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THE INFLUENCE OF ANESTHESIA IN THE CENTRAL NERVOUS SYSTEM STUDY

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ABSTRACT

Central nervous system is a complex machine; it is constituted by more or less 172 billions of cells divided between neurons and glia. This incredible number of cells, particularly neurons, are constantly connected, working 24 hours a day, never stopping. Their activity is maintained even during particular conditions such as sleep, pathological unconsciousness (coma) or general anesthesia.

For all these peculiarities, since ancient times, man has devoted much effort to the study of the most fascinating organ of living beings (mammals in particular).

Nowadays, thanks to the advance in medical technology, many tools are available to "look inside the brain".

Magnetic resonance imaging and, particularly, functional magnetic resonance imaging (fMRI) is a modern biomedical imaging method, which allows a non-invasive assessment of brain function.

The detection of brain activity is based on the coupling between neuronal activity, energy consumption, and blood flow.

Functional connectivity (FC) of brain regions is modulated in various central nervous system diseases, during sleeping and general anesthesia.

Anesthesia during MRI procedures is commonly used in preclinical setting and, sometimes, is required also in clinical setting (uncollaborative patients, children, drugs induced-coma etc).

The study of the relationship between anesthetics and FC presents a double value: allows to distinguish the alterations induced by anesthesia on FC, avoiding possible confounding elements, and permits an in depth investigation of drugs behaviour.

For all these reasons, the main topic of this PhD dissertation is the relationship between anesthesia and central nervous system. We started from theoretical studies in healthy subjects and we arrived to clinical setting, describing the possible application of anesthetics drugs as a treatment of neurologic diseases.

The first study included aims to describe the way in which dexmedetomidine and isoflurane modulate FC in guinea pigs. We analysed the characteristic of cortical, subcortical and cortico-subcortical connectivity under both drugs with resting state fMRI.

The second study presented partial results of a more complex work concerning FC in rats under 4 different anesthetics protocols. Because in FC studies blood flow represents a crucial element we dedicated a part of the work to the study of haemodynamic alterations through the administration

of contrast medium. Dynamic Suceptibility Contrast MRI analysis allows the study of cerebral blood flow and cerebral blood volume of different brain anatomic regions under dexmedetomidine, isoflurane, midazolam-dexmedetomidine and midazolam-isoflurane.

Finally, we moved to clinical setting to describe the successful treatment of 3 dogs suffering from idiopathic epilepsy presented in emergency department in a state of super refractory status epilepticus. They were treated successfully with a continuous infusion of dexmedetomidine and ketamine.

In conclusion, taken together this thesis gives a little contribute to better understand anesthetics behavior at brain level. We believe that it is important to make a "rational choice" when we decide the anesthetic protocol for neuroimaging procedures (both in clinical and preclinical setting) and this "rational choice" could be make only if we have a widespread literature that describe the highest number of anesthetic protocol and their interaction on central nervous system. Finally, thanks to the continuous improvement of our understanding of anesthetics mechanism of action, especially from a molecular and functional point of view, is it possible to use different anesthetics as a therapy for different neurologic conditions, particularly the one based on neurotransmitters imbalance.

INTRODUCTION

The living organism is constantly exposed to external and internal stimuli (Seiferle 1975). Transduction, transmission, transformation (modulation), translation (perception), and the response to stimulation are ongoing processes that are essential for the adaptation of the organism to changing conditions and daily survival (Seiferle 1975). All of these processes require the specialized nervous system, which, along with the endocrine system, mediates the adjustments and reactions of the organism to its internal and external environments (Dorland 1982).

General anesthesia has been defined as a drug-induced, reversible state characterized by amnesia, unconsciousness, analgesia, immobility, and muscle relaxation (Antognini and Berg 1995; Zecharia and Franks 2009;). In order to achieve these goals, anesthetic agents must alter function in numerous parts of the nervous system. So, brain could be considered the most important organ for all anesthetic drugs.

It has been postulated that general anesthetics act at multiple sites within the central nervous system (CNS) by decreasing the transmission of information ascending from the spinal cord to the brain (Angel 1993). The major end- points of general anesthesia such as amnesia and unconsciousness are most likely the result of anesthetic impeded neurotransmission at supraspinal sites, including the brainstem (Eger et al.1971), thalamus (Franks 2008), and cerebral cortex (Antognini and Berg 1995; Fiset et al. 1999; Purdon et al. 2009). Sedative and anesthetic agents modulate brain activity altering CNS hemodynamic and metabolism in a different way. In order to provide effective assistance to diagnosis, prognosis, and assess potential treatments, it is crucial to understand the CNS physiological alterations induced by different anaesthetic agents both in healthy and pathological subjects.

Advanced neuroimaging techniques have significantly expanded our knowledge of neural correlates of consciousness level and different pathological conditions in human and animal brain.

Advances in functional magnetic resonance imaging (fMRI) methods have enabled non-invasive investigations of functional brain networks (Biswal et al. 1995). Task-free resting-state fMRI (rs-fMRI) studies have shown that functional connectivity (FC) is modulated in various CNS diseases, such as epilepsy, Alzheimer's disease, bipolar disorder, depression, autism, multiple sclerosis, and

schizophrenia (Fox and Raichle 2007; Lu and Stein 2014; Smucny et al. 2014), and in different arousal states, such as during sleep and anesthesia (Nallasamy and Tsao 2011).

Therefore, it is crucial to characterize the effects of different anesthetics at brain level in order to promote a better interpretation of diagnostic procedure, avoiding potential confounds, to identify which drugs suite better to different neurological condition and to recognise any therapeutic property.

THE CENTRAL NERVOUS SYSTEM: A COMPLEX MACHINE

NEURAL COMMUNICATION

Neural tissue is composed of two categories of cells: neurons and glia (Bear et al. 2001). The numbers of these cells (~85 billions of each) has been suggested to be roughly equal in the adult human brain (Azevedo et al. 2009). Neurons are responsible for detecting changes in the body and its environment, reacting to these changes by communicating with other neurons, and transmitting responses to the detected changes. Glial cells are mainly supporting cells providing insulation and energy substrates for neurons (Bear et al. 2001), but, according to emerging evidence, they also participate in neuronal signalling (Kettenmann and Verkhratsky 2008).

Soma, axon, and dendrite(s) are the typical parts of a neuron (Bear et al. 2001). The conduction of information in neurons, which typically occurs in axons, is based on electrical excitability. At rest, a voltage gradient is maintained across the cell membrane. If a stimulus occurs, the ions on the both sides of the membrane become redistributed, leading to a decrease in the voltage gradient. If the decrease, or depolarization, exceeds a predefined voltage threshold, an action potential will be triggered. After full depolarization, the original electric potential is gradually restored in a process called repolarization. Once initiated, the action potential passes down the nerve's cell membrane until it reaches the end of the axon. As the function of the CNS is based on the complex interplay between neurons, it is essential to transfer the information further in the large-scale network. The signalling between neurons is called synaptic transmission (Bear et al. 2001). The synapse is a specialized junction, which typically occurs between the pre-synaptic axonal terminal and the post-synaptic dendrite (Pakkenberg et al. 2003).

Synapses of animals' brain can be divided in electrical and chemical (Bear et al. 2001). The majority of the synapses in adult mammalian brain involve the release of chemicals. In the chemical synapse, the electric information arriving at the presynaptic site is converted into a chemical form and this is delivered to the postsynaptic site across a synaptic cleft, across an intercellular gap. The presynaptic cellular site contains synaptic vesicles, which store the chemical compounds, the neurotransmitters, required for the signal transmission across the synapsis. The release of neurotransmitters into synaptic cleft is triggered by the incoming action potential. Subsequently, the released

neurotransmitters bind to specific receptor proteins located in the postsynaptic membrane, inducing receptor-specific intracellular changes in postsynaptic activity. There are more than 100 chemical substances involved in synaptic transmission (Bear et al. 2001). These substances can be divided in amino acids (e.g., gamma-amino butyric acid (GABA), glycine, and glutamate) and amines (e.g., acetylcholine, dopamine, norepinephrine (NE), and serotonin. The nature of neurotransmitter targeting directly the electrical excitability of the postsynaptic neuron can be categorized as either excitatory or inhibitory (Bear et al. 2001). For instance, glutamate mediates the majority of the excitatory neurotransmission in the brain, while GABA is involved in most of the inhibitory actions. Another important excitatory neurotransmitter is represented by the noradrenergic system. Finally, talking about the most important neurotransmitters of the CNS is mandatory to remember also the cholinergic, the dopaminergic and the serotoninergic system. The study of neurotransmission is particularly important for health sciences since several CNS diseases are associated with imbalances in neurotransmitter levels.

NEURAL FUNCTIONAL CONNECTIVITY- THE RESTING STATE

In this paragraph we are going to introduce FC giving an overview on it from a physiological point of view. A future paragraph will be dedicated to rs-fMRI from technical standpoint.

Brain is a complex network of functionally and structurally interconnected regions. Functional communication between brain regions is likely to play a key role in complex cognitive processes, thriving on the continuous integration of information across different regions of the brain. This makes the examination of FC of high importance, providing new important insights in the core organization of brain. Functional connectivity is defined as the temporal dependency between spatially remote neurophysiological events (Aertsen et al. 1989; Friston et al. 1993). Areas of the brain which exhibit signal fluctuations correlated in time are assumed to be functionally connected. In the contest of functional neuroimaging, FC is suggested to describe the relationship between the neuronal activation patterns of anatomically separated brain regions, reflecting the level of functional communication between regions.

Interestingly, around 15 years after the invention of fMRI, studies started to examine the possibility of measuring FC between brain regions as the level of co-activation of spontaneous functional MRI

time-series, recorded during rest (Biswal et al. 1997; Greicius et al. 2003). During these resting-state experiments, volunteers (human) were instructed to relax and not to think of something in particular, while their level of spontaneous brain activity was measured throughout the period of the experiment. Biswal and colleagues were the first to demonstrate that during rest the left and right hemispheric regions of the primary motor network are not silent, but show a high correlation between their fMRI BOLD (Blood Oxygenation Level Dependent) time-series (Biswal et al. 1995; Biswal et al. 1997), suggesting ongoing information processing and ongoing FC between these regions during rest (Biswal et al. 1997; Greicius et al. 2003). In their study the resting-state time-series of a voxel in the motor network was correlated with the resting-state time-series of all other brain voxels, revealing a high correlation between the spontaneous neuronal activation patterns of these regions. Several studies have replicated these pioneering results, showing a high level of FC between different brain areas (Biswal et al. 1997; Greicius et al., 2003; De Luca et al. 2005; Damoiseaux et al. 2006; Fox and Raichle 2007; Van den Heuvel et al. 2008).

Besides the eponymous “resting” condition, organized spontaneous activity in the fMRI signal has also been demonstrated under various states of consciousness including sleep (Tagliazucchi and Laufs 2014; Mitra et al. 2015), anesthesia (Kiviniemi et al. 2000), coma, and minimally conscious state (Heine et al. 2012). A major compelling aspect of the fMRI signal has been the organized spontaneous brain activity across different species including mice (Jonckers et al. 2011), rats (Hutchison et al. 2010), rabbits (Schroeder et al. 2016), dogs (Berns et al. 2012), with monkeys (Mantini et al. 2011), mice, and rats representing the largest fraction of “resting-state” rs-fMRI investigations if humans are excluded.

CEREBRAL BLOOD FLOW

The human brain (as the other mammalian brain), despite its relatively small proportion of body size, has high metabolic activity, requiring 20% of total basal oxygen consumption. Constant cerebral blood flow (CBF) is required to satisfy the brain’s oxygen needs. The brain receives 15% of resting cardiac output in adults. The average cerebral blood flow is 50 ml/100 g/min, however, there is a great variation between white and grey matter of the brain. A decrease in CBF to 20-25 ml/100 g/min exposes the brain tissue to ischemia. Cerebral blood flow of less than 10 ml/100 g/min will

result in infarction within a few minutes (Scheinberg and Stead 1949; Lassen 1959). Cerebral blood flow is partly regulated by the brain's complex intrinsic mechanism called flow metabolic coupling, which optimally matches oxygen delivery and consumption. An increase in local metabolic activity will result in higher CBF in that area (Kety and Schmidt 1945; Scheinberg and Stead 1949).

In normal circumstances, CBF autoregulation describes the ability of the brain to maintain a stable CBF despite fluctuations in cerebral perfusion pressure (CPP). Cerebral perfusion pressure is calculated as mean arterial pressure (MAP) minus intracranial pressure (ICP), or central venous pressure if higher. A change in MAP, and consequently in CPP, results in changes in cerebrovascular resistance (vasodilatation or constriction in cerebral arteries). It is believed that autoregulation works when systemic mean arterial blood pressure varies between 50 and 150 mmHg. More recent findings suggest, however, that the lower threshold might actually be higher. Outside these thresholds, CBF is directly related to CPP (Sokoloff et al. 1957; Lassen et al.1969).

Autoregulation is further divided into dynamic and static autoregulation. Dynamic autoregulation acts as a rapid response to pressure pulsations in systemic blood pressure, whereas static autoregulation reflects long-term changes in MAP (Cohen et al 1967). Traumatic brain injury (TBI), subarachnoid haemorrhage, brain tumours, and various other neurosurgical conditions may alter the regulatory mechanics of CBF.

$PaCO_2$ is a strong regulator of CBF and a linear-like correlation exists between CBF and $PaCO_2$. Hyperventilation results in lower $PaCO_2$ and vasoconstriction in cerebral arteries, thus reducing blood flow, whereas higher $PaCO_2$ increases CBF by vasodilation of the cerebral arteries. Low $PaCO_2$ value caused by hyperventilation may result in brain tissue hypoxemia due to intense vasoconstriction (Lassen 1976).

CEREBRAL METABOLISM

Brain function is intimately related to both cerebral perfusion and metabolism. The characteristic features of cerebral metabolism include (1) high cellular energy demands utilizing adenosine triphosphate (ATP) energy obtained from aerobic glucose oxidation, (2) no oxygen and minimal glucose and glycogen substrate reserves relative to consumption rates, and (3) low concentrations

of high-energy phosphate compounds. All these characteristics render the brain highly dependent upon adequate blood flow for minute-to-minute delivery of oxygen and glucose (Lassen 1959; Siesjö 1978).

The mean global cerebral metabolic rate of oxygen ($CMRO_2$) of the normal awake human brain is about 3.0–5.5mL/100g/min (Kety and Schmidt 1945; Scheinberg and Stead 1949). Approximately 60% of the available oxygen is expended in subserving the external work of the brain as represented in the electroencephalogram (EEG). In the absence of external work (i.e., an isoelectric EEG), the healthy normothermic brain will continue to consume about 40% of the normal energy and oxygen. This basal metabolism is necessary for maintaining neuronal and glial cell integrity, including the energy requirements for maintaining ionic gradients, biosynthesis, and axonal transport (Michenfelder 1988). The basal metabolic state can be reversibly produced by higher doses of anesthetic agents (Michenfelder 1974) and hypothermia (Steen et al. 1983).

ANESTHESIA

Every year, several millions of patients are exposed to general anaesthetics, drugs that remove the most precious human but also animal attribute — consciousness. The ability of the anaesthetist to induce safe and reversible loss of consciousness in patients has proved to be of inestimable value; however, it has also posed one of the most long-standing and baffling pharmacological puzzles (Franks 2008). Anesthesia in human medicine is not only required for surgery but also for a wide range of diagnostic procedures. In addition to their wide use in human subjects, anesthetics have been exploited in veterinary medicine and preclinical animal experiments for similar reasons (Lukasik and Gillies 2003). Additionally, the prevention of motion of animals is essential in several imaging modalities, such as in MRI, as motion severely deteriorates the image quality (Masamoto and Kanno 2012). Awake and restrained animals are likely to experience significant stress during such measurements in the noisy environment of the device (Lahti et al 1998) and thus stress and the effect of stress on results can be minimized by using anesthesia (Lukasik and Gillies 2003). Furthermore, anesthesia represents a therapy in different neurological conditions such as status epilepticus, sleep deprivation syndrome or Parkinson disease.

Nowadays, one of the most important instrument to study the brain and its activity is magnetic resonance imaging (MRI), particular fMRI.

The majority of the preclinical MRI studies and a considerable number of human MRI (children, uncooperative patients, claustrophobic patients etc..) are conducted under general anesthesia, mainly to prevent the motion and stress of subjects during scanning (Murphy and Brunberg 1997; Lukasik and Gillies 2003).

The use of anesthesia in fMRI experiments, however, induces a fundamental level conflict; the ultimate intention is to investigate and localize brain activity, but brain activity has been modulated by the anesthesia, leading to a non-responding behavioural state. General anesthetics are known to suppress both spontaneous and evoked electrical brain activity (Steward et al. 2005). Anesthetics can cause interference with fMRI measurements at different levels. Anesthetics can modulate the 1) baseline neural processing, including spontaneous activity, metabolism, and blood flow, 2) neural responses to various stimuli, 3) neurovascular coupling mechanisms, and 4) vascular reactivity (Masamoto and Kanno 2012). There are some obvious differences between conscious and

anesthetized subjects but variability in fMRI results, however, is not limited to these two main conditions; neural activity, physiological state, and subsequent hemodynamic responses vary greatly even among different anesthesia protocols (Haensel et al. 2015).

In this work we will focus the attention to the relationship between anesthetics and CNS. In the next paragraphs we present a literature overview regarding the effect of dexmedetomidine, midazolam, isoflurane and ketamine on CNS on both human and animal studies.

ANESTHESTICS: BRAIN IS THE TARGET

The mechanism of anesthetics agents is continuously debated and many hypotheses are postulated. Nowadays, it is clear that all anesthetic drugs act selectively at the molecular level; numerous ion channels, enzymes, and receptor systems have been systematically investigated, only a few of them appear to be directly involved in the mechanisms of anesthesia (Franks 2008).

With the knowledge that anaesthesia likely results from CNS depression, it can be hypothesized that anaesthesia results from either enhanced inhibitory transmission or reduced excitatory transmission. Two main targets have been extensively described; $GABA_A$ receptors and N-methyl-D-aspartate (NMDA) glutamate receptors. When GABA binds to $GABA_A$ receptors an influx of Cl ions produces a hyperpolarization. With few exceptions, the majority of anaesthetic agents potentiate GABA-mediated conductance. On binding of the main excitatory transmitter glutamate, NMDA receptors gate an influx of Ca2þ and Naþ. Ketamine, xenon and nitrous oxide inhibit this ion movement to depress excitatory transmission.

First, many years ago it was postulated that inhibitory GABA receptors, particularly the subtype A ($GABA_A$), could be considered as potential binding sites for anesthetics, and subsequently mediate the anesthetic effects (Nicoll 1978). GABA is the major inhibitory neurotransmitter in the mammalian brain. Indeed, there is substantial evidence supporting the fact that the GABAergic system is involved in anesthetic mechanisms, as almost all general anesthetics potentiate GABAergic neurotransmission, and directly bind and activate GABA receptors at higher concentrations (Franks 2008). On GABA binding to $GABA_A$ receptors an influx of Cl ions results to produce a hyperpolarization. With the exception of ketamine, $alpha_2$-agonist,s xenon and nitrous oxide and

other few molecules, all anaesthetic agents potentiate GABA-mediated conductance (Lambert 2011).

