Differential effects of propofol on gamma-band activity across cortical and thalamic sites in the rat, *in vivo*

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April, 2012

This thesis was submitted to McGill University in partial fulfillment of the requirements of the degree of Masters in Neuroscience

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Abstract

Objective. Gamma (γ) oscillations (30-200 Hz) are intrinsic brain rhythms that are important for arousal and conscious processes. Although general anesthetics (GAs) cause concentration-dependent reductions of γ activity evoked by stimulation, their effect on spontaneously occurring γ oscillations remain largely unclear. The author investigated the concentration-dependent effects of propofol on behavior and spontaneous γ-oscillations.

Methods. Adult male Long-Evans rats (n=9) were chronically implanted with electrodes in the barrel cortex and ventroposteromedial thalamus. Spontaneous γ-power was assessed over different propofol plasma-concentrations (0, 3,6,9,12 µg/ml, and recovery) and frequency ranges (30-50, 51-75, 76-125, 126-500 Hz). Behavior was scored based on rearing, ambulation and jumping (n=6). Anesthesia was defined as loss of righting reflex.

Results. Anesthesia occurred at the 9µg/ml concentration. Three patterns of γ-power change were identified. The first one was an inverted-U shaped pattern found within cortical (30-75 Hz) and thalamic (30-50 Hz) recordings. The second was a plateau-drop pattern, representing no change across sub-anesthetic concentration, followed by a drop with onset of anesthesia. This occurred in the cortex (76-200 Hz) and thalamus (51-75 Hz). Finally a concentration-dependent linear decline pattern was observed in the thalamus across the 76-125 Hz and 126-200 Hz range. Increased ambulation and jumping was observed at sub-anesthetic concentrations coinciding with the inverted-U shape pattern.

Conclusions. These results demonstrate that the effects of GAs effect on spontaneous γ oscillations depend on drug concentration, recording site and frequency range investigated.
Résumé

Objectif. Les oscillations gamma (γ) sont des rythmes cérébraux intrinsèques importants pour la vigilance et les processus conscients. Bien que les anesthésiques généraux (AG) atténuent l'activité γ provoquée par une stimulation sensorielle, l'effet des AG sur l'activité γ spontanée demeure largement inconnu. L'auteur a caractérisé les courbes concentration-réponse du propofol sur le comportement et sur les oscillations γ spontanées.

Méthodes. Des rats adultes (n=9) ont eu une implantation chronique d'électrodes dans le cortex en baril et dans le noyau ventral postéro-médial du thalamus. La puissance γ spontanée a été mesurée en présence de 4 concentrations plasmatiques de propofol (3,6,9,12 µg/ml, et réveil) et sur 4 bandes de fréquence (30-50, 51-75, 76-125, 126-500 Hz). Le comportement a été évalué sur la base du cabrage, de la marche et des sauts (n=6).

Résultats. La perte de conscience est survenue à la concentration de 9µg/ml. Trois profils de modification de la puissance de l'activité γ furent trouvés. Le premier profil en forme de U inversé a été observé dans le cortex (30-75 Hz) et le thalamus (30-50 Hz). Le second profil, de type plateau-chute, était caractérisé par une absence de changement pour les concentrations subanesthésiques et d'une diminution abrupte au moment de l'anesthésie. Ce profil a été observé dans le cortex (76-200 Hz) et le thalamus (51-75 Hz). Enfin, un troisième profil, représentant une diminution linéaire reliée à la concentration, a été observé dans le thalamus (76-200 Hz). Une augmentation de la marche et des sauts a été observée en présence de concentrations subanesthésiques et coïncidait avec le profil de U inversé.

Conclusions. Ces résultats démontrent que les effets des AG sur γ dépendent de la concentration de l’agent anesthésique, du site d'enregistrement et de la bande de fréquence.
Acknowledgements

I am most indebted to my supervisor, Dr. Gilles Plourde, who not only guided me during this thesis, but became a personal mentor in helping me develop a sharp and critical mind when engaging in scientific pursuits. His passion for the fields of neuroscience, anesthesiology, and philosophy, has encouraged me to continue my intellectual pursuits without losing sight of the bigger picture. I am also grateful to my committee members, Dr. Barbara Jones and Dr. Abbas Sadikot for the time taken to review my work and offer constructive comments throughout my degree.

I would also like to thank the lab technician, Monsieur René Derroussent, who constructed the testing chamber, installed our EEG acquisition unit, and did innumerable other things during the labs setup. His unparalleled technical skill made testing possible. Further, I am grateful to the McGill Cancer Institute for the histological preparations, and to Dr. David Mumby of Concordia University for the behavioural aspects of my experiment. Thank you also to Dr. Richard Courtemanche of Concordia University for serving as external reviewer and offering numerous helpful comments. I would also like to thank the Montreal Neurological Institute’s Animal Care Committee for their support in aiding me whenever I had questions concerning surgical or overall care of the animals.

This work was supported in part by the Fonds de Recherche en Santé du Québec (FRSQ), the McGill Entrance Bursary, as well as from grants from the Canadian Anesthesiologists’ Society and Association des Anesthésiologistes du Québec to Gilles Plourde.
Introduction

The importance of understanding how general anesthetics (GA) act on the central nervous system cannot be overstated. Each year over 21 million patients receive GAs (Sebel et al., 2004), and whilst most cases are uneventful, there are significant risks of its use. One such risk is intraoperative awareness, which occurs when a patient unexpectedly regains consciousness, but cannot communicate because muscle relaxants are co-administered during surgery (Avidan et al., 2008). Most studies have estimated its occurrence at 1 or 2 per 1000 cases, with incidence higher in pediatric cases (Lopez et al., 2007).

The mechanisms by which GAs suppress consciousness remains unclear (Franks, 2008). As a result, clinicians rely on a series of clinical parameters - heart rate, blood pressure, movements, etc.- to gauge depth of anesthesia. One fundamental depth marker which also guides inhaled anesthetic administration is the minimum alveolar concentration (MAC). Introduced in 1965, MAC is defined as the end-tidal anesthetic concentration where 50% of the population cease to move in response to noxious stimuli (Eger et al., 1965). However researchers have questioned MACs ability to adequately reflect anesthetic depth. Motion in anesthetized patients for example, can be elicited during intubations and laryngoscopies, and is mediated by brainstem and spinal reflexes rather than the forebrain (Katoh et al., 1999). Indeed, anesthetic-induced immobility largely results from actions on the spinal cord (Sonner et al., 2003). MAC has nevertheless remained a key descriptor for anesthetic depth for inhalants. Additionally, MAC is only applicable to inhalant anesthetics, and while
a similar concept can be applied to intravenous drugs using plasma concentrations, these parameters are not available during routine anesthesia. Thus, the MAC approach is not a feasible measure of anesthetic-depth across the full spectrum of GAs.

Intraoperative electroencephalogram (EEG) maybe a more reliable index of a patient’s state, as it can provide real-time information concerning cortical activity in response to pharmacodynamic changes in the brain. Indeed, the EEG has been used within research for decades to assess pharmacological effects of anesthetics (Kiersey et al., 1951; Galla et al., 1958). More recently, monitoring devices have been developed to help assess anesthetic depth and decrease the risk of intraoperative awareness. One such index is the Bispectral Index Monitor (BIS; Aspect Medical Systems, MA), which uses proprietary weighted-parameters derived from EEG recorded from electrodes placed on the forehead to yield a single value which indicates the probability of adequate anesthesia (Liu et al., 1997). Even though critics of the technology have argued that it is difficult to gauge its efficacy (O'Connor et al., 2001), and have pointed to medical conditions that may skew readings (Duarte and Saraiva, 2009), the BIS still remains used in 60% of operating rooms in the United States (Orser, 2008). One parameter assessed in the computation of BIS, are the gamma (γ) range (up to 47 Hz) of the EEG (Miller et al., 2004).

Gamma oscillations (30-200 Hz) reflect high-frequency rhythmic synchronizations of neurons (Fries et al., 2007). They have been noted in a number of species from insects (Stopfer et al., 1997) to humans (Tallon-Baudry et al., 1996; Howard et al., 2003; Tanji et
al., 2005), and have been long considered an important element of ongoing activity of the waking brain, reflecting the depolarization of thalamic and cortical neurons (Steriade, 1993; Steriade et al., 1996b). This is substantiated by their role in working-memory maintenance (Pesaran et al., 2002), sensory perception (Gray et al., 1989), attention (Fries et al., 2001), etc. Gamma oscillations are thought to provide an adequate temporal scaffold in which spatially disparate elements could converge (Buzsáki and Draguhn, 2004; Jensen et al., 2007; Alkire et al., 2008a).

