Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see 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	Provide the title of the project.	10.2.g

2 Categories

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

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

3 General description of the project

3.1 Background

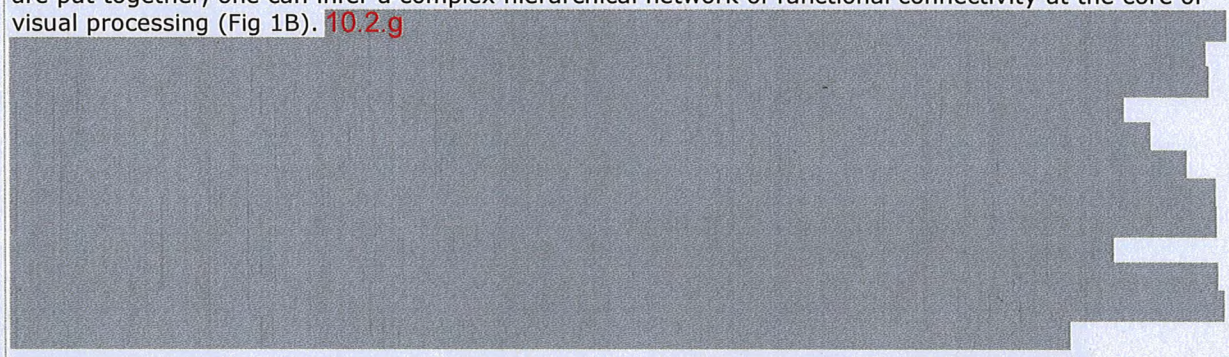
Describe the project (motivation, background and context) with respect to the categories selected in 2.1.

The experiments described in this proposal are designed to understand how processing in a large distributed network of brain areas contributes to fundamental visual cognitive processes. The experiments will be performed in 2, and maximally 3 monkeys.

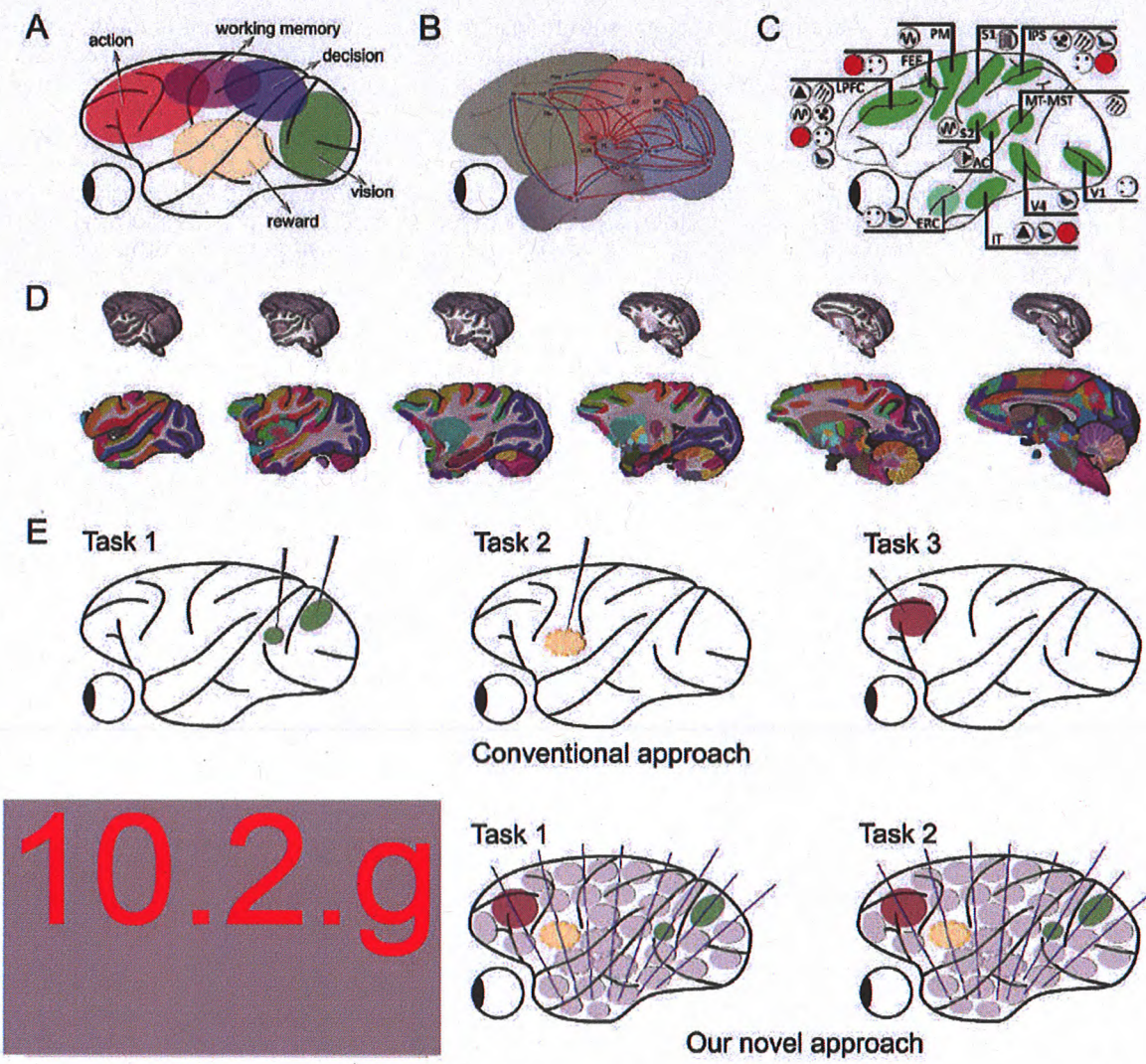
Visual information processing is central to human behaviour. Not only do we rely heavily on our visual system for the perception and analysis of our environment, but vision also strongly interacts with cognitive processes like attention, decision-making, memory, learning, and action selection 10.2.g The importance of the visual modality is reflected in the functional organization of the human