Glutamatergic system is the second molecular site of anesthetics (Franks 2008) with the NMDA. It represents the major excitatory neurotransmitter in the mammalian CNS. Several inhalation anesthetics induce inhibitory effects on NMDA receptors (Franks 2008), and NMDA antagonists, such as ketamine, can induce loss of consciousness (Carter 2013).

Another molecular target for anesthetic drugs is the is a group of two-pore-domain K+ ion channels (2PK) (Nicoll and Madison 1982). It has been found that 2PKs are modulated by the inhalation anesthetics (Patel et al. 1999) and the genetic modification of 2PK channels can furthermore modulate the anesthetic effects of these agents (Heurteaux et al. 2004). The exact role of 2PKs in brain function is not fully understood, but they are thought to modulate neuronal excitability (Franks 2008). Therefore, any change in 2PK function could hypothetically hyperpolarize the cell membrane, and subsequently disturb the propagation of neuronal signal. 2PKs, however, are not a common target for all anesthetics as several intravenous anesthetics have no effect on 2PK functions.

Finally, anesthetics drugs play their action also at level of noradrenergic, cholinergic, dopaminergic and serotoninergic system.

The adrenergic system, a major neuromodulator system that regulates arousal, attention, mood, learning, memory, and stress response, has been a drug target for diseases such as attention-deficit/hyperactivity disorder (Cinnamon Bidwell et al. 2010). Cholinergic signalling arises from several nuclei, particularly the striatum and nucleus accumbens in the basal forebrain, can modulate neural excitability, synaptic plasticity and coordinated activity, and is involved in attention, learning and memory (Picciotto et al., 2012). Dopamine is involved in reward, aversion, cognitive control and motor function. The nigrostriatal dopamine pathway that projects from the substantia nigra is crucial for motor function, and the mesolimbic and mesocortical dopamine pathways which arise from the ventral tegmental area are important for motivational function (Wise, 2004). Serotonin, which is produced in the raphe nuclei and distributed across the brain, regulates mood, appetite and sleep and has been widely used as a drug target for the treatment of depression (Berger et al. 2009).

DEXMEDETOMIDINE

OVERVIEW AND MOLECULAR PATHWAY

Dexmedetomidine (DEX) is a potent and highly selective alpha$_2$-adrenoceptor agonist with sympatholytic, sedative, amnestic, and analgesic properties (Venn et al. 1999: Carollo et al. 2008), which has been described as a useful and safe adjunct in many clinical applications. It is the most recently developed and commercialized agent in this pharmacological class. It provides a unique "conscious sedation" (patients appear to be asleep, but are readily roused), analgesia, without respiratory depression. It decreases CNS sympathetic outflow in a dose- dependent manner and has analgesic effects best described as opioid-sparing. There is increasing evidence of its organ protective effects against ischemic and hypoxic injury including cardioprotection, neuroprotection and renoprotection (Panzer et al. 2009).

A variety of alpha$_2$-adrenergic receptor subtypes have been identified using molecular techniques. Currently, four subtypes are commonly described: α_{2A} receptors are located in the cerebral cortex and brainstem and are the primary source of sedation and supraspinal analgesia in addition to centrally mediated bradycardia; α_{2B} receptors are located in the spinal cord and vascular endothelium with stimulation resulting in spinal analgesia, vasoconstriction, and peripherally mediated bradycardia; α_{2c} receptors are also located in the spinal cord, modulating spinal analgesia and possibly thermoregulation; and α_{2D} receptors have been cloned and are thought to be similar to α_{2A} in function and distribution (Katzung 2004).

The hypnotic effect of DEX is mediated by the hyperpolarization of noradrenergic neurons in the locus ceruleus of the brain stem (a small bilateral nucleus that contains many adrenergic receptors), which is the primary site in modulating wakefulness. When the alpha$_2$ adrenergic receptor is activated, it inhibits adenylyl cyclase. This latter enzyme catalyzes the formation of cyclic AMP (cAMP), a crucial second messenger molecule that acts in many catabolic cell processes. By reducing the amount of cAMP in the cell, DEX favours anabolic over catabolic pathways. Simultaneously, there is an efflux of potassium through calcium-activated potassium channels and an inhibition of calcium entry into calcium channels in nerve terminals (Khan et al. 1999). The change in membrane ion conductance leads to a hyperpolarization of the membrane, which suppresses neuronal firing in the locus ceruleus as well as activity in the ascending noradrenergic pathway (Kamibayashi & Maze

2000). The locus ceruleus is also the site of origin for the descending medullospinal adrenergic pathway, which is known to be a key mechanism in regulating nociceptive neurotransmission.

When a hypnotic dose of DEX was administered to laboratory animals, NE release from the locus ceruleus was inhibited. The absence of inhibitory control over the ventrolateral preoptic nucleus (VLPO) resulted in the release of GABA and galanin, which further inhibited the locus ceruleus and tuberomamillary nucleus (TMN). This inhibitory response also causes a decrease in the release of histamine, which results in a hypnotic response. This response is similar to that found in normal sleep in that the reduction of NE release by the locus ceruleus triggers the release of GABA and galanin by the VLPO. These neurotransmitters further inhibit norepinephrine release by the locus ceruleus and suppress histamine secretion by the TMN. The reduced occupancy of the histamine receptors on the cells of the subcortical areas induces a hypnotic state (Nelson et al. 2001).

FUNCTIONAL CONNECTIVITY

Despite exists evidence that DEX is more suitable than other anesthetics for fMRI studies, based on its effect on functional connectivity and longitudinal studies, its use is still relatively rare (less then 2%) (Haensel et al. 2015).

Akeju and colleagues in 2014 studied the effects of DEX on CNS, particular on metabolism, CBF and connectivity in human subjects. The choice of DEX depends from the fact that this drugs seems to be appealing choice for testing a specific mechanism of how an anesthetic can alter the level of consciousness. Dexmedetomidine selectively targets presynaptic alpha$_2$-adrenergic receptors on neurons projecting from the locus ceruleus to the preoptic area (Correa-Sales et al. 1992; Chiu et al. 1995; Mizobe et al. 1996). This leads to activation of inhibitory outputs to the major arousal centres in the midbrain, pons, and hypothalamus producing a neurophysiological and behavioral state that closely resembles NREM II sleep (Nelson et al. 2003; Huupponen et al. 2008; Akeju et al. 2014). Dexmedetomidine also acts at the locus ceruleus projections to the intralaminar nucleus of the thalamus, the basal forebrain and the cortex (Espana and Berridge, 2006).

In their study they observed first that during both wakefulness and unconsciousness induced by DEX, the default mode network (DMN) and bilateral frontoparietal networks (FPN) were consistently identified. Furthermore, they found no difference in FC between the cortical regions of

the DMN and FPNs, suggesting that cortico–cortico functional connectivity may be maintained during unconsciousness. However, they observed a decrease in FC between the thalamus and the DMN in the unconscious state. This loss of thalamic functional connectivity was observed in a region consistent with intralaminar, midline, mediodorsal, and ventral anterior nuclei.

They also found that FC of the left cerebellar representation of the DMN (Buckner et al. 2011) was significantly reduced during unconsciousness. When they compared the FPNs in these two states, they found that the left and right FPNs showed opposite changes in FC with a cerebellar cluster, likely indicating a switch in hemispheric dominance for cortico-cerebellar functional connectivity during unconsciousness.

In contrast, a study conducted in 2018 by Paasonen and colleagues in rat showed that FC under medetomidine was globally suppressed when compared with the awake condition. They reported that if the nucleus accumbens and hypothalamus were not considered the 89% of the remaining connection were suppressed including the 92% of the thalamo-cortical connections as like FC in cortex and striatum. Also the prefrontal parts of the DMN is suppressed by medetomidine.

CEREBRAL BLOOD FLOW AND CEREBRAL METABOLISM

Generally, alpha$_2$-adrenoreceptors agonist decrease baseline CBF and constrict both pial arteries and vein. In fact, there are evidences supporting a dose-dependent decline in both global and regional CBF after DEX administration, which cannot be merely caused by its impact on systemic hemodynamic effects. Dexmedetomidine seems to have a direct effect on vasoconstriction of cerebral vasculature (Wang et al. 2013). Serial transcranial doppler exams in healthy human volunteers confirm previous findings in animal models showing linear relationship between middle cerebral artery flow velocity and DEX infusion (Tsaousi & Bilotta, 2016). Prielipp and colleagues in 2002 studied the effect of DEX on CBF in healthy human subjects using a positron emission tomography and they reported that both regional and global CBF are decreased after administration of DEX. They showed that CBF was decreased in 13 of 14 ROI (region of interest) as reported in figure 1. They reported a global reduction in CBF of approximately 33%. Zornow et al. (1990) observed dose-related reductions in CBF velocity of up to 28% in human volunteers during DEX administration.

Despite evidence from the past, nowadays it is clear that DEX causes a substantial decrease in cerebral metabolic rate. Furthermore, exists also a significant reduction in the slope of the PaCO2–CBF velocity relation during administration of DEX (Drummond et al.2008).

Drummond and colleagues (2008) in a study conducted on healthy human subject reported that DEX administration resulted in a dose-related decrease in cerebral blood flow velocity (CBFV). Cerebral blood flow velocity decreased by approximately 18% and 32% (both statistically significant) from the baseline value when the plasma DEX concentration was increased to 0.6 ng/ml and 1.2 ng/ml, respectively. A similar dose-related reduction in cerebral metabolic rate (CMR) occurred simultaneously. Cerebral metabolic rate decreased by 26% and 41% (*vs.* presedation) at the 0.6 ng/ml and 1.2 ng/ml serum levels, respectively. The combined effect of these changes in CBFV and CMR was that there was no statistically significant change in the CBFV/CMR ratio in association with DEX administration, although there was a trend toward increases in that ratio.

Akeju and colleagues (2014) investigated the CMR of glucose (CMRglc) in relationship with brain connectivity and they recognized a global decrease in glucose metabolism with a reduction on its uptake in human brain under DEX. They found reduced CMRglc in thalamic, frontal, and parietal brain regions. The difference in CMRglc exhibited a spatial distribution consistent with the previously described DMN (Raichle et al. 2001; Damoiseaux et al. 2006) and both left and right FPNs (Damoiseaux et al. 2006; Vincent et al., 2008).

ROI	Treatment	CBF (mL · 100 g^{-1} · min^{-1})	CBF (95% CI)	*P* value[a]	*P* value[b]
1 (left anterior cortex)	Baseline	93	(74–116)		
	DEX-LOW	66	(52–83)	0.0024	
	DEX-HIGH	67	(54–85)	0.0050	0.83
	DEX-OFF	69	(52–91)	0.0330	
2 (right anterior cortex)	Baseline	89	(69–115)		
	DEX-LOW	67	(51–87)	0.0060	
	DEX-HIGH	63	(49–82)	0.0036	0.53
	DEX-OFF	67	(50–90)	0.0481	
3 (left superior cortex)	Baseline	98	(76–127)		
	DEX-LOW	70	(54–91)	0.0007	
	DEX-HIGH	62	(48–81)	<0.0001	0.20
	DEX-OFF	74	(55–99)	0.0280	
4 (right superior cortex)	Baseline	91	(73–113)		
	DEX-LOW	63	(50–79)	0.0014	
	DEX-HIGH	61	(49–76)	0.0008	0.75
	DEX-OFF	62	(48–81)	0.0073	
5 (left posterior cortex)	Baseline	87	(68–111)		
	DEX-LOW	67	(52–86)	0.0073	
	DEX-HIGH	62	(49–80)	0.0104	0.46
	DEX-OFF	60	(45–80)	0.0259	
6 (right posterior cortex)	Baseline	86	(68–110)		
	DEX-LOW	62	(48–79)	0.0056	
	DEX-HIGH	62	(49–79)	0.0096	0.97
	DEX-OFF	61	(45–82)	0.0284	
7 (midline anterior cortex)	Baseline	123	(97–157)		
	DEX-LOW	88	(69–113)	0.0053	
	DEX-HIGH	78	(61–99)	0.0043	0.23
	DEX-OFF	76	(57–102)	0.0130	
8 (midline posterior cortex)	Baseline	107	(87–133)		
	DEX-LOW	76	(61–94)	0.0010	
	DEX-HIGH	79	(64–98)	0.0048	0.64
	DEX-OFF	74	(58–96)	0.0083	
9 (left caudate nucleus)	Baseline	51	(41–64)		
	DEX-LOW	38	(30–48)	0.0086	
	DEX-HIGH	40	(32–51)	0.0323	0.55
	DEX-OFF	37	(28–49)	0.0225	
10 (right caudate nucleus)	Baseline	54	(44–68)		
	DEX-LOW	41	(33–52)	0.0168	
	DEX-HIGH	45	(36–55)	0.0659	0.46
	DEX-OFF	42	(32–55)	0.0564	
11 (left thalamus)	Baseline	137	(104–181)		
	DEX-LOW	79	(60–105)	0.0003	
	DEX-HIGH	73	(56–97)	<0.0001	0.55
	DEX-OFF	89	(64–125)	0.0139	
12 (right thalamus)	Baseline	131	(101–171)		
	DEX-LOW	83	(64–109)	<0.0001	
	DEX-HIGH	74	(57–97)	<0.0001	0.21
	DEX-OFF	87	(64–118)	0.0112	
13 (left white matter)	Baseline	50	(38–67)		
	DEX-LOW	40	(30–53)	0.0053	
	DEX-HIGH	38	(28–51)	0.0227	0.50
	DEX-OFF	43	(31–59)	0.2994	
14 (right white matter)	Baseline	53	(41–68)		
	DEX-LOW	42	(32–55)	0.0419	
	DEX-HIGH	39	(29–49)	0.0145	0.32
	DEX-OFF	37	(27–50)	0.0355	
Global CBF	Baseline	91	(72–114)		
	DEX-LOW	64	(51–81)	0.0002	
	DEX-HIGH	61	(48–76)	0.0006	0.50
	DEX-OFF	63	(49–83)	0.0120	

Figure 1: Prielipp et al.2002 " Geometric Least-Square Means of Cerebral Blood Flow (CBF) with 95% Confidence Interval (CI) by Each Region of Interest (ROI) in human volunteers after administration of two doses of dex (low 0.2 µg/kg/h; high 0.6 µg/kg/h)

MIDAZOLAM

OVERVIEW AND MOLECULAR PATHWAY

Midazolam (MDZ) is a water-soluble benzodiazepine that is widely used for both sedation and induction of anaesthesia in human and in animals because of its minimal systemic hemodynamic effects (Nugent et al. 1982). Benzodiazepines, are used for numerous indications, including anxiety, insomnia, muscle relaxation, relief from spasticity caused by central nervous system pathology, and epilepsy. Benzodiazepines are also used intraoperatively because of their amnesic and anxiolytic properties. Despite a huge use in human and veterinary medicine, benzodiazepine are not used frequently in preclinical setting, particular in preclinical neuroimaging.

They exert their influence on the CNS by enhancing the $GABA_A$ receptor's affinity for GABA, resulting in increased chloride conductance and hyperpolarization of postsynaptic cell membranes. The GABAA receptor is a pentameric combination of homologous subunits with a central pore, spanning the cell membrane. The receptor is frequently described in terms of its α subunit expression. The benzodiazepine binding site is usually located on the α_1, α_2, and γ subunits. Benzodiazepines enhance endogenous GABA binding to the receptor (Goodchild 1993).

GABA receptor are widely distributed in the brain tissue with individual subunits exhibit a distinct but overlapping regional and cellular distribution. Subunits α_1, β_1, β_2, β_3, and γ_2 are found throughout the brain, although differences in their distribution were observed. Subunits α_2, α_3, α_4, α_5, α_6, γ_1, and δ are more confined to certain brain areas. Thus, the α_1 subunit is the most abundant subunit and is ubiquitously distributed throughout the brain. The α_2 subunits are less abundant than α_1 subunits and are preferentially located in forebrain areas. The highest concentrations were found in olfactory bulb, striatum, nucleus accumbens, septum, dentate gyrus, amygdala and hypothalamus. But α_2 subunits were less abundant in thalamus (except reticular nucleus) midbrain and brainstem areas (Pirker et al 2000). α_3 subunits were strongly present in the olfactory bulb, in the inner layers of the cerebral cortex, the reticular thalamic nucleus, the superior colliculus, the amygdala and cranial nerve nuclei. Subunit α_4 was strongly detected in the thalamus, dentate gyrus, olfactory tubercle and basal ganglia. The α_5 subunit immunoreactivity was strongest in Ammon´s horn, the olfactory bulb and hypothalamus, whereas the α_6 subunit was only present in the cerebellum and the cochlear nucleus (Pirker et al 2000). The β subunits are widely distributed. The β_2 subunit is one of the most widely distributed subunits in the brain. The subunit γ_1 is a minor

subunit and exhibits a quite specific distribution in the brain. It is preferentially located in the central and medial amygdaloid nuclei, in pallidal areas, the substantia nigra pars reticulata and the inferior olive. In contrast, the γ_3 subunit is expressed in most brain areas but with low abundancy. The δ subunit is frequently co-distributed with the α_4 subunit , e.g. in the thalamus, striatum, outer layers of the cortex and in the dentatus. In the cerebellum, however, it is co-distributed with the α_6 subunit (Pirker et al 2000).

The response to benzodiazepines is multifactorial. The location of α_1 and α_2 subunits on the receptors, the location of the receptor in the CNS, the specific drug's affinity for the variety of sub receptors on the GABA receptor, lipid solubility, and overall pharmacokinetics all play a role in the clinical effect of a particular drug and dose.

FUNCTIONAL CONNECTIVITY

Compared to other drugs MDZ has not been deeply studied regarding its effect on functional connectivity, specially investigated through fMRI and resting state. To the author knowledge literature doesn't present a study regarding rs-fMRI analysis after MDZ administration in preclinical environment. Regarding human literature Liang et al. in 2015 have demonstrated that the sensory (visual, auditory, and sensorimotor) related networks remain intact under midazolam-induced light sedation while the higher-order (DMN, executive control, salience networks) networks are functionally disconnected. These findings provide direct evidence that higher-order cognitive functions including memory, attention, executive function, and language were impaired prior to lower-level sensory responses during sedation. Furthermore, Kiviniemi and colleagues, in a study conducted in 2005 on twelve healthy human subjects described that the very low frequency (VLF) power of the BOLD signal was elevated significantly after midazolam sedation in the brain; the temporal BOLD signal synchrony elevated significantly in the auditory cortex and borderline significantly in the visual cortex.

CEREBRAL BLOOD FLOW AND CEREBRAL METABOLISM

The use of benzodiazepines induces a decrease in CBF globally, along with a reduction of glucose consumption due to a lower metabolic demand. Based on a study by Veselis et al. (1997), MDZ sedation does not only cause a global decrease in CBF but also produces significant regional changes

A study conducted in 2012 by Liang et al. has demonstrated that the contrast of MRI signal, using arterial spin labelling perfusion before and after midazolam administration, revealed a decrease in CBF in the left dorsolateral prefrontal cortex (DLPFC), left cingulate gyrus and left posterior cingulate gyrus/precuneus (Riad et al., 2000).

The administration of MDZ at the dose of 5.75 mg/kg decreased CBF 51% and $CMRO_2$ 38% in young rats. This depression was significantly less than the 62% decrease in CBF and 59% decrease in CMRO2 produced by midazolam in old rats (Baughman et al. 1987).

