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Evaluating Anesthetic Protocols for Non-Human Primate Functional Neuroimaging

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Abstract

Functional magnetic resonance imaging (fMRI) is a non-invasive technique that can be used to measure a proxy of neural activity *in vivo* with high spatial specificity. One subject can be followed for a long period of time to assess changes in functional brain organization. However, fMRI is extremely sensitive to motion. The challenges of training non-human primates to reduce motion in an MRI scanner motivate the study of anesthesia which is commonly used to substitute for this training. In this thesis, I compare three different commonly used anesthetic protocols: isoflurane, propofol-fentanyl in combination, and fentanyl alone, to test which of these best preserves a Blood Oxygen Level Dependent (BOLD) response to visual and somatosensory sensory stimuli in two rhesus macaque (*Macaca mulatta*) monkeys. In one animal, somatosensory responses were equally robust under propofol-fentanyl and fentanyl anesthesia but in the other animal, these responses were only robust under propofol-fentanyl. Somatosensory and visual responses were not observed under isoflurane anesthesia for either animal. It was under fentanyl and combinations with fentanyl that both sensory modalities appeared to best-elicit sensory related BOLD signals in these animals suggesting they should be further studied in a larger cohort.

Keywords

neuroimaging, macaque, primate, anesthesia, fMRI, fentanyl, propofol, isoflurane, visual, somatosensory

Summary for a Lay Audience

Functional magnetic resonance imaging (fMRI) is a technique that researchers can use to non-invasively measure which areas of the brain are active. This means that animal models, such as monkeys, and humans can easily be used as subjects for studies that use fMRI to measure brain activity during different types of activities. This is especially useful for studying how the brain changes over time after an injury as the brain is known to reorganize its processing functions in order to adapt or to heal. However, studying these injuries is difficult to do in humans because there is usually no before-injury fMRI report available for patients and injuries between patients vary widely in their degree. As a result, animal models, especially animal models such as non-human primates that have very similar sensory systems as human beings, are a valid alternative for such studies. Although NHPs have been successfully used in a variety of studies where they were kept awake, they are very challenging to train to enter an MRI scanner with limited movement. In fMRI studies, even slight motion from the subject can obscure the signal researchers are trying to measure. Anesthesia is commonly used by researchers to overcome this training challenge which would permit large cohorts of animals for studies about brain plasticity, but it is unclear which anesthetic protocol is most optimal to use for these studies. This investigation compares three common anesthetic protocols for non-human primate brain imaging to evaluate the robustness of the sensory signal that can be measured under each protocol. It was found that fentanyl was the anesthetic protocol under which the animals had the most robust brain response to visual and touch stimulation. This investigation offers a model for continued exploration of anesthetics so that the process of anesthetized fMRI studies of primates can be most effectively used for studying how the brain changes following injury and how it heals.

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Thank you.

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List of Abbreviations

7OP = Parietal Operculum
ANTS = Advanced Neuroimaging Tools
BOLD = Blood Oxygenation Level Dependent response
CL = Claustrum
F5 = Ventral Premotor Cortex
fMRI = Functional Magnetic Resonance Imaging
FSL = FMRIB Software Library
FST = Fundus of the Superior Temporal sulcus of the visual cortex
IG = Granular Insula
LGN = Lateral Geniculate Nucleus of the thalamus
LSI = Left primary Somatosensory area
LSII = Left secondary Somatosensory area
MRI = Magnetic Resonance Imaging
MT = Medial Temporal area of the visual cortex
VIP = Ventral Intraparietal Area
VPM = Ventral Posterior Medial nucleus of the thalamus
GLM = General Linear Model
CFMM = Centre for Functional and Metabolic Mapping
STS = Superior temporal sulcus

Introduction

1. Macaque primates are a key tool for neuroscience research

While there are many non-human primate species that are used to study the brain, macaque monkeys have been studied for decades in the field of neuroscience because of their physiologic homologies with human beings (Tsao et al. 2006). Macaque and human brains share features of neuronal architecture (Petrides & Pandya 2002), have similar distribution of neurotransmitter systems (Mansour et al 1998), drug metabolism and pharmacokinetic responses (Reuning et al 1989). Macaque monkeys are especially useful to study sensory systems because of the similarities between human and macaque sensory systems. For example, a comparative analysis of many different kind of primates found that macaques and humans had the greatest density of Meissner's corpuscles present in the distal phalanges of other apes and monkeys (Varendeev et al. 2015). This may mean that compared to other primates, macaques and humans may have more evolutionarily preserved hand-use. Macaques, like humans, also have a very advanced visual system (Dubowitz et al. 2001). Alert macaques have been used to show that the cluster model of visual cortical organization that was observed in humans is also present in macaque MT area (Kolster et al. 2009). Research with macaques has therefore been crucial to understand the complex human visual system (Maunsell et al. 1987, Anderson et al. 1997).

This homology permits us to study in macaques, mechanisms and phenomena that occur in human beings, using techniques that cannot be reasonably used in human beings such as models of nerve injuries. They can be used to study changes in neuronal architecture that may occur following nerve injury, and the efficacy of different pharmacological interventions to help treat such injuries as well. Injury studies are preferably carried out in animals because pre-injury brain functionality information is usually not available for human patients and comparison to a healthy control cohort may be insufficient to study some mechanisms.

2. Benefits of using fMRI for neuroscience research on functional reorganization

By the late 1980s it was known that regional cerebral blood flow increases near areas of neuronal activity based on Positron Emission Tomography (PET) studies that measured radiolabelled oxygen ($H_2{}^{15}O$) changes during tasks (Kanno et al 1987). It was then found that regional cerebral blood flow could be imaged using high resolution magnetic resonance imaging (MRI) (Ogawa et al 1990). Functional magnetic resonance imaging (fMRI) measures the blood oxygen level dependent (BOLD) response in the brain. BOLD imaging uses the magnetic properties of oxygenated and deoxygenation blood to detect regional changes in blood flow.

When neurons in one area of the brain are activated, they consume more oxygen which causes an increase in deoxygenated blood. Deoxyhemoglobin is paramagnetic which results in a lower magnetic resonance signal than oxygenated blood containing oxyhemoglobin, which is diamagnetic (Ogawa et al 1990). Local blood vessels respond to the demand for more oxygenated blood in these areas with an increase in cerebral blood flow and vessel volume. This results in an increase in oxyhemoglobin in excess of the initial oxygen demand. Because oxygenated blood is diamagnetic, there is a local signal increase compared to the surrounding area which is correlated to neural activity in that area (Menon et al 1995). This phenomenon is called the hemodynamic BOLD response and can be used to approximate brain activity in response to different stimuli. This correlation is strong because of neurovascular coupling which is the process that describes how vascular control over different areas of the brain is tightly controlled and is coupled to neural activity.

The BOLD response measured by fMRI can be used to approximate neural function *in vivo* in subjects in an MRI scanner because it is non-invasive. The non-invasive nature of the experiment allows us to study brain function in a longitudinal study. Longitudinal studies are especially important to study phenomena such as functional reorganization of the brain following nerve injuries and brain plasticity in the treatment of these injuries with various therapies. Researchers can use fMRI to take advantage of the homology between macaques and humans and study both using the same modality in order to understand the applicability of conclusions from more invasive neuroscience techniques done in macaques, to human beings.

3. Managing motion is important to do in fMRI experiments

When human subjects are used for fMRI experiments, they are asked to stay as still as possible for the duration of the scan because the fMRI process is very sensitive to motion. This is because the BOLD signal we are interested in investigating is a much smaller signal change than would occur due to even slight movements. Even a minor head movement from the subject can distort the image and create signal intensity changes that can be mistaken for stimulus (Hajnal et al 1994). The activity-driven signal in a brain area is small so even small movements of the brain in the magnetic field produces inconsistencies in phase and amplitude which can generate blurring artifacts much larger than the actual signal (Hajnal et al 1994). Registration of images that use images containing these errors may worsen the error as they interpolate spurious activations into the time series of all images (Grootnook et al. 2000).

Physiologic sources of movement such as due to respiration and breathing can be corrected for because they are predictable signals and therefore a regressor can be created that predicts this movement. Motion correction can be done in data preprocessing which includes a linear registration with six degrees of freedom between each fMRI volume to the middle volume, aligning the volumes together and correcting for gross head movement. This gross head movement can be due to gradual compression of the cushioning used to support the subject's head in the scanner for example. However, motion exceeding the size of a voxel is generally thought to be unacceptable because it would obfuscate task-related BOLD signal (Satterwaite et al. 2012). Even in head-fixed and trained awake monkeys, some runs must be discarded due to unpredictable movements (Dutta et al. 2014). Motion correction algorithms themselves may worsen the effect of motion artifacts in the data (Freire et al 2001). For these reasons, management of motion is very important for fMRI experiments.

4. Awake macaques in fMRI neuroimaging research

Awake monkeys are challenging to use in fMRI research because they cannot simply be told to stay still as a human can. Awake animal studies have many limitations such as shorter experiments, and training times that average from 1-2 years at least to acclimatize the animal to

the MR environment (Goense 2010). The process of using awake monkeys in fMRI has been improved using a variety of techniques such as head-posts, reward systems, body motion tracking to remove noisy runs and the use of contrast agents (Vanduffel et al 2001). Body motion monitors can be used to record motion in anticipated areas and thus can be regressed out of the data in preprocessing stages.

When NHPs are used to study higher cognitive functions, an awake animal is important because to study these higher cognitive functions requires some behaviour from the animal (Peeters et al 2009). As a result of many innovative techniques training and motion monitoring techniques, awake experiments with trained non-human primates have been done with great success and in-fact appear to increase in sophistication and frequency of use in the field of fMRI research. Awake monkeys have been successfully studied in experiments that have shown the area of the brain that is responsible for detecting faces (Ku et al 2011), visual cortical organization (Kolster et al 2009), gustatory behaviours (Kaskan et al 2019) and functional organization of pitch perception (Norman-Haginere et al. 2019) to name a few examples.

5. Awake macaques are not well suited to longitudinal lesion studies

Despite the success of a variety of awake NHP fMRI studies, there are certain cases where using awake animals is not optimal. Anesthetic restraint is preferred for example in longitudinal studies of a large cohort of lesioned animals. While rhesus macaque monkeys can be trained to perform a variety of complex cognitive tasks, an extensive training period is required to train these animals. In cases where a large cohort of macaques must be used for an experiment, the challenge of training this large cohort would make the use of anesthetic restraint a very appealing alternative.

Lesion studies are used in the study of peripheral nerve injuries. Peripheral nerve injuries commonly occur in humans however the nature of the injury is highly variable, and it is difficult to determine the precise extent of injuries in order to decide which patients can reasonably be included together in a cohort to study effects long term (Corbetta et al 2002). Studying the efficacy of recovery from different therapies requires consistency in the type of injury so that

changes in nerve function can be causally linked to the therapy rather than be a result of the different natures of the injuries themselves. In addition, a repeated measures test with a variety of aetiologies of nerve injuries could limit our ability to test and understand the mechanism by which a therapy may be resulting in a recovery. Drop-outs are less likely to occur in animal studies than in human studies, which is especially salient to longitudinal studies as humans subjects may move away or drop-out due to non-compliance (Bhat et al. 2017). As a result, lesioned animal models are a way to study peripheral nerve injuries in a more standardized and thorough way.

Furthermore, it is difficult to compare changes in the brain before and after the injury in human studies because it is highly unlikely that the individual's brain would have been scanned or assessed in any way before the injury. Human studies usually compare injured cohorts to healthy control groups; however, this does not permit researchers to determining whether an effect in an individual's brain is a biological idiosyncrasy that was present before the injury, or if it was a change brought on by the injury. It is also difficult to determine the degree to which an individual regained functionality of a limb or a nerve without an initial healthy state for comparison. Macaque monkeys that are lesioned as part of an experiment are preferable to human subjects as they would have a pre- and post- injury brain function profile to do these comparisons.

In order to longitudinally study the effects of nerve injury on functional reorganization in awake animals, a large cohort of macaques would have to undergo a rigorous, expensive and time-consuming training process and then be potentially irreversibly injured and brain altered by therapy that would limit the animal's utility for other experiments. Thus far, few studies done in awake macaques include a large cohort and the average number for such studies is three animals. There are also few studies investigating large scale reorganization of the brain after spinal cord injury even though it has been demonstrated that there is reorganization in the somatosensory cortex in NHPs (Jain et al 2008, Chen et al 2012, Dutta et al 2014) and brain reorganization has been shown to occur in the normal recovery from peripheral nerve injuries (Ramachandran & Rogers-Ramachandran 2000). The unrealistic prospect of applying controlled nerve injuries to a

large cohort of MRI trained macaques motivates the study of other restraint methods such as anesthesia.

Physical restraints used in an awake animal that has not been trained can cause a great deal of anxiety which can interfere with the data. The use of a paralytic agent such as curare has been shown to induce even greater anxiety because the paralysis of respiratory muscles necessitates intubation of the animal while it is otherwise awake (Anderson 2010). Anesthetic restraint provides more reliable motion control than the techniques employed in awake animals, so it permits researchers to remove the possibility that motion artifacts may be contaminating signal that is observed in awake NHP studies.

6. Anesthetic restraint has been used successfully in fMRI experiments

Despite the attenuation of signal recorded from anesthetized animals, there is evidence to suggest that fMRI experiments done in anesthetized animals can still provide useful conclusions. In higher visual areas, like the superior temporal sulcus (STS), it was found that the functional BOLD signal was reduced in the anesthetized animal compared to the awake one (Goense et al 2008a, Rainer 2002) but the BOLD time course was not significantly different between the anesthetized and awake condition in the primary V1 area of NHPs (Goense et al 2008b). Visual stimuli activate the same cortical areas in monkeys anesthetized with remifentanil and mivacurium, as in awake monkeys (Ku et al 2011). Resting state networks, which are coherent, system level neural oscillations, have been shown to be preserved in non-human primates under isoflurane anesthesia even if they are attenuated (Hutchinson et al 2011, Vincent et al 2007). In experiments done in four macaques anesthetized at low doses of ketamine + xylazine in combination, researchers were able to trace somatotopic reorganization of somatosensory area 3b following dorsal column lesions of the spinal cord (Dutta et al 2014). Although the anesthetics used in this study were at low enough doses that the animals had to be head-fixed and still undergo some training to reduce motion, the study had a larger cohort than most awake macaque studies and demonstrates that brain changes can not only be recorded using fMRI in anesthetized macaques, but also that they can be traced over time. A further investigation of anesthetic

protocols would reveal whether or not complete sedation can be used to repeat this type of experiment, and then therefore be used to expand such an experiment to a larger cohort.

7. Anesthetic restraints may limit interpretability of fMRI results

Anesthesia causes many changes in the brain in many ways that limit us from assuming that it perfectly models the awake condition of an animal (Masamoto & Kanno 2012). Neurovascular coupling is a process that involves three brain cell types: neurons, supporting cells and vascular cells. Neural activation causes supporting cells to send vasoactive signals to vascular cells to alter cerebral blood flow in order to fulfil the oxygen and nutrient demands of the active neurons. Anesthetic agents are known to affect vascular and metabolic parameters such as cerebral blood flow (CBF), cerebral blood flow (CBF), cerebral blood volume (CBV) that are integral to the neurovascular coupling process resulting in alterations to the BOLD signal measured under anesthesia (Sicard et al. 2003). This is because the BOLD response relies on the changes in the ratio of oxygenated to deoxygenated blood which relies on tight coupling between brain activity and accompanying vascular changes.

If all anesthetics altered blood flow in the same way, we would easily be able to compare between experiments that utilize different anesthetic protocols for the animals involved; however, this is not the case (Aladj et al 1991, McPherson et al 1985). Functional connectivity is altered in different ways for different types of anesthetic protocols in rats (Passonen et al 2018). Different anesthetics used at different doses produced disparate results in fMRI experiments measuring functional connectivity in rats (Williams et al 2010). Propofol causes dose dependent hypotension, and volatile anesthetics as well as benzodiazepines cause a decrease in cerebral metabolic rate and cerebral blood flow (Ludbrook 1997). Anesthetics can depress spontaneous and evoked neuronal activity as well as cerebral metabolism (Ruskin et al 2013) which is an important component of neurovascular coupling (Masamoto et al 2012). The nitric oxide pathway which influences cerebral blood flow, was shown to be dominant in anesthetized rats but did not play a major role in awake rats (Nakao et al 2001).

It is also unclear to what degree and in what ways each anesthetic agent alters the brain of a subject from the awake condition. This means it is unclear how much we can extrapolate observations from an anesthetized animal to make conclusions about an awake animal. It is imperative therefore to compare anesthetics in their ability to preserve functional activity in order to choose the one best suited to fMRI investigations of non-human primates. Finding the most effective anesthetic protocol for macaque fMRI and making that the standard would make comparison between experiments more reliable.

8. Mechanism of Action of Anesthetics

There are many different types of anesthetic agents that are commonly used in veterinary procedures to anesthetize animals for MRI studies. Different anesthetics work through different mechanisms of action that are not entirely elucidated today at the molecular and whole brain level. An anesthetic agent attenuates the degree of the sensory signal that is able to reach the cortex and be processed there. There are certain brain areas that are known to be involved in the processes that mediate anesthetic endpoints. The ventrolateral preoptic area is known to be asleep-promoting area and these nuclei are extensively affected by clinical concentrations of hypnotic drugs like isoflurane (Leung et al. 2014).

The thalamus is another area that is thought to be crucially involved in the mechanism of action of anesthesia. It is a bilateral structure in the diencephalon comprising 50 nuclei and subnuclei with rich interconnections to other structures in the brain (Mashour 2013). Increasing concentrations of propofol cause a decrease in activity, regional cerebral blood flow and metabolism in the thalamus (Fiset et al. 1999). The thalamus has been shown to switch from a tonic firing mode to a burst firing mode under anesthetic sedation (Alkire et al. 2000) and forms the basis of the hypothesis that the thalamus is the switch that causes a loss of consciousness under anesthesia. The injection of nicotine (Alkire et al 2007) or antibodies blocking voltage gated potassium channels to reverse hyperpolarization (Alkire et al. 2009) into the centromedial thalamus of anesthetized rats has been shown to restore consciousness. This is evidence that the thalamus is a central player in loss of consciousness; however, there is insufficient evidence to demonstrate that the thalamus is driving the loss of consciousness or merely responding to it.

The reason that functional studies, and not just resting state studies, can be accomplished at all in anesthetized animals (Goense et al 2008, Ku et al 2011, Dutta et al 2014) tells us that we do not fully understand how anesthesia accomplishes its sedative endpoints. Functional signals are attenuated but not completely ablated in fMRI BOLD studies done on anesthetized subjects meaning that anesthesia does not ablate all sensory processing pathways to achieve its sedative endpoints. However, even though functional responses to sensory stimuli are active under anesthesia, it is unclear to what degree and in what ways they are the altered from the awake condition.

Most anesthetics such as isoflurane, propofol and benzodiazepines act through the upregulation of GABAa receptor mediated suppression of signal potentiation (Ludbrook 1997). It has also been shown that GABAa receptor density is higher in the neocortex than in subcortical structures (Henstschke et al 2005). The greater sensitivity of the neocortex to common anesthetic effects as compared to the thalamus and caudate nucleus for example, may contribute to why function of structures that relay primary sensory information and primary sensory cortical activity may still be observed under anesthesia that causes loss of consciousness, amnesia and loss of motor responses.

The thalamus is known to be brain structure that is important for consciousness and arousal. Anesthetized macaques were shown to be roused by stimulation of the central lateral area of the thalamus (Redinbaugh et al 2020). The thalamus was shown to be more sensitive to consciousness changes than frontal areas of the brain, providing evidence for its regulatory role in consciousness (Redinbaugh et al 2020). It has been shown that the non-specific nuclei of the thalamus, responsible for integration of cortically processed information, are more affected by anesthesia than the specific nuclei of the thalamus, which are responsible for relaying afferent sensory information to higher cortical areas (Liu et al. 2013). This was determined by measuring thalamo-cortical connectivity between different nuclei and higher areas of the cortex both during deep sedation and in the awake condition in human subjects. This preservation of thalamo-cortical connectivity could be the reason why activation can be seen in primary sensory areas in response to stimulation in anesthetized animals. Different drugs may alter this thalamo-cortical

connection to varying degrees based on their mechanism of action which are not completely understood. Seed-based connectivity analysis comparison of awake monkeys to monkeys anesthetized with 1% sevoflurane anesthesia reveals that functional connectivity was preserved in the primary somatosensory, visual and auditory cortices (Vincent et al. 2007). Sensory evoked potentials can be recorded in humans at concentrations well in excess of that required to achieve anesthetic endpoints such as immobility and loss of consciousness (Rehberg et al. 1998) and motor nerves are still firing under general anesthesia induced immobility (Merrill AW et al. 2006). In fact, transmission of impulses through the dorsal horn of rats continues during halothane, isoflurane and propofol anesthesia protocols (Barter et al. 2008).

Three anesthetic protocols were selected for study in this investigation: isoflurane, propofol+fentanyl in combination and fentanyl alone. The following sections will outline specific properties of these anesthetics to elaborate on why each should be studied to investigate its suitability for NHP fMRI BOLD experiments.

8.1 Isoflurane

Isoflurane and other inhaled anesthetics attenuate the signal by inhibiting NMDA receptors and to a lesser extent by acting as an agonist for GABAa receptors (Antkowiak 2001). NMDA receptors bind to the neurotransmitter glutamate which modifies sodium channels to increase the polarization of the cell and cause potentials to propagate along the neuron. Thus, NMDA receptors enhance the transfer of information. Glutamate is the major excitatory neurotransmitter in the central nervous system. It acts on both ionotropic (NMDA) and metabotropic (AMPA) receptors however NMDA has higher permeability to Ca2+ which regulates several intracellular pathways. It has been shown that selective blockade of excitatory glutamatergic neurotransmission via the NMDA receptors in the central nervous system can induce general anesthesia, which includes analgesia, loss of consciousness and immobility (Irifune et al. 2007). The NMDA receptor is inhibited by most major anesthetics to varying degrees. However, it has been shown that NMDA receptor antagonism is not sufficient to cause anesthesia. An NMDA antagonist more potent than Ketamine and Isoflurane, called dizocilpine, alone was able to cause ataxia but not loss of consciousness (Kelland et al. 1993). NMDA receptor antagonism forms a

necessary part of the mechanism of function of isoflurane anesthesia but is not sufficient to explain its mechanism.

Glycine is one of the major neurotransmitters responsible for inhibition of signal propagation in the central nervous system. It binds to GABA receptors, thus, agonism of GABA receptors is another mechanism of action of anesthetics such as isoflurane. The analgesic effects of anesthesia are thought to be achieved by the modulation of spinal GABAa receptors thus depressing ventral root potentials in the spinal cord (Nadeson et al. 1997). The anesthetic effects are achieved by attenuation of signal propagated at the level of the thalamus and cortex. However, GABA agonism alone is insufficient to achieve immobility required for complete anesthesia (Zhang et al. 2004).