brain, where many brain regions contribute to vision. Traditionally, researchers have assigned primary functions to distinct regions of the brain and investigated their neuronal correlates with dedicated tasks and recordings in one or a few of those specific areas (Fig 1A,E). More recent evidence, however, suggests that cognitive tasks, like perceiving a visual stimulus or planning an action, require interactions between many brain regions, including the cortex but also many subcortical structures. While progress in the field of visual neuroscience has been substantial, understanding of the role of many subcortical regions has been lacking. Indeed, although we have learned about a subset of cortical areas (mainly in the occipital and temporal lobes) that seem primarily dedicated to the processing of visual information, vision is known to depend on or be modulated by neural activity in numerous other areas, including a large variety of subcortical regions.

The first stages of visual information processing already take place in the retina (Baden et al., 2020), which projects to the thalamus where the lateral geniculate nucleus (LGN) relays visual information to the primary visual cortex (V1) in the occipital lobe. Neurons in V1 have small receptive fields and are sensitive to elementary aspects of visual stimuli such as contrast and orientation. From V1, a cascade of feedforward projections to cortical areas result in neurons with larger receptive fields and more complex tuning properties. Feedback projections from these later cortical areas back to earlier areas modulate neural activity, causing effects on neuronal activity that psychologists relate to attention, memory, conscious perception and other cognitive processes. When the results of many different experiments, each with their own unique paradigm and recordings from a limited number of (mostly cortical) areas, are put together, one can infer a complex hierarchical network of functional connectivity at the core of visual processing (Fig 1B). 10.2.g



Whereas the function of several cortical visual areas is rather well-documented, the involvement of many *subcortical* areas (Fig 1D) is less well understood and sometimes completely unknown. In addition to the LGN, there is evidence indicating that some areas like the pulvinar, superior colliculus, amygdala, basal ganglia, and midbrain play a substantial role in vision. A few studies started to investigate the role of the superior colliculus and pulvinar in the context of attention, suggesting a causal involvement through complex subcortical-cortical connections or interactions with the basal ganglia (Krauzlis et al., 2013) which are more commonly investigated for their role in reward processing and reinforcement learning (Yasuda et al., 2012). The involvement of other subcortical areas in visual processing is often only investigated in tight relation to what is considered the area's primary function. The amygdala for instance is primarily investigated in the context of emotion, which inspired investigation of its role in the processing of emotional visual stimuli. This approach of studying different areas with fundamentally different tasks makes it difficult to unify recordings from these areas into a common framework of the neurobiology of visual cognition. Further evidence for a role of subcortical areas stems from observations that deficits in visual processing, attentional control, and goal-directed behavior in a range of human disorders such as ADHD, autism spectrum disorders, schizophrenia, and Alzheimer's disease are associated with deviant responses in subcortex and atypical subcortical-cortical connectivity (Leuba and Saini, 1995; Butler et al., 2007; Cerliani et al., 2015; Richard and Lajiness-O'Neill, 2015; Mills et al., 2018).