ISOFLURANE

OVERVIEW AND MOLECULAR PATHWAY

Isoflurane (ISO) is the most commonly used anesthetic drug in preclinical study and in veterinary medicine. Sevoflurane and desflurane, that belonging to the same family, are the most common agents to anesthesia maintenance in human patients.

In preclinical field ISO is one of the standard anesthetics for structural imaging studies, but it is also being increasingly exploited in functional studies (Lukasik and Gillies 2003). For instance, almost 44 % of the preclinical pharmacological fMRI studies were conducted under ISO anesthesia until 2013 (Haensel et al. 2015).

Inhalation anesthetics are unique among the anesthetic drugs because they are administered, and in large part removed from the body, via the lungs. Their popularity arises in part because their pharmacokinetic characteristics favour predictable and rapid adjustment of anesthetic depth.

ISO induces sufficient muscle relaxation without evoking convulsions (Eger 1984, Lukasik and Gillies 2003). Nevertheless, inhalation anesthetics cause vasodilation and decrease heart rate, mean arterial blood pressure, and breathing rate (Van Aken and Van Hemelrijck 1991, Lukasik and Gillies 2003, Masamoto and Kanno 2012).

In general, inhalation agents potentiate inhibitory cell targets such as $GABA_A$ receptors, glycine receptors, and two-pore domain potassium channels; these same agents inhibit excitatory cell targets such as NMDA receptors, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors, nicotinic receptors, and voltage-gated sodium channels. Distribution of GABA receptor in the CNS are previously described in the chapter of midazolam; in the following part we will report only literature regarding inhalation agents mechanism of action.

Their anesthetic effect is predominantly mediated by $\alpha_1$subunit of the $GABA_A$ (Mihic and Wick 1997; Jenkins et al. 2001). Isoflurane, desflurane, and sevoflurane all enhance the response at the $GABA_A$ to endogenous GABA and prolong the duration of GABA-mediated synaptic inhibition. At overclinical concentrations there is 'direct activation' of the $GABA_A$, whereby there is opening of the receptor's anion channel in the absence of GABA (Garcia et al. 2010).

FUNCTIONAL CONNECTIVITY

Literature concerning the effect induced by ISO on FC is huge and detailed particular in animal models. The popularity of ISO in MRI experiments originates from its several advantageous properties: administration is easy, both induction and recovery are fast, the level of anesthesia is easily controllable and stable throughout measurements, and there do not appear to be any clear contraindications preventing follow- up studies (Lukasik and Gillies 2003). Nevertheless, decades of studies in this field revealed that ISO cause a severe suppression of neuronal activities decreasing brain connectivity (Fukuda et al., 2013) and promotes the inhibition of neurotransmission-modulated vasodilation (Toda et al. 1992). These drawbacks are significant confounding factors in fMRI, as the anesthesia-induced vasodilation decreases the relative vascular response to neural activation or other vasoactive compounds (Sicard et al. 2003, Sicard and Duong 2005), subsequently hindering the detection of fMRI signal changes (Masamoto and Kanno 2012).

Generally, alteration of FC seems to depend from the depth of anesthesia level; this concept is true both for injectable and inhalant anesthetics despite the different neural mechanism. One recent study examined the distributed intrinsic FC of macaques across six isoflurane levels, and found stable FC patterns between 1.00% and 1.50%, but a decrease of interhemispheric cortical FC strength during moderate to high doses (Hutchison et al 2014). These findings support that the functional repertoire of brain state is related to the depth of anesthesia. A more recent study investigated alterations in cerebral regional activity and FC simultaneously in rhesus monkeys anesthetized at three different concentrations of ISO with a minimum alveolar concentration (MAC) of 1.0, 1.3 and 1.6 using resting-state rs-fMRI. Generally, an increase in the ISO dose from 1.0 MAC to 1.3 MAC decreased the amplitude of low-frequency fluctuations (ALFF) in the cerebellum, visual area and the cortico-subcortical net-work. A further increased from 1.0 MAC to 1.6 MAC decreased ALFF in a more widespread area, including the arousal system, cerebellum, sensory and visual areas, the cortico-subcortical and DMN.

Furthermore, the same study revealed disrupted FC between neurons distributed in cortico-cortical and thalamo-cortical networks. An increase in ISO from 1.0 MAC to 1.3 MAC reduced FC of the DMN, frontal-parietal, cortico-subcortical, motor, sensory, auditory and visual areas. An increase in isoflurane from 1.0 MAC to 1.6 MAC decreased FC in a more widespread area, including the default mode network, frontal-parietal and cortico-cerebellar circuits, motor, sensory auditory, and visual areas, especially in regions of the cortico-subcortical networks and limbic systems. These results support the hypothesis that higher order areas, such as the DMN, FPN and sensory networks, tend to be the most susceptible to isoflurane dose.

A recent study conducted by Paasonem and colleagues in 2018 on rats shows that the administration of 1.3% of ISO causes an increase of FC in the fronto-cortical region, while the majority of the thalamo-cortical and intrasubcortical connections are suppressed as the thalamic activity.

CEREBRAL BLOOD FLOW AND CEREBRAL METABOLISM

Studies performed in humans and in animals indicate that the administration of ISO results in cerebral vasodilatation and has evident effects on CBF, cerebral blood volume (CBV), permeability, neurovascular coupling, neuron functionality. Increased CBF and reduced $CMRO_2$ consumption have been observed in animal and human studies (Cucchiara et al. 1974; Olsen et al. 1994). Also, previous canine, baboon, and human studies have shown that the CBF autoregulation mechanism was disturbed under high dose isoflurane(McPherson and Traystman 1988; Olsen at al. 1994).

ISO induces alteration in CBF in a dose depended manner: it has been demonstrated that ISO induces vasoconstriction in the low concentrations (~0.5% or less) and vasodilatation in the high concentration (~0.95% or more), indicating that there exist transition doses in the range of 0.5% to 0.95%. Effects of ISO on regional CBF studied in monkeys are reported in figure 2.

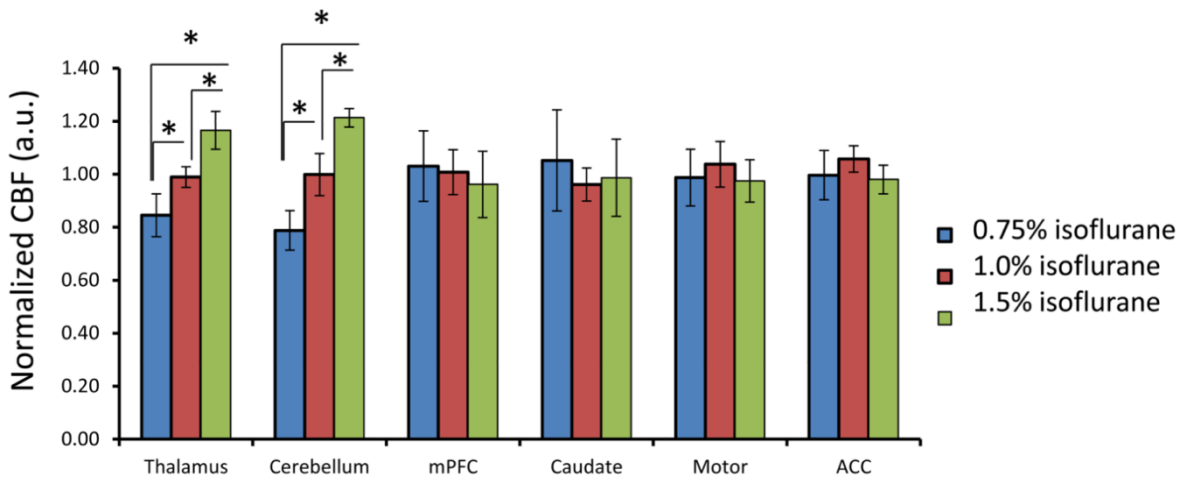

Figure 2:Chun-xia Li et al. 2013 "Dose-dependence effect of ISO on mean CBF in global, cortical and subcortical regions of anesthetized macaque monkeys (mean±SD). CBF in each ROI is normalized to the mean CBF value of three dose levels: 0.75%, 1.0%, 1.5%.: p<0.05"*

KETAMINE

OVERVIEW AND MOLECULAR PATHWAY

Ketamine is a non-competitive NMDA glutamate receptor antagonist with a complex profile of pharmacological effects that has made it an important target in biomedical and neuroscience research. It belongs to the family of dissociative anesthetics. High doses of ketamine have long been used medically to produce anesthesia (Haas and Harper, 1992) in both human and veterinary subjects. Recently, ketamine has emerged as a potential treatment for multiple psychiatric disorders. Sub-anesthetic doses of ketamine have shown efficacy for treating postoperative pain (Schmid et al., 1999), neuropathic pain (Schwartzman et al., 2009), treatment-resistant depression (Berman et al., 2000; Krystal et al., 2013), and suicidal ideation (Ballard et al., 2014; Price and Mathew, 2015). Furthermore, ketamine is used for the treatment of super-refractory *status epilepticus* (SRSE) (Fang and Wang 2015). This last use of ketamine will be deeply described in a chapter of this dissertation. Ketamine exerts its action binding to the phencyclidine-binding site, which prevents glutamate, an excitatory neurotransmitter, from binding. Prevention of glutamate binding results in depression of the thalamocortical, limbic, and reticular activating systems. When administered at high doses ketamine has a direct negative cardiac inotropic effect (Diaz et al 1976),

but it is usually overcome by central sympathetic stimulation. Intravenous administration of ketamine increases systemic and pulmonary arterial pressure, heart rate, cardiac output, myocardial oxygen requirements, and cardiac work (Haskins et al. 1985). It is likely that these changes are the result of direct stimulation of the CNS leading to increased sympathetic nervous system outflow (Wong and Jenkins 1974). Ketamine also inhibits NE reuptake into postganglionic sympathetic nerve endings, leading to an increased concentration of plasma catecholamines. Unlike other injectable anesthetics, ketamine does not cause significant respiratory depression. Ventilatory responses to hypoxia and carbon dioxide are maintained in animals receiving ketamine as the sole anesthetic agent (Soliman et al. 1975). Ketamine causes and increase of muscle tone when administered at anesthetics doses; this reason it is frequently administered whit drugs promoting muscle-relaxation (e.g. benzodiazepines).

FUNCTIONAL CONNECTIVITY

Rs-fmri studies on ketamine were done on both human and animals. A study performed on human brain showed that ketamine although shares some similarities with other hypnotic anesthetic agents, such as the effect on frontal-parietal connectivity is different in some aspects. Salient finding regarding FC induced by ketamine discovered in a study by Bonhomme and colleagues (2016) in human subjects are reported in figure 3.

Network	Effects on Connectivity
DMn	Breakdown of DMn by ketamine, discreet during light sedation, more pronounced during loss of responsiveness
	The main disconnection is frontal
	Decreased thalamic connectivity during ketamine-induced loss of responsiveness, but still present
	Ketamine increases connectivity between DMn and several other brain regions
	Anticorrelation with DMn is markedly reduced by ketamine, even during light sedation
LECn an	Tenuous effect of ketamine on LECn and RECn
RECn	Ketamine increases connectivity between RECn and right primary sensory cortex
	Ketamine increases connectivity between RECn and right insula
	Absence of significant interactions between LECn and RECn during ketamine-induced loss of responsiveness to command
SALn	Few changes during light ketamine sedation
	Breakdown during ketamine-induced loss of responsiveness to command
	Heterogeneous alteration within the network
AUDn	No remarkable effect
VISn	No cross modal interaction inhibition
SMn	

AUDn = auditory network; DMn = default mode network; LECn = left executive control network; RECn = right executive control network; SALn = salience network; SMn = sensorimotor network; VISn = visual network.

Figure 3: Bonhomme et al. 2016. Summary of Salient Findings regarding Connectivity in Studied Networks at Different Depths of Ketamine Sedation

CEREBRAL BLOOD FLOW AND CEREBRAL METABOLISM

Administration of both ketamine and s-ketamine (subanaesthetic doses) in human subject seem to causes an increase of whole brain CBF with no corresponding change in CMR of both oxygen and glucose. CMR of glucose increased only in thalamus.

Subanesthetic doses of ketamine seem to principally increase human CBF and glucose metabolic rate, with only minor effects on cerebral metabolic rate of oxygen (L˚angsjo et al. 2003; L˚angsjo et al. 2004). However, pure anesthesia with racemic ketamine has been associated with increased whole brain CBF with no changes in either of the metabolic components ($CMRO_2$ and CMR_{glc}) in humans (Takeshita et al. 1972).

DIAGNOSTIC IMAGING FOR DRUGS STUDY: FUNCTIONAL MRI AND DYNAMIC SUSCEPTIBILITY CONTRAST (DSC) MRI

In the past 20 years, fMRI has gained widespread acceptance as a powerful tool for mapping brain function. Furthermore, fMRI has been recognized as an in important tool for the study of drugs behavior at brain level.

Typically, pharmacologic MRI (ph-MRI) studies attempt to localize activation sites and temporal dynamics of drug responses, e.g., in the characterization of a drug´s pharmacodynamics, Blood Brain Barrier-permeability, or in finding appropriate dose ranges of a novel drug candidate (Leslie and James 2000; Jenkins 2012; Jonckers et al. 2013). Importantly, the phMRI signal was found to correlate better with drug actions in CNS and their consequences (secondary neurotransmission, behavior, etc.) than with absolute drug concentration in tissue, a clear advantage of this approach compared to other forms of functional imaging (Stein et al. 1998; Jenkins 2012). Anesthesia plays a central role in ph-MRI studies; it can represents the goal of the study or it can plays a role as a "nuisance" if the goal is a different drugs. Indeed, anesthesia can have considerable effects on fMRI studies by disturbing neurovascular coupling and neural activity; drugs under investigation may undergo direct interactions with anesthetics, or drugs may share the same cellular level targets as the anesthetics, and these mechanisms can significantly modulate the observed phMRI response. Therefore, a knowledge of the pharmacodynamics of both the drug being studied and the anesthetic agent used to immobilize the animal is essential in phMRI study design. Furthermore, it is crucial to understand the effect induced by anesthesia in different neurologic conditions in order to obtain the best design limiting potential cofounding elements.

fMRI is typically used to study small changes in the blood BOLD signal which are induced by performance of a task or by administration of a stimulus. In the past decades, research efforts concentrated on identifying regions specialized in given cognitive tasks, but more recently the interest has shifted toward a wider prospective aiming at understanding how brain multiple regions interact with one another and how this leads to behavioural phenomena. The recent advances in functional neuroimaging have provided new tools to study the brain, according to this view, that is, as a network of interacting regions. Functional connectivity represents a novel approach of fMRI which enables to investigate the neural activity of regions that are functionally connected even

when they are anatomically distant. Functional connectivity can be defined as the synchrony of neural activity among regions. Areas of the brain which exhibit signal fluctuations correlated in time are assumed to be functionally connected. These BOLD signal fluctuations occur at low frequencies (<0.1 Hz) and have been observed throughout the brain. Functional connectivity can be studied during the performance of active tasks, such as finger tapping or visual stimulation, as well as during resting state, a condition in which the participant is not performing any active task and is simply instructed to remain still, with eyes closed or open while fixating a cross (Rosazza and Minati 2011). In this dissertation we are going to focused our attention to the resting state.

BOLD SIGNAL

The stimuli-induced changes in local neuronal spiking rate and energy consumption are, however, relatively small (up to 10 % but typically less than 5 %) compared to task- or stimuli- free state (Scholvinck et al. 2008; Attwell et al. 2010; Raichle, 2015). Such small changes in neuronal activity would be difficult to detect but, fortunately, the increase in blood flow is roughly 4-fold greater than required to meet the needs of the neurons (Lin et al. 2010), facilitating the indirect detection of neural activity by fMRI.

As its name suggests, the BOLD contrast mechanism alters the T2∗ parameter mainly through neural activity–dependent changes in the relative concentration of oxygenated and deoxygenated blood. Deoxyhemoglobin (dHb) is paramagnetic (Pauling and Coryell 1936) and influences the MR signal (Brooks et al. 1975) unlike oxygenated Hb. In the presence of dHb, the T2 value decreases quadratically with field strength, as expected from the dynamic averaging owing to diffusion in the presence of field gradients (Thulborn et al. 1982; Thulborn et al 1992). The effects of dHb on T2∗ are even stronger, as first noticed by Ogawa et al. in their seminal studies on the rat brain in high fields (Ogawa et al. 1990). Specifically, Ogawa & Lee observed that blood vessel contrast varied with changes in blood oxygen demand or flow (Ogawa et al. 1990[1]). They attributed the contrast increase to a magnetic susceptibility effect associated with the paramagnetic deoxyhemoglobin in red cells (Ogawa et al. 1990). The neuronal activity increases local CBF excessively (delivery of oxygen > consumption of oxygen), which increases the proportional amount of local Hb. As local CBF is dependent on local CBV (Grubb et al. 1974), neuronal activity also increases local blood volume as well as the absolute amount of diamagnetic Hb, emphasizing even more the local changes in the magnetic field and the BOLD signal (Huettel et al. 2004).

RESTING STATE FMRI- BRAIN NETWORK

It is well known that under resting conditions the brain is engaged in spontaneous activity which is not attributable to specific inputs or to the generation of specific output, but is intrinsically originated. The brain under normal physiological conditions is never idle, but always remains neuro-electrically and metabolically active. Therefore, it was postulated that rsfMRI could be the long-awaited tool allowing researchers to non-invasively investigate brain as a dynamically active system consisting of connections between regions and/or networks (Smucny et al. 2014). there are extensive advantages associated with rsfMRI and the technique also has many applications. The acquisition of rsfMRI data is technically relatively fast and easy to perform and the measurement is conducted during rest; no external task or stimulus is required from the subject (Fox and Raichle 2007). Therefore, rsfMRI data can be obtained from patients that are unwilling or incapable to perform tasks, and from animals that cannot perform similar tasks as humans during fMRI (Fox and Raichle 2007, Smucny et al. 2014). Additionally, the rsfMRI provides information of whole-brain activity, whereas task-based fMRI experiments only focus on certain brain region(s).

Multiple analysis techniques are used to look at the datasets. The most commonly employed ones are the ROI based analysis and the independent component analysis (ICA). The first is based on the extraction of the time-course of the BOLD signal from a pre-defined ROI and subsequent identification of the regions showing a significant correlation with the ROI. This time-course correlation analysis produces functional connectivity maps showing which areas are connected with the given ROI and to what extent. This method represents the most straightforward way to study the FC, as the results are relatively clear and simple to interpret (Greicius et al. 2003; Fox and Raichle 2007). ICA, by contrast, is a statistical technique that does not involve any a priori assumption and allows the exploration of multiple whole-brain networks. When applied to fMRI, ICA is able to extract from the BOLD time series a number of independent components which are spatial maps associated with the time courses of the signal sources (McKeown et al. 1998). Each component can be interpreted as a network of similar BOLD activity.

RESTING STATE COMPONENTS IN HUMAN

Using both ROI-based analysis and ICA, FC studies have reported a number of networks that result to be strongly functionally connected during rest. In human fMRI there are some component that seems to be always present: the default mode network (DMN), the sensorimotor component, the executive control component, up to three visual components, two lateralized frontoparietal components, the auditory component and the temporo-parietal component. As already reported, these resting-state net-works consist of anatomically separated, but functionally connected regions displaying a high level of correlated BOLD signal activity.

