Centrale Commissie Dierproeven

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 on the project proposal, see the Guidelines to the project licence application form for animal procedures on 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'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 3.4.4.1 Type of animal procedure: 10.2.g recordings from awake-behaving monkeys

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.

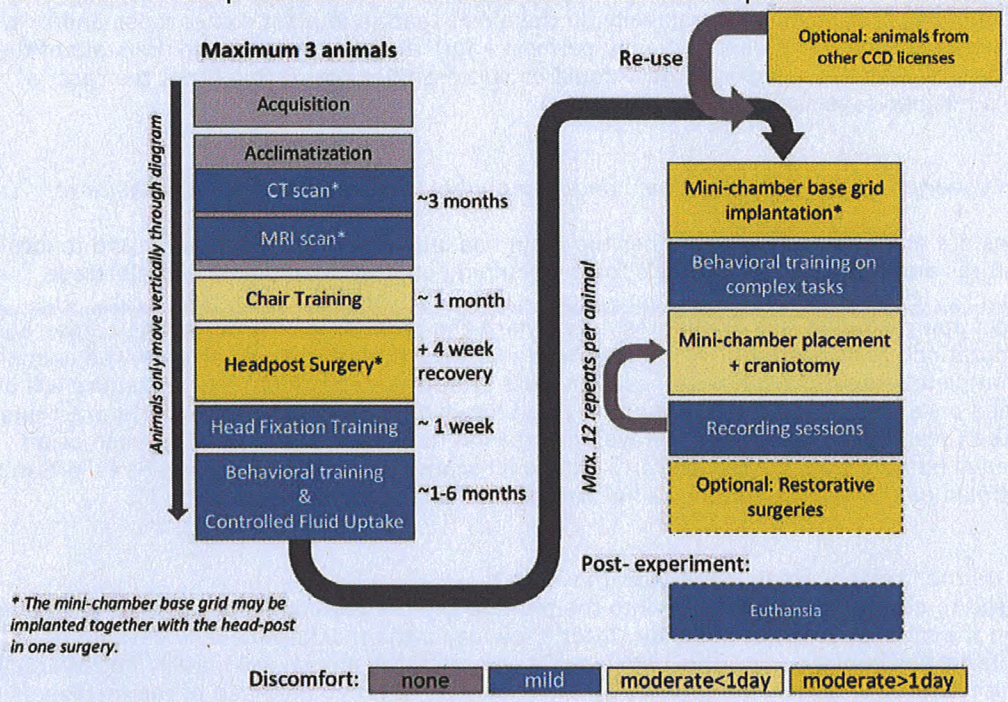
The experiments described in this appendix are designed to address how distributed brain circuits process information in a visual cognitive task.
Our approach involves in the following steps: 1) Using neuroimaging techniques to create a custom 3D printed head-post, base-grid, 10.2.g . 2) Implant the head-post and the base-grid (in either a single or separate procedures) and allow time for integration with the skull. 3) 10.2.g
4) Making a series of electrophysiological recordings using 10.2.g while the monkey performs the same visual cognition tasks. 5) Repeating steps 3 and 4 to gain coverage of many different cortical and sub-cortical brain regions (maximally 12 times, covering a full hemisphere). The whole procedure (steps 2-5) will take approximately 2-3 years.

The main outcome parameters are i) The behaviour of the animal on the visual task including eye-movements, accuracy and reaction times ii) Single-neuron activity from multiple brain areas during performance of the task.

The data will be analysed by relating the neural activity to the behaviour using advanced regression, machine learning and deep learning techniques. The timeline of an animal through the procedures described in this appendix is shown in the flow diagram below. The maximum of 12 repetitions in the righthand column of the scheme will span approximately 2-3 years.

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

An overview of the procedures and the associated discomfort is presented in the flow chart



Acquisition and housing
Monkeys (F2 or later generation) will be obtained from a licensed breeding facility. In all cases, we will first try to obtain animals from a 10.2.g (most likely 10.2.g Only under exceptional circumstances (no monkeys available at the primate center) we will get them from a licensed importer. Monkeys will be housed in the primate facility of our institute. All animals are male and typically between 3-5 years of age when they arrive. This is the age at which young male monkeys typically leave their social group. New animals are paired with established members of the group whenever possible to maximize social housing. For this process, we consult an ethologist from a national primate centre who advises us on appropriate pairings. This can be desirable to form stable pairings or larger groups (if the social character of the animals allows it). Our facility contains large cages, and the monkeys will have access to a floor-to-ceiling play cage, which allows them to climb and swing. The play cage also contains a 'look-out' platform where the monkeys can view other monkeys in the facility. The environment will be enriched with toys (e.g. boxes filled with nuts or sweets, which the monkeys can fiddle out) and access to natural daylight. A TV screen shall be running in front of the cages during the day. A logbook will be maintained individually for each of the monkeys, carefully monitoring their general appearance, their eating behaviour, weight, and the performance during the training sessions.

Acclimatization
Discomfort: Mild or none
The monkeys will be adapted to the animal housing facility and the staff. This includes but is not limited to an initial period in which the animal will be housed with a partner, will receive daily food treats from the staff, and will have access to toys in his cage and television. Previously acquired monkeys in the facility have successfully undergone this period of adaptation and interact well with the staff and do not exhibit signs of stress due to their environment. During this period, the monkey will receive a CT and MRI scan (see below). During one of these procedures, the monkey will also be fitted with a collar, which is later used for guiding the monkey into a primate chair.

CT scanning
Discomfort: Mild (recovery from brief anaesthesia), once per animal (unless complications require re-assessment)
A CT scan is obtained to allow 3D models of the monkey's skull to be constructed. These are used to custom design surgical implants which perfectly fit the skull of the animal. The monkey is anesthetized in its home cage with an intramuscular injection, and then transferred to the CT scanner. The scanning procedure lasts less than 5 minutes. The monkey is then returned to his home-cage, where it recovers from anesthesia. The

total duration of the procedure is approximately 30 minutes. When there are no complications, this procedure is only done once per animal. Occasionally, in the event that an implant comes loose and/or we suspect a problem with the underlying bone, we may perform a further CT scan to assess the state of the bone tissue and inform us whether a repair surgery could be successful or not. If this is not the case, a humane end point is reached (see section I).

MRI scanning
Discomfort: Mild (recovery from brief anaesthesia), once per animal (unless complications require re-assessment)
Structural MRI scans are obtained to check whether the brain has any anatomical anomalies, and to localize specific brain structures and (larger) blood vessels for the planning of surgical implants and electrode trajectories. The monkey is anesthetized in its home cage and then transferred to the MRI facility. The anatomical scan lasts approximately 15-30 minutes, after which the monkey is returned to his home-cage to recover. The total duration of the procedure is approximately 60-90 minutes and we will allow the animal at least 24 hours to completely recover from anesthesia. If there are no complications, this procedure will only need to be performed once per animal. For the most-detailed localization of blood vessels, a contrast agent-agent can be injected (described in SOP: MRA). If we have reason to suspect brain damage, such as an infection or intracranial hemorrhage, we can perform additional scans for diagnostic properties to inform a potential treatment plan and/or assess whether a humane endpoint is reached (see section I).