With the exception of ketamine (Plourde et al., 1997), all other GAs have been shown to cause concomitant reductions in 40 Hz auditory-evoked responses - gamma rhythm driven by sensory stimuli- when given at concentrations just sufficient to cause hypnosis (Gilron et al., 1998; Meuret et al., 2000; Plourde et al., 2003a). However, there is currently little direct evidence describing whether GAs disrupt spontaneously occurring γ-activity in humans or animals. Indeed, the majority of the literature has focused on stimulation-induced manifestations of γ (Plourde et al., 1998; Imas et al., 2005; Imas et al., 2006) or in-vitro paradigms in animal models (Faulkner et al., 1998). Concerning the studies evaluating the effects of anesthetics on spontaneously occurring γ, there are many contradictions in the literature as to whether anesthetics have any appreciable effects during hypnosis. Results have ranged from no observable changes (Hudetz et al., 2011), to significant increases (Murphy et al., 2011) and decreases (Breshears et al., 2010; Hudetz et al., 2011) in γ-activity. Interestingly, despite the paradoxical changes in γ recorded in both Breshears et al. (2010) and Murphy et al. (2011), both showed similar increases in low frequency delta,
theta, alpha, and beta waves upon induction of anesthesia. However, the frequency ranges used to define $\gamma$ rhythms and the methods used to quantify the changes are not consistent. Lastly, no study has addressed how propofol influences $\gamma$ across systematic step-wise increases in anesthetic blood-concentration, particularly in an animal model. Many studies have relied on conditions comparing different behavioural states (e.g. awake vs. anesthesia), which only provide subjective assessments to a concentration-effect drug profile. Further, basic neurophysiological data on $\gamma$-oscillations (Barth and MacDonald, 1996; Steriade et al., 1996a) are limited by the lack of information provided concerning level of anesthesia and drug-dosages used on their animal subjects (Plourde, 2007). Thus there is a need to examine the effects of GA on spontaneous cortical and thalamic (corticothalamic) $\gamma$-activity by determining concentration-effect relationships.

The author thus proposes examining the effects of propofol (Diprivan, Zeneca Pharmaceuticals) on spontaneously occurring $\gamma$-activity in the rat primary somatosensory barrel-field (S1Bf) and its corresponding thalamic nucleus, the ventroposteriomedial thalamus (VPM). Local field potentials (LFPs) will be recorded with chronically implanted intracerebral electrodes to characterize fast neuronal oscillations under conditions ranging from normal waking to sedation and deep anesthesia. Using multiple recording sites, and a thorough analysis of the entire $\gamma$ spectrum, this study will also help explain the existing contradictions in the literature.
Anesthesia and EEG

It is currently unclear how anesthetics cause unconsciousness (Franks, 2008). Although much research has been done from ligand-oriented perspectives to in-vivo studies (Franks and Lieb, 1998; Rudolph and Antkowiak, 2004), the chemical heterogeneity of GAs poses a major difficulty for understanding their mechanisms of action. For example, commonly used inhalants such as sevoflurane or isoflurane are relatively complex molecules compared to the noble gas anesthetic, xenon. However despite their chemical diversity, they all elicit unconsciousness. The ability to cause unconsciousness is an essential feature for the inclusion in this drug category.

General anesthetics have been shown to target at least 30 different ion channels (Campagna et al., 2003), and a host of receptor family-types including GABA, acetylcholine, catecholamines, glutamate, and glycine, with each agent having its own individual affinities and selectiveness (Mashour et al., 2005). However, even though GAs display significant chemical heterogeneity there exist many similarities at the level of global and regional brain activity (Franks and Lieb, 1998; Franks, 2008).

Recordings of the electrical activity of the brain date back to the seminal work of Hans Berger (1929), followed by Alfred Loomis et al. (1937) who distinguished various EEG states as a function of sleep. Later work revealed that during states of arousal, cortical, and thalamic neurons manifest a low-amplitude, highly disynchronous behaviour (Moruzzi and
Magoun, 1949; Steriade et al., 1993; Destexhe et al., 1999) whereas during states of slow-wave sleep or anesthesia, neurons become progressively hyperpolarized, and exhibit high-amplitude, synchronous activity (Steriade et al., 1993).

General anesthetics role in suppressing consciousness at clinically relevant concentrations would suggest a monotonic, dose-dependent decline in thalamocortical activity, especially when considering that these drugs have an exceptional affinity to GABAa receptors (Bonin and Orser, 2008). However, GAs exert a bidirectional effect on thalamic and cortical systems (thalamocortical), which was first detailed by Winters (1976). Regardless of class, most GAs induce a certain level of excitation at low doses, causing highly disynchronous, low-amplitude cortical EEG, as well as prominent motor activity and ataxis (Winters, 1976; Sloan, 1998). Following that, anesthetics can either cause a shift towards a depressive state or paradoxically manifest proconvulsant behaviours from cataplectic anesthesia to status epilepticus. Further, although proconvulsant effects of GAs are uncommon, they have been noted in most conventional compounds such as sevoflurane (Constant et al., 2005), ketamine (Ferrer-Allado et al., 1973), opioids (Kearse et al., 1993), as well as propofol, which is regarded as a anticonvulsant at higher doses (Walder et al., 2002).

Alternatively, GAs also elicit the expected effect of thalamocortical depression towards an isoelectric EEG state (Medullary Paralysis/Death). This is denoted by the appearance of spike-like bursts and high-amplitude slow waves (Winters, 1976). As the depth of anesthesia increases, so to do the time intervals of these bursts, thus eliciting a pattern of
burst-suppression (Amzica, 2009). Adding further complexity is the possibility that burst-suppression may itself be a form of epilepsy (Amzica, 2009) as it has been shown to promote hyperexcitability in response to sensory stimuli (Kroeger and Amzica, 2007). Of these two possible shifts (excitatory or depressant), most GAs induce an initial excitatory stage followed by a gradual descent towards isoelectricity.

Thus, with few exceptions such as ketamine and nitrous oxide - which do not depress cortical activity or impede sensory information flow (Schwender et al., 1993; Hirota, 2006) - most GAs, such as propofol, elicit global cortical depression and cerebral blood-flow reduction at clinical relevant concentration. This is particularly evident in arousal-linked brain areas like the medial-thalamus and midbrain structures (Fiset et al., 1999b). The precise concentration-effect relationship of propofol on EEG however still remains unclear. Although studies have investigated this relationship in the past (Fiset et al., 1999a; Freeman et al., 2000; Murphy et al., 2011), information regarding intracranial recordings have not yet been rigorously studied, particularly in animal models. One of the only studies to explore this in rodents had subjects given a single high-target concentration injection of propofol, where any changes to the recorded EEG was determined offline and linked to the estimated concentration based on propofol’s pharmacokinetics (Dutta et al., 1997).
Unfortunately, this methodology failed to take into account the time needed for the concentration of propofol in the brain to match that of the systemic concentration. Further, the authors did not investigate if there were any significant changes across the frequency bands of the EEG. Thus there exists a need to assess the specific EEG component changes
to clinically relevant concentrations of propofol anesthesia to better understand its hypnotic properties.

The benefit of an intracerebral approach would allow for a more accurate characterization of neuronal activity without the burden of spatial summation and phase cancellation that is inherent in the scalp EEG paradigm (Pfurtscheller and Cooper, 1975).

*Anesthetic influences on γ-rhythms*

Raw EEG is aperiodic, nonlinear, and is difficult to correlate with any specific brain state, since it is the superposition of sine waves of different frequencies. Indeed, with the exception of isoelectricity and burst suppression, raw EEG provides little qualitative characteristics that allow for a quantitative analysis of anesthetic depth. However the underlying frequency components (or rhythms) that compose the raw EEG can be correlated to specific behaviours. Indeed, one brain rhythm that has long been associated with arousal-mediated behavioural and cortical processes, has been γ (30-200 Hz) (Adrian, 1942; Sauvé, 1999; Fries et al., 2007). Early observations of γ rhythms were in the 30-80 Hz frequency range however recent developments have drawn attention to the 80-200Hz range (Crone et al., 2011). As a result, there is now a trend to use the 30-200Hz range in the study of gamma oscillations although these rhythms may reflect different entities depending on the specific frequency range (Uhlhaas et al., 2011).
As mentioned above, $\gamma$ is particularly interesting in arousal/anesthesia research. Its manifestation is neither a uniquely human phenomenon either. It has been found in many species from insects (Stopfer et al., 1997) to humans (Tallon-Baudry et al., 1996), suggesting an important role for its conservation in evolution. In humans it has been found across all major cortical areas including the visual (Engel et al., 1991a; Engel et al., 1991b), auditory (Brosch et al., 2002), and somatosensory cortex (Bauer et al., 2006).