Isoflurane gas anesthesia has been widely used for anesthetized fMRI studies in animals. It can almost entirely eliminate movement artifacts but has been shown to also significantly blunt BOLD responses in both non-human primates and human subjects (Zhang et al 2000). This could be because as a sedative agent, it acts on a variety of receptors involved in the anesthetic pathway and thus elicits more potent anesthesia.

8.2 Propofol

Propofol is the chemical 2,6-diisopropylphenol and is a commonly used intravenous anesthetic developed by John Glen and Roger James in 1977 and commercially launched in the late 1980s (Sahinovic et al 2018). It binds to the beta subunit of the post synaptic gamma aminobutyric acid (GABAa) receptor causing hyperpolarization of the post-synaptic membrane and thus inhibits neuronal depolarization. Neuronal depolarization is how action potentials, the carriers of information in a neuron, pass information from one neuron to the next. If the membrane of a neuron is hyperpolarized, the threshold for depolarization signal received from the previous neuron increases, thus limiting information transfer (Borman et al. 2000). Although the mechanism of action of propofol at the cellular level is understood it is as yet unclear how this translates to greater effects on brain function such as loss of consciousness. Although total cerebral metabolism is reduced in every region of the brain under propofol anesthesia by 30-

70%, it is unclear that the reduction in cerebral metabolic rate corresponds to reduced consciousness. Ketamine anesthesia for example raises cerebral metabolic rate even while the animal appears to be functionally sedated (Hudetz et al. 2012). Thus, propofol's reduction in cerebral metabolic rate may not be the cause for its ability to diminish cortical responses.

8.3 Fentanyl

Fentanyl is an opioid anesthetic that acts primarily on the mu-opioid receptors and produces anesthetic effects that are distinct from isoflurane or propofol. At lower doses, fentanyl achieves analgesia, but at higher doses, it can achieve loss of consciousness, loss of movement and amnesia, as well as analgesia. Because it is not thought to affect NMDA and GABA receptors, except indirectly, we believe that it would spare the sensory cortical response to a greater degree than the other anesthetic protocols. It is known that fentanyl significantly decreases the propofol concentration required for loss of consciousness (Kazama et al. 1997). This is incredibly valuable because at higher doses, fentanyl has the risk of causing asphyxiation as the muscles around the lungs become inactivated. In addition, animals can develop a tolerance to fentanyl over time if it is used for longitudinal experiments in animals with repeated scanning under anesthesia. Using a combination of fentanyl to lower the dose of other anesthetics such as propofol means that there is a greater probably for signal to be spared as the different drugs act on different receptors and consequently each receptor type would not be saturated. Fentanyl may also permit us to better extrapolate conclusions from animal fMRI studies to humans because studies in pharmacological MRI have shown that macaques and humans have very similar distribution of mu-opioid receptors in the brain (Seah et al. 2014).

9. Choosing the optimal anesthetic candidate for MRI research

Different anesthetics may be better suited to preserve activity in different species of animals. Remifentanyl (a version of fentanyl) is a mu-opioid agonist which decreases cerebral metabolism and intracranial pressure with minimal cerebral perfusion changes. It is known to have little effect on the BOLD signal in anesthetized monkeys (Dubowitz 2004) but not in rats where isoflurane is superior for preserving this function (Hendrich et al, 2001). This means that

anesthesia studies in one species cannot be reliably translated between species without considering changes in anesthesia effect due to differing physiology. Rat brains may be more sensitive to cerebral pressure changes due to opioids and may have more sensitive, or a larger number of these receptors in key areas regulating cerebral pressure compared to macaques for example.

Different anesthetics may be better suited to preserving different types of functions of the brain. It was found that when dogs were sedated with in propofol, isoflurane or fentanyl anesthesia, they were able to produce significant univariate BOLD responses to visual stimuli in the subcortical, LGN, area of the brain but not in the striatum (Willis et al. 2001). However, for macaques, isoflurane attenuates the BOLD signal more severely in the visual cortex than in the lateral geniculate for any given dose (Dubowitz et al 2004). Isoflurane is able to preserve a large number of resting state networks in macaques (Hutchinston et al, 2011) but it not able to preserve the BOLD signal of sensory functions to the same degree. Propofol anesthesia could be a valid candidate because it has been shown that functional connectivity in the propofol sedated rat better mimicked the functional connectivity of the awake rat compared to isoflurane, urethane and medetomidine protocols (Passonen et al 2018).

Combination anesthetic protocols may be more ideal than individual drugs in some cases. While anesthesia is known to significantly attenuate the BOLD response measured, fentanyl in combination with propofol reduces the dose of propofol needed to achieve sedation endpoints and thus preserves greater somatosensory BOLD response in the common marmoset than when propofol is used alone (Liu et al. 2013). In a study comparing isoflurane (inhaled) and fentanyl (opioid) anesthesia protocols in rabbits, it was found that fentanyl anesthesia used alone preserved a greater percentage of the awake BOLD signal than isoflurane used alone or in combination with fentanyl protocols (Askenov et al. 2015). Isoflurane and propofol act on similar receptors so they may have an additive effect on loss of consciousness when combined (Sebel et al. 2006) whereas propofol and fentanyl in combination do not.

10. Physiology of Sensory Systems in Macaques

In order to study the ability of suitability of different anesthetic protocols for studying sensory systems in macaques, simple and reliable stimuli were chosen that have been shown to elicit robust univariate responses in macaques in the past. A complex stimulus would not be suitable here because the different features of the sensory system are not relevant to this investigation, but rather the comparison of the anesthetic protocol under which it is tested. The macaque sensory systems stimulated are the somatosensory system and the visual system, both of which have many similarities with human beings.

10.1 The Somatosensory system

The somatosensory response is highly developed in primates as it is used for a variety of functions such as foraging and the manipulation of tools. It is thought that the sensitive touch receptors on the hands of primates form a "tactile fovea" in the primate brain, that is a high-resolution representation of the world through touch (Hoffmann et al. 2004). There are many different types of touch receptors in the glabrous (hairless) skin of primate fingers. Two major categories of mechanoreceptor afferents have been established based on their response to skin indentation. Rapidly adapting receptors respond to transient skin deformation whereas slowly adapting receptors respond to prolonged skin deformation. Rapidly adapting receptors include Meissner's corpuscles (Type 1) and Pacinian corpuscles (Type 2) whereas slowly adapting receptors include Merkel's endings (Type 1) and Ruffini's corpuscles (Type 2) (Pare et al. 2002). Proprioceptive information from the passive movement of joints for example that are detected by the golgi tendon organs are carried by group II afferents as well.

Sensory afferent signals travel through the dorsal column medial lemniscus pathway to the medulla where second order neurons carry the information to thalamus (Catala et al. 2013). Different nuclei of the thalamus are specialized to relay specific modalities of sensory afferent information to different sensory cortices in the brain. Sensory afferents from mechanoreceptors in the skin travel from the VPM and VPLc nuclei of the thalamus to the primary somatosensory cortex (SI). This has been divided into four cytoarchitectonic areas called Brodmann's areas

which are: area 1, 2, 3a and 3b (Powell & Mountcastle 1959). In the most anterior section, 3a, neurons respond to stimulation of join, muscle or other types of deep structures in the fingers, hand or forearm such as the manipulation of finger joints. Posterior to 3a is area 3b where neurons are mainly activated by light contact of the glabrous skin at the distal finger segments. Area 1 and 2 receive information from areas 3a and 3b (Iwamura et al. 1983). The same body region is thought to activate neurons in different regions of SI based on the receptor type (Kaas et al. 1979). A somatotopic map of the body is preserved in area SI and in area SII.

This investigation explores the sensory response to passive joint flexion so, rapidly adapting mechanoreceptors as well proprioceptive receptors on the glabrous skin of the hand should be activated and a signal change should be observed in the hand area of SI. The contralateral SI area should show greater signal change than the ipsilateral area because the nerve carrying somatosensory signal from one side of the body decussates at the medulla (Hayashi et al. 1999) although it is known that there is some ipsilateral processing of somatosensory stimuli as well. This sensory response is straightforward and has been observed (Iwamura et al 1983) so the main question will be: under which of the anesthetic protocols does the stimulus elicit the most robust response.

10.2 The Visual System

Afferent sensory signals travel from the retina to specific nuclei of the thalamus based on their modality. Visual inputs are routed through the lateral geniculate (LGN) nucleus of the thalamus. Visual sensory stimulus information travels to a variety of brain areas including visual cortices from V1-5. V5 is also known as the medial temporal area (MT) which is responsible for detecting motion from the visual field. Both macaques and humans have complex visual systems. Complex visual systems are needed to read social cues which is important for social interactions (McFarland et al 2013) and considering both humans and macaques are highly social, this homology makes is logical. Functional MRI studies indicate some key functional areas in the brain that are involved in the processing visual information. The visual cortex includes striatal and extrastriatal areas. Visual cortical areas include V1, V2, V3 and V3a, the middle temporal area (MT), the medial superior temporal area (MST) and the superior temporal polysensory area

(STP). It is possible to collect visually-evoked responses in fMRI studies of awake macaques (Stefanacci et al 1998, Dubowitz et al 2001) as well as in anesthetized macaques (Logothetis et al 1999, Willis et al, 2001) and at all life stages (Kourtzi et al 2006). The areas that were consistently activated in these experiments were the LGN, MT and V1 areas. The LGN, a nucleus of the thalamus, is a part of the subcortical visual system and relays information to V1, MT(V5) and other areas of the visual cortex. Indeed, in a study that compared visual cortex activation in macaques and humans, homologous brain areas were activated in each species (Dubowitz et al 2001) contributing to the fact that macaques are an ideal animal model to study this system.

In this investigation, the Random Dot Kinetogram stimulus (see Methods) was chosen as the visual sensory stimulus because it is a robust, simple and reliable stimulus that has elicited visual activity in anesthetized macaques in the past. Activity was specifically expected in the MT region of the brain as it is known to process information about the movement of shapes the visual field (Zeki et al 1974) and is also known to process the speed of these objects and their arrangement in a 3D space for the purposes of producing eye-tracking and smooth pursuit eye movements (DeAngelis et al 2003, Takemura et al 2003).

11. Rationale and Research Questions

Macaque lesion models offer researchers an opportunity to systematically study sensory systems and neuroplasticity that is relevant to human beings. However, there are many investigations which cannot currently be done because anesthetic restraint of macaques during fMRI is not completely optimized. Anesthetic restraint is preferable to awake studies in situations where lesion studies must be done on a large cohort. Although many researchers successfully use awake macaques in fMRI studies, training a large lesion cohort for fMRI is unrealistic and has not yet been done successfully; the average macaque cohort reported in publications is two to three animals at most.

Furthermore, researchers who use anesthetic restraint for animal models in fMRI face the limitation that many of the signals they wish to measure and, thus the processes they wish to

study, are altered or severely attenuated by anesthetics. In addition, the diversity of anesthetic effects prevents researchers from making clear comparisons between studies in which different anesthetic protocols were used. Standardization in fMRI protocols would permit researchers to compare and combine data from multiple labs and create more powerful meta-analytical studies. The standardization procedure would require first the evaluation of common anesthetic protocols to understand which anesthetic protocols are best suited for different sensory modalities. One of the parameters that can approximate the suitability of an anesthetic to study a sensory modality is the robustness of the sensory response BOLD signal that can be elicited while the animal is anesthetized. Many anesthetics attenuate or ablate sensory response signal entirely, so isolating those protocols under which a BOLD response can be reliably elicited is important step toward identifying the optimal anesthetic protocol for NHP imaging studies.

The investigation in this thesis begins this standardization procedure by evaluating three commonly used anesthetic protocols for non-human primate fMRI studies of sensory stimulation: isoflurane gas, propofol and fentanyl in combination and fentanyl alone. The objectives of the study are to determine first whether a univariate response can be elicited under each anesthetic protocol and if so, what the nature of the univariate response is. The brain areas that are activated will be compared to brain areas that were predicted to be active in response to stimuli based on literature. For example, at the simplest level, the somatosensory stimulus to the right hand will be expected to elicit a response in the left primary somatosensory area (LSI) and the visual stimulus is expected to elicit a response in the fundus of the superior temporal (FST) area. This will tell us the degree to which the brain responds in predictable ways under each anesthetic protocol. It will also be investigated whether or not, and to what degree, salient thalamic nuclei will respond to the sensory stimulus while the animal is fully sedated. Thalamic activity has been recorded before in sedated rodents (Shroeter et al 2014).

An ideal anesthetic protocol is one under which the animal responds to sensory stimuli in a very similar way to an awake monkey. As this investigation does not include an awake monkey, this will be impossible to determine. Nevertheless, as there are so many possible anesthetic restraint candidates, the results from this investigation may be used to narrow down which anesthetics should be studied further and compared to an awake animal. As such, the results from this

investigation present an important initial step in developing the large-scale macaque studies that are lacking from fMRI macaque literature and will undoubtedly provide important insights into sensory neuroplasticity.

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Methods

This study was conducted at the Centre for Functional and Metabolic Mapping (CFMM) at the Robarts Research Institute at The University of Western Ontario. All experiments were performed in the morning between 8am and 1pm. Two adult male rhesus macaque monkeys were used (*M. mulatta* G and N, weighing 13 and 14 kg respectively and between the ages of 10-12). The experimental protocol was approved by the Western University Animal Care Committee (protocol #: 2019-004) according to the guidelines set out by the *Canadian Council of Animal Care*. A registered veterinary technician skilled in the healthcare and maintenance of non-human primates (NHPs) supervised all aspects of animal care.

1. Experimental Protocol

The experimental protocol consisted of three conditions, visual and somatosensory stimulation as well as rest. The experimental conditions in each run were presented in a block-design in the same order for all sessions. All blocks were 15 seconds in duration.

Each run began with 15s of rest following a trigger from the scanner console. Rest was followed by a visual stimulus followed by 15s of rest, then 15s of somatosensory stimulus, and 15s of rest. This sequence was repeated five times for each run. One run lasted 5 minutes and 25seconds and included 5 repetitions of each condition. It lasted 315 volumes with a TR of 1s. An average of 10 runs (7-11) were collected for each session. An average of 50 repetitions of each condition was collected in each session. A trigger was sent from the scanner to begin each run. During each session, the animal was wrapped in a blanket to ensure that it was warm in the scanner.

The stimuli were delivered using the PsycoPy3 (Pierce et al 2019) system, which automatically delivered the appropriate stimulus at pre-set time points. The stimuli were triggered to begin a run by the MRI scanner, right after the discarded steady state acquisition scans were collected. The discarded scans are carried out to allow the MRI system's longitudinal and transverse magnetizations to reach steady-state levels. The time at which each run started was also recorded in a separate data file for reference. These timings were used to ensure that the experimental

design timings input into the GLM matched the experimental timings. No slice-time correction was used in the data preprocessing because the TR was 1000ms. The PsychoPy3 setup is shown in Fig 1. A schematic of the full experiment is shown in Fig 2.

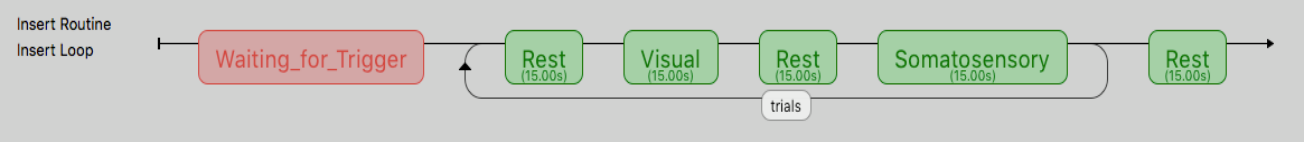

Fig 1. The PsychoPy3 stimulation protocol. First the system waits for a trigger from the MRI scanner to begin a run. It then moves to a 15second rest block, followed by visual stimulation, then rest, then somatosensory stimulation, then rest again. This loop repeats 5 times for one run and the end of the run is followed by another 15 seconds of rest.

1.1 Visual Stimulation

The subject was exposed to visual stimuli in the form of the Random Dot Kinetogram which consists of moving black dots on a white screen delivered using PsychoPy3 (Fig 1). The dots occurred at 60% density. There were 90 dots were moving at a constant speed setting of 0.25deg/s, at a size setting of 80deg, and a dot lifetime setting of 200ms. There was coherence of 0 for the map. The Random Dot Kinetogram stimulus has been used previously to stimulate the MT area in non-human primates (Priebe NJ et al. 2003).

The animal's eyes were kept open using medical tape holding the eyelids open. Optixcare eye, a sterile carbomer gel, specifically formulated for veterinary use as an eye lubricant (CLC Medica, Ontario, Canada) was placed on each eye to make sure that it did not dry out over the course of the experiment. An adjustable mirror was attached to the front of the head coil to ensure that the screen displaying visual stimuli during the experiment would be visible to the animal. The mirror was adjusted by looking from the other side of the wooden bore where the screen was located, and we confirmed that the animal would be able to see the screen if its eyes were visible in the mirror from this location.

1.2 Somatosensory Stimulation

The somatosensory stimulus was delivered via one metre wooden dowels taped to the animal's right hand prior to the scan, onto the glabrous skin at the distal interphalangeal joints of digits 2,3 and 4. The dowels were moved forwards and backwards at a steady rhythm moving once per second, causing flexion of the animal's fingers. The rhythm was set by a 60 beats per minute metronome sound in the experimenter's ear. The stimulus lasted for 15s causing 15 hand flexions in one trial.

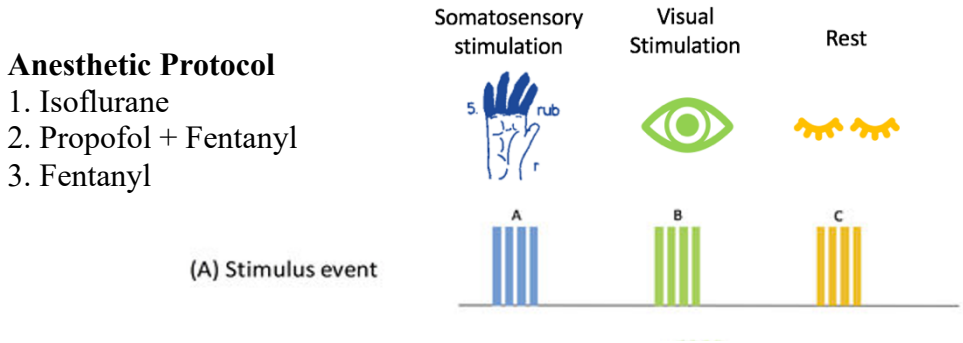

Fig 2. The stimulus design for each experiment. This was repeated five times for each run such that there were five iterations of somatosensory stimulation and five iterations of visual stimulation. One run lasted 5 minutes and 25 seconds.

1.3 Anesthesia Protocols

Access to food was withdrawn at least 12h before an experimental session. Each animal underwent three experimental sessions, one for each of the three different anesthetic protocols being compared in this investigation. There was at least one month between scans. Table 1 details the scanning schedule for each monkey.

Table 1. Anesthesia Protocol experiment dates for each monkey

Anesthetic Protocol	**Monkey N**	**Monkey G**
Isoflurane	September 10, 2019	October 14, 2019
Propofol + Fentanyl	November 21, 2019	December 4, 2019
Fentanyl	December 5, 2019	November 19, 2019

There was a total of six scanning sessions, with three sessions for each animal (Monkey N and Monkey G). In each of the three sessions, the animal was anesthetized using one of three candidate anesthesia protocols. The protocols are as follows:

1) A ketamine induction agent (10-15mg/kg IM) and a pre-medication of acepromazine (0.1mg/kg IM) was given to permit safe handling and transportation of the animal to the MRI suite. Following proper placement of the animal in the MRI bed in the sphinx position (laying down on its stomach), monitoring equipment was attached to measure respiration rate, heart rate and blood O_2 saturation. The animal was then given a constant rate infusion of Isoflurane gas alone (1-2%) delivered in oxygen (1.5L/min). This protocol is indicated in the results section as the "Isoflurane" protocol.

2) This protocol is referred to in the results as the "Propofol-Fentanyl" protocol. In this protocol, the animal was given a premedication of ketamine (1-3mg/kg IM) to transport it to the MRI suit and then a Ketamine induction agent (5-10mg/kg) with a bolus of fentanyl (5mcg/kg IM). The animal was then given a constant rate infusion of fentanyl (7-10mcg/kg/hr IV) and Propofol (0.1-0.7mg/kg/min IV with 1.5-2L/min oxygen) with all of the appropriate monitoring equipment and stimulus equipment attached.

3) This protocol is referred to in the results as the "Fentanyl" protocol. In this protocol, the animal was given a Ketamine induction agent (5-10mg/kg) to transport it to the MRI suite and then given a bolus of fentanyl (5mcg/kg IM) while it was being prepared with the monitoring equipment. When all of the equipment was set up, it was given a constant rate infusion of Isoflurane gas (0.25-1% delivered in 1.5L/min oxygen) with Fentanyl (7-10mcg/kg/hr IV). Isoflurane was weaned off and was absent during the 7 runs of this experiment such that it was only anesthetized using fentanyl.

The animal's heartrate, respiratory rate and O_2 saturation was monitored throughout the experiment. If the heart rate increased or respiratory rate increased beyond baseline, then the isoflurane level was increased in order to prevent the animal waking up in the scanner and

becoming distressed. The respiratory rate was monitored and if it fell below a certain level 90% saturation then the fentanyl level was decreased as this drug can depress respiratory function. The O_2 saturation level was limited in its utility because it relied on the animal having a light patch of skin on which to attach the monitoring clip.

A more detailed description of anesthesia protocols as well as the physiologic parameters measured during the scan can be found in the Appendix of this document (Tables 2-14).

1.4 MRI Scanning Protocol

Data were acquired on an actively shielded 7T Siemens MAGENTOM Step 2.3 58-cm horizontal bore scanner (Erlanger, Germany) operating at a slew rate of 300mT/m/s. An in-house designed and manufactured 8-channel transmit, 24-channel receive primate head radiofrequency coil was used for all MR image acquisitions (Gilbert et al. 2016). Magnetic field optimization (B_o shimming with shims up to 4^{th} order) was performed using an automated 3D mapping procedure over the whole brain. For each animal, there were three sessions. In each session, we acquired seven to eleven, 5 minutes 25 second runs of 350 $T2^*$ weighted continuous multi-band echo-planar imaging (EPI) functional volumes (TR = 1000ms, TE = 18.0ms, flip angle = 40^o, slices = 34, matrix size = 350 x 350, field of view = 96 x 34mm, acquisition voxel size = 1.0 x 1.0 x 1.0mm). EPI functional volumes were acquired with GRAPPA at an acceleration factor of 2.

For each animal, at least one MP2RAGE image was also collected for registration purposes (TE/TR = 3.88ms/6500ms, TI = 2700ms, flip angle = 5^o, field of view = 128 x 128mm, slices = 128, slice thickness = 0.5mm, resolution = 256).

