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 l cence applicat on form for animal procedures on our webs te (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 ~~eco di gs f o awake behavi g o keys~~Procedures for the preparation and execution of high-density electrode recordings in 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 techn ques to create a custom 3D printed head-post, base-gr d, and mini-chambers. 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) Placing a mini recording chamber on one of the grid pos tions, making a series of small cran otomies during the placement operation 4) Making a series of electrophysiolog cal 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 reg ons 10.2.g The whole procedure (steps 2-5) will take approximately 2-3 years.

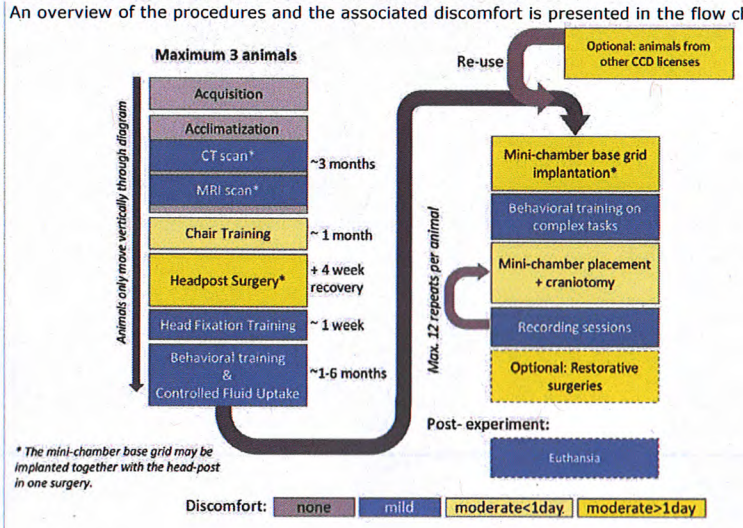
The main outcome parameters are i) The behaviour of the animal on the visual task including eye-movements, accuracy and reaction times ii) Single-neuron activity from multiple brain areas during performance of the task.

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 durat on of the treatment. Provide justif cations 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 facil ty. 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 enr ched 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 indiv dually for each of the monkeys, carefully monitoring their general appearance, their eating behaviour, weight, and the performance during the training sess ons.

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 per od 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 f tted with a collar, wh ch 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 surg cal implants which perfectly fit the skull of the animal. The monkey is anesthetized in its home cage w th an intramuscular inject on, 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 complicat ons, this procedure is only done once per animal. Occas onally, 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 sect on 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 specif c brain structures and (larger) blood vessels for the planning of surg cal implants and electrode trajectories. The monkey is anesthetized in its home cage and then transferred to the MRI facil ty. The anatom cal scan lasts approximately 15-30 minutes, after which the monkey is returned to his home-cage to recover. The total durat on 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 sect on 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 l quid 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 s tting 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 inst tute. Specialist anaesthet c 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 t tanium 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. Add tional analges cs 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 operat on may be combined with the implantat on 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 t 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 behav oral react on 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 typ cally 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 flu d ration during the training session. During the training per ods, animals are typically in the setup 5 days per week, 1-4 h per day (typ cal is 2-3 h, but we use a maximum of 4 h) during waking hours. Training on the in tial 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 per od, 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 rap d 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 fixat on dot changes colour) before making his eye movement. Most animals are also trained to make hand-movements in response to part cular visual stimuli during the training period. The duration of this training per od varies from 1 to 6 months depending on the aptitude of the monkey. In our experience, all monkeys are able to learn these tasks w thin 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 restrict on' (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 wh ch 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 repetit ons 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 Br tish NC3R center (National Center for Replacement, Refinement and Reduction of Animals in Research) (Prescott et al., 2010). These alternatives comprise 1) positive reinforcement w th fru t juice, without controlled fluid uptake; 2) food-based reinforcement; 3) electrical stimulat on of reward centers in the brain. Reinforcement w th ju ce in the absence of controlled fluid uptake works well in the early stages of training when training sess ons 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 qu ckly 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 stimulat on of the reward centers involves an extra surgery with the accompanying risks of complicat ons and direct electrical stimulation of the basal ganglia may interact with the neural processes that are the main focus of this applicat on.