Chair training
Discomfort: Moderate the first 1-2 times, none after this.
The collar will be used to gently pull the monkey into the primate chair. Food and liquid rewards will be used in order to condition the monkey to enter the chair. After a few days, the monkeys usually get into the primate chair voluntarily and rapidly. Once this behaviour is acquired, the animal will initially be rewarded with fruit or fruit juice for sitting quietly in the chair for short periods of time. The head of the monkey is not fixated at this stage. The time spent in the chair will gradually be increased as the animal becomes ever more comfortable and will be adjusted according to the animal's behavioural reaction. We consider chair training to be complete when the animal can calmly sit in the chair for more than an hour.

Surgery: Head-post implantation
Discomfort: Moderate for 1-2 days, becoming mild for 2-3 days.
All surgeries are performed in the purpose-built primate operation room within the primate facility of our institute. Specialist anaesthetic equipment is available and the surgeries are performed by trained staff. In order to head-fix our monkeys during training, a head-post is attached to the skull. After induction of anaesthesia, an incision is made in the skin, and the skin is gently pulled aside, exposing the area of the skull above the cortex. We have refined the method by 3D printing the head-post in titanium according to the shape of the skull as determined with the CT. As a result, the procedure is short (lasting approximately 1-2 hours) and the implant usually becomes integrated in the bone. Once the head-post is attached the skin is sutured closed. Analgesics are given during the surgery. At the end of the surgery, the animal is monitored and kept warm while waking up. Additional analgesics are given during the recovery period. Following the surgery, training will be discontinued for at least four weeks so that the animal may recuperate. After this time, the head-post is solidly fixed to the animal's skull. Note that this operation may be combined with the implantation of the base grid described below, in which case it is expected to take approximately 2-3 hours. The choice of whether we can combine the procedures depends on the animal's history and our initial experience with the implantation. It is conceivable that animals that are re-used from a different project will already have a head-post. Furthermore, since the base-grid implantation constitutes a new type of procedure we will likely implant it in a dedicated procedure the first time. Based on this experience, we will consider whether it would be feasible to implant the head-post and base-grid in a single procedure in other animals.
After every surgical procedure, the animal is closely monitored for clinical signs and if necessary, a veterinarian is consulted and appropriate action is taken (see also H).

Head-fixation training
Discomfort: Mild (decreasing to none after 1-2 training sessions), session lasting up to one hour.
The animal will receive food and juice rewards for sitting calmly in the chair with their head fixated via the implanted head-post. The amount of time spent fixated in the chair will increase progressively and will be modulated according to the behavioral reaction of the individual animal. Once the animal quietly sits in the chair with his head fixed for a sufficient period of time (0.5 hours), the animal will begin training on the basic experimental tasks. This step usually takes about a week, with the discomfort of the procedure being mild for the first one or two times that the animal is fixated, and lower after this.

Behavioural training on basic tasks

Discomfort: Mild

To motivate monkeys to perform their task, they are placed on a fluid-control regime (described below). During training, the monkey is presented with sensory stimuli and responds with an eye movement and/or hand movement. We use positive reinforcement to train the animals, correct responses are followed by a fluid reward and the animals can work until satiated. The size of the reward is individually determined and is adapted throughout the training session to ensure that the monkeys remain motivated to work. No negative reinforcement is used, incorrect trials are typically followed by a lack of reward, and in some cases a small 'timeout' (5-10 s) may be given. As the monkeys learn the paradigm and their performance increases, we gradually make the task more challenging. Task difficulty is adjusted to ensure that the monkeys can obtain their full fluid ration during the training session. During the training periods, animals are typically in the setup 5 days per week, 1-4 h per day (typical is 2-3 h, but we use a maximum of 4 h) during waking hours. Training on the initial tasks takes between 2-6 months depending on the monkey. An example of a training task is to have the monkey direct their gaze to a very small region of a computer screen for 1 s (known as 'fixation'). The difficulty of the task slowly increased by gradually decreasing the size of the area that the animal must fixate upon while slowly increasing the duration of the fixation. At the end of the training period, the animal is expected to be able to fixate in a 1º diameter window for at least 400 ms and perform delayed saccade-tasks. A saccade is a very rapid eye-movement that monkeys and humans make approximately 3 times per second to direct the eyes to objects of interest. In a delayed-saccade task the animal must wait for a 'go' cue (e.g., the fixation dot changes colour) before making his eye movement. Most animals are also trained to make hand-movements in response to particular visual stimuli during the training period. The duration of this training period varies from 1 to 6 months depending on the aptitude of the monkey. In our experience, all monkeys are able to learn these tasks within 6 months.

Controlled fluid uptake

Discomfort: Mild

To motivate the animals to work their access to fluid is controlled in accordance with the NCad guideline on 'motivation through restriction' (2018) (also described in SOP: Controlled Fluid Uptake Protocol). The main reason why we use controlled fluid- uptake is that we need to obtain a sufficiently large number of trials per session, for two reasons. First, we need reliable measures of the animals' perception which demands a large number of trials. Second, we obtain a larger number of trials to study the activity of neurons. The activity of neurons is inherently stochastic, i.e., the responses of a cell to repetitions of the same stimulus are variable, a stochasticity that is inherent to proper brain function. Controlled fluid access is by the far the most common method to motivate animals to perform cognitive tasks. We note that only healthy and cooperative monkeys that are at ease will perform these tasks in which they make eye or arm movements. Alternative methods have been explored as described by a workgroup for the British NC3R center (National Center for Replacement, Refinement and Reduction of Animals in Research) (Prescott et al., 2010). These alternatives comprise 1) positive reinforcement with fruit juice, without controlled fluid uptake; 2) food-based reinforcement; 3) electrical stimulation of reward centers in the brain. Reinforcement with juice in the absence of controlled fluid uptake works well in the early stages of training when training sessions are short and tasks simple, but it is insufficient to motivate the animal to perform more difficult tasks or a larger number of trials. Food reinforcement with treats like raisins or peanuts is used in our lab to reward an animal for compliant behavior (for instance, for coming to the correct compartment of the cage to interact with a researcher). It can also be used as reinforcement for short and simple tasks at the start of training. However, animals satiate quickly when rewarded with food and chewing movements cause artifacts in our recordings, which makes this type of reinforcement unsuitable for sessions that require many trials and precise recording of neural activity. Electrical stimulation of the reward centers involves an extra surgery with the accompanying risks of complications and direct electrical stimulation of the basal ganglia may interact with the neural processes that are the main focus of this application.

Controlled fluid-uptake is thus the only viable method available to obtain sufficient numbers of trials to be able to reliably measure behavior and neural activity. We implement controlled fluid-uptake in a gradual fashion that adapts the level of fluid control to the behavior of each individual. We begin with positive reinforcement using fruit juice without any controlled fluid uptake. We only use fluid control regimes if the animal is not sufficiently motivated to perform the task with no fluid control. We gradually introduce the fluid control with the aim to have the animals drink as much fluid as possible. Nevertheless, in the majority of animals it is necessary to restrict access to fluid to some level to obtain enough trials on the complex behavioral tasks described in the application. The amount of fluid control is individually determined for each monkey and we always begin by training animals without any fluid control. Most animals require some level of restricted access to fluid to motivate to perform behavioral tasks, and almost all animals require restricted access to motivate them to work on complex tasks. Our aim is to allow the animal to drink fluid

during performance on the behavioral task until they are satiated.

