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Master's Thesis:

Effects of chronic corticosterone administration on endogenous cortical network activity, physiology and behaviour in adolescent mice

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Abstract

Chronic stress can have a severe impact on the human body and brain, resulting in cognitive deficiencies and anxiety-related disorders like major depression. In order to investigate the effects of stress at the neurobiological and behavioural level, multiple animal models -especially rodents- have been used extensively. Thanks to animal research it has been possible to identify mechanisms through which endogenous stress hormones like glucocorticoids (cortisol for humans, corticosterone for rodents) affect almost every organ of the body resulting, when in excess, in diseases or anxiety-related disorders (60). Different stress protocols have been used to induce specific phenotypes and the anatomical and physiological features of the animals have been measured and linked to the respective behaviours. Also, extensive research has been done to examine the effects of glucocorticoids on the levels of other hormones (serotonin, adrenaline, noradrenalin etc.) and on the neuronal anatomy of important brain areas like the hippocampus (64), the amygdala (70) and the prefrontal cortex (125). In parallel, through neuroimaging techniques in humans, we have been able to link brain activity to specific stress symptoms and the subjective experience of the individual. However, the intermediate level of the local brain networks, or microcircuits has hardly been investigated.

In the present thesis we investigated the effect of chronic corticosterone administration on the spontaneous cortical network electrical activity of adolescent mice, while at the same time monitoring characteristic aspects of their anatomy, physiology and behaviour. Chronic exogenous corticosterone administration has been proposed as a relatively simple model of depression and anxiety (76) and as such could be useful in the investigation of stress effects on

cortical network activity. C57/Bl6 male mice (n=24) received corticosterone in their water (50 μg/ml, diluted in 1% ethanol) for two weeks starting at postnatal day 28 (P28), while their body weight, food and liquid consumption were measured daily. A second control group received regular water with ethanol for the same period. On day 13 of administration (P41), mice were examined on the Elevated Plus Maze in order to assess their behaviour and trace any anxiety traits. On the following days, mice were sacrificed, their brains extracted, cut in slices and put in a submerged chamber filled with oxygenated artificial cerebrospinal fluid.

Cortical network activity was recorded using the in vitro model of the spontaneously active brain slice (41, 42). In this preparation, the isolated cortical network generates spontaneous periods of depolarization (Up states), interspersed with quiescent periods (Down states). This activity is exhibited in the absence of pharmacological or electrical stimulation, and is considered the default activity of the cortical circuits, reflecting endogenous connectivity. Spontaneous network activity was assessed by means of local field potential (LFP) recordings which were obtained from cortical layers II/III using low impedance (~0.5 MΩ) glass pipettes filled with ACSF. Network activity events were analyzed and characterized as three different types of events (upstates, biphasics or multiphasics). Additionally, on the day of sacrifice the adrenal glands of mice were extracted and weighted. A subset of the initial group of treated mice, after receiving the corticosterone treatment for 2 weeks, were then left for 4 weeks with regular water to investigate if the corticosterone effects would disappear (n=8, washout group). After washout, they were also tested in the EPM, sacrificed in the following days and used in electrophysiology experiments.

We found that corticosterone administration had obvious (and anticipated) effects on the anatomy, physiology and behaviour of treated mice. CORT mice manifested a generally more anxious phenotype with reduced weight gain, while consuming equal amounts of food and significantly more liquid than the control group. Their adrenal glands were lighter than those of the control group, their tails were shorter and thinner and during the 2-week period of administration they produced more fecal boli and urine. In the Elevated Plus Maze, the CORT group covered significantly less distance and spent increased time in the closed arms compared to the control group. In spite of these clear signs of being under stress, the various parameters of Up state activity of the CORT group did not differ significantly from the control animals. During the washout period of the experiment, the CORT group recovered and all parameters returned to control levels. These results suggest that the endogenous cortical activity is a robust phenomenon under tight homeostatic regulation and is not significantly affected by corticosterone levels.

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1. Introduction

1.1. Choice of paradigm and methodology

Behaviour is the internally coordinated responses (actions or inactions) of whole living organisms (individuals or groups) to internal and / or external stimuli, excluding responses more easily understood as developmental changes (1). According to the previous definition, all animals behave. Behaviour is observable, quantifiable and thus measurable. Psychologists and psychophysicists have identified different types of behaviour and measured how different groups of subjects react to various kinds of tasks by counting reaction times and number of mistakes. These methods have managed to associate various stimuli with corresponding responses and have found differences among groups and individuals depending on age, gender, expertise or training, pathophysiology, previous experiences etc. However, the real challenge for Cognitive Science and Biological Sciences of the Brain and Mind is to understand the biological mechanisms through which behaviour emerges. How does a stimulus become a percept, a volition become action, a thought become sentence? All these processes are extremely complex and require the investigation of multiple levels of anatomy, physiology and activity of the brain and nervous system. We need to explain how activity at each level translates to the next one: how nerve cells communicate with each other, how this communication is integrated into network activity, how this activity is transmitted to different areas of the brain and in turn to different parts of the body and how these parts produce behaviour.

Levels of description and methods of analysis

A. The single neuron Neuroscience has come a long way in deciphering the anatomy and function of the neuron -which is the functional unit of the nervous system- by generating electrical signals that transmit information (100, p.37). Even though there exist various types of neurons that differ in morphology, all share some general features. First of all, each neuron has a cell body containing a nucleus and organelles like mitochondria, ribosomes and endoplasmic reticulum which are essential to the function of all cells. Also, because neurons' specialization is the intercellular communication, they are equipped with appendages that serve as transmitters (axon) and receivers (dendrites). The number of dendrites in each neuron is a very important determinant of neuronal function, since it indicates the amount of input from other neurons (100, p.4-5). Axons, on the other hand, are unique extensions from the cell body to the axon terminal that may travel close or very far, depending on the type of neuron and organism, in order to transmit the signal to other cells. The axon terminal of the transmitter (pre-synaptic) neuron reaches close to a dendrite of a receiving (post-synaptic) neuron and releases chemical molecules called neurotransmitters, which the post-synaptic neuron receives through specialized receptors. Neurons in humans have a resting (membrane) potential of approximately -70 milliVolts (mV), meaning that when not excited there are more positive ions in the extracellular fluid than inside the neuronal membrane. A signal received on the dendrites of a neuron is called post-synaptic potential and can be inhibitory (IPSP) when it is such as to make the membrane potential more negative or keep it stable, or excitatory (EPSP) when it tends to make the membrane potential more positive. If there are sufficient EPSPs, the membrane potential surpasses a certain level called threshold potential and an action potential

is emitted from the axon hillock. The action potential or spike is a brief (about 1ms) change of membrane potential from negative to positive and it has a specific amplitude, independent of the current that generated it (100, p.37-38). The action potential, the EPSPs & IPSPs, the synapse and all mechanisms related to the single neuron and the neuron-to-neuron communication have been and still are investigated.

B. Whole Brain approaches. Another level of description of behaviour and related brain activity are the various anatomical areas like the Prefrontal Cortex, Thalamus, Hypothalamus, Hippocampus and Amygdala. Each of these areas (and sub-areas) have been associated with specific behaviours and disorders through the use of a multitude of methods like Electroencephalography (EEG), Magnetoencephalography (MEG), functional Magnetic Resonance Imaging (fMRI) and Positron Emission Tomography (PET). All four of these methods are used to measure brain activity of a living organism extracranially and each has its own merits and limitations. The most critical factors that determine the utility of each of these methods are signal specificity and spatiotemporal resolution. Signal specificity expresses the amount of certainty that the recorded signal reflects actual network activity (112). fMRI and PET measure blood flow in the brain, in order to detect and estimate (indirectly) brain activity. fMRI has provided important insight into the localization of brain activity while the subject is responding to a stimulus, thus expressing a behaviour (114). However, it has a very low temporal resolution and is highly susceptible to movements of the subjects' heads (a troublesome factor in children research). PET uses a radioactive isotope (typically Fludeoxyglucose - ^{18}F, to identify areas with higher concentration of glucose -i.e. metabolism- or other substances). In the case of glucose this means that the technique is capable of detecting areas with higher metabolic activity and

indirectly neuronal activity. PET has an inferior spatial analysis than fMRI, is much more expensive and uses radioactive substances and that is why it is used much less than fMRI in both animal and human research.