Controlled flu d-uptake is thus the only viable method available to obtain suff cient numbers of trials to be able to reliably measure behavior and neural activity. We implement controlled fluid-uptake in a gradual fash on that adapts the level of fluid control to the behavior of each indiv dual. We begin with positive reinforcement using fru t ju ce without any controlled fluid uptake. We only use flu d 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 flu d 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

behav oral tasks described in the appl cation. The amount of fluid control is indiv dually determined for each monkey and we always begin by training animals w thout 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 'motivat on through restrict on' (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 per od, the animals must receive a minimum of 35mg per kilogram metabolic weight per day, this number is based on recommendations by the Br tish 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 flu d. This is a minimum amount and the animals typ cally receive much more flu d 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 sess on, the liqu d 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 flu d control, the researchers and animal caretakers monitor ts 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 tens on, 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 w th ad libitum access to fluid until t 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 behav or, which ind cates that the animals' wellbeing can be stably ensured during training sessions w th a proper protocol. Indeed, from their general appearance, it is very diff cult, 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 condit on, 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 ind cations of impaired kidney function. Hence, our own experience is in accordance with the literature, wh ch indicates that a careful protocol of controlled fluid uptake is a safe and effective manner to motivate animals to perform the required cogn tive 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 t tanium structure wh ch 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 stabil ty of the recording chamber as the chamber is fastened to the base-gr d 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-gr d. The total durat on 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 on one of the locations on the base grid. The animal receives appropriate analgesics during and after the procedure. The skin above the selected base-grid posit on 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 plast c pegs. 10.2.g The total duration of the procedure is less than 1 hour. We will record from a chamber for an average of 2-4 weeks and then replace the chamber at another pos tion. 10.2.g

In prev ous projects, we have used standard single recording chambers with a large craniotomy that are typically implanted for a period of many months. 10.2.g The combination of small burr holes w th 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 w thin the recording chamber.

Awake recording
Discomfort: Mild, 1-5 times a week.
The awake electrophys ological recording sessions are similar to the behavioural training sess ons, 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 per od of mild discomfort (in a small percentage of animals). The probe is then slowly lowered through the gu de-tube into the brain which causes no discomfort. A recording session will start w th a brief standard procedure to characterize some properties of the recorded neurons, such as their receptive fields. The animals will then perform the task as described above. The durat on 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 histolog cal 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 penetrat ons will be about 120-240 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. Histolog cal verification of the trajectories of similar probes in mice required the usage of a dye because the tissue damage was too minimal to observe the trajectories in unmarked tissue (Steinmetz et al, 2018). The NHP probes are furthermore already being successfully used in non-human primates in several labs around the world that report that their insertion is comparable to convent onal electrodes.

Surgery: Restorative surgeries
Discomfort: Moderate for 1-2 days, becoming mild for 3-4 days.
In rare cases the head-post or base-gr d 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 durat on 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 indiv dual 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 w th 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 facil ty 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, wh ch takes 10-15 minutes per animal.

References

Gray et al., 2016. Physiological, Behav oral, and Scientific Impact of Different Fluid Control Protocols in the Rhesus Macaque (*Macaca mulatta*). eNeuro 3(4).
Hage, S.R., Ott, T., Eiselt, A.-K., Jacob, S.N., Nieder, A., 2014. Ethograms indicate stable well-being during prolonged training phases in rhesus monkeys used in neurophysiological research. Lab. Anim. 48, 82–87.
Netherlands Nat onal Comm ttee for the protect on of animals used for scientif c purposes, 2018. Motivation by restr ction?: 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 motivat onal tools for macaques used in behavioural neuroscience research: Report of a Working Group of the NC3Rs. J. Neurosci. Methods 193, 167–88
10.2.e
University of California Davis, 2001. Pol cy statement: water restriction in rhesus behaviour studies. UC Davis Office of Environmental Health and Safety.

