivo* MRI	**no**	**moderate**	**4**
1x [redacted] of [redacted] + repetitive [redacted] of [redacted] + behavior + 1x non-recovery *in vivo* MRI	**no**	**moderate**	**15**
Behavior + 1x non-recovery *in vivo* [redacted]	**no**	**mild**	**6**
Behavior + 1x non-recovery *in vivo* MRI	**no**	**mild**	**6**
Behavior + non-recovery stroke	**yes**	**mild**	**34**
Stroke + behavior + non-recovery *in vivo* MRI	**yes**	**severe**	**6**
Stroke + behavior + 1x non-recovery *in vivo* [redacted]	**yes**	**severe**	**6**

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Euthanasia of animals after the final experimental procedure is necessary to collect brains for histology.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

[redacted]

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1

General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	11500
1.2	Provide the name of the licenced establishment.	UMCU
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Phase 1: [redacted] manipulation and MRI in healthy rats and rats with experimental stroke.

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

In phase 1 we will validate the [redacted] manipulation [redacted] within the [redacted] network, by looking at the acute effects of [redacted] manipulation on behavior and on brain activation (an acute [redacted] with the [redacted] during an MRI scan, and investigating the repetitive effects of [redacted] manipulation (Fig. 1). The primary aim of this study is to identify feasible and safe [redacted] stimulation therapies. We will investigate this using:

- Different stimulation paradigms: i.e. facilitatory and inhibitory paradigms, with a maximum of four different paradigms.
- Two different stroke models: a photothrombotic model to induce cortical stroke and a transient MCAO model to induce predominantly subcortical stroke. Both models will be used in this phase to determine which neuronal connections in the [redacted] network would be viable targets based on the type of induced stroke.

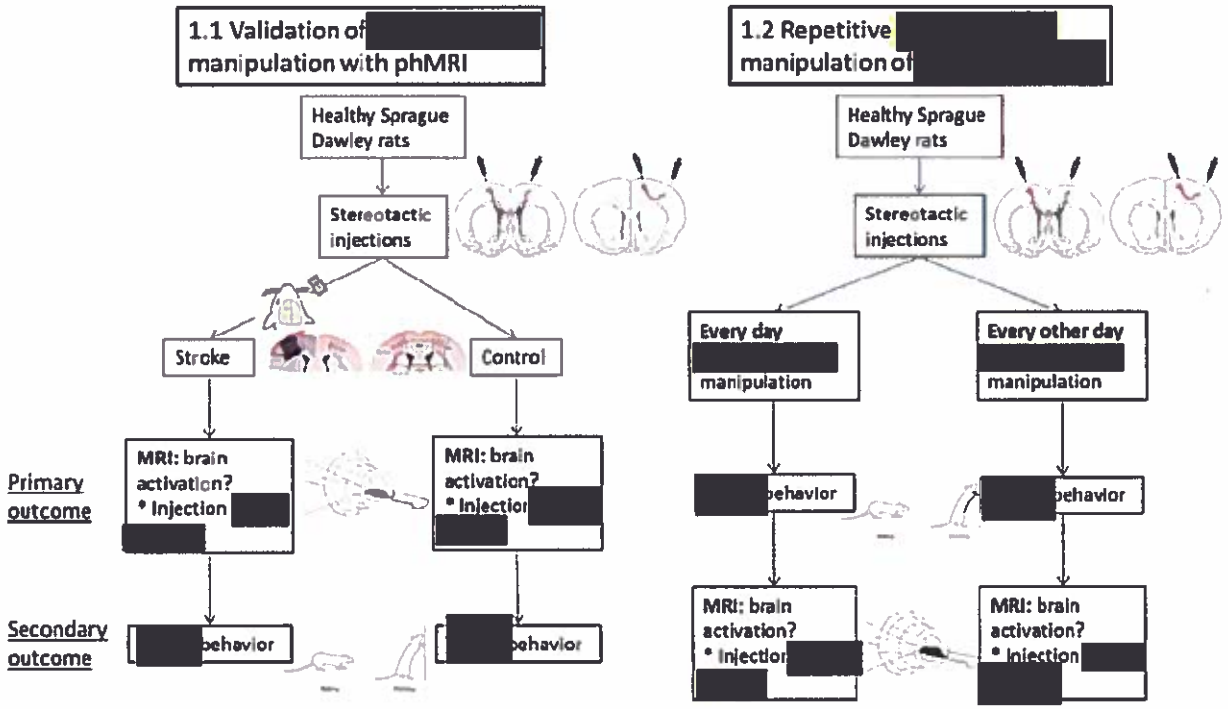


Figure 1: Phase 1 will be subdivided into two sub-phases, corresponding to the experiments that we would like to perform:

- In phase 1.1 we would investigate whether acute [redacted] manipulation [redacted] is able to activate or deactivate [redacted] connections in the [redacted] network, using behavior and MRI parameters, in control and in two models of experimental stroke.
- In phase 1.2 we would investigate the feasibility and safety of repetitive [redacted] with the [redacted] with respect to [redacted] internalization, using behavior and MRI parameters.

The primary outcome measure for phase 1.1 (Fig. 1), in which we investigate the acute effects of [redacted] manipulation by [redacted] with an [redacted] on the [redacted] network, will be brain activation or deactivation induced by this [redacted] upon administration during pharmacological MRI. This [redacted] brain activation will show whether we will be able to activate a [redacted] connection within the [redacted] network using [redacted] manipulation [redacted]

The primary outcome parameter for phase 1.2 (Fig. 1) is the [redacted] behavior induced by the [redacted] of the [redacted] This [redacted] behavior will be measured using the [redacted] to assess [redacted] and behavioral activity levels. This [redacted] behavior will show whether the network can be stimulated to the same extent, using a fixed amount at each [redacted] time point, or whether behavioral responses upon [redacted] manipulation will decrease potentially due to [redacted] of the [redacted]

The secondary outcome parameters for phase 1.1 are 1) [redacted] increases or decreases in [redacted] behavior observed in the [redacted] and 2) MRI-based measures of functional connectivity. These parameters will be used to further validate our approach of using [redacted] manipulation [redacted] to stimulate [redacted] connections in the [redacted] network.

The secondary outcome parameters for phase 1.2 are MRI-based measures of 1) [redacted] brain activation or deactivation during pharmacological MRI; 2) functional connectivity. These parameters will be used to investigate whether repetitive [redacted] with an [redacted] (either daily or every-other-day) will influence the [redacted] brain activity and functional connectivity.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Phase 1.1 and 1.2 will only start when phase 0.3 and 0.7 of the preparation phase has been established.

Animal models of stroke

Photothrombotic stroke

Photothrombotic stroke will be induced in naïve Sprague Dawley rats. In this model, a cortical infarct is induced by the systemic injection of a photosensitive dye (Rose-Bengal) in combination with the focal illumination of the skull (Watson et al. 1985). The illumination leads to the local activation of Rose-Bengal, which results in the disturbance of endothelial function and local thrombosis in small cortical vessels. The advantages of this model are the relatively small and reproducible infarct size, the ability to place the infarct within the desired sensorimotor subdivisions of the cortex, the minimal surgical manipulation of the animal and high survival rate (Fluri et al. 2015).

Transient middle cerebral artery occlusion (tMCAO) stroke

To increase translation of promising brain stimulation techniques to the clinic, we will also include another stroke model resulting in a predominantly subcortical stroke, which produces a pattern of damage (i.e. striatum and cortex) similar to humans (Corbett et al., 2017). Stroke will be induced in naïve Sprague Dawley rats, by the transient occlusion of the middle cerebral artery (tMCAO model) with an intraluminal filament (Longa et al. 1989). This second model is chosen based on similarities in pathology with stroke patients (caused by the focal occlusion of a large cerebral artery), high reproducibility and low invasiveness (Fluri et al. 2015; Kumar and Gupta 2016). However, there is a relatively large (12%) rate of subarachnoid hemorrhage which can reduce the blood flow bilaterally, and there could be potential difficulties in eating and weight loss.

The stroke induction in both models may take 3 hours at maximum. If the procedure takes longer, rats will be euthanized directly following surgery. In case the surgery is considered successful, and performed within 3 hours, rats will be allowed to recover and will be subjected to the rest of the study.

We included both the photothrombotic and tMCAO model in this appendix, because the models may demonstrate different affected connections, which may influence the possibility to activate/inhibit a [redacted] pathway and the efficacy of this [redacted] manipulation.

[redacted] manipulation

manipulation

manipulation [redacted] is a more invasive brain stimulation technique. [redacted] neuronal pathways will be targeted in healthy rats by a [redacted] approach consisting of (1) [redacted] of an [redacted] carrying an [redacted] into a [redacted] area in the [redacted] network, and (2) [redacted] of a [redacted] into the receiving area of the neurons targeted by (1). This will result in the [redacted] in only those neurons that [redacted] from the [redacted] area to the receiving area in the [redacted] network. This [redacted] may take 4 weeks at maximum. After the stereotactic [redacted] rats are allowed to recover, and further experimental procedures will be performed after at least two weeks, to make sure the [redacted] is [redacted] Optimal timing of the stereotactic [redacted] before other experimental procedures will be established in phase 0.3.

For [redacted] manipulation purposes, rats will be subjected to [redacted] manipulation one time just before the behavioral assessment (before the MRI session) and during the MRI session in phase 1.1 or repeatedly in phase 1.2. This [redacted] manipulation involves the [redacted]

MRI protocol

Phase 1.1 & Phase 1.2

The MRI protocol will allow 1) assessment of [redacted] brain activity (pharmacological MRI) and 2) assessment of functional connectivity within one single imaging session of 3 hours at maximum. MRI will be performed once per animal in phase 1.1 and twice per animal in phase 1.2. Animals will be under inhalation or infusion anesthesia during MRI and may therefore require an intravenous catheter in the tail vein. Infusion of the otherwise [redacted] [redacted] during pharmacological MRI requires an intravenous or intraperitoneal catheter. Animals will be killed directly after MRI at the last time-point and

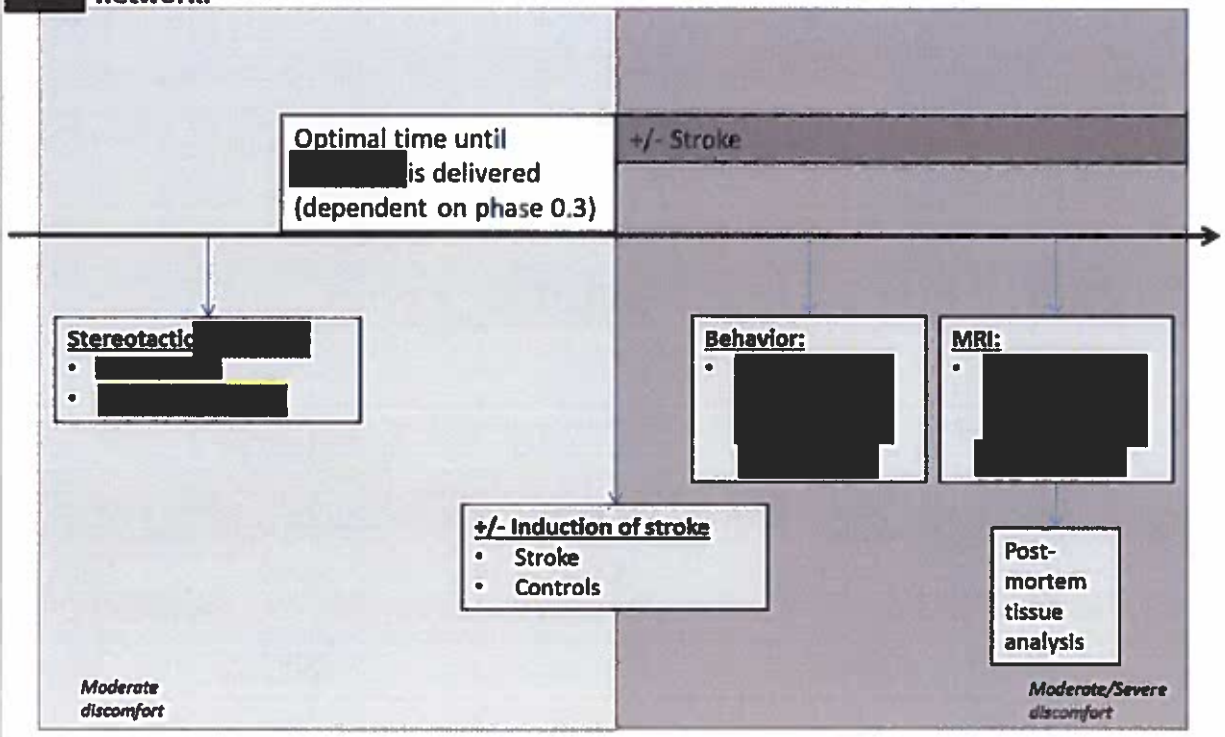
the brains will be harvested for histological assessment.

Behavior

[redacted] activation of the [redacted] in the [redacted] network will be assessed behaviorally using the [redacted] This test provides opportunities to assess different generalized [redacted] and behavioral activity levels, and since it has been demonstrated to be able to discriminate between stroke in the left and right hemisphere (Robinson 1979), it may be sensitive for hemispheric imbalance.

Behavioral tests will be performed in phase 1.1 once, in combination with a [redacted] of the [redacted] at least two days before the MRI session to validate whether the [redacted] manipulation of the [redacted] network results in behavioral effects. In phase 1.2, behavioral assessment will be performed every day during the [redacted] manipulation period, also when the [redacted] is given every other day. Using a [redacted] test, the efficacy of the [redacted] manipulation can be evaluated, and potential [redacted] internalization due to repetitive manipulation may be seen as decreased [redacted] behavior.

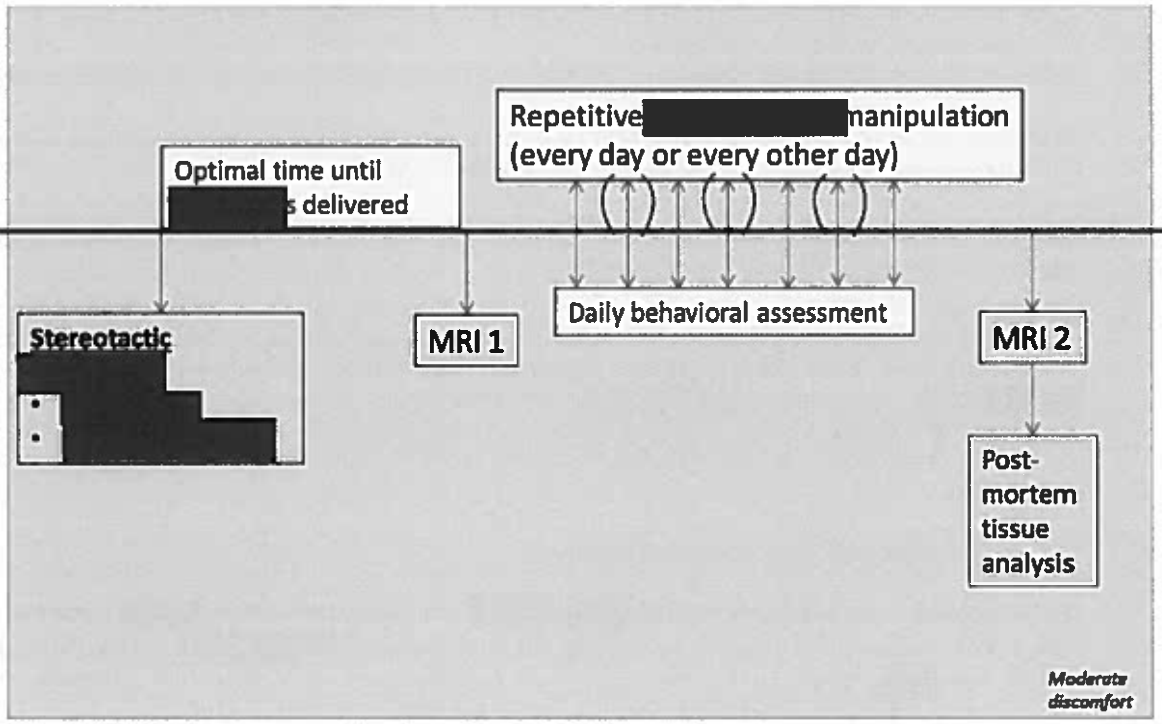
Sub-phase 1.1: Prospective outline of study to validate [redacted] manipulation of the [redacted] network.



- We will use two different rat models of experimental stroke and a healthy control group. We will perform the study in both males and females.
- We will use different brain stimulation paradigms, i.e. facilitatory and inhibitory with a maximum of four different stimulation paradigms.
- Next to the four manipulation groups, we need two control groups; 1) No [redacted] + [redacted] and 2) [redacted] + [redacted].
- This makes in total: 3 x 2 x (2+4) = 36 groups.
- Before stroke induction, two stereotactic [redacted] are performed to express the [redacted] in a [redacted] connection in the [redacted] network. Timing of these [redacted] prior to the induction of stroke depends on phase 0.3, so the exact time-point cannot be specified yet.
- Stroke will be induced using both the photothrombotic (cortical infarct) and tMCAO (predominantly subcortical infarct) stroke models.
- Behavior will be an [redacted] to assess [redacted] and behavioral activity levels.

- The *in vivo* MRI experiment will be performed for 1) quantitative assessment of brain activity induced by [redacted] manipulation during scanning (pharmacological MRI) and 2) quantitative assessment of functional connectivity. *In vivo* MRI will take 3 hours at maximum. MRI is the imaging method of choice because of potential clinical translation.
- Directly after the last MRI session, rats will be killed and brains will be excised for histology, to check [redacted] and availability of the [redacted]

Sub-phase 1.2: Prospective outline of longitudinal study to investigate the feasibility and safety of repetitive [redacted] manipulation of the [redacted] network.



- In this sub-phase, we will only use control, healthy Sprague Dawley rats, both males and females
- Rats will be subjected to two different repetitive stimulation paradigms: every day or every other day.
- We will use two different [redacted] manipulation paradigms; a facilitatory or inhibitory paradigm (as determined in phase 0.3 and 1.1) and a sham stimulation paradigm (rats that receive [redacted] instead of the [redacted]
- This makes in total: 2x2x2=8 groups.
- Timing of the stereotactic [redacted] prior to the first MRI session depends on phase 0.3, so the exact time-point cannot be specified yet.
- Length of the [redacted] manipulation (number of [redacted] with the [redacted] will be comparable to the length of the TMS and tDCS stimulation protocols, as determined in phase 0.1 and 0.2; and will not last longer than 14 days. [redacted] manipulations with [redacted] will be performed every day or every other day. A manipulation session consists of the [redacted] of the [redacted] followed by the [redacted] (behavior)
- The *in vivo* MRI experiment will be performed for 1) quantitative assessment of brain activity induced by [redacted] manipulation during scanning (pharmacological MRI) and 2) quantitative assessment of functional connectivity. *In vivo* MRI will take 3 hours at maximum. MRI is the imaging method of choice because of potential clinical translation.
- Behavioral assessment will be performed with the [redacted] to assess [redacted] and behavioral activity. Animals will be subjected to these behavioral tests every day, even when [redacted] manipulation is performed every other day.
- Directly after the last MRI session, rats will be killed and brains will be excised for histology, to check [redacted] and availability of the [redacted]

References

Corbett D, Carmichael ST, Murphy TH, et al (2017) Enhancing the alignment of the preclinical and clinical stroke recovery research pipeline: Consensus-based core recommendations from the Stroke Recovery and Rehabilitation Roundtable translational working group. Int J Stroke 12:462–471. doi: 10.1177/1747493017711814

Fluri F, Schuhmann MK, Kleinschnitz C (2015) Animal models of ischemic stroke and their application in clinical research. Drug Des Devel Ther 9:3445–3454. doi: 10.2147/DDDT.S56071

Kumar A, Gupta V (2016) A review on animal models of stroke: An update. Brain Res Bull 122:35–44. doi: 10.1016/j.brainresbull.2016.02.016

Longa EZ, Weinstein PR, Carlson S, Cummins R (1989) Reversible Middle Cerebral Artery Occlusion Without Craniectomy in Rats. Stroke 20:84–91.

Robinson RG (1979) Differential Behavioral and Biochemical Effects of Right and Left Hemispheric Cerebral Infarction in the Rat R. Science 205:707–710.

Watson BD, Dietrich WD, Busto R, et al (1985) Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann Neurol 17:497–504. doi: 10.1002/ana.410170513

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

To minimize the number of animals needed in these studies, we have included the preparation phase (phase 0) as animal appendix 1. In these preparation sub-phases, training of personnel to perform photothrombotic and tMCAO stroke induction, optimization of existing MRI protocols, setup of [redacted] manipulation [redacted] and optimization of behavioral tests will be done within the same animals where possible, without unnecessary discomfort. In addition, training for the induction of both photothrombotic and tMCAO stroke models, leads to refinement of the procedure, and will reduce animal loss.

Power calculations (Performed in G*power):

Sub-phase 1.1: Study to validate [redacted] manipulation of the [redacted] network

The primary aim of this study is to validate the application of [redacted] manipulation [redacted] within the [redacted] network. We will perform an ANOVA-test for the difference between more than two independent means (6 groups, per sex and per stroke model/control (6 times)).

Control rats: [redacted] brain signal intensity changes (as measured with pharmacological MRI) versus sham stimulation and [redacted] Based on our previous study, we expect to find an average difference in the total BOLD response (measured with MRI) upon [redacted] manipulation or sham manipulation of 166.7, with a variability of 105.7 [redacted] Using a significance level of 5% and a power of 80%, this results in a sample size of 12 rats per group.

Stroke rats: [redacted] brain signal intensity changes (as measured with pharmacological MRI) versus sham stimulation and [redacted] Since no [redacted] manipulation experiments have been performed in stroke animal models before, we will use the same effect size of [redacted] manipulation on brain signal intensity changes as in control animals. This results in a sample size of 12 rats per group.

Sub phase 1.2: Longitudinal study to investigate the feasibility and safety of repetitive [redacted] manipulation of the [redacted] network

The primary aim of this study is to investigate the effects of repetitive [redacted] manipulation of the [redacted] network on [redacted] and behavioural activity, with respect to overstimulation resulting in [redacted] internalization. We will perform an ANOVA test for the difference between more than two independent means (4 groups, per sex).

Control animals: changes in [redacted] and behavioral activity, induced by the either every day or every other day [redacted] with the [redacted] compared to [redacted] Based on previous studies investigating the effects of [redacted] manipulation on behavior [redacted] we expect a difference in the total distance moved (centimeters) upon [redacted] manipulation or sham manipulation of 9131 with a variability of 4319. Using a significance level of 5% and a power of 80%,

this results in a sample size of 6rats per group.

Since there is no available literature about the effects of [redacted] manipulation in female rats, we will use the power calculations of the male rats to determine female sample sizes.

References

[redacted]

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Adult male and female Sprague Dawley (Sprague Dawley/CRL:CD(SD) rats from Charles River will be used in this part of the study. The rat's cerebral anatomy and blood supply are well described, and our lab has extensive experience using these animals in stroke models.

Emerging data have suggested that stroke incidence, mortality and severity may be sex dependent (Ahnstedt et al. 2016). For example, fluctuating levels of estrogen have been linked to the extend of brain damage after experimental stroke (Carswell et al. 2000), and young female animals demonstrate less ischemic damage than young males (Manwani et al. 2013). Biological sex may also influence how patients/animals respond to stroke treatments, since the mostly applied treatment for stroke (thrombolysis) has been suggested to be more effective in women than men (Kent et al. 2005).