This is achieved by:

- The difficulty of the task is adjusted on each day so that the animal is able to receive fluid at a high rate, motivating him to work for more trials, and drink more fluid in total.
- The rate of fluid delivery is slowly increased during a training session to ensure that the animals drinks throughout the session.
- If the training session has to be aborted, for example due to a technical fault, then the animal receives fluid equivalent to the average intake during a training session.
- We investigate the preferences of each animal for particular rewards e.g., apple juice, different types of fruit syrup, or water, and use a reward that is appealing to the animal

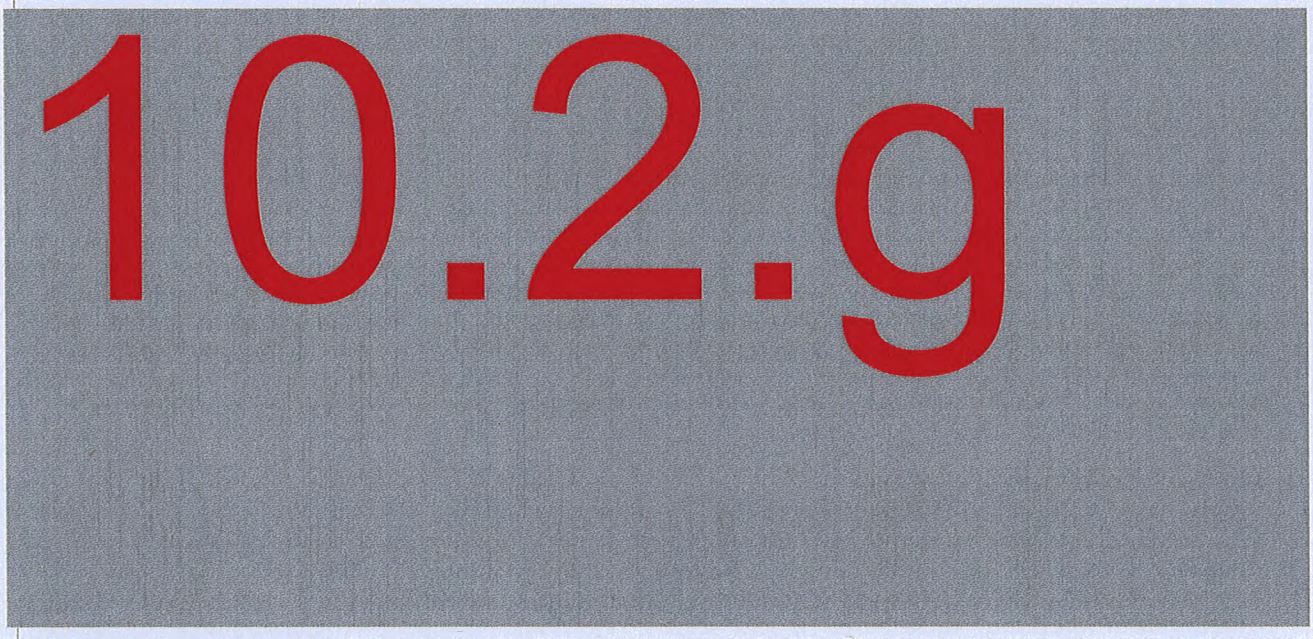
In accordance with the NCad-guideline on 'motivation through restriction' (2018), we take a number of measures to prevent dehydration:

- The monkeys always receive a minimum of 17 ml per kilogram metabolic weight per day. Metabolic weight is weight in kilograms raised to the power of 0.75 and more accurately reflects the monkey's fluid requirements as heavier monkeys require proportionately less water than lighter monkeys. For example, a 10 kg animal must receive a minimum of: $10^{0.75}$ x 17 = 96ml of fluid per day. If this amount is not reached during the training session, it is supplemented.
- Averaged over a three-day period, the animals must receive a minimum of 35mg per kilogram metabolic weight per day, this number is based on recommendations by the British N3CR (National Center for the Replacement, Refinement, and Reduction of Animals in Research) (Prescott et al., 2010) and the primate facility of UC Davis (2001). For example, a 10kg animal must receive a minimum of: $10^{0.75}$ x 35 = 197ml of fluid per day, averaged over the previous three days. If this average is not achieved, the animal is supplemented with fluid. This is a minimum amount and the animals typically receive much more fluid than this.
- Fluid intake, both received during training and supplemented in the cage, is monitored daily and logged in an electronic system accessible by researchers, caretakers and inspectors.
- The animal is provided with fruit after the training session, the liquid content of the fruit is not counted towards the minimum amount.
- During breaks in the training schedule of more than one day (e.g., weekends) the monkey receives a full water bottle of at least 700ml, animals over 15kg receive an extra bottle. If the break is only one day, then the animal receives an amount of fluid equal to what it would typically receive during a training session.
- While the animal is under fluid control, the researchers and animal caretakers monitor its appearance and behaviour carefully every day, with checks by the animal caretakers during the weekend. We weigh the monkey before and after training and compare the weight to the average weight during the last week. The weight is also checked over longer intervals to prevent a slow loss of weight. We check the monkey for any signs of dehydration such as reduced skin tension, sunken eyes, either increased or reduced activity, dry faeces. If any of these welfare criteria is abnormal, the monkey is taken out of training and provided with ad libitum access to fluid until it has recovered. In that case, the Animal Welfare Body will be informed so that they can check the animal. This has not happened in the previous 10 years. These criteria (weight, fluid consumed per day) are logged in an electronic system for each monkey so that the history is accessible.
- The animal receives a non-working period once every 9 weeks (on average over a year). During this period the animal is not trained and receives a full bottle each day (>700ml).

The British NC3R center investigated in 2010 the use of controlled fluid regimes in brain research with macaque monkeys (Prescott et al., 2010). Their conclusion was that, when a controlled-fluid protocol is carefully applied and monitored, there are no negative consequences for the health of the animal. Follow-up research from the University of Newcastle (Gray et al., 2016) showed that controlled fluid uptake for 7 days per week did not lead to abnormal blood values or signs of dehydration. Another study (Hage et al., 2014) analyzed a broad range of behaviors over several months during fluid control and found no evidence for alterations in behavior, which indicates that the animals' wellbeing can be stably ensured during training sessions with a proper protocol. Indeed, from their general appearance, it is very difficult, if not impossible, to distinguish between monkeys under fluid control and monkeys with ad libitum access to water. Furthermore, the animals are seen regularly by a veterinarian to inspect their general condition, and we investigate measures of kidney function during the yearly checkups by means of a blood test that measures creatinine and urea concentrations. We have never obtained indications of impaired kidney function. Hence, our own experience is in accordance with the literature, which indicates that a careful protocol of controlled fluid uptake is a safe and effective manner to motivate animals to perform the required cognitive tasks.