EEG records electrical activity of the brain through electrodes that are placed on the skull. It has a good temporal resolution compared to PET and fMRI but the amount of noise introduced (because of its extracranial nature) is a very limiting factor. Apart from that, the EEG has minimal capability of distinguishing the exact origin of the activity within the brain. Another important methodological tool for investigating the brain activity is Magnetoencephalography (MEG). In MEG, multichannel superconducting quantum interference device (SQUID) gradiometers record and measure the weak magnetic fields that are produced by electric currents flowing in neurons. Its temporal resolution is better than 1ms while its spatial resolution is, at best, 2-3mm for sources in the cerebral cortex (101). Comparison of the Signal to Noise Ratio (SNR) of EEG and MEG from different cortical areas has showed that the SNR of EEG was more uniform than that of MEG while MEG was found to have a high SNR when the source of the signal was superficial but it worsened significantly for deeper sources (102). Spatial resolution can be improved for both methods by increasing the number of recording channels to more than 32 (103). MEG has also been used as a tool for the investigation of resting-state activity of the human brain and it has given similar results with fMRI concerning spatial coordinates. MEG poses an intriguing alternative to fMRI because of its superior temporal resolution and due to the fact that, unlike fMRI, it is not based on assumptions concerning the electrical activity and haemodynamics (104, 111, 113). On the other hand, EEG is the most direct method because it records electrical activity and not phenomena related to it

but its inferior spatial resolution creates a problematic barrier. Researchers have also managed to combine some of these four methodologies in order to accumulate simultaneous temporal and spatial recordings achieving a significant insight on brain areas that are responsible for specific behaviours (2, 107, 108, 109, 110).

Besides the typical stimulus - response scheme, many researchers have focused on spontaneous brain activity through resting-state fMRI and/or EEG in awake subjects (106) or from subjects during sleep (13,17), sedation (18) and patients under comma (19). This investigation has revealed the existence of a network of brain areas that show increased activity in the absence of external stimuli and reduced activity when stimuli are present. This group of areas has been named Default Mode Network (DMN) and is an intensely investigated field (2,3). Differences in baseline activity of the DMN among patients and normal population has been linked to Alzheimer's disease (4), Autism (5,6), Epilepsy (7,8), Post Traumatic Stress Disorder (9), Depression (10,11), Schizophrenia and Bipolar Disorder (12), Parkinson's disease (14) and drug addiction (15). In addition, different patterns of activity of the DMN among normal individuals have been associated with past and prospective memory and empathy (16). The Default Mode Network is considered to be an important marker of various disorders and it is considered to exist also in rodents (20) and nonhuman primates (21). Regardless of the particular regions that may be part of the DMN, resting state activity has been used extensively and still remains a valuable tool for the investigation of the network activity of the brain.

All of the previously mentioned methods (fMRI, PET, EEG and MEG) have been implemented in order to measure the electrical brain activity and especially the resting state activity. However, in order to achieve a higher level of resolution and examine the underlying

mechanisms we need to gain access to the internal part of the brain and there are very rare instances which give the opportunity to record from a live human brain. One legendary case of a researcher who managed to surpass this obstacle was the neurosurgeon Wilder Graves Penfield (1891-1976) who invented the Montreal Procedure, a surgical operation to treat epilepsy. Penfield aimed to destroy epileptogenic brain tissue in order to stop epileptic seizures. He gave local (instead of general) anesthesia to patients before the operation so as to keep them awake during surgery. Before operating he stimulated different parts of the brain and asked the patients what they felt. This way he was able to target the epileptogenic areas and avoid traumatizing important brain areas related to motion and language (among others). Through this method he also managed to create maps of sensory and motor cortices of the brain, which are still broadly used. Apart from special cases like the Montreal procedure, there are not a lot of instances in which a researcher is permitted to record through electrodes directly attached to the brain of a living patient and even in these cases there are great limitations into what can be investigated, for how long and for what purpose.

An additional problem is that the recorded electrical activity consists of a variety of different types of electrical charges and currents. The measured signal consists mainly of synaptic transmembrane current while Na^+ and Ca^{2+} spikes, ionic fluxes through voltage, intrinsic membrane oscillations and action potentials also contribute (105). Due to the large scale nature of the non-invasive methods described above, it is not possible to access small neuronal populations and differentiate among different sources.

C. Brain networks (local or global). One method that has been used extensively in the previous years and has given valuable insight on the network dynamics of cortical activity is

electrophysiological recordings of Local Field Potential (LFP) which can be implemented both in vivo in and ex vivo (22, 24). LFP activity represents the slow voltage fluctuations caused by ionic currents near the recording electrode (<200 Hz). It contains a broad spectrum of neural oscillators ranging from under 1 Hz to over 100 Hz that can be analyzed in different band-limited components (e.g. delta, theta, alpha, beta and gamma bands) which provide a valuable insight into the integrative excitatory and inhibitory synaptic processes at the network level. Also, through LFP signals we are able to empirically examine distinct and potentially independent information channels in neural processing. LFP recordings can be implemented simultaneously with intracellular recordings, thus allowing us to observe possible interrelated patterns and eventually giving us insight into circuit mechanisms generating neuronal activity (111).

Local Field Potential recordings in brain slices have revealed a characteristic slow-wave type of spontaneous cortical network activity resembling the slow oscillation that occurs during deep sleep. During slow-wave sleep, cortical and sub-cortical networks interact to generate rhythmic patterns of activity. One type of activity that appears systematically is a 'slow rhythm', interrupted every three to five seconds by a recurrent tonic activity in cortical neurons that consists of a relatively steady barrage of EPSPs and IPSPs and the discharge of both excitatory and inhibitory neurons called 'Upstate', followed by a period of hyperpolarization and quiescence called 'Downstate'. Upstates and Downstates are considered to depend at least partially on cortico-cortical connections and it has been proposed that this activity is generated by recurrent excitation among large networks of cortical neurons, interrupted by periods of ‘disfacilitation’ in which the recurrent activity fails (24). However, this activity has been

recorded through LFP electrophysiological recordings in brain slices of mice (41, 42), ferrets (23) and cats (24), which indicates that this type of activity can also be generated and maintained in smaller networks of neurons (24, 127). In general, slow oscillations have been proposed as the default cortical activity when the cortex is disconnected from the outside world (127). Therefore, Up- and Downstates' characteristics (duration, occurrence, amplitude and different frequency components) have been proposed as a single-metric index of the functional maturation and regional differentiation of the mouse cerebral cortex (41). Therefore, in our experiment we used this ex vivo paradigm to examine the effects of chronic corticosterone on endogenous cortical activity. Moreover, since it has been shown that upstates are more robust (higher occurrence, larger duration) during puberty than in adulthood (41), we performed our experiments in slices of pubertal mice kept in oxygenated aCSF and used the Upstates' components as a metric of cortical network dynamics.