Most of the experimental work has been done in male animals, despite the higher clinical burden of female stroke patients. To increase translational possibilities, and because of the above mentioned sex differences, we willl include both males and females but perform separate analyses for both sexes.

Maximum number of rats: 528.

Sub-phase 1.1: maximum 480 rats (240 males and 240 females).

Groups and maximum group sizes (males) :

1) Control animals:
   a. [redacted] manipulation 1 (n=12)
   b. manipulation 2 (n=12)
   c. manipulation 3 (n=12)
   d. manipulation 4 (n=12)
   e. Sham manipulation (n=12)
   f. [redacted] (n=12)

2) Phototrombotic stroke:
   a. [redacted] manipulation 1 (n=12)
   b. manipulation 2 (n=12)
   c. manipulation 3 (n=12)
   d. manipulation 4 (n=12)
   e. Sham manipulation (n=12)
   f. [redacted] (n=12)

3) tMCAO stroke:
   a. [redacted] manipulation 1 (n=12)
   b. manipulation 2 (n=12)
   c. manipulation 3 (n=12)
   d. manipulation 4 (n=12)
   e. Sham manipulation (n=12)
   f. [redacted] (n=12)

With the photothrombotic stroke, we do not expect any animal loss based on our own experience and literature stating that the mortality rate in this stroke model is very low (Fluri et al. 2015).
In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss due to tMCAO, resulting in 16 rats per group.
This results in a total amount of 12x6 + 12x6 + 16x6 = 72 + 72 + 96 = 240 male rats.

We will need the same amount of female animals.

Sub-phase 1.2: maximum of 48 rats (24 males and 24 females)
Groups and maximum group sizes (males):

1) Every day [redacted] manipulation with [redacted] (n=6)
2) Every day [redacted] manipulation with [redacted] (n=6)
3) Every other day [redacted] manipulation with [redacted] (n=6)
4) Every other day [redacted] manipulation with [redacted] (n=6)

This results in 4x6 = 24 male rats.
We will need the same amount of female rats.

References

Ahnstedt H, Mccullough LD, Cipolla MJ (2016) The Importance of Considering Sex Differences in Translational Stroke Research. Transl Stroke Res 7:261–273. doi: 10.1007/s12975-016-0450-1

Carswell HVO, Dominiczak AF, Macrae IM (2000) Estrogen status affects sensitivity to focal cerebral ischemia in stroke-prone spontaneously hypertensive rats. Am J Physiol Hear Circ Physiol 278:290–294.

Kent DM, Price LL, Ringleb P, et al (2005) Sex-Based Differences in Response to Recombinant Tissue Plasminogen Activator in Acute Ischemic Stroke A Pooled Analysis of Randomized Clinical Trials. Stroke 36:62–65. doi: 10.1161/01.STR.0000150515.15576.29

Liu F, Yuan R, Benashski SE, McCullough LD (2009) Changes in experimental stroke outcome across the lifespan. J Cereb Blood Flow Metab 29:792–802. doi: 10.1038/jcbfm.2009.5

Manwani B, Liu F, Scranton V, et al (2013) Differential effects of aging and sex on stroke induced inflammation across the lifespan. Exp Neurol 249:1–25. doi: 10.1016/j.expneurol.2013.08.011

C. Re-use

Will the animals be re-used?

X No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☐ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:
Animals are required to study the effects of [redacted] manipulation of the [redacted] network, both in control and stroke animals, as the complex interaction between different cells, growth factors and functional connectivity etc. of the brain can never be mimicked completely *in vitro*. In addition, an intact animal is needed to study the stimulation effects on behavioral and clinical relevant parameters.

Reduction:
The two studies described in this appendix to validate [redacted] manipulation of the [redacted] network are designed to only be started when the corresponding preparation phases will be established. During these preparation phases, initial experiments to start-up and test different [redacted] manipulation paradigms will be performed to detect the most promising ones that will be used in the currently described studies. This leads to a reduction in the number of stimulation paradigms, and hereby a reduction in the amount of animals used in these experiments.

In addition, during the preparation phase, the induction of stroke will be trained, to reduce animal loss during the longitudinal studies.

MRI is the main imaging method of choice because it allows the verification of our results from animal studies to clinical outcomes. In our laboratory, we have a long history of neuro-MRI in rodents, leading to a reduction in the number of animals used.

Refinement:
All surgeries and imaging experiments will be performed under anesthesia. During these procedures, animal physiology (temperature, heart rate, respiration rate, oxygenation and temperature) will be continuously monitored and kept within physiological range. In case physiological parameters during surgery or imaging indicate that the animals are experiencing unexpected discomfort, animals will be euthanized.

Where possible, animals will be housed socially, with at least two animals per cage. Only in exceptional circumstances animals will be housed individually, i.e. when a cage mate dies during an experiment, or when animals have an epicranial device (tDCS setup) fixed to the skull and a suitable cap cannot be designed to protect the structure from gnawing damage. If animals were to be housed individually, it would be for a maximum of 5 weeks.

Moreover, in case animals reach the humane endpoints described in this proposal, animals will be euthanized.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

All animals will have the appropriate anesthesia and analgesics pre- and post-operatively in case of stroke induction, stereotactic [redacted] and imaging. All rats will be carefully monitored for any adverse side effects. During MRI, rats will be under anesthesia, and the physiology parameters of the animal (heart rate, respiration rate, temperature) will be continuously monitored and kept within physiological range. Furthermore, animals will be supplemented with subcutaneous saline before and after each imaging session to prevent dehydration during imaging.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

X No

Yes > If this may adversely affect animal welfare, describe how the animals will be housed and

provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

X Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

While the animal is deeply anesthetized, lidocaine will be applied on the skin before an incision is made for the stereotactic injection surgery. Lidocaine will also be applied on the skin before induction of stroke and at suture wounds upon closure. Analgesia will be administered 2x 24h after stereotactic injection and stroke induction surgery.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Due to the photothrombotic and tMCAO stroke procedures, animals may expose unusual behavior such as a failure to groom, a hunched posture, and/or inappetence. Moreover, animals may experience partial paralysis, explaining behavioral deficits and reduced ability to eat. Animals are expected to lose weight, up to 10% of pre-stroke body weight within 7 days after stroke, based on previous experiments of post-stroke rats compared to sham-controlled animals. Beyond the time window of 7 days post stroke, animals are expected to gain weight.

Explain why these effects may emerge.

The expected effects are a direct consequence of stroke surgery.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Liquid food and/or food pellets will be placed inside the cage. Furthermore, animal cages will be placed on a heating mattress. In case of exacerbated weight loss (more than 10%) Ringer Lactate will be subcutaneously injected.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

In case stroke surgery takes longer than 3 hours the animal will directly be killed.

In the event that any animal shows a progressive weight loss of over 20%, compared to pre-stroke weights, the animal will be killed.

In addition we will use an animal motility score to idenfity the humane endpoint for animals suffering from stroke
The following motility scores will be assessed in their home cage:
(0) Normal exploratory behavior;
(1) Slightly reduced exploratory behavior;
(2) Moving limbs without proceeding;
(3) Moving only to stimuli;
(4) Unresponsive to stimuli, with normal muscle tone;
(5) Severely decreased tone, premortal signs.

Animals with a motility score of 5 will be killed. Animals with a motility score of 4 will be observed 2 times daily. In case no progression is observed within 2 days, the animal will be killed.

Indicate the likely incidence.

In post-stroke studies, this is a rare occurrence in fewer than 5% of the entire study population.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Subphase 1.1

	Stroke	Discomfort	Estimated % of animals
Stereotactic [redacted] + behaviour + [redacted] manipulation (1x) + 1x in vivo MRI.	No	Moderate	30%
Stereotactic [redacted] + behaviour + [redacted] manipulation (1x) + 1x in vivo MRI.	Yes	Severe	70%

Subphase 1.2

	Stroke	Discomfort	Estimated % of animals
Stereotactic [redacted] + repetitive behaviour + repetitive [redacted] manipulation + 2x in vivo MRI.	No	Moderate	100

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Euthanasia of animals after the final serial MRI is necessary to collect brains for histology.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

[redacted]

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1

General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	11500
1.2	Provide the name of the licenced establishment.	UMCU
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 3 Type of animal procedure: Phase 2: Brain stimulation and multiparametric MRI in healthy rats and rats with experimental stroke.

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

In phase 2 we will investigate the efficacy of different brain stimulation therapies in stroke recovery. The primary aim of this study is to find promising stimulation therapies and paradigms that improve functional recovery after experimental stroke. We will test this using:

- Different brain stimulation paradigms per technique (rTMS, tDCS and [redacted] manipulation [redacted] i.e. facilitatory, inhibitory and sham stimulation paradigms, with a maximum of four different stimulation paradigms per technique.
- Two different time-points to start brain stimulation after stroke: sub-acute and sub-chronic.
- Two different stroke models: a photothrombotic stroke model to induce cortical stroke and a transient MCAO stroke model to induce subcortical stroke. Experiments will first be performed in the cortical stroke model, because we have extensive experience with the photothrombotic stroke model in our lab. When promising stimulation paradigms have been identified, their efficacy will also be tested in the subcortical stroke model (tMCAO model).
- Phase 2 will be subdivided into three sub-phases, each corresponding to the stimulation technique applied, i.e. 2.1 using TMS, 2.2 using tDCS and 2.3 using [redacted] manipulation [redacted] These three sub-phases can be performed in parallel, if corresponding phases in phase 0 have been established.

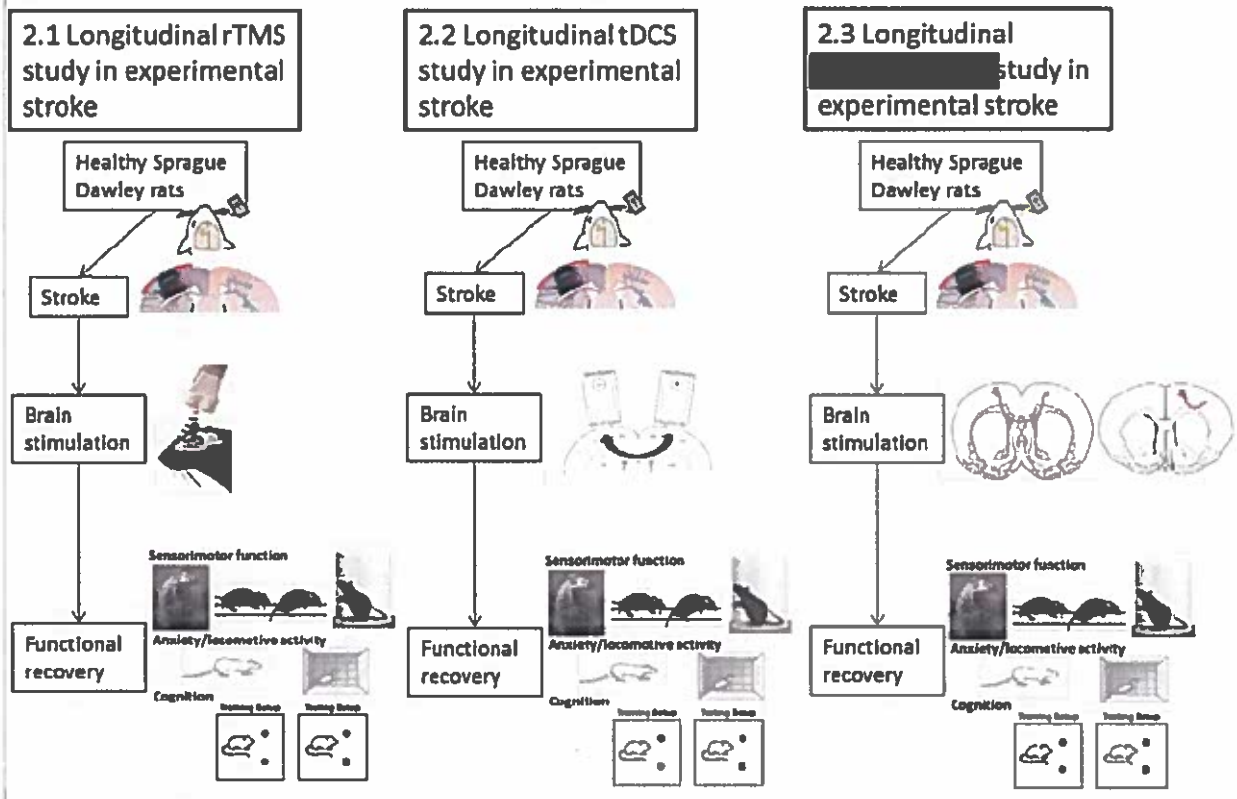


Figure 1: Phase 2 will be subdivided into three sub phases, corresponding to the experiments that we would like to perform:

- In phase 2.1 we would investigate the efficacy of different rTMS protocols to promote functional recovery after stroke, using behavior and MRI parameters, in two models of experimental stroke.
- In phase 2.2 we would investigate the efficacy of different tDCS protocols to promote functional recovery after stroke, using behavior and MRI parameters, in two models of experimental stroke.
- In phase 2.3 we would investigate the efficacy of different [redacted] manipulation protocols to promote functional recovery after stroke, using behavior and MRI parameters, in two models of experimental stroke.

All three stimulation techniques will either be combined with standard environment or sensorimotor therapy, using enriched environment or constrained-induced movement therapy (CIMT), since literature has shown that combination therapies may be most efficient to improve functional recovery after stroke (Takeuchi and Izumi 2015). The recovery of animals in the standard environment will be compared to those housed in the enriched environment.

The primary outcome measure for phase 2.1, 2.2 and 2.3 (Fig.1) is behavior. We will combine different behavioral tests to assess sensorimotor function, anxiety/locomotive activity and cognition (as determined in phase 0.6). These parameters will allow the monitoring of disease progression and the efficacy of brain stimulation to improve functional recovery after experimental stroke.

The secondary outcome parameters for phase 2.1, 2.2 and 2.3 are MRI-based measures of structural and functional connectivity, brain activity and infarct size. These parameters will be used to investigate underlying mechanisms of different brain stimulation techniques and paradigms by investigating its effect on structural and functional connectivity and stimulation-induced activity. In addition, it allows monitoring of disease progression and stroke outcome by measuring infarct size. With these secondary parameters we aim to identify MRI-based markers that can predict stroke outcome (behavior).

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Phase 2 will only start when corresponding sub-phases in the preparation phase (phase 0) have been established:

- Phase 2.1 (rTMS): phase 0.1, 0.6, 0.7 and 0.8.
- Phase 2.2 (tDCS): phase 0.2, 0.6, 0.7 and 0.8.
- Phase 2.3 ([redacted] manipulation [redacted] phase 0.3, 0.6, 0.7, 0.8 and phase 1.

Animal models of stroke

Photothrombotic stroke

Photothrombotic stroke will be induced in naïve Sprague Dawley rats. In this model, a cortical infarct is induced by the systemic injection of a photosensitive dye (Rose-Bengal) in combination with the focal illumination of the skull (Watson et al. 1985). The illumination leads to the local activation of Rose-Bengal, which results in the disturbance of endothelial function and local thrombosis in small cortical vessels. The advantages of this model are the relatively small and reproducible infarct size, the ability to place the infarct within the desired sensorimotor subdivisions of the cortex, the minimal surgical manipulation of the animal and high survival rate (Fluri et al. 2015).

Transient middle cerebral artery occlusion (tMCAO) stroke

To increase translation of promising brain stimulation techniques to the clinic, we will also include another stroke model resulting in a predominantly subcortical stroke, which produces a pattern of damage (i.e. striatum and cortex) similar to humans (Corbett et al., 2017). Stroke will be induced in naïve Sprague Dawley rats, by the transient occlusion of the middle cerebral artery (tMCAO model) with an intraluminal filament (Longa et al. 1989). This second model is chosen based on similarities in pathology with stroke patients (caused by the focal occlusion of a large cerebral artery), high reproducibility and low invasiveness (Fluri et al. 2015; Kumar and Gupta 2016). However, there is a relatively large (12%) rate of subarachnoid hemorrhage which can reduce the blood flow bilaterally, and there could be potential difficulties in eating and weight loss.

The stroke induction in both models may take 3 hours at maximum. If the procedure takes longer, rats will be euthanized directly following surgery. In case the surgery is considered successful, and performed within 3 hours, rats will be allowed to recover and will be subjected to the rest of the study.

Brain stimulation techniques

Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is a noninvasive brain stimulation technique, which involves a magnetic coil inducing currents in the brain. While animals are exposed to TMS, they will be anesthetized when determining the optimal positioning of the animal for receiving target specific stimulation. Anesthesia will either be administered via inhalation or infusion. Electromyography in combination with TMS will be used to determine the required dose of stimulation for each animal. By inserting needle electrodes into the limbs of the animal we can measure muscle responses induced by the stimulation, as a measure of cortical activity.

Rats will be subjected to TMS according to promising stimulation protocols as determined in phase 0.1, which may involve daily stimulation sessions. The stimulation sessions may take 2.5 hours at maximum per day, for a total of maximum 14 days.

Transcranial direct current stimulation

Transcranial direct current stimulation (tDCS) is a noninvasive brain stimulation technique, which requires placed electrodes on the scalp of the animal. Animals will be anesthetized and fixed in a stereotact for the placement of the electrode cannula/s. A vertical incision will be made on the skull to expose the cranium. The periosteum will be removed, to ensure that the skull is completely dry for the proper fixation of the cannula/s. The cannula/s will be fixed on the skull using dental cement and screws. After the cement has dried, the skin will be sutured around the fixed cannula/s. This procedure will take a maximum of 2 hours.

Rats will be subjected to tDCS according to promising stimulation protocols as determined in phase 0.2, which may involve daily stimulation sessions and immobilization of the animal by anesthetics when needed. The stimulation sessions may take 2.5 hours at maximum per day, for a total of maximum 14 days. tDCS stimulation may be applied as a combination therapy with stimulation during a behavioral task.

manipulation manipulation is a more invasive brain stimulation technique. neuronal pathways will be targeted in healthy rats by a approach consisting of (1) of an carrying an into a area in the network, and (2) of a into the receiving area of the neurons targeted by (1). This will result in the in only those neurons that from the area to the receiving area in the network. This may take 4 weeks at maximum. After the stereotactic , rats are allowed to recover, and further experimental procedures will be performed after at least two weeks, to make sure the is Optimal timing of the stereotactic before other experimental procedures will be established in phase 0.3.

For stimulation purposes, rats will be subjected to manipulation according to promising stimulation protocols as determined in phase 1, which involves of the These simulation sessions may be repeated every day or every-other day, depending on phase 1, for a total of maximum 14 days.

MRI protocol

The MRI protocol will allow 1) quantitative assessment of structural connectivity; 2) quantitative assessment of functional connectivity; 3) quantitative assessment of stimulation-induced brain activity and 4) quantitative assessment of infarct size within one single imaging session of 2.5 hours at maximum. MRI will be performed four or five times per animal. Animals will be under infusion or inhalation anesthesia during MRI and may therefore require an intravenous catheter in the tail vein. In addition, MRI protocols may require the administration of contrast agents via an intravenous catheter. Animals will be killed directly after MRI at the last time-point.

Behavior

Phase 0.6 will establish the most sensitive behavioral test for stroke-induced changes in sensorimotor function and cognition, which include different behavioural tests for assessment of sensorimotor function (e.g. forelimb asymmetry test, skilled reaching test, beam walk, neurological severity score, and grip strength), anxiety/locomotive activity (open field test) and cognition (e.g. Barnes maze and novel object recognition). Behavioral test will be performed regularly to assess post-stroke recovery and efficacy of brain stimulation therapy for 2-6 times per month, with a maximum of 2 hours per day.

These behavioural tests may include tests which will cause more than mild distress to the animals, because of food restriction protocols; for example, the skilled reaching test. To get the animals sufficiently motivated to participate in this task, animals are typically food deprived and kept on 85-90% of their bodyweight. The skilled reaching test can measure skilled movements that are similar to movements in human patients (Whishaw et al. 1992). Since the deficits in paw-reaching are associated with the infarct size, it is suggested that this test is sensitive and useful to evaluate treatments effects over a long period of time (Graboswki et al. 1993).

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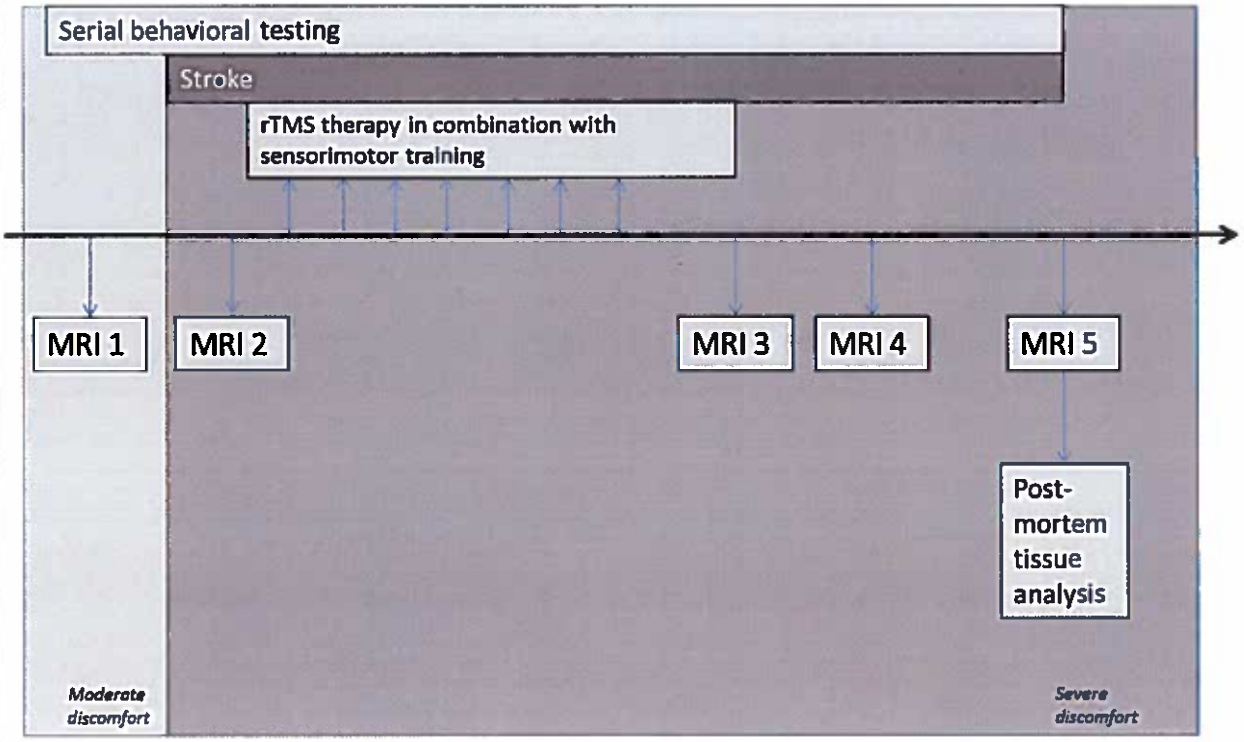
Sub-phase 2.1: Prospective outline of longitudinal study to investigate the efficacy of different TMS stimulation paradigms to improve functional recovery after experimental stroke.