For each animal, within each session, three 10 minutes runs of resting-state data (rsfMRI) were collected. Each 10-minute run consisted of 600 T2*- weighted continuous multi-band echo-planar imaging (EPI) functional volumes (TR = 1000 ms, TE = 18 ms, flip angle = 40°, slices = 42, matrix size = 96 x 96, field of view = 96 x 96 mm, acquisition voxel size = 1 x 1 x 1 mm). Resting state data was also collected to measure the blood-oxygenation level-dependent (BOLD) signal at rest. This scan also avoids potential confounds; in task-based fMRI it would be difficult

to distinguish, for example, whether the change in functional connectivity (FC) between two brain areas was due to the stimulus or due to the anesthetic protocol.

2. Image Analysis

2.1 Pre-Processing

MR image preprocessing was implemented using the FMRIB Software Library (FSL; http://www.fmrib.ox.ac.uk). First the MP2RAGE image, was brain extracted using FSL's brain extraction tool. The resultant image was the animal's brain without the surrounding muscle and bone around it. This image was turned into a binary mask which was then applied to the functional images.

Functional data were then pre-processed using FSL's MCFLIRT motion correction (12-parameter affine transformation) (Jenkinson et al., 2002), no spatial smoothing, and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting with $\sigma = 100$ s). Functional images were re-labelled using the "mri_convert" function so that the orientation labels would align between the functional images and the atlas. All of the functional images within a session were registered together using the FLIRT function in the FMRIB toolbox.

2.2 Analysis of Functional Data using the GLM

Each voxel that makes up the image experiences some signal change in the MRI scanner. A general linear model (GLM) was used to determine how much of the signal change can be attributed to the experimental condition applied and the probability that the signal change is due to the paradigm and not due to chance or noise. To do this, design paradigm regressors are created and convolved with the expected hemodynamic response function of the animal. In this analysis, the double gamma hemodynamic response function was convolved with the design paradigm for regressor 1 (tactile stimulus) and for regressor 2 (visual stimulus). The paradigm consists of the timings when the stimulus was on and when it was off for one complete run. The GLM then compares this paradigm to the activity measured in the voxel and measures beta

weights. Beta weights are the constant by which activity in each voxel would have to be multiplied in order to match with the expected model. If the voxel's activity pattern behaved exactly as we expect it would if it were responding to the stimulus perfectly, its beta weight would be one because there would be no difference between its activity and the expected pattern.

The general linear model was implemented using FSL's FEAT (fMRI Expert Analysis Tool, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT) where the BOLD signal time course was used as a predictor in a multiple regression model for each individual functional run. At the individual subject level and for each time point, a fixed effects analysis was performed across all functional runs. Corrections for multiple comparisons were implemented at the cluster level with Gaussian random field theory with $z > 2.3$ and a cluster significance of $p < 0.05$.

Two regressors were created: one to represent the presence of visual stimuli for each run and one to represent the presence of somatosensory stimuli for each run. Each run was analyzed for each session. This within-subject, within-session analysis produced a contrast of parameter estimates (COPE) image for each time point that showed positive correlations of each voxel's signal change relative to baseline. A statistical parametric map, also called a "t-statistic map," was produced for each regressor. The t-statistic maps for created using the visual stimulus regressor were averaged across all runs for a given subject within one session. The same was done for the t-statistic maps created using the somatosensory stimulus regressor. These images were overlaid onto the averaged functional echo-planar image (EPI) for the respective session. Thus, the results include a t-statistic contrast that shows areas of activity during the visual stimulation, and one to show areas of activity during the tactile stimulation for each session (under each anesthetic protocol). In total, six t-statistic contrast maps were produced for Monkey N and six for Monkey G.

The t-statistic map is the result of multiple tests to determine the likelihood that the activity in the voxel was due to the paradigm and not due to chance. It was determined for each voxel by dividing the beta weight for a given paradigm (tactile or visual), by the standard error of these beta values across the whole brain. This relationship is illustrated in the following formula where

"*t"* is *t*-stat value, "$c\beta$" represents the beta weight for a given paradigm—also known as contrast, and "*SE*" represents standard error of the beta weight values.

$$t = \frac{c\beta}{SE(c\beta)}$$

T-statistic maps do not report amplitude of activation, but rather spatially localized regions of the brain that show a significant effect different from zero. To adequately evaluate the t-statistic maps, they were visualized in FSLeyes from the FMRIB Software Library and the thresholds were set to 2<t<10 such that only voxels more likely to have significant activity can be seen. Clusters of high t-value voxels in these t-statistic maps, therefore, indicate areas that are likely to have activity in response to the regressor that was used to generate it. The visual t-statistic map therefore was explored for clusters that would indicate BOLD activity in response to visual stimulation. The somatosensory t-statistic map was explored for clusters that would indicate BOLD activity in response to somatosensory stimulation BOLD activity is used as an approximation of brain activity.

Average t-statistic maps were calculated for each experiment and each animal. These maps show the activity in different voxels where a colour code is used to indicate t-value. Warmer colours indicate a higher t-value which means that the activity observed in the given voxel was more likely due to the experimental paradigm and not due to noise. Higher t-value voxels clustered together indicate an area of positive BOLD activity. All of these t-statistic maps are reported in the axial, coronal and sagittal planes. The slice in each t-statistic map has been chosen to include those brain areas that were anticipated to show activity in response to the stimulation.

2.3 Region of Interest Identification

The D99 macaque atlas (Reveley et al. 2017) was non-linearly registered using the Advanced Neuroimaging Tools (ANTs) software (Avants et al. 2011) to the mean functional EPI image for each animal and thus was aligned to all of the functional images. This atlas which is constructed based on original histological slices from male rhesus macaque monkey by Saleem and Logothetis (2012). The registered atlas was then overlaid onto the t-statistic contrasts (the statistical parametric maps) and the mean functional image and this was used to then

qualitatively identify the regions that suggested some clusters of activity for each session. An area that had a cluster of voxels with high t-statistic values would be inferred as having a high chance that the signal change in that area was correlated to the experimental paradigm and thus had neural activity that responded to the stimulus. For example, the average t-statistic contrast under regressor 1 (the tactile stimulation pattern) for Monkey N during its "Fentanyl" session, was overlaid with the non-linearly registered D99 atlas. This atlas was used to identify the region that corresponded to any clusters of high t-value voxels in the t-statistic map. The identified regions of interest (ROI) were then used to create binary region of interest masks. The regions of interest identified in the tactile t-statistic contrast maps were as follows: left secondary somatosensory area (LSII), bilateral ventral premotor cortex (F5), bilateral granular insula (IG), bilateral parietal operculum (7op), claustrum (CL) and the ventral parietal medial nucleus of the thalamus (VPM). The ROIs identified in the visual t-statistic contrast maps were medial temporal of the visual cortex (MT), lateral geniculate nucleus of the thalamus (LGN), fundus of the superior temporal visual area (FST) and the ventral intraparietal VIP area.

The atlas was non-linearly registered to each animal's mean functional image, for each experiment separately. This registered atlas was then used to create masks for the regions of interest initially identified using the high value clusters found in the t-statistic maps (as listed above). These masks were then used to calculate the percent BOLD change for each run, for each animal and in each anesthetic condition. For example, the LSI area was found to have some activity in the t-statistic map overlaid with the macaque atlas. Thus, each registered atlas was used to create an LSI mask specific to each experiment. The appropriate LSI mask was used to calculate the percent BOLD change in each run and runs were averaged for each experiment. These values were represented in a bar graph with error bars representing standard deviation. This procedure was repeated for every ROI mentioned above.

2.4 Isolating the Hand Area Functional Region of Interest

Isolating the hand area of SI was done by using data from another rhesus macaque monkey who had undergone a similar tactile stimulus protocol that had been used to generate a distance map indicating the exact finger area in its brain. This other monkey's T1 anatomical images

(MPRAGE) were registered to Monkey N and Monkey G's anatomical (T1) images, and then this matrix was used to bring the other monkey's distance map to both our subject's T1 images and T2* images using the non-linear registration software, ANTs. Once the images were aligned, the distance map were multiplied with the SI map from the D99 atlas, such that the resulting map included the distance map bounded by the SI area. I calculated the 90th percentile voxels in the distance map, which indicate those voxels which are different than 90% of the rest of the voxels in the map and would thus indicate the most likely location of the hand area. We used this dataset available from neurovault to complete this analysis: https://identifiers.org/neurovault.collection:3864 (Gorgolewski et al. 2015).

3. *Analysis of Percent Signal Change*

Percent signal change is calculated by dividing the time series by the mean baseline signal over time. The brain is always active and has a baseline of activity that is also registered in an fMRI scan intended to measure task-related activity. The mean baseline activity might be different in different experiments, so it is useful to calculate this value to compare activity between experiments.

$$\%\ BOLD\ change = \frac{c\beta}{mean} \times 100$$

Percent BOLD change was calculated as it is a quantitative measure of the amount of signal change during the experiment in response to stimulation. It was calculated for ROIs that were expected to be active during each type of stimulation. The medial temporal area of the visual cortex (MT) was expected to be activated in response to visual stimulation and the primary contralateral somatosensory area (LSI) was expected to be activated in response to somatosensory stimulation. In addition, regions that were qualitatively observed to have significant clusters of activity on the t-statistic map were identified by overlaying the D99 Macaque atlas (Reveley et al. 2017) onto the map. The non-linearly registered atlas was then used to create binary masks of areas showing activity in the t-statistic maps and the percent BOLD change was calculated for these areas in all experiments.

To calculate percent BOLD change, the BOLD image was calculated by dividing the $c\beta$ image by the mean of rest volumes and multiplying that image by 100. Next, this image was analyzed using "fslstats" function of FSL to report the average % BOLD change of all the voxels within the provided mask for an ROI. There was a %BOLD change value calculated for each run and these values were averaged and reported in the results as bar graphs.

Two questions were asked for each ROI identified: 1) did the activity change from baseline under each type of anesthetic protocol for the animal? 2) was the activity in the ROI different for the animal across the three anesthetic protocols? First, we wanted to determine whether there was reliable percent signal change in the expected areas of interest, MT area for the visual stimulation and LSI area for the tactile stimulation, for each animal under each type of anesthetic protocol. To do this, the percent BOLD change was calculated for this area for all of the runs in each session, and in each animal. The percent BOLD change for each ROI was averaged within a session for an animal and reported in a bar graph that showed the average signal change in each session. A two-tailed t-test was performed to determine whether this percent BOLD change was significantly different from zero in each session. Next the activation for an ROI was compared for one animal across all three sessions using an ANOVA test to see whether the activity in a particular ROI such as the LSI area, changed across the three sessions (under different anesthetic protocols). Although normally, doing multiple t-tests requires us to do a multiple comparison's correction to reduce the false positive rate that may arise, this was not done for this investigation as it was largely exploratory in nature. The two animals could not be meaningfully compared to each other as there were only two animals available for analysis.

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Results

During tactile stimulation, activity was anticipated in the left primary somatosensory area (LSI) of the brain and during visual stimulation, activity was anticipated in the bilateral medial temporal area (MT) of the visual cortex but was actually mainly observed in the fundus of the superior temporal sulcus (FST) area. Monkey G and Monkey N responded in different ways to sensory stimulation under different anesthetic protocols. These responses were measured using statistical parametric maps and percent BOLD change in specific regions of interest.

The anesthetics were delivered in each experiment, at the minimal dose at which the animal was still sedated based on physiologic parameters measured. This meant that an exact dose of the anesthetic was not delivered for each experiment, but rather a range of doses. It was found that these changes in doses did not result in significant variance in physiologic parameters such as heart rate, respiratory rate, temperature, oxygen saturation level (SPO2) and CO2 values (see Appendix Tables 2-13). Stability in these parameters indicates that the animal was sufficiently sedated during all runs and for each experiment.

1.1 Functional Response of LSI to somatosensory stimulation of the right hand

Statistical parametric maps were made using the somatosensory stimulus regressor in the GLM and averaged across all runs for each experiment and for each animal. These maps are shown in

Fig 3. The t-statistic maps were first investigated for activity in the anticipated brain area (LSI). Figure 3 reveals that for Monkey N, during both the propofol-fentanyl and the fentanyl anesthetic protocols, there is a clear cluster of high t-value voxels indicating activity in the LSI area (Fig 3B, 3C). From a qualitative assessment, the response during the fentanyl condition appears stronger than during any of the other conditions. This is because the activated voxels have a higher t-value in the fentanyl map than in the propofol-fentanyl map and are clustered more closely together in the left SI area. Additionally, the fentanyl condition for Monkey N reveals that many other areas of the brain appear to have been activated in response to somatosensory stimulation (Fig 3C).

The t-statistic maps are reported for Monkey G in Figure 3D-F. These maps revealed that, of the three anesthetic conditions, only during the propofol-fentanyl condition (Fig 3E) was somatosensory stimulation able to qualitatively elicit a cluster of high t-statistic value voxels and this response appears to be clustered in the LSI area. Clusters of high t-value voxels were not identified in other regions in Monkey G in response to somatosensory stimulation, based on a qualitative assessment of these statistical parametric maps.

1.2 Percent BOLD change response to somatosensory stimulation

In order to determine the amount of activity in different brain areas during each anesthetic protocol, the percent signal (BOLD) change was calculated for specific regions of interest. The D99 atlas was non-linearly registered to each animal's pre-processed average EPI image using ANTs software. The registered atlas was then used to create masks of relevant regions of interest (see Methods). The region of interest that was explored first for somatosensory stimulus response was the primary SI area. The whole SI area rather than just the hand area was assessed in this analysis. The mask for this ROI and the resultant percent BOLD change for each anesthetic protocol in this region is shown in Figure 4. Each bar represents the % BOLD change averaged over 7-10 runs for the experiment.

In Figure 4A, the % BOLD change in response to tactile stimulation in the LSI area is reported for Monkey N. We observed that the BOLD change response was significantly different from baseline for Monkey N during the propofol-fentanyl ($p < 0.01$), the fentanyl ($p < 0.01$) and during the isoflurane protocols ($p = 0.03$); however, during the isoflurane protocol, the % BOLD change was actually reduced from the baseline. We also found that the percent BOLD change in the left (contralateral) somatosensory area was significantly different than that in the right (ipsilateral) somatosensory area for all anesthetic conditions in Monkey N, based on a two tailed t-test between left and right hemisphere BOLD values (Isoflurane $p = 0.15$, Propofol-fentanyl $p < 0.01$, Fentanyl $p < 0.01$). A hemisphere specific response supports the assertion that the response was indeed due to the hemisphere specific stimulation: only the right hand was stimulated, and it is well established that the contralateral area of the sensory cortex should be disproportionately activated (Nihashi et al 2005). The sensory response in the left SI during both the propofol-fentanyl and the fentanyl conditions was also shown to be significantly greater than that during the isoflurane condition (ANOVA test $F(2) = 15.5$, $p < 0.001$, with Tukey's post-hoc, $p < 0.01$, $p < 0.001$ respectively). No significant differences were observed between the BOLD changes observed in SI under the propofol-fentanyl protocol and the fentanyl protocol (ANOVA test $F(2) = 15.5$, $p < 0.001$ with Tukey's post-hoc, $p_{tukey} = 0.775$).

The same analysis is reported for Monkey G in Figure 4B. We observed that the % BOLD change was significantly greater than baseline for the isoflurane ($p < 0.003$) and propofol-fentanyl conditions ($p = 0.044$) but not for the fentanyl condition in the left SI area ($p = 0.09$). Hemispheric differences were found in each condition as well: the left SI area showed greater activity than the right SI (Iso $p < 0.01$, Propofol-Fentanyl $p < 0.01$, Fent $p < 0.01$), providing evidence that the response was indeed to the somatosensory stimulus applied to the animal's right hand. However, no significant differences were observed between the three anesthetic conditions for Monkey G where significance was measured with a p value < 0.05 (ANOVA test $F(2) = 6.02$, $p = 0.004$). Tukey's post hoc reports p_{tukey} value for difference between Fentanyl and Isoflurane is $p = 0.153$, between Fentanyl and Propofol-Fentanyl is $p = 0.403$, and between Isoflurane and Propofol-Fentanyl is $p = 0.986$. All three p_{tukey} values are greater than 0.05 indicating no significant difference. The significance reported in the ANOVA were due to hemispheric differences alone.

1.3 Functional response in hand area of LSI to right hand stimulation

The SI area receives inputs from somatosensory receptors from all over the body, however, in this investigation, we stimulated only the fingers on the right hand. This would stimulate both cutaneous and proprioceptive receptors that would be processed only in a small area of SI. While there was no difference between the propofol-fentanyl and fentanyl condition tactile responses in Monkey N, the t-statistic map shows a difference in the distribution of activation. The fentanyl condition appears to have stronger more localized response to somatosensory stimulation in Monkey N (Fig 3C) than the propofol fentanyl condition (Fig 3B), but it is unclear whether this localized response is within the hand area of SI. The somatotopic organization of the SI area of both the macaque and human brain is well known. In order to more precisely analyse the tactile stimulus response, therefore, a mask of the hand area alone was isolated and the BOLD changes within this more specific ROI were assessed (see Methods). The quality of the alignment of the functional hand area ROI taken from another macaque to the subjects used in this investigation are shown in Figure 5B for Monkey N and Figure 5D for Monkey G.

This, now very specific, map of the macaque hand area was used to calculate the percent BOLD change in response to the tactile stimulus. In the Monkey N (Fig 5A), the BOLD change responses of the hand area to somatosensory stimulation that were observed under the propofol-fentanyl and fentanyl anesthetic protocols were significantly increased from the baseline (two tailed t-test with $p < 0.01$, and $p = <0.01$ respectively) and were significantly greater than the tactile BOLD response observed under isoflurane protocol (ANOVA, $F(9) = 34.1$, $p < 0.001$ with Tukey's post hoc test). The BOLD change activity measured in the left SI hand area in response to somatosensory stimulation under isoflurane was not significantly increased or decreased from baseline (two-tailed t-test $p = 0.22$). There was no significant difference observed between the BOLD change observed under the propofol-fentanyl and the fentanyl protocols (ANOVA $F(2) = 34.154$, $p < 0.001$ with Tukey's post hoc $p_{tukey} = 0.806$). However BOLD change in the hand area under both the propofol-fentanyl and fentanyl protocols was greater than that observed in the hand area under isoflurane for Monkey N ($p_{tukey} < 0.001$ and $p_{tukey} < 0.001$ respectively).

In Monkey G (Fig 3B), the BOLD change responses to somatosensory stimulation in the hand area was only observed to be significantly increased from the baseline in the propofol-fentanyl condition (two tailed t-test with $p < 0.01$ $df = 10$) and the signal under propofol-fentanyl was significantly greater than the signal observed in the fentanyl and isoflurane protocol for this animal (ANOVA test $F(2) = 5.1$, $p < 0.02$ with Tukey's post-hoc $p_{tukey} < 0.05$ and $p_{tukey} < 0.05$ respectively). Unlike when the whole LSI area was analyzed, when only the hand area of LSI was investigated, there was no significant BOLD change observed under the isoflurane condition for Monkey G (two tailed t-test $p = 0.17$). There was no significant difference between the BOLD change observed in the hand area of LSI under the isoflurane protocol and the fentanyl protocol (Tukey's post-hoc, $p_{tukey} = 0.929$).

We then investigated whether the BOLD change pattern was different when just the hand area of left SI was assessed as compared to the entire left SI area. We found that in Monkey N, under all anesthetic protocols, the left SI hand area showed a significantly greater percent BOLD change than the BOLD change observed under the left SI area as a whole (two tailed t-tests, Isoflurane $p < 0.001$, Propofol-Fentanyl $p < 0.001$, Fentanyl $p < 0.001$) (Fig 6A). In Monkey G, only under the propofol-fentanyl condition, the percent BOLD change in the left-hand area was greater than the signal change in the left SI area as a whole (two-tailed t-test, Propofol-Fentanyl $p < 0.001$) (Fig 6B). The BOLD change under isoflurane and under fentanyl protocols, however, was not observed to be significantly changed from baseline (two-tailed t-tests, $p = 0.17$, and $p = 0.72$ respectively).

1.4 Functional response in other ROIs observed to be active during somatosensory stimulation

As previously mentioned, Figure 3C shows that other areas in addition to SI may have been activated in response to somatosensory stimulation of the right hand, at least in Monkey N. To assess this quantitatively, the same non-linearly registered D99 atlas was overlaid onto the t-statistic contrast map and the functional image to determine what areas correspond to these observed clusters of activation. The atlas labels determined a collection of areas of the brain that show high t-statistic value voxels clustered together. Masks for these regions were created using the atlas and were used to calculate the average % BOLD change across the runs for each

experiment. The regions of interest were as follows: left secondary somatosensory area (LSII), bilateral ventral premotor cortex (F5), bilateral granular insula (IG), bilateral parietal operculum (7OP), claustrum (CL). These areas are all reported to be involved in the detection or processing of tactile information and their functions are expanded further in the discussion section of this report.

We observed that for Monkey N (Fig 7), during the fentanyl condition, all of these areas (the third bar in Fig 7A-E) showed a % BOLD change signal that is significantly increased from baseline in response to somatosensory stimulation (two tailed t-tests, $p < 0.05$). During the propofol condition (the second bar in Fig 7A-E), all areas report a significant increase in % BOLD change except for the IG (two-tailed t-test, $p = 0.763$) (Fig 7C). Finally, during the isoflurane condition (the first bar in Fig 7A-E), there is a significant decrease in % BOLD change reported in all areas except the 7OP and CL ($p = 0.196$ and $p = 0.43$ respectively) (Fig 7D). In all somatosensory areas assessed, it was revealed that the % BOLD change observed under the fentanyl condition was significantly greater than under the other anesthetic protocols (ANOVA test $F(2) = 6.8$, $p = 0.04$ with Tukey's post hoc, $p < 0.001$). The pattern of these results may elucidate different mechanisms of action of anesthetic sedation that will be further elaborated in the Discussion.

For Monkey G, the same areas were assessed for % BOLD change during the different anesthetic conditions (Fig 8). Under the fentanyl protocol (the third bar in Fig 8A-E), the response to somatosensory stimulation was significantly increased from baseline in SII ($p < 0.05$) and F5 ($p < 0.05$), whereas it was decreased significantly in 7OP ($p < 0.01$) and CL ($p < 0.01$) and did not change in the IG area ($p = 0.41$) (Fig 8C). During the propofol-fentanyl protocol (the second most bar in Fig 8A-E), the response was significantly decreased in F5 ($p < 0.01$), IG ($p < 0.05$) and CL ($p < 0.01$) regions but was not significantly different from baseline in the SII and 7OP regions ($p = 0.31$ and $p = 0.64$ respectively). During the isoflurane protocol (the first bar in Fig 8A-E), the response in Monkey G was not significantly changed from baseline in any region assessed here (two-tailed t-test, SII $p = 0.21$, F5 $p = 0.39$, IG $p = 0.17$, 7OP $p = 0.73$, CL $p = 0.48$).