Animals will be on this detailed controlled fluid uptake protocol for the duration of their training and the recordings, which are estimated to add up to 3-4 years.

Surgery: Base-grid implantation
Discomfort: Moderate for 1-2 days, becoming mild for 1 week.
A surgical procedure is carried out under general anaesthesia to implant a custom 3D printed base-grid onto the skull. The base-grid is a thin titanium structure which is designed to integrate into the skull, underneath the skin (Figure 1A-B), and forms the basis for later placements of miniaturized recording chambers ('mini-chambers', Figure 1C-D). The use of a grid greatly improves the stability of the recording chamber as the chamber is fastened to the base-grid using titanium screws. After induction of anaesthesia, an incision in the skin will expose the skull. The base-grid will be attached to the skull using screws and the skin will be re-sutured above the base-grid. The total duration of the procedure is approximately 2-3 hours. Note that the placement of the grid may be combined with the implantation of the head-post to reduce the total number of surgeries. The first time we implant a base-grid we will likely do it in a dedicated procedure (which could be in a re-used animal that already has a head-post). Based on this initial experience, we will consider whether it would be feasible to implant the head-post and base-grid in a single procedure in other animals. After every surgical procedure, the animal is closely monitored for clinical signs and if necessary, a veterinarian is consulted and appropriate action is taken (see also H).



Behavioural training on complex tasks
Discomfort: Mild, 1-5 times a week
Once the animals have completed their initial training, specialized training will begin on the texture-defined curve-tracing task. The animal will be trained to either make an eye-movement towards the end of the target curve or make a hand-movement depending on the colour/shape of a target located at the end of the curve. There will also be control conditions in which the animal will perform a different task presented close to the fixation point and ignore the curves. The animals are expected to learn new tasks in 1-6 months. When animals are trained to execute a new task, we aim to keep their performance well-above chance to keep them motivated while we make incremental changes to a known task. Training progress is discussed in weekly meetings and any lapses in performances are typically addressed with small changes that make the current task easier or more familiar to the animal.

Surgery: Mini-chamber placement and craniotomy
Discomfort: Moderate for 1 day, becoming mild for 1-2 days.
A surgical procedure is carried out under general anaesthesia to place a mini-chamber 10.2.g The animal receives appropriate analgesics during and after the procedure. The skin above the selected base-grid position is opened and the mini-chamber is screwed into the base-grid. A series of 5-15 small holes of 1-2 mm ('craniotomies') in diameter are then drilled into the skull, leaving the

underlying *dura mater* intact. The burr-holes will be sealed with sterile plastic pegs. 10.2.g [redacted] . The total duration of the procedure is less than 1 hour. 10.2.g [redacted]

In previous projects, we have used standard single recording chambers with a large craniotomy that are typically implanted for a period of many months. Maintenance of these conventional chambers requires dura thinning procedures under general anaesthesia, 10.2.g [redacted] This chamber 10.2.g procedure requires only a small incision of the skin and has a comparable discomfort level as the dura thinning procedure. The combination of small burr holes with a titanium implant is furthermore expected to give rise to a healthy interface between the skin and the implant and a lower probability of infections within the recording chamber.

Awake recording
Discomfort: Mild, 1-5 times a week.
The awake electrophysiological recording sessions are similar to the behavioural training sessions, with the exception that a 10.2.g is lowered to a predetermined depth in the brain at the start of the session and the animal is connected to the recording equipment. The plastic peg sealing the burr-hole will be removed and the dura will be penetrated with a guide-tube which causes a very brief period of mild discomfort (in a small percentage of animals). The probe is then slowly lowered through the guide-tube into the brain which causes no discomfort. The animals will then perform the task as described above. The duration of each session will be between one and *maximally four hours*. The precise duration will be adjusted based on animal motivation and performance. On some sessions we may dip the probe in a coloring agent to facilitate post-experiment histological verification of trajectories. Animals are generally either in a recording session or being trained (on variants of the task, or to maintain performance) 5 days a week. The total phase of the recording sessions will be approximately 2-3 years, depending on animal performance and recording success. Over the course of the project, the number of penetrations will be 10.2.g This is in the same order of magnitude as is traditionally done with a single large recording chamber and metal electrodes, but distributed over a larger volume of brain tissue and with much thinner probes, both aspects that reduce potential tissue damage.

Surgery: Restorative surgeries
Discomfort: Moderate for 1-2 days, becoming mild for 3-4 days.
In rare cases the head-post or base-grid may become loose. A repair surgery is then performed to prevent failure of the implant. The repair surgery is performed under general anaesthesia with appropriate analgesia. The nature and duration of the repair surgery depends upon the type of implant and the extent of the problem which may be assessed by a CT scan. An individual monkey can undergo a maximum of two restorative surgeries per implant during the course of these procedures, which could result in maximally 4 procedures. Repair surgery will always be performed in consultation with the Animal Welfare Body (IvD) and (if necessary) the veterinarian.

Perfusion
Discomfort: Mild or none
Histological analysis needs to be performed to verify the location of electrodes. The monkey will be euthanized at the end of the experiment. The animals are euthanized by an overdose of barbiturates. The monkeys are cardially perfused with fixative and the brain isolated for further analysis of probe trajectories.

Annual health-check
Discomfort: Mild (recovery from brief anesthesia).
Once per year, each animal in our facility is checked by the veterinarian to assess their general health and appearance and to take blood/urine samples for further testing. In this way, the long-term health of the animals is closely monitored. The animal is lightly anesthetized during this procedure, which takes 10-15 minutes per animal.

References

Gray et al., 2016. Physiological, Behavioral, and Scientific Impact of Different Fluid Control Protocols in the Rhesus Macaque (*Macaca mulatta*). eNeuro 3(4).
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Netherlands National Committee for the protection of animals used for scientific purposes, 2018. Motivation by restriction?: Starting points for controlled fluid and food intake in neurocognitive research from a 3Rs perspective. Opinion Report.
Prescott M.J., Brown V.J., Flecknell P.A., Gaffan D, Garrod K, et al., 2010. Refinement of the use of food and fluid control as motivational tools for macaques used in behavioural neuroscience research: Report of a Working Group of the NC3Rs. J. Neurosci. Methods 193, 167–88
University of California Davis, 2001. Policy statement: water restriction in rhesus behaviour studies. UC Davis Office of Environmental Health and Safety.

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

We ask permission to use three monkeys. In neurophysiological primate research, the absolute minimum number of animals that can be used to check for consistency across animals is two, which is also the broadly accepted norm. Statistics are performed across neurons. Due to the use of 10.2.g we anticipate recording from many 10.2.g in an individual animal yielding an extremely high statistical power. In some cases, it is possible that we observe differences in the cognitive strategy of the animal when performing the task, or that different (contradictory) neural results are found in the two animals. In such cases, we require the addition of a third animal to verify the results. In addition, although we have good experience with most of our monkeys, some individual circumstances may preclude a monkey from being used for a specific experiment (e.g., if he is not able to learn a behavioural task). In such cases, we will also apply for an additional animal. Applications for an extra animal beyond the initial two will be submitted to the IvD of the institute for evaluation and permission.