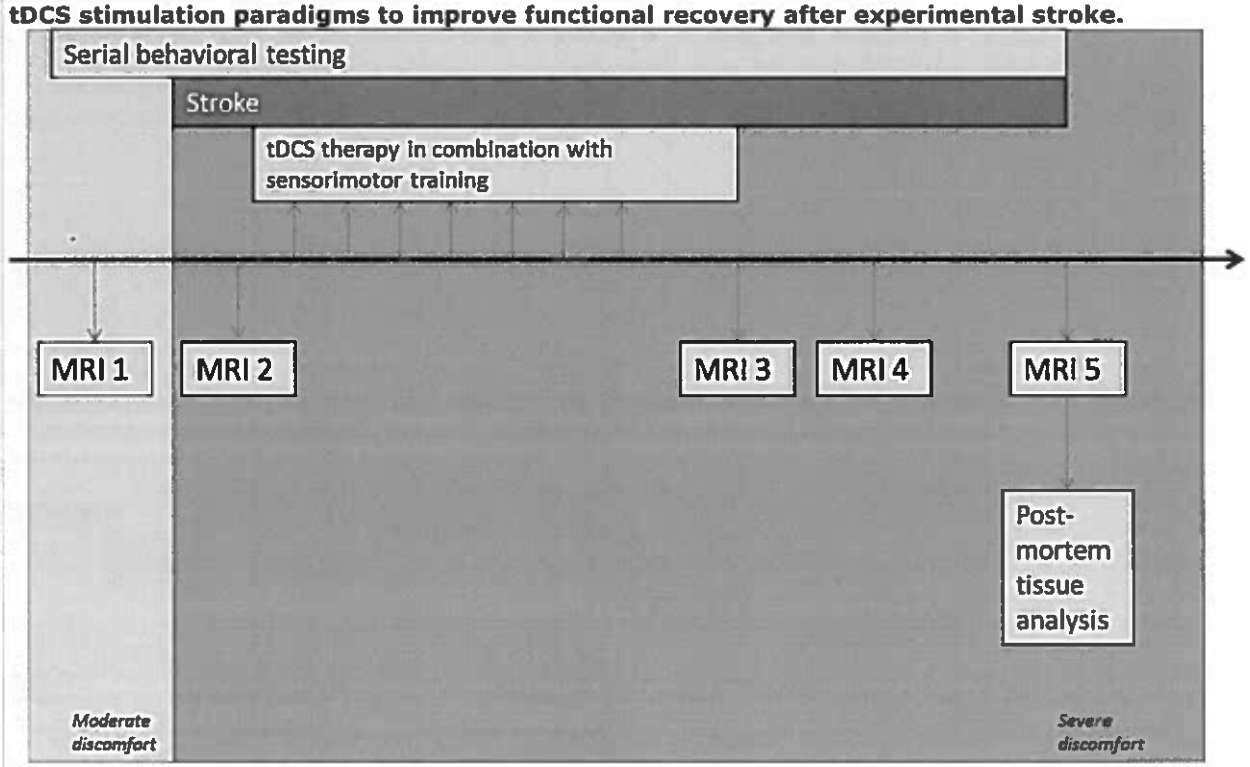


- We will use different brain stimulation paradigms in combination with different environments (standard/enriched environment): i.e. facilitatory, inhibitory and sham stimulation, with a maximum of five different paradigms. A similar study is currently being conducted in our lab and based on the functional recovery influences from the respective environments; a decision will be made whether animals will be housed in the standard or the enriched environment in prospective experiments.
- We will start stimulating at two different time-points after stroke: sub-acute and sub-chronic.
- We will test the efficacy of TMS in two different rat models of experimental stroke. When promising TMS treatment protocols are developed in the photothrombotic stroke model, they will be applied in the tMCAO stroke model.
- We will perform the study in both males and females.
- This makes in total: 2x5x2=20 groups per stroke model (total of 40 groups).
- Stroke will be induced using both the photothrombotic (cortical infarct) and the transient occlusion of the middle cerebral artery (tMCAO; predominantly subcortical infarct) stroke model.
- Behavioral tests for assessment of sensorimotor function will be performed on a regular basis (2-

6x per month) during the entire time course of the experiment.

- The TMS stimulation paradigms will be dependent on phase 0.1. Therefore, the amount, length and duration of the stimulation protocol cannot be determined yet, but it will involve daily repetitive stimulation sessions which may involve repetitive anesthesia when needed, with a maximum of 14 days. The stimulation sessions will have a maximum duration of two hours.
- Serial MRI will be performed 4 or 5 times per animal to cover the functional recovery after experimental stroke and the effects of brain stimulation on this functional recovery:
    - Time 1: Before stroke.
    - Time 2: After stroke but before stimulation (dependent on the stimulation time-points after stroke: sub-acute or sub-chronic.
    - Time 3: Directly after stimulation protocol.
    - Time 4: After stimulation has been stropped.
    - Time 5: After stimulation has stopped, to determine the lasting effects of stimulation.
- The *in vivo* MRI experiment will be performed for 1) quantitative assessment of structural and functional connectivity 2) quantitative assessment of brain activity and 3) high resolution imaging of edema to identify ischemic lesions. In vivo MRI will take 2.5 hours at maximum. MRI is the imaging method of choice because of potential clinical translation and because all parameters can be assessed *in vivo* longitudinally.
- Directly after the last MRI session (MRI 5), rats will be killed and brains will be excised for histology.

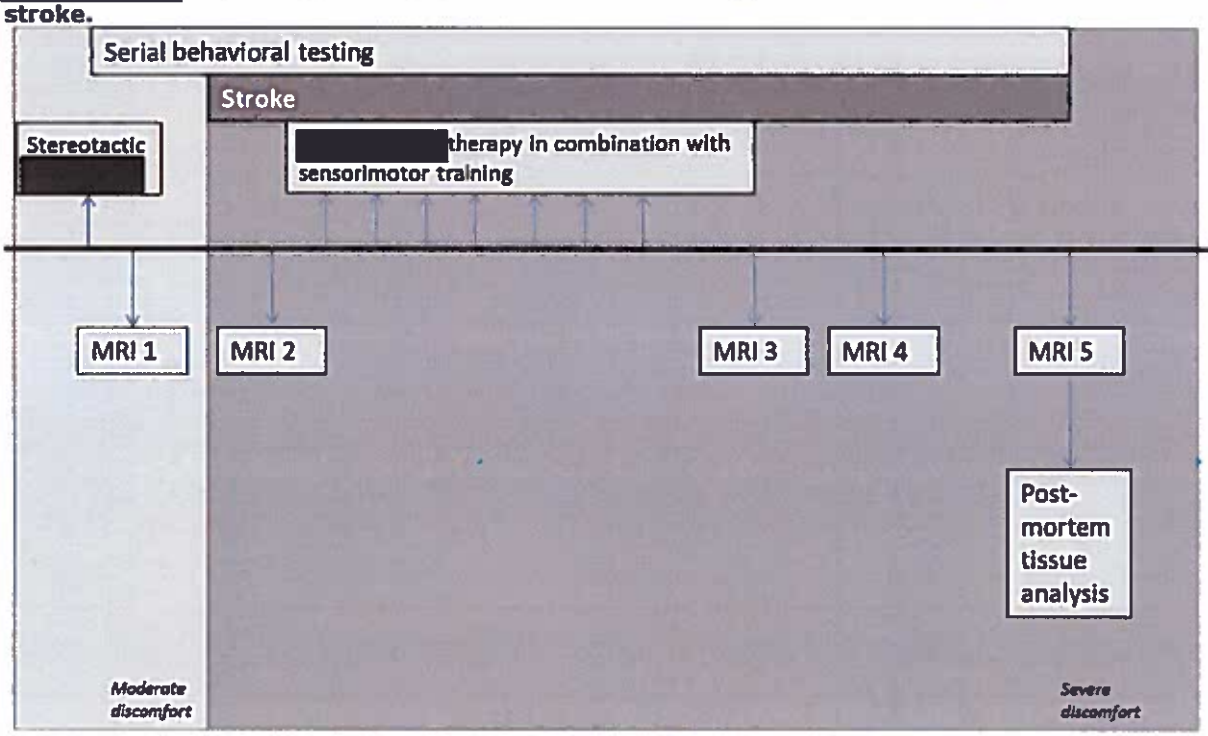
Sub-phase 2.2: Prospective outline of longitudinal study to investigate the efficacy of different tDCS stimulation paradigms to improve functional recovery after experimental stroke.



- This study will use different tDCS stimulation paradigms (facilitatory, inhibitory, sham and a combination therapy with a maximum of four different paradigms) and the same two time-points after stroke to start stimulation therapy (sub-acute and sub-chronic) as sub-phase 2.1. In addition, the efficacy of tDCS therapies will be first investigated in the photothrombotic stroke model, followed by studies in the tMCAO model, and studies will be performed both in male and female rats.
- This makes in total: 2x4x2=16 groups per stroke model (total of 32 groups).
- There will be two surgeries, one surgery to induce stroke and one surgery in which electrode cannula/s will be placed on the scalp of the animal.
- The tDCS stimulation protocols will be dependent on phase 0.2. Therefore, the amount, length and duration of the stimulation protocol cannot be determined yet. However, it will involve daily repetitive stimulation sessions which may involve repetitive anesthesia when needed, with a

maximum of 14 days. The stimulation sessions will have a maximum duration of two hours. The same MRI protocols will be performed as in sub-phase 2.1, at the same time points.

Sub-phase 2.3: Prospective outline of longitudinal study to investigate the efficacy of different [redacted] manipulation paradigms to improve functional recovery after experimental stroke.



- This study will use different [redacted] manipulation paradigms (with a maximum of four different paradigms) and the same two time-points after stroke to start stimulation therapy (sub-acute and sub-chronic) as sub-phase 2.1 and 2.2. In addition, the efficacy of [redacted] manipulation therapies will first be investigated in the photothrombotic stroke model, followed by studies in the tMCAO stroke model, and studies will be performed both in male and female rats.
- This makes in total: 2x4x2=16 groups per stroke model (total of 32 groups).
- Before stroke induction, two stereotactic [redacted] are performed to [redacted] target a connection to [redacted] Timing and location of these [redacted] depend on phase 0.3, so the exact time-point cannot be specified yet, although the [redacted] need at least two weeks to become fully [redacted]
- The [redacted] manipulation paradigms will be dependent on phase 0.3 and phase 1. Therefore, the amount, length and duration of the stimulation protocol cannot be determined yet, although it will involve repetitive stimulations using systemic [redacted] with a [redacted] [redacted] with a maximum of 14 days. Stimulation sessions may be performed every day or every other day, and will consists of a [redacted] with the [redacted] [redacted]
- The same MRI protocols will be performed as in sub-phase 2.1 and 2.2, at the same time points.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

To minimize the number of animals needed in these studies, we have included the preparation phase (phase 0) as animal appendix 1. In these preparation sub-phases, training of personnel to perform photothrombotic and tMCAO stroke induction, optimization of existing MRI protocols, setup of [redacted] manipulation [redacted] optimization of non-invasive brain stimulation therapies with TMS and tDCS and optimization of behavioral tests will be done within the same animals where possible, without unnecessary discomfort. In addition, training for the induction of both photothrombotic and tMCAO stroke model leads to refinement of the procedure, and will reduce animal loss.

Power calculations (Performed at www.statstodo.com):
For the power analyses of the treatment study, we used a power of 85%, to increase the chances to find a true treatment effect. Recent research has demonstrated that to increase the translation of preclinical work to the clinic, statistical power of experimental studies needs to be increased, to reduce false positives/negatives and inflated/underestimated effect sizes (Dirnagl 2016). Because of a different number of groups per analyses, power analyses with identical values (significance level, power, difference and variance) may result in different samples sizes.

Longitudinal study 2.1
The primary aim of this study is to identify promising TMS stimulation paradigms, starting at two different time points after stroke, to improve functional recovery after stroke and to investigate underlying mechanisms of brain-plasticity. We will investigate these different paradigms within the different stroke models separately. Therefore, we will perform an ANOVA-test for the difference between more than two independent means (5 groups per stroke model, for 2 different time points after stroke). We will perform this ANOVA-test per stroke model, resulting in in total 20 groups.

The effect of a maximum of three different TMS paradigms on functional recovery after stroke, compared to sham stimulation, started at two different time points after stroke. Since there is not much literature about the efficacy of TMS on functional recovery after stroke in laboratory animals, we determined the effect size for the power calculation based on the effects of tDCS stimulation (Yoon et al. 2012). In this study during the subchronic stroke phase, tDCS-treated animals showed an improvement of 31% in their sensorimotor functions compared to sham-treated animals. In our laboratory, sensorimotor functions are tested with i.e. the sensorimotor performance score (SPS) (van Meer et al. 2012). The standard deviation of these behavioural tests in animals with a lesion in the sensorimotor cortex after stroke is around 29%. Therefore, using a significance level of 5% and a power of 85%, in 5 groups, with the largest difference to be detected = 0.31 and within group standard deviation = 0.29, this results in a sample size of 11 rats per group.

Longitudinal study 2.2
The primary aim of this study is to identify promising tDCS stimulation paradigms, starting at two different time points after stroke, to improve functional recovery after stroke and to investigate underlying mechanisms of brain-plasticity. We will investigate these different paradigms within the different stroke models separately. Therefore, we will perform an ANOVA-test for the difference between more than two independent means (4 groups per stroke model, with two different time points after stroke). We will perform this ANOVA-test per stroke model, resulting in in total 16 groups.

The effect of a maximum of three different tDCS paradigms on functional recovery after stroke, compared to sham stimulation, started at two different time points after stroke. The effect size we used for the power calculations is based on literature (Yoon et al. 2012). In this study during the subchronic stroke phase, tDCS-treated animals showed an improvement of 31% in their sensorimotor functions compared to sham-treated animals. In our laboratory, sensorimotor functions are tested with i.e. the sensorimotor performance score (SPS) (van Meer et al. 2012). The standard deviation of these behavioural tests in animals with a lesion in the sensorimotor cortex after stroke is around 29%. Therefore, using a significance level of 5% and a power of 85%, in 4 groups, with a largest difference to be detected = 0.31 and within group standard deviation = 0.29, this results in a sample size of 12 rats per group.

Longitudinal study 2.3
The primary aim of this study is to identify promising [redacted] manipulation paradigms, starting at two different time points after stroke, to improve functional recovery after stroke and to investigate underlying mechanisms of brain-plasticity. We will perform an ANOVA-test for the different between more than two independent means (4 groups per stroke model, with 2 different time points after stroke). We will perform this ANOVA-test per stroke model, resulting in in total 16 groups.

The effect of a maximum of three different [redacted] manipulation paradigms on functional recovery

after stroke, compared to sham stimulation, started at two different time points after stroke. Since there is not much literature about the efficacy of [redacted] manipulation on functional recovery after stroke in laboratory animals, we determined the effect size for the power calculation based on the effects of tDCS stimulation (Yoon et al. 2012). In this study during the subchronic stroke phase, tDCS-treated animals showed an improvement of 31% in their sensorimotor functions compared to sham-treated animals. In our laboratory, sensorimotor functions are tested with i.e. the sensorimotor performance score (SPS)(van Meer et al. 2012). The standard deviation of these behavioural tests in animals with a lesion in the sensorimotor cortex after stroke is around 29%. Therefore, using a significance level of 5% and a power of 85%, in 4 groups, with a largest difference to be detected = 0.31 and within group standard deviation = 0.29, this results in a sample size of 12 rats per group.

Since there is no available literature about the effects of the different stimulation techniques in female rats, we will use the power calculations of the male rats to determine female sample sizes.

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Yoon KJ, Oh B, Kim D (2012) Functional improvement and neuroplastic effects of anodal transcranial direct current stimulation (tDCS) delivered 1 day vs . 1 week after cerebral ischemia in rats. Brain Res 1452:61–72. doi: 10.1016/j.brainres.2012.02.062

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Adult male and female Sprague Dawley (Sprague Dawley/CRL:CD(SD) rats from Charles River will be used in this part of the study. The rat's cerebral anatomy and blood supply are well described and our lab has extensive experience in using these animals in stroke models.

Emerging data have suggested that stroke incidence, mortality and severity may be sex dependent (Ahnstedt et al. 2016). For example, fluctuating levels of estrogen have been linked to the extend of brain damage after experimental stroke (Carswell et al. 2000), and young female animals demonstrate less ischemic damage than young males (Manwani et al. 2013). Biological sex may also influence how patients/animals respond to stroke treatments, since the mostly applied treatment for stroke (thrombolysis) has been suggested to be more effective in women than men (Kent et al. 2005).

Most of the experimental work has been done in male animals, despite the higher clinical burden of female stroke patients. To increase translational possibilities, and because of the above mentioned sex differences, we willl include both males and females but perform separate analyses for both sexes.

Maximum number of rats: 1436.

Longitudinal study 2.1: TMS: maximum of 540 rats (270 male, 270 female).

Groups and maximum group sized for the photothrombotic stroke model:

1) Sub-acute brain stimulation:
    a. Standard environment, Stimulation paradigm 1 (n=11)
    b. Standard environment, Stimulation paradigm 2 (n=11)
    c. Enriched environment, Stimulation paradigm 1 (n=11)
    d. Enriched environment, Stimulation paradigm 2 (n=11)
    e. Sham stimulation (n=11)

2) Sub-chronic brain stimulation:
    a. Standard environment, Stimulation paradigm 1 (n=11)
    b. Standard environment, Stimulation paradigm 2 (n=11)
    c. Enriched environment, Stimulation paradigm 1 (n=11)
    d. Enriched environment, Stimulation paradigm 2 (n=11)
    e. Sham stimulation (n=11)

We do not expect any loss of animals due to the photothrombotic stroke based on our own experience and literature showing the low mortality in this stroke model (Fluri et al. 2015).
This results in a total of 10 groups with 11 rats per group = male 110 rats.

Groups and maximum group sized for the tMCAO stroke model:

1) Sub-acute brain stimulation:
    a. Standard environment, Stimulation paradigm 1 (n=11)
    b. Standard environment, Stimulation paradigm 2 (n=11)
    c. Enriched environment, Stimulation paradigm 1 (n=11)
    d. Enriched environment, Stimulation paradigm 2 (n=11)
    e. Sham stimulation (n=11)
2) Sub-chronic brain stimulation:
    a. Standard environment, Stimulation paradigm 1 (n=11)
    b. Standard environment, Stimulation paradigm 2 (n=11)
    c. Enriched environment, Stimulation paradigm 1 (n=11)
    d. Enriched environment, Stimulation paradigm 2 (n=11)
    e. Sham stimulation (n=11)

In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss due to tMCAO = 16 rats per group.
This results in a total of 10 groups with 16 rats per group = 160 male rats.

This results in a total number of 110 + 160 = 270 male rats
We need the same number of female rats.

Longitudinal study 2.2: tDCS: maximum of 448 rats (224 male, 224 female).
Groups and maximum group sized for the photothrombotic stroke model:

1) Sub-acute brain stimulation:
    a. Stimulation paradigm 1 (n=12)
    b. Stimulation paradigm 2 (n=12)
    c. Stimulation paradigm 3 (n=12)
    d. Sham stimulation (n=12)
2) Sub-chronic brain stimulation:
    a. Stimulation paradigm 1 (n=12)
    b. Stimulation paradigm 2 (n=12)
    c. Stimulation paradigm 3 (n=12)
    d. Sham stimulation (n=12)

We do not expect any loss of animals due to the photothrombotic stroke based on our own experience and literature showing the low mortality in this stroke model (Fluri et al. 2015).
This results in a total of 8 groups with 12 rats per group = 96 rats.

Groups and maximum group sized for tMCAO stroke model:

1) Sub-acute brain stimulation:
    a. Stimulation paradigm 1 (n=12)
    b. Stimulation paradigm 2 (n=12)
    c. Stimulation paradigm 3 (n=12)
    d. Sham stimulation (n=12)
2) Sub-chronic brain stimulation:
    a. Stimulation paradigm 1 (n=12)
    b. Stimulation paradigm 2 (n=12)
    c. Stimulation paradigm 3 (n=12)
    d. Sham stimulation (n=12)

In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss due to tMCAO = 16 rats per group.
This results in a total of 8 groups with 16 rats per group = 128 rats.

This results in a total number of 96 + 128 = 224 male rats
We need the same number of female rats.

Longitudinal study 2.3: [redacted] manipulation: maximum of 448 rats (224 male, 224 female).

Groups and maximum group sized for the photothrombotic stroke model:

1) Sub-acute brain stimulation:
    a. Stimulation paradigm 1 (n=12)
    b. Stimulation paradigm 2 (n=12)
    c. Stimulation paradigm 3 (n=12)
    d. Sham stimulation (n=12)
2) Sub-chronic brain stimulation:
    a. Stimulation paradigm 1 (n=12)
    b. Stimulation paradigm 2 (n=12)
    c. Stimulation paradigm 3 (n=12)
    d. Sham stimulation (n=12)

We do not expect any loss of animals due to the photothrombotic stroke based on our own experience and literature showing the low mortality in this stroke model (Fluri et al. 2015).
This results in a total of 8 groups with 12 rats per group = 96 rats.

Groups and maximum group sized for the tMCAO stroke model:

1) Sub-acute brain stimulation:
    a. Stimulation paradigm 1 (n=12)
    b. Stimulation paradigm 2 (n=12)
    c. Stimulation paradigm 3 (n=12)
    d. Sham stimulation (n=12)
2) Sub-chronic brain stimulation:
    a. Stimulation paradigm 1 (n=12)
    b. Stimulation paradigm 2 (n=12)
    c. Stimulation paradigm 3 (n=12)
    d. Sham stimulation (n=12)

In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss due to tMCAO = 16 rats per group.
This results in a total of 8 groups with 16 rats per group = 128 rats.

This results in a total number of 96 + 128 = 224 male rats
We need the same number of female rats.

References

Ahnstedt H, Mccullough LD, Cipolla MJ (2016) The Importance of Considering Sex Differences in Translational Stroke Research. Transl Stroke Res 7:261–273. doi: 10.1007/s12975-016-0450-1

Carswell HVO, Dominiczak AF, Macrae IM (2000) Estrogen status affects sensitivity to focal cerebral ischemia in stroke-prone spontaneously hypertensive rats. Am J Physiol Hear Circ Physiol 278:290–294.

Kent DM, Price LL, Ringleb P, et al (2005) Sex-Based Differences in Response to Recombinant Tissue Plasminogen Activator in Acute Ischemic Stroke A Pooled Analysis of Randomized Clinical Trials. Stroke 36:62–65. doi: 10.1161/01.STR.0000150515.15576.29

Liu F, Yuan R, Benashski SE, McCullough LD (2009) Changes in experimental stroke outcome across the lifespan. J Cereb Blood Flow Metab 29:792–802. doi: 10.1038/jcbfm.2009.5

Manwani B, Liu F, Scranton V, et al (2013) Differential effects of aging and sex on stroke induced inflammation across

the lifespan. Exp Neurol 249:1-25. doi: 10.1016/j.expneurol.2013.08.011

C. Re-use

Will the animals be re-used?

X No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☐ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:
Animals are required to study the effects of brain stimulation therapies in the post-stroke recovering brain in vivo, as the complex interaction between different cells, growth factors, structural and functional connectivity etc. of the brain can never be mimicked completely in vitro. In addition, an intact animal is needed to study the stimulation effects on behavioral and clinical relevant parameters.

Reduction:
The three longitudinal studies in this appendix are designed to only be started when the corresponding preparation phases will be established. During these preparation phases, different brain stimulation paradigms will be tested, to detect the most promising ones that will be used in the currently described longitudinal studies. This leads to a reduction in the number of stimulation paradigms, and hereby a reduction in the amount of animals used in these longitudinal experiments.

In addition, during the preparation phase, training of personnel in the induction of stroke using the photothrombotic model and the tMCAO model, to reduce animal loss during the longitudinal studies.