10.2.g

Figure 1. Background and strategy of large-scale neurophysiological recordings with high-density NHP probes. ***(A)*** *Conventional view of the functional organization of the primate brain assigning a main function to a particular region of the brain, such as visual processing in the occipital lobe (green), reward processing in the subcortical basal ganglia (yellow), or action planning in frontal cortex (red).* ***(B)*** *Current knowledge of the brain networks underlying visual processing in primates describes sophisticated feedforward (blue arrows) and feedback (red arrows) connectivity between numerous cortical areas and a few subcortical thalamic regions (Adapted from Gilbert and Li 2013). The involvement of many other areas remains unexplored.* ***(C)*** *A broad range of brain areas (green) with neural correlates of (visual) working memory have been revealed with many different stimuli and paradigms (icons). Adapted from Christophel et al. (2017).* ***(D)*** *Combined cortical (Reveley et al., 2017) and subcortical atlases (Hartig et al., 2021) of the rhesus macaque displayed on macaque brain template (Jung et al., 2021). Different areas are drawn in different colors on six sagittal slices spanning the left hemisphere. Note the abundance of subcortical areas, many of which have not yet been recorded from in the context of visual cognition.* ***(E)*** *Conventional approach of recording neuronal responses from one or a few areas in the context of a task specifically designed for the area's presumed function (see panel A).* (10.2.g

The notion that the apparent involvement of a brain area in a cognitive function strongly depends on the behavioral context (task and stimulus) can be illustrated by the well-studied phenomenon of visual working memory – the crucial ability to keep information in mind. Combining results from a wealth of studies, we have argued that visual working memory mechanisms can be seen in a large range of cortical and subcortical brain areas, including the thalamus and basal ganglia (10.2.e [redacted] (Fig 1C). The same is true for visual perception; interactions between visual cortex and subcortical areas form the basis of visual cognitive operations 10.2.e [redacted] Taken together, the current view is that a better understanding of visual processing in the context of cognitive behaviour (attentional control, decision-making, goal-directedness, etc) requires investigation of both cortical and subcortical neuronal substrates, and that for a coherent view of the potential involvement of many unexplored (sub)cortical areas it is important to study them in the same behavioural context.

Studies investigating the neural mechanisms of visual perception in humans and animals have so far focused primarily on areas of the visual cortex and systematic approach to understand the role of the many regions of the subcortex has been lacking. This omission has an important technological origin as it has been difficult to record the activity of large cell assemblies deep in the brain, especially in combination with simultaneous cortical recordings. However, with the development of new technologies to record neural activity at a larger scale, it is becoming feasible to study the role of deeper and more widely distribute brain areas in visual perception.

10.2.g [redacted]

Though informative, there are several limitations in the study of cognitive processes in rodents. First, 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. Thirds, other thought processes (such as the capacity to memorize information and hence the cognitive ability of rodents) are less well developed 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 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, including humans, do not have their counterpart in rodents (Van Essen et al., 2019). Similar 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. For these reasons it is indispensable to conduct these studies in monkeys to reach the aim of our project, which is to describe the contributions of subcortical and cortical areas to fundamental visual cognitive processes including object recognition, scene segmentation, attentional selection, working memory and consciousness.

Our laboratory has a good track-record in the study of visual cognition, and our experience will allow us to perform experiments that target these important neuronal mechanisms. Electrophysiological recordings in different brain areas will be made while the monkey performs specific behavioural/visual tasks to assess the functional involvement of these brain areas. For the recordings we will use novel

10.2.g [redacted] which allows large-scale recording from

subcortical and cortical areas of the macaque brain. Combined with high-precision probe placement (using CT and MRI-guided planning techniques), this approach will provide insights into the distributed neural mechanisms of perception, cognition, and consciousness. In particular, we aim to record from the thalamus, the basal ganglia and various other subcortical structures, cataloguing the activity of neurons in sparsely studied brain territory whose involvement in perception, attention and consciousness has remained unclear.

High-density recordings of distributed neural mechanisms of visual cognition

Previous studies targeted one or only a few brain regions during cognitive tasks and could, due to the technical limitations, only touch the tip of the iceberg. The systematic mapping of brain regions supporting visual cognition was simply not achievable. T10.2.g The use of the novel will allow us to simultaneously record hundreds of neurons across multiple brain areas along the shank of an electrode (Fig 1F). With multiple probe insertions/trajectories across the brain on different recording sessions, we will be able to carry out a systematic mapping of a much larger number of regions of the monkey brain (Fig 1G). Because recording sites span the entire shank of the probe, we will furthermore be able to record from multiple areas at the same time, which will yield valuable information about the relative contributions and timing of different brain areas during different cognitive processes. This adds to the more conventional 'pseudo-population' approach wherein neuronal activity from different recording sessions is temporally aligned to external events such as the timing of task components or a visual stimulus. This allows network-like analyses from non-simultaneously recorded brain areas. Tissue damage from this type of recordings will be minimal, because the probes are very thin, and their high channel count allows sampling of many more neurons with a single insertion than has previously been possible with many penetrations of conventional metal electrodes. As a result, we will obtain an amount of data that would otherwise require tens of monkeys.