Met opmaak: Inspringing: Links: 0 cm, Verkeerd-om: 0,39 cm
heeft opmaak toegepast: Lettertype: Niet Cursief
heeft opmaak toegepast: Lettertype: Niet Vet
heeft opmaak toegepast: Lettertype: (Standaard) Times New Roman, 12 pt, Nederlands (standaard)

Describe wh ch 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 ant cipate recording from many hundreds to thousands of neurons 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 addit on of a third animal to verify the results. In add tion, although we have good experience w th most of our monkeys, some indiv dual circumstances may preclude a monkey from being used for a specific experiment (e.g., if he is not able to learn a behav oural task). In such cases, we will also apply for an additional animal. Applications for an extra animal beyond the initial two will be subm tted to the IvD of the institute for evaluat on and permiss on. Historically, the inclusion of an additional animal has been required in ~25% of studies.

B. The animals

Specify the species, origin, life stages, estimated numbers, sex, genet c 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 l censed importer	>3 years (typically 3-5 years)	2 (max 3)	male	no	n/a

Provide justif cations 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 cognit on. This approach requires a species with comparable neuroanatomy to the human and the abil ty to perform cogn tive 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 in rodents and cats from ~~from~~ those in primates. Primates have a region of their retina with extremely high spatial resolution known as the fovea wh ch 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 reg ons 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. Cats also lack a retina. Third, other thought processes (such as the capacity to memorize information and hence the cogn tive ability of rodents) are more primitive in rodents and cats than in primates. Fourth, some brain structures have a different organizat on in primates than in rodents or cats. 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 hierarch cal levels (Wang et al, 2011). As a result, many brain reg ons 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 cort cal areas in primates, many of which do not exist in rodents or cats. The same is true for the interactions between the large and well-developed prefrontal cortex in primates and ts connections to the basal ganglia, which are less developed in rodents and cats.
Origin	All monkeys are obtained from a nat onal primate center, or in except onal 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 or a humane endpoint is reached before all recordings have been completed) 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 complicat ons 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 t is unlikely that there are differences between the sexes in basic cognitive behav our 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 w th 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 specif c justifications for these choices

It is our intention to house monkeys in pairs. Following an implantat on 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 behav oural 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 wh ch other adverse effects on the animals' welfare may be expected?

1. Infect ons: 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 m crobial 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 w th experts in macaque behav our) 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 superf cial, damage, but sometimes it may be necessary to clean a wound and stitch t 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. Insuff cient 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 w thin minutes with no ill effects. Very rarely (<5%) the bleed may be larger leading to neurolog cal 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 reject on, or unwanted growth of granulation tissue that prevents the implant from integrating w th 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 phys ology. 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 off cer. If necessary, treatment will be initiated (topically or systemically applied medicat on). 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 dynam c feature and changes in dominance do occur over time, for instance w th aging. When problems start to arise, we re-assess the situation and change the social housing structure if necessary. In these s tuat ons, we ask advice from an expert in the social interact ons between monkeys.

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

5. We mon tor 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 mon tor their long-term health.

6. Beside the reduction in invasiveness of the proposed implants (point 1) we have made several add tional 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 medicat on is given immediately and the underlying cause is treated, e.g. with high-dose corticostero ds 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 wh ch 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.

Ind cate the likely inc dence.

Each animal that undergoes surgery will be monitored for clin cal parameters. The monkey will be mon tored for its general appearance and activ ty level. If a monkey has an appearance that gives cause for concern (e.g., signs of infect on 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 w thin 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 decis on 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 w th 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 dec de 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 infect ons. While large trauma or severe infections are reasons to euthanize the animals immediately, smaller traumas or infect ons 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 informat on 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 qu ckly 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 mini-chamber and burr holes; 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 implantat on 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 rap dly. 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 gr d 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. Prev ous research indicated that there is no ind cation 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 (P ckard, 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 possibil ty 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' med cal condition), we will ask the opin on 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 surg cal procedures (implantation of head post and base-grid) are followed by a minimum of four weeks of recovery. In pract ce, 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 enr ched with toys and puzzles and the animals engage in their natural behav ors such as grooming, climbing and foraging for food (e.g., for peanuts h dden 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 opin on 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 evaluat ons 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 enr chments 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 at any moment there is a threat that the future cumulative discomfort level of an indiv dual 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 ever occurring.