MRI is the main imaging method of choice because it allows verification of our results from animal studies to clinical outcomes. In our laboratory, we have a long history of neuro-MRI in rodents, leading to a reduction in the number of animals used. Furthermore, longitudinal assessment of animals using MRI, required less animals than cross-sectional studies.

Refinement:
All surgeries and imaging experiments, and stimulation therapies when necessary, will be performed under anesthesia. During these procedures, animal physiology (temperature, heart rate, respiration rate, oxygenation and temperature) will be continuously monitored and kept within physiological range. In case physiological parameters during surgery or imaging indicate that the animals are experiencing unexpected discomfort, animals will be euthanized.

Where possible, animals will be housed socially, with at least two animals per cage. Only in exceptional circumstances animals will be housed individually, i.e. when a cage mate dies during an experiment, or when animals have an epicranial device (tDCS setup) fixed to the skull and a suitable cap cannot be designed to protect the structure from gnawing damage. If animals were to be housed individually, it would be for a maximum of 5 weeks.

Moreover, in case animals reach the humane endpoints described in this proposal, animals will be euthanized.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects

on the environment.

All animals will have the appropriate anaesthesia and analgesics (pre-operatively and post-operatively) in case of brain stimulation, stroke induction and imaging. All animals will be carefully monitored for any adverse effects. During brain stimulation and MRI, animals will be under anaesthesia, and the physiology parameters of the animal (temperature, heart rate, respiration rate) will be continuously monitored and kept within physiological range. Furthermore, animals will be supplemented with subcutaneous saline before each imaging session, in order to prevent dehydration during imaging.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

X No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

X Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Animals will be monitored closely after surgical procedures While the animal is deeply anesthetized, lidocaine will be applied on the skin before an incision is made for the stereotactic injection surgery.

Lidocaine will also be applied on the skin before induction of stroke and at suture wounds upon closure. Analgesia will be administered 2x 24h after stereotactic injection and stroke induction surgery.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Due to the photothrombotic and tMCAO stroke procedures, animals may expose unusual behavior such as a failure to groom, a hunched posture, and/or inappetence. Moreover, animals may experience partial paralysis, explaining behavioral deficits and reduced ability to eat. Animals are expected to lose weight, up to 10% of pre-stroke body weight within 7 days after stroke, based on previous experiments of post-stroke rats compared to sham-controlled animals. Beyond the time window of 7 days post stroke, animals are expected to gain weight.

Explain why these effects may emerge.

The expected effects are a direct consequence of stroke surgery.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Liquid food and/or food pellets will be placed inside the cage. Furthermore, animal cages will be placed on a heating matrass. In case of exacerbated weight loss (more than 10%) Ringer Lactate will be subcutaneously injected.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

In case stroke surgery takes longer than 3 hours, or if one of the blood vessels at the surgical site ruptures, the animal will directly be killed.

In the event that any animal shows a progressive weight loss of over 20%, compared to pre-stroke weights, the animal will be killed.

In addition we will use an animal motility score to idenfity the humane endpoint for animals suffering from stroke.
The following motility scores will be assessed in their home cage:
(0) Normal exploratory behavior;
(1) Slightly reduced exploratory behavior;
(2) Moving limbs without proceeding;
(3) Moving only to stimuli;
(4) Unresponsive to stimuli, with normal muscle tone;
(5) Severely decreased tone, premortal signs.

Animals with a motility score of 5 will be killed. Animals with a motility score of 4 will be observed 2 times daily. In case no progression is observed within 2 days, the animal will be killed.

Indicate the likely incidence.

In post-stroke studies, this is a rare occurrence in fewer than 5% of the entire study population.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Subphase 2.1

	Stroke	Discomfort	Estimated % of animals
Repetitive behaviour + 5x In vivo MRI + Repetitive TMS stimulation	Yes	Severe	100

(which may include anesthesia)			

Subphase 2.2			
	Stroke	**Discomfort**	**Estimated % of animals**
Repetitive behaviour + 5x In vivo MRI + Repetitive tDCS stimulation (which may include anesthesia)	Yes	Severe	100

Subphase 2.3			
	Stroke	**Discomfort**	**Estimated % of animals**
Repetitive behaviour + 5x In vivo MRI + Repetitive [redacted] manipulation (without anesthesia)	Yes	Severe	100

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Euthanasia of animals after the final serial MRI is necessary to collect brains for histology.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

[redacted]

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1

General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	11500	
1.2	Provide the name of the licenced establishment.	UMCU	
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number	Type of animal procedure
		4	Phase 3: Working mechanisms of brain stimulation and multiparametric MRI in healthy rats and rats with experimental stroke.

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

In phase 3, the working mechanisms of rTMS, tDCS and [redacted] manipulation protocols will be investigated in stroke animals. The working mechanisms of the respective stimulation techniques/protocols will be evaluated by using advanced MR protocols to measure brain activity [redacted], brain [redacted] and functional activity/connectivity using optical [redacted] imaging [redacted].

The primary aim of this phase is to elucidate the underlying working mechanisms of plasticity-enhancing brain stimulation protocols that effectively promote functional recovery after experimental stroke. We will investigate this:

- For the most effective brain stimulation paradigms and protocols (rTMS, tDCS and [redacted] stimulation).
- In two different stroke models: the photothrombotic stroke model and transient MCAO stroke model.

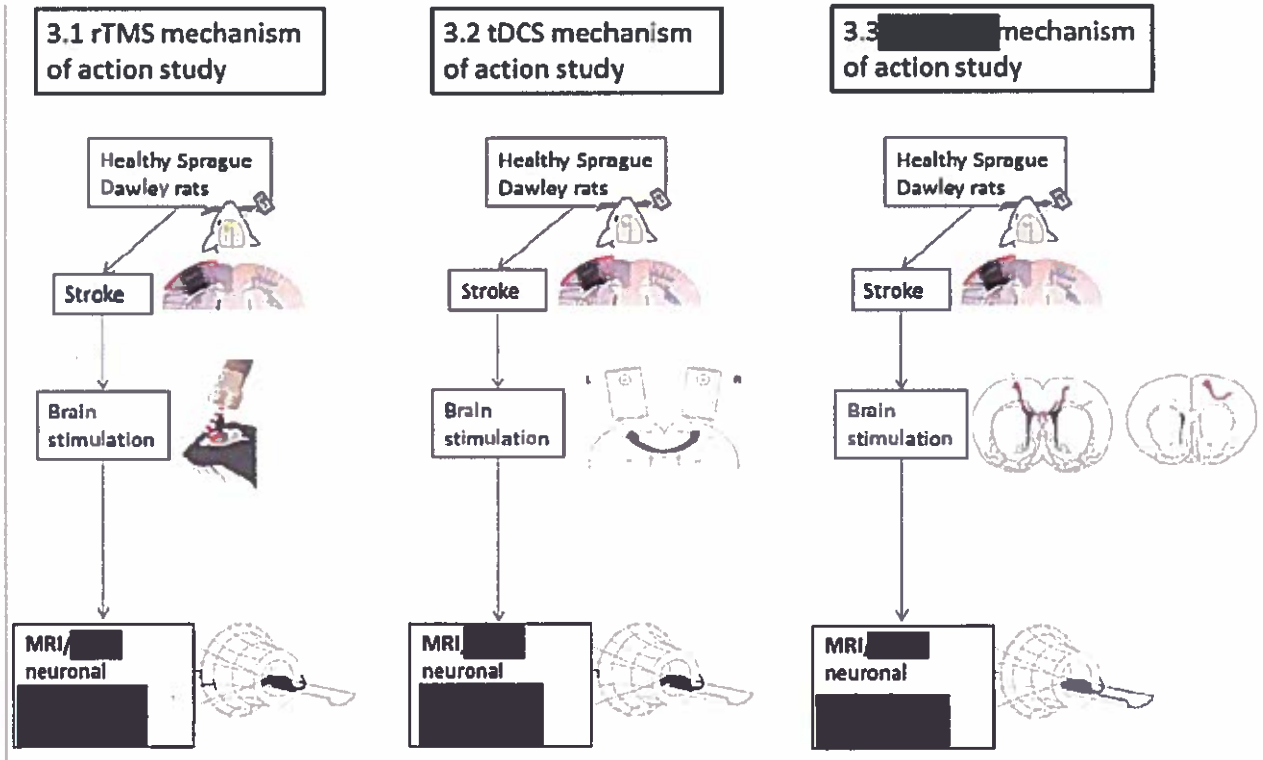


Figure 1: Phase 3 will be subdivided into three sub-phases, corresponding to the experiments that we would like to perform:

- In phase 3.1 we would investigate the working mechanisms of promising rTMS protocols in two models of experimental stroke.
- In phase 3.2 we would investigate the working mechanisms of promising tDCS protocols in two models of experimental stroke.
- In phase 3.3 we would investigate the working mechanisms of promising [redacted] manipulation protocols in two models of experimental stroke

Phase 3 will be subdivided into three sub-phases, each corresponding to the most optimal stimulation protocol/s, i.e. 3.1 using rTMS, 3.2 using tDCS and 3.3 using [redacted] manipulation [redacted] Firstly, we will look into the mechanisms of action of rTMS protocols, because of the three stimulation techniques, rTMS has shown great therapeutic potential. As for tDCS and [redacted] manipulation, we will look into the most optimal treatment protocols, as established in Appendix 3.

The primary outcome parameters for phase 3.1, 3.2 and 3.3 (Fig. 1) are MRI-based measures of structural and functional connectivity, neuronal activation, brain [redacted] and infarct size. These parameters will be used to investigate underlying mechanisms of the most optimal brain stimulation techniques and protocols. In addition, it allows monitoring of disease progression and stroke outcome by measuring infarct size. With these parameters we aim to identify MRI-based markers that can predict functional recovery after stroke.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Sub-phases 3.1-3.3 will only start when corresponding sub-phases in phase 2 have been established:

- Phase 3.1 (rTMS): phase 2.1
- Phase 3.2 (tDCS): phase 2.2
- Phase 3.3 ([redacted] manipulation [redacted] phase 2.3

Animal models of stroke

Photothrombotic stroke

Photothrombotic stroke will be induced in naïve, male, Sprague Dawley rats. In this model, a cortical infarct is induced by the systemic injection of a photosensitive dye (Rose-Bengal) in combination with the focal illumination of the skull (Watson et al. 1985). The illumination leads to the local activation of Rose-Bengal, which results in the disturbance of endothelial function and local thrombosis in small cortical vessels. The advantages of this model are the relatively small and reproducible infarct size, the ability to place the infarct within the desired sensorimotor subdivisions of the cortex, the minimal surgical manipulation of the animal and high survival rate (Fluri et al. 2015).

Transient middle cerebral artery occlusion (tMCAO) stroke

To increase translation of promising brain stimulation techniques to the clinic, we will also include another stroke model resulting in a predominantly subcortical stroke, which produces a pattern of damage (i.e. striatum and cortex) similar to humans (Corbett et al. 2017). Stroke will be induced in naïve Sprague Dawley rats, by the transient occlusion of the middle cerebral artery (tMCAO model) with an intraluminal filament (Longa et al. 1989). This second model is chosen based on similarities in pathology with stroke patients (caused by the focal occlusion of a large cerebral artery), high reproducibility and low invasiveness (Fluri et al. 2015; Kumar and Gupta 2016). However, there is a relatively large (12%) rate of subarachnoid hemorrhage which can reduce the blood flow bilaterally, and there could be potential difficulties in eating and weight loss.

The stroke induction in both models may take 3 hours at maximum. If the procedure takes longer, rats will be euthanized directly following surgery. In case the surgery is considered successful, and performed within 3 hours, rats will be allowed to recover and will be subjected to the rest of the study.

Brain stimulation techniques

Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is a noninvasive brain stimulation technique, which involves a magnetic coil inducing currents in the brain. While animals are exposed to TMS, they will be anesthetized to receive target specific stimulation. Anesthesia will either be administered via inhalation or infusion. Electromyography in combination with TMS will be used to determine the required dose of stimulation for each animal. By inserting needle electrodes into the limbs of the animal we can measure muscle responses induced by the stimulation, as a measure of cortical activity.

Rats will be subjected to TMS according to promising stimulation protocols as determined in phase 2.1, which may involve daily stimulation sessions. The stimulation sessions may take 2.5 hours at maximum per day, for a total of maximum 14 days.

Transcranial direct current stimulation

Transcranial direct current stimulation (tDCS) is a noninvasive brain stimulation technique, which requires placed electrodes on the scalp of the animal. Animals will be anesthetized and fixed in a stereotact for the placement of the electrode cannula/s. A vertical incision will be made on the skull to expose the cranium. The periosteum will be removed, to ensure that the skull is completely dry for the proper fixation of the cannula/s. The cannula/s will be fixed on the skull using dental cement and screws. After the cement has dried, the skin will be sutured around the fixed cannula/s. This procedure will take a maximum of 2 hours.

For stimulation purposes, rats will be subjected to tDCS based on promising stimulation protocols as determined in phase 2.2, which may involve daily stimulation sessions and immobilization of the animal by anesthetics when needed. The stimulation sessions may take 2.5 hours at maximum per day, for a total of maximum 14 days.

[redacted] *manipulation* [redacted]

[redacted] manipulation [redacted] is a more invasive brain stimulation technique. [redacted] neuronal pathways will be targeted in healthy rats by a [redacted] approach consisting of (1) [redacted] of an [redacted] carrying an [redacted] into a [redacted] area in

the [redacted] network, and (2) [redacted] of a [redacted] into the receiving area of the neurons targeted by (1). This will result in the [redacted] in only those neurons that [redacted] from the [redacted] area to the receiving area in the [redacted] network. This [redacted] may take 4 weeks at maximum. After the stereotactic [redacted] rats are allowed to recover, and further experimental procedures will be performed after at least two weeks, to make sure the [redacted] is [redacted] Optimal timing of the stereotactic [redacted] before other experimental procedures will be established in phase 0.3.

For stimulation purposes, rats will be subjected to [redacted] manipulation according to promising stimulation protocols as determined in phase 1 and 2.3, which involves [redacted] of the [redacted] These simulation sessions may be repeated every day or every-other day, depending on phase 1, for a total of maximum 14 days.

MR protocols

The MRI protocols will allow 1) quantitative assessment of structural connectivity; 2) quantitative assessment of functional connectivity; 3) quantitative assessment of stimulation-induced brain activity or [redacted] changes and 4) quantitative assessment of infarct size within one single imaging session of 4 hours at maximum. MRI will be performed four times per animal. Animals will be under infusion or inhalation anesthesia during MRI and may therefore require an intravenous catheter in the tail vein. In addition, MRI protocols may require the administration of contrast agents/[redacted] via an intravenous catheter. Animals will be killed directly after MRI at the last time-point.

Optical imaging [redacted] protocol

Optical imaging will allow for the quantitative assessment of acute stimulation-induced brain activity. The imaging procedure will take 1.5 hours at maximum. Animals will be under infusion or inhalation anesthesia during imaging and may therefore require an intravenous catheter in the tail vein. Animals will be euthanized after the last imaging time-point.

References

Corbett D, Carmichael ST, Murphy TH, et al (2017) Enhancing the alignment of the preclinical and clinical stroke recovery research pipeline: Consensus-based core recommendations from the Stroke Recovery and Rehabilitation Roundtable translational working group. Int J Stroke 12:462–471. doi: 10.1177/1747493017711814

Fluri F, Schuhmann MK, Kleinschnitz C (2015) Animal models of ischemic stroke and their application in clinical research. Drug Des Devel Ther 9:3445–3454. doi: 10.2147/DDDT.S56071

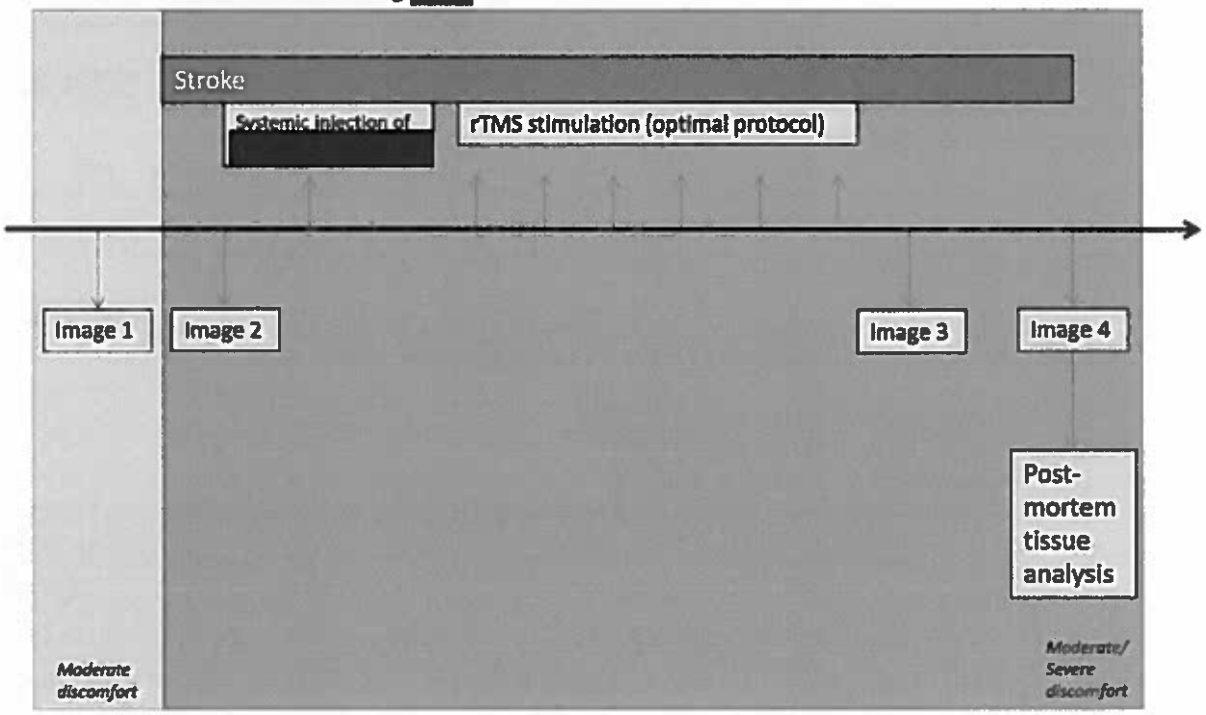
Kumar A, Gupta V (2016) A review on animal models of stroke: An update. Brain Res Bull 122:35–44. doi: 10.1016/j.brainresbull.2016.02.016

Longa EZ, Weinstein PR, Carlson S, Cummins R (1989) Reversible Middle Cerebral Artery Occlusion Without Craniectomy in Rats. Stroke 20:84–91.

Watson BD, Dietrich WD, Busto R, et al (1985) Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann Neurol 17:497–504. doi: 10.1002/ana.410170513

Sub-phase 3.1: Longitudinal study to investigate the mechanism of action of the most optimal rTMS paradigm/s to improve functional recovery after experimental stroke.

3.1.1 Mechanism of action using [redacted]
3.1.2 Mechanism of action using [redacted]
3.1.3 Mechanism of action using [redacted]

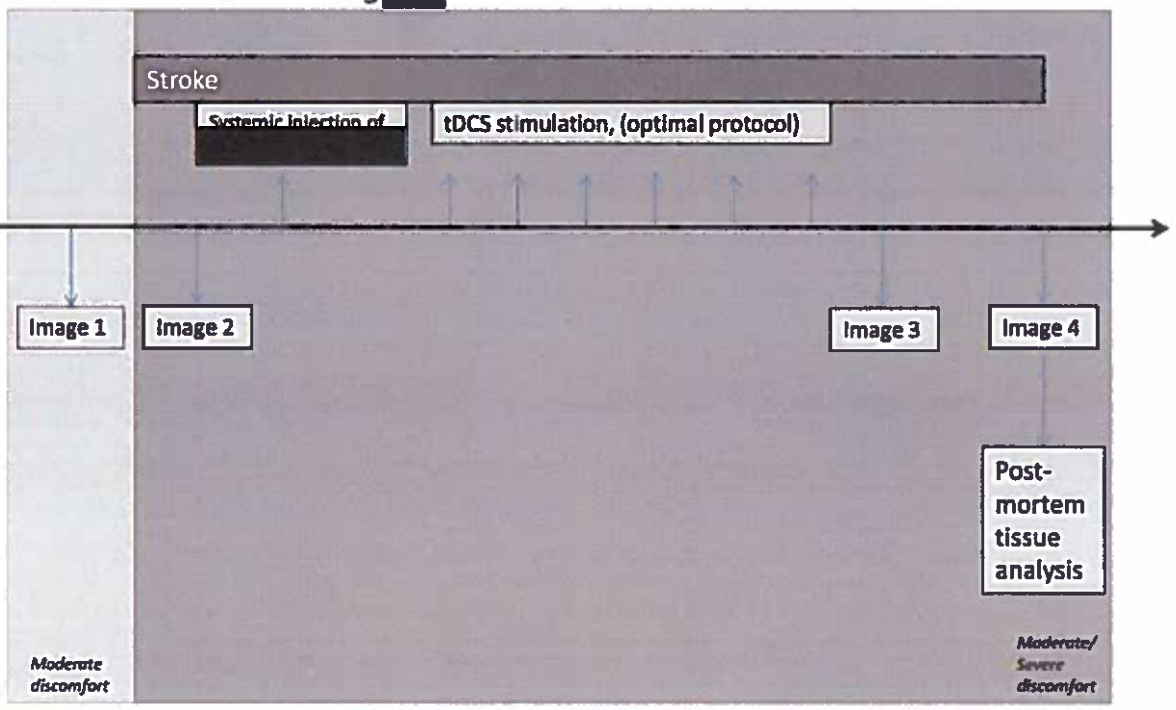


- We will use the most optimal brain stimulation paradigm from literature or determined with our current study (with a maximum of 2 different paradigms, of which one is sham stimulation).
- We will start stimulation at the most optimal time-point (1 time-point) after stroke, as determined in phase 2.
- We will use 3 advanced imaging protocols to measure brain activity ([redacted] 3.1.1), brain [redacted] ([redacted] 3.1.2) and functional activity/connectivity using optical [redacted] imaging ([redacted]: 3.1.3).
- We will investigate the working mechanisms of rTMS in two different rat models of experimental stroke: the photothrombotic and tMCAO stroke model.
- We will perform the study in both males and females.
- This makes in total: 2x1x3x2 = 12 groups per stroke model (total of 24 groups).
- Stroke will be induced as the photothrombotic stroke model (cortical infarct) or by transient occlusion of the middle cerebral artery (tMCAO; predominantly subcortical infarct).
- The rTMS paradigm will be dependent on phase 0.1 and 2.1. Therefore, the amount, length and duration of the stimulation protocol cannot be determined yet, but it will involve repetitive stimulation sessions which may involve repetitive anesthesia when needed, with a maximum of 14 days. The stimulation sessions will have a maximum duration of two hours.
- Serial MRI will be performed 4 times per animal to elucidate the plasticity mechanisms of action of repetitive TMS after experimental stroke:
    - Time 1: Before stroke.
    - Time 2: After stroke but before stimulation (dependent on the stimulation time-points after stroke: sub-acute or sub-chronic).
    - Time 3: Directly after stimulation protocol.
    - Time 4: 1 month after stroke, namely 1 week after stimulation has stopped.
- The *in vivo* imaging experiments will be performed for:
    - **3.1.1 Mechanism of action using [redacted]**
        - 1) quantitative assessment of structural and/or functional connectivity; 2)

quantitative assessment of neuronal activation [redacted], and 3) high resolution imaging of edema to identify ischemic lesions. In vivo MRI will take 2.5 hours at maximum.
    - To measure neuronal activation [redacted] animals are exposed to repetitive [redacted]
  - **3.1.2 Mechanism of action using [redacted]**
    - 1) quantitative assessment of structural and/or functional connectivity; 2) quantitative assessment of brain [redacted] and 3) high resolution imaging of edema to identify ischemic lesions. In vivo MRI will take 2.5 hours at maximum.
    - To measure the brain [redacted] animals receive a [redacted] either before, or during MR acquisition.
  - **3.1.3 Mechanism of action using [redacted]**
    - 1) quantitative assessment of functional connectivity and 2) quantitative assessment of cortical activity. The imaging procedure will take 1.5 hours at maximum.
- Directly after the last imaging session (session 4), rats will be killed and brains will be excised for histology.