Not surprisingly, the role of $\gamma$ oscillations has been attributed to a number of high-level neurocognitive processes including general arousal (Gross and Gotman, 1999), sensory processing (Gray et al., 1989), and attentional selection (Fries et al., 2001). Further, anomalies of $\gamma$-rhythms have been linked to a number of general psychiatric disruptions of reality including schizophrenia and Alzheimer's disease (Uhlhaas et al., 2008). Disruption of $\gamma$ has also been shown to be sufficient to impede behaviour, as selective blocking of 30 Hz inhibitory neurons in the mushroom body of the honeybee significantly disrupts odor discrimination (Stopfer et al., 1997).

The many correlates of $\gamma$-oscillations, however, do not explain why high frequency oscillatory synchrony may maintain a selective advantage above other frequencies. One prominent theory is that $\gamma$-frequencies may act as a suitable scaffold onto which information can be carried across (Fries et al., 2007). Neurons in sensory cortical areas receive a plethora of convergent information. During times when a sensory cortical area is not processing specific information, there is a low signal/noise ratio (Fries et al., 2007;
Jensen et al., 2007). For example, if two neurons are converging onto a single primary sensory pyramidal cell, and fire at low frequencies, two problems arise. Primarily, the probability of coincident arrival becomes less as these two converging cells fire with decreasing frequency. This in effect would not improve the signal/noise ratio. Secondly, the slow arrival of synaptic input reduces the chance that it will facilitate a large enough excitatory post-synaptic potential (EPSP) within the cell soma to reach the firing threshold for an action potential (Jensen et al., 2007).

Neurophysiologically, the ultimate purpose of $\gamma$-activity within this theory is to act as a facilitator, ensuring that information is properly spread across areas. This then provides a reasonable mechanism to account for the phenomenon of sensory binding - the integration of sensory information from disparate brain areas. Indeed, modal specific cortical areas show increased $\gamma$-activity across both primary and secondary structures (Engel et al., 1991b). Further, $\gamma$ synchrony between bilateral homologous structures can be disrupted following a callosotomy (Engel et al., 1991a), suggesting there exists a tight coupling even across large cortical distances.

A key mediator of cortical activity is the thalamus. Its sensory gating circuits and diffuse reciprocal connections with the neocortex may provide a key for mediating cortical responsiveness to information (Huguenard and McCormick, 2007). Indeed, injections of GABAa agonists into the thalamus has been shown to mimic anesthetic effects with corresponding slowing of cortical activity (Miller and Ferrendelli, 1990). Further, Alkire et
al, (2007) have shown that intralaminar injections of nicotine can antagonize sevoflurane anesthesia in rats. The same anesthetic has also been shown to decrease thalamic metabolism whilst patients undergo anesthesia (Alkire et al., 2008b). In addition, behavioural improvements to minimally conscious patients can be partially recovered following thalamic stimulation (Schiff et al., 2007). Thus the thalamus plays an important role mediating cortical and behavioural arousal.

One global mechanism by which GAs may attenuate arousal is through the interference of $\gamma$-generation. EEG recordings from the scalp suggest the induction of anesthesia is often accompanied by a decline in $\gamma$-power in bilateral frontal lobes and between the frontal and parietal regions (John and Prichep, 2005). Coherence between fronto-occipital regions are also disturbed following anesthesia (Imas et al., 2006). This is further supported by studies showing that 40 Hz auditory-steady state responses - sustained cortical responses to rapidly delivered auditory stimuli- in human subjects are significantly reduced following sufentanil (Gilron et al., 1998), sevoflurane (Plourde et al., 2003b), and propofol (Meuret et al., 2000) anesthesia- with the exception of ketamine (Plourde et al., 1997). These studies showing a concomitant decline in $\gamma$-activity with hypnosis have led to the view that GAs may interfere with arousal by attenuating $\gamma$ within the EEG spectra as well as breaking down long-range coherence, thereby disrupting the integrative processes needed for consciousness (Alkire et al., 2008a). However studies investigating the effects of GAs on spontaneously occurring $\gamma$-activity often show contradictory results. Whereas some studies have shown significant decreases in $\gamma$-activity across anesthetic concentrations in both sevoflurane (Uchida et al.,
2000) (30-150 Hz) and propofol (Fiset et al., 1999a; Freeman et al., 2000; Breshears et al., 2010) in human electrocorticography - electrodes placed directly on the cortical surface -

(ECoG) and EEG recordings, others have shown significant increases in recorded gamma activity during propofol hypnosis (Murphy et al., 2011)(25-40 Hz). In addition, studies investigating spontaneously occurring gamma using multiple acquisition techniques and recordings sites have often found contradictory results, suggesting there are selective effects across the brain. Fell et al (2005) for example, found significant decreases in gamma (32-48 Hz) recorded through scalp EEG paired with significant increases in subcortical electrode recordings in the same human subjects. This was also true of Verdonk et al (in preparation) who found significant decreases in gamma (62-90 Hz) in implanted ventroposteromedial electrodes but no change across cortical dural recordings in humans. Further, these differential effects of anesthetics on gamma activity may not solely be a result of different recording sites, but may be a consequence of gamma definition used. Indeed, isoflurane has been shown to elicit differential effects on gamma activity within cortical regions depending on which segments of the gamma spectrum is investigated (Hudetz et al., 2011).

Thus there are striking inconsistencies across studies investigating spontaneously occurring gamma, concerning the acquisition technique used, the brain sites investigated, as well as the frequency interval used to define gamma. Further, many anesthetics do not follow a simple monotonic course of progressive sedation (Winters, 1976), and thus strict control of plasma-concentration is also an important factor. There is therefore a need to establish the
precise concentration-dependent relationship with corticothalamic activity for the purposes of establishing the impact of propofol on the brain, as well as to better characterize markers for arousal in clinical settings.

**Proposed Resolution of Problem and Experimental Protocol**

This thesis will aim to study the effects of concentration-dependent changes produced by a conventional anesthetic, propofol, on cortical and thalamic LFPs, with a specific focus on $\gamma$ power. It is to my knowledge the first experiment of its kind to establish a precise concentration-effect relationship of propofol on spontaneously occurring gamma activity in an animal model. It will also attempt to provide the first detailed analysis of propofol’s effects on gamma activity across its entire spectrum with step-wise increases in propofol concentration. Further, there are two advantages in using intracranial animal models in lieu of humans in this experiment. Primarily, animal subjects can be instrumented with intracranial implants without having an underlying neurological impairment— in contrast to human subjects, which invariably have epilepsy or other brain pathologies. Secondly, intracranial implants offer a more reliable index of high-frequency cortical activity since it does not suffer from restrictive spatial summation notable in scalp EEG (Pfurtscheller and Cooper, 1975), and reduces the likelihood of electromyogram (EMG) contamination. This is due to the fact that each brain site can be instrumented with a dual-prong (or bipolar) electrode, which generates a differential signal between the two poles, which eliminates any signal common between them.
To examine the effects on fast neuronal oscillations, we will record local field potentials (LFPs) from the primary somatosensory barrel cortex (S1Bf) and its corresponding thalamic nuclei, the ventroposteromedial nucleus (VPM) in rats with chronically instrumented recording electrodes and a venous catheter for the administration of propofol.

The S1Bf remains an ideal structure to assess corticothalamic activity, owing to its size and its well structured cytoarchitectural representation of the mystacial pad (Deschênes et al., 1998; Jones and Barth, 1999a). This disproportionately large vibrissae representation in the brain is a compensatory measure for the rats other weak senses like vision (Petersen, 2007). The whiskers representations exist as cluster of “barrels” found in layer 4 which are large columnar cellular aggregates that topographically correspond to a specific whisker along the mystacial pad (Petersen, 2007). Upon the activation of primary sensory neurons from a rat’s vibrissae, these barrels compute spatiotemporal relationships when the whisker is in contact with objects in its environment (Alloway, 2008). Interestingly, this specific relationship is maintained downstream in the somatosensory pathway, whereupon the glutamatergic VPM neurons from corresponding “barrelloids” also respond to a principal whisker and project only to a specific barrel formation, forming a one-to-one relationship between barrels and barreloids (Deschênes et al., 1998).

The S1Bf is also an attractive neural model for investigation because γ activity has been shown to increase in response to manual vibrissal stimulation in un-anesthetized rats free from restraint (Jones and Barth, 1999b). This spontaneous γ -activity is likely a result of
fast spiking neurons within the cortex and plays a key role in mediating sensory responses (Cardin et al., 2009).