A multi-dimensional task to probe several aspects of visual cognition

One of the tasks we have selected to study, is known as the texture-defined curve-tracing (TDCT, Figure 2). The task 10.2.g, 10.2.e is ideal for our purposes as it requires animals to sequentially solve sub-problems which each involves a different cognitive process. In the task, the monkey sees two sausage-shaped objects which need to be segregated from the background before they can be perceived. Figure-ground segregation depends on a motion difference: pixels in the objects move in one direction for a short duration (<0.5s) whereas the pixels in the background move in the other direction and they can only be seen when the pixels start to move. The monkey has to attend one of these shapes and ignore the other one, remember it across a brief delay and plan an eye movement to a circle on the attended object. The advantage of this task is that it gives insight into the neuronal mechanisms underlying (1) motion perception, (2) object recognition, (3) figure-ground segregation, (4) the selection of an object by attention, (5) memory of the object when it is no longer visible because the motion of the pixels stopped, and (6) the transformation of the perception/memory into an action. Such a complex task involves processing in multiple brain areas and can only be performed by primates. With our new approach we will, for the first time, be able to systematically map out the contributions of cortical and subcortical brain regions to all these cognitive processes.

In another task, modelled after the "Threshold for Conscious Report" (CRT) task 10.2.e we will probe conscious awareness. In this CRT task, weak stimuli were presented that were sometimes reported as perceived and sometimes remained subliminal. Previous recordings from a few cortical areas suggest that the extent of signal propagation throughout the visual cortical hierarchy determines whether a stimulus is perceived. Combining such a task with simultaneous recordings from hundreds of neurons in multiple brain areas 10.2.g will reveal how neural activity unfolds in different brain areas when a stimulus is perceived or not. This makes it possible to describe which brain regions make the distinction between stimuli that are consciously perceived or not and advance our understanding of how the brain solves complex cognitive problems and give rise to visual perception.

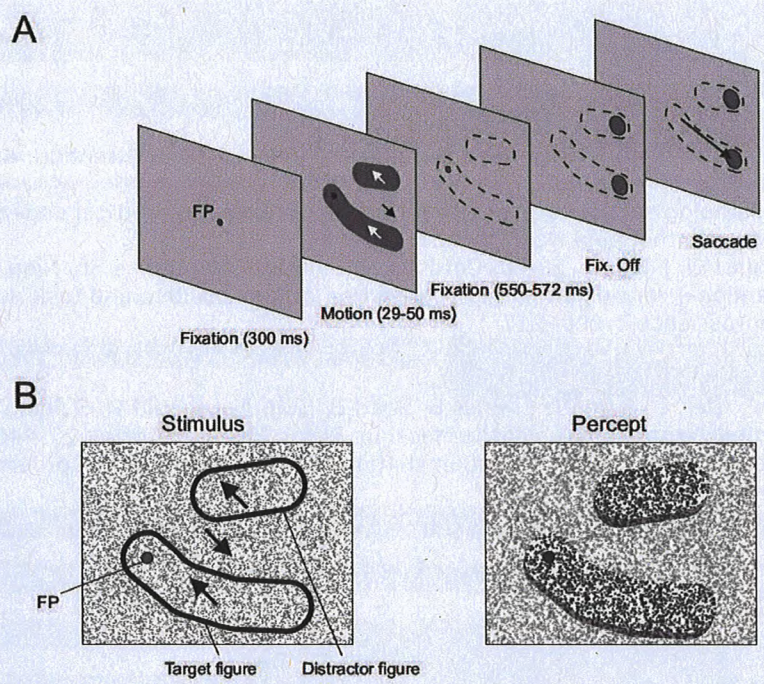


Figure 2. Texture-defined curve-tracing. (A) *The monkeys view a computer-screen and fixates their eyes on a central fixation point (FP). Two curves defined by moving textures appear briefly and then disappear. One of the curves, the target curve, is connected to the fixation point and the monkey's task is to mentally trace this curve (i.e., without moving their eyes) while ignoring the distracter curve. They have to remember the curve for a brief period before a go-cue is given (fixation dot turns off) then make a rapid eye-movement (saccade) to the end of the remembered target curve.* ***(B, left)*** *The curves are defined by a brief movement of a texture made of a random-dot pattern. The dots on the curves move in the opposite direction to the background. It is also possible to define the curves using different static textures.* ***(B, right)*** *A cartoon of the monkey's perception with the target curve (lower curve) and the distractor curve (upper curve) that segregates from their background (here based on a difference in contrast).*

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3.2 Purpose

3.2.1 Describe the project's immediate and ultimate goals. Describe to which extent achieving the project's immediate goal will contribute to achieving the ultimate goal.