1.2. Stress and chronic Corticosterone administration in mice

1.2.1. Definitions

Every event that could be potentially harmful or life-threatening for an organism induces a series of physiological responses as a reaction to the danger. Multiple situations in everyday life can be interpreted as threatening to an individual: an unknown stray dog heading towards us, an unexpected letter by the bank, an earthquake, a sudden pain in the abdomen or one's girlfriend asking him to break up, are all situations that can be considered stressful. However, as noted by Bruce McEwen (26), the term *'stress'* is not universally defined the same way. Some researchers identify stress as the stressor event while others consider it to be the physiological

response of the body. In some cases the term stress is used to describe a chronic status of inability to cope with stressful situations. It is therefore important to define what we refer to as stress. A beautiful and classic conceptualization of stress is the one by Chrousos and Gold (28):

> Living organisms survive by maintaining an immensely complex dynamic and harmonious equilibrium, or *'homeostasis'*, that is constantly challenged or outright threatened by intrinsic or extrinsic disturbing forces or *'stressors'*. The steady state required for successful adaptation is maintained by counteracting/reestablishing forces, or adaptational responses, consisting of an extraordinary repertoire of physical or mental reactions that attempt to counteract the effects of the stressors in order to reestablish homeostasis. In this context, we define *'stress'* as a state of disharmony, or threatened homeostasis.

This definition uses the important term of homeostasis (first coined by Walter Bradford Cannon in his book 'The Wisdom of the Body', published in 1932) as the state of dynamic equilibrium in the body. However, as noted by Bruce McEwen, homeostasis should only refer to a limited number of physiological variables (PH, body temperature, glucose levels and oxygen tension) which have to remain within strict boundaries around their set-points in order for the organism to remain alive. Apart from these primary systems, there are many other (hormones of the HPA axis, catecholamines, cytokines and others) which are also important for the survival and well-being of the individual but can diverge from their normal levels. In this case, we say that the variables are in an *'allostatic state'* and we call *'allostasis'* the process of actively maintaining homeostasis by managing the levels of the secondary systems (26). Also, If the stress response

is inadequate or excessive and prolonged, the cost of reinstating homeostasis might become too high, which is a condition that is termed *'allostatic load'* (29). Even though the definition by Chrousos & Gold illustrates sufficiently the context of a stressful circumstance, the term 'state' is itself a little ambiguous. In order to have a more precise and intuitively valid definition of stress we can turn to the one given by Joëls & Baram (27):

> Any actual or potential disturbance of an individual's environment — a *'stressor'* — is recognized or perceived by specific brain regions. The subjective state of sensing potentially adverse changes in the environment is called *'stress'* and leads to the release of molecules that we here call *'stress mediators'*, which bind to receptor targets. Each of these mediators acts on specific neuronal populations, resulting in unique downstream effects. Together, these effects constitute the *'stress response'*, which enables the animal to adapt to the changing environment.

Since both definitions capture different aspects of the term, we will attempt to give a new one, so as to combine the two: *stress is the subjective experience of a potentially harmful event (stressor) that is threatening the homeostasis of the organism.*

This definition allows also for the distinction between eustress and distress first articulated in 1936 by Hans Hugo Bruno Selye (30). Eustress and distress have common neurobiological features. What discriminates them is that *'eustress'* is a stress response that plays an adaptive role towards homeostasis, *experienced as agreeable or beneficial* (30, p.39) which can *enhance human growth and development at the emotional and intellectual level* (31, p.8) while *'distress'* is the state under which the organism is not capable of reinstating homeostasis, thus resulting

to pathological conditions (31). We can deduce that the difference between eustress and distress is primarily a matter of quantity and not quality: 'Too much of a good thing can be a bad thing'. In the words of McEwen (32):

> 'Good stress,' in popular jargon, generally refers to those experiences that are of limited duration and that a person can master and which leave a sense of exhilaration and accomplishment, whereas 'bad stress' or 'being stressed out,' in the vernacular, refers to experiences where a sense of control and mastery is lacking and which are often prolonged or recurrent, irritating, emotionally draining, and physically exhausting or dangerous.

Another important distinction is the one between acute and chronic stress. The stress response has evolved as a way for the organism to face sudden changes of the environment. So*, 'acute stress'* refers to an event that is limited in time and therefore not particularly threatening to the organism. On the contrary, *'chronic stress'* can have a long duration spanning from days to years and has been shown to result in pathological changes in both anatomy and physiology (29). Moreover, an individual that suffers from chronic stress will have lower resistance against stressful situations and will react in a less adaptive way (as in the case of chronic sleep deprivation), a state called *'stressed out'* (32).

1.2.2. The neurobiology of stress

When a stressor is perceived by our sensory organs (eyes, ears, skin etc.) a cascade of physiological changes take place inside the body in response to the potential threat. In the

Central Nervous System (CNS), arousal, alertness, attention, vigilance, cognition and aggression are facilitated while vegetative functions like reproduction, feeding and growth are inhibited. In the Peripheral Nervous System (PNS), oxygenation, metabolism, cardiovascular tone and respiration, nutrition of brain, heart and skeletal muscles are enhanced (33). All these changes serve the *'fight-or-flight'* response, a term coined by Walter Bradford Cannon to describe the two main behavioural reactions of animals when under threat (as described in 31).

The neurobiological substrate of the stress response contains a multitude of components that can be divided in central and peripheral.

The central components include:

(i) the parvocellular neurons, which secrete corticotropin-releasing hormone (CRH);

(ii) the neurons of the paraventricular nuclei (PVN) of the hypothalamus that secrete arginine vasopressin (AVP);

(iii) the CRH neurons, which form the paragigantocellular and parabrachial nuclei of the medulla and the LC, and

(iv) other neural groups in the medulla and pons (LC/NE system) mostly secreting NE (LC/NE stands for Locus caeruleus /norepinephrine)

The peripheral components include:

(i) the neuroendocrine HPA axis (Hypothalamus, Pituitary glands, Adrenal glands)

(ii) the efferent systemic sympathetic-adrenomedullary systems, and

(iii) components functioning under the control of the parasympathetic system.

(list adopted by 31, p.8)

The stress response acts in two waves. The first wave begins within seconds and most importantly involves the increased release of epinephrine and NE from the sympathetic nervous system and the secretion of CRH from the hypothalamus into the hypophysial portal system inducing increased release of adrenocorticotropic hormone (ACTH). The second wave is much slower and involves the production of glucocorticoids (or corticosteroids) as a result of the increased ACTH. Corticosteroids affect brain functioning through delayed, genomic (but also through rapid, non-genomic) mechanisms (32). The central mechanism of the stress response is the HPA axis. The primary hypothalamic regulator of the HPA axis is the CRH. The neurons of the PVN of the hypothalamus secrete and release CRH and AVP. CRH then induces the secretion of ACTH by the anterior lobe of the pituitary gland, which is then released into the blood circulation and eventually reaches the adrenal glands, causing them to secrete the so-called 'stress hormones': glucocorticoids (corticosterone in rodents, cortisol in human) and catecholamines (adrenaline and noradrenaline). AVP acts synergistically with CRH for the secretion of ACTH, however does not have significant ACTH secretagogue activity on its own. Also, CRH and AVP in the hypothalamus have a positive reciprocal interaction with CRH stimulating the secretion of AVP and vice versa. When glucocorticoids are secreted, they activate the two types of steroid hormone receptor family of transcription factors, the Glucocorticoid Receptors - GR and the Mineralocorticoid Receptors - MR (35). Cortisol in humans influences up to 20% of the expressed genes and affects all major homeostatic systems of the body (33). Glucocorticoids in both humans and animals play a key regulatory role on the basal activity of the HPA axis and on the termination of the stress response through a negative feedback loop. When glucocorticoids are released in the blood, a portion reaches the pituitary

glands inhibiting the ACTH secretion and eventually glucocorticoids secretion, thereby minimizing the deteriorating effects these hormones have on the metabolism, reproduction and immune system (63). In addition to the main terminating loop, the hippocampus has connections which inhibit the release of CRH from the hypothalamus (115). In the absence of stress, CRH and AVP are produced and released in an oscillatory mode every 20-30 minutes with variations in amplitude depending on the time of day / night, changes in lighting, feeding schedule and activity. However, when a stressor is perceived, CRH and AVP secretion becomes more intense and synchronized and, as a result, ACTH and cortisol levels rise (34), with all the aftereffects previously described.