There were no significant differences measured between anesthetic protocols for any of these regions of interest. An ANOVA test was done ($F(2) = 4.1$, $p < 0.01$) with Tukey's post hoc test. The SII response under isoflurane was not different than under propofol-fentanyl ($p_{tukey} = 1.0$) or from fentanyl ($p_{tukey} = 1.0$) and there was no difference between propofol-fentanyl and fentanyl response ($p_{tukey} = 1.0$). The F5 response under isoflurane was not different than under propofol fentanyl ($p_{tukey} = 0.62$), or from fentanyl ($p_{tukey} = 0.98$), and there was no difference between propofol-fentanyl and fentanyl response ($p_{tukey} = 1.0$). The IG response under isoflurane was not significantly different than under propofol-fentanyl ($p_{tukey} = 0.99$), or from fentanyl ($p_{tukey} = 0.99$) and there was no difference in the response between propofol-fentanyl and fentanyl ($p_{tukey} = 1.0$). The 7OP response under isoflurane was not significantly different than under propofol-fentanyl ($p_{tukey} = 1.0$), or from fentanyl ($p_{tukey} = 1.0$) and there was no difference in the response between propofol-fentanyl and fentanyl ($p_{tukey} = 1.0$). The CL response under isoflurane was significantly greater than under propofol-fentanyl ($p_{tukey} < 0.001$), and from fentanyl ($p_{tukey} < 1.0$) but there was no difference in the response between propofol-fentanyl and fentanyl ($p_{tukey} = 0.11$). This is because under the CL condition, both the propofol-fentanyl and fentanyl BOLD responses were decreased from baseline for Monkey G.

2. Functional Response to Visual Stimulation

The averaged t-statistic maps showing response to visual stimulation for Monkey N and Monkey G under each anesthetic condition, are reported in Figure 9. For Monkey N, the visual stimulus was able to elicit a significant BOLD change effect only in the FST area of the brain during the fentanyl condition (Fig 9C) but was not able to elicit activity that can be qualitatively observed in any regions during the isoflurane (Fig 9A) or propofol fentanyl (Fig 9B) conditions. For Monkey G, Fig 9D-F shows that a response to the visual stimulus was not observed under any of the anesthetic protocols when the visual areas of the animal's brain were inspected based on the absence of clusters of voxels with a high t-statistic value.

2.1 Percent BOLD change response to visual stimulation in the MT area

The visual areas were assessed for the amount of BOLD change activity in specific ROIs. First, the area that was expected to respond to this type of visual stimulus, the medial temporal area of the visual cortex (MT), was investigated. A mask of this area was created using the D99 atlas and non-linearly registered to the average functional image for each animal. Fig 10B for Monkey N and Fig 10D for Monkey G shows the quality of these masks. The percent BOLD change was calculated using these masks. It was observed that the MT area did not show any significant activity above the baseline for Monkey N (Fig 10A) under any of the anesthetic protocols (two tailed t-tests, Iso $p = 0.43$, Prop-Fent $p = 0.35$, Fent $p = 0.82$), and for Monkey G (10C, two tailed t-tests, Iso $p = 0.34$, Prop-Fent $p = 0.21$, Fent $p = 0.10$). There was also no reliable difference found between anesthetic protocols for Monkey N (ANOVA $F(2) = 0.149$, $p > 0.05$) or for Monkey G (ANOVA $F(2) = 0.414$, $p > 0.05$).

2.2 Functional response in other ROIs observed to be active during visual stimulation

Additional regions of interest (ROI) were determined by subjective assessment of high t-statistic value clusters qualitatively observed in the average t-statistic maps in Monkey N (Fig 9C). This was done in much the same way as was done to find additional ROIs for assessment of the somatosensory response. The ROIs explored in response to visual stimulation in this investigation were: the fundus of the superior temporal visual area (FST) and the ventral intraparietal (VIP) area. Percent BOLD change in response to visual stimulation, in each monkey and under each anesthetic condition for the appropriate ROIs were calculated.

For Monkey N, the quantitative assessment of activity in the visual area ROIs is reported in Fig 11. In Fig 11D, the ROI mask used for the FST area was overlaid onto Monkey N's mean functional EPI image in Fig 10B and for the VIP area. Under the fentanyl condition, the FST (11A) and VIP (11C) areas showed significant activation (FST $p = 0.001$, VIP $p = 0.04$). In addition, the activation level in the FST (11A) area during the fentanyl protocol was found to be greater than that observed during both the propofol-fentanyl and during the isoflurane condition (ANOVA test $F(2) = 35$, Prop-Fent $p < 0.001$ and Iso $p < 0.001$). None of the other anesthetic conditions, propofol-fentanyl or isoflurane, showed any observable change in activity from baseline in either the FST or the VIP areas (two-tailed t-test, Prop-Fent $p = 0.34$, Iso $p = 0.07$).

For Monkey G, the quantitative assessment of activity in the visual area ROIs is reported in Fig 12. The ROI mask used for the FST area was overlaid onto Monkey G's mean functional EPI image in Fig 12B and for the VIP area in Fig 12D. No reliable signal change was observed in the FST area for Monkey G under any anesthetic protocol (Fig 12A, two tailed t-test, Iso $p = 0.21$, Prop-Fent $p = 0.47$, Fent $p = 0.33$). Only under the isoflurane protocol was there any reliable signal change, which was in the VIP area (Fig 12C, two-tailed t-test, $p = 0.02$). There was no reliable signal change in the VIP area for Monkey G under propofol-fentanyl or fentanyl protocols (Fig 12C, two tailed t-test, $p > 0.05$).

3. Thalamic Responses to Somatosensory and Visual Stimulation

Specific thalamic nuclei are responsible for relaying different modalities of sensory information to higher cortical areas. The ventral posterior medial (VPM) nucleus relays primarily somatosensory information to the primary somatosensory areas and the lateral geniculate (LGN) nucleus relays primarily visual sensory information to the primary visual areas. The VPM and LGN area masks were created for each animal and are shown overlaid on each animal's mean functional EPI image below the relevant percent BOLD change analysis.

In Monkey N, there was no change in the signal observed in salient thalamic nuclei in response to visual or somatosensory stimulation. The percent BOLD change in the VPM area for Monkey N was calculated in response to somatosensory stimulation and it was found that there was no reliable signal change from baseline observed under any of the anesthetic protocols except for the Iso condition where the BOLD change was decreased from baseline (two-tailed t-test, Iso $p < 0.01$, Prop-Fent $p = 0.53$, Fent $p = 0.29$) (Fig 13A). The percent BOLD change in the LGN area for Monkey N was calculated in response to visual stimulation and it was found that there was no signal change from baseline observed under any of the anesthetic protocols (two-tailed t-test, Iso $p = 0.33$, Prop-Fent $p = 0.09$, Fent $p = 0.21$) (Fig 13B).

In Monkey G, there was also no change in the signal observed in salient thalamic nuclei in response to visual or somatosensory stimulation. The percent BOLD change in the VPM area for Monkey G was calculated in response to somatosensory stimulation and it was found that there was no signal change from baseline observed under any of the anesthetic protocols except during the Isoflurane condition where it was increased (two-tailed t-test Iso $p < 0.001$, prop-fent $p = 0.54$, Fent $p = 0.26$) (Fig 13C). The percent BOLD change in the LGN area for Monkey G was calculated in response to visual stimulation and it was found that there was no signal change from baseline observed under any of the anesthetic protocols (two-tailed t-test Iso $p = 0.16$ $df = 9$, Prop-Fent $p = 0.11$ $df = 10$, Fent $p = 0.19$ $df = 9$) (Fig 13D).

4. Summary of Main Results

In monkey N, a robust BOLD response to somatosensory stimuli was observed under propofol-fentanyl and under fentanyl anesthetic protocols but not under isoflurane (Fig 3A, B,C). This response was seen specifically in the LSI hand area (Fig 5A) and many other ROIs salient to the somatosensory response (Fig 7). In response to visual stimuli, however, a significant increase in BOLD activity was only observed under fentanyl anesthesia both in t-statistic maps of the response to visual stimulation (Fig 9A, B, C) and in percent BOLD change calculations of the FST area (Fig 11A) and VIP areas (Fig 11B) for monkey N. An analysis of relevant thalamic nuclei activity revealed that the VPM area for monkey N had a significant percent BOLD change in response to somatosensory stimulation, but the LGN area did not have a significant BOLD change response to visual stimulation (Fig 13A, B). Even though the normal pathway for sensory information flow was not robustly active for Monkey N, there was robust activity seen in response to both the somatosensory and visual modalities. This means that another pathway was likely active under anesthesia.

In Monkey G, a robust BOLD response was only seen in response to the somatosensory stimulus under propofol-fentanyl anesthesia (Fig 3E). A BOLD response to visual stimulation was not observed in Monkey G under any anesthetic protocol in any of the ROIs explored (Fig 9 D, E,

F). An analysis of relevant thalamic nuclei revealed that neither the VPM area was significantly active in response to somatosensory stimulation, nor was the LGN area significantly active in response to visual stimulation (Fig 13 C, D). This means the normal pathway for sensory information flow that goes through the thalamus was not observed to be active for Monkey G but was active somewhat for Monkey N.

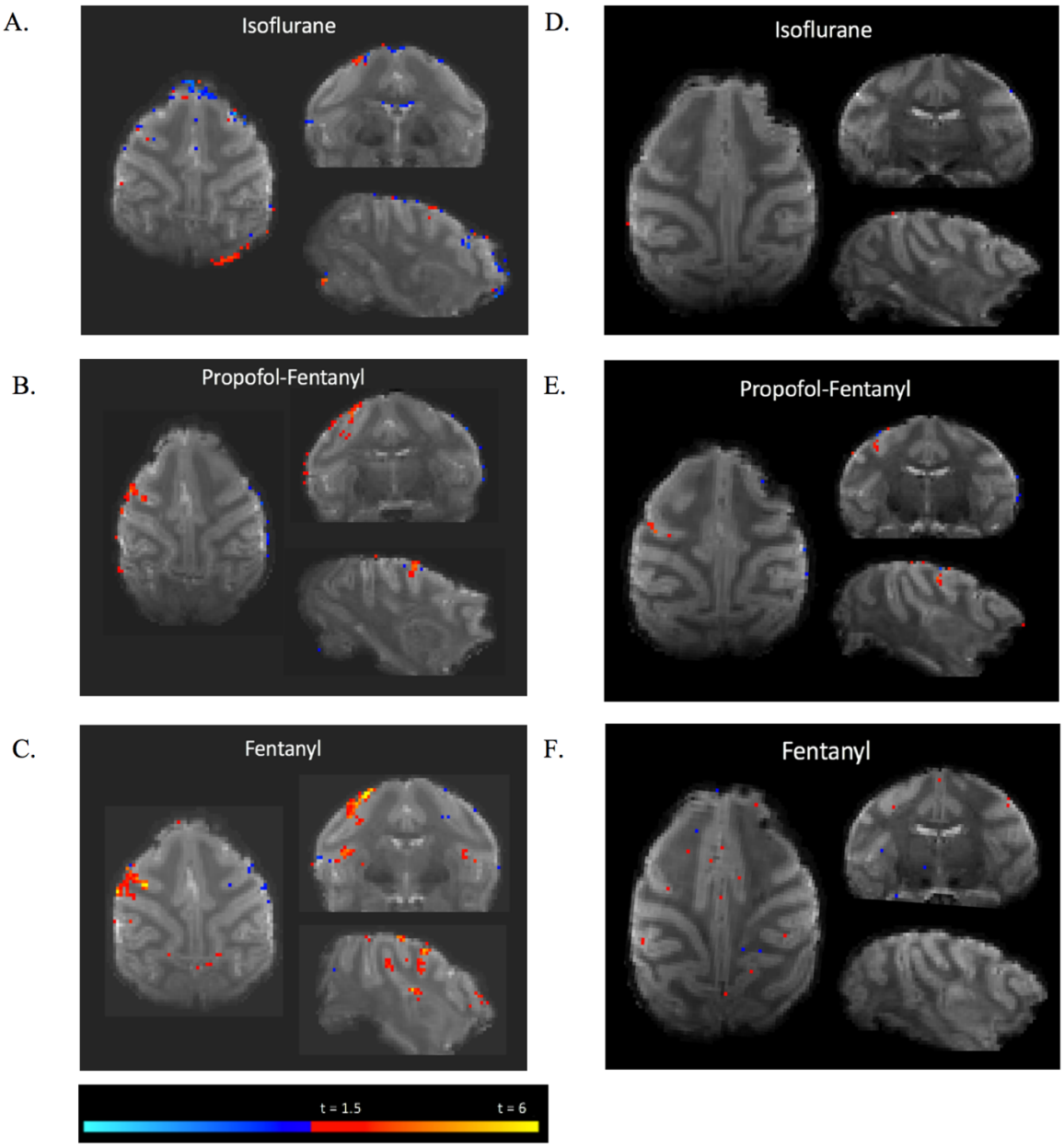


Fig 3. Statistical parametric maps of somatosensory stimulation response under three anesthetic protocols

The t-statistic maps were calculated using FSL software and overlaid onto the average functional image for that animal from that particular experiment. This image shows coronal, sagittal and axial views for Monkey N during somatosensory stimulation under the three different types of anesthetic protocols: (A) Isoflurane (B) Propofol+Fentanyl and (C) Fentanyl alone. The t-statistic maps of response to somatosensory stimulation are also shown for Monkey G under Isoflurane (D), Propofol+Fentanyl (E) and Fentanyl (F) anesthesia. Activity in each voxel represents a change in the signal from baseline. Values that are further from t=0 indicate a greater probability that the reported activity in the voxel is not due to chance. A number of high t-value voxels clustered together indicates that area was more likely to be functionally responding to the stimulation.

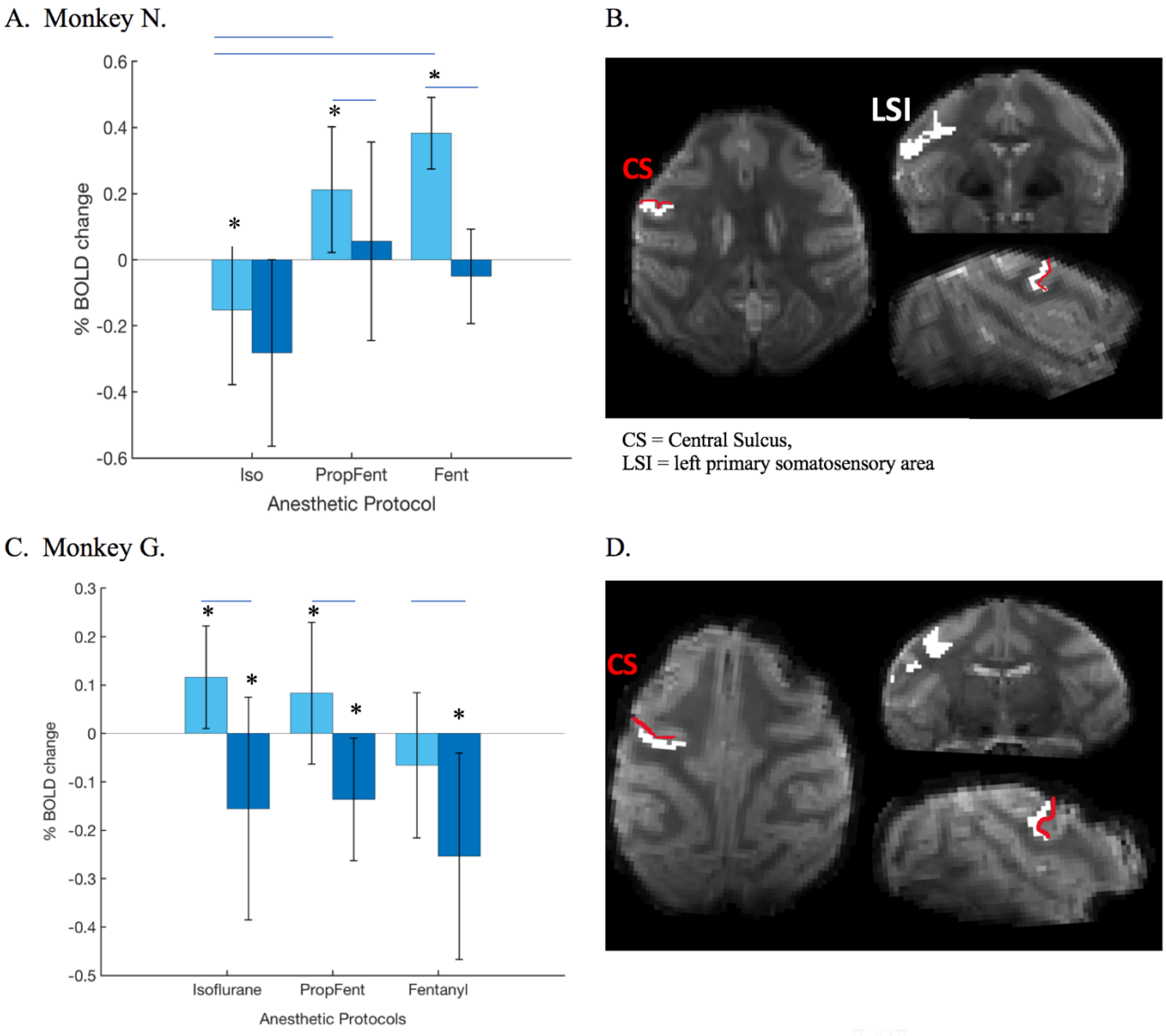


Fig 4. SI BOLD response to right hand somatosensory stimulation under different anesthetics

The percent BOLD change was calculated for left (light blue) and right (dark blue) primary somatosensory (SI) areas in two rhesus macaque monkeys: Monkey N and Monkey G, in response to flexion and extension of the right-hand. This experiment was done while the animal was anesthetized using isoflurane, propofol + fentanyl, or fentanyl alone protocols. Animals were scanned in a 7T MRI. Each bar represents the average percent BOLD change response in the LSI or RSI for one animal under a particular anesthetic protocol, over 7-11 runs of somatosensory stimulation. Stars indicate significant increase from baseline based on a two-tailed t-test ($p < 0.05$). Error bars show standard deviation. Horizontal lines indicate significant differences between anesthetic protocol groups according to an ANOVA test. (A) shows the average responses in the left and right hemispheres for each anesthetic protocol in Monkey N. (B) shows the region of interest that was used for this analysis overlaid on Monkey N's average functional image. (C) shows the average responses in the left and right hemispheres for each anesthetic protocol in Monkey G. (D) shows the region of interest that was used for this analysis overlaid on Monkey G's average functional image.

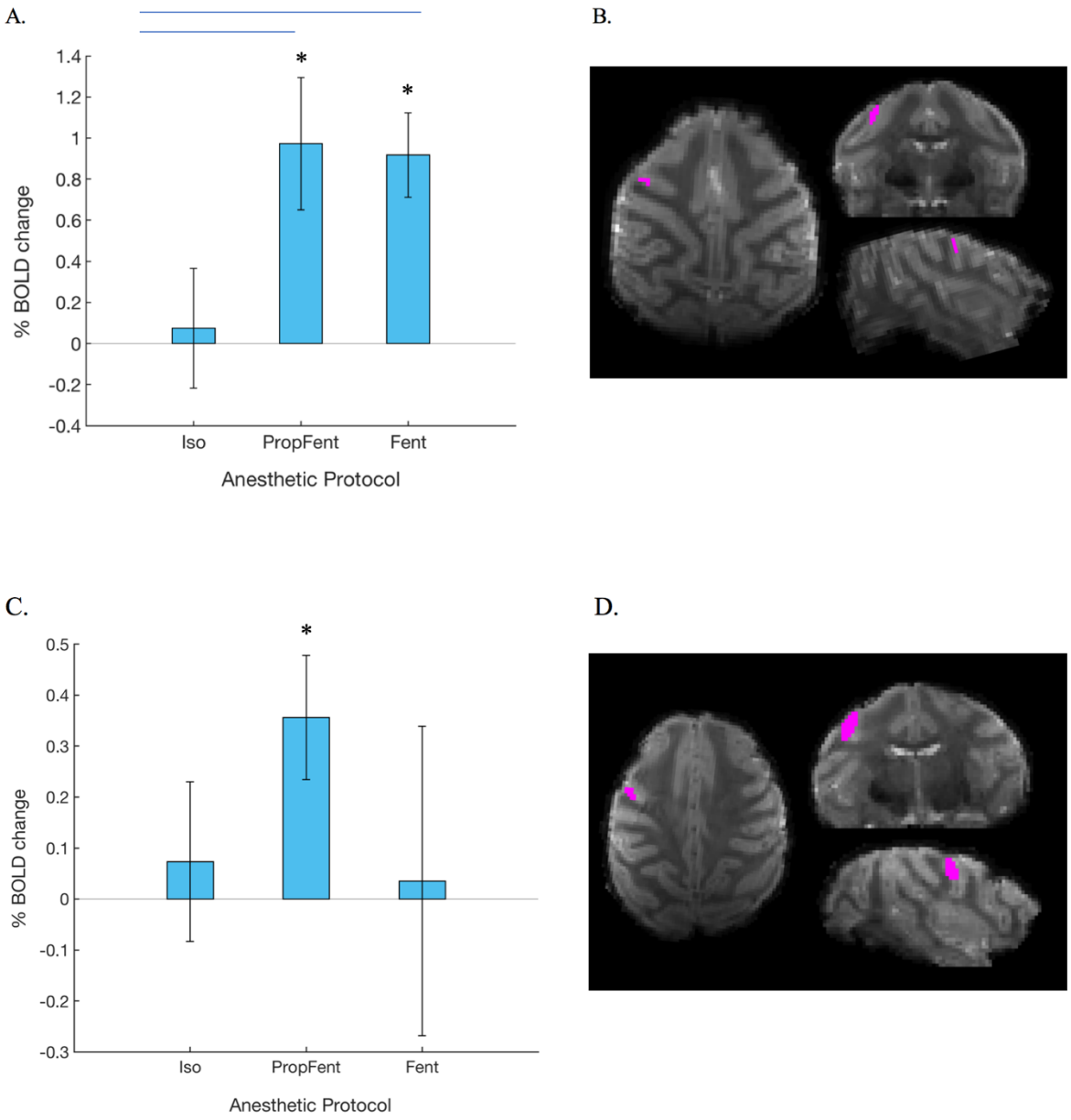


Fig 5. The hand area ROI of the macaque was isolated using a distance map generated in a different macaque monkey through multivariate fMRI analysis of finger stimulation response. This map was non-linearly registered to monkey N and monkey G using ANTs software and is shown overlaid on the respective monkey's mean functional EPI image (Fig B for Monkey N and Fig D for Monkey G). The hand area is a more specific ROI than using the entire somatosensory area as an ROI. The percent BOLD change in response to somatosensory stimulation of the hand by wooden dowels attached to the hand and moved at 1Hz was calculated within the hand area of SI for monkey N (Fig A) and monkey G (Fig C). Horizontal lines represent a significant difference in responses observed under different anesthetic conditions within the same animal, based on an ANOVA test.