B. The animals

Specify the species, origin, life stages, estimated numbers, sex, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Sex	Genetically altered	Strain
1	rhesus macaque	national primate center or licensed importer	>3 years (typically 3-5 years)	2 (max 3)	male	no	n/a

Provide justifications for these choices

Species	We will use rhesus macaque monkeys (*Macaca mulatta*) in these experiments. The main aim of this application is to understand the neural processes that underlie visual cognition. This approach requires a species with comparable neuroanatomy to the human and the ability to perform cognitive tasks. The ability of rodents to recognize objects and to perceptually segregate them from the background is limited. Second, eye movements and shifts of attention are very different from those in primates. Primates have a region of their retina with extremely high spatial resolution known as the fovea which is used for all detailed daytime vision. Primates constantly (3 times per second) make saccadic eye-movements so that the fovea of each eye are pointed at interesting regions of the visual scene. Importantly, primates are able to withhold the actual eye-movement allowing them to covertly attend a particular object while keeping their eyes fixated on a different object. In rodents, the control of the eyes is very different. The rodent retina is broadly speaking uniformly sensitive, they have no fovea. This means there is no need to move the eyes to fixate particular regions in the visual scene. Rodents very rarely make eye-movements and if they do these appear to be related to movements of the head or changes in arousal state. It appears therefore that rodents lack a mechanism for visuospatial attention, making it impossible to study this process in these species. Third, other thought processes (such as the capacity to memorize information and hence the cognitive ability of rodents) are more primitive than in primates. Fourth, some brain structures have a different organization in primates than in rodents. For example, the hierarchy of visual cortical areas with its many levels in the monkey is similar to humans. It differs in rodents, in which the

	higher visual areas form a ring around the primary visual cortex and there are only few hierarchical levels (Wang et al, 2011). As a result, many brain regions in primates do not have their counterpart in rodents (Van Essen et al., 2019). Equally large differences are to be found in the subcortex. For example, the subregions of the primate pulvinar, a region of the (subcortical) thalamus, are reciprocally connected to the many visual cortical areas in primates, many of which do not exist in rodents. The same is true for the interactions between the large and well-developed prefrontal cortex in primates and its connections to the basal ganglia, which are less developed in rodents.
Origin	All monkeys are obtained from a national primate center, or in exceptional circumstances (i.e. if no animals are available from the primate center) from a licensed importer. In all cases, monkeys are F2 (or later) generation animals.
Life stages	Monkeys are typically acquired aged 3 years or older.
Number	We estimate that to obtain reliable results we will need data from at least two animals. In cases where the behavioral or neural data is contradictory between the two animals or we are unable to use an individual animal (e.g., they cannot learn the task) we will apply to the institute's Animal Welfare Body (ivD) for the use of a third animal.
Sex	We exclusively use male monkeys in these studies. Our facility houses only male monkeys as males adapt better to living in paired social housing than females and there are no possible complications with breeding that would be present if we would house both male and female animals. The choice for males will not affect the results of the study as it is unlikely that there are differences between the sexes in basic cognitive behaviour and perceptual mechanisms. Males are therefore chosen to allow us to maintain 100% male animals in our facility.
Genetic alterations	
Strain	

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☒ Yes

☐ No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

It is our intention to house monkeys in pairs. Following an implantation surgery, monkeys will be housed solitarily for several days to ensure full rest and recovery. It is also possible that an animal will be housed solitarily for behavioural reasons, i.e. if there is no suitable cage-mate available and attempts at paired housing result in fights. Behavioural dynamics are however continuously monitored (periodically with the help of an expert monkey ethologist) to evaluate potential pairings and minimize solitary housing. In all cases, the cage environment will be enriched by bedding material, swings, toys, and treats. We will monitor their weight and appearance on a daily basis.

Their fluid uptake will be controlled. The measures we take to reduce adverse effects of restricted fluid uptake have been described above.

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

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

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

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

During surgery, anaesthesia and analgesia will be applied as described above. Also, post-surgery analgesics will be administered.

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

1. Infections: There is a rare possibility of infection around the wound area. In these cases, we will apply additional analgesics and/or antibiotics. Visible signs of microbial infection will be monitored. The following will be considered as signs of an unhealthy state of the animal: (a) aberrant behaviour; (b) dehydration; (c) weight loss.

2. Wounds as a result of fighting with other animals: the social housing structure in the animals' home-cage environment is carefully determined (together with experts in macaque behaviour) and closely monitored. However, fights do sometimes occur between animals to assert dominance, either between cage-mates or between animals in neighbouring cages. These fights usually do not cause any, or only minor superficial, damage, but sometimes it may be necessary to clean a wound and stitch it up. We do believe, however, that the benefit of social housing outweighs the discomfort associated with such occasional fights.

3. The occurrence of weight loss due to the controlled fluid uptake and the measures that we take to prevent dehydration have been described above.

4. Insufficient recovery after surgery: applicable if an animal shows permanent weight loss (more than 15%-20% of the weight immediately after surgery for more than 10 days). This has not occurred in the past 10 years.

5. Loosening of an implant.

6. Seizures. In very rare cases (<5%) it may be possible an animal suffers from a seizure, which may be due to brain-swelling after an operation or a side-effect of an infection.

7. Sub-dural bleeding. During operations or electrode penetrations it can occur that a blood-vessel is damaged. Small bleeds typically cease within minutes with no ill effects. Very rarely (<5%) the bleed may be larger leading to neurological symptoms. These typically disappear within a few days. In the case of persistent severe neurological symptoms, the animal is euthanized.

Explain why these effects may emerge.

Surgical implantation of cranial and brain implants is accompanied by risk of microbial infection, tissue rejection, or unwanted growth of granulation tissue that prevents the implant from integrating with the body. The causes of the other adverse effects are described above.

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

1. We expect that the risks of adverse effects will be reduced by the mini-chambers and tiny burr holes which cause much less tissue damage than state-of-the-art recording chambers. Hence, the new type of recording that we will introduce is a form of refinement.

2. We constantly monitor the animal's behaviour, liquid intake, wound area, and physiology. Surgeries are performed under sterile conditions and without any unnecessary delays to minimize the amount of time the animal spends under anaesthesia. Animals will be monitored daily and if adverse effects are present, this will be discussed with the veterinary officer. If necessary, treatment will be initiated (topically or systemically applied medication). Interfaces between biological tissue and implants are cleaned regularly to and recording chambers cleaned every few days to minimize the risk for infection.

3. The social housing structure in the animals' home-cage environment is carefully determined (together with experts in macaque behaviour) and closely monitored. Cage-mates are matched based on age (difference), physical appearance and character to avoid ambiguous dominance structures as much as possible. Social structure, however, is a dynamic feature and changes in dominance do occur over time, for instance with aging. When problems start to arise, we re-assess the situation and change the social housing structure if necessary. In these situations, we ask advice from an expert in the social interactions between

monkeys.

4. The measures to prevent dehydration due to controlled fluid uptake have been described above.

5. We monitor animals carefully after surgery. They are placed under heat-lamps during recovery and given post-operative analgesics. Food and water are freely available in the home-cage. The animals are temporarily housed alone after the surgery to allow proper recovery. All animals are checked once per year by the veterinarian to monitor their long-term health.