In conclusion, the findings will not only contribute to a better understanding of the basic neurophysiological understanding of the mechanisms of GAs, but may have implications concerning the validity of the BIS index monitor which uses changes in EEG as a measure of arousal (Miller et al., 2004). The development of a general marker for anesthesia is critical in biomedical research, and this will help establish a guide for γ-activity’s effectiveness. The relationship between behaviour and γ-activity will also be assessed.

The author hypothesizes that step-wise increases in propofol concentration will yield biphasic changes in observed behaviour and γ power. However given the contradictions in previous research, it is also predicted that these changes will not be consistent across γ’s entire spectrum. Given its uncharacteristically large spectrum and the non-unitary changes observed in Hudetz et al. (2011) with isoflurane, this would partially resolve these conflicts. In addition, these inconsistencies may in part be explainable by recording site used to investigate these effects. Thus it is also likely that the S1Bf and VPM will show differential patterns of gamma activity across propofol concentrations.
Methods

All surgical procedures and experimental protocols adhered to the guidelines of the Canadian Council on Animal Care as well as the Montreal Neurological Institute’s Animal Ethics Board. Male Long-Evans rats (300 - 320g, n = 9) were acquired from Charles River Laboratories (Senneville, Quebec), and arrived with a right jugular catheter. Animals were housed in individual cages with full access to food and water and ample enrichment. A normal light-cycle was used throughout the duration of the experiment (light on from 07:00 to 19:00 Hrs).

Surgery

All animals were anesthetized with ketamine (50mg/kg, ip) / xylazine (5mg/kg, ip) and secured to a stereotaxic frame which was equipped with a heating pad and rectal temperature monitor. A midline incision was made and the periosteum of the skull was retracted with hemostats. Bipolar, teflon-coated stainless-steel wires (125µm exposed tips. 1.0mm tip separation) were positioned in the VPM (A/P: -3.5 , L/M: 2.7, V/D: -6.6, relative to bregma) and S1Bf (A/P: -2.3 , L/M: 5.0, V/D: -3.6) for LFP recording. This was done to generate a differential bipolar recording which minimizes the possibility of myogenic contamination in the EEG, since no EMG recording was possible (see below).

Following their instrumentation, the cortical electrodes (0.008µm exposed tips) were adjusted vertically to optimize the amplitude of the field EPSPs (fEPSP) evoked via VPM stimulation. Thicker wires (0.011µm exposed tips) soldered to stainless steel screws were
placed in the contralateral parietal bone and the ipsilateral frontal bone to serve as the reference and ground electrodes, respectively.

Although the catheter was instrumented in the right jugular vein, the tubing was extended across and out the back of the animal for ease of drug administration. Unfortunately, this precluded the possibility of an EMG electrode in the clavotrapezius neck muscle.

Electrode leads were then fastened to gold-plated Amphenol pins and inserted into a nine-pin connector. This assembly was then secured to the skull using acrylic dental cement. One week was then allowed for recovery and observation, whilst the catheter was flushed every 3-5 days with a gentamycin / heparin solution.

**Design**

There was a single testing session per rat with six conditions (Baseline - 3µg propofol / ml blood- 6µg/ml - 9µg/ml- 12.0 µg/ml - Recovery). During each session, rats were placed in a 40x40x60cm airtight Plexiglass chamber. Propofol was administered through the right jugular vein catheter with with a Harvard-22 syringe pump controlled by the Stanpump software developed by Steven L. Shafer and colleagues (Department of Anesthesiology, Stanford University, CA) using pharmacokinetic parameters derived from Knibbe et al. (2005). This software uses a three-compartment pharmacokinetic model to calculate the infusion rate required to achieve the desired plasma concentration of a propofol and drives the Harvard-22 pump accordingly. The algorithm relies on an initial bolus injection to
quickly reach the desired plasma concentration followed by an exponentially-decreasing rate of infusion to maintain the plasma concentration steady. This software was required because bolus or fixed rate infusion of propofol (or any other drug) do not yield stable plasma concentrations. Prior to each testing session, the rat’s weight, length, age, and sex are entered into the software, yielding an individual profile for drug delivery.

Behaviour was assessed through the use of a 3x3 white grid along the floor of the chamber. Using a video-recorder, this allowed us to quantify activity levels as propofol concentration increases.

The experimental sessions were preceded by three forty-minute acclimation sessions, where the animal will be allowed to explore the testing chamber. During this time the animal’s head assembly was attached to the commutator, but no recording was done. This reduced any anxiety-provoked excitement that would confound the results.

The initial baseline condition consisted of recording LFPs and behaviour while the rats explored the environment. Following that, the Stanpump program delivered an initial steady concentration of 3µg of propofol (the condition expected to cause mild sedation/excitation). After a 15 minutes drug equilibration period, behaviour and LFPs were re-recorded. This was repeated for 6, 9, and 12µg/ml (expected to cause moderate sedation/excitation, unconsciousness, and deep anesthesia, respectively). The entire spectrum of concentrations given provided a measure of light sedation to deep anesthesia. Finally, the
propofol administration was terminated and the animals were given time to recover before the final recovery condition.

**Behavioural Analysis**

The testing chamber was equipped with a 3x3 grid floor which was used to quantify *orienting, impulsivity, and general activity* adapted from Colorado et al (2006) and with the support of the David Mumby’s memory laboratory (Centre for Studies in Behavioural Neurobiology, Concordia University, Montreal). Unpublished data from our laboratory suggests propofol may elicit both cortical and behavioural hyperactivity at sub-anesthetic dosages in rats. Further, unpublished observations from Fiset et al. (1999) have noted that human patients exhibit excitation and inappropriate behaviour when given 1.5µg/ml of propofol, which equates to approximately 3.0µg/ml in rodents. Anesthesia was defined as a loss of righting reflex as well as an absence of any attempt to right.

Orienting behaviour occurs when a rat stands on its hind-legs, which is associated with non-selective attention. The parameters used were: *rear counts, duration*, as well as *jumping* counts.

Indexing impulsive behaviour is one method way to monitor hyperactivity in rat models. Although ambulation velocity is a key marker, the chamber’s commutator and dimensions precludes the possibility of installing a camera within the cage, pointing directly down. In lieu of this, *number of zone crossings* - both peripheral and central- along the 3x3 grid, and
time spent in the centre of the grid were quantified. The latter of which is an important measure since rats will not normally venture into open space, and will instead prefer peripheral orientations to avoid predation (Treit and Fundytus, 1988).

**Electrophysiology Recordings**

EEG from the cortex and thalamus were amplified (0.1 Hz to 475 Hz band pass), digitized at 3000 Hz, and stored for offline analysis. A successful recording was defined as an artifact free EEG of at least 2 minutes for each condition. The two monopolar recordings acquired from each brain site were subsequently formatted offline to generate a differential “bipolar” recording (superficial - deep contact), which were used for all statistics and reporting. This is because monopolar EEG is subject to contamination from volume-conducted myogenic artifacts.

**Statistical Analysis**

Behaviour was coded offline from recorded video using a computer-based event recorder (Best Software, Educational Consulting Inc. (BEST), Las Vegas, NV, USA), to identify peripheral / centre zone crossings, centre duration, rearing frequency and duration, and jumping. The data was normalized to the baseline value of each respective variable. Using SPSS version 19 (SPSS: An IBM company, Chicago, IN, USA), data was evaluated for normality using the Kolmogorov-Smirnov test to determine if parametric statistics were appropriate. If passed, a repeated-measures ANOVA with Tukey’s HSD for post-hoc
analysis was used. If the data failed normality, a Friedman test was conducted, with a
Wilcoxon signed rank-test for post-hoc comparisons.

The spectral power was computed using Fast-Fourier Transform on the entire EEG sample
with the Matlab <spectrum> function (MathWorks Inc. Natick, MA) using 2-second long
non-overlapping segments and a Hamming window. The $\gamma$ frequency segments were
defined as: 30-50, 51-75, 76-125, 126-200 Hz. Pairwise differences in spectra power over
selected frequency ranges between concentrations were assessed with a permutation test
(Blair and Karniski, 1993). One Permutation test was conducted across each condition for
every gamma segment in both recording sites (S1Bf & VPM), yielding a total of 8
permutation tests. To minimize the impact of interference from external electrical sources,
power at 59-61 Hz, 119-121 Hz, and 179-181 Hz were excluded from analysis.