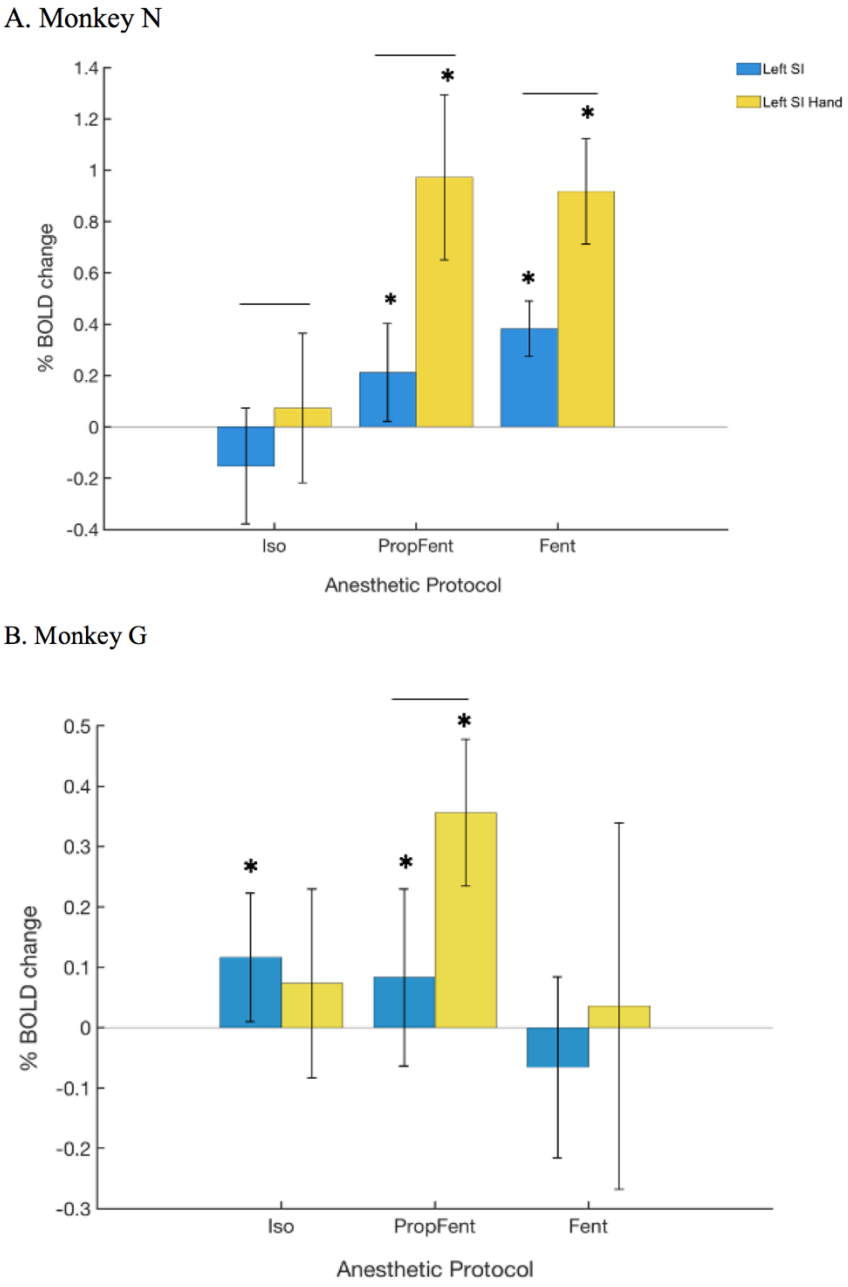
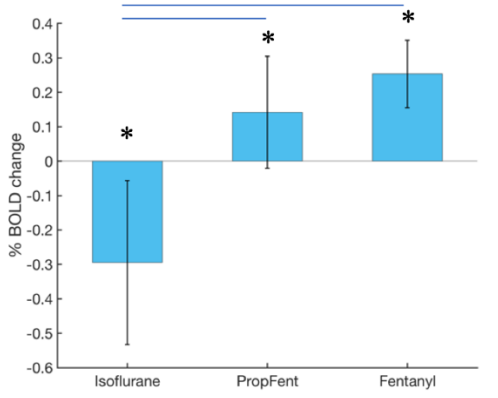
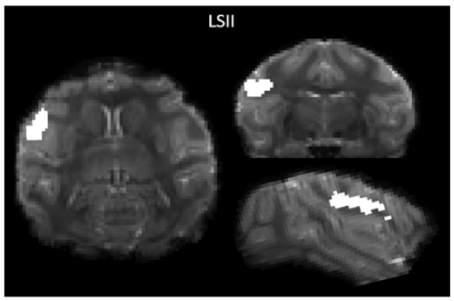


Fig 6. Comparison of BOLD response in whole LSI area to BOLD response in hand area of LSI

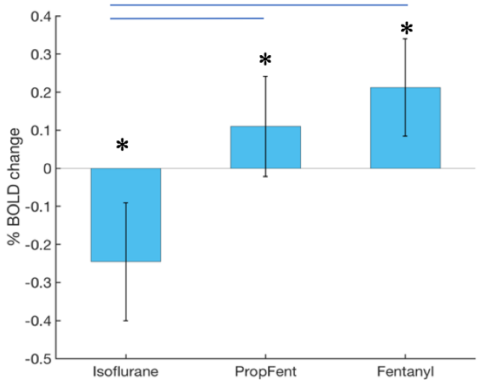
The percent BOLD change response to somatosensory stimulation was compared between the left SI area as a whole (blue bars) and the left SI hand area derived from multivariate analysis of macaque hands in 7T MRI (yellow bars). The analysis was done for Monkey N (A) and for Monkey G (B). Each bar represents an average percent BOLD change signal across 7-11 runs of somatosensory stimulation of the animal's right hand in a 7T MRI system under three different experimental conditions: Isoflurane anesthesia, Propofol-Fentanyl anesthesia or Fentanyl anesthesia. Stars indicate an average percent BOLD change significantly different from the baseline (two-tailed t-test, $p < 0.05$). Horizontal lines indicate significant difference between the two bars (two-tailed t-test, $p < 0.05$).

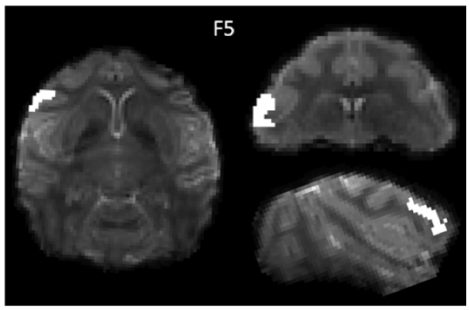
A. LSII



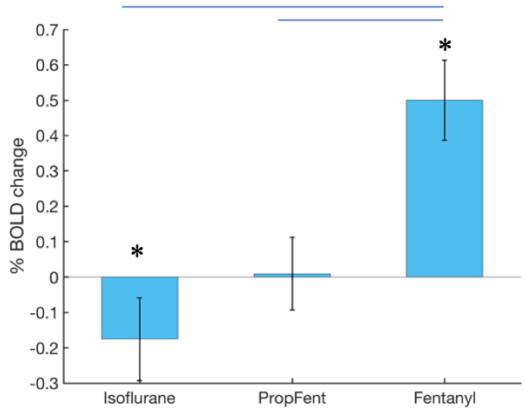


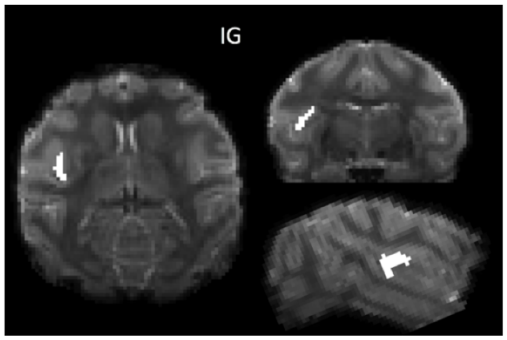
B. F5



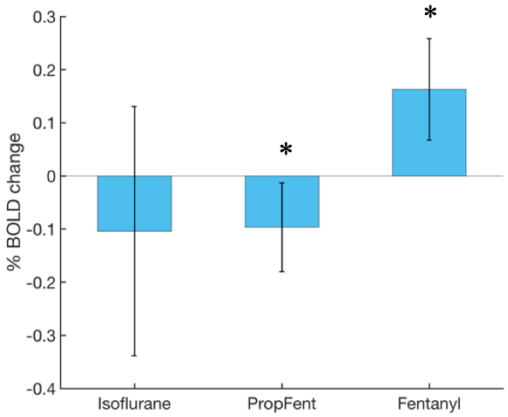


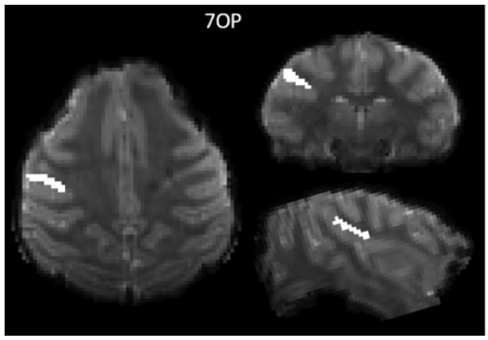
C. IG





D. 7OP





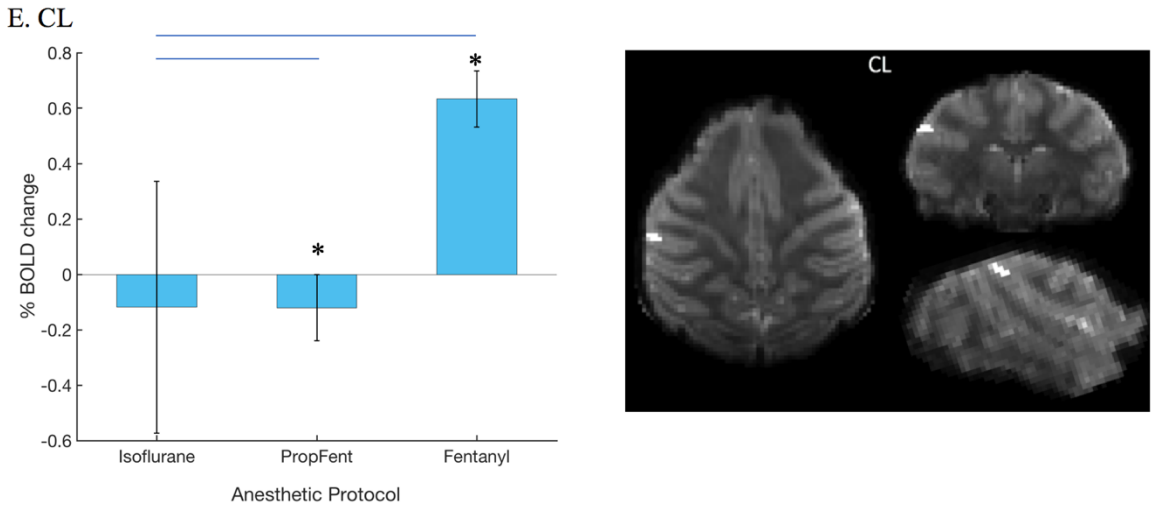
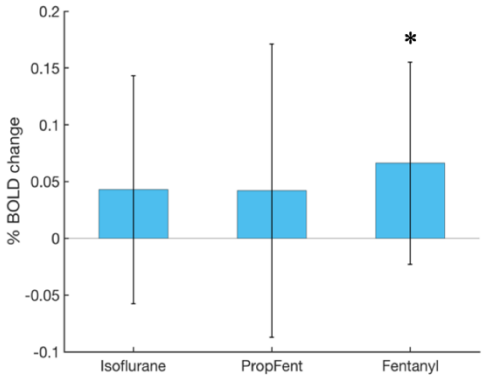
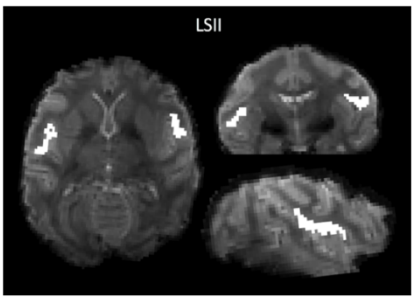


Fig 7. Somatosensory area BOLD response to right hand stimulation for Monkey N

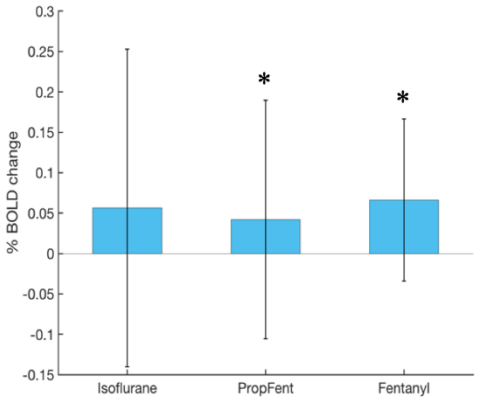
Somatosensory area BOLD response to stimulation of the left-hand fingers of the **Monkey N** is shown here. The ROIs explored here are the left secondary somatosensory area (LSII) in Fig A, area (F5) in Fig B, area (IG) in Fig C, area (7op) in Fig D, area (CL) in Fig D. The ROIs were extracted from the D99 Macaque Atlas and registered to the functional images for each animal using ANTs software. Each bar represents an average of % BOLD change calculated for 7-11 runs of data collected for each anesthetic condition. Stars represent significant change in the %BOLD change from baseline according to a two-tailed t-test ($p<0.05$). A pink horizontal line across two different conditions represents a significant difference in the % BOLD change between the two anesthetic conditions in that ROI with Tukey's post hoc. Adjacent to each bar graph comparing the average percent BOLD change in each anesthetic protocol, is a map showing the region of interest mask used overlaid on the animal's average functional EPI image.

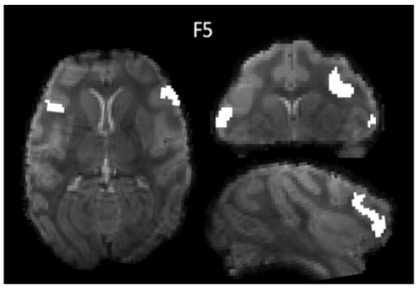
A. LSII



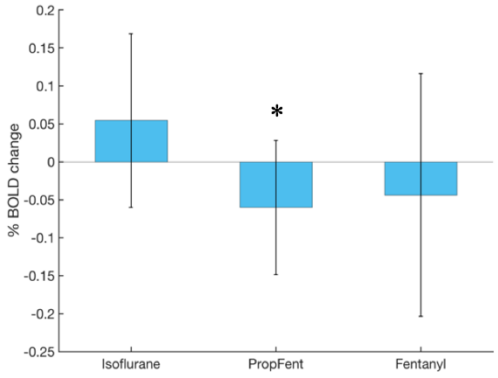


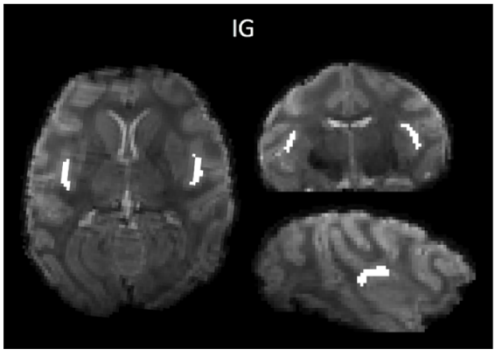
B.F5



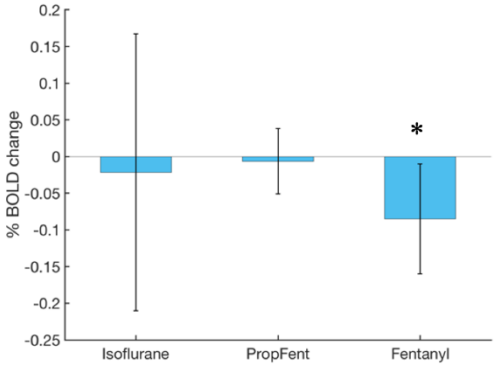


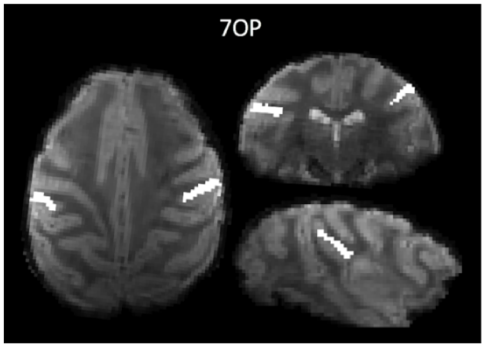
C. IG





D. 7OP





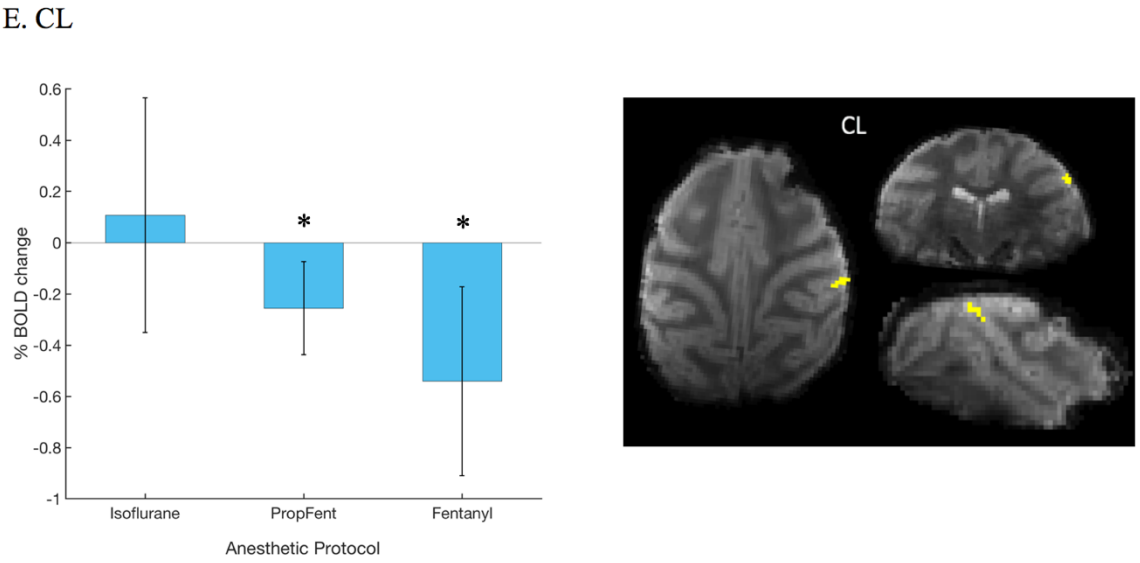


Fig 8. Somatosensory area BOLD response to right hand stimulation for Monkey G

Somatosensory area BOLD response to stimulation of the left-hand fingers of the **Monkey G** is shown here. The ROIs explored here are the left secondary somatosensory area (LSII) in Fig A, area (F5) in Fig B, area (IG) in Fig C, area (7op) in Fig D, area (CL) in Fig D. The ROIs were extracted from the D99 Macaque Atlas and registered to the functional images for each animal using ANTs software. Each bar represents an average of %BOLD change calculated for 7-11 runs of data collected for each anesthetic condition. Stars represent significant change in the %BOLD change from baseline according to a two-tailed t-test ($p<0.05$). A pink horizontal line across two different conditions represents a significant difference in the % BOLD change between the two anesthetic conditions in that ROI with Tukey's post hoc. Adjacent to each bar graph comparing the average percent BOLD change in each anesthetic protocol, is a map showing the region of interest mask used overlaid on the animal's average functional EPI image.

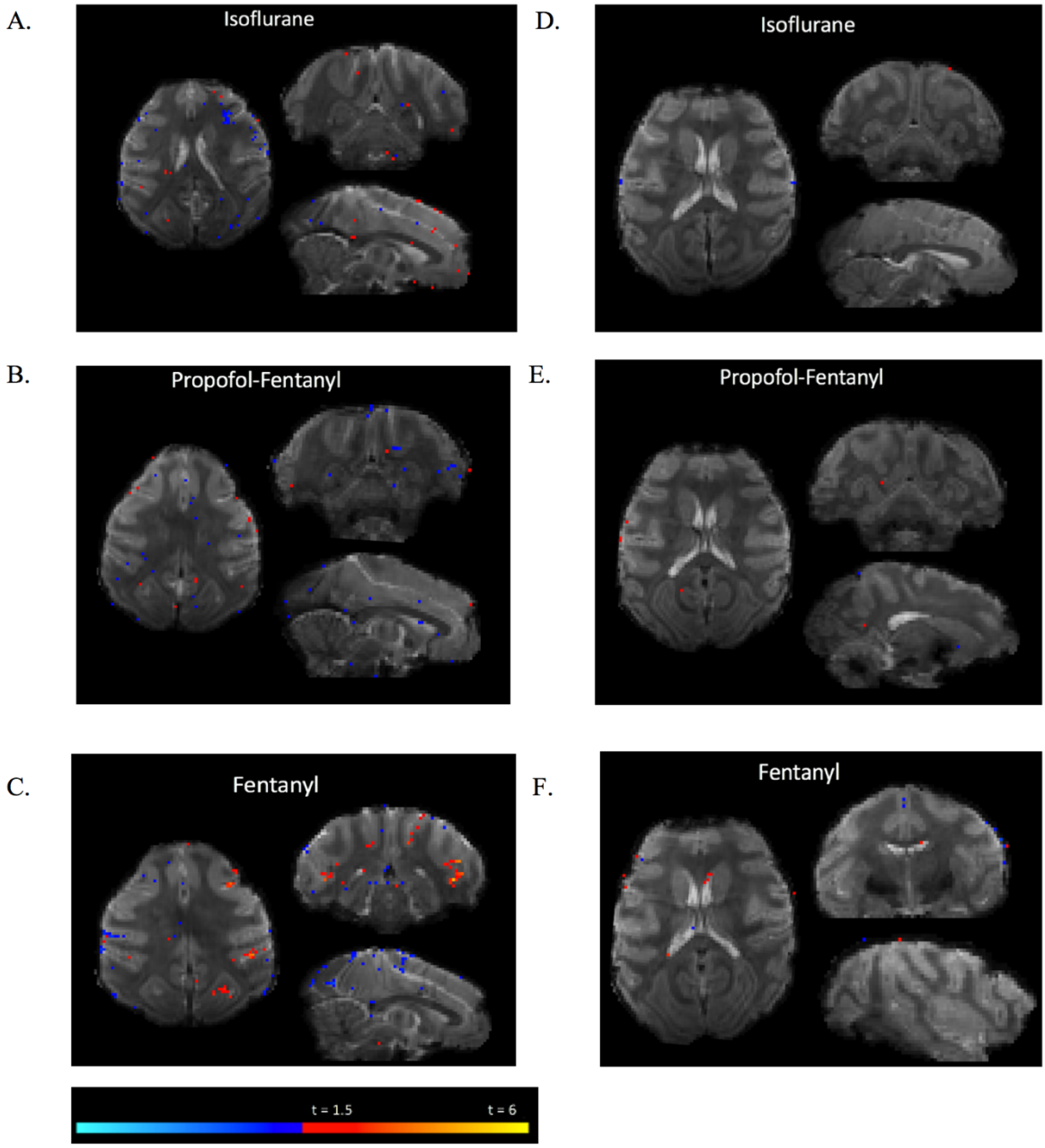


Fig 9. Statistical parametric maps of visual stimulation response under three anesthetic protocols

The t-statistic maps were calculated using FSL software and overlaid onto the average functional image for that animal from that particular experiment. This image shows coronal, sagittal and axial views for Monkey N during somatosensory stimulation under the three different types of anesthetic protocols: (A) Isoflurane (B) Propofol + Fentanyl and (C) Fentanyl alone. The t-statistic maps of response to somatosensory stimulation are also shown for Monkey G under Isoflurane (D), Propofol + Fentanyl (E) and Fentanyl (F) anesthesia. Activity in each voxel represents a change in the signal from baseline. Values that are further from t=0 indicate a greater probability that the reported activity in the voxel is not due to chance. A number of high t-value voxels clustered together indicates that area was more likely to be functionally responding to the stimulation.