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 appl cation 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 ts spatial resolution does not allow the monitoring of activ ty of single neurons. The spatial resolution of EEG/MEG is not sufficient to localize the neural activ ty to particular brain regions. To fully understand the neural mechanisms of perception and cogn tion 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 surg cal investigat ons of epileptic patients who are implanted with depth electrodes to localize the source of the epilept c activ ty (our lab carries out such studies when possible). In these studies, the location of the electrodes is based purely on clin cal criteria, and they are very rarely placed in the visual cortex or in areas involved in attent onal 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 sect on B. Briefly, rodents and cats are unable to perform the complex tasks, which are essential to understanding the mechanisms of cogn tion. Furthermore, some cognitive processes, such as the specific types of eye movements made by humans and monkeys, are absent in rodents and cats. Given these cons derations, 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 , wh ch 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 percept on and attent on 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 tradit onal technology.
Refinement	The proposed innovative method 10.2.g

	[redacted] 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, [redacted] are much thinner than trad tional 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) behav our 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 condit oning 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-surg cal analges cs 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 antibiot cs are given to minimize discomfort and the risk for infect ons.

We constantly mon tor the welfare of trained animals, assessing their appearance and behaviour every weekday, w th 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 irregular ties 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 w th 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 verif cation of recording sites, which prohibits additional re-use.

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

☒ No

☐ Yes > Prov de specific justificat ons 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 dupl cation is required.

The proposed experiments are novel and an important step towards understanding visual cognit on. The applicants' lab is the frontrunner in this field and the appl cants are very familiar w th 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 l cenced 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 prov de justificat ons for this choice.

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

☐ No > Describe the method of killing.

☒ Yes > Describe the method of killing that will be used and prov de justif cations for this choice.

The animals will be euthanized by an overdose of barbiturates followed by perfusion w th 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 t is not feasible to rehome the animals.

14

Van: info@zbo-ccd.nl
Aan: 10.2.g
Cc: 10.2.e 10.2.g
Onderwerp: Aanhouden AVD10.2.g202114411
Datum: woensdag 3 maart 2021 13:50:57

Geachte 10.2.e

Op 18-01-2021 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Large scale networks for visual perception and awareness in the primate brain." met aanvraagnummer AVD10.2.g02114411. 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 moet een realistisch weergave zijn van het project. In de ogen van de CCD worden de welzijnsaantastingen onder het kopje "expected impacts/adverse effects on the animals" niet geheel realistisch/volledig weergeven. Kunt u hier nog vermelden dat de dieren restricties in vochtopname hebben en kunt u kort toelichten wat er bij de metingen gebeurt met de apen?

Onduidelijkheden
De NTS moet een realistische weergave zijn van het project. In de ogen van de CCD worden de welzijnsaantastingen onder het kopje "expected impacts/adverse effects on the animals" niet geheel realistisch/volledig weergeven. Kunt u hier nog vermelden dat de dieren restricties in vochtopname hebben en kunt u kort toelichten wat er bij de metingen gebeurt met de apen?

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 dit bericht op. U kunt dit aanleveren via NetFTP.

Uw aanvraag zal worden besproken tijdens de CCD vergadering van 12 maart. Indien uw antwoord uiterlijk 11 maart is ontvangen zal dit worden meegenomen in de beoordeling.