6. Beside the reduction in invasiveness of the proposed implants (point 1) we have made several additional refinements to our cranial implants and we continuously review and refine the design of our implants. In the unlikely event that a head post or recording chamber becomes loose or detached, the animal is closely inspected, and may undergo a CT scan to allow us to assess the condition of the bone. If an implant becomes loose/detached from the skull, we reattach the implant in a repair surgery. The repair surgery is performed under anaesthesia and with analgesia in an identical fashion to the original attachment surgery. We estimate the discomfort to be moderate during recovery from the anaesthesia (1 day) becoming mild for 1-2 days. In rare cases (once in the past 10 years), the skull becomes infected, causing moderate discomfort. In these cases, the monkey is immediately euthanized under anaesthesia. We minimize the occurrence of headpost failures by slowly adapting the monkey to being head-fixed in the set-up so that it is relaxed and does not exert strong forces on the headpost while fixed in the setup.

7. If the animal suffers a seizure, anti-seizure medication is given immediately and the underlying cause is treated, e.g. with high-dose corticosteroids or antibiotics. The veterinarian is informed.

8. All operations are performed as precisely as possible by trained staff.

E. 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 F

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

Indicate the likely incidence.

Each animal that undergoes surgery will be monitored for clinical parameters. The monkey will be monitored for its general appearance and activity level. If a monkey has an appearance that gives cause for concern (e.g., signs of infection around the wound, weight loss, or reduction in activity level), we will notify the IvD and evaluate the animal together with the veterinarian. Similarly, if the animal does not recover well from anaesthesia (i.e., does not return to normal behaviour within a few days), we will evaluate the animal together with the veterinarian.

In addition, the weight of the animal will be monitored and if the animal loses 10-15% of their weight in a few days or if the animal loses more than 20% of their weight throughout the course of the experiment then the veterinarian will be contacted, and a decision will be made whether a humane endpoint has been reached. In practice, this point is highly unlikely to be reached. Severe weight loss will almost always occur in combination with changes in behaviour or general appearance, and bases on these parameters, we will consult with the veterinarian at a much earlier point to establish a treatment plan or decide that a humane endpoint is reached.

If a cranial implant breaks off and infection or severe damage to the skull is sustained, the monkey will be immediately euthanized. A detailed assessment of skull integrity can be done on the basis of a CT scan (see above), while MRI can be used to diagnose intracranial trauma or infections. While large trauma or severe infections are reasons to euthanize the animals immediately, smaller traumas or infections might be treated or given time to heal. In that case it may be necessary to obtain multiple CT/MRI at different time-points to follow healing progress or treatment success and re-assess whether a human endpoint is reached.

Based on experience with similar types of experiments in NHP as described here, humane endpoints are

expected to be met in 0-5% of the animals tested within the time frame of the experiments.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

Acclimatization
Discomfort: Mild or none

CT scanning
Discomfort: Mild (recovery from brief anaesthesia)

MRI scanning (initial)
Discomfort: Mild (recovery from brief anaesthesia)

Chair training
Discomfort: Moderate the first 1-2 times, none after this.
The animal experiences some stress the first one or two times, but they very quickly learn to associate the chair with rewards and voluntarily enter the chair after this phase.

Surgery: Head-post implantation
Discomfort: Moderate for 1-2 days, becoming mild for 2-3 days.

Head-fixation training
Discomfort: Mild (but decreasing after 1-2 times)

Behavioural training on basic tasks
Discomfort: Mild

Controlled fluid uptake
Discomfort: Mild

Behavioural training on complex tasks
Discomfort: Mild

Surgery: Implantation of titanium base-grid
Discomfort: Moderate for 1-2 days, becoming mild for 1 week.

Surgery: Placement of 10.2 g
Discomfort: Moderate for 1 day; 10.2 g .

Awake electrophysiological recording
Discomfort: Mild
At this point, the animals are cooperative and experience no discomfort from performing the tasks. They may experience brief mild discomfort from entering electrodes through the dura in electrophysiological recording sessions. Being head-fixed for several hours might also cause mild discomfort, but the animals are gradually accustomed to this experience and generally remain either cooperative (performing their task) or fall asleep.

Surgery: Restorative surgeries
Discomfort: Moderate for 1-2 days, becoming mild for 3-4 days.
Restorative surgeries cause considerably less discomfort than the original implantation surgery as there is no need to cut through the skin and muscle tissue, which have already been removed during the original implantation. Hence, the overall tissue damage is minimal, and the animals recover rapidly. The surgery and recovery from surgery is classified as moderate discomfort (for 1-2 days), becoming mild (for 3-4 days). There is a maximum of two restorative surgeries per implant. The probability is small given that the

titanium base grid integrates well with the bone.

Perfusion
Discomfort: Mild or none

Annual health check
Discomfort: Mild (recovery from brief anaesthesia)

Cumulative discomfort
The monkeys will undergo several surgical procedures, which lead to discomfort at the lower end of moderate that is mostly associated with the recovery from anesthesia, while using an effective pain killing regime, and lasts a maximum period of 1-2 days. Previous research indicated that there is no indication that successive procedures cause cumulative suffering that is more than that caused by the individual procedures and that there is no increase in discomfort through incomplete recovery between events ('stacking') or potentiation of adverse effects and suffering by earlier procedures (Pickard, 2013). Many animals instead showed signs of diminished responses to repeated procedures such as restraint and handling.

Furthermore, we have taken several measures to exclude the possibility that the cumulative discomfort can exceed the moderate level:

(1) Animals are typically pre-selected based on their character and (social) behaviour at the primate center to minimize acclimatization and living-in-captivity distress, and optimize the potential for successful social housing and cooperation in the cognitive tasks.

(2) The behavior and health of the animal is carefully monitored by the researchers, the care-takers and experienced vets and entered in an electronic database, which includes the general appearance of the animal, its weight and the amount of food/drink. To exclude the longer-term ethological and psychological adverse effects on the animals' wellbeing (i.e., not to the animals' medical condition), we will ask the opinion of an expert monkey ethologist on a regular basis. If there is a threat that the future cumulative discomfort level of an individual animal might exceed moderate (for medical or psychological/ethologic reasons), the animal will be taken out of the experiment (rehomed or euthanized) thereby excluding the possibility of severe cumulative discomfort.

(3) Larger surgical procedures (implantation of head post and base-grid) are followed by a minimum of four weeks of recovery. In practice, the interval between successive larger surgeries is a few months, ensuring that the monkeys have fully recovered after the surgeries before any further interventions take place.

(4) Social housing of a high standard. The animals live in stable social pairs in large floor-to-ceiling cages with natural daylight. The cages are enriched with toys and puzzles and the animals engage in their natural behaviors such as grooming, climbing and foraging for food (e.g., for peanuts hidden in the sawdust on the floor). We see no evidence for stereotypical movements or any evidence that long-term housing causes any suffering for the animals.

(5) Controlled fluid protocol approach. Our approach to controlled fluid uptake is to use the mildest form of fluid control necessary to achieve the desired performance of the animal. In the answer to the question above we outlined why the amount of discomfort associated with this procedure is maximally mild.