Sub-phase 3.2: Longitudinal study to investigate the mechanism of action of the most optimal tDCS paradigm/s to improve functional recovery after experimental stroke.

3.2.1 Mechanism of action using [redacted]
3.2.2 Mechanism of action using [redacted]
3.2.3 Mechanism of action using [redacted]

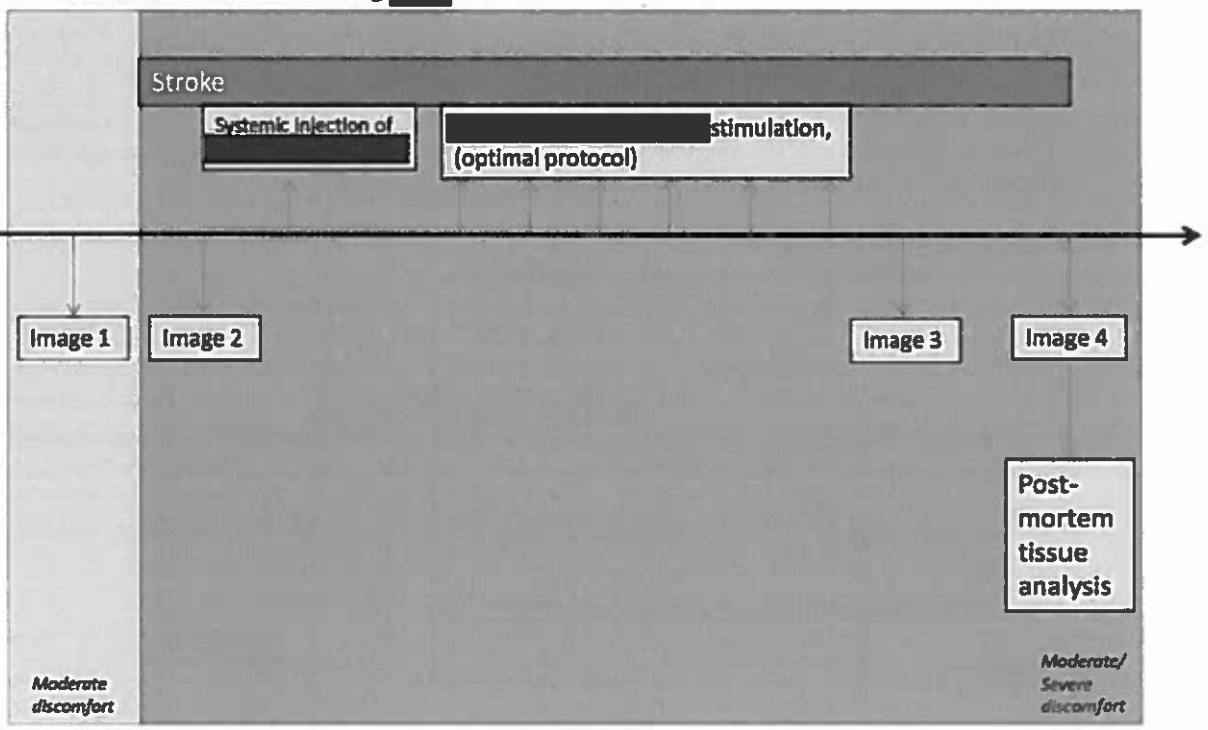


- We will use the most optimal brain stimulation paradigm as determined in phase 2.2 (with a maximum of 2 different paradigms, of which one is sham stimulation).
- We will start stimulation at the most optimal time-point (1 time-point) after stroke, as determined in phase 2.
- We will use 3 advanced imaging protocols to measure brain activity ([redacted]: 3.1.1), brain [redacted] ([redacted] 3.1.2) and functional activity/connectivity using optical [redacted] imaging ([redacted] 3.1.3).
- We will investigate the working mechanisms of rTMS in two different rat models of experimental stroke: the photothrombotic and tMCAO stroke model.

- We will perform the study in both males and females.
- This makes in total: 2x1x3x2 = 12 groups per stroke model (total of 24 groups).
- Stroke will be induced as the photothrombotic stroke model (cortical infarct) or by transient occlusion of the middle cerebral artery (tMCAO; predominantly subcortical infarct).
- Before or after the stroke surgery cannula(s) and electrodes will be placed on the scalp of the animal.
- The tDCS stimulation protocols will be dependent on phase 0.2 and 2.2. Therefore, the amount, length and duration of the stimulation protocol cannot be determined yet. However, it will involve repetitive stimulation sessions which may involve repetitive anesthesia when needed, with a maximum of 14 days. The stimulation sessions will have a maximum duration of two hours.
- The same MRI [redacted] and optical imaging ([redacted] protocols will be performed as in sub-phase 3.1.

Sub-phase 3.3: Longitudinal study to investigate the mechanism of action of the most optimal [redacted] manipulation [redacted] paradigm/s to improve functional recovery after experimental stroke.

3.3.1 Mechanism of action using [redacted]
3.3.2 Mechanism of action using [redacted]
3.3.3 Mechanism of action using [redacted]



- Similar to the study in 3.2, this study will use the same two stroke models (photothrombotic and tMCAO stroke model), but an optimal [redacted] manipulation paradigm (compared to the tDCS stimulation paradigm in 3.2).
- We will use 3 advanced imaging protocols to measure brain activity ([redacted] 3.1.1), brain [redacted] ([redacted] 3.1.2) and functional activity/connectivity using optical [redacted] imaging ([redacted] 3.1.3).
- This study will also be performed in males and females.
- Therefore, this study also consists of: 2x3x2 = 12 groups per stroke model (total of 24 groups).
- Before or after the stroke surgery cannula(s) and electrodes will be placed on the scalp of the animal.
- The [redacted] manipulation protocols will be dependent on phase 0.3, 1 and 2.3. Therefore, the amount, length and duration of the stimulation protocol cannot be determined yet. However, it will involve repetitive stimulation sessions by [redacted] of the [redacted] with a maximum of 14 days.

- The same MRI [redacted] and optical imaging ([redacted]) protocols will be performed as in sub-phase 3.1.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

To minimize the number of animals needed in these studies, we have included the preparation phase (phase 0) as animal appendix 1. In these preparation sub-phases, training of personnel to perform photothrombotic and tMCAO stroke induction, optimization of existing MRI protocols, setup of [redacted] manipulation [redacted] optimization of non-invasive brain stimulation therapies with TMS and tDCS and optimization of behavioral tests will be done within the same animals where possible, without unnecessary discomfort. In addition, training for the induction of both photothrombotic and tMCAO stroke model leads to refinement of the procedure, and will reduce animal loss

Power calculations (Performed at www.statstodo.com):
Longitudinal studies 3.1 – 3.3
The primary aim of these studies is to elucidate the underlying working mechanisms of the most optimal plasticity-enhancing rTMS, tDCS, and [redacted] stimulation [redacted] protocols respectively, that effectively promotes functional recovery after experimental stroke. We will investigate each of the optimal stimulation treatment protocols, compared to sham stimulation, within two stroke models, using three different imaging techniques [redacted] respectively. Therefore, we will perform a t-test for the difference between two independent means (stimulation vs. sham). We will perform this t-test per stroke model and per imaging technique, for each stimulation technique separately. This results in a total of 12 groups per stimulation technique, namely 4 groups (stimulation vs. sham, cortical and subcortical stroke) per imaging modality [redacted]

Longitudinal study 3.1

3.1.1 Mechanism of action using [redacted]

The effect of the optimal rTMS paradigm on brain activity after stroke, compared to sham stimulation, will be elucidated. Since there is limited literature available about the *in vivo* effect of rTMS on brain activity in stroke animal models, we determined the effect size for the power calculation based on the effects of whisker stimulation using [redacted] In this study, the authors show a 35% increase in brain activity (increase in signal intensity) following whisker stimulation, compared to the control condition. The standard deviation of the changes in activity (signal intensity) is around 19%. Therefore, using a significance level of 5% and a power of 80%, in 2 groups, with the smallest difference to be detected = 0.35 and within group standard deviation = 0.19, this results in a sample size of 6 rats per group.

3.1.2 Mechanism of action using [redacted]

Since there is limited information available about the *in vivo* [redacted] effects of rTMS on brain [redacted] in stroke animal models, we determined the effect size for the power calculation based on the effects of tDCS in a traumatic brain injury model using [redacted]. In this study, the authors show a change of 28% in [redacted] after stimulation, compared to the sham condition. The standard deviation of the changes in [redacted] is around 17%. Therefore, using a significance level of 5% and a power of 80%, in 2 groups, with the smallest difference to be detected = 0.28 and within group standard deviation = 0.17, this results in a sample size of 7 rats per group.

3.1.3 Mechanism of action using [redacted]

Since there is limited literature available about the *in vivo* working mechanism of rTMS in stroke animal models, we determined the effect size for the power calculation based on the effects of single pulse TMS stimulation in naïve rats using optical imaging (Murphy et al., 2016). In this study, the authors show and 32% change in brain activity following TMS, compared to the control condition. The standard deviation of the changes in activity (signal intensity) is around 15%. Therefore, using a significance level of 5% and a power of 80%, in 2 groups, with the smallest difference to be detected = 0.32 and within group standard deviation = 0.15, this results in a sample size of 5 rats per group.

Since there is no available literature about the effects of differnt brain stimulation techniques in female stroke rats, we will use the power calculations of the male rats to determine female sample sizes.

Longitudinal study 3.2
The effect sizes and calculated group sizes described for Longitudinal study 3.1 and sub-phases (3.1.1, 3.1.2, 3.1.3) has also been applied for Longitudinal study 3.2 and sub-phases (3.2.1, 3.2.2, 3.2.3).

Longitudinal study 3.3
The effect sizes and calculated group sizes described for Longitudinal study 3.1 and sub-phases (3.1.1, 3.1.2, 3.1.3) has also been applied for Longitudinal study 3.3 and sub-phases (3.3.1, 3.3.2, 3.3.3).

References
Murphy, S.C., Palmer, L.M., Nyffeler, T., Müri, R.M., Larkum, M.E., 2016. Transcranial magnetic stimulation (TMS) inhibits cortical dendrites. Elife 5. doi:10.7554/eLife.13598

[redacted]

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Adult male and female Sprague Dawley (Sprague Dawley/CRL:CD(SD) rats from Charles River will be used in this part of the study. The rat's cerebral anatomy and blood supply are well described and our lab has extensive experience in using these animals in stroke models.

Emerging data have suggested that stroke incidence, mortality and severity may be sex dependent (Ahnstedt et al. 2016). For example, fluctuating levels of estrogen have been linked to the extend of brain damage after experimental stroke (Carswell et al. 2000), and young female animals demonstrate less ischemic damage than young males (Manwani et al. 2013). Biological sex may also influence how patients/animals respond to stroke treatments, since the mostly applied treatment for stroke (thrombolysis) has been suggested to be more effective in women than men (Kent et al. 2005).

Most of the experimental work has been done in male animals, despite the higher clinical burden of female stroke patients. To increase translational possibilities, and because of the above mentioned sex differences, we willl include both males and females but perform separate analyses for both sexes.
Maximum number of rats: 516.

Longitudinal study 3.1: TMS: maximum of 172 rats (86 male, 86 female).

3.1.1 Mechanism of action using [redacted] (56 rats: 28 male, 28 female)

1) Groups and maximum group size for photothrombotic stroke:
    a. Optimal stimulation paradigm 1 (n=6)
    b. Sham stimulation (n=6)

We do not expect any loss of animals due to the photothrombotic stroke based on our own experience and literature showing the low mortality in this stroke model (Fluri et al. 2015).
This results in a total of 2 groups with 8 rats per group = 12 male rats.

2) Groups and maximum group size for tMCAO stroke:
    a. Optimal stimulation paradigm 1 (n=6)
    b. Sham stimulation (n=6)

In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss due to tMCAO = 8 rats per group. This results in a total of 2 groups with 8 rats per group = 16 male rats.

This results in a total of 28 male rats.

The working mechanism of the optimal rTMS protocol and a sham stimulation protocol will also be tested in females.

1) Groups and maximum group size photothrombotic stroke:
   a. Most optimal stimulation protocol (n=6)
   b. Sham stimulation protocol (n=6)
2) Groups and maximum group size for tMCAO stroke:
   a. Most optimal stimulation protocol (n=8)
   b. Sham stimulation protocol (n=8)

This results in a total of 28 female rats.

3.1.2 Mechanism of action using [redacted] (68 rats: 34 male, 34 female)

1) Groups and maximum group size for photothrombotic stroke:
   c. Optimal stimulation paradigm 1 (n=7)
   d. Sham stimulation (n=7)

We do not expect any loss of animals due to the photothrombotic stroke based on our own experience and literature showing the low mortality in this stroke model (Fluri et al. 2015).
This results in a total of 2 groups with 7 rats per group = 14 male rats.

2) Groups and maximum group size for tMCAO stroke:
   c. Optimal stimulation paradigm 1 (n=7)
   d. Sham stimulation (n=7)

In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss due to tMCAO = 10 rats per group. This results in a total of 2 groups with 10 rats per group = 20 male rats.
This results in a total of 34 male rats.

The working mechanism of the optimal rTMS protocol and a sham stimulation protocol will also be tested in females.

1) Groups and maximum group size for photothrombotic stroke:
   c. Most optimal stimulation protocol (n=7)
   d. Sham stimulation protocol (n=7)
2) Groups and maximum group size for tMCAO stroke:
   c. Most optimal stimulation protocol (n=10)
   d. Sham stimulation protocol (n=10)

This results in a total of 34 female rats.

3.1.3 Mechanism of action using optical imaging [redacted] (48 rats: 24 male, 24 female)

1) Groups and maximum group size for photothrombotic stroke:
   a. Optimal stimulation paradigm 1 (n=5)
   b. Sham stimulation (n=5)

We do not expect any loss of animals due to the photothrombotic stroke based on our own experience and literature showing the low mortality in this stroke model (Fluri et al. 2015).
This results in a total of 2 groups with 5 rats per group = 10 male rats.

2) Groups and maximum group size for tMCAO stroke:
   c. Optimal stimulation paradigm 1 (n=5)

d. Sham stimulation (n=5)

In the subcortical stroke (tMCAO) groups, we need to add 25% at maximum to compensate for animal loss due to tMCAO = 7 rats per group. This results in a total of 2 groups with 7 rats per group = 14 male rats.

This results in a total of 24 male rats.

The working mechanism of the optimal rTMS protocol and a sham stimulation protocol will also be tested in females.

1) Groups and maximum group size for photothrombotic stroke:
   a. Most optimal stimulation protocol (n=5)
   b. Sham stimulation protocol (n=5)
2) Groups and maximum group size for tMCAO stroke:
   c. Most optimal stimulation protocol (n=7)
   d. Sham stimulation protocol (n=7)

This results in a total of 24 female rats.

Longitudinal study 3.2: tDCS: maximum of 172 rats (86 male, 86 female).
For the mechanism of action study performed in 3.2 and sub-phases, the exact power calculations were used as for 3.1. Therefore, animal numbers per group are the same.

Longitudinal study 3.3: [redacted] manipulation [redacted] maximum of 172 rats (86 male, 86 female).
For the mechanism of action study performed in 3.3 and sub-phases, the exact power calculations were used as for 3.1. Therefore, animal numbers per group are the same.

References

Ahnstedt H, Mccullough LD, Cipolla MJ (2016) The Importance of Considering Sex Differences in Translational Stroke Research. Transl Stroke Res 7:261–273. doi: 10.1007/s12975-016-0450-1

Carswell HVO, Dominiczak AF, Macrae IM (2000) Estrogen status affects sensitivity to focal cerebral ischemia in stroke-prone spontaneously hypertensive rats. Am J Physiol Hear Circ Physiol 278:290–294.

Kent DM, Price LL, Ringleb P, et al (2005) Sex-Based Differences in Response to Recombinant Tissue Plasminogen Activator in Acute Ischemic Stroke A Pooled Analysis of Randomized Clinical Trials. Stroke 36:62–65. doi: 10.1161/01.STR.0000150515.15576.29

Liu F, Yuan R, Benashski SE, McCullough LD (2009) Changes in experimental stroke outcome across the lifespan. J Cereb Blood Flow Metab 29:792–802. doi: 10.1038/jcbfm.2009.5

Manwani B, Liu F, Scranton V, et al (2013) Differential effects of aging and sex on stroke induced inflammation across the lifespan. Exp Neurol 249:1–25. doi: 10.1016/j.expneurol.2013.08.011

C. Re-use

Will the animals be re-used?

X No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☐ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:
Animals are required to set up and test the mechanisms of action of effective brain stimulation therapies with imaging modalities *in vivo*, as the complex interaction between different cells, growth factors etc. of the brain can never be mimicked completely *in vitro*. In addition, an intact animal is needed to study the stimulation effects on behavioral and clinical relevant parameters.

Reduction:
The three longitudinal studies in this appendix are designed to only be started when the corresponding preparation phases will be established. During these preparation phases different brain stimulation paradigms will be tested, to detect the most promising ones that will be used in the currently described longitudinal studies. This leads to a reduction in the number of stimulation paradigms, and hereby a reduction in the amount of animals used in these longitudinal experiments.

In addition, during the preparation phase, personnel will be trained in the induction of stroke using the photothrombotic model and the tMCAO model, to reduce animal loss during the longitudinal studies.

MRI is the main imaging method of choice because it allows verification of our results from animal studies to clinical outcomes. In our laboratory we have a long history of neuro-MRI in rodents, leading to a reduction in the number of animals used. Furthermore, longitudinal assessment of animals using MRI, requires less animals than cross-sectional studies.

Refinement:
All imaging experiments, surgeries and and stimulation therapies (where necessary) will be performed under anaesthesia. During these procedures, animal physiology (temperature, heart rate, respiration rate, oxygenation and temperature) will be continuously monitored and kept within physiological range.
In case physiological parameters during surgery or imaging indicate that the animals are experiencing unexpected discomfort, animals will be euthanized.

Where possible, animals will be housed socially, with at least two animals per cage. Only in exceptional circumstances animals will be housed individually, i.e. when a cage mate dies during an experiment, or when animals have an epicranial device (tDCS setup) fixed to the skull and a suitable cap cannot be designed to protect the structure from gnawing damage. If animals were to be housed individually, it would be for a maximum of 5 weeks.

Moreover, in case animals reach the humane endpoints described in this proposal, animals will be euthanized.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

All animals will have the appropriate anaesthesia and analgesics (pre-operatively and post-operatively) in case of brain stimulation, stroke induction and imaging. All animals will be carefully monitored for any adverse effects. During brain stimulation, [redacted] and MRI, animals will be under anaesthesia, and animal physiology (temperature, heart rate, respiration rate) will be continuously monitored and kept within physiological range. Furthermore, animals will be supplemented with subcutaneous saline before each imaging session, in order to prevent dehydration during imaging.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

X No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

X Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Animals will be monitored closely after surgical procedures. While the animal is deeply anesthetized, lidocaine will be applied on the skin before an incision is made for the stereotactic injection surgery. Lidocaine will also be applied on the skin before induction of stroke and at suture wounds upon closure. Analgesia will be administered 2x 24h after stereotactic injection and stroke induction surgery.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Due to the photothrombotic and tMCAO procedures, animals may expose unusual behavior such as a failure to groom, a hunched posture, and/or inappetence. Moreover, animals may experience partial paralysis, explaining behavioral deficits and reduced ability to eat. Animals are expected to lose weight, up to 10% of pre-stroke body weight within 7 days after stroke, based on previous experiments of post-stroke rats compared to sham-controlled animals. Beyond the time window of 7 days post stroke, animals are expected to gain weight.

Explain why these effects may emerge.

The expected effects are a direct consequence of stroke surgery.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Liquid food and/or food pellets will be placed inside the cage. Furthermore, animal cages will be placed on a heating mattress. In case of exacerbated weight loss, Ringer Lactate will be subcutaneously administered.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

In case stroke surgery takes longer than 3 hours, or if one of the blood vessels at the surgical site ruptures, the animal will directly be killed.

In the event that any animal shows a progressive weight loss of over 20%, compared to pre-surgery weights, the animal will be killed.

In addition we will use an animal motility score to idenfity the humane endpoint for animals suffering from stroke. The following motility scores will be assessed in their home cage:

(0) Normal exploratory behavior;
(1) Slightly reduced exploratory behavior;
(2) Moving limbs without proceeding;
(3) Moving only to stimuli;
(4) Unresponsive to stimuli, with normal muscle tone;
(5) Severely decreased tone, premortal signs.

Animals with a motility score of 5 will be killed. Animals with a motility score of 4 will be observed 2 times daily. In case no progression is observed within 2 days, the animal will be killed.

Indicate the likely incidence.

In post-stroke studies, this is a rare occurrence in fewer than 5% of the entire study population.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Subphase 3.1			
	Stroke	**Discomfort**	**Estimated % of animals**
4x In vivo MRI/imaging + Repetitive TMS stimulation (which may include anesthesia)	Yes	Severe	100

Subphase 3.2			
	Stroke	**Discomfort**	**Estimated % of animals**
4x In vivo MRI/imaging + Repetitive tDCS stimulation (which may include anesthesia)	Yes	Severe	100

Subphase 3.3			
	Stroke	**Discomfort**	**Estimated % of animals**
4x In vivo MRI/imaging + Repetitive [redacted] manipulation (without anesthesia)	Yes	Severe	100

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Euthanasia of animals after the final experimental procedure is necessary to collect brains for histology.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?
☐ No > Describe the method of killing that will be used and provide justifications for this choice.
X Yes

DEC UTRECHT

A. Algemene gegevens over de procedure

1. Aanvraagnummer : 2017.I.540.020
2. Titel van het project : Mechanisms of action of therapeutic brain stimulation therapies after experimental stroke
3. Titel van de NTS : Werkingsmechanismen van hersenstimulatie therapieën na een experimentele hersenberoerte

4. Type aanvraag:
   - ☒ nieuwe aanvraag projectvergunning
   - ☐ wijziging van vergunning met nummer :

5. Contactgegevens DEC

Naam DEC	: DEC Utrecht
Telefoonnummer contactpersoon	: 088 – 75 59 247
Emailadres contactpersoon	: dec-utrecht@umcutrecht.nl

6. Adviestraject (data dd-mm-jjjj):
   - ☒ ontvangen door DEC: 20-09-2017
   - ☐ aanvraag compleet:
   - ☒ in vergadering besproken: 04-10-2017
   - ☐ anderszins behandeld:
   - ☒ termijnonderbreking(en) van / tot : 11-10-2017/18-10-2017
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met max. 15 werkdagen:
   - ☐ aanpassing aanvraag:
   - ☒ advies aan CCD: 24-10-2017

7. De aanvraag is afgestemd met de IvD en deze is hiermee akkoord.

8. Eventueel horen van aanvrager
   - Datum:
   - Plaats:
   - Aantal aanwezige DEC-leden:
   - Aanwezige (namens) aanvrager:
   - Gestelde vragen en verstrekte antwoorden:
   - Het horen van de aanvrager heeft geleid tot aanpassing van de aanvraag.