1.2.3. Chronic stress effects

If an individual experiences constantly or repeatedly stressful circumstances, then we say that he is under 'chronic stress', which means that the stress response becomes maladaptive and causes various health problems to the organism. When someone is under chronic stress, the excessive and continuous secretion of CRH, NE and glucocorticoids may produce fatigue, nausea, headaches and other pains. The systematic release of cortisol due to chronic stress in developing children has been associated with suppressed growth as a result of a hypo-functioning growth hormone axis (33). Also, glucocorticoids antagonize insulin and increase blood pressure, thus increasing the risk for diabetes, hypertension and arterial disease (63). Furthermore, in cases of adults with a vulnerable background, chronic hypersecretion of glucocorticoids can lead to excessive visceral fat, reactive insulin hyper secretion, low growth-hormone secretion and hypogonadism (33). Another important mechanism leading to various

types of pathology is the impairment of the immune response due to persistent activation of the HPA axis. This can result in infections, autoimmune disorders and allergic diseases (33), delayed or inefficient wound healing (72) and even cancer (36). Additionally, in cases of chronically stressed animals, the effects of stress can also appear even in the absence of a stressor, as the animals seem to develop an inability to terminate the glucocorticoid loop, an effect possibly caused by damage in the hippocampus due to excessive glucocorticoids (115).

In parallel with all the anatomical and physiological symptoms, chronic stress has also been related to a plethora of cognitive dysfunctions, psychiatric disorders and substance use disorders (113). The interaction between post-traumatic stress disorder (PTSD) and the HPA axis has been thoroughly investigated in human patients (war veterans, rape victims) and animal models, bearing, however, unclear results (115). Epilepsy is one of the most severe disorders that have been found to be affected by stress in multiple ways. Early life stress or chronic stress in adults increase seizure occurrence and lower seizure threshold, a fact that is also self-reported by epilepsy patients. Also, exogenous administration of stress hormones like CRH or corticosterone to rodents induce seizure activity, increase seizure rate and discharge levels (37). Lastly, learning and memory have been found to decline earlier in life in cases of chronic stress, possibly binding stress to Alzheimer's Disease. Prolonged exposure to stress or glucocorticoids can have deleterious effects in memory anatomy and function (25). For example, chronic immobilization of transgenic mice models of AD resulted in accelerated onset and severity of cognitive dysfunctions and increased neurodegeneration and tau phosphorylation in the hippocampus (116).

A possible mechanism responsible for these effects could be the interaction of the stress system and three brain areas that regulate emotional processing and are also involved in learning and memory. Indeed, chronic stress has been found to cause significant effects on the hippocampus, the amygdala and the prefrontal cortex. All three of these areas contain glucocorticoid receptors which makes them subject to stress effects (63). For instance, the stress system has a mutually reinforcing interaction with the amygdala. The central nucleus of amygdala has its own CRH system which gets activated by the stress system while, in turn, amygdala stimulates the stress system. CRH, norepinephrine, cortisol and other hormones activate the amygdala causing anxiety and increased or decreased appetite. The importance of the amygdala - stress interaction expands to emotional memories as it has been found that, without glucocorticoids or noradrenalin, recall of emotional memories was impaired. Another synergistic mechanism is the interaction of the stress hormones and the reward system, which may result in depression and cravings for food. This effect could be explained by the reciprocal corticosteroid - serotonin interaction which has been broadly investigated (117). Finally, stress mediators also obstruct sleep, causing insomnia, loss of sleep and drowsiness (33).

1.2.4. Justification of stress induction protocol: corticosterone treatment

As already mentioned, corticosterone administration is a protocol that has been used extensively to investigate the effects of stress on brain anatomy and function. It has been well documented that exogenous corticosterone administration has similar effects with chronic stress while at the same time bypassing the various behavioural parameters that increase the variability of other stress protocols. Chronic corticosterone treatment by injection or by passive

administration in the drinking water are both able to cause dendrites to retract in CA3 hippocampus (39, 64), or even cause neuronal death in hippocampal pyramidal cells (69). Repeated high-dose glucocorticoid treatment mimics chronic stress and induces dendritic lengthening in Basolateral Amygdala (BLA) (38) while, 21 days of corticosterone treatment in rats cause atrophy of apical dendrites of hippocampal CA3 pyramidal neurons (64) and retraction in mPFC neurons (125). Moreover, many researchers agree that, despite any possible differences in the underlying mechanism, chronic corticosterone treatment and chronic restraint or immobilization stress both cause impairment of hippocampal-dependent memory tasks (39). Hence, although it does not replicate all aspects of the stress response, corticosterone administration has several advantages: it is a simpler, 'cleaner' and less variable protocol which, depending on the dosage, may lead to robust effects. Indeed, corticosterone administration is often used as a first method to investigate chronic stress (58) and as such it was the protocol we chose for stress induction.

To the best of our knowledge, this is the first study to specifically investigate the effects of chronic oral corticosterone administration on the spontaneous cortical network activity of mice. We are aware of two experimental paradigms in the past that have focused on the relation between stress or corticosterone administration to rodents and spontaneous brain network activity. In 2002, the research group of Krugers and Jöels investigated whether a brief in vitro administration of 100 nM corticosterone to hippocampal slices from mice with low basal corticosterone levels resulted in altered synaptic potentiation (46). However, this paradigm is very different from our own and its results cannot be compared to our own. More recently, Jöels and her colleagues carried a series of experiments that had much more relative research

goals to our own (123). The researchers used the Chronic Immobilization Stress protocol on male rats, aged 3 months old. The stressed group remained under complete immobilization for 2 hours per day for 10 days, without access to food or water while the control animals remained with no access to food or water for the same period of time in their home cage. One day after the end of the stress protocol 8 rats from each group were sacrificed, their brains were removed quickly and cut coronally to examine the mPFC, amygdala and hippocampus. Their tissues were golgi-stained and a selection of neurons was morphologically analyzed. The rest of the rats (10 per group) were subjected to resting state fMRI scanning on day 11 in order to assess functional connectivity patterns in their brain. Through independent component analysis and comparing the emerging areas with known neuroanatomical regions, 10 components were identified as anatomically and functionally meaningful networks and were considered to represent the somatosensory, motor, visual cortex and others. To investigate the connectivity among these areas, they created subject-level spatial maps and time-course for each map and subjected them to voxel-wise statistical testing. After that, they calculated an overall average activity for each area and the pairwise correlation among the 10 components. This analysis showed an increased connectivity between the somatosensory cortex, the visual cortex and the DMN of the stressed animals compared to control. The relationship of these results to our own will be analyzed in the discussion.

2. Materials & Methods

2.1. Animal model - C57/Bl6 mice

C57/Bl6 male mice were bred in the animal facility of the Center for Experimental Surgery of the Biomedical Research Foundation of the Academy of Athens (BRFAA). The facility is registered as a breeding and experimental facility according to the Presidential Decree of the Greek Democracy 160/91, which harmonizes the Greek national legislation with the European Council Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. The present study was approved by the Regional Veterinary Service, in accordance to the National legal framework for the protection of animals used for scientific purposes. All efforts were made to minimize animal suffering and reduce the number of animals. 48 male mice were weaned after P21 (P22-P25), marked through ear clipping, weighted and divided in two groups (CORT and control) matched for weight. Each group was then put in separate 1800 X 545 X 1860 MM cages (TECNIPLAST 1291H, IVC type, SEALSAFE series), 3 or 6 animals per cage, supplied with bedding material and one tissue as nesting material (and also to provide a mildly enriched environment and reduce aggression, 119). They were kept at a 12:12-h dark/light schedule (lights out at 7pm). Temperature remained stable at 24±2^{o}C and humidity was 45-65% at all times. Food and water was provided *ad libitum*.