The (cumulative) discomfort level classification **'moderate'** is also in accordance with (a) the opinion of our vets, who have ample experience with experiments in monkeys and rate the cumulative discomfort as moderate, (b) we inspected the retrospective assessments of the discomfort experienced by monkeys in similar experiments of the previous five years, and found that they have always been in the moderate category, (c) the opinion of the IvD and (d) the welfare evaluations of comparable procedures have resulted in moderate discomfort level. The discomfort has lowered over the years, due to refinements of the implants, anesthesia protocols, measurement techniques and enrichments in the monkeys' environment and we expect that the proposed method to use mini-chambers and tiny burr holes provides a further refinement. Even though this is unlikely, if there is a threat that the future cumulative discomfort level of an individual animal might exceed moderate (for medical or psychological/ethologic reasons), the animal will be taken out the experiment, thereby excluding the possibility of severe cumulative discomfort.

G. 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	The main aim of this application is to understand how distributed brain circuits create the visual percept and solve cognitive tasks. Many studies have approached this question by using cognitive neuroscientific techniques in humans. These studies have provided valuable information about the brain areas which are involved in visual cognition and have led to a number of proposed models. Unfortunately, the temporal resolution of fMRI is not sufficient to track activity in cognitive tasks and its spatial resolution does not allow the monitoring of activity of single neurons. The spatial resolution of EEG/MEG is not sufficient to localize the neural activity to particular brain regions. To fully understand the neural mechanisms of perception and cognition we need a technique with both high temporal and spatial resolution such as invasive electrophysiological recordings. Invasive recordings can only rarely be performed in humans, typically in surgical investigations of epileptic patients who are implanted with depth electrodes to localize the source of the epileptic activity (our lab carries out such studies when possible). In these studies, the location of the electrodes is based purely on clinical criteria, and they are very rarely placed in the visual cortex or in areas involved in attentional control such as the FEF or parietal cortex. They are hardly ever placed in the subcortical structures that will be targeted by us. This makes it impossible to collect sufficient neural data to gain an understanding of the underlying neural mechanisms. It is therefore not possible to replace the experiments described here with experiments in humans. It is also impossible to replace the monkeys in these experiments with rodent models or cats for the reasons outlined above in section B. Briefly, rodents and cats are unable to perform the complex tasks, which are essential to understanding the mechanisms of cognition. Furthermore, some cognitive processes, such as the specific types of eye movements made by humans and monkeys, are absent in rodents. Given these considerations, no alternative to the macaque monkey is available.
Reduction	The number of animals we want to use is the minimum number with which reliable results can be obtained, and no further reduction is possible. The use of 10.2.g which allow recordings from multiple brain areas and capture the activity of hundreds of cells simultaneously, means that we are able to generate a large amount of data in one study. The use of such high-yield techniques will ultimately lead to a reduction in the number of animals that need to be used across different labs. We aim to publish the full data set of the present study online, thereby opening it up for other researchers. Hence, our approach to study the activity across many brain structures in a task that gives insight into perception and attention shifts is expected to become a general resource for other researchers in this field. It will reduce the need of other labs to carry out similar experiments in various brain structures. By introducing a method that allows the sampling of neurons across many brain structures, the protocol will allow us to generate data in 2-3 monkeys that would have required tens of monkeys using more traditional technology.
Refinement	The proposed innovative method 10.2.g is a refinement over the state-of-the-art larger recording chambers, which are usually affixed to the skull using acrylic cement. The titanium integrates with the bone and the mini-chambers and tiny burr holes cause little tissue damage, less than the state-of-the-art larger recording chamber. These state-of-the-art larger chambers also require the occasional thinning of the dura under anaesthesia (~12 times per implant). Dura thinning will not be necessary with the new mini-chambers, 10.2.g are much thinner than traditional metal electrodes reducing the risk of tissue damage. In addition, the location of (larger) blood vessels can be estimated based on MRI scans so that they can be avoided when probes are lowered into the brain. All other procedures (including housing) are refined to minimise discomfort for the animals as much as possible, using the latest knowledge and techniques in animal welfare. We take several measures to refine our experiments. Animals are typically pre-selected based on their character and (social) behaviour at the primate center. This reduces acclimatization times and stress upon arrival in our facility. We further

	decrease the amount of stress by gradually introducing all aspects of the behavioural tasks, and careful conditioning of the monkeys to any novel aspects of our behavioural experiments. We moreover implement an elaborate set of measures (described above) to prevent adverse effects of the controlled fluid uptake. All surgeries are carried out by persons who are well trained and antagonists are used to minimize the discomfort of recovering from anaesthesia. Finally, animals are housed socially in an enriched environment in order to keep them engaged, reduce the discomfort of living in a cage, and improve their cognitive abilities.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☐ No

☒ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

During surgeries, analgesics and anaesthesia are used to minimize pain and suffering. Breathing and temperature will be registered, and level of anaesthesia and warmth of heating-pad will be adjusted as such. Post-surgical analgesics will be administered, and animals will be kept on a warm blanket or under a warm lamp until they wake up. Food and fluids are placed in the home cage to facilitate easy access to food and water. They will be allowed to recover for several weeks following surgery. During a recovery period of at least 7 days post-surgery, behaviour, wound area, and appearance will be monitored daily. In this period, additional analgesics and antibiotics are given to minimize discomfort and the risk for infections.

We constantly monitor the welfare of trained animals, assessing their appearance and behaviour every weekday, with checks by the animal caretakers during the weekend. During periods of behavioural training, the animals are weighed daily and all details concerning weight, appearance, fluid intake and any irregularities are recorded in an electronic database which can be viewed by researchers, caretakers and inspectors.

In the training phase, we gradually habituate the animal to the experimental set-up (chair, experiment room, etc.) to reduce potential stress. Likewise, the duration of recording and training sessions are adjusted to the animal's behavior and motivational state.

H. Re-use

Will animals be used that have already been used in other animal procedures?

☐ No > Continue with question I.

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

The animals used in this project can potentially be transferred from other protocols. We will only consider animals for re-use that were not previously implanted with intracranial implants. Animals that only received a head-post can potentially be selected for re-use. In such cases, re-use is preferable to the acquisition and training of a new animal as it leads to a reduction in the total number of animals. Re-used animals will generally be older than newly acquired animals (>5 years old) and their suitability for re-use will always be evaluated by the IvD.

At the end of the experiments in this project, animals will be sacrificed for histological verification of recording sites, which prohibits additional re-use.

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.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

The proposed experiments are novel and an important step towards understanding visual cognition. The applicants' lab is the frontrunner in this field and the applicants are very familiar with the research literature and the present set of experiments are ground-breaking and have not been performed previously. The research described here is not legally required.

J. Location where the animals procedures are performed

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

☒ No > Continue with question K.

☐ 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.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

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

After the experiments, we will carry out extensive histological analysis to verify the recording locations.

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.

☒ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☒ Yes > Describe the method of killing that will be used and provide justifications for this choice.