- If applicable, describe all subobjectives

The main objective of this study is to reveal the contributions of subcortical and cortical areas to fundamental visual cognitive processes including object recognition, scene segmentation, attentional selection, working memory and consciousness. Previous studies focused on understanding neural processing in specific brain areas in various tasks, comparing the activity of at most three regions within a task. Here, we will adopt the opposite approach of systematically studying neural activity in multiple cortical and sub-cortical brain areas, within a single task.

Within the five years covered by this application, we will 1) establish a recording and data-analysis set-up 10.2.g , and 2) sample neural activity from an extensive set of brain areas in monkeys during tasks that are specifically designed to address fundamental aspects of visual cognition. The texture-defined curve-tracing task and the threshold-for-conscious-report task described above are two prominent examples of such tasks.

3.2.2 Provide a justification for the project's feasibility.

We have a lot of experience in training macaques to perform complex visual tasks. Specifically, we have experience in training monkeys to perform tasks that demand attention shifts and to keep information in working memory, tasks that are composed of subtasks (e.g., figure-ground segregation combined with attention shifts), and tasks in which monkeys make eye or hand-movements. The two tasks described in detail above (TDCT & TCR) have been executed in the lab in combination with more conventional recordings in the past. We have many years of experience in performing the surgical procedures required to achieve our goal and with performing fMRI with macaques. 10.2.g

and we have started additional collaborations 10.2.g

Our lab is situated in an excellent research environment, where the facilities that are required for this project are available, including a state-of-the-art primate facility and surgical room. Our lab utilizes a variety of related techniques. These include fMRI and psychophysics in humans and non-human primates; electrophysiology in humans, non-human primates and mice; optical imaging in mice; brain stimulation in non-human primates and mice; and computational modelling of neural mechanisms. In recent years, the acquisition of large datasets from optical imaging, MRI, and large-scale electrophysiological recordings in our lab has led to significant improvements in data storage, data curation and open data sharing infrastructures. This development will continue and expanded upon with the acquisition of large 10.2.g data-sets.

The lab has an outstanding track record with high impact publications (Nature, Science, Nature Neuroscience, Neuron, PNAS, etc., see 10.2.g The work proposed here is funded through 10.2.g .

3.2.3 Are, for conducting this project, other laws and regulations applicable that may affect the welfare of the animals and/or the feasibility of the project?

☒ No

☐ Yes > Describe which laws and regulations apply en describe the effects on the welfare of the animals and the feasibility of the project.

3.3 Relevance

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

Scientific relevance
Our understanding of vision and visual cognition has made great advances through the use of cognitive neuroscientific techniques in humans such as fMRI, EEG and MEG. These techniques have identified brain areas and networks of brain areas that appear to be involved in perceptual organisation, the selection of behaviorally relevant items, the shifting of attention and working memory. Previous studies indicate that these cognitive operations require the interaction between many brain areas of the cortex and subcortical structures. Unfortunately, these non-invasive techniques cannot provide cellular resolution of activity and our understanding of the neural circuits that solve cognitive tasks and produce visual percepts is still largely incomplete. The proposed study will advance our knowledge of these circuits, by systematically mapping activity in most of them, and the results will inform theories of brain function concerning visual perception, figure-ground segregation, attention, working memory, decision making and cognitive control. The use of 10.2.g in monkeys is novel and the data will be the first of its kind in terms of the scale of the number of areas recorded from. Traditional electrophysiological studies with single electrodes or chronically implanted electrode arrays typically focus on a limited set of brain areas, potentially missing out on contributions from a broad range of additional areas. With 10.2.g we can sample a much larger set of cortical and subcortical brain areas with cellular resolution and obtain an unprecedented broad yet detailed view of the neuronal mechanisms of visual perception. We will address central and unresolved questions in cognitive neuroscience about the formation of visual percepts and the control of attention. These problems are of interest to a broad field involving neuroscientists, cognitive neuroscientists, psychologists and computer vision researchers, and our results will be of great importance for all of these fields.