9. Correspondentie met de aanvrager
   - Datum vragen: 11-10-2017
   - Datum antwoord: 18-10-12017
   - Gestelde vragen en antwoorden:

Projectvoorstel

- 3.4 Onderzoeksstrategie: In afbeelding 3 staan bij 'functional recovery' testen vermeld die niet worden beschreven in de bijlage. Graag verhelderen.
  De verschillende 'functional recovery' testen zijn toegevoegd aan de tekst in 3.4.. De testen die in afbeelding 3 stonden waren illustratief bedoeld, maar zijn aangepast en gematcht aan de 'functional recovery' testen die nu ook in de tekst genoemd staan.
- 3.4 Onderzoeksstrategie: De DEC vraagt zich het volgende af: In figuur 3 van het projectvoorstel en figuur 1 van bijlage 2, wordt geïllustreerd dat de verbinding tussen beide hemisferen wordt geactiveerd/geïnhibeerd, maar het is de DEC nog niet helder welke groep neuronen wordt getarget en waarom, en hoe dit het gewenste effect kan bewerkstelligen. In de referenties verwijst u naar een studie betreffende het mesocortico limbische systeem, maar vereist het model dat u in deze studies gebruikt niet andere typen [redacted] Graag uw uitleg.
  Met de [redacted] manipulatie zullen verschillende connecties tussen gebieden in het [redacted] netwerk worden getarget. Dit kunnen connecties zijn die een verbinding vormen tussen beide hemisferen, of connecties binnen één hemisfeer, maar het zullen altijd connecties zijn binnen het [redacted] netwerk, getarget op [redacted] Figuur 3 van het projectvoorstel en figuur 1 van bijlage 2 zijn aangepast en geven nu beide mogelijkheden (tussen of binnen hemisferen) weer. Na een hersenberoerte is er sprake van een disbalans tussen de activiteit in de aangedane en niet-aangedane hemisfeer, waarbij de activiteit in de aangedane hemisfeer omlaag gaat en de activiteit in de niet-aangedane hemisfeer omhoog. Het stimuleren (aangedane hemisfeer) of inhiberen (niet-aangedane hemisfeer) van verbindingen binnen één hemisfeer zou mogelijk de disbalans tussen beide hemisferen kunnen herstellen. Bovendien is, door deze disbalans, de invloed van de aangedane naar de niet-aangedane hemisfeer verlaagd, en de invloed van de niet-aangedane naar aangedane hemisfeer verhoogd. Door deze verbindingen tussen beide hemisferen respectievelijk te stimuleren of te inhiberen hopen we de balans tussen beide hemisferen te herstellen, en hierbij ook het herstel na een beroerte te bevorderen. Deze uitleg over welke connecties tussen gebieden in het [redacted] netwerk beïnvloedt kunnen worden is toegevoegd aan het projectvoorstel in de tekst onder 3.4.2 (in groen).
  Er zijn geen andere type [redacted] nodig om connecties in het [redacted] te manipuleren vergeleken met manipulatie van connecties in het mesocortico limbisch systeem. Wat wel kan veranderen is de coat van het [redacted] wat invloed heeft op hoe goed het [redacted] de neuronen in bepaalde gebieden binnen kan dringen. Deze coat kan [redacted] specifieker maken om bepaalde cellen wel of niet binnen te dringen. In de pilot experimenten die beschreven staan in bijlage 1 zal dit onderzocht worden en zal het meeste efficiënte [redacted] geselecteerd worden, dit is nu ook beschreven in bijlage 1.

- De antwoorden hebben geleid tot aanpassing van de aanvraag.

10. Eventuele adviezen door experts (niet lid van de DEC)
    - Aard expertise:
    - Deskundigheid expert:
    - Datum verzoek:
    - Strekking van het verzoek:
    - Datum expert advies:
    - Advies expert:

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet).
2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.
4. Er zijn geen DEC-leden betrokken bij het betreffende project.

C. Beoordeling (inhoud):

1. Hersenbloeding, en een al dan niet transient herseninfarct zijn aandoeningen waarbij de bloedvoorziening van grotere of kleinere hersengebieden acuut verstoord wordt. Ze hebben een hoge sociaal-economische impact omdat ze in veel gevallen leiden tot ernstige invaliditeit en langdurige afhankelijkheid van revalidatieprogramma's.
   Momenteel bestaan er twee effectieve therapieën om na een acuut herseninfarct de bloedvoorziening in de aangedane hersendelen te herstellen: medicamenteuze trombolyse of operatieve verwijdering van het bloedstolsel. Echter, bij 85-95% van de patiënten zijn deze behandelingen niet toepasbaar, omdat ze alleen direct na een infarct (binnen 6-7 uur) effectief zijn. Is een snelle behandeling niet mogelijk, dan ontstaat een niet meer te herstellen hersenschade. Maar ook als de behandeling wel op tijd kan worden toegepast ervaren vele patiënten nog langdurige invaliditeit. Recentelijk hebben *proof-of-principle* studies uitgewezen dat hersenstimulatie significante verbeteringen te weeg kan brengen in zowel gedrag als motorisch functioneren bij herstellende herseninfarctpatiënten. Twee, niet-invasieve technieken om hersenactiviteit te stimuleren, dan wel te remmen en daardoor functioneel herstel te bevorderen zijn resp. *'transcranial magnetic stimulation'* (TMS) en *'transcraniaal direct-current stimulation'* (tDCS). In het project wordt daar een derde techniek aan toegevoegd: een [redacted] behandeling onder de naam [redacted]. Er bestaan echter nog een groot aantal onzekerheden in de beoordeling van deze technieken die nader pre-klinisch proefdieronderzoek vereisen: o.a. wanneer te beginnen met de behandeling na het infarct, wat is het werkingsmechanisme van TMS en tDCS en wat is de potentie van [redacted]. De effecten van de drie behandelingen zullen worden bestudeerd d.m.v. een viertal neuro-imaging technieken: MRI [redacted] en [redacted] (optical [redacted] imaging), met voor iedere techniek een duidelijk omschreven doelstelling. Het project is systematisch verdeeld in een viertal, sterk van elkaar afhankelijke fasen, die gedeeltelijk overlappend en voor een deel opeenvolgend zullen worden uitgevoerd.

De DEC-Utrecht is van mening dat het hier gaat om een toetsbaar, hypothese-gedreven project dat qua inrichting het meest overeenkomt met voorbeeld 1 uit de handreiking Definitie Project.

2. Voor zover de DEC bekend, is er geen mogelijk tegenstrijdige wetgeving die het uitvoeren van de dierexperimenten in de weg zou kunnen staan.

3. De in de aanvraag aangekruiste doelcategorie sluit aan bij de hoofddoelstellingen.

Belangen en waarden

4. Het directe doel van het project is pre-klinisch onderzoek met ratten naar de ontwikkeling en effectiviteit van één nieuwe, en de effectiviteit en werkingsmechanisme van een tweetal al bekende methoden om hersenactiviteit gericht en goed gelokaliseerd te stimuleren, dan wel te remmen na een (geïnduceerd) herseninfarct. Daarmee samenhangend verbetering en doorontwikkeling van neuro-imaging technieken om de effecten van de behandelingen te kunnen bestuderen.
   Het uiteindelijke doel van dit onderzoek is bij patiënten in de herstelfase na een herseninfarct middels gerichte hersenstimulatie verstoorde gedrags- en motorische functies te herstellen. Van de twee reeds bekende behandelmethoden (TMS en tDCS) is de effectiviteit in *'proof-of-principle'* onderzoek aangetoond, maar er zijn nog teveel onzekerheden (bv met betrekking tot aanvang en duur van de behandeling) en gebrek aan kennis van het werkingsmechanisme. Deze inzichten zijn nodig om tot klinische toepassing over te gaan. De effectiviteit en de klinische toepasbaarheid van de nieuwe [redacted]-methode moet nog bewezen worden.
   De DEC is van mening dat er in voldoende mate een relatie is tussen het directe doel en het uiteindelijke doel.

5. De belangrijkste belanghebbenden in dit onderzoeksproject zijn de proefdieren en de patiënten die getroffen zijn door een herseninfarct en de onderzoekers.
   Gerelateerd aan het directe doel van het onderzoek zijn het de proefdieren. Behalve de beperkingen, inherent aan het proefdier-zijn, zal een belangrijk deel van de dieren ernstig ongerief ondervinden van het induceren van het herseninfarct, de gevolgen daarvan, en de ingrepen die nodig zijn voor de herstelbehandelingen. Voor de proefdieren zijn er dus slechts ernstige, negatieve morele waarden in het geding.
   Samenhangend met het uiteindelijke doel van het onderzoek komen positieve morele waarden die bevorderd worden vooral ten goede aan patiënten die getroffen zijn door een herseninfarct. Dit onderzoek kan in de toekomst behandelmethoden opleveren die bijdragen aan het herstel van gedrags- en motorische functies die beschadigd zijn door een herseninfarct en die zonder behandeling tot invaliditeit van de patiënt leiden.
   Ook de onderzoekers kunnen als belanghebbende worden beschouwd. Succesvol onderzoek resulteert in prestige en carrièremogelijkheden. Bovendien opent het mogelijkheden voor fondsverwerving voor nieuw onderzoek. De DEC is echter van mening dat deze overwegingen

geen rol behoren te spelen bij de ethische afweging voor het gebruik van proefdieren afgezet tegen het directe en uiteindelijke doel van het onderzoek.

6. De DEC ziet geen aanleiding om de in de aanvraag beschreven effecten op het milieu in twijfel te trekken.

Proefopzet en haalbaarheid

7. De onderzoeksgroep heeft ruime ervaring met de technieken voor inductie van een herseninfarct bij ratten en het bestuderen van de gevolgen daarvan. Met name op het gebied van neuro-imaging heeft de groep veel expertise en bekendheid. Het gaat hier om MRI voor het zichtbaar maken van neuro-vascularisatie, functionele en structurele neuronale netwerken, [redacted] hersenactiviteit, infarctgrootte en –lokalisatie; [redacted] voor [redacted] niveaus van [redacted] pathways; [redacted] voor neurale activering en [redacted] voor corticale activiteit en functionele verbindingen. De DEC acht kennis en kunde van de onderzoeksgroep en andere betrokkenen m.b.t. de dierproeven, en de technieken om de effecten te screenen ruim voldoende en verwacht dat de doelstellingen behaald kunnen worden, en dat voorkomen kan worden dat mens, dier en milieu onnodige negatieve effecten ondervinden als gevolg van de dierproeven. De lange ervaring met de te gebruiken herseninfarctmodellen en de neuro-imagingtechnieken maken het mogelijk dat de onderzoekers aan de 3V-beginselen kunnen voldoen

8. Het project is goed en gefaseerd opgezet. De eerste fase (fase 0) is met name bedoeld de imagingtechnieken voor de diverse parameters verder te ontwikkelen. Voor zover mogelijk zal dit vooraf aan kadavermateriaal worden uitgevoerd. De verdere voorgestelde experimentele opzet en fasering zijn logisch en helder, de uitkomstparameters sluiten naadloos aan bij de aangegeven doelstellingen en de gekozen strategie. De experimentele aanpak kan leiden tot het behalen van de doelstelling binnen het kader van het project.

Welzijn dieren

9. Er is geen sprake van de volgende bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren:
   - ☐ Bedreigde diersoort(en) (10e lid 4)
   - ☐ Niet-menselijke primaten (10e)
   - ☐ Dieren in/uit het wild (10f)
   - ☐ Niet gefokt voor dierproeven (11, bijlage I EU richtlijn)
   - ☐ Zwerfdieren (10h)
   - ☐ Hergebruik (1e lid 2)
   - ☐ Locatie: buiten instelling vergunninghouder (10g)
   - ☐ Geen toepassing verdoving/pijnbestrijding (13)
   - ☐ Dodingsmethode niet volgens bijlage IV EU richtlijn (13c lid 3)

10. De dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van de EU richtlijn.

11. Het cumulatieve ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Met name het induceren van het herseninfarct en de gevolgen daarvan, en dat geldt voor 82,9% van de dieren, wordt naar de mening van de DEC terecht ingeschat als het toebrengen van ernstig ongerief. Daarnaast treedt matig ongerief op als gevolg van operaties onder narcose (10,7%) en licht ongerief voor die dieren die alleen een gedragsexperiment hebben ondergaan en/of onder narcose worden gedood na een of meer niet-invasieve handelingen vooraf (6,4%).

12. De ernstigste aantasting van de integriteit van de dieren wordt veroorzaakt door het opzettelijk aanbrengen van een herseninfarct. Dat overkomt ruim 80% van de proefdieren. De dieren kunnen daarna voor kortere of langere tijd verlammingsverschijnselen vertonen en kan hun normaal gedrag verstoord zijn. Daarnaast vereist de [redacted]-techniek [redacted] en ondergaan alle dieren een of meerdere malen een narcose. Uiteindelijk worden alle dieren gedood

13. De humane eindpunten zijn in de bijlage dierproeven goed gedefinieerd in een schaal van 1 tot 5. Dieren die maximaal 2 dagen score 4 vertonen en dieren met score 5 worden uit het experiment gehaald. Het percentage dieren dat naar verwachting een humaan eindpunt bereikt (5%) is goed ingeschat.

3V's

14. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn. Het werkingsmechanisme en effectiviteit van hersenstimulatietherapieën kan uitsluitend bestudeerd worden in diermodellen die het mogelijk maken langdurige effecten van hersenstimulatie onder gecontroleerde en reproduceerbare omstandigheden in kaart te brengen. M.n. in fase 0 van het project wordt ook kadavermateriaal gebruikt voor de verdere ontwikkeling van neuro-imaging technieken.

15. Het aantal te gebruiken dieren is realistisch ingeschat en er is een heldere strategie om ervoor te zorgen dat tijdens het project met het kleinst mogelijke aantal dieren wordt gewerkt waarmee nog een betrouwbaar resultaat kan worden verkregen. Gedragstesten, hersenstimulatietherapieën en MRI-methodes worden geoptimaliseerd en getest in dieren die ook gebruikt worden voor training van operaties. Mede hierdoor wordt het aantal dieren zoveel als mogelijk beperkt. Daar waar de variatie in parameters bekend is wordt het aantal te gebruiken dieren vooraf statistisch berekend.

16. Het project is in overeenstemming met de vereiste van verfijning van dierproeven en het project is zodanig opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd. De

onderzoeksgroep heeft ruime ervaring met de infarctmodellen en MRI bij de rat. Het welzijn van de dieren wordt de eerste dagen na het aanbrengen van het infarct intensief gemonitord. De dieren krijgen pijnstilling, de kooien worden verwarmd. Vloeibaar voedsel en standaard voer op de bodem van de kooi wordt aangeboden. Bij ernstig gewichtsverlies (20%) of een welzijnsscore 4 of 5 wordt een dier uit het experiment genomen.

17. Er is geen sprake van wettelijk vereist onderzoek.

Dieren in voorraad gedood en bestemming dieren na afloop proef

18. Dieren van beide geslachten zullen in gelijke mate worden ingezet. Dieren van beide geslachten worden echter gescheiden gescoord op de effecten van de hersenstimulatie om te voorkomen dat de variatie binnen de groepen te groot wordt waardoor meer dieren nodig zouden zijn.

19. De dieren worden in het kader van het project gedood. Post-mortem bestuderen van hersenmateriaal is een intrinsiek onderdeel van het project. De dieren worden volgens een, in bijlage IV van de EU richtlijn, genoemde methode gedood.

20. De vraag over hergebruik is niet van toepassing omdat de dieren gedood worden in het kader van het experiment.

NTS

21. De niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd

D. Ethische afweging

1. De uiteindelijke doelstelling van dit project is methoden voor hersenstimulatie te ontwikkelen en/of te vervolmaken om bij patiënten die een herseninfarct hebben gehad gedrags- en/of motorisch functieverlies te herstellen. De DEC kent aan deze doelstelling een groot belang toe. Twee bestaande methoden hebben hun waarde nog niet voldoende bewezen om stelselmatig in de kliniek toe te passen en hun werkingsmechanisme is nog onbekend. Een derde moet nog ontwikkeld worden en de effectiviteit is niet bekend. Om hierin te voorzien wordt het in dit project beschreven onderzoek voorgesteld.
   De morele vraag die de DEC dient te beantwoorden is of het belang van het uiteindelijke doel, de onvermijdelijke aantasting van het welzijn en de integriteit van de gebruikte proefdieren om het directe doel te bereiken kan rechtvaardigen.

2. Er vindt een aanzienlijke aantasting van welzijn en integriteit van de proefdieren plaats, met ernstig ongerief voor ruim 80% van de proefdieren.
   Indien de hierboven genoemde doelstellingen behaald worden, dan zal dit project er uiteindelijk toe bijdragen dat mogelijk verstoord gedrag en motorische functies van herseninfarctpatiënten hersteld of verbeterd kunnen worden. Het is aannemelijk dat de translationele doelstelling

behaald zal worden. Deze aanname is gebaseerd op de uitgebreide ervaring van de onderzoekers met het rattenherseninfarctmodel, en hun expertise op het gebied van neuro-imagingtechnieken. De inzet van proefdieren acht de DEC noodzakelijk voor het bereiken van de directe doelstelling, en daarmee de weg te openen naar de uiteindelijke doelstelling. De onderzoekers doen al het mogelijke om het ongerief voor de dieren en het aantal dieren tot een minimum te beperken.
Dat het voor de onderzoeker(s) van belang kan zijn om aansprekende onderzoeksresultaten te boeken is juist, maar in de uiteindelijke afweging kent de DEC daar weinig gewicht aan toe.

3. Op grond van het bovenstaande is de DEC van oordeel dat het uiteindelijke doel, nl het herstel van gedrags- en/of motorische functionaliteit van herseninfarctpatiënten een substantieel belang vertegenwoordigt en dat dit substantiële belang opweegt tegen de ernstige aantasting van het welzijn en de integriteit van een groot deel van de proefdieren. Het gebruik van de proefdieren zoals beschreven in de aanvraag acht de DEC-Utrecht daarmee gerechtvaardigd.

E. Advies

1. Advies aan de CCD

☐ De DEC adviseert de vergunning te verlenen.

☒ De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden.
- ☒ Op grond van het wettelijk vereiste dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD.
- ☐ Voor de uitvoering van dit project is tevens ministeriële ontheffing vereist
- ☐ Overige door de DEC aan de uitvoering verbonden voorwaarden, te weten...

☐ De DEC adviseert de vergunning niet te verlenen vanwege:
- ☐ De vaststelling dat het project niet vergunningplichtig is om de volgende redenen:...
- ☐ De volgende doorslaggevende ethische bezwaren:...
- ☐ De volgende tekortkomingen in de aanvraag:...

2. Het uitgebrachte advies is gebaseerd op consensus.

3. Er zijn geen knelpunten/dilemma's naar voren gekomen tijdens het beoordelen van de aanvraag en het opstellen van het advies.

Centrale Commissie Dierproeven

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UMC Utrecht
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3501 AA UTRECHT

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0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD1150020173846
Bijlagen
2

Datum 27 oktober 2017
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 26 oktober 2017. Het gaat om uw project "Mechanisms of action of therapeutic brain stimulation therapies after experimental stroke". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD1150020173846. Gebruik dit nummer wanneer u contact met de CCD opneemt.

Wacht met de uitvoering van uw project
Als wij nog informatie van u nodig hebben dan ontvangt u daarover bericht. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn bijgeschreven op de rekening van de CCD. U ontvangt binnen veertig werkdagen een beslissing op uw aanvraag. Als wij nog informatie van u nodig hebben, wordt deze termijn opgeschort. In geval van een complexe aanvraag kan deze termijn met maximaal vijftien werkdagen verlengd worden. U krijgt bericht als de beslisperiode van uw aanvraag vanwege complexiteit wordt verlengd. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Factuur
Bijgaand treft u de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te voldoen, zodat we uw aanvraag in behandeling kunnen nemen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan uw aanvraag buiten behandeling worden gesteld. Dit betekent dat uw aanvraag niet beoordeeld wordt en u uw project niet mag starten.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
27 oktober 2017
Aanvraagnummer:
AVD1150020173846

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
27 oktober 2017
Aanvraagnummer:
AVD1150020173846

Gegevens aanvrager

Uw gegevens

Deelnemersnummer NVWA:	11500
Naam instelling of organisatie:	UMC Utrecht
Naam portefeuillehouder of diens gemachtigde:	[redacted]
Postbus:	12007
Postcode en plaats:	3501 AA UTRECHT

Gegevens verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	[redacted]
Afdeling:	Biomedical MR Imaging and Spectroscopy Group, Center for Image Sciences, UMC Utrecht
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Datum:
27 oktober 2017
Aanvraagnummer:
AVD1150020173846

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	Postdoctoraal onderzoeker
Afdeling:	Biomedical MR Imaging and Spectroscopy Group, Center for Image Sciences, UMC Utrecht
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Over uw aanvraag

Wat voor aanvraag doet u?	[x] Nieuwe aanvraag [ ] Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn [ ] Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Over uw project

Geplande startdatum:	1 januari 2018
Geplande einddatum:	1 januari 2023
Titel project:	Mechanisms of action of therapeutic brain stimulation therapies after experimental stroke
Titel niet-technische samenvatting:	Werkingsmechanismen van hersenstimulatie therapieën na een experimentele hersenberoerte
Naam DEC:	DEC Utrecht
Postadres DEC:	Postbus 85500 3508 GA Utrecht
E-mailadres DEC:	dec-utrecht@umcutrecht.nl

Betaalgegevens

De leges bedragen:	€ 1.684,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Verplichte bijlagen:	[x] Projectvoorstel [x] Beschrijving Dierproeven [x] Niet-technische samenvatting
Overige bijlagen:	[x] DEC-advies

Ondertekening

Naam: [redacted]

Functie: [redacted]

Plaats: Utrecht

Datum: 26 oktober 2017

Datum:
27 oktober 2017
Aanvraagnummer:
AVD1150020173846

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD1150020173846
Bijlagen
2

Datum 27 oktober 2017
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 27 oktober 2017
Vervaldatum: 26 november 2017
Factuurnummer: 173846
Ordernummer: CB.841910.3.01.011

Omschrijving	Bedrag
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD1150020173846	€ 1.684,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL29INGB 070.500.1512 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 93144, 2509 AC te 's Gravenhage.

From: [redacted]
To: [redacted]
Cc: Instantie voor Dierenwelzijn Utrecht; [redacted]
Subject: Re: FW: Aanhouden AVD1150020173846
Date: woensdag 22 november 2017 14:00:00
Attachments: image001.png
format-nts-brainstimulation_CCD_final.docx
Response_CCD_feedback.docx

Dear [redacted]

Thank you for your suggested edits on the NTS, this helped to shorten text significantly! Please find attached our responses to the CCD (Response_CCD_feedback doc) and the new NTS, which are now ready for submission to the CCD.

In the Response_CCD_feedback doc I also included a short cover letter.

Please let me know if there is anything else that I should do.

Kind regards,

[redacted]

2017-11-21 16:32 GMT+01:00 [redacted]

> Dear [redacted]
>
> Thank you for sending me the NTS.
>
> Attached, you will find the NTS. Although the 3.1 section is shortened drastically, I still think the informative value is sufficient. I included some suggestions for some small adaptions and I also tried to shorten it a little bit further.
>
> Best regards,
>
> [redacted]
>
> [redacted] • Head of the Animal Welfare Body Utrecht • Bolognalaan 50, 3584 CJ Utrecht • [redacted] • PO Box 12007, 3501 AA Utrecht • [redacted] • tel: [redacted] • [redacted] •

Instantie voor
Dierenwelzijn
Utrecht

From: [redacted]
Sent: maandag 20 november 2017 14:02
To: [redacted]
Subject: Re: FW: Aanhouden AVD1150020173846

Dear [redacted]

I have now worked through all of the questions from the CCD, awaiting approval from my supervisor before I send everything to you.