DMN is the most studied component of fMRI studies. This signal component is identifiable in:

- precuneus/ posterior cingulate,
- lateral parietal cortex,
- mesial prefrontal cortex.

This set of regions is typically observed to be more intensely activated during the rest and relatively deactivated during the demanding tasks requiring focused attention such as working memory tasks and visuo-spatial tasks (Corbetta and Shulman 2002, Greicius et al. 2003).

RESTING STATE COMPONENTS IN RODENTS

Despite the technical challenge and differences in neuroanatomical and functional organization between species (Van den Heuvel et al., 2016), it has been demonstrated that similar Resting State Networks (RSN), such as the bilateral connectivity in sensory and motor networks, can be consistently identified in the anesthetized rat (Pawela et al. 2008; Zhao et al. 2008; Biswal and Kannurpatti 2009; Majeed et al. 2009), awake rat (Becerra et al. 2011; Liang et al. 2011), and, more recently, anesthetized mouse (Grandjean et al. 2014; Nasrallah et al. 2014; Sforazzini et al. 2014). Particularly, DMN-like network has been found in both the rat (Lu et al. 2012) and mouse brain (Sforazzini et al. 2014), indicating the evolutionally preservation of this large-scale network. Almost the majority of rodents' studies are performed under sedation/general anesthesia substantially for ethic concerns.

Three kinds of FC patterns have been consistently identified in the sedated, anesthetized and awake rat brain;

1. Bilaterally symmetric connectivity within functional modules. This includes the primary and secondary somatosensory, motor, and visual cortices, hippocampus and subcortical areas, such as the caudate putamen, thalamus, superior colliculus, and hypothalamus (Pawela et al. 2008; Zhao et al. 2008; Majeed et al. 2009; Hutchison et al. 2010).
2. Anteroposterior connectivity along the midline. This is found between the cingulate and retrosplenial cortices (Hutchison et al. 2010; Jonckers et al. 2011).
3. Large-scale cross-modular connectivity. The DMN-like connectivity is of this type. It was initially only detected in the well-habituated awake rat brain, and comprised the cingulate (homologous to the human anterior cingulate cortex), retrosplenial (homologous to the human posterior cingulate cortex) and parietal cortices and hippocampus (Upadhyay et al. 2011). However, with a more stable anesthesia regimen that combined medetomidine and light isoflurane, a more extensive network consisting of the orbital cortex (homologous to the human orbital frontal cortex), prelimbic and cingulate cortex, retrosplenial cortex, posterior parietal cortex, auditory/temporal associated cortex, and dorsal hippocampus could be detected (Lu et al. 2012).

DYNAMIC SUSCEPTIBILITY CONTRAST (DSC) MRI PERFUSION

Dynamic Suceptibility Contrast MRI (DSC – MRI) and Dynamic Contrast Enhanced MRI (DCE – MRI) are the two exogenous techniques to study the hemodynamic properties of the tissue. DSC-MRI (Ostergaard et al. 1996), the most commonly technique in clinical settings, uses T2*/T2 signal decay caused by the passage of the contrast to indirectly estimate a) CBV that indirectly represents the vascular density of the capillary bed; b) CBF, the blood flow inside the capillary bed; c) Mean Transit Time (MTT) that represents the time necessary to the contrast to pass through the capillary bed. DCE-MRI instead is used to specifically study the Blood Brain Barrier dynamic and uses the T1 signal increase to estimate the flow or the rate of exchange between the endovascular wall.

The area under the signal (ΔR2*) versus time curve approximates cerebral blood volume. The mean transit time of the bolus can also be derived from the signal versus time curves and CBF is determined using CBV divided by mean transit time.

Various techniques for perfusion measurement are available, involving endogenous or exogenous markers. The most commonly used exogenous markers are gadolinium chelates. Contrast medium is injected IV as a bolus. Dynamic images are acquired before, during, and after contrast medium injection. From the acquired images, signal intensity–time curves are generated, which allow for the calculation of concentration-versus-time curves (Barbier et al. 2001).

These techniques acquire a time series of T*2-weighted images after an intravenous bolus injection of an MR contrast agent and measurements of CBF, CBV, and mean transit time (MTT) are made by applying indicator dilution theory. These values can be quantitative (i.e., expressed in absolute units) if the arterial input function (AIF) can be measured and used to deconvolve the tissue curves in the case of CBF or used to normalize the integral of the tissue curves in the case of CBV.

CEREBRAL BLOOD FLOW AND CEREBRAL BLOOD VOLUME

The contrast in DSC MRI is typically based on the administration of an intravascular contrast agent, a small superparamagnetic iron oxide, which similarly to paramagnetic dHb increases the magnetic susceptibility, reduces transverse relaxation time, and decreases the fMRI signal (van Bruggen et al. 1998; Huettel et al. 2004). The contrast agent, which typically has a half-life of many hours, distributes into the blood circulation, and decreases the fMRI signal in the vasculature to a baseline level. During neural activity, the local increase in blood volume can be detected as a further decrease in the MRI signal, because of the local increase of CBV and paramagnetic contrast agent. Even though the variation in CBV is relatively small compared to changes in CBF (Grubb et al. 1974), the use of a contrast agent enhances the blood volume changes to a detectable level, mainly because of the large surface of capillary bed expressing the changes in magnetic susceptibility (van Bruggen et al. 1998).

Cerebral blood flow is one of the parameters generated by perfusion techniques. Cerebral blood flow is defined as the volume of blood passing through a given amount of brain tissue per unit of time, most commonly milliliters of blood per minute per 100g of brain tissue (Petrella and Provenzale 2000).

Although the exact relations between the different MRI contrasts remain unclear, CBV and CBF are known to be tightly coupled (Grubb et al. 1974; Shen et al. 2008). It is also known that the different contrasts or sequences emphasize different vascular components (Shen et al. 2008), which may be necessary to be taken into account in experimental design and data interpretation.

BRAIN DISEASES: WHEN IMBALANCE IN NEUROTRANSMISSION IS THE CAUSE

As we can deduct from the previous paragraph of this dissertation, giving the vastity and the complexity of the human and animal brain, that alterations in neurotransmission could give rise to many different CNS pathologic conditions.

Disorders or substances that alter the production, release, reception, breakdown, or reuptake of neurotransmitters or that change the number and affinity of receptors can cause neurologic or psychiatric symptoms and cause disease (e.g., epilepsy and status epilepticus, Parkinson disease, depression). Drugs that modify neurotransmission can alleviate many of these disorders. In this contest anesthetics need be took under consideration not only as drugs strictly used for induction and maintenance of general anesthesia but also as a real therapy able to solve or improve different neurologic disease. In table 1 we present a literature report concerning the most common diseases induced by neurotransmission imbalance and the relative therapy. The next section of this thesis will be completely dedicated to status epilepticus and its relative treatment as an explicative example of neurologic condition that found in general anesthesia the therapy.

DISORDER	PATHOPHISIOLOGY	TREATMENT
Alzheimer disease	Extracellular beta-amyloid deposits, intracellular neurofibrillary tangles, and senile plaques, particularly in the limbic system (eg, hippocampus), in the association area of the cortex, and in neurons that synthesize and use acetylcholine (eg, in the basal nucleus of Meynert and its wide projections to the cortex)	Cholinesterase inhibitors (donepezil, rivastigmine, galantamine) delay synaptic degradation of acetylcholine and thus modestly improve cognitive function and memory. Memantine, an NMDA-receptor antagonist, may slow progression of the disease and increase autonomy..
Anxiety	May reflect reduced activity of GABA, perhaps due to imbalance of	Benzodiazepines increase the probability of opening chloride channels

	endogenous inhibitors, stimulators of the GABA receptor, or both May also involve imbalances in norepinephrine and 5-HT responses	modulated by GABA through GABA-A receptor activation. SSRIs are the drugs of choice for long-term treatment because tolerance to benzodiazepines can develop.
Brain injury	injury (eg, trauma, hypoxia, prolonged seizures) stimulating excessive release of excitatory neurotransmitters (eg, glutamate) and accumulation of intracellular calcium, which contribute to neuronal death	in experimental models of ischemia and injury, calcium channel blockers, glycine, and older NMDA-receptor antagonists (eg, dextromethorphan, ketamine) may reduce the extent of neuronal loss, but these drugs are not effective in people. Memantine, a newer NMDA-receptor antagonist, is under study.
Depression	Complex abnormalities in cholinergic, catecholaminergic (noradrenergic, dopaminergic) and serotonergic (5-HT) transmission Possible involvement of other hormones and neuropeptides (eg, substance P, dopamine, acetylcholine, GABA)	Antidepressants downregulate receptors indirectly or directly by inhibiting reuptake of 5-HT (as with SSRIs) and norepinephrine or dopamine or by blocking MAO. Blockade of 5-HT2A/2C (a type of 5-HT receptor abundant in the prefrontal area) may increase the efficacy of SSRIs (eg, trazodone).
Seizure disorders	Seizures consisting of sudden synchronous high-frequency firing by localized groups of neurons in certain brain areas, perhaps caused by increased activity of glutamate or reduced activity of GABA	Phenytoin, lamotrigine, carbamazepine, valproate, topiramate, and some other antiseizure drugs (eg, zonisamide, oxcarbazepine) stabilize voltage-dependent sodium channels. Ethosuximide and gabapentin decrease certain calcium currents. Phenytoin also reduces excessive neurotransmitter release.

		Lamotrigine may decrease levels of glutamate and aspartate. Phenobarbital and benzodiazepines enhance GABA activation by affecting the GABA-A receptor–chloride channel complex. Tiagabine blocks GABA glial uptake. Valproate increases levels of GABA. Topiramate increases GABA activity.
Huntington disease (chorea)	Major neuronal damage in the cortex and striatum due to polyglutamine expansion (encoded by CAG repeat), produced by an abnormal gene on chromosome 4 (the abnormal gene overproduces the protein huntingtin, which may combine with molecules that induce excessive stimulation of cells by excitatory amino acid neurotransmitters such as glutamate)	No specific treatment exists, but drugs that block NMDA receptors may block the toxic effects of excess glutamate. GABA-mimetic drugs are ineffective.
Mania	Increased norepinephrine and dopamine activity, reduced 5-HT levels, and abnormal glutamate neurotransmission	Lithium is the traditional first choice. It reduces norepinephrine release and increases 5-HT synthesis. Valproate and lamotrigine are beneficial, possibly by normalizing glutamate transmission. Topiramate blocks voltage-dependent sodium channels, augments GABA activity at some subtypes of the GABA-A receptor, antagonizes the AMPA/kainate subtype of the glutamate receptor, and inhibits the

		carbonic anhydrase enzyme, particularly isozymes II and IV. Gabapentin is thought to bind to the alpha-2/delta subunit (1 and 2) of the voltage-dependent calcium channel in the CNS. Carbamazepine and oxcarbazepine stabilize voltage dependent sodium channels.
Pain	Tissue injury, which causes release of substance P and glutamate in the posterior horn of the spinal cord and release of other macromolecules that mediate pain signals, such as CGRP (which can dilate cranial blood vessels and lead to migraine pain), neurokinin A, and bradykinin, which are localized primarily in the lamina II and IV of the spinal cord Further modulation of these signals by endorphins (in the spinal cord) and by 5-HT and norepinephrine (in the descending pathways that originate in the brain)	NSAIDs inhibit prostaglandin synthesis selectively (with COX-2 inhibitors—eg, celecoxib, parecoxib) or nonselectively (with COX-1 and -2 inhibitors—eg, ibuprofen, naproxen) and reduce pain impulse formation. Opioid analgesics (eg, morphine) activate endorphin-enkephalin (mu, delta, and kappa) receptors, reducing pain impulse transmission. New treatments that can block CGRP receptors can attenuate dilation of the cranial blood vessels and prevent migraine pain.
Parkinson disease	Loss of dopaminergic neurons of the pars compacta in the substantia nigra and other areas, with reduced levels of dopamine and metenkephalin, altering the dopamine/acetylcholine balance and resulting in striatal acetylcholine overactivity	Levodopa reaches the synaptic cleft, is taken up by the axon through presynaptic nigral neurons, and is decarboxylated to dopamine, which is secreted into the cleft to activate dendritic dopamine receptors. Amantadine increases the presynaptic release of dopamine; dopamine

		agonists stimulate dopamine receptors, although bromocriptine, pramipexole, and ropinirole bind only to D2, D3, and D4 dopamine receptor subtypes. Anticholinergic drugs reduce activity of the cholinergic system, restoring the balance of dopamine and acetylcholine. MAO-B inhibitors prevent reuptake of dopamine, increasing its levels. Selegiline, an MAO-B inhibitor, blocks dopamine breakdown and thus prolongs the response to levodopa and allows the dosage of carbidopa/levodopa to be reduced. Catechol O-methyltransferase (COMT) inhibitors also inhibit dopamine breakdown.
Schizophrenia	Increased presynaptic release, synthesis of dopamine, sensitivity or density of postsynaptic dopamine receptors, or a combination	Antipsychotic drugs block dopamine receptors and reduce dopaminergic overactivity to normal. Haloperidol preferentially blocks D2 and D3 receptors (high affinity) and D4 receptors (low affinity) in mesocortical areas. Clozapine has a high affinity for binding D4 and 5-HT2 receptors, suggesting 5-HT system involvement in the pathogenesis of schizophrenia and its response to treatment. Clozapine has a significant risk of leukopenia.

		Olanzapine and risperidone, similar to haloperidol, also have high affinity for 5-HT2 and D2 receptors.

Table 1:resume of the most common diseases induced by neurotransmission imbalance and the relative therapy. MSD manual 2019.

WHEN ANESTHESIA IS THE THERAPY: THE STATUS EPILEPTICUS

Status epilepticus (SE), refractory status epilepticus (RSE), and super-refractory status epilepticus (SRSE) are life-threatening conditions that require a prompt, aggressive and dynamic treatment approach. This pathology affects both human and animal patients. Status epilepticus can be defined as a continuous convulsive seizure lasting more than 5 minutes or the occurrence of 2 or more epileptic seizures between which there is incomplete recovery of consciousness (Berendt et al. 2015). Refractory SE is defined as SE unresponsive to first-line anticonvulsant therapy that requires general anesthesia to control seizures; SRSE is defined as SE continuing or reoccurring more than 24 hours after initiation of anesthetic treatment (Shorvon and Ferlisi 2012). SE can also be described as either convulsive or nonconvulsive depending on the clinical presentation. However, the type of SE likely influences the outcome, so distinctions are important. For instance, excitotoxic damage is higher risk in convulsive SE than in some forms of non-convulsive SE. Convulsive SE also stresses other organ systems, including cardiac and pulmonary systems, from generalized convulsions potentially causing aspiration pneumonia, pulmonary edema, stress-induced cardiac injury, or myocardial ischemia, as well rhabdomyolysis and renal failure. On the other hand, non-convulsive status epilepticus, which can be detected only with electroencephalography, is often associated with considerable delay in detection and initiation of treatment, and subsequently tends to be more medically refractory. To discuss pharmacological management of SE, it is important to understand how seizures become refractory. First, seizures are sustained by either imbalance of neuronal excitation and inhibition or failure of normal inhibitory mechanisms. GABA is the commonest inhibitory neurotransmitter, preventing neurons from excess excitation by activation of the $GABA_A$ receptor, and glutamate is the commonest excitatory neurotransmitter, and mediates excess excitation via NMDA receptors. Second, SE can become self-sustaining, with neuronal damage and pharmacoresistance becoming apparent after 30 minutes of continuous seizure activity in both experimental and clinical studies (Fujikawa 1996, Kapur and Macdonald 1997, Mazarati et al.1998,). As SE continues, there is a progressive development of pharmacoresistance to benzodiazepines (Kapur and Macdonald 1997), and NMDA receptor blockers such as ketamine can remain effective late in the course of SE (Mazarati and Wasterlain 1999). This is likely explained by intensified “receptor trafficking,” in which the number of glutaminergic receptors at the cell surface increases

and the number of GABA receptors decreases (Arancibia-Carcamo and Kittler 2009) leading to a reduction in GABAergic activity. There are likely many other mechanisms contributing to the development of RSE and SRSE, which may each be potential targets of therapy. Reported mechanisms include (1) mitochondrial failure or insufficiency (Cock et al.2002), (2) inflammatory processes (Marchi et al.2011) resulting in decreased integrity of the blood–brain barrier and higher potassium levels (David et al.2009), and (3) changes in gene expression (Henshall2013).

SE and its progression is a typical neurologic condition raising from a severe imbalance of brain neurotransmission. The knowledge of anesthetics behavior match with the enteropathogenesis of SE is the start point to understand why this class of drugs represents the best therapy option. In emergency, benzodiazepine are indicated during the first phases in order and enhances the action of the $GABA_A$ receptor. Secondly, different antiepileptic drugs (AED) are introduced (for drugs and relative mechanism of action see table 1). If SE does not respond to this kind of medication general anesthesia is recommended starting with drugs acting on $GABA_A$ receptors (e.g. propofol) moving to NMDA antagonist if patient does not respond to GABA-ergic drugs. Recently, one experimental study has described also the use of $alpha_2$-agonist for the treatment of benzodiazepine-refractory nerve agent-induced status epilepticus (McCarren et al. 2018). Reports in the literature support the ability of the noradrenergic nervous system to regulate seizure activity, especially through $alpha_2$-adrenoreceptors. The lack of norepinephrine or $alpha_{2A}$-adrenoreceptors results in increased seizure activity in rodents. It has been speculated that stimulation of $alpha_{2A}$-postsynaptic receptors is responsible for the anticonvulsant response, possibly suppressing the release of excitatory neurotransmitter in regions such as the hippocampus, cortex or amygdala (Szot et al. 2004). Furthermore, DEX owns neuroprotective qualities and is able to decrease metabolic demand.

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AIMS OF THE STUDY

The general aim of this PhD thesis was to implement data available on anesthetics behavior at central nervous system level. Particularly, the study of functional connectivity represent the focal point of this study.

Anesthesia is frequently required during diagnostic procedures; it is indispensable in uncollaborative patients such as children, in case of claustrophobia or psychiatric disease, or for all patients with any condition incompatible with the persistence into the magnetic resonance scanner. Furthermore, anesthesia is always necessary in veterinary patients especially in preclinical setting. Because the most anesthetics recognize in central nervous system their target this kind of drugs could introduce potential pitfall especially in neuroradiology. Fortunately, the fact that anesthesia is able to modulate CNS transmission could be useful to treat different neurologic conditions specially based on neurotransmission imbalance.

The following specific aims were investigated:

1. To investigate FC with rs-fMRI under isoflurane and dexmedetomidine in guinea pigs
   As discussed earlier, the common requirement of anesthesia is one of the most problematic factors in preclinical fMRI experiments. Therefore, the main aim of the study was to compare fMRI results obtained under different anesthesia protocols, and explain the results in the light of the present neuropharmacological knowledge. The study was performed in a species not yet studied: the guinea pigs.
2. To utilize DSC to evaluate brain hemodynamic responsiveness in anesthetized rats with 4 different anesthetic protocols (isoflurane, dexmedetomidine, midazolam + isoflurane and midazolam +dexmedetomidine). In this study also FC with rs-fMRI was performed but data analysis is steel ongoing.
3. To describe one possible application of anesthetics drugs (dexmedetomidine and ketamine) as a main treatment for severe neurologic condition such as super refractory status epilepticus.