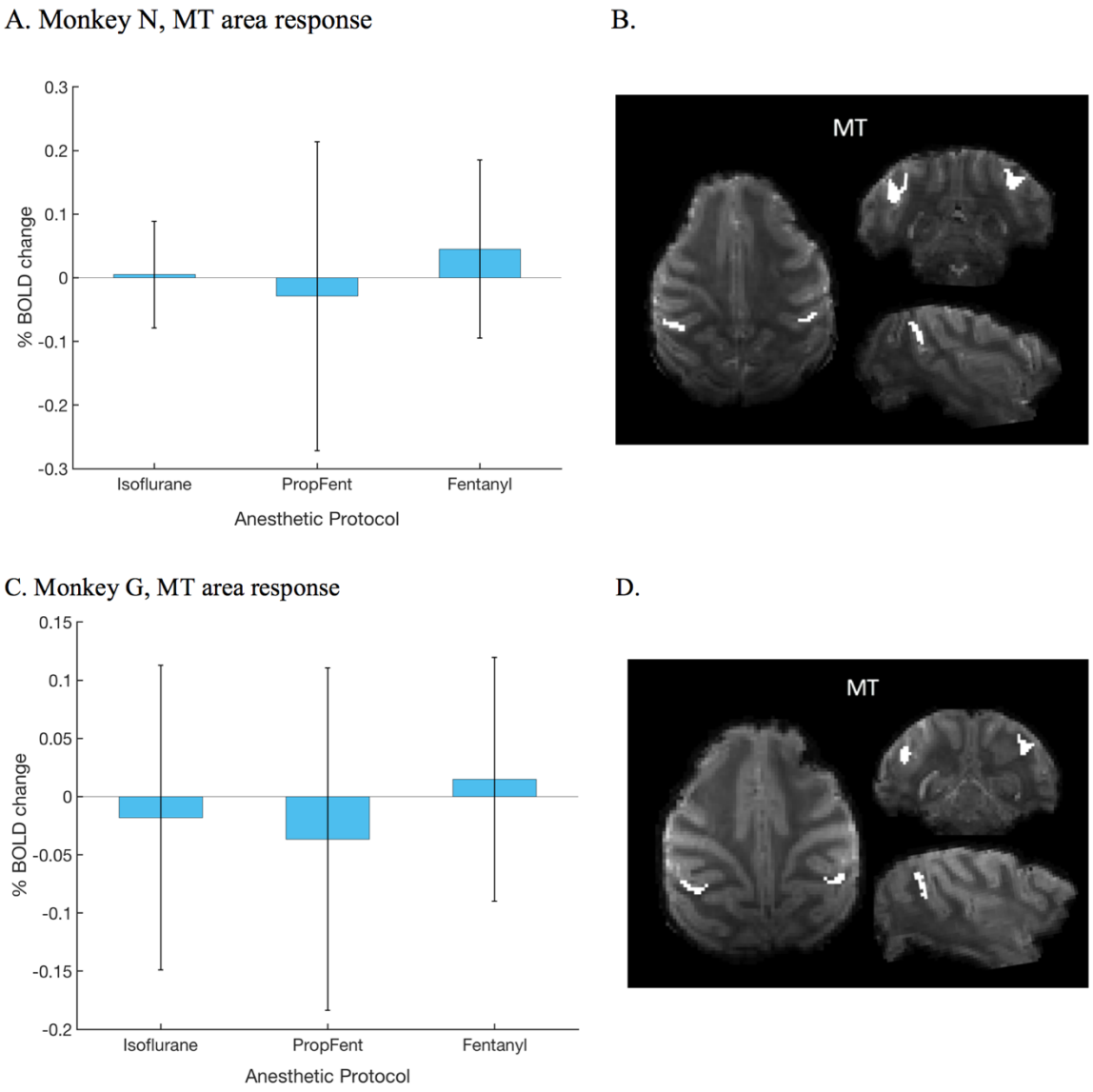


Fig 10. Visual stimulation response in MT area for Monkey N and Monkey G.

The percent BOLD change response of the medial temporal area of the visual cortex was calculated for Monkey N (10A) and Monkey G (10C) in response to a random dot kinetogram stimulus generated using PsychoPy3 software and exposed to the animal in a blocked design of 15s stimuli in a 7T MRI system. The animal was exposed to the stimulus under three types of anesthetic protocols: Isoflurane, Propofol+Fentanyl, or Fentanyl alone and the MT area responses under each of these anesthetics is shown here. The responses were calculated using MT area masks generated by the D99 atlas and non-linearly registered to each animal's mean functional EPI image. The quality of this registration is shown for Monkey N (10B) and Monkey G (10D).

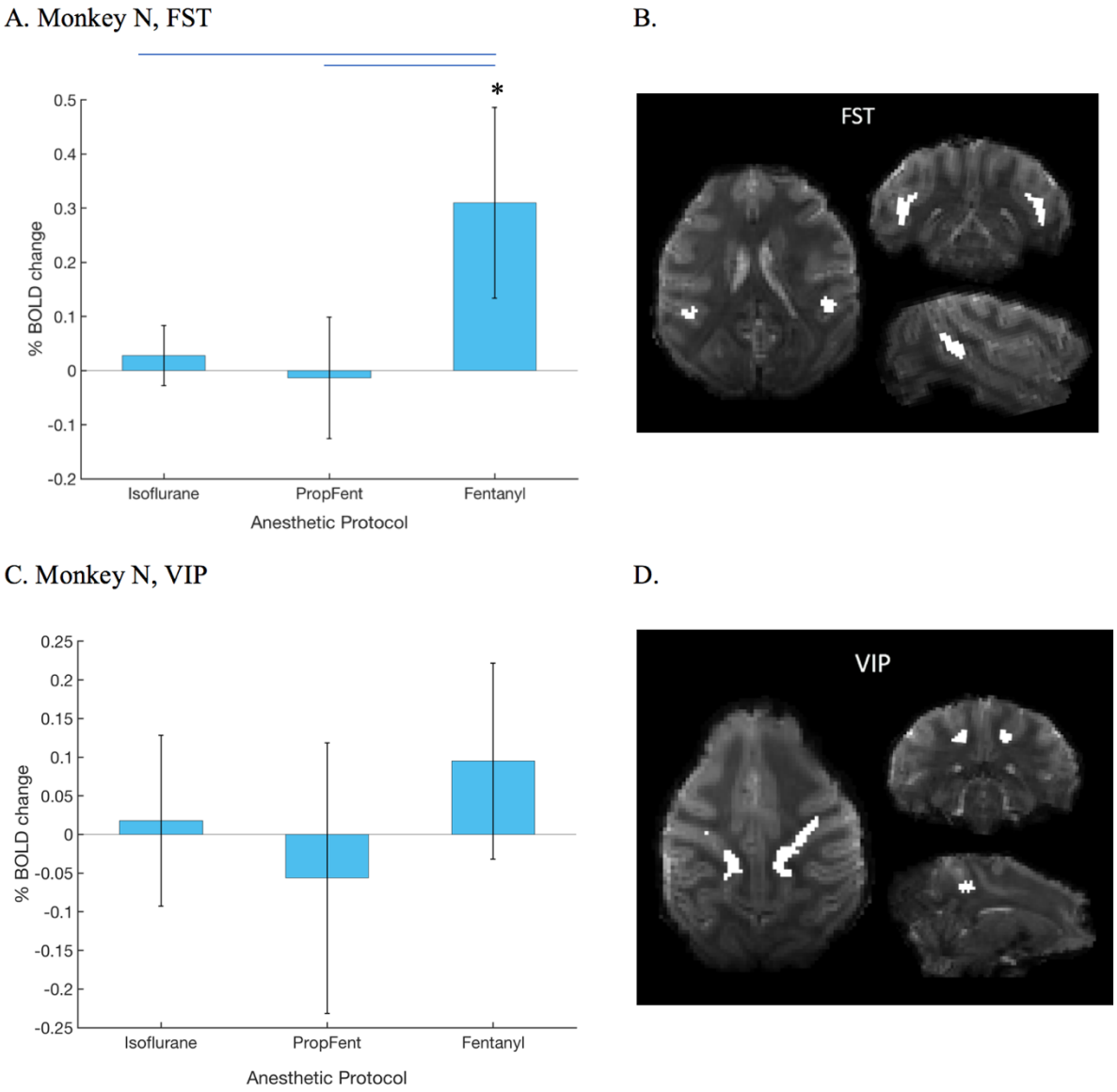


Fig 11. Visual stimulation response in FST and VIP areas for Monkey N

The percent BOLD change response of the FST area (11A) and the VIP area (11C) was calculated for **Monkey N** in response to a Random Dot Kinetogram stimulus generated using PsychoPy3 software and exposed to the animal in a blocked design of 15s stimuli in a 7T MRI system. The animal was exposed to the stimulus under three types of anesthetic protocols: Isoflurane, Propofol+ Fentanyl, or Fentanyl alone and the MT area responses under each of these anesthetics is shown here. The responses were calculated using masks generated by the D99 atlas and non-linearly registered to each animal's mean functional EPI image. The quality of this registration is shown for the FST area (11B) and VIP area (11D).

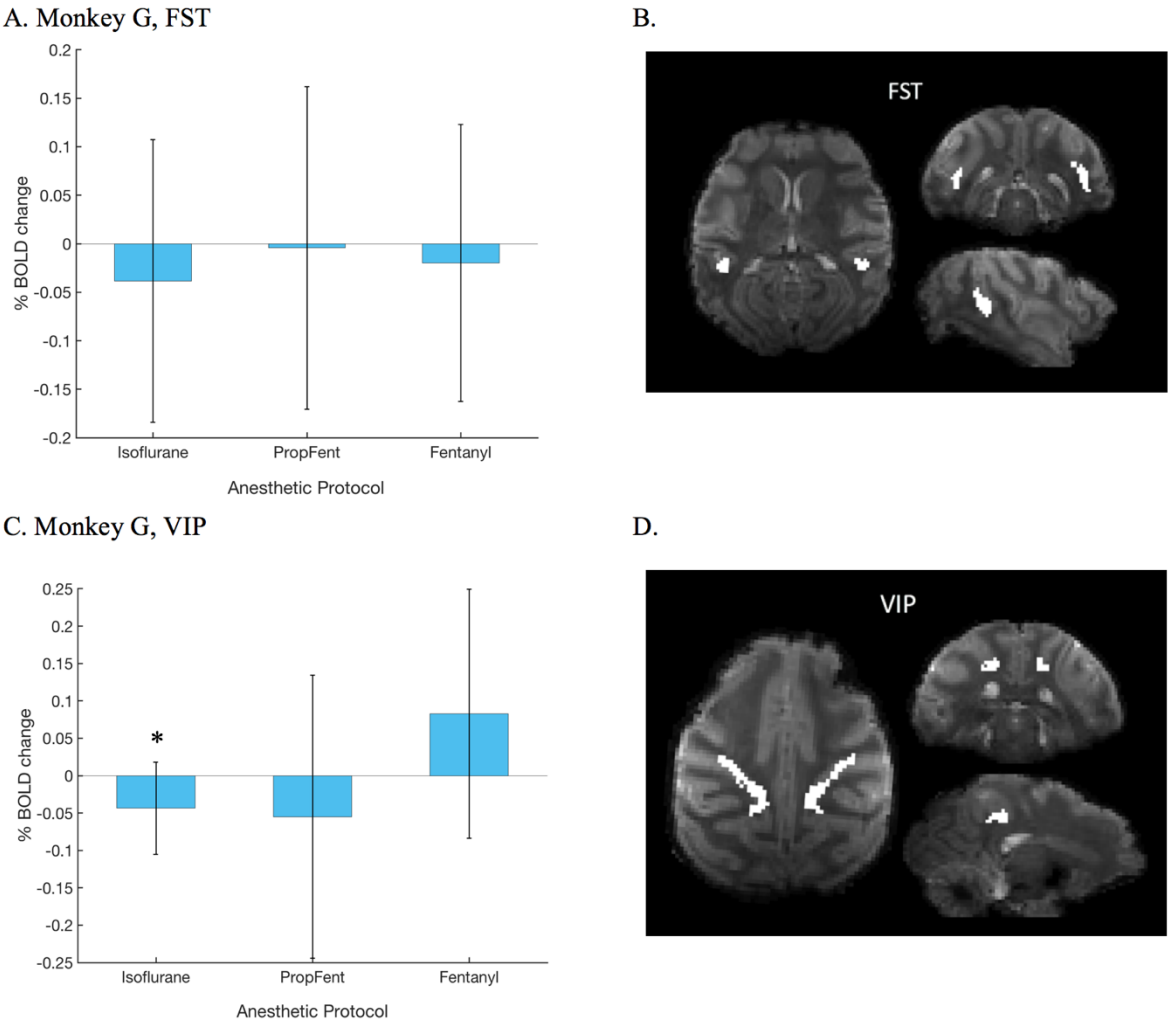
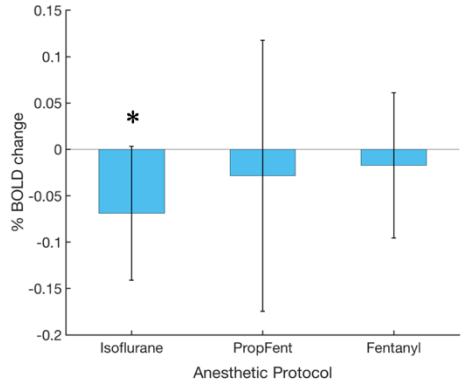


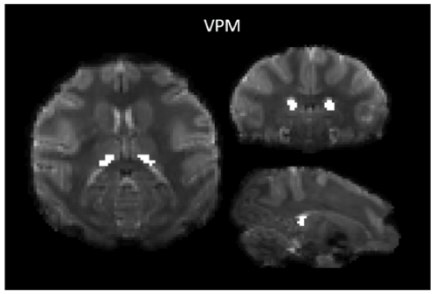
Fig 12. Visual stimulation response in FST and VIP areas for Monkey G

The percent BOLD change response of the FST area (12A) and the VIP area (12C) was calculated for **monkey G** in response to a Random Dot Kinetogram stimulus generated using PsychoPy3 software and exposed to the animal in a blocked design of 15s stimuli in a 7T MRI system. The animal was exposed to the stimulus under three types of anesthetic protocols: Isoflurane, Propofol + Fentanyl, or Fentanyl alone and the MT area responses under each of these anesthetics is shown here. The responses were calculated using masks generated by the D99 atlas and non-linearly registered to each animal's mean functional EPI image. The quality of this registration is shown for the FST area (12B) and VIP area (12D).

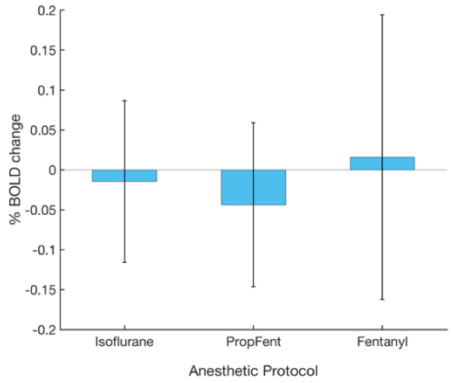
Monkey N, Thalamic Response

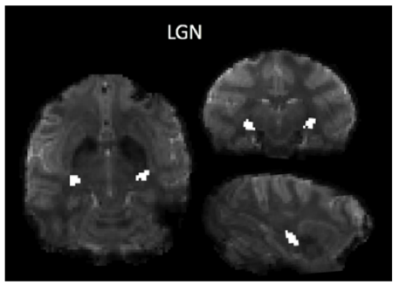
A. VPM





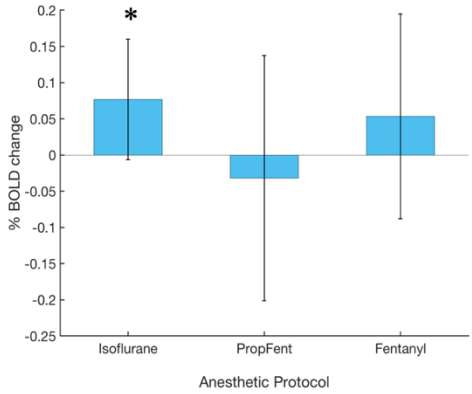
B. LGN

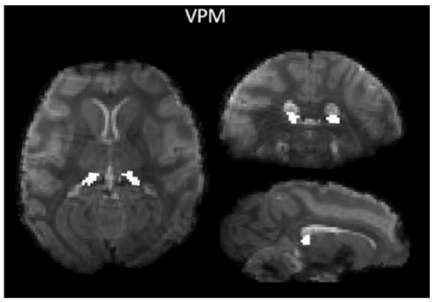




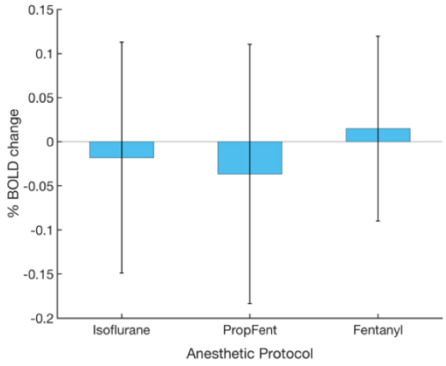
Monkey G, Thalamic Response

C. VPM





D. LGN



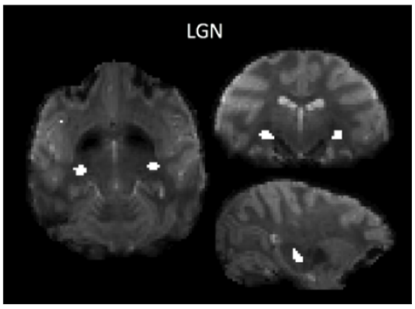


Fig 13. Thalamic percent BOLD change response to visual and somatosensory stimulation

The percent BOLD change response of the bilateral VPM nucleus of the thalamus in response to somatosensory stimulation of the right hand in a 7T MRI system was recorded for two rhesus macaques, Monkey N (13A) and Monkey G (13C). The percent BOLD change response of the bilateral LGN nucleus of the thalamus in response to Random Dot Kinetogram visual stimulation in a 7T MRI system was recorded for two rhesus macaques, Monkey N (13B) and Monkey G (13D). The animal was exposed to the stimuli under three types of anesthetic protocols: Isoflurane, Propofol+Fentanyl, or Fentanyl alone and the responses of the salient thalamic areas that process these stimuli is shown here for each anesthetic protocol. The responses were calculated using masks generated by the D99 atlas and non-linearly registered to each animal's mean functional EPI image. The quality of this registration for each mask is shown below its corresponding percent BOLD change graph.

Discussion

1. Summary of the Experiment

The goal of this investigation was to compare the robustness of the BOLD response to somatosensory and visual stimuli under different anesthetic protocols in non-human primates. Analysis of the fMRI BOLD response was done using the GLM and produced statistical parametric maps. Clusters of high t-value voxels indicate areas of positive BOLD activity. These areas were further analyzed and the percent BOLD change from baseline was calculated (see Methods) and averaged across all runs for each relevant ROI, for each experiment, and for each animal. The response pattern differed between monkeys and between anesthetic protocols. Monkeys were treated as separate case studies in the analysis.

Two rhesus macaque monkeys were stimulated with a visual stimulus of a random dot kinetogram and with a somatosensory stimulus of regular finger flexion of the right hand. These stimuli were delivered for each animal while it was anesthetized using one of three anesthetic protocols: isoflurane alone, propofol + fentanyl in combination or fentanyl alone. The experiment was done in a 7T MRI scanner system in order to measure the animal's fMRI BOLD response to somatosensory and visual stimuli. It was expected that there would be differences in the animal's BOLD response to the stimuli under the three different anesthetic protocols.

2. The Primary Response to Somatosensory Stimulation Under Different Anesthetic Protocols

The first question asked from this investigation is: was a univariate response to somatosensory stimulation observed under each type of anesthetic agent, and for each monkey? During somatosensory stimulation of Monkey G, when the left SI area as a whole was investigated, a reliable increase in BOLD signal was observed under both the isoflurane and the propofol-fentanyl protocols. For Monkey N, a significant BOLD change response to somatosensory stimulation was found in the LSI area as a whole under both the propofol-fentanyl and fentanyl protocols. There was no statistically significant difference between these responses. The LSI response was significantly decreased from baseline under isoflurane for monkey N. The

somatosensory stimulus response observed in Monkey N under fentanyl and under propofol-fentanyl was much more robust than the somatosensory stimulus response observed under isoflurane in monkey G. Note that the range of percent BOLD change values on the y axis of Figure 2, is much greater for Monkey N (2A) than for Monkey G (2B). This means that the two animals had very different sensitivities to the anesthetic protocols administered.

The somatosensory stimulation was limited to the right hand so the sensory information would primarily be processed in only a small portion of the primary left SI area responsible for the hand. In an analysis that assessed the entire left SI area, areas other than those responsible for processing hand stimulation may have been suppressed while areas responsible for the hand alone in SI may have shown significant activity (Williams et al 2003). Thus, a clear picture of the response pattern may have not been revealed in this analysis. There is also evidence that the size of receptive fields changes during anesthesia, varying with depth of sedation (Friedberg et al. 1999) making more functionally precise ROIs even more important to define during analysis.

To investigate this effect, the left SI area was further segmented for a hand-specific ROI, using data from a multivariate analysis of finger stimulation in an experiment with a different monkey that was completed previously by a colleague (see Methods). This permitted a more specific analysis of BOLD change. It was expected that there might be hitherto masked signal that would be revealed or that there would be a signal increase observed or that there would be a difference between the response under propofol-fentanyl and fentanyl alone conditions. The most robust somatosensory response in the hand area of SI was found under propofol-fentanyl for Monkey G, and not isoflurane (Fig 5B). The BOLD change calculated under the hand area alone for Monkey G aligns with the activity found in Monkey G in statistical parametric maps in Fig 3. The t-statistic maps for Monkey G showed a significant somatosensory response under the propofol-fentanyl condition as well and not under any other protocol. For Monkey N, the pattern of activation did not change when the hand area of SI alone was analyzed: the greatest BOLD change was observed under propofol-fentanyl and fentanyl protocols (Fig 3A) and there was no difference in the response between these protocols. Activation observed in the hand-specific ROI in both animals is evidence that hand-relevant somatosensory response was observed.