The animals will be euthanized by an overdose of barbiturates followed by perfusion with a fixative. This is a painless method that allows recovery of the brain tissue for further analysis.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Van: Info-zbo
Aan: 10.2.g
Cc: 10.2.e
Onderwerp: AVD10.2.g202114411
Datum: dinsdag 19 januari 2021 11:34:45
Bijlagen: OntvangstBevestiging.pdf

5

Geachte 10.2.e

In de bijlage treft u de ontvangstbevestiging van uw **aanvraag AVD**10.2.g**202114411** aan, waarnaar wij gemakshalve naar verwijzen.

Met vriendelijke groet,

Centrale Commissie Dierproeven www.centralecommissiedierproeven.nl
Nationaal Comité advies dierproevenbeleid www.ncadierproevenbeleid.nl
.....

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Centrale Commissie Dierproeven

6

> Retouradres Postbus 93118 2509 AC Den Haag

10.2.g

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Onze referentie
Aanvraagnummer
AVD 202114411
Bijlagen
2

Datum 19 januari 2021
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte 10.2.e

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 18 januari 2021. Het gaat om uw project 10.2.g Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD10.2.g202114411. 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, stuur een e-mail naar info@zbo-ccd.nl of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
19 januari 2021
Aanvraagnummer:
AVD102g202114411

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Centrale Commissie Dierproeven

Gegevens aanvrager

Uw gegevens

Deelnemersnummer NVWA:

Naam instelling of organisatie:

Naam portefeuillehouder of diens gemachtigde:

Straat en huisnummer:

Postbus:

Postcode en plaats:

10.2.g

Gegevens verantwoordelijke onderzoeker

Naam:

Functie:

Afdeling:

Telefoonnummer:

E-mailadres:

10.2.e

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:

Functie:

Afdeling:

Telefoonnummer:

E-mailadres:

10.2.e

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:	15 februari 2021
Geplande einddatum:	15 februari 2026
Titel project:	10.2.g
Titel niet-technische samenvatting:	Het in kaart brengen van de hersennetwerken die betrokken zijn bij visuele waarneming in het primaten brein.
Naam DEC:	10.2.g
Postadres DEC:	
E-mailadres DEC:	

Betaalgegevens

De leges bedragen:	€ 1.397,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Verplichte bijlagen:

[x] Projectvoorstel
[x] Beschrijving Dierproeven
[x] Niet-technische samenvatting

Ondertekening

Naam:	10.2.e
Functie:	
Plaats:	

Centrale Commissie Dierproeven

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10.2.g

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Onze referentie
Aanvraagnummer
AVD10.2.g202114411
Bijlagen
2

Datum 19 januari 2021
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 19 januari 2021
Vervaldatum: 18 februari 2021
Factuurnummer: 2114411
Ordernummer: 10.2.e

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD10.2.g202114411	€ 1.397,00

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 te 's Gravenhage.

7

Van: Info-zbo
Aan: 10.2.g
Cc: 10.2.g
Onderwerp: factuur AVD10.2.g202114411
Datum: dinsdag 19 januari 2021 11:37:39
Bijlagen: Factuur AVD10.2.g202114411.pdf

Geachte heer of mevrouw,

In de bijlage treft u de factuur van uw **aanvraag AVD10.2.g202114411** aan, waarnaar wij gemakshalve naar verwijzen.

Met vriendelijke groet,

Centrale Commissie Dierproeven www.centralecommissiedierproeven.nl
Nationaal Comité advies dierproevenbeleid www.ncadierproevenbeleid.nl
.....

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E: info@zbo-ccd.nl

8

Van: info@zbo-ccd.nl
Aan: Kasbeheer
Onderwerp: Betaalgegevens AVD10.2.g202114411
Datum: dinsdag 19 januari 2021 11:29:06

Er is een nieuwe aanvraag ontvangen. Hiervoor is een factuur verstuurd. Hieronder de gegevens t.b.v. het opboeken van de factuur.

NAW-gegevens:
10.2.g

Factuurdatum: 19-01-2021
Factuurnummer: 2114411
Aanvraagnummer: AVD10.2.g202114411
Factuurbedrag: EUR 1.397,00

Met vriendelijke groet,

Centrale Commissie Dierproeven
www.centralecommissiedierproeven.nl

.....
Postbus 93118 | 2509 AC | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl

Van: info@zbo-ccd.nl
Aan: 10.2.g
Onderwerp: Verzoek om advies over projectvergunningsaanvraag AVD10.2.g02114411
Datum: dinsdag 19 januari 2021 11:38:56
Bijlagen: 10.2.g

9

Geachte leden van 10.2.g

De Centrale Commissie Dierproeven (hierna: CCD) verzoekt u in het kader van vergunningverlening advies te geven over het project met als titel: 10.2.g en aanvraagnummer: AVD10.2.g202114411.

Uw commissie wordt verzocht op grond van artikel 10.a.2 van de Wet op de dierproeven de aanvraag te beoordelen en een ethische toetsing uit te voeren waarbij wordt afgewogen of de doelstelling van het project, de verwachte voordelen voor mens, dier of milieu en de haalbaarheid van de doelstellingen, het gebruik van dieren en de schade die zal worden toegebracht aan de dieren in de vorm van lijden, pijn en angst kan rechtvaardigen.

Graag ontvangen wij van u bericht dat deze e-mail goed is ontvangen en wanneer u dit advies in de vergadering gaat bespreken.

Voor het in te dienen advies dient de DEC gebruik te maken van de meest actuele versie van het op de website van de CCD gepubliceerde Format DEC-advies en de toelichting daarbij. U dient deze aanvraag vertrouwelijk te behandelen. Voor de communicatie met de CCD dient u gebruik te maken van FileSecure.

De CCD verzoekt u uiterlijk binnen 20 werkdagen, na 19-01-2021, uw advies bij de CCD in te dienen. Indien de aanvraag door uw commissie niet in behandeling kan worden genomen, dient u dit per ommegaande per e-mail aan de CCD te melden.

Ingeval uw commissie tussentijds aanvullende informatie wil inwinnen bij de aanvrager wordt de termijn opgeschort en geeft u in uw advies aan wanneer dit is geweest. Opschorting van de adviestermijn vindt niet plaats ingeval u ten behoeve van uw advies een onafhankelijk extern expert raadpleegt. Mocht u verwachten door een andere reden dan opschorting uw advies later dan 20 werkdagen na 19-01-2021 bij de CCD in te dienen, dan verzoeken wij u dit direct aan de CCD te melden.

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

Met vriendelijke groet,
Centrale Commissie Dierproeven

www.centralecommissiedierproeven.nl

.....
Postbus 93118 | 2509 AC | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl

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Van: info@zbo-ccd.nl
Aan: 10.2.g
Cc: 10.2.e
Onderwerp: Verzoek om advies AVD10.2.g202114411 verstuurd aan DEC
Datum: dinsdag 19 januari 2021 11:39:17

Geachte meneer, mevrouw,

Op 18-01-2021 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "10.2.g met aanvraagnummer AVD10.2.g202114411.

Uw aanvraag is naar 10.2.g gestuurd. Zij zal hierover advies aan de CCD uitbrengen. Als de DEC vragen heeft, zal zij contact met u opnemen.

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

Met vriendelijke groet,

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