Epilepsy is a common neurologic condition originating from an imbalance on neurotransmission. Anesthesia, acting principally by modulating neurotransmission, could restore physiologic condition controlling this disease.

RESEARCH PAPERS

The papers published/accepted were reported keeping the style indicated by the guidelines of each Journal.

FUNCTIONAL CONNECTIVITY UNDER DEXMEDETOMIDINE AND ISOFLURANE IN GUINEA PIG BRAIN – A PILOT STUDY

(Although it has been almost completed, the manuscript reported here is still in preparation)

FUNCTIONAL CONNECTIVITY UNDER DEXMEDETOMIDINE AND ISOFLURANE IN GUINEA PIG BRAIN – A PILOT STUDY

Abstract

Background: Guinea pigs are of increasing interest as a human model specially in neuroscience. Different neurological conditions find in guinea pig the more translational model (de Curtis et al. 2016)

Resting state functional magnetic resonance imaging (rs-fMRI) is a translational imaging method with a great potential in several neurobiological application. Most preclinical fMRI experiments are carried out under anesthesia to minimize animal movements and distress (Lukasik and Gilles 2003). Anesthesia, inevitably, introduces a confounding effect on functional connectivity (FC) (Gao et al. 2016). Functional connectivity in guinea pig, unlike rats and mice, have not yet been studied. Therefore, we have compared the influence of isoflurane (ISO) and dexmedetomidine (DEX) on rs-fMRI in guinea pigs.

Methods: Fourteen adult female Dunkin Hartley guinea pigs were anesthetized with ISO and, after a 7 days-wash out period, with DEX. Physiologic parameters were recorded continuously. Magnetic Resonance Imaging acquisitions were performed by 7T preclinical instrument. A coronal gradient-echo echo planar imaging was carried out for Rs-fMRI experiments. Seed-based FC analysis was then performed.

Results: Functional connectivity maps displayed localized bilateral cortico-cortical correlation with respect to the cortical seed in DEX group while, under ISO correlation was stronger and widespread across the brain (cortico-cortical and cortico-subcortical). With regard to the thalamus seed, data showed a widespread correlation with other subcortical and cortical region (ipsilateral and contralateral). Under DEX correlation was found confined within the homolateral thalamic region

and the hippocampal region. Results are mostly in accordance with available literature in rodents and in humans.

Conclusions: Data obtained in this work can be considered a good starting point to increase the role of the guinea pig as preclinical model, especially for all neurologic conditions that required FC studies (epilepsy, Alzheimer's disease).

Introduction

Brain is a complex network of functionally and structurally interconnected regions. Functional communication plays a key role in complex cognitive processes, thriving on the continuous integration of information across different brain regions. Nowadays, task-free resting state functional magnetic resonance imaging (fMRI) studies have shown that functional connectivity (FC) is modulated in different central system disease, during anesthesia and sleeping (Fox and Raichle 2007; Lu and Stein 2014; Paasonem et al. 2018). Despite neuroanatomical and functional organization between species, FC has been recognized not only in humans but also in animals (Biswal and Kannurpatti 2009; Belcher et al. 2013; Sforazzini et al. 2014). Animal models provide a powerful way to investigate normal brain function, pathophysiologic mechanism of central nervous system (CNS) diseases, drugs behaviour and much more in a controlled environment. Nowadays, scientific community is at work to find the best animal model respecting the current legislation on animal testing and all the ethical principles of experimentation. Rodents, particularly rats and mice, are the most employed animal models for brain disease (Chuang and Nasrallah 2017). Until now, guinea pig has been not commonly used for brain studies. Nevertheless, guinea pigs are good compact animals that are easy to handle and share many physiological functions with humans (Wagner and Manning 1976; Terril and Clemons; 1998, Lee et al. 2014). Guinea pig brains are a miniature copy of the human brain at many levels including the Circle of Willis which mirrors in detail to that found in humans (Librizzi et al. 1999). Furthermore, in contrast with mice and rats who born at relatively underdeveloped stages, guinea pigs are “precocial” and have a relatively advanced development of the brain like humans at birth (Verley, 1977; Clancey et al., 2000; Clancey et al., 2007). De Curtis and colleagues (2016) studied guinea pig as a model for human epilepsy, finding an high level of fitting between species. For ethical and practical issues, the vast majority of experiments, including FC studies, are conducted under general anesthesia to minimize stress and movement (Lukasik and Gilles 2003). Furthermore, controlled anesthetic conditions offer potential advantages controlling animal movements, irregular breathing, heart rhythm and stress-related physiological parameters that can affected FC analysis (Grandjean et al. 2014). On the other hand, anesthesia is known to disturb neuronal activity, metabolic and hemodynamic responses, neurovascular coupling and FC (Masamoto and Kanno 2012, Gao et al. 2016). Concerning FC, some functional network may be preserved under anesthesia while other are suppressed (Nallasamy and Tsao 2011). So, it is crucial to identify the effect of different drugs on FC. In rodents preclinical setting, isoflurane (ISO) is one of the standard anesthetics for structural imaging studies, but it is

also being increasingly exploited in functional studies (Lukasik and Gillies 2003). The popularity of ISO in magnetic resonance imaging (MRI) experiments originates from its several advantageous properties: easy drug administration, fast induction and recovery, easily controllable and stable level of anesthesia throughout measurements, and no clear contraindications preventing follow- up studies (Lukasik and Gillies 2003). Nevertheless, decades of studies in this field revealed that ISO cause a severe suppression of neuronal activities decreasing brain connectivity (Fukuda et al., 2013) and promotes the inhibition of neurotransmission-modulated vasodilation (Toda et al. 1992).

Dexmedetomidine (DEX) is a potent and highly selective alpha$_2$-adrenoceptor agonist with sympatholytic, sedative, amnestic, and analgesic properties (Carollo et al. 2008), which has been described as a useful and safe drug in many clinical applications including sedation for diagnostic procedure, particularly in children and in non-collaborative subjects (Plambech and Afshari 2015).

Despite exists a lack of coherence concerning the effect of DEX on FC (Paasonem et al. 2018; Chuang and Nasrallah 2017), it seems that this drug could be more suitable for fMRI studies compare to other anesthetics preserving FC, particularly at low doses (Fukuda et al. 2013).

To the authors knowledge, no studies to date have examined FC in guinea pigs. Furthermore, always to the authors knowledge, no studies have investigated FC under ISO and DEX. Therefore, we investigated FC with resting-state fMRI from naïve guinea pigs under ISO and DEX anesthesia.

Materials and methods

Animals

The animal procedures were performed in accordance with the Italian Laws (D.L.vo 26/2014 and following addition), which enforce EU 63/2010 Directive on the approximation of laws, regulations, and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (Ministry of Health authorization number 897/2018-PR released on November 28th 2018).

Fourteen adult female Dunkin Hartley guinea pigs (Charles River Laboratories Italia srl, Italy) weighing 320-360 g were used in the fMRI experiments. Animals were maintained on a 12/12 h light-dark cycle at 21 ± 2 C° with 50-60 % humidity. Food and water were available *ad libitum.* Each animal was used as a control of itself so, they underwent to rs-fMRI exam both with ISO (first session) and, after a 7 days wash out period, with DEX (second session).

Anesthesia

All guinea pigs were initially anesthetized with ISO (IsoFlo 100%, Zoetis Italia s.r.l) (4% induction and 1.6 to 2.0 % maintenance) delivered in a mixture of air and oxygen (30/70) via a nose cone. Exhaled gas from the guinea pigs was actively vacuumed away from the nose cone via a built-in vacuum line. Following induction, anesthesia was continued according to the subsequent scheme:

ISO : during measurements ISO was maintained at 1.6-2 %

DEX: a bolus of 0.5 mg/kg DEX (Dexdomitor 0.5 mg/mL, Vetoquinol Italia, Bertinoro (FC), Italy) was injected subcutaneously (SC) and ISO was discontinued 5 min afterwards. Continuous infusion of DEX (SC) was started 10 min after bolus injection at a dose of 0.5 mg/kg/h to maintain sedation during the procedure. A mixture of air and oxygen was held until the end of the exam.

All procedures were performed under spontaneous ventilation. Heart rate, respiratory frequency as well as rectal temperature were monitored continuously during the procedures (Small Animals Instruments incorporated, NY USA). Animals' temperature was kept at 36.5 ± 0.5 °C by means of a warm-water circuit integrated into the animal holder. At the end of the exam animals anesthetized with DEX received atipamezole SC (same volume of DEX) and time to recovery was provided.

The systemic physiological parameters values for each animals were calculated by averaging the data points acquired during MRI acquisition, starting from 20 min after induction of anesthesia. The mean values of animals per anesthetic protocol were then averaged. Analysis of physiological parameters were performed with Shapiro-Wilk test (for data normal distribution) and then, with Mann-Whitney U test. A value of $p< 0.05$ was considered significant.

Magnetic resonance imaging

MRI acquisitions were performed by a horizontal-bore 7T preclinical instrument (BioSpec 70/20 USR, Bruker, Ettlingen, Germany) with a 20 cm bore diameter. The scanner is equipped with an actively shielded gradient system with integrated shims set up to 2nd order. The maximum gradient amplitude is 440 mT/m. A cross coil configuration was employed for acquisitions. A 72 mm linear birdcage RF coil was used for radiofrequency excitation and a rat brain surface coil was received signal. The scanner is interfaced to an Avance III console controlled by ParaVision 6.0.1 software (Bruker).

Guinea pigs were scanned in prone position, head was placed with the animal's incisors secured over a bite bar and fixated by ear bars. Ophthalmic ointment was applied to the eyes and small earplugs with external gauze pads were accommodated onto the ears to minimize scanner noise.
A set of three orthogonal T2-weighted images were acquired as anatomical reference and for localized shim volume placement. Rapid Acquisition with Refocused Echoes (RARE) sequences were performed with the following parameters: TR = 3000 ms, TE = 39 ms, contiguous Axial / Coronal / Sagittal slice thickness = 1 mm, in-plane resolution = 0.156 × 0.156 mm^2, RARE factor = 8, number of averages NA = 2, acquisition time 3 mins 48 secs.
fMRI experiments were carried out by coronal gradient-echo Echo Planar Imaging (EPI) sequences. EPI sequence parameters were the following: TR = 1400 ms, TE = 20 ms, Receiver Bandwidth = 300 kHz, 20 contiguous slices acquired in reverse interlaced order, slice thickness = 1 mm, FOV 30 × 20 mm^2, in-plane resolution 0.156 × 0.156 mm^2. Before acquisition, first and second order localized shimming procedure was performed. Optimized fat suppression pulses were employed.
1200 volumes without averaging were acquired in a total acquisition time of 28 minutes.

Data preprocessing and analysis

Pre-processing was performed using SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK) for MATLAB© (The MathWorks, Natick, USA). The first 10 time points were dismissed to account for the T1 relaxation effect. First, functional images were realigned using a rigid-body transformation (6 realignment parameters) and then they were normalized to an in-house Proton Density (PD) template created using the all PD anatomical images of guinea-pigs of this sample. Next, a motion parameter regression was applied on realigned normalized images to reduce motion artifacts. The time series were furthered band-pass filtered between 0.01 Hz and 0.5 Hz using an in house Matlab script.
Seed-based FC analysis was then performed with DPABI Rest toolbox (Yan et al. 2016), using the following regions of interest (ROIs) as seeds: bilateral cortex and thalamus. The ROIs were manually drawn by an expert operator (DG) directly on the PD templates to avoid any co-registration errors. The correlation maps for each seed were computed performing a linear correlation of the mean of time series in seed region with all the other voxels in the entire brain. Correlation maps created with DPABI were subsequently introduced in a with an ANOVA for pair measure design using SPM12. Results are presented as color-coded overlap of group means on an PD template image, with a threshold of $p \leq 0.01$ Family Wise Error corrected, to take account for multiple comparison.

Results

Physiology

The physiological parameters, presented in table 1, are in the normal range for anesthetized animals. Statistical comparison indicated no difference in body weight among groups. The heart rate differs among the groups; lower values were detected in DEX group. Additionally, respiratory rate was lower in ISO group compared with DEX group.

Seed-based analysis

The data analysis revealed intra and inter-hemispheric correlation with a delineation of anatomical structures.

Under DEX, the seed in the left cortex showed an ipsilateral and contralateral FC more circumscriptive at cortical level, while under ISO the same seed presented a widespread FC across different brain structures, cortical and subcortical, both ipsilateral and contralateral. The same pattern was observed for seed in the right cortex.

Under DEX, the FC in the left thalamic seed was confined to whole homolateral thalamic region, mainly, and the bilateral hippocampal region. Under ISO, the pattern of FC in the left thalamic seed was more widespread with other subcortical and cortical regions both ipsilateral and contralateral. The pattern for right thalamus was symmetric compared to left thalamic seed (figure 1).

Discussion

The most important reason for using rodents in research is to model aspects of human physiology and function, most notably to advance our understanding of human diseases and maybe, in future, for animals also. Rodent models have proven invaluable for studying many human conditions, although it should equally be recognized that the translational value of all model systems, especially within the field of neuroscience, is still far from perfect (Ellenbroek and Youn 2016). To date, although not yet popular, guinea pigs are of increasing interest as a human model, especially in neuroscience (de Curtis et al. 2016). In fact, this species of rodents seems to provide a superior and more translational model for human brain studies, if compared with mice and rats; particularly, this superiority is expressed in certain specific neurological pathologies such as Alzheimer's disease (Sharman et al. 2013), epilepsy (De Curtis et al. 2016), behavioral alteration and drugs addiction (e.g.

methanphetamines). Furthermore, guinea pigs are neither nocturnal nor diurnal, eliminating the need for a reversed light-dark cycle. Their intermittent activity and sleep patterns might also make them interesting for research on brain processes and sleep physiology and on sleep disorders (Lee et al. 2014).

In the aforementioned neurologic condition, the study of resting state FC seems to be particularly important and nowadays, many efforts are directed in this direction.

Giving the lack in literature, the aim of the present study was to investigate FC in anesthetized guinea pigs in order to provide preliminary information in a para-physiologic condition (under anesthesia that is quite inevitable in preclinical setting). The study was conducted under ISO that represents the gold standard anesthetic in preclinical environment in order to compare results obtained in mice and rats. Furthermore, DEX was introduced giving its popularity in human medicine particularly in neurology and neuroradiology (Stuart and Sury 2016). Recently, DEX has acquired growing popularity in neuranesthesia thanks to its neuroprotective quality both in young and elderly patients (Wu et al. 2019). Additionally DEX seems to represents an interesting choice in fMRI study in preclinical environment (Williams et al. 2010, Fukuda et al. 2013). Finally, DEX and ISO seem to influence FC in a very different way (Williams et al. 2010). Also physiological parameters are modulated in a different way by this two anesthetic. As expected heart rate was lower in DEX group than in ISO while respiratory rate was higher. Bradycardia is a known side effect of alpha$_2$-agonists (Lukasik and Gilles 2003). Furthermore, DEX induced a lighter level of sedation compared with ISO and does not cause respiratory depression explaining why guinea pigs presented an higher respiratory frequency.

To date, there are no doubts that anesthesia in general, and specifically different anesthetics, affect neural activity, metabolism, cerebrovascular tone, perfusion pressure and cerebral autoregulation altering FC during rs-fMRI studies in a different way (Grandjean et al. 2014).

In fact, data obtained in the present study show a different anesthetic-specific map induced by DEX and ISO on guinea pig FC according with data available in literature on mice and rat (Zhao et al. 2008; Grandjean et al. 2014).

With respect to the cortical-seed, analysis (both left and right) revealed the presence of a widespread inter-hemispheric FC of cortical region under ISO. Subjects anesthetized with DEX displayed a significant intra-hemispheric (ipsilateral) cortico-cortical connectivity, similar to ISO, while inter-hemispheric connectivity resulted weaker and more localized . Data of the present study are in agreement, especially concerning ISO, with results obtained both in mice and in rats;

Grandjean and colleagues (2014), as well as Guilfoyle one year before (2013), reported bilateral connectivity between corresponding cortical regions in mice under iso anesthesia. Similar results emerged also in rats, in which cortico-cortical correlation values obtained under ISO are much more widespread and encompass the entire cortex if compared to medetomidine, an analogue of DEX, in which correlation is high but extremely localized (Williams et al. 2010). Bilateral cortical connectivity under alpha$_2$-agonists is not reported by all authors; Jonckers et al. (2011) and Nasrallah et al. (2014) described a unilateral cortical connectivity in mice using high dose of medetomidine. The explanation of this discrepancy could reside in the band-pass filter applied by the author (0.01-0.1 HZ) or in the dose of medetomidine; lower dosages are associated with the presence of bilateral connectivity while an high dose is correlated with the persistence of only unilateral FC (Nasrallah et al. 2014). Finally, regarding cortico-cortical connectivity for both data obtained in the present study on guinea pigs and data available in literature on other rodents (e.g. Williams et al. 2010), results under DEX seem to be similar to the one obtained using a seed-based correlation in awake humans (Cordes et al.2001).

Furthermore, in guinea pigs anesthetized with ISO emerges a widespread (ipsilateral and contralateral) correlation between cortical and subcortical regions with respect to the seed in cortex; this correlation was not present in DEX group. These data are partially in agreement with analysis performed by Grandjean and colleagues (2014) in which correlation between somatosensory cortex and thalamus was weaker.

According to the vast majority of literature on different species, including mice (Grandjean et al. 2014), rats (Zhao et al. 2008, Fukuda et al.2013), and humans (Akeju et al. 2014), no resting state FC was detected between cortical regions and the seed in the thalamus under DEX. This seems to be an hallmark of FC alterations induced by alpha$_2$-agonist. The specific pharmacological mechanism of each anesthetics elicits specific neural response. The effects of alpha$_2$-agonist on neural function are different from those of the other anesthetics and this could reflect the unique feature on FC pattern. The target of this pharmacological class of drugs is the alpha$_2$ adrenoreceptor, whose density is different across the brain. Thalamus, for example, shows an high density of this receptor while other region display intermediate (cortex) or low (caudate putamen) concentration. This distribution may explain why thalamo-cortical FC is frequently suppressed under DEX while, for example, bilateral cortex connectivity is maintained (Grandjean et al. 2014). Furthermore, in the present study, the largest part of the correlation between thalami in the two hemispheres is suppressed and this was also reported in another study (Zhao et al. 2008). This seems to support

the idea that, under DEX, also in guinea pigs, the thalamus in completely "blocked" and FC is suppressed in all direction. However, it is important to remember that a lack of FC does not mean a lack of network connection (Zhao et al. 2008).

In contrast with behavior under DEX, both thalamocortical and interthalamic FC was detected bilaterally under ISO; unregressed analysis show a widespread pattern of correlation across cortical and subcortical regions in ISO group. A quite similar finding was described in rodents (Williams et al. 2010) . Furthermore, a similar FC pattern was described by Grandjean and colleagues in 2014 under propofol, a GABA-ergic drugs used to induced and maintained general anesthesia both in humans and in animals. Compared to ISO and/or propofol, anesthetic depth is lower under DEX. Dose-dependence studies using rs-fMRI combined with electrophysiological recording performed in rats under ISO (Liu et al., 2013b) and under propofol (Liu et al., 2013a) substantiate the concept that, in deeply anaesthetized animals, the spatial confinement of correlated regions disappears at the expense of unspecific widespread synchronicity across large cortical areas. Despite the suppression on neural activity expected in rodents under 2% of ISO (doses importantly above the minimum alveolar concentration in all rodents), widespread correlation was observed in MRI signal. William and colleagues (2010) tried to explain this phenomenon justifying that a general increase in the amplitude of cerebrovascular fluctuations could result in correlation less localized then in medetomidine-anesthetized rats.