Mice remained in the same cage until day P46 without any cleaning. In the cages with 3 mice, animals were put through the Elevated Plus Maze (EPM) task on day P41 and were sacrificed in days P42-46. In the cages with 6 animals, 3 of the animals were introduced to the EPM on day P41 and 2 of them were sacrificed on days P42-44. After sacrifice, the adrenal glands of mice

were carefully excised from the surrounding adipose tissue and weighed. The 4 mice left remained in the cage drinking CORT until P46 when they were transferred to a new clean cage with a new tissue as nesting material, with regular drinking water and food provided *ad libitum* for another 4 weeks. On P74 they were introduced in the EPM and were then sacrificed on days P74-80. It has been suggested that mice should be reintroduced to water slowly after CORT treatment (79). However, because we investigated for the first time the effects of CORT in cortical network dynamics we wanted the effect to be as robust as possible, therefore we did not include a transitional stage post CORT treatment.

Even though the rat has been the model of choice for the investigation of stress and anxiety in previous decades, in recent years the use of mouse as a model has gained a lot of supporters (mainly but not only) due to the newly emerged technology of genetic engineering (57, 66) and its unique applicability to the mouse compared to other animals (53). Specifically, the C57/Bl6 strain is the background mouse strain used for all behavioural experiments in transgenic and knockout research (76). Therefore, the animal model chosen for our experiments was the C57/Bl6 mouse which has been used in various experimental settings and its anatomical, physiological and behavioural reaction to multiple protocols of acute or chronic stress (maternal deprivation, 65; restraint, 73) or corticosterone (in vitro, 46; and in vivo, 53, 59, 76) have been thoroughly investigated (47). Behaviourally, the C57 strain manifests high levels of locomotion (56), a characteristic that is useful in order to detect any changes as an effect of stress. More importantly, recent research both from our lab (41,42) and from others, has focused on the spontaneous cortical network dynamics of the C57/Bl6 mouse in vitro (43,44)

and in vivo (45). The combination of these elements warrant the C57/Bl6 a suitable model for our research.

Only male mice were chosen as subjects since it has been shown that corticosterone effects are more robust in males than females (49, 66) and the characterization of spontaneous network activity has been performed mostly on male mice (41,42). Animals' age was chosen based on a) observations that suggest pre-puberty, puberty and adolescence are the most vulnerable periods to stress and more probable to sustain effects throughout the lifespan (48) and b) results from our own lab which show that spontaneous network electrical activity along the lifespan of the mouse is more robust in puberty (41).

2.2. Corticosterone treatment

Exogenous chronic Corticosterone administration (CORT) for several weeks is considered an efficient and practical protocol that provides a good model of chronic stress (58, 62), depression (76), anxiety (79) and metabolic syndrome (59). There are multiple ways of administering exogenous corticosterone. It can be injected daily, diluted in the drinking water or inserted in a pellet through operation. We opted for the second method which seems to be chosen by most laboratories. The drinking water protocol is technically less demanding, non-invasive and allows for the maintenance of the naturally occurring diurnal rhythm of the hormone secretion (76). There are two main protocols for diluting CORT in water described in detail. The first one, which we decided to follow has been previously described by the Karatsoreos group (59). The second one, as described by Goorley and colleagues (79), also

remains a valid protocol. Possible discrepancies and differential effects among the two protocols are discussed in the discussion section.

In our experiment, drinking water was replaced by a solution of 50 µg/ml free-CORT (Sigma-Aldrich, St. Louis, Missouri, USA) dissolved in 100% ethanol (because CORT is hydrophobic) and then diluted in regular tap water to a final ethanol concentration of 1% or a 1% ethanol solution alone (vehicle for control group). Animals had *ad libitum* access to the drinking solution for 2 weeks (P28-43). Higher doses have been used in other experiments (100 µg/ml: 59, 62, 71, 76) but in these setups mice were older than in our case, so we reasoned that the selected dose would bring the expected anatomical and physiological effects without becoming dangerous for the survival of the animals. After weaning (P22-25), mice were kept undisturbed for 3 to 5 days to adapt to the new cage and the new life stage. After this habituation period, at the age of P28, the water of the experimental group was replaced by CORT solution while food remained the same. The control group had its water replaced with liquid containing ethanol. Water bottles containing CORT were covered with aluminium tinfoil to avoid light-induced degradation of the compound. Water quantity was monitored every day by measuring the amount of liquid left in the bottle. The bottles had been marked so that we could measure the level of the liquid inside in order to avoid spilling liquid. Liquid was renewed or supplemented every 4-5 days. The mice drunk from this solution for 12 days during which they remained undisturbed, apart from daily measures of body weight, food and liquid consumption. On the 13th day (P41), mice were introduced to the Elevated Plus Maze for 5 minutes each and then returned to their cages.

The first mouse from every cage was sacrificed on day P42 and the second on day P44. Therefore, all mice from both groups received on average 15 days of CORT treatment. As mentioned earlier, mice were either housed in groups of 3 or 6 per cage. In the cages with only 3 mice, the third mouse of each cage continued drinking from the solution until it was sacrificed on day P46, its brain extracted, postfixed in 4% paraformaldehyde solution and stored in Phosphate Buffered Saline (PBS) for further analysis. In the cages housing 6 mice, the first 2 were sacrificed as described on days P42-44, while the other 4 were moved to a new cage with regular water for 4 weeks (washout period).

2.3. Body weight, food - liquid consumption & adrenal glands

Body weight was measured daily for each mouse from the day they were taken from the dam (P22-25) until the day of sacrifice (P42-44) while food and water consumption per cage was also measured daily until the day of the Elevated Plus Maze (P41). Food and water intake is strongly related to stress because part of the normal stress response is the inhibition of vegetative functions. Therefore, it is of great importance to monitor any fluctuations in food and water intake as a lower than normal consumption could be a sign of excessive stress that could eventually lead to bad health and disease. On the contrary, a larger consumption of food could have indicated that mice were not particularly affected by the CORT treatment and this in turn could be a sign that CORT was not being properly administered. Food and liquid consumption was calculated by measuring the remaining quantities of each day and then subtracting them from the previous one. The result indicated the daily food and water consumption per cage, which was then divided with the number of mice in each cage in order to estimate the average

daily consumption for each animal. This procedure is called periodic food and water weighing (75) and is widely used in research.

Apart from periodic weighings, it has been proposed that food and water consumption should be monitored in detail by dividing each meal in bouts (episodes of uninterrupted feeding or drinking, having a start time, duration, and amount consumed) in order to capture the feeding patterns of the animal (75). However, even though this protocol could be more informative, it has not been broadly established yet and requires a specific device and appropriate software, none of which is publicly available. Still, it remains a promising and potentially useful idea which might be able to capture behavioural traits that allude us at this point in research.

Measuring body weight, food and water consumption on a daily basis was considered to be necessary since our research investigated for the first time the effect of chronic CORT administration to mice at a very young age (beginning at 4 weeks old). Previous research has investigated the effects of various concentrations of oral corticosterone administration to mice of different ages: at 5 weeks old for 4 weeks (59, 62), at 6 weeks old for 3 weeks (81) / 25 days (53) / 4 weeks (84) / 6 weeks (76) / 8 weeks (66), at 6-8 weeks old for 2 weeks (78), at 10 weeks old for 4 weeks (77), at 12 weeks old for 2 weeks (79, 80). During washout period (P46-73), the body weight, food and water consumption were measured approximately every 4 days.