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

10.2.e
www.centralecommissiedierproeven.nl

Postbus 93118 | 2509 AC | Den Haag

T: 0900 2800028
E: info@zbo-ccd.nl

NIET-TECHNISCHE PROJECTSAMENVATTING

Naam van het project	Het in kaart brengen van de hersennetwerken die betrokken zijn bij visuele waarneming in het primaten brein
NTS-identificatiecode	NTS-NL-804471 v.1
Nationale identificatiecode van de NTS *Veld wordt niet gepubliceerd.*	
Land	Nederland
Taal	nl
Indiening bij EU *Veld wordt niet gepubliceerd.*	nee
Duur van het project, uitgedrukt in maanden.	60
Trefwoorden	aandacht cognitie hersenschors diepe hersenen Neuropixels
Doel(en) van het project	Fundamenteel onderzoek: Zenuwstelsel

DOELSTELLINGEN EN VERWACHTE VOORDELEN VAN HET PROJECT

Beschrijf de doelstellingen van het project (bijvoorbeeld het aanpakken van bepaalde wetenschappelijke onduidelijkheden, of wetenschappelijke of klinische behoeften).	We leven in een complexe wereld waarin we onze aandacht moeten verdelen over vele prikkels en taken. Hersennetwerken verwerken deze prikkels, bepalen waarop we onze aandacht richten, en hoe we reageren op de prikkels. Hiervoor is de samenwerking tussen verschillende gebieden van de hersenen erg belangrijk. De controle van aandacht zorgt er bijvoorbeeld voor dat we ons kunnen concentreren op iets belangrijks, maar ook dat we soms snel kunnen schakelen en onze aandacht op iets anders richten. Aandacht is daarnaast ook de 'poortwachter' van het geheugen. We onthouden de informatie waarop we de aandacht richten en vergeten de rest. De hersennetwerken die visuele prikkels verwerken en deze informatie omzetten in bewustzijn, aandacht en gedrag zijn grotendeels onbegrepen. Zelfs bij de meest eenvoudige taken blijken netwerken van hersencellen in vele gebieden van de hersenen samen te werken. Een aantal van deze gebieden liggen diep in het brein en zijn niet goed toegankelijk voor het meten van de activiteit van de zenuwcellen. Om de rol van deze diepe hersengebieden in de verwerking van visuele prikkels beter te begrijpen, willen we in resusapen de activiteit van grote groepen zenuwcellen in diverse hersengebieden registreren tijdens visuele taken. Dit onderzoek levert gedetailleerde nieuwe inzichten in de circuits die betrokken zijn bij het verwerken van wat we zien. De studie brengt de activiteit van gebieden in de hersenschors en die daaronder in kaart op een wijze die voorheen niet mogelijk was. De gegevens worden toegankelijk gemaakt voor alle onderzoekers zodat zij er hun voordeel mee kunnen doen.
Welke potentiële voordelen kan dit project opleveren? Leg uit hoe de wetenschap vooruit kan worden geholpen of mensen, dieren of het milieu uiteindelijk voordeel kunnen hebben bij het project. Maak, waar van toepassing, een onderscheid tussen voordelen op korte termijn (binnen de looptijd van het project) en voordelen op	Menselijk gedrag wordt in belangrijke mate bepaald door het verwerken van prikkels. Een beter begrip van hoe deze prikkels worden verwerkt, hoe ze onze aandacht kunnen vangen en hoe ze het bewustzijn bereiken is van belang voor beter begrip, en op langere termijn ook het beter behandelen, van neurologische en psychiatrische aandoeningen. De opgedane kennis is voornamelijk fundamenteel wetenschappelijk van aard Deze studie is de eerste waarbij gedetailleerde informatie over de rol van zenuwcellen in een groot aantal samenwerkende hersengebieden tijdens belangrijke hersenprocessen zal worden geregistreerd. Dit zal nieuwe inzichten geven in de hersencircuits voor waarneming, bewustzijn en aandacht. Deze nieuwe kennis zal van belang zijn bij de ontwikkeling van prothesen om bijvoorbeeld blinden een vorm van zien terug te geven. Daarnaast zal kennis over de nog weinig onderzochte hersengebieden helpen bij het begrip van hoe aandoeningen van deze gebieden leidt tot problemen bij de waarneming en tot

lange termijn (die mogelijk pas worden bereikt nadat het project is afgerond).	stoornissen van aandachtsprocessen zoals bij ADHD en autisme. Tot slot onderzoeken we ook hoe een prikkel het bewustzijn bereikt, wat van groot belang is om een onderscheid te kunnen maken tussen de verschillende vormen van bewustzijnsstoornissen die optreden in comapatiënten.