This study represents a preliminary investigation on FC under anesthesia in guinea pigs. This rodent species, still poorly studied, seems to be a good alternative for FC studies. Several features reported from human rs-fMRI investigations are reproduced also in this study; Stamatakis et al. (2010) observed loss of spatial confinement of the default mode network and motor networks linked to anesthesia, similar to our observations of widespread cortical synchronization with higher doses of ISO while Akeju and colleagues (2014) reported that during DEX induced-unconsciousness cortico-cortical FC is maintained while thalamo-cortical FC is lost.

In the present work, guinea pigs were not mechanically ventilated and consequent not paralyzed; this choice was made attempting to reproduce MRI conditions in humans. The vast majority of MRI studies that required sedation (children, uncollaborative adults, drugs-induced coma etc..) are performed in a state of mild sedation with the patient in spontaneous ventilation (Stuart and Sury 2016).

The literature on this filed, despite in continuous growing, remains controversial with regards to some aspects. Data obtain in the present study are broadly in agreement with what previously

described in rats and mice, indicating the presence of a FC common general trend among rodents under anesthesia. However, before drawing conclusions, more studies, investigating more complex connectivity networks, different dosages of the same drugs and different combinations of drugs are required.

In the light of the data obtained, authors can conclude that, although no major macroscopic differences were found regarding FC compared to rats and mice, the guinea pigs showed peculiarities of species that, could deserve future studies in order to expand the literature also in this species which, as mentioned above, for anatomical and physiological differences could represents a model closer to humans.

	Weight (g)	Heart rate (bpm)	Respiratory rate (bm)
Iso	319±35	255±23	31±5
Dex	328±40	186±14	60±9

Table 1: *physiologic parameters obtained from guinea pigs that underwent resting-state functional magnetic resonance imaging (bpm = beats per min; bm= breaths per min)*

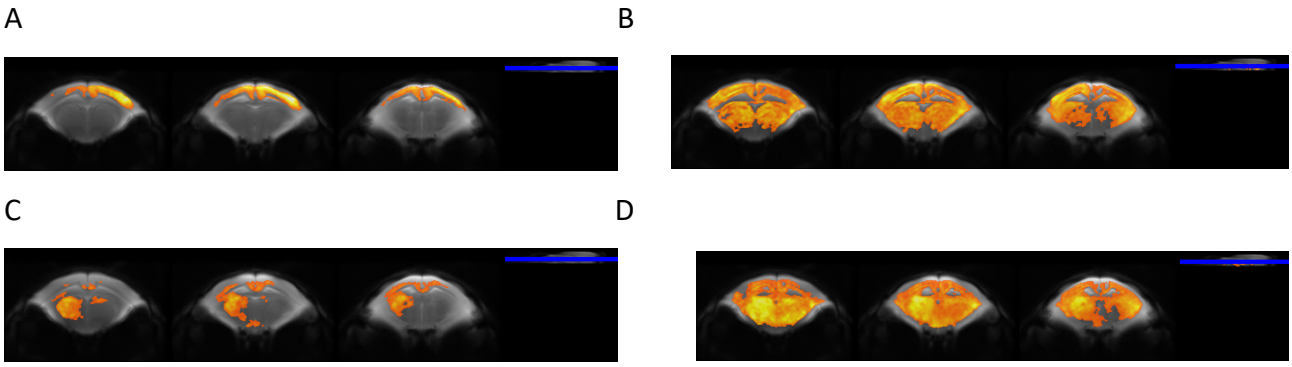

Figure 1*: seed-based analysis: data for the different anesthetics regimes. Images display region with correlation coefficients exceeding the threshold value for seed in the cortex (A and B) and in the thalamus (C and D). Analysis of the dexmedetomidine group for cortical seed (right cortex in the picture A) revealed circumscriptive ipsilateral and contralateral correlation. In isoflurane group (right cortex seed in picture B) a widespread correlation across the cortex and with the subcortical regions was detected. Concerning thalamus seed (left thalamus in the picture C) for dexmedetomidine, the correlation were found confined within the ipsilateral thalamic region and the hippocampus. In isoflurane group a widespread correlation across most brain structures was detected.*

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REGIONAL CEREBRAL BLOOD FLOW AND CEREBRAL BLOOD VOLUME UNDER FOUR ANESTHETIC PROTOCOLS MEASURED WITH DYNAMIC SUSCEPTIBILITY CONTRAST MAGNETIC RESONANCE IMAGING IN RATS

Regional cerebral blood flow and cerebral blood volume under four anesthetic protocols measured with dynamic susceptibility contrast magnetic resonance imaging in rats

This is a part of work concerning functional connectivity under four anesthesia protocols in rats brain. Data analysis is still ongoing. For this reason, here are presented only data concerning dynamic susceptibility contrast MRI in a form of short communication

Short communication

Measurements of brain hemodynamic parameters, an indirect tools for determination of neuronal functionality, can be performed by using tools based on magnetic resonance imaging (MRI), positron emission tomography (PET) or single photon emission computed tomography (SPECT) (Waelbers et al. 2012).

Dynamic Susceptibility Contrast MRI (DSC – MRI), an exogenous technique used to study the hemodynamic properties of the tissue (Ostergaard et al., 1996), is the most common diagnostic technique apply in clinical settings. This particular imaging approach uses T2*/T2 signal decay caused by the passage of the contrast to indirectly estimate a) the cerebral blood volume (CBV), which indirectly represents the vascular density of the capillary bed; b) the CBF, the blood flow inside the capillary bed; c) the mean transit time (MTT) which represents the time necessary for the contrast medium to pass through the capillary bed (Ostergaard et al., 1996).

Rodents are important tools for translational neuroscience and neurology. Although MRI studies with awake animals have been performed (Jonkers et al. 2014; Paasonem et al.2018), this approach is often tied to ethical and technical restrictions that heavily restrict practical application. Therefore, rodents MRI is commonly performed under anesthesia. However, anesthesia protocols are known to influence cerebral hemodynamics (Munting et al.2018). In order to more clearly relate MRI measures of brain hemodynamics obtained from rat models to clinical data, and for meaningful

comparison with other preclinical studies, it is important to understand the hemodynamic influence of the different anesthesia protocols used.

Isoflurane (ISO) is the most widely used anesthetic in rodents imaging study (Munting et. 2018); it is reported that ISO, due to its vasodilating effect, can increase CBF and CBV (Drummond et al. 1986). In contrast, dexmedetomidine (DEX), an alpha$_2$-adrenoreceptor agonist, seems to decrease CBF and CBV thank to its vasoconstrictive property (Grandjean et al. 2014). Nevertheless, recently, with the introduction of more specific and precise methods for brain investigation, some contradictions concerning DEX action on CBF are introduced (Waelbers et al. 2012).

The use of benzodiazepines, such as midazolam (MDZ) induces a decrease in CBF and CBV globally, along with a reduction of brain glucose consumption due to a lower metabolic demand. Based on a study by Veselis et al. (1997), MDZ sedation does not only cause a global decrease in CBF but also produces significant regional changes that are likely to represent the neural structure affected by this drug.

The purpose of the present study, was to investigate the influence of four different anesthetic protocols on regional CBF and CBV through the administration of gadolinium based contrast medium (GD-CM) in experimental rats. For this purpose, 24 adults female Sprague Dawley rats (Charles River Laboratories Italia srl, Italy) weighing 190-220 g were used. Animals were maintained on a 12/12 h light-dark cycle at 21 ± 2 C° with 50-60 % humidity. Food and water were available *ad libitum.* The animal procedures were performed in accordance with the Italian Laws (D.L.vo 26/2014 and following addition), which enforce EU 63/2010 Directive on the approximation of laws, regulations, and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (Ministry of Health authorization number 897/2018-PR released on November 28th 2018). Rats were divided in four different groups depending on the anesthetic protocol as describe below.

All rats were initially anesthetized with ISO in a 60% O_2/40% air mixture: 3.5% for induction and 2 % during animals preparation. A 24 G cannula was placed in the tail vein for intravenous (IV) GD-CM administration. Subsequently, anesthesia was switch, depending to the group, to one of the following protocols:

- Isoflurane: during measurements ISO (IsoFlo 100%, Zoetis Italia s.r.l) was maintained at 1.5%
- Dexmedetomidine: a bolus of 0.1 mg/kg DEX (Dexdomitor 0.5 mg/mL, Vetoquinol Italia) was injected subcutaneously (SC) and ISO was discontinued 5 min afterwards.

- Midazolam + isoflurane: a bolus of 10 mg/kg MDZ (Midazolam-Accord 5 mg/ml, Accord health care, italia s.r.l) was injected SC and ISO was decreased to 0.5% and was maintained at this value during measurements.
- Midazolam + dexmedetomidine: a bolus of 10 mg/kg MDZ (Midazolam-Accord 5 mg/ml, Accord health care, italia s.r.l) in association 0.05 mg/kg of DEX (Dexdomitor 0.5 mg/mL, Vetoquinol Italia) was injected SC and ISO was discontinued 5 min afterwards.

All procedures were performed under spontaneous ventilation. Heart rate, respiratory frequency as well as rectal temperature were monitored continuously during the procedures (Small Animals Instruments incorporated, NY USA). Animals' temperature was kept at 36.5 ± 0.5 °C by means of a warm-water circuit integrated into the animal holder. At the end of MRI exams an arterial blood gas analysis was perform for $PaCO_2$ monitoring (caudal artery).

For DSC analysis a GD-CM (Gd-HP-DO3A) ProHance® (Bracco Imaging Italia spa Milan, Italy) was injected IV at the dose of 0.1 mmol/kg while running a gradient-echo Echo Planar Imaging (EPI).

The systemic physiological parameters values for each animals were calculated by averaging the data points acquired during MRI acquisition (every 5 min), starting from 15 min after induction of anesthesia. The mean values per anesthetic protocol were then averaged (table 1).

MRI acquisitions were performed by a horizontal-bore 7T preclinical instrument (BioSpec 70/20 USR, Bruker, Ettlingen, Germany) with a 20 cm bore diameter. The scanner is equipped with an actively shielded gradient system with integrated shims set up to 2nd order. The maximum gradient amplitude is 440 mT/m. A cross coil configuration was employed for acquisitions. A 72 mm linear birdcage RF coil was used for radiofrequency excitation and a rat brain surface coil was received signal. The scanner is interfaced to an Avance III console controlled by ParaVision 6.0.1 software (Bruker).

Dynamic Suceptibility Contrast MRI experiments were carried out by coronal gradient-echo Echo Planar Imaging (EPI) sequences.

Echo Planar Imaging sequence parameters were the following: TR = 750 ms, TE = 21 ms, Receiver Bandwidth = 200 kHz, 12 contiguous slices acquired in reverse interlaced order, slice thickness = 1 mm with 0.250 mm gap, FOV 18 × 13 mm^2, in-plane resolution 0.2 × 0.2 mm^2. Linear *k*-space filling was performed in 2 segments. Before acquisition, first and second order localized shimming procedure was performed. Optimized fat suppression pulses were employed.

100 volumes without averaging were acquired in a total acquisition time of 2 mins 30 secs.

Gadolinium-based contrast medium was injected immediately after a 10 volume baseline acquisition.

The DSC volumes were analyzed with a Matlab proprietary software. The software corrected the raw data for motion (using a rigid-body transformation) and distortion artifacts, masked the animal brains and, with an automatic method, calculated the Arterial Input Function (AIF), i.e. the concentration-time curve of the arteries, necessary to estimate the perfusion values. From the corrected data two maps were estimated: i) CBV and ii) CBF.

Regions of interest (ROI) were drawn by an expert neuroradiologist on the T2w volumetric images of the brains, previously coregistered with the DSC data. Regions of interest were drawn on cortex, thalamus, striatum, hippocampus and in the contralateral white matter. Region of interest are based on literature and adapt for the present study (Grandjean et al. 2014) (figure 1).

Values of DSC and physiologic parameters, including $PaCO_2$, are expressed as a mean and standard deviation (table 1 and table 2).

Values of CBV, CBF were paired compared for each anatomic region between different anesthetic protocols. Data were analyzed using a Wilcoxon rank-sum test for independent data with a significance set at $p < 0.05$.

Statistical analysis of CBV and CBF did not show any significative differences between the four protocols (table 2).

Physiologic parameters remain in a range of normality for sedated rats.

Despite, nowadays, there are a growing interest in functional MRI (fMRI) studies and despite hemodynamic component represents a fundamental of fMRI investigation, to date, hemodynamic studies remain almost not widely explored, especially in rodents. Furthermore, because anesthesia could introduce a potential confounding elements, a better understanding of the physiological influence of different anesthetics is required. Despite our results seem to be in contrast with literature, especially for DEX, is it true that a real comparison is not always possible: in fact, to the author knowledge, there are only a few number of studies that partially investigated hemodynamic distribution under anesthesia with DSC (Grandjean et al. 2014).

It is commonly reported that DEX reduces CBF, CBV and CBF velocity (Drummond et al. 2008); nevertheless, many studies are conducted in humans subjects and not in rodents and CBF was investigated with different diagnostic techniques (e.g. transcranial doppler, PET, SPECT etc). Furthermore, in the vast majority of the studies, the comparison is between the awake condition and sedation and not between different anesthetics.

The most comparable study, conducted on mice, described lower CBV in mice under medetomidine, an analogue of DEX, compared with propofol, ISO and urethane.

Prielipp and colleagues (2002) described a decrease in both global and regional CBF in humans under DEX if compared to the baseline. Assuming that may exist differences between humans' and rat's brain that can justify the difference in CBF under DEX (e.g alpha$_{2B}$ -receptor density on vassels), another explanation could reside in the fact that, in the study of Prielipp, a loading dose of DEX was administered IV before constant infusion. It is possible that the DEX loading dose maximally stimulated the receptors mediating cerebral vasoconstriction in a more evident way compare to results of the present study in which DEX was administered through a subcutaneous bolus. While a study conducted on mice have reported a significant difference between CBF under ISO and DEX, with lower values for the second one (investigated with arterial spin labeling), data emerging from a study by Waelbers and colleagues (2012) were different. They have studied regional blood flow in cats with SPECT exams under medetomidine alone or in association with ketamine. In this study, the tracer (^{99m}Tc-ECD) presents an higher uptake in cats sedated with medetomidine if compared with awake animals suggesting, perhaps, an higher CBF under sedation. Even though literature is yet poor, Wealbers and colleagues (2012) reported the possibility of medetomidine-mediated alterations of the pharmacokinetics and pharmacodynamics of the tracer. Consequently, it is not to exclude that DEX, such as all other anesthetics used in the present study, could have altered GD-CM distributions.

It was not possible to investigate the effects of MDZ administered alone because, in the present study, it was not able to induce a sufficient level of sedation to perform MRI exam despite different attempt with increasing doses. So, it was administered in association with DEX or ISO. Value of CBF, and CBV in both MDZ groups are quite similar to value obtained under ISO (for MDZ+ ISO) and under DEX (for MDZ+ DEX) in all regions.

A study conducted in 2012 by Liang et al. has demonstrated that the contrast of MRI signal, using arterial spin labelling perfusion, before and after midazolam administration, revealed a decrease in CBF in the left dorsolateral prefrontal cortex, left cingulate gyrus and left posterior cingulate gyrus/precuneus. These results are likely to represent the neural structure affected by this drug.

Again, the comparison is done between awake and sedation and a reduction of hemodynamic parameters is reasonable in sedated patient, if compared with the awake one, suggesting a decrease in cerebral activity. Furthermore, in the present study, because MDZ was not administered alone, is it possible to see only the sum of the effects induced by MDZ in association with ISO or DEX.

This study presents several limitations; data presented are partially incomplete. A more specific interpretation will be possible when results will be compared with data resulting from a fMRI analysis. The hemodynamic response mediated via neurovascular coupling depends on the cerebrovascular baseline state, which is typically affected by the anesthetic regimen. The knowledge of hemodynamic alterations is fundamental for a better interpretations of fMRI results. Theoretically, vasodilation (e.g. ISO) may reduce the adaptive capacity of vessels, i.e. the amplitude of blood oxygenation level dependent (BOLD) fMRI signal fluctuations and, consequently, the apparent functional connectivity. In contrast, the vasoconstriction effect of alpha$_2$-agonists reduce vascular reactivity. The compromised vascular reactivity could translate into smaller amplitudes of BOLD signal fluctuations and, consequently, reduced functional connectivity values. Nevertheless, the study conducted by Grandjean et al. (2014) shows that this assumption is not always true: reduction in CBV (as they have detected in medetomidine group) does not correspond to a decrease in functional connectivity.

Finally, $PaCO_2$ is a stronger regulator of CBF and a linear-like correlation exists between CBF and $PaCO_2$. In the present study arterial blood gas analysis was performed at the end of the procedure. $PaCO_2$ remains between physiological range in all anesthetics group, so, its contribute in CBF regulation is not remarkable. Supporting data was obtained in forepaw stimulation experiments, where BOLD and CBF responses with mild acidosis (pCO2 49 mmHg, pH 7.30) were comparable to those under normal conditions (Sicard and Duong 2005). In contrast, the response has been almost entirely eliminated in animals exhibiting severe acidosis (pCO2 70 mmHg, pH 7.15) (Sicard and Duong 2005). In the present work, rats were not mechanically ventilated and consequent not paralyzed; this choice was made attempting to reproduce MRI conditions in humans. The vast majority of MRI studies that required sedation (children, uncollaborative adults, drugs-induced coma etc..) are performed in a state of mild sedation with the patient in spontaneous ventilation (Stuart and Sury 2016). Arterial blood gas parameters suggest good gas exchange in all anesthetic protocols despite spontaneous ventilation. Furthermore, also lungs and peripheric perfusion seems to be preserved; $PaCO_2$, SO%, and lactate are in the physiologic range.

To conclude, all anesthetics protocols used in the present study, seem able to preserve CBF and CBV in a comparable way. More study are required to confirm these preliminary results.

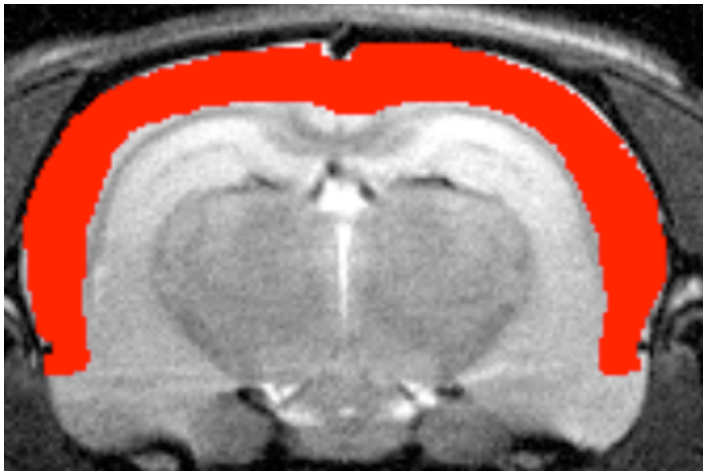

Figure 2: example of a ROI drawn on the cortex on the T2w volumetric images of the rat brains, previously coregistered with the Dynamic Susceptibility Contrast magnetic Resonance Imaging data.