Lastly, we determined whether the effect of propofol on spectral power followed a linear,
quadratic or step decrease (sudden drop at concentration causing unconsciousness with no
or only modest changes at lower or higher concentrations) function (Kirk, 1995). A linear
trend would reveal phenomena that are affected in a concentration-dependent manner by
propofol, an clear indication or pharmacological sensitivity. A quadratic trend would
highlight the possible biphasic effect propofol has on parts of the $\gamma$ spectrum, as is the case
for global EEG. A step decrease would highlight phenomena that best correlate of the
level of consciousness. This analysis provided an assessment if there were segments of
specific frequency bands (30-50, 51-75, 76-125, 126-200Hz) within the entire $\gamma$-spectrum
that exhibited differential effects to propofol anesthesia. This was accomplished by analyzing the averaged power across all concentrations (excluding the recovery period) through a trend analysis for each γ segment on a repeated-measures within-subject ANOVA using SPSS. Each frequency range was tested for both linear and quadratic functions, and their categorization was based on the resulting significance level. The selection of the step decrease function was based on demonstration of a significant decrease between the 6 and 9 µg/ml concentration (all animals were conscious at 6 µg/ml and none were at 9 µg/ml) in the absence satisfactory (P<0.05 with normally distributed residuals) linear trend over the 0, 3, 6, 9 and 12 µg/ml concentrations as well as over the 0, 3 and 6 µg/ml concentrations.

Histology

All rats were euthanized after experimentation with Urethane (1000mg/kg, I.P) and transcardially perfused with 200ml heparinized saline followed by 500ml of 10% neutral buffered formalin. Histological preparations for the first four rats were conducted within the lab but yielded poor tissue unsuitable for microscopy. The brains from the last five rats were processed by the Goodman Cancer Research Centre’s Histology Facility at McGill University. They were post-fixed in 4% paraformaldehyde followed by a paraffin embedding. Slices were made in 25µm step-sections at a thickness of 6µm at the location where the electrode indents (or dimples) could be seen on the surface of the cortical tissue. Finally, the collected tissue was stained using cresyl violet. The accuracy of the electrode tracks was verified through microscopy. Barrel cortex and ventroposteromedial nucleus
placements were verified in 4 animals (See Appendix II). In the remaining animal, gross
distortion of brain tissue around the electrode sites prevented definitive identification of the
target structures.
Results

Behavioural Analysis

Results from the behavioural data had a smaller sample size (n=6) because the changes in behaviour were only noted after testing begun.

During baseline, animals exhibited relaxed behaviour and ambulation across the chamber. Relative to baseline, the 3µg/ml propofol concentration resulted in slightly slower behaviour, however it was not statistically significant. The 6µg/ml concentration however, led to an overall increase in excitatory behaviour relative to the 3µg/ml condition, and was the only condition where jumping was documented.

Of the behavioural variables coded for agitation, peripheral zone crossings yielded a main effect of propofol concentration (F (2,10) = 5.36, p = 0.026). Post-hoc Tukey HSD comparisons found a significant difference between 3µg/ml (M = 19.5 ± 8.7) and 6µg/ml concentrations (M = 57.7 ± 43.8, p <0.05) (see Figure 1A). This was also true of the centre zone crossings, where there was a significant main effect of propofol (F(2,10)=6.94, p = 0.013), with a significant difference between the 3µg.ml (M = 2 ± 2.40 and 6µg/ml (M = 8.0 ± 6.3) conditions (Tukey HSD, p <0.05)(Figure 1B). There was also a significant effect of propofol on total zone crossings (F (2,10) = 5.53, p = 0.024). Tukey's HSD revealed a significant increase from the 3µg/ml (M = 21.5 ± 10.8) and 6µg/ml (M = 65.7 ± 49.4) concentration (Tukey HSD, P<0.05)(Figure 1C). Although the time spent in the centre zone
increased from the 3 (M = 0.3 ± 0.4) to 6µg/ml (M = 0.7 ± 0.3) condition, the difference was not significant (p > 0.05) (Figure 1D).

One other potential marker for anxiety was the orienting behaviour of rearing. Although there were clear mean increases in rearing instances at the 6µg/ml condition (M = 16.8 ± 16.5) relative to baseline ((M = 5.2 ± 6.6) and 3 µg/ml (M = 4.2 ± 3.4), they were not statistically significant (F (2,10) = 3.13, p = 0.088)(Figure 2A). Nor were the durations of the rearings (F (2,10) = 1.60, p = 0.245)(Figure 2B). Jumping was the final measure of hyper-excitability and was only noted during the 6µg/ml conditions (Friedman test , χ²(2) = 10.00, p = 0.007)(Figure 2C).

**Electrophysiology Data**

As predicted, propofol elicited biphasic changes in raw EEG in both the S1Bf and VPM from which the power spectra was compiled from (Figure 3). Subanesthetic concentrations (3-6µg/ml) caused an increase in low-amplitude, high frequency EEG. This is contrasted with the typical slowing of EEG waves during hypnosis (9-12µg/ml), followed by a rebound in activity similar to that of the baseline condition.

The effects of propofol on high-frequency power followed one of three patterns of activity in response to propofol concentrations depending on the recording site (cortex or thalamus) and frequency range assessed (Figure 4): Inverted-U shape, plateau-drop, or linear-decline effect. The first pattern consisted of an increase from baseline to the 6µg/ml concentration,
followed by a decrease at the 9 and 12µg/ml levels. This pattern will be referred to the inverted-U shape effect. The second pattern consisted of no change from baseline to the 6µg/ml concentration, followed by an abrupt decrease at 9 and 12µg/ml. This pattern will be referred as the plateau-drop. The last pattern consisted of a linear concentration-dependent decline in gamma power from baseline to 12µg/ml concentration. This will be referred to as the linear-decline effect.

The inverted-U shaped pattern (Figure 4A) was observed in the cortex within the 30-50 Hz ($p = 0.044$) and 51-75 Hz ranges ($p = 0.034$, Quadratic Trend analysis, within-subjects ANOVA, Figure 5A-B), as well as within the thalamus (30-50 Hz) ($p = 0.057$) (Figure 5C).

The permutation analysis revealed the following pairwise differences (see Appendix for complete comparisons). In the cortex, there was a significant increase of gamma activity at 30-50 Hz and 51-75 Hz ranges following 6µg/ml, relative to baseline ($p = 0.01$, $p = 0.05$, respectively), thus forming a peak in gamma activity. This was followed by an abrupt and significant drop in gamma power at the 9µg/ml concentration- where LORR occurred- that was significantly lower than the previous 6µg/ml condition ($p = 0.05$, 0.01). Interestingly however, in the 30-50 Hz, gamma was still significantly higher than baseline ($p = 0.005$). The gamma power range then continued to decline until 12mg/ml where it was significantly lower than from peak activity during 6µg/ml in both conditions ($p = 0.05$, 0.01) as well as baseline across 51-75 Hz ($p = 0.05$).
The second pattern displayed a plateau-drop - or resistance to change- across sub-anesthetic concentrations which was followed by a sharp decline in hypnotic ranges (9,12 µg/ml) (Figure 4B, 6). This occurred in high cortical (76-125 and 126-200 Hz, Figure 6A-B) and low thalamic frequency range (51-75 Hz, Figure 6C). The absence of change from baseline to the 6µg/ml concentration was also confirmed by non-significant ($p > 0.05$) linear contrast. The sudden drop in gamma power following hypnosis was significantly lower than all previous conditions (permutation test, $p < 0.05$). This was followed by an additional significant drop in gamma at 12µg/ml was that was also significantly lower than all previous conditions across both frequency ranges ($p<0.05$).

The last pattern expressed a continual decline in gamma power across all propofol concentrations (Figure 4, 7). This was observed in the thalamus across high gamma ranges 75-125 ($p = 0.01$) and 126-200 Hz ($p = 0.029$, Figure 7). Gamma power was significantly lower in both 3µg ($p = 0.05$) and 6µg/ml ($p = 0.05$) relative to baseline. Similar to both previous distributions, 9µg/ml was met with an abrupt drop, which was significantly lower than all previous conditions across both ranges ($p < 0.05$). This was also true of the highest 12µg/ml condition ($p < 0.05$).

Figure 7 shows the averaged individual power spectra for each recording site from which the data shown in Figure 5-7 were obtained. The inverted-U shape illustrated in Figure 4 & 5 can be in Figure 8 A1&B1, while the plateau-drop is highlighted in Figure 8 A2,B2, where there is a resistance (or clustering) of power spectra across sub-anesthetic
concentrations. Finally, the linear-decline effect is noted within Figure B2 where there is a step-wise decline in power with increasing propofol concentration. A power spectra from a representative animal is shown in Figure 9. Note the similarity with Figure 8.
Discussion

Summary

The purpose of this experiment was to characterize the effects of propofol, a commonly used IV general anesthetic, on γ oscillations in the cortex and thalamus of freely moving rats. Until now, it has remained unclear how systematic increases in propofol concentrations influence γ activity, and whether propofol’s conflicting effects of γ oscillations in the literature could be explained.