Lastly, adrenal glands were excised from the surrounding adipose tissue and weighed. In order to acquire a more meaningful measurement index we also expressed the adrenal gland weight as a ratio of body weight: [adrenal weight (g) / body weight(g)] (66).

2.4. Elevated Plus Maze

The test chosen to explore the effect of CORT on behaviour was the Elevated Plus Maze (EPM). Our apparatus was a platform made out of PVC that consisted of two open and two closed arms, arranged in such a way that each arm was opposite to its identical and with a 90^{o} angle from the other two. The platform was raised to a height of 50cm above a steady white desk. The floor of the maze was covered by thick white paper which was replaced regularly while the walls of the closed arms were made out of grey Plexiglas. The arms' length was 65cm and width was 6cm while the height of the wall was 14cm for the closed arms and 0.5cm for the open arms. The center of the maze was a square of 6cm x 6cm. Each closed arm had the same light source.

On day P41, in the morning (09.00 - 11.00), mice were taken to the experimental room 30 minutes prior to testing, to allow habituation. After habituation, each mouse was placed in the center of the maze, with its head facing the same open arm, and was allowed to freely explore the maze for 5 minutes. In between trials, the maze was thoroughly cleaned with water mixed with 70% ethanol. Mice were video tracked and the total distance covered, the number of entrances and the total time spent in closed and open arms were quantified with the use of appropriate software (Ethovision XT8.5 specialized video tracking software). Also, the percentage of time spent in the open arms divided by the time spent to the closed ones was calculated. A mouse was considered to enter an arm if all its legs were inside.

In the cages housing 6 mice, only 3 were subjected to the EPM on day P41, 2 of which were sacrificed in the following 3 days. The remaining EPM naive animals (plus the one not sacrificed)

were introduced to the EPM on day P74. It has been documented that, on the second exposure of a mouse to the EPM, the animal displays decreased activity on the open arms of the maze compared to the first (82, 83), a phenomenon called one-trial tolerance (OTT). However, we considered this not to pose a problem for two reasons. First, this handling was adopted for the exact same number of mice for each group, which means that any effect would be the same for both groups. Second, previous studies suggest that if there is a 3-week period between tests (82) and even more if the inter-trial interval is raised to 28 days (83),it is possible to test rodents more than once without a drop in baseline open arm exploration. Our inter-trial interval was 32 days, so we reasoned that any baseline drop would be insignificant.

There is a vast variety of animal models of stress and anxiety (60) each with its own advantages and disadvantages. Popular behavioural tests like the Forced Swim Test (FST) are based in a premise that is problematic, namely, that a mouse which stops swimming early is showing a depressive response. It is not quite clear why such a behaviour should be interpreted as depression and not as an adaptive response to an inescapable situation. On the other hand, the EPM is a very simple protocol that is based on the natural inclination of rodents to avoid brightly lit areas in favor of darker one (74). Furthermore, it has been used as a measure of anxiety of rodents for over three decades and its advantages have been analyzed in length (71). We therefore chose EPM because it is a simple test that measures elementary behaviour (exploring patterns) without being invasive (ecological validity), it is not harmful or particularly stressful (ethical) and it does not include uncontrollable parameters (47, 52, 71). In particular, the mouse behaviour in the elevated plus maze has been properly analyzed as a measure of anxiety (55, 56). The C57/Bl6 has been found to enter the open and closed arms more than the

BALB/c (61), a characteristic that provides greater possibilities of changes as a response to CORT. Furthermore, the behaviour of pubertal C57/Bl6 in the EPM in response to chronic CORT administration has already been investigated (62) and it was found that CORT treated mice displayed anxiety-like behaviour. Further research in other protocols (Elevated Zero Maze, Forced Swimming Test, Open Field Test) has indicated that chronic oral administration of CORT to young adult male C57/Bl6 mice induced longer immobility times in the Forced Swimming Test (76).

2.5. Brain slice preparation

Brain slice preparation was performed as previously described from our lab (41, 42). On the 14th day of CORT administration (P42), the first mouse of each cage was sacrificed by cervical dislocation, its brain was extracted carefully and immediately put in a 50 ml glass beaker containing oxygenated (95% O_2–5% CO_2) ice-cold dissection buffer composed of, in mM: KCl 2.14; $NaH_2PO_4.H_2O$ 1.47; $NaHCO_3$ 27; $MgSO_4$ 2.2; D- Glucose 10; Sucrose 200; and $CaCl_2.2H_2O$; pH: 7.4.

Following extraction, a cut was made at the level of the cerebellum in order to create a flat surface on the caudal part of the cortex. This surface was then glued in a platform which was immediately submerged in oxygenated dissection buffer. Coronal brain slices (400μm) from primary somatosensory cortex were prepared using a vibratome (VT 1000S, Leica). Afterwards, slices were placed in a holding chamber with artificial cerebrospinal fluid (aCSF) and were left to recover at room temperature (RT: 24–26◦C) for at least 1 h before use. The aCSF contained, in

mM: NaCl 126.0; KCl 3.53; $NaH_2PO_4.H_2O$ 1.25; $NaHCO_3$ 26.0; $MgSO_4$ 1.0; D- Glucose 10.0 and $CaCl_2.2H_2O$ 2.0; pH: 7.4.

2.6. In Vitro Electrophysiology

Following recovery, slices were transferred to a submerged chamber (Luigs and Neumann), where they were gravity-perfused at high flow rates (10–15ml/min) to ensure optimal oxygenation of the cortical tissue (85, 86, 88). Recordings were performed in 'in vivo like' ACSF (composition as above but with 1mM CaCl2), since this ionic buffer is thought to better mimic cerebrospinal fluid in vivo (89) and our lab and others have previously shown that under these conditions cortical slices are spontaneously active (41, 42, 24, 90, 91, 92, 93). Recordings were performed at RT after at least 30min of incubation in 1mM [CaCl2] ACSF buffer. To stabilize slices we used a modified submerged type of chamber that included a surface of transparent silicone onto which up to four slices could be pinned. The advantage of this modification was that we could perform simultaneous recordings from 2 different animals, one of each group

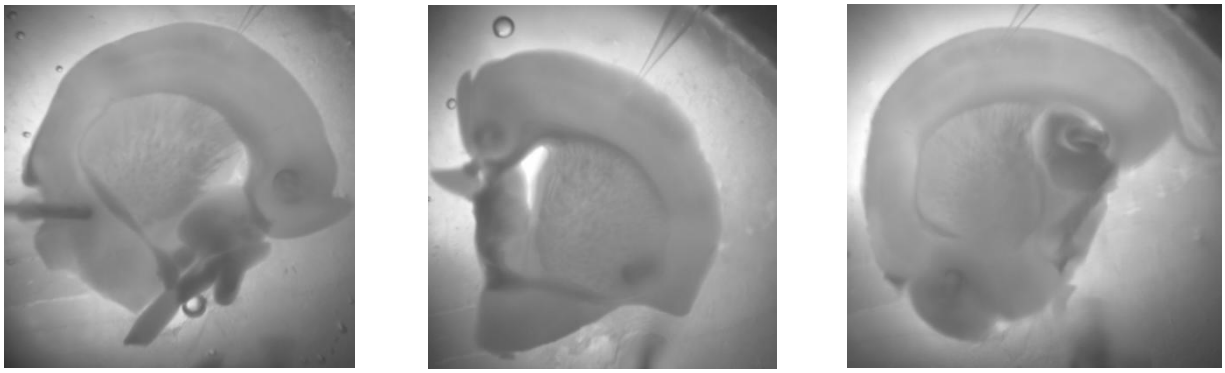


Image 2.1: Coronal mouse brain slices during recordings. The glass pippette is probbed in the primary somatosensory cortex of the whiskers ('barrel cortex', S1BF).

with two slices each. This way we could be certain that the conditions for both groups were identical.