Would you be willing to take a look at our NTS again? Based on the request from the CCD below, we had to significantly shorten section 3.1. I am however, not sure if it is now too technical. Do you think that it would be alright to also shorten some additional sections of the NTS, otherwise we would loose too much of our project description in 3.1. Currently, the NTS is at 538 words, my supervisor will try to reduce the word count further to 500.

-De Niet-technische samenvatting (NTS) bevat ruim 800 woorden in plaats van de in de richtlijn aangegeven 500 woorden. Wij verzoeken u sectie 3.1 van de samenvatting in te korten en de nieuwe versie van de NTS toe te sturen.

Thank you in advance for your help!

Kind regards,

[redacted]

2017-11-14 15:44 GMT+01:00 [redacted]

Dear [redacted]

As you will see, the CCD has some additional questions.

I think that most of them are rather easy to answer.

Please, formulate your draft answers and forward them to us. When needed, you can consult us.

At last the IvD will upload the answers to the CCD.

Best regards,

[redacted]

[redacted] • Head of the Animal Welfare Body Utrecht • Bolognalaan 50, 3584 CJ Utrecht • [redacted] • PO Box 12007, 3501 AA Utrecht • [redacted] • [redacted] • [redacted] • [redacted] •

Instantie voor Dierenwelzijn Utrecht

From: info@zbo-ccd.nl [mailto:info@zbo-ccd.nl]
Sent: dinsdag 14 november 2017 13:30
To: Instantie voor Dierenwelzijn Utrecht
Cc: [redacted]
Subject: Aanhouden AVD1150020173846

Geachte [redacted]

Op 26-10-2017 hebben wij uw aanvraag voor een projectvergunning dierproeven

ontvangen. Het gaat om uw project "Mechanisms of action of therapeutic brain stimulation therapies after experimental stroke" met aanvraagnummer AVD1150020173846. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In dit bericht leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

Welke informatie nog nodig
Wij hebben de volgende informatie van u nodig om uw aanvraag verder te kunnen beoordelen:

Niet technische samenvatting
De NTS bevalt meer dan 500 woorden

Onduidelijkheden
-Voor translatie doeleinden worden experimenten in bijlages 3.4.4.2, 3.4.4.3 en 3.4.4.4 herhaald met hetzelfde aantal vrouwelijke muizen. Kunt u onderbouwen waarom het noodzakelijk is om alle experimenten in vrouwelijke dieren te herhalen? Zou translatie ook behaald kunnen worden door slechts een deel van de proeven in het andere geslacht uit de voeren/te herhalen?

-In bijlages 3.4.4.2, 3.4.4.3 en 3.4.4.4 bij sectie B. wordt benoemd dat bij het tMCAO infarct model een uitval van maximaal 25% wordt verwacht. Om hiervoor te compenseren worden 25% extra dieren toegevoegd aan de tMCAO groepen. Door welke elementen/onderdelen van het tMCAO-model ontstaat deze uitval?
De CCD berekent voor 3.4.4.2 dat in de tMCAO groepen n=12 + 25% (12*0,25= 3) = 15 dieren nodig zijn per groep. Echter in de berekening wordt een groepsgrootte van n=16 vastgesteld. Kunt u uitleggen hoe u tot een groepsgrootte van 16 bent gekomen? In bijlages 3.4.4.3 en 3.4.4.4 vindt de CCD soortgelijke onduidelijkheden in de groepsgrootte van het tMCAO model. Wij verzoeken u alle tMCAO-infarct-model-groepsgroottes te onderbouwen.

-In de in vivo [redacted] worden dieren blootgesteld aan een vastenperiode tijdens de nacht. Kunt u uitleggen waarom deze vastenperiode noodzakelijk is voor de [redacted]

- In sectie D van elke bijlagen wordt benoemd bij verfijning dat dieren individueel gehuisvest worden in de tDCS experimenten omdat een instrument op het cranium is geplaatst dat mogelijk door de kooigenoot kan worden beschadigd. Het is voor de CCD niet duidelijk of dit uitzonderlijk is of alle dieren in de tDCS setup groep individueel gehuisvest worden. U wordt verzocht te verhelderen om hoeveel dieren het gaat.

-De Niet-technische samenvatting (NTS) bevat ruim 800 woorden in plaats van de in de richtlijn aangegeven 500 woorden. Wij verzoeken u sectie 3.1 van de samenvatting in te korten en de nieuwe versie van de NTS toe te sturen.

Zonder deze aanvullende informatie kan de beslissing nadelig voor u uitvallen omdat de gegevens onvolledig of onduidelijk zijn.

Opsturen binnen veertien dagen
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. U kunt dit aanleveren via NetFTP.

Wanneer een beslissing
De behandeling van uw aanvraag wordt opgeschort tot het moment dat wij de aanvullende informatie hebben ontvangen. Als u goedkeuring krijgt op uw aanvraag,

kunt u daarna beginnen met het project.

Mocht u vragen hebben, dan kunt u uiteraard contact met ons opnemen.

Met vriendelijke groet,
Namens de Centrale Commissie Dierproeven

[redacted]
www.centralecommissiedierproeven.nl

.....
Postbus 20401 | 2500 EK | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl

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Kind regards,

[redacted]

PhD Candidate | Biomedical MR Imaging & Spectroscopy Group | Center for Image Sciences

University Medical Center Utrecht | Building Nieuw Gildestein | Yalelaan 2 | 3584 CM UTRECHT

E-mail [redacted]

--
Kind regards,

[redacted]

PhD Candidate | Biomedical MR Imaging & Spectroscopy Group | Center for Image Sciences
University Medical Center Utrecht | Building Nieuw Gildestein | Yalelaan 2 | 3584 CM UTRECHT
E-mail [redacted]

22 November 2017

Application number: AVD1150020173846

Project title: Mechanisms of action of therapeutic brain stimulation therapies after experimental stroke

Dear CCD members,

Herewith, we submit our responses for your consideration, based on the feedback received on 14 November 2017. We hope that our responses below (questions 1-6) address the suggested uncertainties sufficiently. Additionally, find attached a new and shortened version of the NTS.

Please let us know if we can be of further assistance.

Kind regards,

[redacted]

1.) **Voor translatie doeleinden worden experimenten in bijlages 3.4.4.2, 3.4.4.3 en 3.4.4.4 herhaald met hetzelfde aantal vrouwelijke muizen. Kunt u onderbouwen waarom het noodzakelijk is om alle experimenten in vrouwelijke dieren te herhalen? Zou translatie ook behaald kunnen worden door slechts een deel van de proeven in het andere geslacht uit de voeren/te herhalen?**

To determine the mechanisms of action of the different treatment protocols, we rely on the functional outcome measures of the treatment protocols in both male and female animals, as described in phase 2 (Appendix 3, Longitudinal stroke study) of this study. Since it is still unclear, due to limited reseach in female animals, we don't know how these animals will respond to the stimulation studies.

Therefore, in phase 3 of the this study, we also opted for investigating the mechanisms of action in both males and females, because these mechanisms might be different between the sexes due to hormonal fluctuations in the female animals. For example, fluctuating levels of estrogen have been linked to the extent of brain damage after experimental stroke (Carswell et al., 2000), and young female animals demonstrate less ischemic damage than young males (Manwani et al., 2013). Biological sex may also influence how patients/animals respond to stroke treatments, since the mostly applied treatment for stroke (thrombolysis) has been suggested to be more effective in women than men (Kent et al., 2005).

Carswell HVO, Dominiczak AF, Macrae IM (2000) Estrogen status affects sensitivity to focal cerebral ischemia in stroke-prone spontaneously hypertensive rats. Am J Physiol Hear Circ Physiol 278:290–294.

Kent DM, Price LL, Ringleb P, et al (2005) Sex-Based Differences in Response to Recombinant Tissue Plasminogen Activator in Acute Ischemic Stroke A Pooled Analysis of Randomized Clinical Trials. Stroke 36:62–65. doi: 10.1161/01.STR.0000150515.15576.29

Manwani B, Liu F, Scranton V, et al (2013) Differential effects of aging and sex on stroke induced inflammation across the lifespan. Exp Neurol 249:1–25. doi: 10.1016/j.expneurol.2013.08.011

2.) **In bijlages 3.4.4.2, 3.4.4.3 en 3.4.4.4 bij sectie B. wordt benoemd dat bij het tMCAO infarct model een uitval van maximaal 25% wordt verwacht. Om hiervoor te compenseren worden 25% extra dieren toegevoegd aan de tMCAO groepen. Door welke elementen/onderdelen van het tMCAO-model ontstaat deze uitval?**

For the induction of experimental ischemic stroke using the transient middle cerebral artery occlusion (tMCAO) model, an intraluminal filament is inserted into the internal carotid artery and it is advanced into the blood vessel to block blood flow to the middle cerebral artery (Kumar and Varun Gupta, 2016. Brain Research Bulletin). Animal loss can occur, when a blood vessel is ruptured during the insertion of the intraluminal filament, leading to subarachnoid hemorrhage. The intraluminal suture may also block the hypothalamic blood supply leading to hypothalamic infarction and subsequently hyperthermia. These shortcomings arise as a result of the incorrect insertion of the suture and/or filament, which could result in animal loss.

3.) **De CCD berekent voor 3.4.4.2 dat in de tMCAO groepen n=12 + 25% (12*0,25= 3) = 15 dieren nodig zijn per groep. Echter in de berekening wordt een groepsgrootte van n=16 vastgesteld. Kunt u uitleggen hoe u tot een groepsgrootte van 16 bent gekomen? In bijlages 3.4.4.3 en 3.4.4.4 vindt de CCD soortgelijke onduidelijkheden in de groepsgrootte van het tMCAO model. Wij verzoeken u alle tMCAO-infarct-model-groepsgroottes te onderbouwen.**

In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss. The numbers of animals per group were calculated as follow:

Original group size X 25% = Additional animals
Original group size + Additional animals + (Additional animals X 25%) = True group size

Different from the calculations of the CCD, we also anticipate an animal loss of 25% in the additional animals that are included in each group. See detailed calculations for animals in the tMCAO groups below.

Longitudinal study 3.1: TMS: maximum of 172 rats (86 male, 86 female).

3.1.1 Mechanism of action using [redacted]

2) Groups and maximum group size for tMCAO stroke:
   a. Optimal stimulation paradigm 1 (n=6)
   b. Sham stimulation (n=6)

   In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss.

   6 animals per group X 25% = 1.5
   6 + 1.5 + (1.5 X 25%) = 7.9
   7.9 is rounded up to 8 animals per group.
   This results in a total of 2 groups with 8 rats per group = 16 male rats.

   The working mechanism of the optimal rTMS protocol and a sham stimulation protocol will also be tested in females. In the female tMCAO group, we will use exactly the same number of animals, as calculated for the male animals.

2) Groups and maximum group size for tMCAO stroke (female rats):
   a. Most optimal stimulation protocol (n=8)
   b. Sham stimulation protocol (n=8)

3.1.2 Mechanism of action using [redacted]

2) Groups and maximum group size for tMCAO stroke:
   a. Optimal stimulation paradigm 1 (n=7)
   b. Sham stimulation (n=7)

   In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss.

   7 animals per group X 25% = 1.75
   7 + 2 + (2 X 25%) = 9.5
   9.5 is rounded up to 10 animals per group.
   This results in a total of 2 groups with 10 rats per group = 20 male rats.

   The working mechanism of the optimal rTMS protocol and a sham stimulation protocol will also be tested in females. In the female tMCAO group, we will use exactly the same number of animals, as calculated for the male animals.

2) Groups and maximum group size for tMCAO stroke (female rats):
   a. Most optimal stimulation protocol (n=10)

b. Sham stimulation protocol (n=10)

3.1.3 Mechanism of action using optical imaging [redacted]

2) Groups and maximum group size for tMCAO stroke:
   a. Optimal stimulation paradigm 1 (n=5)
   b. Sham stimulation (n=5)

   In the subcortical stroke (tMCAO) groups, we need to add 25% at maximum to compensate for animal loss.

   5 animals per group X 25% = 1.25
   5 + 1.25 + (1.25 X 25%) = 6.5625
   6.5625 is rounded up to 7 animals per group.
   This results in a total of 2 groups with 7 rats per group = 14 male rats.

   The working mechanism of the optimal rTMS protocol and a sham stimulation protocol will also be tested in females. In the female tMCAO group, we will use exactly the same number of animals, as calculated for the male animals.

2) Groups and maximum group size for tMCAO stroke (female rats):
   a. Most optimal stimulation protocol (n=7)
   b. Sham stimulation protocol (n=7)

Longitudinal study 3.2: tDCS: maximum of 172 rats (86 male, 86 female).

For the mechanism of action study performed in 3.2 and sub-phases, the exact power calculations were used as for 3.1. Therefore, animal numbers per group are the same.

3.2.1 Mechanism of action using [redacted]

2) Groups and maximum group size for tMCAO stroke (male rats):
   a. Optimal stimulation paradigm 1 (n=8)
   b. Sham stimulation (n=8)

   In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss.
   The working mechanism of the optimal tDCS protocol and a sham stimulation protocol will also be tested in females. In the female tMCAO group, we will use exactly the same number of animals, as calculated for the male animals.

2) Groups and maximum group size for tMCAO stroke (female rats):
   a. Most optimal stimulation protocol (n=8)
   b. Sham stimulation protocol (n=8)

3.2.2 Mechanism of action using [redacted]

2) Groups and maximum group size for tMCAO stroke (male rats):
   a. Optimal stimulation paradigm 1 (n=10)
   b. Sham stimulation (n=10)

   In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss.
   The working mechanism of the optimal rTMS protocol and a sham stimulation protocol will also be tested in females. In the female tMCAO group, we will use exactly the same number of animals, as calculated for the male animals.

2) Groups and maximum group size for tMCAO stroke (female rats):
   a. Most optimal stimulation protocol (n=10)
   b. Sham stimulation protocol (n=10)

3.2.3 Mechanism of action using optical imaging [redacted]

2) Groups and maximum group size for tMCAO stroke (male rats):
   a. Optimal stimulation paradigm 1 (n=7)
   b. Sham stimulation (n=7)

In the subcortical stroke (tMCAO) groups, we need to add 25% at maximum to compensate for animal loss.

The working mechanism of the optimal rTMS protocol and a sham stimulation protocol will also be tested in females. In the female tMCAO group, we will use exactly the same number of animals, as calculated for the male animals.

2) Groups and maximum group size for tMCAO stroke (female rats):
   a. Most optimal stimulation protocol (n=7)
   b. Sham stimulation protocol (n=7)

Longitudinal study 3.3: [redacted] manipulation [redacted] maximum of 172 rats (86 male, 86 female).

For the mechanism of action study performed in 3.3 and sub-phases, the exact power calculations were used as for 3.1. Therefore, animal numbers per group are the same.

3.3.1 Mechanism of action using [redacted]

2) Groups and maximum group size for tMCAO stroke (male rats):
   a. Optimal stimulation paradigm 1 (n=8)
   b. Sham stimulation (n=8)

   In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss.

   The working mechanism of the optimal rTMS protocol and a sham stimulation protocol will also be tested in females. In the female tMCAO group, we will use exactly the same number of animals, as calculated for the male animals.

2) Groups and maximum group size for tMCAO stroke (female rats):
   a. Most optimal stimulation protocol (n=8)
   b. Sham stimulation protocol (n=8)

3.3.2 Mechanism of action using [redacted]

2) Groups and maximum group size for tMCAO stroke (male rats):
   c. Optimal stimulation paradigm 1 (n=10)
   d. Sham stimulation (n=10)

   In the tMCAO stroke model, we need to add 25% at maximum to compensate for animal loss.

   The working mechanism of the optimal rTMS protocol and a sham stimulation protocol will also be tested in females. In the female tMCAO group, we will use exactly the same number of animals, as calculated for the male animals.

2) Groups and maximum group size for tMCAO stroke (female rats):
   a. Most optimal stimulation protocol (n=10)
   b. Sham stimulation protocol (n=10)

3.3.3 Mechanism of action using optical imaging [redacted]

2) Groups and maximum group size for tMCAO stroke (male rats):
   c. Optimal stimulation paradigm 1 (n=7)
   d. Sham stimulation (n=7)

   In the subcortical stroke (tMCAO) groups, we need to add 25% at maximum to compensate for animal loss.

   The working mechanism of the optimal rTMS protocol and a sham stimulation protocol will also be tested in females. In the female tMCAO group, we will use exactly the same number of animals, as calculated for the male animals.

2) Groups and maximum group size for tMCAO stroke (female rats):
   a. Most optimal stimulation protocol (n=7)
   b. Sham stimulation protocol (n=7)

4.) **In de in vivo [redacted] worden dieren blootgesteld aan een vastenperiode tijdens de nacht. Kunt u uitleggen waarom deze vastenperiode noodzakelijk is voor de [redacted]**

Appendix 1: Preparation Phase, section 2A

Magnetic resonance [redacted] will be performed to look at changes in brain [redacted] which involves looking at the [redacted] of the brain. The use of fasted animals for this experiment is beneficial in creating a relatively stable blood glucose level amongst animals, reducing variability of blood glucose levels between test animals. Additionally, this would allow us to compare our findings with other studies that have followed a similar [redacted] protoco[redacted]

[redacted]

5.) **In sectie D van elke bijlagen wordt benoemd bij verfijning dat dieren individueel gehuisvest worden in de tDCS experimenten omdat een instrument op het cranium is geplaatst dat mogelijk door de kooigenoot kan worden beschadigd. Het is voor de CCD niet duidelijk of dit uitzonderlijk is of alle dieren in de tDCS setup groep individueel gehuisvest worden. U wordt verzocht te verhelderen om hoeveel dieren het gaat.**

Animals that will receive transcranial direct current stimulation (tDCS) will only be housed solitarily when the tDCS cannula/s on the cranium cannot be protected well from damage that can be induced by the cage companion. The likelihood of solitary housing of the animals is low, since we have in the meantime developed a metal cap to cover the cannulas. Up until know, this metal cap is working well in a preliminary tDCS trial. All tDCS animals could potentially be housed individually, if this metal cap is not durable enough (Appendix 1 = 46 animals; Appendix 3 = 448 animals; Appendix 4 = 172 animals).

6.) **De Niet-technische samenvatting (NTS) bevat ruim 800 woorden in plaats van de in de richtlijn aangegeven 500 woorden. Wij verzoeken u sectie 3.1 van de samenvatting in te korten en de nieuwe versie van de NTS toe te sturen.**

Section 3.1 has been shortened as requested. However, to reduce the NTS to 500 words or less, we also shortened some of the other sections of the NTS. This was done, because by removing all additional text from Section 3.1, it would significantly limited the understanding of what we will be doing. The NTS has been reduced to 484 words.

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD1150020173846
Bijlagen
1

Datum 8 december 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 26 oktober 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Mechanisms of action of therapeutic brain stimulation therapies after experimental stroke" met aanvraagnummer AVD1150020173846. Wij hebben uw aanvraag beoordeeld.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a lid 1 van de Wet op de dierproeven (hierna: de wet).

U kunt met uw project starten. De vergunning wordt afgegeven van 1 januari 2018 tot en met 31 december 2022. Deze termijn is anders dan in uw aanvraag, omdat een vergunning een looptijd van maximaal 5 jaar kan hebben.
De onderbouwing van deze beslissing vindt u onder 'Overwegingen'.

Procedure

Advies dierexperimentencommissie
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie (DEC) DEC Utrecht gevoegd. Dit advies is ontvangen op 26 oktober 2017. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a lid 3 van de wet. Het advies van de DEC is betrokken bij de behandeling van uw aanvraag.

Nadere vragen aanvrager
Op 14 november 2017 hebben wij u om aanvullingen gevraagd. U heeft tijdig antwoord gegeven. De aanvullingen hadden betrekking op het inkorten van de NTS, de onderbouwing voor het gebruik van vrouwelijke dieren in het project, de berekening van de groepsgrootte voor het tMCAO infarct model, een onderbouwing voor de vastenperiode voorafgaand aan de [redacted] en een onderbouwing voor de individueel gehuisveste dieren. Uw antwoord is betrokken bij de behandeling van uw aanvraag.

Overwegingen
Alle hierboven genoemde stukken liggen ten grondslag aan ons besluit.

Wij kunnen ons vinden in de inhoud van het advies van de DEC, inclusief de daaraan ten grondslag liggende motivering.

Beoordeling achteraf
Na afloop van het project zal er een beoordeling plaatsvinden, zoals bedoeld in artikel 10a1 lid 1 sub d en artikel 10a1 lid 3 van de wet. De reden van deze beoordeling achteraf is dat in dit project dieren ernstig ongerief ondergaan. Deze beoordeling zal uiterlijk december 2023 plaatsvinden. Meer informatie over de eisen die gesteld worden bij de beoordeling achteraf vindt u in de bijlage 'Weergave wet- en regelgeving'.

Bezwaar
Als u het niet eens bent met deze beslissing, kunt u binnen zes weken na verzending van deze brief schriftelijk een bezwaarschrift indienen.
Een bezwaarschrift kunt u sturen naar Centrale Commissie Dierproeven, afdeling Juridische Zaken, postbus 20401, 2500 EK Den Haag.

Bij het indienen van een bezwaarschrift vragen we u in ieder geval de datum van de beslissing waartegen u bezwaar maakt en het aanvraagnummer te vermelden. U vindt deze nummers in de rechter kantlijn in deze brief.

Bezwaar schorst niet de werking van het besluit waar u het niet mee eens bent. Dat betekent dat dat besluit wel in werking treedt en geldig is. U kunt tijdens deze procedure een voorlopige voorziening vragen bij de Voorzieningenrechter van de rechtbank in de woonplaats van de aanvrager. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisend belang.

Voor de behandeling van een voorlopige voorziening is griffierecht verschuldigd. Op

http://www.rechtspraak.nl/Organisatie/Rechtbanken/Pages/default.aspx kunt u zien onder welke rechtbank de vestigingsplaats van de aanvrager valt.

Datum:
8 december 2017
Aanvraagnummer:
AVD1150020173846

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Centrale Commissie Dierproeven
namens deze:

i.o.

[redacted]

Algemeen Secretaris

Bijlagen:
- Vergunning
  Hiervan deel uitmakend:
  - DEC-advies
  - Weergave wet- en regelgeving

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam:	UMC Utrecht
Adres:	Postbus 12007
Postcode en plaats:	3501 AA UTRECHT
Deelnemersnummer:	11500

deze projectvergunning voor het tijdvak 1 januari 2018 tot en met 31 december 2022, voor het project "Mechanisms of action of therapeutic brain stimulation therapies after experimental stroke" met aanvraagnummer AVD1150020173846, volgens advies van Dierexperimentencommissie DEC Utrecht.
De functie van de verantwoordelijk onderzoeker is [redacted]
Het besluit is gebaseerd op de volgende (aangepaste) stukken:

1 een aanvraagformulier projectvergunning dierproeven, zoals ontvangen op 26 oktober 2017
2 de bij het aanvraagformulier behorende bijlagen:
   a Projectvoorstel, zoals ontvangen op 26 oktober 2017;
   b Bijlagen dierproeven
      - 3.4.4.1 Phase 0: Preparation phase to optimize and establish stimulation, imaging, stroke and behaviora techniques., zoals ontvangen op 26 oktober 2017;
      - 3.4.4.2 Phase 1: [redacted] manipulation and MRI in healthy rats and rats with experimental stroke. zoals ontvangen op 26 oktober 2017;
      - 3.4.4.3 Phase 2: Brain stimulation and multiparametric MRI in healthy rats and rats with experimental stroke., zoals ontvangen op 26 oktober 2017;
      - 3.4.4.4 Phase 3: Working mechanisms of brain stimulation and multiparametric MRI in healthy rats and rats with experimental stroke., zoals ontvangen op 26 oktober 2017;
   c Niet-technische Samenvatting van het project, zoals ontvangen op 22 november 2017;
   d Advies van Dierexperimentencommissie zoals ontvangen op 26 oktober 2017
   e De aanvullingen op uw aanvraag, ontvangen op 22 november 2017.