Gamma oscillations have been repeatedly demonstrated to be involved in several perceptual and cognitive operations from general arousal to conscious processes (Gross and Gotman, 1999). Although a number of studies have addressed how anesthetics influence γ oscillations, most have focused on the 40 Hz-components of sensory-evoked potentials (Plourde, 2006), and have found a systematic decline in recorded γ during hypnosis across most GAs (Plourde, 1993; Gilron et al., 1998; Plourde et al., 1998; John and Prichep, 2005; Imas et al., 2006). This helped established the theory that anesthetics may suppress consciousness through dampening γ’s activity in the brain (see (Alkire et al., 2008a)).

Research investigating the effect of anesthetics on spontaneously occurring gamma however have found contradictory evidence, ranging from no observable changes (Hudetz et al., 2011), to significant increases (Murphy et al., 2011) and decreases (Breshears et al., 2010). However as shown in Table 1, the frequency ranges used to define γ and techniques used to acquire EEG have been inconsistent across these papers, which may explain the variable findings. Further, no study has addressed how propofol influences γ across

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systematic step-wise increases in anesthetic plasma-concentration, particularly in an animal model. The majority of papers have focused on behavioural states condition markers (e.g. awake vs. unconscious) in lieu of more objective conditions, such as plasma-concentrations.

Since concentration-effect relationships provide the best assessment of drug effects on brain activity, we investigated how step-wise increases in propofol concentrations influenced gamma oscillations, over a frequency range (30-200 Hz) sufficiently wide to encompass the ranges used in the current literature.

The results revealed that the effects of propofol on γ power depended on the frequency range and on the recording site (cortex vs. thalamus). Three patterns of activity were identified across the conditions: *Inverted-U shape, Plateau-Drop Effect, and Linear-Decline effect*. Furthermore, the findings revealed the characteristic sequence of CNS excitation during sub-hypnotic concentration followed by depression during anesthesia (Winters, 1976). This sequence was evident in the global EEG activity, the Inverted-U shape pattern of γ power, and mirrored the behavioral changes (excitation followed by hypnosis) caused by propofol. Although EMG was not recorded, the author is confident that any myogenic artifacts would have been removed through the differential signal of the bipolar recording.
Differential Patterns of $\gamma$ Activity: The Three Patterns

The inverted-U shape pattern consisted of significant increases in $\gamma$ power until achieving an apex of activity at the last sub-anesthetic concentration (6µg/ml) followed by a significant decrease in power once anesthesia (and LORR) was achieved (9 & 12µg/ml).

The plateau-drop effect displayed no significant changes in $\gamma$ activity across subanesthetic concentrations followed by an abrupt and significant decline once 9µg/ml (LORR) was achieved, and a further decline during deep anesthesia (12µg/ml). The last pattern of activity was that of a linear-decline effect, which showed a consistent and steady decline in proportion to increasing propofol concentrations.

In all instances except one (cortex, 30-50 Hz), there was a significant increase in $\gamma$ power during recovery in comparison with the preceding two periods during which the animals were unconscious, as revealed by complete absence of any attempts to right (9 & 12µg/ml).

Inverted-U Shape Pattern

The inverted-U shape activity pattern was found within the lower-end of the $\gamma$ spectrum in both the cortex (30 -50 & 51-75 Hz) as well as the thalamus (30-50 Hz). Thus across both cortex and thalamus, low-range $\gamma$ power responded non-linearly to step-wise increases in propofol concentration. This pattern of activity seemed to best reflect both the biphasic changes seen in global EEG as well as the sequence of behavioral excitation preceding hypnosis. The behavioural variables assessed in this report have been previously demonstrated to reflect both agitation and disinhibition in rats (Colorado et al., 2006).

Specifically, the increases in global and peripheral zone frequency crossings as well as
jumping suggest that propofol elicited significant agitation or hyperactivity. The increases in centre-zone crossings suggest that propofol also caused disinhibition, since rats have a natural avoidance to open spaces to avoid predation (Treit and Fundytus, 1988). Lastly, the increases in rearing which reflects orienting behaviour and/or agitation, observed across sub-anesthetic concentration failed to reach significance. This is likely due to the small sample size (n=6). Thus, the increases in behavioural agitation seen across all animals matched best with the progressive and significant increases seen in the inverted-U shaped pattern of activity in both cortical and thalamic sites. The sample size was too small, for using regression analysis to quantify the relationship between behaviour and EEG. It is nevertheless reasonable to conclude that the increase in $\gamma$ power observed with the inverted-U shape pattern likely reflects behavioral excitation. The results also suggest that $\gamma$ power in the cortex in the 30-50 & 51-75 Hz ranges are not an adequate marker of hypnosis. At the 9 $\mu$g/ml concentration, where hypnosis occurs, cortical $\gamma$ power was significantly higher than during baseline within the 30-50 Hz range, while being not significantly different from 51-75 Hz. Further, at the deep hypnotic concentration of 12 $\mu$g/ml, cortical $\gamma$ power in the 30-50 Hz range was not significantly different from baseline. Thalamic power in the 30-50 Hz range did provide a better measure of hypnosis since power was significantly less than during baseline for both the 9 $\mu$g/ml and 12 $\mu$g/ml concentrations. These observations demonstrate that $\gamma$ power does not respond to propofol homogeneously across its entire spectrum.
Plateau-Drop Effect

The second pattern of $\gamma$ activity, the plateau-drop effect, was seen in the cortex within the high-$\gamma$ range (76-125 Hz & 126-200 Hz), and the thalamus in the medium $\gamma$ range (51-75 Hz). This might reflect a step-decrease of arousal within both the cortex (mid-high range $\gamma$) and thalamus (mid-range $\gamma$). Indeed, loss of consciousness appears as the main determinant of power. Furthermore, there was no relationship with any variables reflecting behavioural excitation (e.g. frequency crossings, jumping) Thus during subanesthetic concentrations, although the animals were agitated, they were indeed still awake. However once 9µg/ml was achieved, loss of consciousness occurred and a significant decline in $\gamma$ power was observed, followed by an increase in $\gamma$ power once the animal recovered from hypnosis. Thus unlike the inverted-U shape pattern of activity which may reflect changes in overall behavioural agitation, this plateau-drop effect may signal changes in general arousal, and the animals transition to a state of anesthesia.

Linear-Decline effect

The thalamus showed a third pattern, concentration-dependent linear-decline, which was exclusively within the high-$\gamma$ ranges (76-125 Hz & 126-200 Hz). This pattern was not observed in the cortex. The linear-decline pattern shared a similarity with the two previous patterns, in that there was a significant decrease in power from the 6 $\mu$g/ml to the 9µg/ml concentration, which caused loss of consciousness. The concentration-dependent feature of the linear-decline pattern suggests a pure pharmacological effect of propofol acting in and around the VPM. Specifically, propofol may yield this effect by its actions on the reticular
thalamic nuclei (RTN) GABAergic neurons, which provide a large amount of inhibition to the VPM (Ghaszanfar et al., 2001). Indeed, Ying & Goldstein (2005) have found that propofol inhibits RTN SK channels (Ca-activated K channels) which in turn increases GABA release, thus potentiating inhibition onto the VPM. Thus, increasing RTNs inhibitory drive could depress the VPMs ability to establish high-frequency oscillation, and thus account for the linear-decline effect in gamma power activity. In summary, this linear-decline pattern may reflect a pure pharmacological effect of propofol on the VPM.

Recapitulation

Taken together, these differential effects of propofol on γ activity may suggest that gamma is not a unitary brain rhythm, but a series of related but distinct entities. There are many behavioural and cognitive phenomena which influence only very high frequency γ oscillations (Tanji et al., 2005; Ray et al., 2008). This idea is further substantiated by the findings presented here showing three individual patterns of activity within conventionally defined γ oscillations. Consequently it may be a misnomer to suggest all of γ is involved in arousal. Indeed, the three patterns - which exist within arbitrarily-defined, non-overlapping frequency bands - seem to reflect related, yet distinct processes of arousal. The Inverted-U shape pattern best reflected behavioral excitation; the Plateau-Drop pattern best reflected loss of consciousness at onset of anesthesia; the Linear-Decline pattern reflected a concentration dependent pharmacological effect.
**Proposed Resolution to Literature Contradictions**

The research concerning the effects of GAs on spontaneously occurring $\gamma$ resulted in conflicting outcomes. There are many contradictions, which include effects spanning from no significant change in $\gamma$ power during hypnosis (Hudetz et al., 2011) to significant increases and decreases in gamma power (Fell et al., 2005). Three major factors which may explain why anesthetics seems to have variable effects on spontaneously occurring $\gamma$ oscillations: specific drug, methodology, and gamma interval definition.