VOORSPELDE SCHADE

In welke procedures worden de dieren gewoonlijk gebruikt (bijvoorbeeld injecties, chirurgische procedures)? Vermeld het aantal en de duur van deze procedures.	Voor de resusapen zal het ongerief matig zijn. Dit matige ongerief ontstaat omdat in de loop van de proef meerdere operaties onder anesthesie nodig zijn. Dit niveau van matig ongerief is telkens van korte duur. De rest van de tijd dat een aap in de proef zit, wordt het dier getraind om gedragstaken uit te voeren en worden metingen gedaan aan hersencellen. Hierbij is het ongerief geclassificeerd als licht.
Wat zijn de verwachte gevolgen/nadelige effecten voor de dieren, bijvoorbeeld pijn, gewichtsverlies, inactiviteit/verminderde mobiliteit, stress, abnormaal gedrag, en wat is de duur van die effecten?	Negatieve gevolgen voor het welzijn van proefdieren zijn 1) ongerief als gevolg van de chirurgische ingrepen verricht onder volledige anesthesie en adequate pijnbestrijding, 2) het ondervinden van stress tijdens het aanleren en uitvoeren van de taken onder gecontroleerde vochtopname. We laten de apen stap voor stap wennen aan alle procedures om stress te verminderen. In dit project zal in een periode van 5 jaar in verschillende hersengebieden van dezelfde dieren worden gemeten. Het ongerief zal met name in de beginperiode van het project optreden als het meetimplantaat chirurgisch wordt aangebracht en de dieren voor het eerst getraind worden. Trainingen en metingen in het verdere verloop van het project geven licht ongerief. In periodes waarin de dieren getraind worden of er metingen plaatsvinden krijgen de apen water of vruchtensap voor het uitvoeren van de taak. Door een zorgvuldig protocol zorgen de onderzoekers ervoor dat de apen altijd voldoende vocht krijgen. Tijdens trainingen voeren dieren taken uit door naar een scherm te kijken en met oog- of handbewegingen te reageren op wat zij zien. Tijdens metingen voeren de dieren dezelfde taken uit, maar wordt via dunne elektrodes in de hersenen de activiteit van hersencellen gemeten. De dieren ervaren geen pijn omdat hersenen zelf geen pijnreceptoren hebben. Na de meting wordt de elektrode weer verwijderd uit de hersenen.
Welke soorten en aantallen dieren zullen naar verwachting worden gebruikt? Wat zijn de verwachte ernstgraden en de aantallen dieren in elke ernstcategorie (per soort)?	

Soort:	*Totaal aantal*	*Geraamde aantallen naar ernstgraad*			
		Terminaal	*Licht*	*Matig*	*Ernstig*
Rhesusapen (Macaca mulatta)	3	0	0	3	0

Wat gebeurt er met de dieren die aan het einde van de procedure in leven worden gehouden?	*Soort:*	*Geraamd aantal te hergebruiken, in het habitat-/houderijsysteem terug te plaatsen of voor adoptie vrij te geven dieren*		
		Hergebruikt	*Teruggeplaatst*	*Geadopteerd*

Geef de redenen voor het geplande lot van de dieren na de procedure.	Om inzicht te verkrijgen in de locaties van elektrodes in het hersenweefsel worden de dieren na afloop van de experimenten onder diepe narcose gebracht en gedood om de hersenen te kunnen onderzoeken onder de microscoop.