Aanvraagnummer:
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Naam proef	Diersoort/ Stam	Aantal dieren	Ernst
3.4.4.1 Phase 0: Preparation phase to optimize and establish stimulation, imaging, stroke and behavioral techniques.			
	Ratten (Rattus norvegicus) / Sprague Dawley/CRL:CD(SD)	325	12,0% Ernstig 33,0% Matig 55,0% Licht
3.4.4.2 Phase 1: [redacted] manipulation and MRI in healthy rats and rats with experimental stroke.			
	Ratten (Rattus norvegicus) / Sprague Dawley/CRL:CD(SD)	528	64,0% Ernstig 36,0% Matig
3.4.4.3 Phase 2: Brain stimulation and multiparametric MRI in healthy rats and rats with experimental stroke.			
	Ratten (Rattus norvegicus) / Sprague Dawley/CRL:CD(SD)	1.436	100,0% Ernstig
3.4.4.4 Phase 3: Working mechanisms of brain stimulation and multiparametric MRI in healthy rats and rats with experimental stroke.			
	Ratten (Rattus norvegicus) / Sprague Dawley/CRL:CD(SD)	516	100,0% Ernstig

Voorwaarden

Beoordeling achteraf
In dit project worden dierproeven toegepast die vallen in de categorie ernstig volgens artikel 10b van de wet en wordt daarom voorzien van beoordeling achteraf. Deze beoordeling zal uiterlijk december 2023 plaatsvinden. Er zal dan beoordeeld worden of de doelstellingen van het project werden bereikt. Daarnaast wordt bekeken of de schade die de dieren hebben ondervonden, het aantal en soorten proefdieren en de ernst de dierproeven conform de vergunning waren.

Ter informatie
Onderstaande informatie is opgenomen op grond van artikel 1d lid 4, artikel 10a1 lid 2, artikel 10 lid 2 en/of artikel 10a3 van de wet.
- Go/ no go momenten worden voor aanvang van elk experiment afgestemd met de IvD.

Aanvraagnummer:
AVD1150020173846

- Het is verboden een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is.
- Het is verboden dierproeven te verrichten voor een doel waarvan het belang niet opweegt tegen het ongerief dat aan het proefdier wordt berokkend.
- Overige wettelijke bepalingen blijven van kracht.

Centrale Commissie Dierproeven

Aanvraagnummer:
AVD1150020173846

Weergave wet- en regelgeving

Dit project en wijzigingen
Volgens artikel 10c van de Wet op de Dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.
Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister een ontheffing is verleend.

Verzorging
De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.
In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.
Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

Pijnbestrijding en verdoving
In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn

kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

Einde van een dierproef
Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijven schade zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.
Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13d volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13c is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.

Beoordeling achteraf
Volgens artikel 10a1, lid 1d en lid 3 van de wet worden projecten waarbij niet-menselijke primaten worden gebruikt, projecten die als ernstig ingedeelde dierproeven omvatten of een dierproef die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, achteraf beoordeeld worden.

Centrale Commissie Dierproeven

Formulier
Beoordeling achteraf
(Versie 11 juli 2018)

- Dit formulier gebruikt u om uw beoordeling achteraf te schrijven.
- Gebruik bij het invullen van dit formulier de Toelichting op het formulier 'Beoordeling achteraf'.
- Meer informatie over de beoordeling achteraf vindt u op de website www.centralecommissiedierproeven.nl.

1 Algemene gegevens

1.1	Vul het AVD nummer in.		AVD1150020173846
1.2	Vul de gegevens in van de instellingsvergunning-houder in.	Naam instelling of organisatie	UMC Utrecht
		E-mailadres contactpersoon	
		(Optioneel) e-mailadres Instantie voor Dierenwelzijn	info.IVD@uu.nl
1.3	Vul hier de gegevens van de verantwoordelijk onderzoeker in.	Titel, voorletters en achternaam	[redacted]
		Telefoonnummer	[redacted]
		E-mailadres	[redacted]
1.1	Vul de titel van het project in.		Werkingsmechanismen van hersenstimulatie therapieën na een experimentele stroke.

2 Gebruik dieren

2.1	Geef per bijlage en per diersoort aan hoeveel dieren u heeft gebruikt. -Indien niet alle aangevraagde diersoorten gebruikt zijn, licht dit toe. -Indien dit afwijkt van het aantal dieren in de vergunning, verklaar het verschil.	Appendix 1: 97 rats gebruikt Appendix 2: 0 gebruikt Appendix 3: 41 gebruikt Appendix 4: 0 gebruikt
2.2	Geef per bijlage en per diersoort aan: het aantal dieren dat terminaal, licht, matig of ernstig ongerief heeft ondergaan. -Indien dit afwijkt van het vooraf ingeschatte cumulatieve ongerief, verklaar het verschil.	Appendix 1: Preparation phase to optimize and establish stimulation, imaging, stroke and behavioral techniques. Terminaal: 51 ratten (52.6%) Licht: 16 ratten (16.5%) Ernstig: 30 ratten (30.9%) De nadruk bij deze fase van het onderzoek lag op het optimaliseren van een aantal invasieve technieken waardoor relatief meer dieren ernstig ongerief hebben ervaren. Gebaseerd op de oorspronkelijke berekeningen was 12% van 325 dieren ingeschat op ernstig ongerief, hetgeen overeenkomt met 39 dieren. Uiteindelijk zijn er dus netto minder dieren met ernstig ongerief gebruikt. Appendix 2 : Chemogenetic manipulation and MRI in healthy rats and rats with experimental stroke. Geen ratten gebruikt. Deze experimenten zijn nieuw in het lab. Hoewel ze bijzondere en nieuwe inzichten kunnen opleveren hebben de andere projecten prioriteit gekregen en ontbrak uiteindelijk voldoende tijd om ze uit te voeren. Appendix 3 : Brain stimulation and multiparametric MRI in healthy rats and rats with experimental stroke. Ernstig: 41 ratten (100%) Geen verschillen met het vooraf ingeschatte ongerief. Appendix 4 : Working mechanisms of brainstimulation and multiparametric MRI in healthy rats and rats with experimental stroke. Geen ratten gebruikt. Door het vertrek/de promotie van de betrokken PhD studenten konden deze experimenten geen doorgang vinden.

3 De 3V's

3.1	<u>Vervanging</u> Zijn er tijdens het project voor het project relevante mogelijkheden voor vervanging naar voren gekomen? - Zo ja, welke? - Zo ja, in hoeverre heeft u deze kunnen toepassen in het project? -Zo ja, in hoeverre zijn deze mogelijkheden relevant voor toekomstig onderzoek?	Tijdens het project zijn er geen relevante mogelijkheden voor vervanging naar voren gekomen.

3.2	Vermindering Zijn er tijdens het project voor het project relevante mogelijkheden voor verdere vermindering naar voren gekomen? - Zo ja, welke? - Zo ja, in hoeverre heeft u deze kunnen toepassen in het project? - Zo ja, in hoeverre zijn deze mogelijkheden relevant voor toekomstig onderzoek? - Was het vooraf ingeschatte aantal dieren per proefgroep optimaal voor betrouwbare statistische analyse?	Tijdens het project zijn er geen relevante mogelijkheden voor verdere vermindering naar voren gekomen. Het gebruikte aantal dieren per proefgroep was voldoende voor onze statistische analyse.
3.3	Verfijning - Zijn er tijdens het project voor het project relevante mogelijkheden voor verdere verfijning naar voren gekomen? - Zo ja, welke? - Zo ja, in hoeverre heeft u deze kunnen toepassen in het project? - Zo ja, in hoeverre zijn deze mogelijkheden relevant voor toekomstig onderzoek? - Is de monitoring van het dierenwelzijn adequaat gebleken? - Kunnen de criteria voor humane eindpunten verfijnd worden?	Ja. Tijdens het project zijn maatregelen getroffen om uitval en het bereiken van een humaan eindpunt na de operatie waarbij een beroerte wordt geïnduceerd, terug te dringen (wellicht naar aanleiding van de IMPROVE richtlijnen). Ten eerste het standaard aanbieden van 'weekvoer' na de operatie. Daarna een extra verscherpte monitoring na de operatie. Beiden zijn ingevoerd in overleg met de IvD. Hierbij zorgt het weekvoer voor beter herstel en gewichtstoename na een operatie, en de verscherpte monitoring zorgt ervoor dat problemen met het dier (inclusief, maar niet enkel, het gewichtsverlies na operatie) tijdig herkend en erkend worden, en er overlegd kan worden met dierenarts en/of IvD hoe te handelen. De verscherpte monitoring en het preventief aanbieden van weekvoer wordt nu toegepast in alle modellen van een beroerte binnen ons lab. De verscherpte monitoring is in deze studie adequaat gebleken. De humane eindpunten zijn voor de huidige studie aangescherpt, en worden momenteel toegepast voor alle modellen met een beroerte in ons lab.

4 Strategie

4.1	Voldeden de diermodellen aan de verwachtingen? Licht uw antwoord toe. -Indien de diermodellen niet voldeden, beschrijf wanneer dit werd gesignaleerd, of de modellen zijn aangepast en of besloten is de proeven (tijdelijk) te stoppen?	Ja, er is al ruime ervaring met de beschreven diermodellen voor beroerte in het lab. Echter, dit werkprotocol was ook bedoeld om een aantal nieuwe procedures/modellen te testen. Zo werd er een techniek getest om canules op de kop van de rat te plaatsen waardoor de hersenen gestimuleerd konden worden. Het boren van openingen in de schedel voor dit doeleinde moet nog beter geoptimaliseerd worden. Er zijn meerdere verbeteringen gesuggereerd, maar verdere experimenten in die richting zijn gestopt.
4.2	Waren de in de aanvraag beschreven keuzemomenten en de criteria op basis waarvan keuzes werden gemaakt voldoende specifiek om onnodig gebruik en/of	Ja

	ongerief van dieren te voorkomen?	

5 Verworvenheden (wat heeft het project opgeleverd?)

5.1	In hoeverre zijn de directe doelen van het projectvoorstel bereikt? Indien de directe doelen niet (volledig) bereikt zijn, licht toe waarom niet.	**Appendix 1: Preparation phase to optimize and establish stimulation, imaging, stroke and behavioral techniques.** Een aantal dieren zijn gebruikt om operaties te oefenen, zowel het aanbrengen van een MCAO als het plaatsen van een canulle en andere diertechnische handelingen waarbij ervaring moest worden opgedaan. Eén studie werd uitgevoerd bij 22 ratten naar de effecten van repetitive transcranial magnetic stimulation (rTMS) op neurale activatie door het gebruik van MRI met signaal versterking door mangaan (MEMRI). Mn^{2+} ionen zijn een biologische vervanging van Ca^{2+} ionen. De laatste spelen een rol bij het openen van de ion-kanalen bij neuronale activatie. Mn^{2+} ionen veroorzaken, in tegenstelling to Ca^{2+} ionen, een contrast dat te zien is op een MRI. In eerste instantie werd uitgezocht wat de optimale dosis $MnCl_2$ was voor een optimaal protocol (60 mg/kg zonder bij-effecten). Daarna werd bepaald wat het optimale tijdspunt was voor MEMRI na mangaan toediening (na 24 uur). Vervolgens werd bestudeerd of er veranderingen waren in het MEMRI contrast met en zonder rTMS. Ook hier speelt de timing van de toediening van mangaan en stimulatie met rTMS een grote rol. Er is gedemonstreerd dat rTMS de neuronale activatie in zowel de gestimuleerde als de niet-gestimuleerde hemisfeer van de rat beinvloed. Daarmee is het doel van deze pilotstudie bereikt. Een andere studie onderzocht de mogelijkheden van het plaatsen van een cannule op de kop van de rat om transcraniele direct current stimulatie (tDCS) mogelijk te maken terwijl de betreffende rat bij bewustzijn is (17 ratten). tDCS is een mogelijke nieuwe behandeltherapie na beroerte. De mogelijkheid om een gedragstaak te laten uitvoeren door een rat terwijl gelijktijdig tDCS behandeling plaatsvind werd getest. Er werden op dat moment geen veranderingen in de functionele connectiviteit van de hersenen gevonden ten gevolge van tDCS. Het doel van de studie was echter bereikt, een extra techniek om te stimuleren was gerealiseerd. Bij een andere (vervolg)studie met een eigen CCD is vervolgens de techniek opnieuw toegepast hetgeen heeft geleid tot een publicatie. De derde studie betrof de toepassing van een nieuw MR techniek waarmee het glucosemetabolisme in de hersenen kan worden bestudeerd door middel van het injecteren van ^2H gelabeled glucose (57 ratten). Dit wordt net als normaal glucose opgenomen in de hersenen en vervolgens omgezet in glutamaat en/of glutamine via de normale citroenzuurcyclus. In het geval van een beroerte is dit normale metabolisme door gebrek aan zuurstof echter veranderd en produceren de hersenen ook lactaat. Met deuterium spectroscopic imaging kan de hoeveelheid omgezet glucose en de snelheid van de omzetting worden bestudeerd. Een aantal experimenten zijn uitgevoerd om vast te stellen of deze techniek in ons lab kon worden ingezet. Daarna is een grotere studie opgezet (waarbij ook dieren zijn gebruikt die onder Appendix 3 vallen) waarbij onderzocht werd in hoeverre het normale glucosemetabolisme verstoord blijft na een beroerte gevolgd door reperfusie. De resultaten zijn zichtbaar in onderstaande figuren:

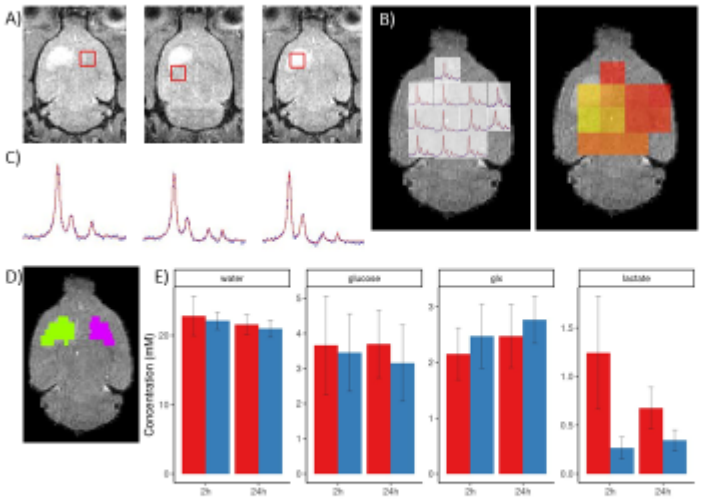

Figure 2: Active glucose metabolism 2 and 24 hours after 90 minutes MCA occlusion in rats

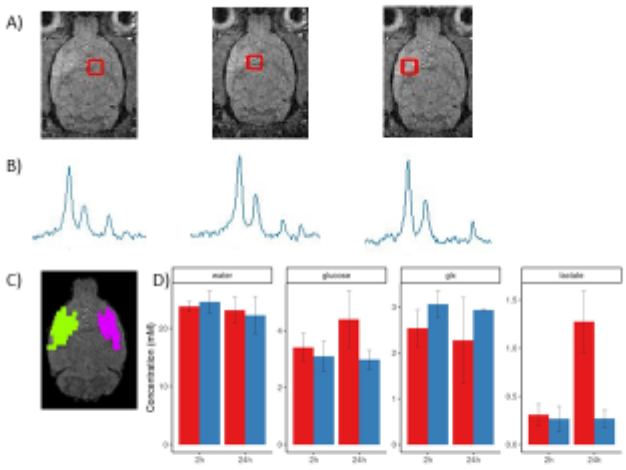

Figure 1: Active glucose metabolism 2 and 24 hours after 45 minutes MCA occlusion in rats

Daarmee zijn de doelen van dit project bereikt.

Appendix 3 : Brain stimulation and multiparametric MRI in healthy rats and rats with experimental stroke.

Ook bij de projecten horend bij deze appendix zijn dieren gebruikt om hands-on ervaring op te doen met de benodigde stroke-inductie en/of gedragstaken voordat er een studie is begonnen.

Zoals eerder vermeld is een deel van de dieren gebruikt voor het hiervoor beschreven deuterium MR project (57 ratten).

Een ander deel van de dieren is gebruikt voor een project waarbij rTMS werd gebruikt om het herstel van de ratten te bevorderen na een beroerte (14 ratten). De mate van herstel werd vastgesteld door met functionele MRI veranderingen in de connectiviteit van de hersenen te meten ten opzichte van een controle groep. Deze experimenten zijn afgerond en hebben hun doel behaald.

Tijd en middelen ontbraken om de overige doelen in het projectvoorstel (met name beschreven in Appendix 2 en 4) te realiseren.

5.2 Zijn er nog andere waardevolle opbrengsten van het project te vermelden?

De resultaten zijn vastgelegd in een drietal MSc thesis en hebben bijgedragen aan de opleiding en training van studenten.
Daarnaast hebben de experimenten tot de volgende meeting abstracts geleid:

[redacted]

En daarnaast de volgende publicaties:

[redacted]

Er wordt nog gewerkt aan verdere analyse van de data. De verwachting is dat deze zullen leiden tot het afronden van twee proefschriften [redacted] en daaraan verbonden wetenschappelijke publicaties.

6 Overige aspecten

6.1 Heeft u nog verdere opmerkingen die volgens u relevant zijn voor de beoordeling achteraf van het project?

Neen

7 Leerpunten

7.1 Beschrijf wat voor u de belangrijkste leerpunten zijn met betrekking tot het ontwerp van en de uitvoering van toekomstige projecten.

Deze CCD aanvraag was een van de eerste grote aanvragen die door onze groep werd gedaan, waarbij veel verschillende onderzoeksprojecten werden gecombineerd en veel onderzoekers betrokken waren.
Doordat verantwoordelijkheden voor (met name) het afronden van de CCD werden doorgeschoven en veel betrokkenen in de tussentijd elders werk hebben gevonden is deze beoordeling achteraf te laat en minder compleet dan gewenst ingeleverd omdat expertise verloren is gegaan.
Er is in de afgelopen jaren al gewerkt aan het beter vastleggen van experimenten/projecten die op het lab worden uitgevoerd door middel van het invoeren van standard operating procedures (SOPs) voor heel

veel uiteenlopende handelingen en het opzetten van een structuur om data op te slaan volgens de FAIR principes, waardoor we ze in de toekomst eenvoudiger beschikbaar kunnen stellen in een ‘open’ repository.
Nu blijkt dat er met name in de beginfase en de eindfase van een project nog meer aandacht moeten worden besteed aan de plaats die de CCD aanvraag, de werkprotocollen en de afsluiting van het project binnen deze data structuur krijgen. Informatie hierover moet binnen het lab eenvoudig te vinden en te begrijpen zijn en digitaal beschikbaar. We gaan toekomstige data management plannen hierop aanpassen. Daarnaast is er meer interne controle nodig wat betreft het vastleggen van deze informatie. Over de vorm daarvan wordt nog nagedacht, maar toekomstige projecten moeten resistent worden tegen het verdwijnen van uitvoerenden.

8 Ondertekening

8.1

Ondertekening door de portefeuillehouder namens de instellingsvergunninghouder. De ondergetekende verklaart:

- dat de beantwoording van de vragen in het formulier Beoordeling achteraf is afgestemd met de Instantie voor Dierenwelzijn.
- dat het formulier volledig en naar waarheid is ingevuld.

Naam

Datum

Handtekening

Centrale Commissie Dierproeven i.o.

Aanvulling Niet-technische samenvatting

Beoordeling achteraf 20173846-BA

1 Algemene gegevens

1.1	Titel van het project	Werkingsmechanismen van hersenstimulatie therapieën na een experimentele stroke.

2 Gebruik dieren

2.1	Welke diersoorten zijn gebruikt?	Ratten
2.2	Hoeveel dieren zijn gebruikt?	138
2.3	Wat is het werkelijke ongerief dat de dieren hebben ondergaan?	Terminaal: 51 ratten (37.0%) Licht: 16 ratten (11.6%) Ernstig: 71 ratten (51.4%)

3 Opbrengsten

3.1	Wat zijn de belangrijkste opbrengsten van het project?	Hersenstimulatie kan een verbetering in functioneel herstel na beroerte geven. Veelbelovende technieken zijn transcraniële magnetische stimulatie (TMS), transcraniële direct current stimulatie (tDCS) en chemogenetische manipulatie. Met deze technieken worden hersencellen tijdelijk meer of minder actief door middel van een magnetische veld (TMS), elektrische stroom (tDCS) of door manipulatie van speciale receptoren in het brein (chemogenetische manipulatie). TMS is gebruikt bij een groep ratten om te zien of en waar de behandeling de hersencellen activeert. Na een aantal voorbereidende experimenten, heeft dit uiteindelijk geresulteerd in de waarneming dat dit inderdaad het geval is, afhankelijk van de positie van de TMS spoel. Daarnaast is een groep ratten met een beroerte gedurende de herstelperiode behandeld met TMS. De resultaten van dit onderzoek worden nog bestudeerd. tDCS is mogelijk gemaakt voor toepassing in ratten terwijl er tegelijkertijd gedragstaken door de dieren werden uitgevoerd. Dit zou de effectiviteit van de behandeling groter moeten maken. Helaas werden er

geen effecten van de behandeling op de activering van de hersenen vastgesteld.

Tenslotte zijn er nog experiment uitgevoerd waarbij de omzetting van suikers in de hersenen werd bestudeerd na een beroerte. Omdat de hersenen dan tijdelijk een tekort aan zuurstof hebben gehad worden suikers in het gebied van de beroerte omgezet tot melkzuur. De verwachting dat deze verandering weer zou worden omgekeerd als zuurstof dit deel van de hersenen weer kan bereiken lijkt bij deze experimenten niet waar te zijn. Ook 24 uur na beroerte lijkt er nog steeds melkzuur geproduceerd te worden.

4 Nieuwe inzichten

4.1	Zijn er nieuwe inzichten die kunnen leiden tot vervanging, vermindering en/of verfijning?	Er zijn geen nieuwe inzichten verkregen met betrekking tot vervanging of vermindering. Er zijn wel gedurende het project nieuwe inzichten verkregen met betrekking tot verfijning. Alle dieren krijgen voortaan weekvoer aangeboden na de operatie, waardoor ze beter herstellen. Tevens is er een verscherpte monitoring van het welzijn van de dieren na een operatie ingevoerd. Hiervoor zijn speciale formulieren ontwikkeld, waarmee ook de dierverzorgers in een oogopslag kunnen zien hoe de dieren eraan toe zijn na de beroerte. Op deze manier kan er sneller actie ondernomen worden zodra het welzijn van een dier dreigt te verslechteren.

5 In te vullen door CCD

Publicatie datum	5-9-2024
Andere opmerkingen	