	HR (bpm)	RR (bpm)	T (°C)	PaCO2(T)	pH(T)	lac (mmol/L)	SO2%
ISO	*390±25*	*50±9*	*36.4*	*36±2*	7.44±0.01	*1.1±0.4*	*99.8±0.1*
DEX	*265±22*	*39±10*	*36.7*	*44±2*	7.36±0.03	*0.8±0.1*	*99.9±0.1*
MDZ+ISO	*400±12*	*47±8*	*36.5*	*41±2*	7.42±0.01	*0.8±0.1*	*99±0.1*
MDZ+DEX	*250±14*	*49±12*	*36.4*	*47±3*	7.34±0.01	*0.7±0.1*	*99.8±0.1*

Table 2: Table 1: physiologic parameters obtained from rats underwent DSC-MRI. HR=heart rate express in beats per min; RR=respiratory rate express in breaths per min; T= body temperature express in Celsius degree. Arterial blood gas values are standardized for the temperature (T). lac=lactate

CBF

	VALUES				P-VALUE					
ROI	**ISO**	**DEX**	**MDZ + ISO**	**MDZ + DEX**	**ISO vs DEX**	**ISO vs MDZ + ISO**	**ISO vs MDZ + DEX**	**DEX vs MDZ + ISO**	**DEX v sMDZ + DEX**	**MDZ + ISO vs MDZ + DEX**
cortex	*1.46±0.38*	*1.40±0.24*	*1.23±0.48*	*1.33±0.2*	*0,8665*	*0,3357*	*0,3357*	*0,1949*	*0,5358*	*0,8665*
thalamus	*1.57±0.25*	*1.36±0.24*	*1.43±0.19*	*1.43±0.2*	*0,0721*	*0,2810*	*0,2086*	*0,4418*	*0,6943*	*0,7789*
striatum	*1.25±0.25*	*1.32±0.18*	*1.25±0.21*	*1.30±0.32*	*0,6200*	*0,8665*	*0,9452*	*0,5358*	*0,7308*	*0,6620*
hippocampus	*1.22±0.19*	*1.12±0.26*	*1.13±0.18*	*1.16±0.17*	*0,3829*	*0,3969*	*0,9452*	*0,4634*	*0,8357*	*0,8357*

CBV

	VALUES				P-VALUE					
ROI	**ISO**	**DEX**	**MDZ + ISO**	**MDZ + DEX**	**ISO vs DEX**	**ISO vs MDZ + ISO**	**ISO vs MDZ + DEX**	**DEX vs MDZ + ISO**	**DEX vs MDZ + DEX**	**MDZ + ISO vs MDZ + DEX**
cortex	*1.14±0.2*	*1.13±0.13*	*0.99±0.37*	*1.14±0.13*	*0,9551*	*0,5358*	*1,0000*	*0,2786*	*0,7789*	*0,5358*
thalamus	*1.25±0.10*	*1.12±0.15*	*1.13±0.07*	*1.22±0.18*	*0,1520*	*0,0721*	*0,3829*	*0,8785*	*0,3969*	*0,4634*
striatum	*1.11±0.15*	*1.12±0.1*	*1.11±0.16*	*1.16±0.20*	*0,8000*	*0,6943*	*0,9452*	*0,6943*	*0,9452*	*0,7340*
hippocampus	*1.04±0.13*	*0.98±0.22*	*0.93±0.14*	*1.02±0.14*	*0,3176*	*0,1206*	*0,8357*	*0,9551*	*0,4452*	*0,2284*

Table 2:regional cerbrebral blood flow (CBF) and blood volume (CBV) (mean ± SD) assessed with Dynamic Susceptibility Contrast magnetic Resonance Imaging in rats, with a comparison between four different anesthetic protocols. P-values for the comparison of different anesthetic protocols are reported.

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KETAMINE-DEXMEDETOMIDINE COMBINATION AND CONTROLLED MILD HYPOTHERMIA FOR THE TREATMENT OF LONG-LASTING AND SUPER REFRACTORY STATUS EPILEPTICUS IN THREE DOGS SUFFERING FROM IDIOPATHIC EPILEPSY

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Ketamine-dexmedetomidine combination and controlled mild hypothermia for the treatment of long-lasting and super refractory status epilepticus in three dogs suffering from idiopathic epilepsy

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Ketamine-dexmedetomidine combination and controlled mild hypothermia for the treatment of long-lasting and super refractory status epilepticus in three dogs suffering from idiopathic epilepsy

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"Novel treatment for canine status epilepticus"

Abstract

Objective - To describe the use of a ketamine-dexmedetomidine combination and mild hypothermia for the treatment of status epilepticus in 3 dogs that did not respond to GABAergic medication.

Case series summary – Three dogs, each with a diagnosis of idiopathic epilepsy, presented at the emergency department in a state of status epilepticus. The dogs were treated unsuccessfully with benzodiazepine as a first-line therapy that was followed by intravenous propofol anesthesia maintained for at least 12 hours. When general anesthesia was discontinued seizures reoccurred. All 3 dogs then received a bolus of ketamine (1 mg/kg IV) over a period of 5 minutes that was followed by a bolus of dexmedetomidine (3 μg/kg IV) over the same time period and then followed by a continuous infusion for 12 hours of ketamine at a constant rate of 1 mg/kg/h and dexmedetomidine at a variable rate of 3-7 μg/kg/h. Body temperature was maintained between 36.7 and 37.7°C at a state of mild hypothermia throughout treatment. The dogs recovered uneventfully over 48 hours after treatment was discontinued with no evidence of seizures. No notable alterations in physiologic parameters were observed during the drug infusions. All dogs were discharged following examinations that showed normal neurological function.

New or unique information provided – This series of case studies highlights the potential benefits of a ketamine-dexmedetomidine infusion combined with mild hypothermia for the treatment of status epilepticus refractory to GABAergic therapy in dogs suffering from idiopathic epilepsy. After the dogs were weaned from the ketamine-dexmedetomidine infusion, all dogs experienced complete recovery. Thus, this case series introduces a novel approach to treat this intense condition.

Key words*:* status epilepticus, ketamine, dexmedetomidine, hypothermia, dogs

Abbreviations

DEX: Dexmedetomidine

GABA: γ-aminobutyric acid

HR: Heart Rate

IBP: Invasive Blood Pressure

IPPV: Intermittent Positive Pressure Ventilation

KET: Ketamine

MRI: Magnetic Resonance Imaging

NMDA: N-methyl-D-aspartate

PPF: Propofol

RR: Respiratory Rate

RSE: Refractory Status Epilepticus

SE: Status Epilepticus

SRSE: Super-Refractory Status Epilepticus

TIVA: Total Intravenous Anesthesia

Introduction

Status epilepticus (SE), refractory status epilepticus (RSE), and super-refractory status epilepticus (SRSE) are life-threatening conditions that require a prompt, aggressive and dynamic treatment approach. However, pharmacologic management of these seizures represent a challenge in clinical practice[1]. Status epilepticus can be defined as a continuous convulsive seizure lasting more than 5 minutes or the occurrence of 2 or more epileptic seizures between which there is incomplete recovery of consciousness[2]. Refractory SE is defined as SE unresponsive to first-line anticonvulsant therapy that requires general anesthesia to control seizures; SRSE is defined as SE continuing or reoccurring more than 24 hours after initiation of anesthetic treatment[3].

Drug resistance in SE originates from functional and molecular maladaptive changes. There is a loss of inhibition mediated by γ-aminobutyric acid (GABA) and a coexisting upregulation of excitatory N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors for glutamate[4]. GABAergic drugs are commonly used in the initial stages of SE, but their effectiveness tends to decrease over time due to fewer synaptic targets. Therefore, administration of drugs with different mechanisms of action is fundamental to treat RSE and SRSE[1]. Ketamine (KET), an NMDA receptor antagonist, has been used for this purpose in preclinical models and in humans[1,5]. Ketamine decreases excitatory glutamate activity that is overexpressed during RSE and SRSE, and has neuroprotective properties which block NMDA receptor-mediated glutamate excitotoxicity and cell death[6]. In veterinary medicine, there is only one case report in which KET was used to manage RSE in a dog[7]. The noradrenergic nervous system appears to regulate seizure activity, especially through α_2-adrenoreceptors. Both guanfacine and dexmedetomidine, α_2-adrenoreceptor agonists, exhibited anticonvulsant properties in a rodent model[8,9]. Dexmedetomidine (DEX) decreases sympathetic stimulation and attenuates noradrenaline release, lowering brain excitatory neurotransmitters[10]. Moreover, DEX has neuroprotective properties[11] due to its cerebrovascular and cerebral metabolic effects. The cerebral vasoconstriction that DEX induces may control the development of cerebral edema that can be secondary to seizures[12].

The purpose of this case series is to highlight the potential effectiveness of a ketamine-dexmedetomidine (KET-DEX) combination infusion for the treatment of SE refractory to GABA-ergic medication in dogs.

Case 1

A 6-year-old male Bernese Mountain Dog, weighting 43 kg, presented with SE. Clinical history revealed that the dog had suffered from idiopathic epilepsy for 4 years, which was confirmed by normal magnetic resonance imaging (MRI) and CSF exams. For the last 2 years, the dog was receiving phenobarbital[a] therapy (2 mg/kg, PO, q 12 h). During the last 2 months, epileptic seizures had increased in frequency, and 2 days prior to presentation, the patient was having 2-3 generalized epileptic seizures daily followed by ataxia and marked lethargy lasting for at least 1 hour. The medical history stated that the dog had presented with intermittent convulsive epileptic seizures for more than two hours, with incomplete recovery of consciousness. At admission, generalized convulsive seizures, vertical nystagmus, tachycardia (180 beats/min), short capillary refill time, 48 breaths/min and marked sialorrhea were detected. Body temperature was 39.8°C. Diazepam[b] was administered rectally (0.5 mg/kg). Blood gas analysis[c] and CBC[d] did not show remarkable variations and biochemistry[e] showed only an increase in creatine kinase (3199 U/L, reference interval 40-150 U/L) and a mild increase in AST (120 U/L, reference interval 10-44 U/L). Serum phenobarbital concentration[f] was 90.9 µmol/L (21.1 mg/L) (therapeutic range: 43-172 µmol/L [10-40 mg/L]). Echocardiographic examination[g] excluded any cardiac disease. Lactated Ringer's solution[h] (5 ml/kg/h) and supplemental flow-by oxygen were provided. Cooling management was initiated and a urinary catheter[i] was placed. Ten minutes later, seizures occurred again. Diazepam (0.5 mg/kg IV) was administered but failed to control the seizures and the patient remained unresponsive. After obtaining informed consent, endotracheal intubation was accomplished with general anesthesia induced with propofol[j] (PPF) (3.2 mg/kg IV) titrated-to-effect and maintained for 12 hours with total intravenous anesthesia (TIVA) (mean 0.28 ± 0.12 mg/kg/min). Phenobarbital[k] was also administered (2 mg/kg, IM, q 12 h). Two hours later, respiratory depression and hypercapnia occurred ($PaCO_2$ = 65 mmHg, 3 breaths/ min); intermittent positive pressure ventilation (IPPV) was initiated (FiO_2 = 0.4) to maintain end-tidal carbon dioxide and PaO_2 within normal range. No visible seizures were detected during PPF TIVA, but at discontinuation epileptic seizures occurred again in the form of 3 generalized tonic-clonic epileptic seizures lasting between 2 and 4 minutes within 40 minutes. The patient then received a loading dosages of KET[l] (1 mg/kg IV) followed by DEX[m] (3 µg/kg IV) over a 5-minute period. These drugs were maintained as an infusion for 12 hours at 1 mg/kg/h for KET and at a variable rate from 3 to 7 µg/kg/h (mean 4.9 ± 1.2 µg/kg/h) for DEX. The ketamine-dexmedetomidine combination resulted in cessation of visible seizures. During KET-DEX infusion, the dog displayed moderate to deep sedation. However, endotracheal intubation and IPPV were

not required based upon the presence of a swallowing reflex, mild palpebral reflex, and PaO_2 and $PaCo_2$ levels within normal range. During treatments, heart rate (HR), respiratory rate (RR), invasive blood pressure (IBP), and ECG[n] were recorded every 15 minutes. Urinary production, measured every 4 hours, did not show remarkable alterations (Table 1). Arterial blood gas analysis and blood glucose concentration[c], which were checked every 4 hours, remained within normal limits. Body temperature was maintained between 36.7° and 37.7° C. The administration of the KET-DEX infusion was suddenly stopped.

Twelve hours after KET-DEX discontinuation, the patient was seizure-free and able to eat voluntarily. Phenobarbital was administered (2 mg/kg, PO, q 12 h). Motor function recovered completely 24 hours later. Four days after initial hospitalization, the neurologic examination was normal with no evidence of seizures and the dog was discharged with phenobarbital therapy (2 mg/kg PO, q 12 h). Further seizures were not reported, and a follow-up visit 14 days later was unremarkable. Serum phenobarbital concentration was within the therapeutic range.

Case 2

An 8-year-old spayed female Labrador Retriever, weighting 32 kg, presented with multiple epileptic seizures occurring over the previous 2 days. The owner reported occasional seizures (1-2 generalized epileptic seizures/year) since the dog was 2 years of age and no antiepileptic therapy was ever administered. At presentation, the dog was conscious and the physical exam, blood test analysis and echocardiographic exam, were unremarkable. The patient was hospitalized, and an MRI exam was scheduled. One hour after hospitalization, generalized tonic-clonic seizures with loss of consciousness lasting more than 5 minutes were observed. Emergency treatment consisted of midazolam[o] (0.5 mg/kg IV) repeated twice. Lactate Ringer's solution[h] was administered (3 ml/kg/h IV), and a urinary catheter[i] was placed. Forty minutes later, generalized tonic-clonic seizures occurred again, and after obtaining the owner's consent, general anesthesia was induced with PPF[j] (5 mg/kg IV) titrated-to-effect. Rectal body temperature was 39.9° C and cooling management was started to induce mild hypothermia (36.7°-37.7° C). The patient was intubated and mechanically ventilated (IPPV; FiO_2 = 0.4) to maintain end-tidal carbon dioxide and PaO_2 within normal range. General anesthesia was maintained for 24 hours with PPF[j] TIVA (0.44 ± 0.18 mg/kg/min) and resulted in cessation of visible seizures. A decision was made not to perform MRI and CSF exams during the symptomatic phase. A phenobarbital[k] loading dosage was initiated (4 mg/kg, IV, q 6 h, 4 times) and then set at 3 mg/kg IM twice daily. No improvements were recorded when PPF TIVA was

discontinued as persistent twitching and paddling were noted. Therapy with KET-DEX was then initiated through a simultaneous infusion of both drugs for 12 hours. A loading dosage of KET[l] (1 mg/kg IV) and DEX[m] (3 µg/kg IV) was slowly administered over a period of 5 minutes, and then KET was infused at 1 mg/kg/h while DEX was infused at a variable rate between 3 and 5 µg/kg/h (mean 4.4 ± 0,79 µg/kg/h). The ketamine-dexmedetomidine infusion resulted in the cessation of visible seizures, and the dog exhibited moderate to deep sedation; endotracheal intubation and IPPV were not necessary as evidenced by the presence of the swallowing reflex, mild palpebral reflex, and PaO_2 and $PaCo_2$ within normal range. During treatments, HR, RR, IBP, and ECG[n], recorded every 15 minutes, as well as urinary production measured every 4 hours, did not present remarkable alterations (Table 1). Arterial blood gas analysis and blood glucose concentration[c], checked every 4 hours, remained within normal limits. Controlled mild hypothermia was maintained during treatments. The administration of KET-DEX infusion was suddenly stopped.

Twelve hours after KET-DEX discontinuation, the dog was awake, and the physical examination, CBC[d] and serum biochemistry[e] were normal. The dog ate voluntarily and phenobarbital[a] was administered orally (3 mg/kg q 12 h). After 48 hours of observation without further seizures, the dog was discharged. At visits 15 and 45 days later, the owners did not report any neurological signs of seizures, and physical examinations were unremarkable. During the first visit (15 days after discharge), brain MRI and CSF exams were performed under general anesthesia and confirmed the diagnosis of idiopathic epilepsy. Brain MRI was unremarkable and CSF protein content and leucocyte count were within the physiologic range. Serum phenobarbital concentration[f] was within the therapeutic range. Clinical follow-up was interrupted 45 days after discharge due to lack of information from the owner.

Case 3

A 7-year-old mixed-breed male dog, weighting 22 kg, presented with cluster seizures. The owner reported that the dog suffered from idiopathic epilepsy confirmed by a normal MRI and CSF examination. During the previous 3 months, seizures had become more frequent (2-3 tonic-clonic seizures/month). The patient was receiving phenobarbital[a] (2.5 mg/kg, PO, q 12 h) and levetiracetam[p] (20 mg/kg, PO, q 8 h) therapy. At clinical examination, mild tachycardia, moderate tachypnea, lethargy, generalized mild ataxia and absence of menace response were observed. Body temperature was 39.1°C. Crystalloid fluid therapy was initiated (5 ml/kg/h IV). Biochemistry[e], CBC[d] and echocardiography[g] did not show remarkable alterations. Phenobarbital serum concentration[f]

was 54.7 µmol/L (12.7 mg/L) (therapeutic range: 43-172 µmol/L [10-40.0 mg/L]). One hour after hospitalization, the dog showed preconvulsive signs (paddling and twitching) which evolved into a generalized tonic-clonic seizure. The dog was treated with two boluses of diazepam[b] (0.5 mg/kg IV every 15 minutes) unsuccessfully. With the owner's informed consent, general anesthesia was induced (PPF[j] 5.2 mg/kg IV), and the patient was intubated. Anesthesia was maintained for 24 hours (PPF TIVA 0.33 ± 0.12 mg/kg/min), and visible seizures ceased. Phenobarbital[k] intramuscular injections (loading dosage 4 mg/kg at 6, 12 and 24 hours followed by 2 mg/kg every 12 hours) were administered. Levetiracetam[p] was administered transrectally (30 mg/kg q 8 h). Moderate hypothermia was observed (35.5°C) and the patient was actively warmed with hot air flow[q] to 36.7°-37.7°C. Spontaneous ventilation was maintained (FiO_2 = 1) during the first 4 hours ($PaCO_2$ and PaO_2 within normal range). However, during the following 20 hours, IPPV was initiated due to hypercapnia ($PaCO_2$ > 66 mmHg). At PPF TIVA discontinuation, twitching and paddling reappeared, and 20 minutes later, 2 generalized tonic-clonic seizures occurred. The dog was subsequently treated with KET[l] (1 mg/kg IV) followed by DEX[m] (3 µg/kg IV), both as a bolus over a 5-minute period, after which KET (1 mg/kg/h) and DEX (variable rate 3 to 6 µg/kg/h [mean 3.8 ± 1 µg/kg/h]) were infused for 12 hours. The ketamine-dexmedetomidine combination resulted in the cessation of visible seizures, and the dog remained under moderate to deep sedation; endotracheal intubation and IPPV were not necessary due to the presence of the swallowing reflex, mild palpebral reflex, and PaO_2 and $PaCo_2$ that were within the normal range. During treatments, HR, RR, IBP, body temperature and ECG[n] were recorded every 15 minutes, and urinary production was recorded every 4 hours (Table 1). Arterial blood gas analysis and blood glucose concentration, checked every 4 hours, remained within normal limits. Controlled mild hypothermia was maintained during treatments (36.7°-37.7° C). The administration of the KET-DEX infusion was suddenly stopped. A full recovery was observed 24 hours after infusion discontinuation without further seizures. As soon as the dog started to eat voluntarily (18 hours after KET-DEX discontinuation) phenobarbital[a] (3 mg/kg q 12 h) and levetiracetam[p] (20 mg/kg q 8 h) were administered orally. The dog was discharged on day 4 with phenobarbital and levetiracetam therapy. Upon a follow-up visit 7 days later, the dog was healthy with normal neurologic function.