Data sharing
We expect that the study will also provide an important resource to the field. We will make our data sets available online so that other researchers can use them to address their own research questions. We expect that the accessibility and systematicity of our data sets will set an example that will eventually reduce the overall number of invasive experiments necessary in this field (open science). The need for other labs to carry out similar experiments in various brain structures will be reduced and our study will set an example showing how to generate data with two to three monkeys that would have required tens of monkeys with more traditional technologies. Although they are of fundamental nature, the data sets will also be of use in clinical settings. To give one example, some of the subcortical areas that we will record from are used as targets for deep brain stimulation (DBS) in patients suffering from Parkinson's disease and obsessive-compulsive disorders. Linking the function of these areas to processing in the rest of the brain will be useful in understanding the mechanisms of DBS and the causes of unwanted side-effects.

Social relevance
The cognitive processes investigated here are the fundamental building blocks of our cognitive capabilities and are important for virtually every cognitive task performed by humans. Understanding how distributed brain circuits contribute to perception and cognition has wide-ranging social impacts. Our main aim is therefore to generate new fundamental insights. Since the proposed study will be the first of its kind, the results will contribute new fundamental knowledge on the cortical-subcortical mechanisms of visual cognition.

We expect that the new fundamental insights will, in the longer run, lead to applications in various domains. We will give several examples. As a first example, recordings from areas that have been rarely studied in primates will help us understand how lesions caused by, e.g., stroke or brain tumours in these regions can cause problems in the visual or cognitive domains. The second example relates to disorders of consciousness. The expected insight in the cortical and subcortical contributions to conscious perception will indirectly inform about disorders of consciousness (coma) and how different states of impaired consciousness can be distinguished, e.g., minimally conscious state vs. vegetative state. The third example relates to ADHD and autism spectrum disorders. Our results generate insight into the circuits that are responsible for attention shifts and thereby inform about causes of attentional deficits that occur in ADHD and autism spectrum disorders. Fourth, recordings from brain areas that are also targeted by deep brain stimulation in patients suffering from Parkinson's disease or psychiatric disorders

(e.g., Obsessive Compulsive Disorder), and linking the function of these areas to processing in the rest of the brain may be useful in understanding the mechanisms of this technique and the causes of unwanted side-effects. Our team has strong collaborations with the neurosurgery and psychiatry departments of the Amsterdam UMC. The researchers in the psychiatry department investigate how impaired attention control contributes to ADHD and obsessive-compulsive disorder. Our collaboration will ensure optimal clinical use of the fundamental insights expected to be generated by this study.

Our final example is related to the goal of restoring vision in the blind. The neurosurgery department helps us with the design of a neuroprosthetic system to restore a rudimentary form of vision for blind people. A detailed understanding of the neuronal mechanisms of visual perception and visual consciousness will be instrumental by providing new insights in the structure of the visual system and thereby contribute to these efforts. Of special relevance here is insight into the propagation of neural activity from early visual cortical areas to frontal cortex (and the role of subcortical areas therein) where the achievement of a sufficient level of neuronal activity has been shown crucial for conscious perception 10.2.g

Our findings will be communicated to basic scientists, clinicians, clinical researchers, and the general public through scientific and popular scientific publications, conference attendance, and talks. The data will be made available through open access outlets.

3.3.2 Who are the project's stakeholders? Describe their specific interests.

This proposal concerns fundamental (basic) research that will primarily be of interest for the broader field of neuroscientists, cognitive neuroscientists, psychologists and computer vision researchers. Our new approach has the potential to reveal a distributed functional brain organization that is substantially different from current 'text-book' knowledge. In the long-term, the novel knowledge of the underlying neurobiological mechanisms of cognition that is gained from this study may find clinical applications for the development of brain-computer interfaces such as visual prostheses, or the treatment of neuropsychological disorders or psychiatric symptoms.

3.4 Strategy

3.4.1 Provide an overview of the overall design of the project (strategy). If applicable, describe the different phases in the project, the coherence, the milestones, selection points and decision criteria.

Overall design
The aim described above will be addressed in experiments with macaque monkeys. The monkeys will be trained on the texture-defined curve-tracing task, the threshold-for-conscious-report task, and/or other visual cognition tasks until they reach high levels of performance. The animals will then be implanted with a custom-made base-grid for mini recording-chambers which will allow us to target multiple brain areas covering an entire hemisphere in the same animal (described below). Neural recordings can subsequently be made 10.2.g through one of several small burr-hole inside a recording mini-chamber on each recording session (details below under 3.4.2). 10.2.g allow the simultaneous recording of action potentials from hundreds of neurons as well as local field potentials from many brain areas along the electrode trajectory. We will initially record from a single 10.2.g at a time, but with accumulating experience, it may be possible to record from multiple probes and reduce the number of recording sessions necessary to record from all sites. 10.2.g

The activity of the recorded cells will be related to the distinct behavioural epochs of the animal performing the tasks using advanced regression and machine learning techniques. This will allow us to map the spatiotemporal distributed neural correlates of the cognitive behaviours probed by the task, i.e., which areas are correlated with which aspects of the task at which point in time. Such spatiotemporal relations, in turn, will be instrumental in creating dynamic neural network models of visual cognition and deepen our understanding of the brain's distributed cognitive codes.