TOEPASSING VAN DE DRIE V'S

1. Vervanging Beschrijf welke diervrije alternatieven op dit gebied voorhanden zijn en waarom zij niet voor het project kunnen worden gebruikt.	Studies met niet-invasieve technieken bij mensen, zoals fMRI, leveren waardevolle informatie op over de hersengebieden die betrokken zijn bij visuele waarneming, bewustzijn en de controle van aandacht. Met deze technieken kan echter niet de gedetailleerde activiteit van individuele zenuwcellen worden bestudeerd. Dat is alleen mogelijk door de activiteit van deze hersencellen rechtstreeks te meten met elektroden die heel dicht bij de zenuwcellen komen. Incidenteel is dit mogelijk bij epilepsiepatiënten die tijdelijk elektrodes geïmplanteerd krijgen, maar er zijn ons geen studies bekend over de activiteit van hersencellen in de meeste gebieden die door ons zullen worden onderzocht. Voor dit onderzoek is een geschikt proefdier nodig dat de aandacht kan verschuiven zonder oogbewegingen te maken en met een anatomie van de hersenen die vergelijkbaar is met die van de mens. Knaagdieren worden vaak gebruikt voor neurowetenschappelijke studies, maar hun visuele waarneming is beperkt en zij kunnen aandacht niet richten of verschuiven op visuele objecten, waardoor het onmogelijk is deze processen in knaagdieren te bestuderen. Andere dieren, zoals katten of fretten, kunnen niet getraind worden om de aandacht te verschuiven zonder daarbij een oogbeweging te maken. Resusapen kunnen dit wel. Visuele waarneming en aandachtsprocessen in resusapen lijken op die in de mens en ook de anatomie van de betrokken hersengebieden is vergelijkbaar. Er is voor dit onderzoek daarom geen alternatief voor de resusaap beschikbaar.
2. Vermindering Leg uit hoe de aantallen dieren voor dit project zijn bepaald. Beschrijf de stappen die zijn genomen om het aantal te gebruiken dieren te verminderen en de beginselen die zijn gebruikt bij het opzetten van de studies. Beschrijf, waar van toepassing, de praktijken die gedurende het hele project zullen worden toegepast om het aantal dieren die in overeenstemming met de wetenschappelijke doelstellingen werden gebruikt, tot een minimum te beperken. Deze praktijken kunnen bijvoorbeeld bestaan uit proefprojecten, computermodellen, het delen van weefsel en hergebruik.	Het voorgestelde aantal apen (twee, tot mogelijk maximaal drie) is het minimale aantal dat nodig is om statistisch betrouwbare resultaten te verkrijgen. Voor de metingen wordt een nieuwe methode gebruikt die het mogelijk maakt om tegelijkertijd de activiteit van een veel groter aantal cellen te meten, verspreid over diverse hersengebieden. Voorheen was dit niet mogelijk en zouden voor soortgelijke metingen veel meer dieren en experimenten nodig zijn. Door het gebruik van deze nieuwe techniek kunnen derhalve meer resultaten worden behaald in minder dieren.
3. Verfijning Geef voorbeelden van de specifieke maatregelen (bv. verscherpte monitoring, postoperatieve behandeling, pijnbestrijding, training van dieren) die in verband met de procedures moeten worden genomen om de welzijnskosten (schade) voor de dieren tot een minimum te beperken. Beschrijf de mechanismen om gedurende de looptijd van het project nieuwe verfijningstechnieken in gebruik te nemen.	1) We beperken de hoeveelheid stress zoveel mogelijk door de apen geleidelijk te laten wennen aan alle aspecten van de gedragstaken. Dit is ook van belang voor het welslagen van de experimenten, daar gestreste dieren niet of nauwelijks zullen participeren. 2) De dieren worden getraind via een regime met gecontroleerde vochtopname. Ze krijgen de benodigde hoeveelheid vocht tijdens de training. We hanteren een zorgvuldig protocol om negatieve effecten van de gecontroleerde vloeistof opname te voorkomen. In onze ervaring leidt dit protocol tot gering ongerief en zijn er geen negatieve gevolgen voor de gezondheid. 3) Alle operaties worden uitgevoerd onder anesthesie door personen die goed zijn opgeleid en een ruime ervaring hebben. Na de operaties worden pijnstillers gebruikt om postoperatieve pijn te voorkomen. 4) De dieren worden sociaal gehuisvest in tweetallen in een verrijkte omgeving, om zo het ongerief van het leven in een kooi te beperken en om de cognitieve vermogens te verbeteren. Indien aan het einde van de proeven de dieren, moeten worden gedood, gebeurt dit onder volledige anesthesie.
Licht de keuze van de soorten	Voor dit project worden in 5 jaar tijd twee, mogelijk maximaal drie, resusapen gebruikt. Er is veel

en de bijbehorende levensstadia toe	bekend over de visuele hersenschors in de resusaap en de aap is het enige proefdier met een opbouw van de hersenschors en de gebieden onder de hersenschors die goed vergelijkbaar is met die van de mens. Ook lijken de visuele waarneming en de wijze waarop aandacht wordt aangestuurd voldoende op die van de mens.