Discussion

To the authors' knowledge, these are the first reported clinical cases of successful use of a ketamine-dexmedetomidine combination for the treatment of SE in dogs suffering from idiopathic epilepsy.

However, an important limitation of this report is the absence of electroencephalographic monitoring data that could also assess seizures cessation from an electrical standpoint.

All dogs were treated with benzodiazepine, followed by PPF TIVA to induce and maintain general anesthesia for at least 12 hours (12-24 hours) and simultaneously they received phenobarbital and, if possible, a second antiepileptic drug (levetiracetam)[13].

In all 3 dogs, these treatments failed to control seizures, and resistance to GABAergic medication was inferred. In case 1, PPF treatment was stopped after 12 hours and no additional antiepileptic drug was administered in accordance with the pet owner's wishes and due to economic constraints. This first case, therefore, could be considered a case of long-lasting status epilepticus refractory in response to treatment with benzodiazepine and PPF, which contrasts cases 2 and 3 that are dogs with SRSE[3].

Overcoming pharmacoresistance is one of the most challenging issues in long-lasting SE and SRSE management. During prolonged seizures in rats, there is a progressive loss of GABA-mediated inhibition due to an altered $GABA_A$ receptor and a concomitant increase in excitation caused by an overexpression of glutamatergic pathways[4,14]. These phenomena could explain why seizures recurred in all dogs when PPF TIVA was discontinued. In this maladaptive condition, the treatment should address non-GABAergic neurological pathways[1]. KET, a non-competitive NMDA antagonist, currently is often successfully employed in RSE and SRSE in humans[1,5] and reportedly in one dog[7]. Although in the past KET was assumed to be contraindicated in neurological patients at risk for increases in intracranial pressure, nowadays, it has been use safely in humans with neurological illnesses[15].

Regarding SE, the use of NMDA antagonists is mainly recommended for prolonged SE, since during the initial phase NMDA receptors are not activated[16]. Ketamine, by combining with the phencyclidine site inside of the ion channel of the NMDA receptor, can block the flow of Ca^{2+} and Na^{+} and thereby reduce epileptiform burst discharge, inhibiting the conduction of excitation and playing an anticonvulsant role[5].

In the present series, KET was added as third-line therapy in accordance with the current recommendations in human epileptology as well as with the description by Serrano and colleagues (2006)[7] who successfully treated a dog with persistent seizure activities, unresponsive to PPF, with two boluses at 5 mg/kg followed by a constant rate infusion at 5 mg/kg/h. In the present report, however, the dosage (bolus 1 mg/kg IV followed by infusion 1 mg/kg/h) was significantly lower. This was possible due to the simultaneous use of DEX and KET that act at different centers of the brain,

showing synergism with respect to their sedative effects[17]. Another potential benefit of using a low dosage of KET (1 mg/kg IV followed by 1 mg/kg/h) is its neuroprotective effect, i.e., the prevention of cell death caused by an increase of Ca^{2+} influx mediated by glutamate excitotoxicity[6]. In contrast, KET is known to induce neurotoxicity in animal models at high dosages[18].

Dexmedetomidine was added for its anticonvulsant effect[8], for its neuroprotective qualities and for its ability to decrease metabolic demand. Reports in the literature support the ability of the noradrenergic nervous system to regulate seizure activity, especially through α_2-adrenoreceptors. The lack of norepinephrine or α_{2A}-adrenoreceptors results in increased seizure activity in rodents[8]. It has been speculated that stimulation of α_{2A}-postsynaptic receptors is responsible for the anticonvulsant response, possibly suppressing the release of excitatory neurotransmitter in regions such as the hippocampus, cortex or amygdala[8]. Direct application of α_2-adrenoreceptor agonists to the amygdala provided functional protection against seizures in a kitten[19] Dexmedetomidine was able to stop benzodiazepine-refractory nerve agent-induced SE in rats[9]. This study suggested that DEX might also be effective in the early phases. Dexmedetomidine has been used to manage SE in dogs and cats as well[r].

The neuroprotective effects of DEX are attributed to several factors: DEX reduces central noradrenaline release and attenuates CNS sympathetic stimulation; it also decreases the cerebral metabolic rate and oxygen consumption, which dramatically increase during seizures; and it induces direct cerebral vasoconstriction and reduces cerebral edema resulting from seizure activity[12]. Dexmedetomidine also helps to maintain cerebral perfusion preserving MAP within the normal range.

The coadministration of DEX with KET allows a reduction in the KET dosages compared with that in the literature[7]. The variable rate of DEX (3-7 μg/kg/h) permits maintenance of satisfactory sedation and complete muscular relaxation with the lowest dosage required adjusted for each patient.

Respiratory function was preserved in all dogs during KET-DEX infusion, and thus orotracheal intubation and IPPV were avoided as the swallowing reflex was intact and PaO_2-$PaCO_2$ were within normal range. Replacement of the PPF infusion after 24 hours with KET-DEX limited some important side effects related to prolonged PPF infusion[12,20]. Aspiration pneumonia, with subsequent sepsis and disseminated intravascular coagulopathy, has been described in a dog treated for recurrent seizures with prolonged PPF TIVA. Medetomidine infusion added 96 hours after PPF initiation permitted a lighter anesthetic plan to be maintained, facilitating spontaneous breathing without endotracheal intubation[12].

Furthermore, prolonged mechanical ventilation often required during PPF induced-comas can cause lung injuries[21].

In addition to pharmacological treatment, mild hypothermia (36.7°-37.7° C)[22] was maintained in all dogs. All dogs were allowed to cool passively to mild hypothermia by sedative and anesthetic drug administration. Moreover, in cases 1 and 2, additional cooling was instituted with a cooling fan and surface water misting, while dog 3 was actively warmed since his rectal temperature reached a level below the target value. Mild to moderate hypothermia has been shown to reduce seizure activity in experimental animals and in humans[23]; hypothermia activates many anticonvulsant and neuroprotective mechanisms and reduces the increased cerebral metabolic rate during SE. Moreover, mild hypothermia helps to normalize intracranial pressure and reduces the threshold for critical oxygen delivery, calcium-mediated toxicity and glutamate overload-induced excitotoxicity[24]. Mild hypothermia slows the release of excitatory neurotransmitters working in combination with pharmacological treatments[25]. Furthermore, the metabolic reduction induced by hypothermia is helpful to decrease drug metabolism, allowing a decrease in medication administration.

The cases described confirm, clinically, the evidence that ketamine and dexmedetomidine can play a role in the management of long-lasting SE and SRSE. In the current cases, KET-DEX infusion was helpful in treating 3 dogs suffering from idiopathic epilepsy that did not respond to GABAergic medication; hemodynamic stability was preserved and physiologic variables were maintained within normal ranges for the sedated animals. Due to the limited number of cases evaluated, further studies are required to investigate the genuine effectiveness of this protocol compared with conventional therapy and the possible associated complications, and a longer follow-up period should be assessed.

Footnotes

[a] Gardenale, 200 mg tablets, SANOFI S.P.A, Milan (MI), Italy

[b] Valium, 5 mg/ml, Roche S.p.A., Milan (MI), Italy

[c] Nova stat profile pHOx ultra, Diamond Diagnostics Inc, Holliston, Massachusetts, USA

[d] Sysmex Europe, Norderstedt, Germany

[e] Cobas Mira; Roche Diagnostics, Basel, Switzerland

[f] IDEXX Catalyst One Chemistry Analyser, IDEXX Laboratories, Inc., Westbrook, ME, USA

[g] Esaote© MyLab Class C, Firenze, Italy

[h] Ringer Lattato S.A.L.F., S.A.L.F. S.p.A. Laboratorio Farmacologico, Cenate Sotto (BG), Italy

[i] Foley Catheter SurgiVet™, Smiths Medical ASD, Inc., St. Paul, MN, USA

[j] Proposure, 10 mg/ml, Merial Italia S.p.A., Milan (MI), Italy

[k] Luminale, 200 mg/ml, DOMPE' FARMACEUTICI S.p.A., Milan (MI), Italy

[l] Lobotor 100 mg/ml, 10 ml, ACME S.r.l., Cavriago (RE), Italy

[m] Dexdomitor, 0.5 mg/ml, Pfizer Italia S.r.l., Latina (LT), Italy

[n] GE Datex-Ohmeda S/5 Anesthesia Monitor, Soma Technology, Inc., Bloomfield, CT, USA

[o] Ipnovel, 5 mg/ml, Roche S.p.A., Milan (MI), Italy

[p] Keppra 100 mg/ml, UCB pharma, Bruxelles, Belgium

[q] Bair Hugger Warming Unit Total Temperature Management System Model 505, 3M Italia S.r.l., Pioltello (MI), Italy

[r] Rusbridge C, Rubasinska V, Griffiths S et al. Dexmedetomidine in the management of status epilepticus and tremorgenic mycotoxicosis. In: Proceeding of the 26th symposium ESVN-ECVN; 2013: Paris, France. Pp 952-953

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Table 1: Mean values and standard deviations (SD) of heart rate (HR), respiratory rate (RR), mean invasive blood pressure (mIBP) and urinary output (U-O) recorded during propofol total intravenous anesthesia (PPF TIVA) and ketamine-dexmedetomidine (KET-DEX) infusion

	CASE 1		**CASE 2**		**CASE 3**	
Parameter	**PPF TIVA** (mean ± SD)	**KET-DEX** (mean ± SD)	**PPF TIVA** (mean ± SD)	**KET-DEX** (mean ± SD)	**PPF TIVA** (mean ± SD)	**KET-DEX** (mean ± SD)
HR (beats/minutes)	111 ± 16	66 ± 9	120 ± 15	60 ± 4	99 ± 12	70 ± 5
RR (breaths/minutes)	16*	12 ± 2	12*	11 ± 2	14*	9 ± 2
mIBP (mmHg)	60 ± 9	92 ± 6	75 ± 8	89 ± 11	66 ± 10	102 ± 15
U-O (ml/kg/hour)	1.6 ± 0.5	4 ± 1	1.8 ± 0.6	3.7 ± 0.5	2.2 ± 0.2	2.9 ± 0.8

* with IPPV

GENERAL DISCUSSION

How our brain works?

Unfortunately, despite some remarkable advances, the brain remains largely a mystery.

To date, many information exist about anatomy, physiology, pathology but all these data are not sufficient to provide a clear answer to this question.

Brain is a complex network of functionally and structurally interconnected regions (Sporns 2013).

Functional communication between CNS areas is likely to play a key role in complex cognitive processes, thriving on the continuous integration of information across different regions.

For all these reasons the brain is the focal point of this PhD dissertation. Particularly, we have focalized the attention on the relationship between anesthesia and brain functionality, both in healthy and in pathological subjects.

The vast majority of anesthetics drugs recognized in CNS the element of their action. For this reason, anesthesia can introduce some confounding elements especially in neuroimaging. However, thanks to its interaction with neurotransmission it could be used for the treatment of different neurologic condition.

Nowadays, one of the most important tool for the study of CNS is fMRI. The coupling between FC and hemodynamic responsiveness under anesthesia has not been thoroughly investigated, even though there is an emerging evidence that the level of anesthesia and/or the kind of drugs is coupled with parameters in brain connectivity (Nallasamy and Tsao 2011, Paasonem 2018). Therefore, FC could theoretically help to optimize the anesthesia protocol, find the proper temporal window for stimulation studies, provide relevant information to rule out outliers, and subsequently reduce the variance in fMRI data avoiding potential pitfalls. Speaking about improvement concerning the relationship between anesthesia and FC, investigated with fMRI, an important role is played by preclinical setting. Animal models provide a powerful way to investigate normal brain function, pathophysiologic mechanism of CNS diseases, drugs behaviour and much more in a controlled environment (Ellenbroek and Youn 2016). However, should equally be recognized that the translational value of all model systems, especially within the field of neuroscience, is still far from perfect (Ellenbroek and Youn 2016).

In the present work (I) we have investigated FC under anesthesia in guinea pig and in rats (data analysis is still ongoing and in this dissertation we presented only preliminary DSC data).

Until now, many preclinical studies, especially for pathologic models, have been investigated in rats and mice. There are no dubs that the best model for human brain is represented by non-humans primates (Donahue et al. 2018). However, these species present some ethical issue and their use as an experimental model is strictly controlled. Therefore, we have investigated FC in guinea pigs to understand if, for fMRI studies, it could be considered an alternative/superior model compared with rats and mice.

To date, although not yet popular, there is an increasing interest in guinea pigs used as a human model, especially in neuroscience (de Curtis et al. 2016). In fact, this species of rodents seems to provide a superior and more translational model for human brain studies, if compared with mice and rats; particularly, this superiority is expressed in certain specific neurologic pathologies such as Alzheimer's disease (Sharman et al. 2013), epilepsy (De Curtis et al. 2016), behavioural alteration and drugs addiction (e.g. methanphetamines) (Lee et al. 2014). In the aforementioned neurologic conditions, the study of resting state FC seems to be particularly important and nowadays, many efforts are directed in this direction.

The present work compared the effects of different anesthesia protocols on BOLD signal changes (I and partially II). Studies I and II were intended to improve the preclinical fMRI methodology by providing a means to minimize some of the confounding effects induced by anesthetics. The results, together with the literature review, provide a basis for the selection of anesthetic for fMRI experiments (I and II), and a simple tool for measuring the brain hemodynamic responsiveness to external stimuli such as anesthesia (II). Therefore, the findings of studies I and partially II can significantly improve the quality of preclinical fMRI studies, when the use of anesthesia is unavoidable. Particularly, giving the lack of literature, study I gives the start point for FC studies in guinea pigs under anesthesia.

Data emerging in study I are generally in agreement with preclinical literature on mice and rats (Grandjean et al. 2014; Paasonem et al. 2018). Furthermore, many humans FC patterns seem to be preserved in guinea pigs. Because literature is completely lacking in study concerning FC under anesthesia in this animals we started to investigate only a couple of anesthetics.

The study was conducted under ISO that represents the gold standard anesthetic in preclinical environment (Grandjean et al. 2014), in order to compare results obtained in mice and rats. Furthermore, DEX was introduced due to its popularity in human medicine particularly in neurology and neuroradiology (Stuart and Sury 2016). Additionally DEX seems to represents an interesting choice in fMRI study in preclinical environment (Williams et al. 2010, Fukuda et al. 2013).

More studies are required in order to expand the dataset of the influence of anesthesia on FC also in this species as it is in rats and mice (Paasonem et al. 2018; Grandjean et al. 2014). Other drugs need to be studied and at different dosages. Particularly, the association between ISO and DEX deserve to be investigated in guinea pig; in both rats and mice it is indicated as the most suitable protocol for FC studies. Paasonem and colleagues (2018) reported that ISO and DEX association, in rats, exhibited moderate to good correspondence with the awake group, while the two drugs alone showed the most differences compared with the awake condition. The study in object is one of the only that has investigated FC also in an awake group.

At the basis of all the alterations induced by anesthesia, that we can see during fMRI studies, there is the interaction between these drugs and CNS neurotransmitters.

Disorders or substances that alter the production, release, reception, breakdown, or reuptake of neurotransmitters or that change the number and affinity of receptors can cause neurologic or psychiatric symptoms and cause disease (e.g., epilepsy and status epilepticus, Parkinson disease, depression).

Giving this background, is not too difficult to understand why, sometimes, anesthesia could represents not only a tool to induce unconsciousness but also a real therapy.

Therefore, in the last section of this dissertation, in order to complete our "voyage" between anesthesia and CNS we moved to clinical setting. The most important final aim of all preclinical study is to improve humans' and consequently animals' health.

For this reason, we decided to present a report describing how anesthesia could help in the treatment of a common neurologic disease (III). Nowadays, thanks to the advance in the functional knowledge of the brain and of drugs behaviour, allowed largely by fMRI studies, it is possible to introduce new medications to control CNS disorders. Recently many efforts are dedicated to find new treatments for status epilepticus and all its progression. In this context, dexmedetomidine, although studies are still in early stage, attracts many researchers. For all these reasons, in this dissertation, we have presented a clinical report in order to describe the other side of the coin of the relationship between anesthesia and CNS.

We have reported the successful treatment of long-lasting and super refractory status epilepticus through the administration of two drugs commonly used as a sedative/anesthetics in clinical setting.

Status epilepticus represents the summary of “theory and practice”. Each phase of the disease presents a different treatment; the choice of the correct drug can only be made by knowing precisely the functional and molecular changes that causes its development.

As the pathology progress, there is a loss of effectiveness of many therapies and different neurotransmitters need to be modulated.

Drug resistance in SE originates from functional and molecular maladaptive changes. There is a loss of inhibition mediated by γ-aminobutyric acid (GABA) and a coexisting upregulation of excitatory N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid receptors for glutamate (Naylor et al. 2005). GABAergic drugs are commonly used in the initial stages of SE, but their effectiveness tends to decrease over time due to fewer synaptic targets. Therefore, administration of drugs with different mechanisms of action is fundamental to treat RSE and SRSE (Höfler et al.2016). Ketamine, an NMDA receptor antagonist, has been used for this purpose in preclinical models and in humans (Fang et al. 2015 Höfler et al.2016). Ketamine decreases excitatory glutamate activity that is overexpressed during RSE and SRSE, and has neuroprotective properties which block NMDA receptor-mediated glutamate excitotoxicity and cell death (Fujikawa et al.1995). The noradrenergic nervous system appears to regulate seizure activity, especially through alpha2-adrenoreceptors. Both guanfacine and dexmedetomidine, alpha2-adrenoreceptor agonists, exhibited anticonvulsant properties in a rodent model (Szot et al. 2004; Mccarren et al. 2018). Dexmedetomidine decreases sympathetic stimulation and attenuates noradrenaline release, lowering brain excitatory neurotransmitters (Mantz et al. 2011).

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CONCLUSIONS

The present doctorate dissertation includes studies of different nature, but the common thread is represented by the investigation of the central nervous system and its close relationship with anaesthetic drugs.

When we talk about the examination of the central nervous system, especially in the preclinical field or with non-cooperative patients (children, patients suffering from psychiatric or neurodegenerative diseases), we cannot avoid the use of anesthesia.

An accurate knowledge of its effects is essential; from one side, especially in neuroradiology, it is mandatory to distinguish the effects induce by different anesthetics drugs in order to avoid potential pitfall in data interpretation. On the other side, a deep understanding of their mechanism of action, allows a rational administration not only to induce unconsciousness, but also as a therapy for many neurologic condition specially those originating from neurotransmitters imbalance.

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