Components of the project

Newly acquired animals follow a highly standardized training program, which first acclimatizes them to the primate facility and takes the monkeys up to the point where they can perform simple visual tasks in daily training sessions. These initial stages have been carefully optimized and are performed and overseen by the well-trained and experienced technicians and animal caretakers of the facility. This standardization ensures a very high success rate acclimatizing monkeys to the facility and the training routine. Monkeys are obtained from the national primate centre or in exceptional cases from licensed importers if there are no appropriate animals available at the primate centre (this has not been necessary in the past 8 years). In all cases, monkeys are second (F2) or later generation purpose-bred. They are socially housed in the facility, typically in pairs. At an early stage we make a CT scan to map out the shape of the skull and an MRI to know the monkey-specific features of the brain anatomy.

After acclimatization to the new environment the monkeys are trained to move into a primate chair that allows them to be brought comfortably from their home-cage to the experimental set-up. Once the animal is able to calmly sit in the chair for periods of >1 hour, it undergoes the first surgery to implant a head-post. The head-post is used to fixate the position of the head of the monkey during the performance of the visual tasks and the later electrophysiological recordings. It consists of a small vertical rod and horizontal ground-plate part that can have several 'leg-like' protrusions and is form-fitted to the skull of the animal (based on the CT scan). The ground-plate is attached to the skull, allowing head fixation through a connection with the vertical rod. Head-post implantation is an essential step because we train the monkey to control its eye position and these measurements are only possible if the monkey's head position is fixated.

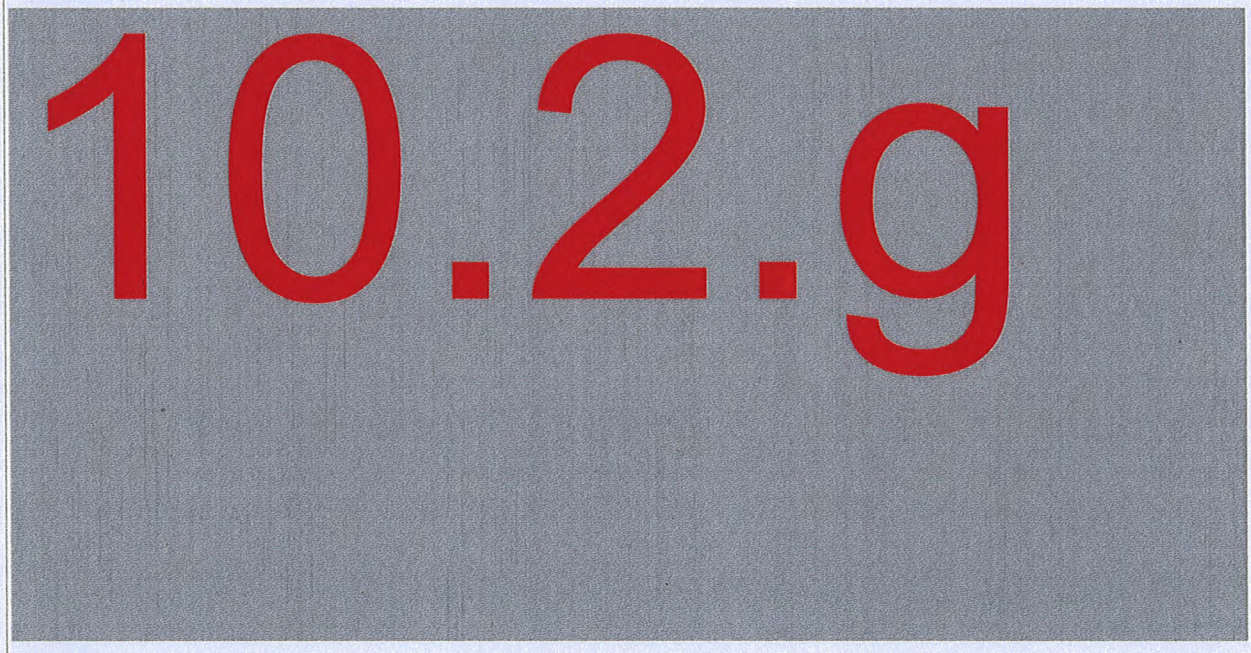
After recovery from the head-post surgery, the animal is first acclimatized to having his head position fixated in the chair. The monkey then begins daily training sessions in which he acquires juice rewards for performing simple eye-movement or hand-movement based tasks. Initially the tasks are very simple, such as directing his gaze ('fixating') on a large dot on a computer screen for a few hundred milliseconds. During this training period, the animals are placed on a controlled fluid-uptake regime. Gradually the difficulty of the tasks is increased by making the dot smaller until the animal can fixate, then make guided eye-movements towards visual targets or hand-movements after the presentation of a 'go' cue. At this stage, the animal is ready to be trained on the actual experimental tasks.