Spontaneous network activity was assessed by means of local field potential (LFP) recordings (sampled at 5 kHz or 10 kHz, band-passed filtered at 1–3000 Hz) obtained from cortical layers II/III using low impedance (~0.5 MΩ) glass pipettes filled with aCSF (1mM [CaCl2]). Signals were acquired and amplified (MultiClamp 700B; Molecular Devices), digitized (InstruTech; ITC-18), and viewed on-line with appropriate software (AxoGraph X, version 1.3.5).

2.7. Data Analysis

For visualization and analysis of spontaneous events, traces were exported from Axograph to MatLab format and then analyzed with LFPAnalyzer, a custom-made software (111) specifically developed to automatically detect the LFP events and mark their onsets and offsets. Data was first pre-processed by low-pass-filtering (at 200Hz; third order Butterworth filter) and the DC offset subtracted. Detection of individual LFP bursts was performed with the following automated method: First, the signal was transformed using the Hilbert Transform to estimate its envelope (94). This is a linear operation that takes a signal u(t) and transforms it to H(u(t)), in the same (time) domain. The Hilbert Transform has been successfully applied for latency analysis (95, 96) in neurophysiological signals and is one of the basic tools in Fourier analysis, providing a concrete means for realizing the harmonic conjugate of a given function or Fourier series. Second, a threshold was applied to detect signal segments with fluctuation values larger than 40% of the Standard Deviation of the entire signal. This threshold was calculated for each trace (data-driven threshold) to ensure that the detection procedure is adjusted to the

corresponding signal-to-noise ratio of each recording and to the specific properties of each time series (e.g., size and frequency of events). Subsequently, the automatically detected LFP events were visually inspected to reject artifacts caused by electrical and/or mechanical noise. Duration of each event was calculated as the time interval between the onset and offset of individual events, while event occurrence was defined as the number of events divided by the duration of the recording session. Events were categorized as Upstates, biphasic bursts or multiphasic events, based on their appearance and spectral power content. The neurobiological substrate of the recorded activity has not been yet fully understood, so it is not clear whether these three types of activity differ qualitatively. The central type of lfp event based on which network activity is measured and compared is the Upstate, defined as a periodic depolarization consisting of barrages of excitatory postsynaptic potentials (EPSPs) and inhibitory post-synaptic potentials (IPSPs) which is displayed as bursts of high and low frequencies' electrical activity (24, 41). The biphasic event is defined as consisting of an initial dip from baseline activity followed by a rise and then a return to baseline, with no high frequencies involved. The multiphasic is considered to be a third category involving events that do not correspond fully to either of the two previous definitions but have characteristics of both.

2.8. Statistics

All data were tested for normality through three interrelated tests: a) distribution histogram examination, b) Shapiro-Wilk test and c) skewness - kurtosis examination. Statistical analyses were performed using SPSS software (version 23). In the comparisons of body weight, adrenal gland weight and behaviour in the EPM, the unit was the mouse. In the comparison of food and

liquid consumption, the unit was the cage. In the electrophysiological recordings, the comparison unit was the individual recording.

The following statistical comparisons were made to examine CORT effects in anatomy and physiology: (1) body weight among groups on P28 and on P41 for all animals (n=24 per group - repeated measures Anova), (2) body weight among groups on P46 and P74 for washout groups (n=8 per group - repeated measures Anova), (3) food and (4) liquid consumption per mouse among groups from days P28 to P41 by dividing the total consumption of each cage with the number of mice per cage (n=6, student's t-test), (5) adrenal glands' weight among initial groups (n=16 for control, n=15 for CORT - student's t-test) and washout groups (n=8 - student's t-test) and (6) normalized adrenal glands' weight after dividing it with the animal's weight (n=8 - student's t-test).

To assess anxiety we examined the behaviour of mice in the EPM by comparing (through t-test among groups): (i) total distance covered in cm, (ii) number of entries in open arms, (iii) number of entries in closed arms, (iv) time spent in open arms, (v) time spent in closed arms and (vi) the index M= $\frac{closed-open}{closed+open}$, where *closed* stands for time spent in closed arms and *open* for time spent in open arms. Higher values in the M index indicate more anxious animals. When data followed normal distribution the comparisons were carried out with independent samples t-test. In the cases where normality criteria were not fulfilled, comparisons were made with the non-parametric test Mann-Whitney. The same comparisons were made for both the initial (n=17 for CORT and n=18 for control) and the washout groups (n=8).

LFP events were initially divided in three categories: upstates, biphasics and multiphasics. For each category of events, a comparison was made among groups for (a) the duration of the event in seconds (based on the automatically detected onset and offset), (b) the occurrence of events in the recording (number of events divided by the duration of the recording session in seconds), (c) maximal negative peak, (d) rectified area and (e) spectral power. Apart from these measures, the power spectrum of each event, estimated on the basis of Fourier Transform coefficients, is presented both as continuous spectra, and in the conventionally described frequency bands: delta (1–4Hz), theta (4–8Hz), alpha (8–12Hz), beta (12–30Hz) and gamma (30–100Hz) range, normalized to the total power of each event in the 1–100Hz range. The normalization procedure allows a direct comparison of the % differences of power, since LFP events within or between recordings can differ significantly in both amplitude and duration and thus in absolute power value.

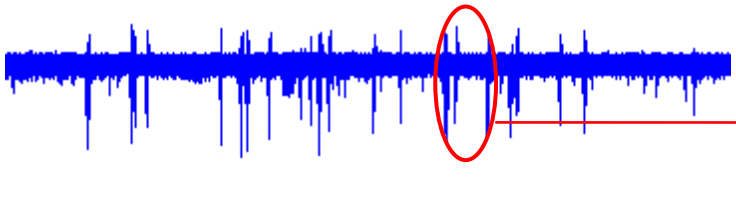


Figure 1a: Spontaneous activity in the form of Up/Down states recorded in a mouse brain slice.

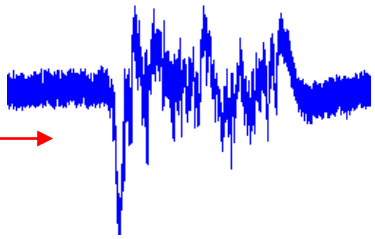
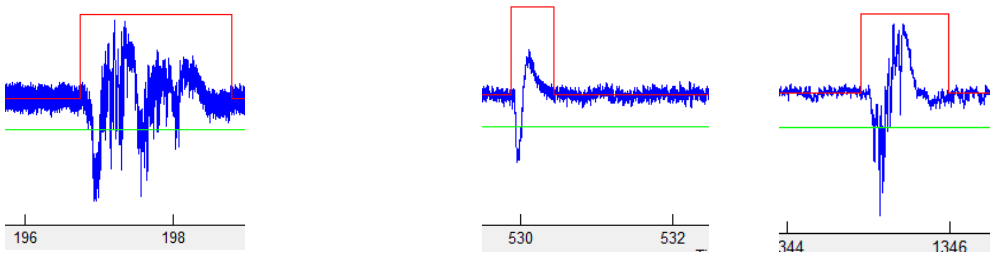


Figure 1b: Close-up of the LFP recording showing an Upstate at higher resolution.



a) Upstates | b) Biphasics or blimps | c) Multiphasics

Figure 2a, b & c: The three event categories

3. Results

3.1. Effects of CORT treatment on animal characteristics

3.1.1. Body weight

We compared the body weight of CORT vs control groups on days P28 and P41 with repeated measures ANOVA (each group consisted of n=24 animals). This comparison showed that on day P28 both groups had identical weights while on day P41 the control group had significantly increased weight compared to CORT (19.00±1.37gr vs 15.88±1.33gr, p<0.001).

	treatment	Mean	Std. Deviation	N
day1	control	12.9250	1.39042	24
	cort	12.9917	1.43555	24
day14	control	19.0000	1.37050	24
	cort	15.8833	1.32523	24

Table 3.1. Descriptive results for CORT vs control body weight (in grams) on days P28 ("day1") and P41 ("day14").