In order to assess how much the signal may have been obfuscated by surrounding areas of SI that were not activated in response to stimulation of the hand alone, the signal change in the hand area alone was compared to the signal change in the whole SI region (Fig 6). In the hand area of LSI, we observed a significant increase in BOLD signal from baseline under every anesthetic protocol for Monkey N, even though the response pattern stayed the same. This indicates that the surrounding SI area was not as responsive to the stimulation and including it in the analysis was diluting the signal.

In Monkey G, a similar pattern was not observed. Applying a hand specific SI mask revealed a significantly greater signal change in the propofol fentanyl condition that was more than three times greater than that measured in the whole SI ROI. However, an increase in signal was not observed in the isoflurane or fentanyl conditions for Monkey G. This could be because in these conditions, a sufficient positive signal was not elicited by the stimulus in the first place, so a more specific ROI could not amplify anything.

This comparison illustrates the necessity of using specific ROIs for signal analysis in task-based fMRI. A similar hand specific ROI was not used for the many other functional areas included in this investigation such as the SII area and the right SI areas because the functional data and distance maps were only available in this dataset for the left SI hand area. This would be useful for future analyses. This investigation was primarily concerned with comparing a broad univariate response to sensory stimuli under different kind of anesthetic protocols. As a result, the analysis also focused on broad responses to match the scope of this investigation.

3. Comparing the robustness of sensory response under different anesthetics

The next question we asked was: which anesthetic agent recorded the most robust fMRI BOLD response to somatosensory stimulation? For monkey G, the results were inconsistent between the whole SI area and the hand-specific SI ROI. It could be that the hand-specific ROI provided a more relevant and reliable measure because results may have been affected by noise or negative activity in surrounding SI areas that were not activated by the hand stimulation. When the hand-specific area was analyzed the most robust somatosensory response was recorded under the

propofol-fentanyl protocol. Both the BOLD change measured in the hand specific SI ROI and the statistical parametric maps for monkey G showed a robust response hand-specific somatosensory response under propofol-fentanyl. This means that based on this investigation, propofol fentanyl protocol may be the most appropriate to record the somatosensory response for this monkey.

For Monkey N, under the propofol-fentanyl and fentanyl conditions, there was a consistent significant univariate response to somatosensory stimulation observed for this animal and are better suited than isoflurane. This response pattern was observed in the whole SI area (Fig 4A) and amplified when just the hand area was analyzed (Fig 6A) indicating that the response was hand-specific and that these anesthetics appear to be both well suited to measuring the somatosensory signal for this animal.

Because the results are inconsistent between Monkey G and Monkey N, it is difficult to say that the BOLD response to somatosensory stimulation is most robust under the propofol-fentanyl or fentanyl anesthetic protocols. Biological differences between the individuals meant that Monkey G was far more sensitive to fentanyl and propofol-fentanyl anesthetic protocols than Monkey N. Similarly, Monkey N may have been more sensitive to isoflurane anesthesia resulting in the differences between the cases that are observed. Although the exact biological differences cannot be determined, it can be clearly observed the in this experiment, a robust somatosensory response could not be recorded in monkeys under isoflurane anesthesia. This is similar to a study that measured percent BOLD change to hind paw electrical stimulation of the mouse and found that the greatest signal increase was observed in propofol-anesthesia when compared signal under to isoflurane, urethane and medetomidine anesthesia (Shroeter et al 2014).

There was no reliable difference between the propofol-fentanyl and fentanyl response for Monkey N. Monkey G only showed a robust response to somatosensory stimulation under propofol-fentanyl in the hand area of LSI rather than LSI as a whole. The safety of each protocol for long-term experimentation can be taken into account for the comparison of these protocols. Fentanyl used alone used as an anesthetic agent requires closer monitoring both during the experiment for respiratory depression, and after consistent experimentation for tolerance to the drug, than a propofol-fentanyl combination protocol (in which a smaller dose of the opioid is

used). For these reasons, propofol-fentanyl may be the most appropriate anesthetic protocol to use in order to record somatosensory responses in anesthetized macaque fMRI studies.

4. Other Regions that Respond to Somatosensory Stimulation Under Anesthesia

The D99 Macaque Atlas (Reveley et al 2001) was nonlinearly registered and overlaid onto statistical parametric maps to determine the regions in which this positive BOLD response was identified. The map revealed that the left SI, left SII, F5, IG, 7OP and CL areas had some clusters of positive BOLD response. The average percent BOLD change from baseline across all runs within an experiment, was calculated for each experiment for each ROI.

There were many other areas that responded to somatosensory stimulation other than the left SI hand area. These ROIs were identified to be involved in somatosensory processing activity and the average BOLD change activity in these ROIs was compared across anesthetic regimens for each animal. For Monkey N, under propofol-fentanyl and fentanyl protocols, the greatest signal change was consistently observed in the LSII, F5 and CL areas whereas under isoflurane some areas were observed to have a significant decrease in BOLD signal from baseline. In the areas 7OP and IG, however, only Fentanyl was able to preserve a significant BOLD change response. This pattern indicates that some brain areas are more sensitive to signal suppression due to fentanyl anesthesia than others. This means that the propofol that was added into the propofol-fentanyl combination had some effect on the pathways to the 7OP and IG areas that fentanyl does not. In order to investigate why that is, the receptors in these areas would have to be assessed for sensitivity to propofol-fentanyl and the calcium signalling in these areas could be measured to see how the propofol-fentanyl combination affects information processing to these areas.

The SII area is a higher processing area for information from the SI area and is a large somatotopically organization structure that integrates information from the SI area (Mathur et al 2014). F5 is a premotor area that is involved in the execution of distal motor acts such as grasping, holding, manipulating and tearing. All neurons in this area have been identified to have a preference for grasping objects in some way (Raos et al. 2006). The CL area (claustrum) forms

a thin folded sheet of grey matter inserted between the striatum and the insular cortex and has direct projections to the cortex. It has similar connectivity as the insula but is not derived from the endopiriform nucleus (Bay and Altman 1991). It is its own structure that is thought to be involved in integrating information from the cortex. It makes sense then that the LSII, F5 and CL areas would be activated in response to flexion of the animal's fingers at the interphalangeal joints as this forms a complex grasping motion that would be processed in these areas. These areas were robustly activated under the propofol-fentanyl and fentanyl anesthetic protocols for monkey N but not under the isoflurane anesthetic protocol.

The 7OP area (the parietal operculum) is known to also be activated with F5 and other areas as a part of the visuo-tactile convergence network (Guipponi et al. 2015) and to judge certain features such as microgeometry (roughness) and microgeometry (shape) of objects (Ledberg et al. 1995). The IG area (granular insula) is a portion of the insular cortex that connects to the cortex to process somatosensory information and also connects to the limbic system (Medulam & Mufson 1982). However, the 7OP and IG areas were only activated significantly under the fentanyl protocol for Monkey N meaning the information that reaches this network is more sensitive to being attenuated or destroyed by the propofol drug. It could be because these areas are more related to functional connectivity of sensory information with other parts of the brain. The effects of propofol anesthesia may accumulate over several receptors resulting in a less powerful signal being detected finally at these areas. It has been shown that the effects of volatile anesthetics such as isoflurane accumulate over the number of synapses as these drugs have been shown to inhibit vesicle exocytosis at the synaptic membrane and thus signal would be attenuated over the number of synapses it has to cross (Hemmings HC et al 2005). A similar effect may be occurring for Propofol-Fentanyl anesthesia but not for Fentanyl. If there is a preservation effect, fentanyl anesthesia appears to preserve the pathway to all of these areas where somatosensory information is processed. Preservation of somatosensory response is a valid prediction for the nature of the response observed under fentanyl anesthesia because a robust positive BOLD response has been observed previously in response to sensory stimulation (Ku et al 2014). In order to test this, however, a comparison to an awake animal would have to be made. It is unclear whether or not accumulation is the reason for attenuated signal in areas that are separated from the principal signal by more synapses. A specific study that investigates and compares the

sensitivity of tissue in different brain areas to different anesthetic regimens would be needed to determine that these areas indeed have lower activity due to sensitivity to anesthesia.

The same somatosensory processing areas were investigated for Monkey G as well. The LSII area only showed a significant increase in percent BOLD signal under the fentanyl condition. The F5 region showed a significant increase in percent BOLD under both propofol-fentanyl and the fentanyl conditions. The IG area only showed one significant change which was a decrease in signal under the propofol-fentanyl condition. The 7OP area similarly only showed one significant change which was a decrease in signal under the fentanyl condition. The CL region showed a significant decrease in signal under both propofol-fentanyl and fentanyl conditions. Negative BOLD activity is an elusive phenomenon that has not yet been fully understood.

5. The Response to Visual Stimulation Under Different Anesthetic Protocols

Average statistical parametric maps of the response to visual activation revealed that for Monkey N, fentanyl was the only anesthetic protocol under which a cortical response to visual stimulation was observed using BOLD fMRI. No significant visual sensory response was recorded in monkey G for any of the ROIs under any of the anesthetic regimens. It has been shown that the visual cortex was active under 0.4% isoflurane anesthetized macaque (Logothetis 1999), however at least 1% anesthetic was observed to be necessary in this investigation to maintain the animal sedated at a level that movement did not occur which could be why visual activity was not observed under isoflurane anesthesia in this study. The ROIs that responded to visual stimulation in the fentanyl area were evaluated for all anesthetic regimens for each animal in addition to the MT area, where activity was anticipated.

Neither animal showed any significant BOLD response in area MT under any of the anesthetic regimens. It was anticipated that area MT would respond to the random dot kinetogram because this area has been demonstrated to have receptive fields for moving visual stimuli (Osborne et al 2004) and has been shown in both humans and macaque fMRI to be stimulated in response to high contrast, moving visual stimuli (Dubowitz et al 2010). Furthermore, it has been demonstrated previously that the BOLD response to visual sensory stimuli can be recorded in

dogs anesthetized under isoflurane, propofol or fentanyl anesthetic regimens (Willis et al. 2001). In the Willis study with dogs, mydriasis was induced at the beginning of each experiment using 0.5% tropamide drops and the eyes were kept open using nonmagnetic insulated copper speculate. In contrast, in our study, mydriasis was not induced, and instead, the eyes were held open using surgical tape that kept the eyelids of the animal pulled back. It could be that mydriasis permitted light to better enter the eye and hit the retina whereas it may not have done so otherwise. Cameras that allow researchers to observe the animal's eye during the experiment could help to determine whether eyes remained open and pupils sufficiently dilated throughout the experiment to observe visual stimuli. Lack of monitoring of the monkeys' eyes in this investigation means that we cannot confirm that the animal's eyes were dilated and looking at the visual stimulus during the experiment, and that there wasn't any nystagmus effect. Another possibility is that the biological differences between dogs and non-human primates make dogs less susceptible to the effect of anesthetics to attenuate or ablate cortical signal in response to visual sensory stimuli. Furthermore, in the Willis study, researchers measured the percentage of significant voxels as a percentage of total voxels in the ROI to control for differences in ROI size, instead of simply measuring percent BOLD change in relevant ROIs. This was not done for this experiment, so ROIs that were much larger may have had significant signal that was localized to a very specific area. Doing both types of analysis in the future may reveal masked effects.

Areas other than the MT area showed activity in response to visual stimulation in the statistical parametric maps. Clusters of high t-value voxels were observed around the intraparietal sulcus and, the V1 and some frontal areas for monkey N under Fentanyl anesthetic (Fig 9C). By overlaying the nonlinearly registered D99 Macaque atlas over this map, these areas were identified to be the FST and VIP areas. The FST area had significant BOLD signal change in response to visual stimulation in Monkey N under fentanyl anesthesia and this change was greater than that observed in any other anesthetic regimen. No significant BOLD change was identified for the VIP area under any anesthetic for Monkey N. For Monkey G however, there was no significant activation observed in the FST area under any anesthetic regimen and the VIP area showed significant decrease in BOLD under the isoflurane condition. Area FST and VIP areas, in addition to the MT area, have been shown to be involved in processing information of

visual motion specifically with dots but not necessarily with moving lines (Vanduffel et al. 2001). The Vanduffel study used awake behaving primates and therefore was able to record many more areas of activation than in this study. Therefore, the FST area may have fewer receptors that are sensitive to signal attenuation from fentanyl in Monkey N. Fentanyl binds to opiate receptors which have been shown to have downstream up-regulatory effects on GABA receptors as well. This may cause an inhibition of signal transmission by increasing the threshold for signal to be transmitted. Although the other anesthetics, isoflurane and propofol, act on GABA receptors as well, they do so directly and so the effects of signal inhibition may be more potent.

There are many reasons that can be used to explain why a response to visual stimulation was not observed for monkey G entirely and for monkey N only under fentanyl anesthesia. However, it is clear that visual stimulation may be more challenging to record in a fully sedated animal using anesthetics such as isoflurane and propofol-fentanyl.

6. Negative BOLD effects observed

There were many negative BOLD effects observed in response to both somatosensory and visual stimulation. These effects were observed in a variety of secondary somatosensory response areas for both monkeys, scattered across all three anesthetic regimens with no clear pattern. It was also observed in the VPM of the thalamus for monkey N in response to somatosensory stimulation (Fig 12A). The lack of pattern to the location of these instances of negative BOLD activation suggests that it was not a function of the type of anesthetic or the specific area (however such a pattern may be revealed in a larger cohort of subjects). Negative BOLD signal intensity indicates the deoxygenated hemoglobin concentration during stimulation rose above that of the rest condition. There are many reasons speculated as to why this occurs. One of them is that anesthesia can induce changes in the blood flow which reduce the local deoxyhemoglobin concentration so the contrast between the area around the ROI responding to a stimulus and the ROI itself, is reduced or even negative (Marcur et al 2006). Another reason is vascular steal, which means that areas adjacent to highly active areas may “steal” the hemoglobin necessary to nourish more active neurons (Wade 2002). This is unlikely in this case because there would have

been a consistent pattern observed in the areas and conditions under which negative BOLD was observed here. In order to determine the correct cause of this negative BOLD phenomenon for this investigation, the study would have to be repeated with a larger cohort of subjects and hemodynamic response functions that are corrected for the vascular effects of each type of anesthetic protocol. There is evidence that a negative BOLD response is not merely an inverse of a positive BOLD response but rather is related to neurovascular coupling phenomena such as the misalignment of cerebral blood flow and cerebral blood volume due to differences in arterial structure in different parts of the brain (Goense et al. 2012). It could be that anesthesia perturbs neurovascular coupling in such a way that negative BOLD effects are observed in brain areas where they would not otherwise be observed in an awake animal. As such, uncovering the nature of these negative BOLD effects would require an awake animal model for comparison, as well as a tracer that can measure CBV vs CBF changes such as that described in Goense et al 2012.

7. Thalamic Response to Sensory Stimulation

Another question that was important to consider was whether thalamic areas responded to the sensory stimulus. It is thought that effects of anesthesia accumulate over the number of synapses and processing that must occur as signal is thought to attenuate as it travels further (Hemmings et al. 2005). Following the logic that areas closer to the incident stimulus should have greater signal, the thalamic areas should have had the greatest signal because they relay information to higher cortical areas. The VPM area of the thalamus relays information to the primary somatosensory area and the LGN area of the thalamus relays information to the visual areas. However, it was found that the VPM and LGN areas did not show significant activity under any anesthetic regimen for either monkey. There were no significant clusters of high-t-value voxels in these areas observed in the statistical parametric activation maps (Fig 3 and Fig 9). In addition, the percent BOLD change in these areas as ROIs was not significant for either animal even under anesthetic regimes where clear cortical activation corresponding to stimulation was observed (Fig 13). The results from this investigation are opposite to the results found by Shroeter et al in 2014 in the mouse where the thalamus was more robustly activated than the S1 area in response to hind-paw electrical stimulation. Perhaps lower doses of anesthesia may be used for mouse

fMRI that permits the animal to retain more function of thalamocortical pathways because it is much smaller than a primate and therefore easier to restrain for scanning procedures.

The thalamo-cortical signal transmission is likely the primary reason for the attenuation of sensory signal that is observed under anesthesia and in fact thalamo-cortical networks have been shown to be distinctly inhibited by anesthetic effects while other networks are preserved (White et al. 2003). The thalamic switch hypothesis (Alkire et al 2000; Mashour, 2006) is the idea that consciousness is regulated primarily at the level of the thalamus and inhibition of thalamo-cortical transmission characterizes the loss of consciousness. In addition, the vascular structure of subcortical areas is different than that of higher cortical areas so the BOLD signal may be affected differently in these areas under anesthesia. Because of these differences, different scanning sequences or analysis methods may be required to detect signal in subcortical areas. The results from this investigation indicate that there are other pathways that information may use to travel to higher cortical areas other than through the thalamus. These other pathways may be less sensitive to anesthetic inhibition of signal transmission.

8. The somatosensory response was more robust than the visual response

The somatosensory response and the visual response cannot be meaningfully compared because they were not matched for intensity. As a result, differences in the magnitude of the BOLD response may simply be due to the fact that the somatosensory stimulation was more intense than the visual stimulus and not because either sensory processing pathway is more or less sensitive to different kinds of anesthetic protocols in macaques. It was observed that the somatosensory response was more robust than the visual stimulus response in the instances where a functional response was observed in both animals. In fact, for monkey G, no visual activation was observed at all. Responses to visual stimulation have been recorded under anesthesia previously in humans (Martin et al 2000) and in dogs (Willis 2001). There is a clear gap in the literature on this subject as it would be interesting to understand the differences in the structure between the visual pathway and the somatosensory pathway that lends the somatosensory pathway perhaps to more robustly preserve activity under sedative doses of anesthesia in primates.

9. Evaluating the Methods of this Investigation

The aim of this investigation was to compare three candidate anesthetics in order to determine their usefulness for task-based fMRI experiments in anesthetized non-human primates. In this section the experimental design of this project is evaluated, and improvements are suggested where necessary.

9.1 Stimuli were not randomized

The experimental design was intended to provide a consistent and robust sensory stimulus to the animal's brain while it was scanned so that the univariate response could be compared between the different types of anesthetic protocols used. Stimuli were chosen that had been demonstrated to produce fMRI responses in anesthetized NHPs in the past. Although in awake scans, the order of a blocked task-based design in fMRI is randomized for each trial, this was not done in this experimental design. Stimuli are randomized in awake subjects so that the subject does not, consciously or unconsciously, memorize and begin to anticipate the stimulus beforehand. As the animals were anesthetized in this experiment to the point where they are no longer thought to be consciously aware of their environment, the problem that randomization solves was not considered relevant and in order to mitigate the complexity of the experimental design, randomization was not done.

9.2 Stimulus intensity was not compared between somatosensory and visual modalities

It was observed that while significant increases in signal were recorded in both monkeys in response to somatosensory stimulation, only monkey N during fentanyl stimulation had any increase in signal in response to visual stimulation. The degree of the increase in signal was also much greater for somatosensory stimulation than for visual stimulation. This could be because the neural pathways to process visual stimulus information in macaques is more sensitive than the neural pathways to process somatosensory stimuli. As a result, a greater visual stimulus intensity may have been needed to elicit the same response in these other anesthetics, if one can be elicited at all. Furthermore, the intensity somatosensory and visual stimulation protocols were

not quantitatively compared and so they may not have been at the same level of intensity. This may be the reason that a clear effect was seen in the different responses to tactile stimulation but not for visual stimulation. Matching stimulus intensities across modalities would be an important next step in a future investigation of anesthetic protocols for fMRI studies in anesthetized NHPs.

9.3 Stimulus intensity was not adjusted for each type of anesthetic agent

Insufficient stimulus intensity may be the reason why a robust response was seen in response to somatosensory stimulation but not for visual stimulation. Different anesthetics have different mechanisms of action to achieve the same endpoints. In an fMRI study on rats, it was found that isoflurane was a viable choice of anesthesia for preserving somatosensory function while maintaining the endpoints of anesthesia. However, the caveat was that the somatosensory stimulus used had to be intensified in current and duration under isoflurane anesthesia to elicit a comparable response to the one recorded under alpha-chloralose anesthesia (Misamoto et al, 2007). Unlike the Misamoto study, in this investigation a consistent stimulus type and intensity was used, within a given modality, for all three anesthetic protocols evaluated. It is possible that each anesthetic protocol may have preserved stimulus response activity if the stimulus had been adjusted to match that drug. However, as it is currently unclear how exactly sensory information is attenuated in each anesthetic protocol, it would be difficult to systematically adjust stimulus parameters to match the anesthetic. The stimulus adjustments would have to be made on a trial-and-error basis and would encompass its own investigation. For example, fentanyl appears to have best preserved sensory functional activity compared to the other anesthetic protocols in Monkey N. It could be that the other anesthetic protocols may perform equally well at preserving sensory function if the nature of the stimulus were to be calibrated to each drug separately. Little to no somatosensory and visual activity was detected under isoflurane anesthesia for both monkeys, but a greater stimulus intensity may be needed to elicit the response.

9.4 The hemodynamic response function may need to be customized for each anesthetic regimen for more accurate results

A prerequisite for the generation of statistical parametric maps is an accurate model of the hemodynamic response function. It has been shown that the hemodynamic response function differs significantly between brain areas for an individual, and also is altered under different anesthetic regimens (Shroeter et al 2014). A study that compared isoflurane, propofol, medetomidine and urethane anesthesia's effects on the BOLD response function in mice found that the response patterns varied greatly between anesthetic agents (Schlegel et al. 2015). The authors suggest it is because the HRF does not account for different peripheral vascular changes under each anesthetic protocol. This is because in different anesthetic agents have different effects on local cerebral blood flow and thus have different baseline hemodynamic response functions. As this beta weights and thus t-statistic values are determined by how closely the HRF convolved with the paradigm predicts the signal observed in each voxel, using an inaccurate HRF could result in mis-estimation of magnitude of activation as well as statistical parametric maps. It is possible that the standard double gamma hemodynamic response function used for analysis between the different anesthetic agents was caused the underestimation of signal from each experiment. In order to mitigate this as a source of error in the future, the proper hemodynamic response function should be measured and used for the different anesthetic regimens and even for different brain areas.

9.5 Ways to Improve the Animal Model

No awake animal model was used as a comparative control in this investigation which limits this type of investigation. In the evaluation of anesthetic agents for non-human primate neuroimaging, a major endpoint is a comparison of anesthetic agents' abilities to preserve a functional response that is closes to the awake response. Ultimately, the conclusions made from studies of anesthetized primates are meant to be meaningfully extrapolated to awake primates and inform hypotheses for human studies. As anesthetized states are demonstrated to be a considerably altered state of the brain, and each anesthetic alters brain function in different ways, it is unclear to what degree such extrapolations can be made from studies done in anesthetized

animals. In this, and many other, studies evaluating anesthetic agents for fMRI studies, anesthetic agents are compared to each other. Although it poses a great limitation that the awake animal would have to go through extensive and lengthy training to be able to enter an MRI scanner with very limited motion, it would be crucial to include this animal in a very thorough and comprehensive comparison of anesthetic agents for fMRI.