One major obstacle in determining how anesthetics cause unconsciousness is their chemical diversity. Indeed, anesthetics share virtually no common molecular similarities, making it difficult to localize functional groups that act on specific channels and receptors (Rudolph and Antkowiak, 2004). As a result, these drugs have slightly different affinities to different protein channels, which has led to the suggestion that anesthetics do not all operate on the same mechanisms to achieve hypnosis. Thus attempting to compare the effects between drugs classes becomes very difficult. Hudetz et al (2011) for example used the common inhalant agent isoflurane, and found no change across $\gamma$ activity within the low 30-50 Hz range recorded in both the frontal cortex, V1, and the hippocampus; while the higher gamma range (70-140 Hz) showed a significant linear decline in response to increasing concentrations of isoflurane. Although our data presented here supported their findings of differential effects of gamma to increasing anesthetic concentrations, it does not reflect the same effects seen in this paper. Within the low $\gamma$ range (30-50 Hz) in the cortex for example, we observed an inverted-U shape pattern, not a linear decline. Thus the
differences seen in how GAs influence spontaneously occurring γ may be due to the fact propofol (C₁₂H₁₈O) and isoflurane (C₃H₂ClF₅O) are two chemically distinct molecules. These discrepancies may also be a result of different recording sites (S1Bf & VPM in present study vs. visual cortex, hippocampus, and frontal cortex in Hudetz et al).

The second issue concerning the discrepancies in the literature is the methodology used, and by extension, the definition of γ. In anesthesia research a compromise must be reached between subject model (human versus non-human) and the degree of invasiveness used in assessing brain activity. Table 1 illustrates the publications that have either directly or indirectly assessed the effects of propofol on spontaneously occurring γ activity. Human research often uses scalp EEG, which although provides researchers with a noninvasive technique to assess electrical activity in cortical regions, it suffers from poor spatial resolution, and risks EMG contamination. In addition, scalp EEG limits the analysis of high frequency oscillations because of spatial summation (Pfurtscheller and Cooper, 1975). As a result, these papers define γ within the low-end of the spectrum, and often include frequencies that are conventionally reserved for beta rhythms.

Murphy et al (2011) for example, using high-density scalp EEG on human subjects, found that γ power doubled during sedation and anesthesia, as compared to baseline at the frontal midline electrode (Fz). Further, source modeling revealed that this γ activity likely originated within the cingulate cortex. This paradoxical activation from anesthesia might be explained by an increase in arterial carbon dioxide resulting from a decrease in lung
ventilation that occurs during anesthesia (Ito et al., 2000). Lastly, the authors used a very conservative definition of gamma (25-40 Hz), which may correspond more with beta rhythms than with $\gamma$. Indeed, closer inspection of the power spectra suggests there may be a decrease at ~40 Hz. Thus if the spectrum of analysis was expanded to include higher frequencies of $\gamma$, it may be likely that there would be a transitioning decline which would match the $\gamma$ power changes found in this current paper.

This would also match the earlier results of Fiset et al (1999) who used scalp EEG to assess the effects of propofol anesthesia on spontaneously occurring $\gamma$ (30 -50 Hz). They showed that $\gamma$ power undergoes a significant decline following hypnosis, as compared to baseline levels. Although this paper helps showcase the need to explore a wider gamma range, Fiset et al (1999) still maintained a restrictive definition of $\gamma$ as a result of the inherent limitation of the scalp EEG technology. Thus the importance in establishing a consistent definition of $\gamma$ cannot be overstated. As seen between Fiset et al (1999) and Murphy et al (2011), activity across brain rhythms can changes across a short spectrum of frequencies, and thus restricting the definition may not reveal the full effect of propofol’s influence on spontaneously occurring $\gamma$ oscillations.

Thus the inherent limitations of scalp EEG restrict the ability to fully assess the effects of GAs on the whole $\gamma$ band spectrum (30-200 Hz). However Breshears et al (2010) using ECoG, they found that hypnotic doses of propofol significantly reduces $\gamma$ power across its entire range (37-45, 75-105, 135-165, 195-200 Hz). Further, although they illustrated how $\gamma$
power changes across the time from baseline to induction, there is no indication of drug concentration. Freeman (2000) showed similar results of propofol’s effect on $\gamma$ (25-100 Hz), but used a very small sample size. However the differences highlighted here cannot only be attributed to EEG technique and $\gamma$ definition. Fell et al. (2005) used both intracranial implants within the hippocampus and rhinal cortex in parallel with scalp EEG in the same patients. The intracranial EEG found that gamma power (32-48 Hz) had a biphasic response between 1/3 and 2/3 of the concentration needed to achieve burst suppression, where there was an apex of increased activity. This was followed by a significant decrease until burst suppression was achieved. This is very similar to the inverted-U shape pattern illustrated in this current paper. However $\gamma$ power (32-48 Hz) recorded from the central midline electrode (Cz) showed a progressed linear-decline effect from awake to burst suppression.

This suggests that not only does $\gamma$ band definition influence the effects seen from propofol anesthesia, but so too does the recording site. Similar results were found by Verdonk et al. (in prep) who found a significant decline in $\gamma$ power (62-90) when recording from electrodes in the VPL in patients with chronic pain, but found no change in gamma power from epidural recordings over the motor cortex.

Thus, the contradictions in the current literature concerning propofol’s influence on $\gamma$ power seem to be multivariate. In all however, there seems to be three general patterns of $\gamma$ activity: inverted-U shape, plateau, and linear-decline effect. Propofol’s influence on
gamma activity can be said to, in general, decrease as concentrations progress towards a state of deep anesthesia or isoelectricity. However this does not exclude the possibility of propofol eliciting a biphasic increase or even no significant changes until concentrations reach a transitory state which seem to be based on different behavioural indexes (general arousal or agitation). Whether or not propofol causes these changes in \( \gamma \) seem to be based on where in the brain these changes are being recorded and how \( \gamma \) is defined. In all, gamma power varies non-linearly to step-wise increases in propofol concentrations in the brain.

**Future Direction**

Future projects are currently being considered to assess the effects of propofol on spontaneously occurring \( \gamma \) on a more refined spatial resolution. Due to the nature of local field potentials (LFPs), the sub-threshold signal gathered from these electrodes resides on the nature of thousands to tens-of-thousands of neurons, in contrast to unit electrodes which allow for the study of single neuron behaviour. Doing unit recordings would also allow the author to assess cross-correlation and coherence between recording sites. Although these two measures were looked at during analysis, they did not yield any useful data, most likely because the S1Bf electrodes where not in line with its corresponding VPM barreloids in the other electrode. As a result, this paper does not attempt to explain how the mutual connections between the S1Bf and VPM, along with the RTN and reticular activating system interact to manifest these biphasic and differential patterns of \( \gamma \) activity.
Additional insight would be granted from assessing γ power during both burst and intraburst periods of the EEG. Indeed, Hudetz et al (2011) found that isoflurane reduces high-frequency γ power (70-140 Hz) within burst period γ power, in contrast to power found within intraburst periods of the EEG. However, it remains unclear if propofol inhibits gamma oscillations in a similar fashion.

Future experiments will also assess if EMG signals influence high-frequency EEG recorded from bipolar electrodes. Although most EEG acquisition techniques account for the possibility of EMG contamination of high-frequency signals, the jugular catheters implanted on the animals for this study excluded the possibility of a EMG electrode. However, bipolar electrodes provide a way to greatly reduce EMG contamination, by way of eliminating any signal that is common to both poles of the electrode. Thus the author is confident that any strong signals from EMG would be eliminated while processing a differential (or bipolar) EEG signal. Further, the risk of EMG contamination is only particularly relevant for baseline levels, since there is considerable attenuation in recorded EMG during hypnosis (e.g. see Murphy et al, 2011 figure 1). It still remains important however to discount the possibility of EMG accounting for the increases in gamma power during behavioural agitation, where the risk of EMG contamination would be high.