VOOR EEN BEOORDELING ACHTERAF GESELECTEERD PROJECT

Project geselecteerd voor BA?	ja
Termijn voor BA	28-02-2027
Reden voor de beoordeling achteraf	
Bevat ernstige procedures	
Maakt gebruik van niet-menselijke primaten	ja
Andere reden	
Toelichting van de andere reden voor de beoordeling achteraf	

AANVULLENDE VELDEN

Nationaal veld 1 *Veld wordt niet gepubliceerd.*	
Nationaal veld 2 *Veld wordt niet gepubliceerd.*	
Nationaal veld 3 *Veld wordt niet gepubliceerd.*	
Nationaal veld 4 *Veld wordt niet gepubliceerd.*	
Nationaal veld 5 *Veld wordt niet gepubliceerd.*	
Startdatum project *Veld wordt niet gepubliceerd.*	
Einddatum project *Veld wordt niet gepubliceerd.*	
Goedkeuringsdatum project *Veld wordt niet gepubliceerd.*	
ICD-code 1 *Veld wordt niet gepubliceerd.*	
ICD-code 2 *Veld wordt niet gepubliceerd.*	
ICD-code 3 *Veld wordt niet gepubliceerd.*	
Link naar de eerdere versie van de NTS buiten het EC-systeem	

16

Van: 10.2.e
Aan: 10.2.e
Onderwerp: DEC advies
Datum: dinsdag 16 februari 2021 08:59:28

Ha beste,

Van aanvraag 10.2.g 2021 14411 is het DEC advies binnen en verwerkt.

Van: 10.2.e
Aan: 10.2.e
Onderwerp: RE: AVD20211441
Datum: maandag 1 maart 2021 21:06:53

Gedaan!
Inderdaad en interessante aanvraag. Ook het punt van de DEC wbt hergebruik. 11.1
Ik zou deze aanvraag ook zeker nog even aan Ferry voorleggen, voor de vergadering. Dan komt ie niet voor verassingen te staan.
Groet!
10.2.e

Van: 10.2.e
Verzonden: maandag 1 maart 2021 17:13
Aan: 10.2.e
Onderwerp: RE: AVD20211441

Is goed, ik zie 'm morgen wel en als er iets onduidelijk is ga ik wel te raden bij één van onze fijne collega's.
Geniet van je vaderschapsverlof de komende weken. Ik hoop dat de bevalling spoedig verloopt en jullie een fijne opstart hebben met z'n vijven.
Groeten 10.2.e

Medewerker behandelen en ontwikkelen
Centrale Commissie Dierproeven www.centralecommissiedierproeven.nl

Postbus 93118 | 2509 AC | Den Haag

T: 0800 7890789
E: info@zbo-ccd.nl

Van: 10.2.e
Verzonden: maandag 1 maart 2021 17:01
Aan: 10.2.e
Onderwerp: RE: AVD20211441

Hoi 10.2.e
Ik ben ermee bezig, ik zet mijn feedback zo dadelijk of vanavond in de map!
Groet! 10.2.e

Van: 10.2.e
Verzonden: woensdag 24 februari 2021 17:03
Aan: 10.2.e
Onderwerp: AVD20211441

Hoi 10.2.e
Ik heb weer een leuke aanvraag waar jij 2e controleur bent:
10.2.g
Ik ben pas maandag weer aan het werk dus je hoeft je niet heel erg te haasten.
10.2.e

Medewerker behandelen en ontwikkelen
Centrale Commissie Dierproeven www.centralecommissiedierproeven.nl

Postbus 93118 | 2509 AC | Den Haag

T: 0800 7890789
E: info@zbo-ccd.nl