A contrast agent could be investigated for use in future investigation of functional neuroimaging in macaques. In an fMRI study of awake, head-fixed macaques, it was found that an iron oxide contrast agent increased percent MR signal changes by a factor of 10 in MT (Vanduffel et al. 2001). The increase in MR signal would permit researchers to measure more signal despite the increase in motion related noise with an awake animal.

In future investigations, more animals should be used in the study in order to offset the effects of individual biological differences between animals. In this investigation, it was found that the BOLD signals observed in Monkey N were much stronger than those observed in Monkey G. Similar patterns have been observed in other investigations (Vanduffel et al 2001) where one monkey does not—perhaps due to biological idiosyncrasy—display the same patterns of activity as other subjects. By investigating a larger number of subjects, patterns can be extrapolated to make conclusions that are applicable to that species of animal at the very least and not just specific to that individual. In this investigation, it is unclear whether the results from Monkey N or Monkey G are more reliable based on internal consistency alone.

Other monkey species may be considered such as marmosets as they are easier to train than macaques; however, macaques have unique physiological homologies to human beings that marmosets do not share. The somatosensory physiology of the hand in macaques is very similar to that in human beings. The differences between marmosets and macaques means that marmosets would respond differently to anesthesia than macaques would and as a result, studying anesthetic protocols in marmosets would not necessarily produce conclusions that could be applied well to macaques.

9.6 Insufficient Resting State Data was collected for completing Resting State Network Analysis

Thirty minutes (600 volumes) of resting state data was collected for each experimental session in order to compare the resting state networks preserved under each type of anesthetic protocol for each animal. Resting state data were also collected because it has been shown previously that these common anesthetic agents preserve resting state networks. Preserving these effects in this project would show that this project was done under replicable conditions. However, literature later revealed that thirty minutes of resting state data are insufficient for proper analysis of resting state networks due to the low signal to noise ratio. As a result, in the future, the full 60-90 minutes of resting state data should be collected so that this comparison of anesthetic effect on resting state data may be collected.

9.7 Analysis methods should be standardized for primates using a pipeline for each type of anesthetic protocol

Each fMRI study in literature, it appears, has a different pipeline with its own unique parameters for analyzing the results from their studies. This has potentially detrimental effects on the ability to compile and understand the results from these sources. From this project, it is clear that a comprehensive and standardized comparison of all anesthetic protocols proposed for use in non-human primates should be carried out. One standardized pipeline for analysis under each type of anesthetic protocol, with its own hemodynamic response function modifications for example, should be used. Different programs such as SPM, AFNI and FSL make use of different pre-sets for their analysis. Using one software for all analysis would standardize the results with the same pre-sets. Most neuroimaging software is also optimized for the analysis of human brains, so the development of such a pipeline would greatly enhance the efficiency and standardization of analysis for all non-human primate imaging researchers. It would also make the analysis more accessible to new trainees who may not have the background in computer science to immediately be able to parse the syntax in each imaging analysis software that is needed to adapt human brain-optimized software for non-human primate analysis.

9.8 Multiple Comparisons Correction was not completed

Due to the exploratory nature of this investigation, a multiple comparisons correction was not completed. Multiple comparisons analysis must be done in an instance where several t-tests are completed in order to eliminate false positives. This is done automatically as a part of the GLM when it produces the statistical parametric maps. However, it must be completed manually when completing further comparisons on this data. Because the comparisons done in this investigation were exploratory in nature rather than informed by a hypothesis that we were trying to disprove, multiple comparisons corrections were not done as it may mask effects that may be more rigorously explored in a future, hypothesis driven study with a larger cohort.

10. The Value of the Results of this Investigation

The results of this investigation serve primarily to inform the methodology of a more comprehensive evaluation of anesthetic agents for non-human primate neuroimaging. In this investigation, based on the pattern of activity from Monkey N, it appears that Fentanyl is the anesthetic protocol that best under which the most robust response to somatosensory stimulation can be observed for both the somatosensory modality and for the visual modality. This can be due to the face that there are more opiate receptors in higher cortical areas than in primary sensory areas whereas there are more GABA receptors in primary sensory areas and fewer in higher processing ones (Henstschke et al. 2005). Fentanyl works primarily through the activation of opiate receptors which inhibit potentiation of signal, whereas propofol and isoflurane through the positive regulation of GABA receptors which also inhibits signal potentiation. There is evidence that opiates activate GABA receptors downstream, but the activity is still indirect. As a result, the receptor distribution could be the reason that primary sensory activity was seen more robustly under fentanyl anesthesia in macaque monkeys exposed to sensory stimulation than under isoflurane anesthesia.

Although the activation pattern observed for Monkey G does not match the pattern observed for Monkey N, it appears that Monkey G did not actually respond to stimulation meaningfully under any of the anesthetic protocols except for Propofol-Fentanyl to somatosensory stimulation.

Propofol-Fentanyl, therefore, may be better suited to study a somatosensory response and Fentanyl may be better suited for the visual response. This could be because there was a difference in the sensitivity of different sensory processing pathway to different types of anesthesia. Differences in the results between monkeys may be due to biological differences between that made Monkey G far more sensitive to anesthesia and thus ablated sensory response at the same dose under which a robust response could be recorded for Monkey N. For future investigations that involve a larger cohort, it would be wise to do pilot studies in each individual primate with simple sensory stimulation at a variety of doses of anesthetic to determine the ideal dose for that animal. In this investigation, dose was determined as the minimal amount required before the animal's heart rate began to rise indicating arousal. However, in the future, head fixation may be used so that motion can be limited with a smaller dose of anesthetic and may preserve more brain activity.

The fact that fentanyl and propofol-fentanyl were the only anesthetics to preserve a robust response and fentanyl preserved a response in both somatosensory and visual modalities tell us something about how the anesthetics may function. Because fentanyl was able to elicit a more robust sensory response even while maintaining anesthetic endpoints such as immobility and sedation, it could be that sedation and sensory processing are pathways that do not perfectly overlap in the brain. Fentanyl may be acting mainly on areas of the brain that induce sedation rather than areas of the brain that transmit primary sensory information. As a result, even though the subject may be consciously unaware and unable to respond, information may still be processed at a primary level, but just not processed at a higher level. This has been demonstrated at sub-anesthetic doses with ketamine anesthesia in macaques (Leopold et al 2002) where macaques were able to carry out binocular rivalry, a basic level of visual processing, even when unresponsive to other cues of consciousness such as the eyelid reflex. Given that anesthesia was first utilized to inhibit sensory information transmission to the brain in order to make surgeries easier for both doctor and patient, it is interesting that a subject can be sedated but sensory processing can still take place at a basic level. This has been demonstrated at a small scale in this study to occur in both the somatosensory and visual modalities at least under fentanyl anesthesia in one animal. It has been shown that when propofol is reduced in the propofol-fentanyl combination anesthetic regimen, the BOLD response function better matches the awake

condition (Liu et al. 2013). This provides further evidence that propofol has a greater inhibitory effect on cortical sensory response.

Although propofol-fentanyl was able to preserve some somatosensory stimulus response in both animals (Fig 3), it was not able to preserve visual stimulus response. This indicates that perhaps the combination of propofol-fentanyl is better suited to the somatosensory neural pathway because that pathway is less sensitive to anesthetic inhibition than the visual neural pathway. When fentanyl was used alone, a visual response was observed in Monkey N, but when fentanyl was used in combination with propofol, the visual response in Monkey N was not observed, indicating that the visual pathway is more sensitive to propofol than it is to fentanyl. As propofol and fentanyl work by different pathways to achieve anesthetic endpoints, it was hypothesized that neither drug's receptors would be completely saturated thus some signal would be permitted to travel by both types of receptors. However, as it was found that the visual response was not at all preserved under propofol-fentanyl protocol. Therefore, it is clear that even a smaller dose of propofol (as it is combined with fentanyl to achieve anesthesia, so a lower dose is used of each drug), is sufficient to inhibit the visual sensory pathway. There is a gap in the literature regarding studies that specifically investigate and compare the receptor profile of different brain areas and what receptors different pathways in the brain rely on. This would be crucial to elucidate why one function in the brain may be more sensitive to ablation or attenuation under some forms of anesthesia but not others.

A combination drug anesthetic protocol could be preferable to a single anesthetic drug protocol because it reduces the changes for a tolerance to build in the animal over time to fentanyl. Furthermore, fentanyl can cause severe respiratory depression and therefore a combination with propofol can permit a reduction of this risk as the deleterious physiological effects of fentanyl on the animal can be diluted with propofol. According to these data, it would be useful to explore the propofol-fentanyl combination anesthesia protocol for use in somatosensory but not for visual fMRI studies in NHPs.

11. Conclusions

The important observation that sensory activity can be detected in an anesthetized macaque permits us to use fMRI to study brain changes longitudinally and *in vivo* in untrained animals. The extensive biologic similarity between the sensory systems of macaques and humans make them an ideal subject to study plasticity in the brain. Functional MRI is a powerful tool to explore almost real-time brain function *in vivo* and its high spatial resolution makes it well-suited to answer questions about plasticity which requires the tracing of local changes in activity patterns. The downside to fMRI is that unlike other brain analysis tools, it requires more strict management of motion in the subject. This means that animals must be at a deeper level of sedation than can be used for EEG for example. It is unclear the degree to which and in what specific ways, brain functions are altered under the influence of anesthesia that may limit the external validity of fMRI studies done in anesthetized animals. This study sets the groundwork for future work that should therefore continue to evaluate the suitability of anesthetic protocols for fMRI studies in non-human primates. Specifically, this investigation reveals that fentanyl and opiate based anesthetic protocols should be more thoroughly investigated. This study also highlights that pathways other than the main thalamo-cortical pathway, such as the putamen and other structures of the brainstem, may be used to process sensory information and these pathways may be strengthened under anesthetic sedation. Future studies should quantitatively match the intensity of sensory stimuli applied, include more animals in the investigation and use functionally validated atlas ROIs to look at specific ROIs rather than general areas of interest. Additionally, future studies should invest in the training and inclusion of at least one awake NHP so that sensory responses under sedation can be compared to “100% preservation of sensory response”. It would also permit researchers to understand in what ways anesthesia alters the sensory response pathways. Anesthesia is an excellent way for us to improve our ability to use both non-human primates and functional neuroimaging to answer important questions about neuroplasticity and longitudinal changes in the brain. A thorough evaluation and optimization of our use of anesthetics can greatly enhance our ability to design more powerful experiments and make more useful conclusions from fMRI studies in non-human primates.

12. References

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Appendix

Table 2. Monkey G Anesthetic Protocol for Isoflurane Experiment

Oct 24/2019	**Isoflurane Experiment**			
Animal ID	09042231			
Weight	12.4kg			
		Dose	**Total**	**Time**
Pre- Medication	Acepromazine	0.1mg/kg	0.12ml	8.17am
Induction Agent	Ketamine	10mg/kg	1.24ml	8:51am
Other Medication	Propofol	2.5-5mg/kg	3-6.2ml	9:11am
	Cerenia	0.1mg/kg	1.3ml	
	Start time:	9:52am	**Completed scan**	Unknown

Table 3. Monkey G Physiologic parameters measured during Isoflurane Experiment

Time	**O2 L/min**	**SPO2**	**Iso %**	**CO2**	**Resp Rate breaths/min**	**Temp °C**	**Heart Rate**
9:20	1	99	1.5	31	37	30.7	90
9:25	1	99	1.5	31	37	30.7	89
9:35	1.5	99	1.5	10	37	37.4	100
9:45	1.5	98	1.5	10	38	37.4	100
9:55	1.5	98	1.5	10	40	37.3	100
10:00	1.5	98	1.5	10	25	37.3	110
10:05	1.5	98	1.25	10	27	37.3	109
10:10	2	98	1.25	23	45	37.3	110
10:15	2	98	1.25	21	47	37.2	110
10:20	2	98	1.25	20	46	37.3	109
10:25	2	98	1.25	20	46	37.3	100
10:30	2	98	1.25	20	45	37.3	100
10:35	2	98	1.25	20	47	37.3	100
10:40	2	98	1.25	20	45	37.3	100
10:40	2	99	1.25	20	47	37.3	100
10:45	2	99	1	20	47	37.3	99
10:50	2	99	1	22	49	37.3	99
10:55	2	99	1	20	50	37.4	99
11:00	2	99	1	21	48	37.3	100
11:05	2	99	1	20	50	37.3	100
11:10	2	99	1	20	49	37.3	100

11:15	2	99	1	20	47	37.2	100
11:20	2	100	1	21	45	37.3	100
11:25	2	100	1	20	48	37.6	100
11:30	2	100	1	20	46	37.7	100
11:35	2	100	1	20	47	37.5	100
11:40	2	100	1	20	49	37.5	100

Table 4. Monkey N Anesthetic Protocol for Isoflurane Experiment

Sept 11/2019	**Isoflurane Experiment**			
Animal ID	07100391			
Weight	13.3kg			
		Dose	Total	Time
Pre-medication	Acepromazine	0.1mg/kg	0.13ml	8:07am
Induction agent	Ketamine	10mg/kg	1.3ml	8:44am
Other Medication	Propofol	2.5-5mg/kg	3.3-6.6ml	9:02am
	Cerenia	0.1mg/kg	1.3ml	

Table 5. Monkey N Physiologic parameters measured during Isoflurane Experiment

Time	**O2 L/min**	**Iso %**	**SPO2**	**CO2**	**Resp Rate breaths/min**	**Temp °C**	**Heart Rate**
8:55	2	1	100				130
9:06	1.5	1	100				110
9:30	1.5	1	98	30	13	35.9	100
9:45	1.5	1	99	26	11		98
9:55	1.5	1	98	27	10	36.1	95
10:00	1.5	1	98	27	11	36	98
10:10	1.5	1	98	21	13	36.1	95
10:20	1.5	1	98	22	15	36.1	95
10:30	1.5	1.5	99	18	18	36.1	95
10:40	1.5	1.25	99	18	23	36.2	98
10:50	1.5	1.25	98	18	24	36.2	100
11:00	1.5	1	99	14	27	36.2	100
11:10	1.5	1.25	98	14	30	36.3	100
11:20	2	1.25	99	14	27		100

11:30	2	1.25	98	14	28	*	100
11:40	2	1.25	99	14	26		100
11:50	2	1.25	97	14	28		100
12:00	2	2	99	13	27		100
12:10	2	1.25	99	16	27		100
12:20	2	1.25	98	17	29		100
12:30	2	2	100	14	24		100
12:40	1.5	1.25	98	15	25		100
12:50	1.5	1.25	97	14	26		100
1:00	1.5	1.25	99	14	28	37	100

* Indicates that the temperature remote stopped working during these runs.

Table 6. Monkey G Anesthetic protocol for Propofol-Fentanyl Experiment

Nov 21/2019	**Propofol-Fentanyl Experiment**			
Animal ID	09042231			
Weight	12.3kg			
		Dose	**Total**	**Time**
Pre-medication	Acepromazine	0.1mg/kg	0.13ml	8:03am
Induction agent	Propofol	2.5-5 mg/kg	3-6ml	9:15am
Other Medication	Ketamine	10mg/g	1.2ml	8:45am
	Fentanyl	5mcg/ml	1.3ml	9:42am
	Cerenia	0.1 ml/kg	1.23ml	
	Start scan:	9:54am	**Completed Scan**	11:35am

Table 7. Monkey G Physiologic parameters measured during Propofol-Fentanyl Experiment

Time	**O2 L/min**	**SPO2**	**CO2**	**Resp Rate breaths/min**	**Temp °C**	**Heart Rate**	**Propofol ml/hr 5mg/ml**	**Fentanyl ml/hr 5mcg/ml**
9:00		100				120		
9:10		100				100		
9:25	1.5	95		26	37.2	115		
9:35	1.5	98	29	26	37.2	99		
9:41	1.5	100	28	26	32.1	79		6
9:42	1.5	99	23	19	37.1	80		6
9:43	1.5	99	29	15	37.1	90		6
9:45	1.5	99	30	11	37.1	89	14	6

9:49	1.5	99	34	9	37	85	13	6
9:50	1.5	99	23	12	37	85	13	5
10:00	1.5	99	22	12	36.9	80	12	4
10:10	1.5	99	22	13	36.8	80	11	4
10:20	1.5	99	21	18	36.7	80	11	4
10:30	1.5	99	22	21	36.6	80	11	4
10:40	1.5	99	19	21	36.6	90	11	4
10:50	1.5	99	20	25	36.6	90	11	4
11:00	1.5	99	20	23	36.6	95	11	4
11:10	1.5	99	20	24	36.6	95	11	4
11:20	1.5	99	20	24	36.6	95	11	4
11:30	1.5	99	20	26	36.5	95	11	4
11:45	1.5	100				95	0	0
11:53	1.5	100				115	0	0
12:05	0	98				75	0	0

Table 8. Monkey N Anesthetic protocol for Fentanyl Experiment

Nov 24/19	**Propofol-Fentanyl Experiment**			
Animal ID	07100391			
Weight	13kg			
		Dose	**Total**	**Time**
Pre-medication	Acepromazine	0.1mg/kg	0.13ml	8:07am
Induction agent	Propofol	2.5-5 mg/kg	3-6ml	9:15am
Other Medications	Fentanyl	5mg/ml	13ml	9:40am
	Cerenia	0.1mg/kg	1.3ml	
	Start scan:	9:59am	**Completed Scan**	11:42am

Table 9. Monkey N Physiologic parameters during Fentanyl experiment

Time	**O2 L/min**	**SPO2**	**CO2**	**Resp Rate breaths/min**	**Temp °C**	**Heart Rate**	**Propofol ml/hr**	**Fentanyl ml/hr**
9:00am	1.5	100		36		130		
9:10am	1.5	100		36		119		
9:20am	1.5	100		16	37.6	100		
9:30am	1.5	100	42	16	37.5	100		

9:40am	1.5	99	20	11	37.5	95	13	8
9:49am	1.5	99	20			95	15	0
9:52am	1.5	99	37	14	37.4	98	15	0
10:00am	1.5	99	31	15	37.4	95	13	2
10:10am	1.5	99	20	18	37.3	95	13	2
10:20am	1.5	99	19	19	37.3	100	13	2
10:30am	1.5	99	18	23	37.4	100	15	4
10:40am	1.5	99	18	23	37.4	100	15	4
10:50am	1.5	99	18	23	37.4	100	13	6
11:00am	1.5	99	17	23	37.4	100	13	6
11:10am	1.5	99	18	21	37.4	100	13	6
11:20am	1.5	99	18	22	37.4	100	13	6
11:30am	1.5	99	18	23	37.5	100	13	6
11:40am	1.5	99	18	22	37.5	100	13	6
11:50am	1	100		20	37.5	5	0	0

* Had to shut off propofol + fentanyl after fentanyl bolus stopped breathing before the scan began. Animal was bagged, decreased fentanyl and propofol, then turned off fentanyl and further decreased the propofol until the animal was breathing again. Turned propofol back on and the animal's respiratory rate and heart rate remained stable. Increased fentanyl to 4 ml/hr.

Table 10. Monkey G Anesthetic protocol for Fentanyl Experiment

Dec 5/2019	**Fentanyl Experiment**			
Animal ID	09042231			
Weight	12.1kg			
		Dose	**Total**	**Time**
Pre-medication	Acepromazine	0.1mg/kg	0.12ml	8:05am
Induction agent	Propofol	2.5-5 mg/kg		9:10am
Pre-Medication	Ketamine	10mg/g	1.2ml	8:48am
Other medication	Fentanyl	5mcg/ml	1.2ml	9:35am
	Cerenia	0.1 ml/kg	1.2ml	
	Start scan:	10:00am	**Completed Scan**	11:48am

Table 11. Monkey G Physiologic parameters during Fentanyl Experiment

Time	O2 L/min	Iso %	SPO2	CO2	Resp Rate breaths/min	Temp °C	Heart Rate	Fentanyl ml/hr
9:15	2	1.5	100				110	5mcg/ml
9:20	1.5	1.5	100	28	27	37.3	100	
9:35	1.5	1.5	99	23	27	37.3	90	16
9:40	1.5	1	99	27	7	37.2	85	16
9:50	1.5	1	100	29	8	37.2	85	16
10:00	1.5	0.75	98	27	9	37.2	90	16
10:10	2	0.5	99	26	11	37.3	95	16
10:20	2	0.5	100	25	13	37.2	95	16
10:27	2	0.25	100	25	14	37.1	95	16
10:40	2	0.25	100	25	14	37.1	95	16
10:50	2	0.25	100	25	15	37	90	16
11:00	2	0.25	100	23	16	36.9	90	16
11:10	2	0	100	23	16	36.9	95	16
11:20	2	0	100	23	19	37	100	16
11:30	2	0	100	22	20	37	100	16
11:40	2	0	100	22	23	37	100	16

Table 12. Monkey N Anesthetic protocol for Fentanyl Experiment

Dec 5/ 2019	**Fentanyl Experiment**			
Animal ID	07100391			
Weight	13kg			
		Dose	**Total**	**Time**
Pre-medication	Acepromazine	0.1mg/kg	0.13ml	8:10am
Induction agent	Propofol	2.5-5 mg/kg	2-5ml	8:50am
Premedication	Ketamine	10mg/kg	1.3ml	8:40am
Other medications	Fentanyl	5mcg/kg	1.3ml	9:22am
	Cerenia	0.1ml/kg	1.3ml	
	Start scan:	9:29am	**Completed Scan**	11:40am

Table 13. Monkey N Physiologic parameters measured during the Fentanyl experiment

Time	O2 L/min	Iso %	SPO2	CO2	Resp Rate breaths/min	Temp °C	Heart Rate	Fentanyl ml/hr
8:40	1.5	1.5	100		30		130	5mcg/ml
8:50	1.5	1.5	100		31		120	ml/hr
9:00	1.5	1	100		20	38.4	110	
9:10	1.5	1.5	100		19		110	
9:15	1.5	1	100	48	16	38	100	
9:22	1.5	0.25	99			37.9	90	18
9:30	1.5	0.25	99	37	15	37.8	90	18
9:40	1.5	0	100	24	16	37.6	90	18
9:50	1.5	1	100	23	25	37.8	109	18
10:00	1.5	0	100	23	15	37.7	89	18
10:10	1.5	0	100	24	23	37.8	110	20
10:20	1.5	0	100	23	23	37.8	110	20
10:30	1.5	0	100	23	22	37.8	99	20
10:40	1.5	0	100	20	21	37.9	95	19
10:50	1.5	0	100	20	20	38	95	18
11:00	1.5	0	100	20	19	38.1	90	18
11:10	1.5	0	100	23	20	38	100	19
11:20	1.5	1.5	100	19	21	38.1	100	19
11:30	1.5	1	100	23	17	38.1	95	19
11:40	1.5	1	100	20	15	38.1	89	19

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