Lastly, the author is currently investigating the effects of propofol on spontaneously occurring brain rhythms below the γ spectrum. Although this thesis focused on γ rhythms due to its relation with arousal and consciousness, other brain rhythms have also been shown to be intimately tied to γ’s presence. Indeed, gamma amplitude is often coupled with
delta and theta oscillations (Buzsáki and Draguhn, 2004; Canolty et al., 2006), and its coupling is often related to task-demands (Schroeder and Lakatos, 2009). Further, beta, delta, and theta brain rhythms have been shown to change as a function of depth of anesthesia (Schwender et al., 1996; Mahon et al., 2008; Sheeba et al., 2008). Using the strict control afforded to animal model use, it is the current goal to establish a holistic profile of EEG changes across step-wise increases of propofol concentrations.
Conclusion

The results shown here are the first detailing propofol’s effects on spontaneously occurring \( \gamma \) activity (30-200 Hz) within the rat, as well as the concentration-effect relationship between propofol and \( \gamma \) activity across step-wise increases in target site concentration across gamma’s entire frequency spectrum. The author concludes that propofol elicits frequency-specific biphasic effects on \( \gamma \) activity in response to step-wise increases in anesthetic concentration. These frequency-specific effects across gamma’s frequency spectrum were categorized into three states as a function of drug concentration: Inverted-U, plateau, and linear-decline. These results suggest the current contradictions in literature concerning gammas role in anesthesia may be explained by the brain region investigated, as well as the frequency range used to define \( \gamma \). Further research is needed to properly understand the function significance of these three patterns, and which, if any of these frequency intervals within \( \gamma \), most closely relate to the biphasic changes seen in behaviour and arousal as a result of propofol anesthesia.
References


Figure 1. General Activity of animals (n=6) following step-wise increases in propofol concentration (0, 3, & 6 µg/ml). Following baseline, 3 µg/ml elicited a small but non-significant decrease in peripheral (A), centre (B), and overall zone crossings (C). This was followed by a significant increase compared to 3µg/ml once 6µg/ml was achieved (p<0.05). Centre Duration (D) did not significantly change across any propofol concentration.
Figure 2. Orienting and jumping behaviour of animals (n=6) following step-wise increases in propofol concentration (0, 3, & 6 µg/ml). Both rearing frequency (A) and duration (B) increased at the 6µg/ml concentration but the difference was not significant (p>0.05). (C) During baseline and 3µg/ml, no jumping occurred across any subject, whereas the 6µg/ml condition elicited a significant increase (p<0.05).
Figure 3. Raw EEG traces for all conditions (propofol concentrations: 0 - 12μg/ml) from a representative animal (same animal as Figure 8). Each condition illustrates a 5 sec epoch (top two traces) for cortex and thalamus recordings and a 1sec epoch (bottom two traces). All traces sampled at 3.0 kHz after 0.1 - 475 Hz analog bandpass filtering. Biphasic changes in EEG waveforms during sub-anesthestic concentrations (3-6μg/ml), compared to baseline levels. This increased in EEG activity is contrasted with an attenuation of high-frequency activity and an increased in amplitude during hypnotic concentrations (9-12μg/ml). This is followed by a partial rebound in EEG activity during recovery.
Figure 4. Idealized schematic representation of the three patterns of propofol-mediated changes of gamma activity across cortical and thalamic sites. (A) Inverted U-shape curve illustrates peak activity at 6µg/ml followed by a downward shift towards deep anesthesia. (B) Plateau-effect shows no change across subanesthetistic concentrations followed by an abrupt descent within hypnotic ranges. (C) Linear-decline effect across all propofol concentrations. Behavioral agitation peaked at the 6 µg/ml concentration. Loss of righting reflex (LORR) was observed at 9 µg/ml and above (shaded area).

Figure 5. Averaged power over selected frequency ranges of cortical (A-B) and thalamic (C) recordings illustrating an inverted-U shaped pattern of activity. Note the increase in gamma power across subanesthetistic concentrations, followed by an abrupt decline once LORR is achieved (9µg/ml). Lines above bars illustrate significant (p<0.05) differences (permutation test) between successive periods. For clarity, only significant differences for sequential comparisons are shown. See appendix I for full list of pair-wise comparisons. Quadratic trend analysis for A, B, and C yielded a p-value of .04, .03, and .05 respectively. The trend analysis included all periods except recovery.
Figure 6. Averaged power over selected frequency ranges of cortical (A-B) and thalamic (C) recordings illustrating a plateau pattern of activity. There is no significant changes across subanesthestic concentrations until LORR is achieved (9μg/ml). Lines above bars illustrate significant (p<0.05) permutation comparison only for successive conditions. See appendix I for full list of comparisons. Quadratic and linear trend analysis for A, B, and C yielded p >0.05. The trend analysis included all periods except recovery.

Figure 7. Averaged power over selected frequency ranges of thalamic recordings illustrating a linear-decline effect across conditions. These power spectra illustrate a steady linear-decline to step-wise increases of propofol concentrations. Lines above bars illustrate significant (p<0.05) permutation comparison only for successive conditions. See appendix I for full list of comparisons. Linear trend analysis for A, and B yielded a p-value of 0.01 and 0.02, respectively. The trend analysis included all periods except recovery.
Figure 8. Averaged power spectra across all animals of barrel field cortex (A) and VPM (B) recordings for all conditions. Power across all frequencies are displayed, with selectively magnified representations for 0-50 Hz (A1,B1) and 51-200 Hz (A2,B2). Note γs biphasic response to increasing propofol concentrations from subhypnotic levels (3-6μg/ml) vs. hypotonic (9-12μg) levels, relative to baseline (blue) in A1-B1. Further, there are differential effects between the cortex A2 (clustering) and thalamus B2 (step-wise declines) across conditions.
Figure 9. Power spectra from a representative animal of barrel field cortex (A) and VPM (B) recordings for all conditions. Power across all frequencies are displayed, with selectively magnified representations for 0-50 Hz (A1,B1) and 51-200 Hz (A2,B2). Note the similar changes in γ power compared to Figure 7.
Table. All investigations on the effects of general anesthetics on spontaneously occurring gamma activity, sorted by: acquisition technology, gamma definition, as well as the resulting contradictions of the agent's effect on gamma.

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Agent</th>
<th>Gamma definition</th>
<th>Acquisition, Technique</th>
<th>Species used</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feld, et al.</td>
<td>2005</td>
<td>Propofol</td>
<td>Decrease in Gamma Power during hypnosis</td>
<td>ECG / Scalp EEG</td>
<td>Human</td>
<td>2.4±18</td>
</tr>
<tr>
<td>Peres, et al.</td>
<td>1999</td>
<td>Propofol</td>
<td>Gamma decrease with protocol concentration</td>
<td>Scalp EEG / ERP</td>
<td>Human</td>
<td>3.0±0.7</td>
</tr>
<tr>
<td>Vercoucke, et al.</td>
<td>2011</td>
<td>Propofol</td>
<td>Increase in Gamma, increase in Thalamus (6-90Hz)</td>
<td>Scalp EEG</td>
<td>Human, Brain</td>
<td>25-40</td>
</tr>
<tr>
<td>Muyties, et al.</td>
<td>2011</td>
<td>Propofol</td>
<td>Increase in Gamma with protocol concentration</td>
<td>Scalp EEG / Interictal Impuls</td>
<td>Human</td>
<td>[30-50]/70-140</td>
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<tr>
<td>Hackett, et al.</td>
<td>2011</td>
<td>Propofol</td>
<td>Increase in Gamma (70-140Hz)</td>
<td>Scalp EEG / Interictal Impuls</td>
<td>Human</td>
<td>[30-50]/70-140</td>
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<tr>
<td>Rees, et al.</td>
<td>2000</td>
<td>Propofol</td>
<td>Decrease in Gamma Power during hypnosis</td>
<td>ECG / Scalp EEG</td>
<td>Human</td>
<td>2.5-100</td>
</tr>
<tr>
<td>Brettmann, et al.</td>
<td>2010</td>
<td>Propofol</td>
<td>Gamma decrease with protocol concentration</td>
<td>Scalp EEG / ERP</td>
<td>Human</td>
<td>3.7±0.125-1.150</td>
</tr>
</tbody>
</table>
Appendix I

Complete permutation tests for SI Barrel Cortex (Cortex) and VPM (Thalamus) across all gamma segments. Significant comparisons are denoted by their p-value, with the sign (+/-) designating significant changes of row conditions relative to column conditions. Significant baseline column conditions (e.g., power between 30-50Hz within the Cortex at the 6μg/ml condition was significantly higher than baseline).
Complete permutation tests for S1 Barrel Cortex (Cortex) and VPM (Thalamus) across all gamma segments. Significant comparisons are denoted by their p-value, with the sign (+/-) designating significant changes of row conditions relative to column conditions (e.g., Power between 30-50Hz within the Cortex at the 6μg/ml condition was significantly higher than baseline).
Histology illustration of the electrode placements (n=4). All verified placements were successfully implanted into the S1Bf (left) and VPM (right). Furthest depth is denoted by a marker (●).
Histology illustration of the electrode placements (n=4). All verified placements were successfully implanted into the S1Bf (left) and VPM (right). Furthest depth is denoted by a marker (●).