To enable brain-wide sampling of brain areas 10.2.g we will design a custom-made, 3D printed titanium 'base-grid' that will be implanted on the skull of the monkeys in a second surgical procedure (i.e. several months after the headpost implantation, Figure 3A,B). The skin is closed over the base-grid and we let the implant integrate with the bone to create a strong connection (once we have gained some experience with this new technique it may be possible to combine the base-grid and head-post implantation in a single surgical procedure). We have a great deal of experience in designing and 3D printing custom made titanium implants based on a co-registration of the CT-scan and the MRI scan of the animal 10.2.e . This method has two important advantages. First, it allows the planning of electrode trajectories and entry points based on the individual subject's anatomy. Second, since the implants closely fit the shape of the individual's skull the surgical procedures are faster, the animals recover faster, and the implants are less prone to infections. 10.2.g At the start of a recording period, we will perform a small third surgical procedure (under light anaesthesia) to place the mini-chamber(s). We make a small incision in the skin at the sites of selected mount-point locations and connect the mini-chambers to the base-grid, creating a tight seal that isolates the inside of the chamber from the outside. With the use of small custom-fabricated grid we will drill small holes (<2 mm; 5-15 holes) in the skull inside the chamber. The creation of these small burr holes is a fast process (other labs have previously carried them out in awake animals). The burr holes can be kept open for the duration of the recording period with sterile plastic pins (and close within weeks if these pins are taken out). The chamber will then be closed off with a cap and the skin fits tightly around it.

During a recording session while the animal is awake, the high-density probes will be carefully inserted through one of the burr-holes with a micromanipulator and pass the dura mater with the help of a short guide-tube. Labs that are already using the high-density probes in monkeys report that inserting them is highly comparable to the procedures with conventional electrodes, which is something the lab has many

years of experience with. -A typical recording session will start with a brief characterisation of the recorded units (e.g., receptive fields), proceeds with the actual task under study, and lasts until the monkey has received enough reward and stops performing the behavioural task. In some sessions, we may use a colouring agent in conjunction with the probe to label electrode trajectories for post-hoc histological verification. Histological verification in rodent studies showed that the tissue damage inflicted by these electrodes was so minimal that such colouring is necessary to observe the electrode trajectories (Steinmetz et al., 2018). At the end of the session, the probe and guide tube will be retracted, and the adaptor and manipulator will be removed. The mini-chamber will be closed and sealed before the monkey returns to its home cage. The interior of the chamber will be cleaned and kept as sterile as possible. A recording session including preparation and post-recording procedures will typically take between two and five hours. 10.2.g

The small diameter of the burr-holes avoids brain swelling, minimizes tissue growth, and the bone will close again within days or weeks when left alone. 10.2.g



At the end of the experimental period, the animal will be euthanized with an overdose of barbiturates followed by perfusion with a fixative and extraction of the brain for histological verification of recording sites (which will initially be estimated based on a combination of information from MRI/CT scans, extensive cortical and subcortical brain atlases, and the identification of grey and white matter boundaries from neural activity).

Coherence

The experiments described here form one coherent approach to study how cortical and subcortical brain areas contribute to perception, attention, working memory and conscious perception. The project contains many state-of-the-art techniques, such as MRI guided 3D printing of cranial implants, mini-chambers for targeting of multiple brain areas and 10.2.g to allow massive sampling of neural activity, which combined together will allow us to achieve our aims. Given our ample experience,

we anticipate that the monkeys will learn the tasks. Only in case the animal does not adapt well to the training procedures (which happens for <5% of monkeys) the animal would not be selected.

3.4.2 Provide a justification for the strategy described above.

The level of cognitive processes we investigate require a monkey model (see 3.1). The use of novel high-density probes allows the recording of large-scale neural activity at cellular resolution and from multiple functional brain areas simultaneously. The use of the base-grid with mini-chambers allows us to sample from a large proportion of the brain with reduced surgical impact. Together, this approach will yield highly detailed and rich neural data from a greatly reduced number of animals compared to traditional recording methods.

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

Serial number	Type of animal procedure
1	Procedures for the preparation and execution of high-density electrode recordings in awake-behaving monkeys10.2.g ~~recordings from awake-behaving monkeys.~~