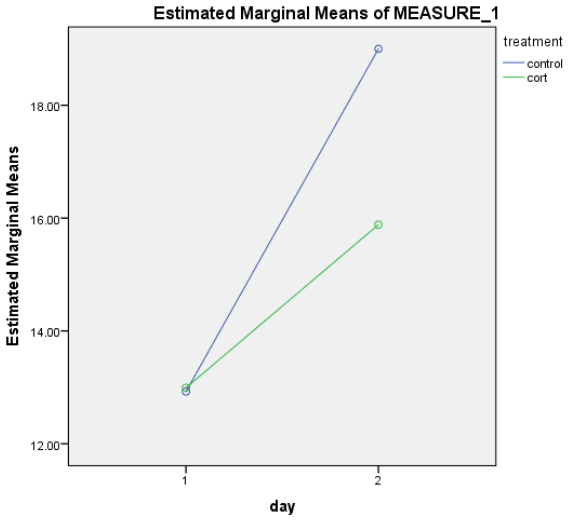


Figure 3.1. Body weight comparison (in grams) with repeated measures ANOVA for CORT vs Control on days P28 and P41.

Source		Mean Square	F	Sig.
day	Sphericity Assumed	482.407	592.832	.000
day * treatment	Sphericity Assumed	60.802	74.720	.000
Error(day)	Sphericity Assumed	.814		

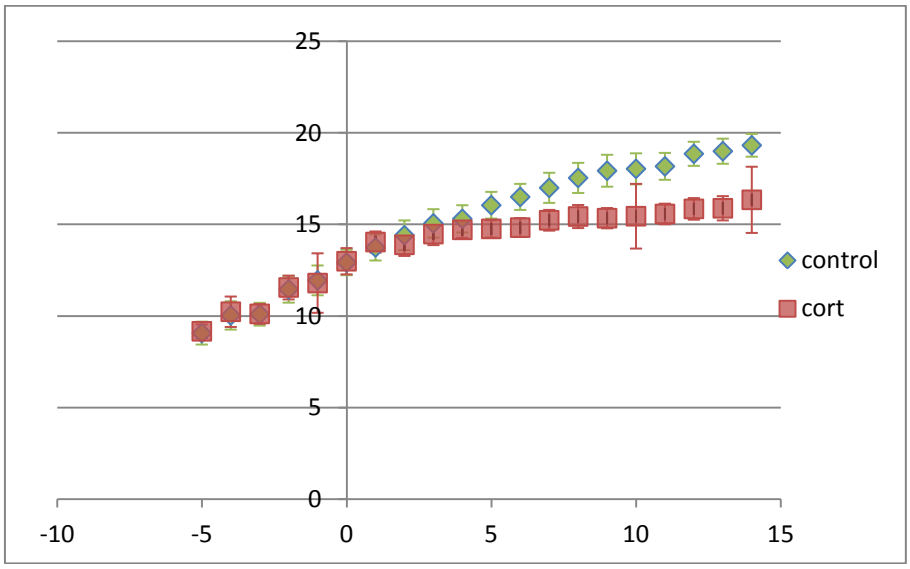
Table 3.2. Within subjects effects & interactions

Source	Mean Square	F	Sig.
Intercept	22179.840	7391.941	.000
treatment	55.815	18.602	.000
Error	3.001		

Table 3.3. Between subjects effects

Daily measurements of body weight from days P23 to P41 showed that during the acclimation days (P23-27, prior to CORT administration) both groups maintained identical weights while during days P28 (1st day of administration) - P41 (day of EPM) the CORT group displayed reduced weight gain every day compared to control (figure 3.2).

Figure 3.2.
Daily weight comparison (in grams) among CORT and control groups. Days -5 to 0 are during habituation and days 1 to 14 are during CORT administration



This effect was reversed during the washout period. Comparison of body weight at the beginning of washout (P46) and on the day of the second EPM (P74) with repeated measures ANOVA showed that there was a significant effect of day and a significant interaction of day*group (figure 3.3). Indeed, independent samples t-test revealed that on P46 the CORT group had significantly lower weight than control (19.15±1.80gr vs 15.85±0.71gr, $p<0.001$) while on P74 there was no significant difference among groups (tables 3.4, 3.5, 3.6).

Taken together, these results show that 2 weeks of CORT administration to pubertal male mice (P28-41) resulted in significantly reduced weight, an effect that was reversed after 4 weeks of washout. This can be displayed clearly in figure 3.4 which shows weight averages for the 8 mice per group that took part both in the initial experiment and in washout.

	treatment	Mean	Std. Deviation	N
day18	control	19.150	1.7976	8
	cort	15.850	.7151	8
day46	control	22.950	1.8447	8
	cort	21.850	1.7130	8

Table 3.4: Average weight (in grams) of CORT vs control on days P46 ("day 18") and P74 ("day 46").

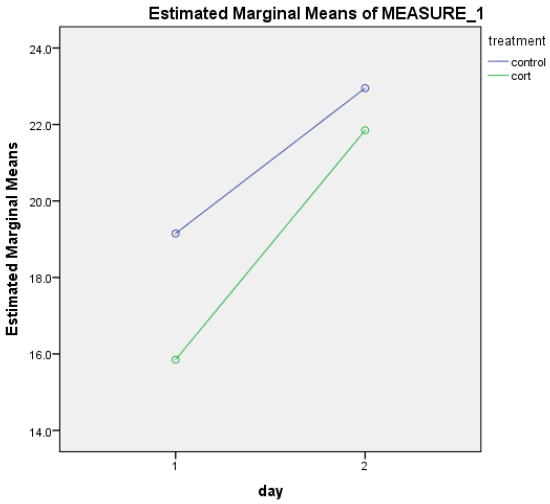


Figure 3.3: ANOVA comparison of CORT vs control body weight (in grams) on days P46 and P74.

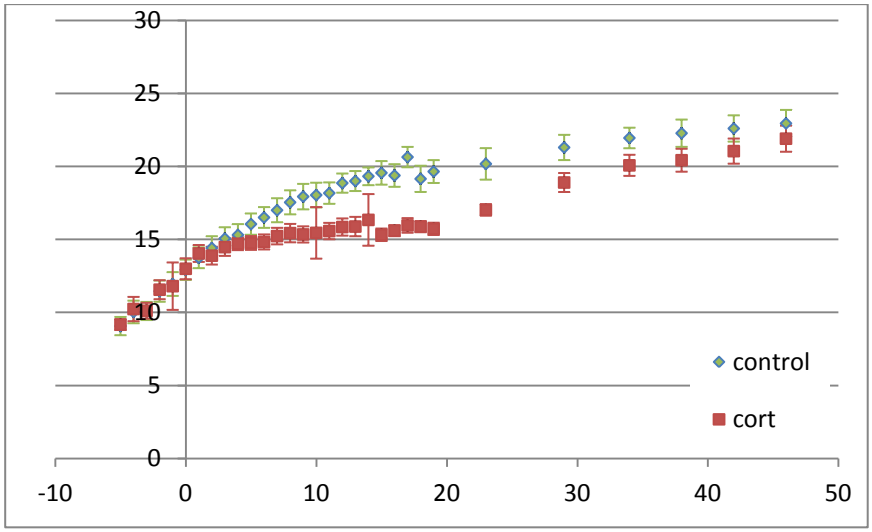
Source		Mean Square	F	Sig.
day	Sphericity Assumed	192.080	367.366	.000
day * treatment	Sphericity Assumed	9.680	18.514	.001
Error(day)	Sphericity Assumed	.523		

Table 3.5: Washout within-subjects effects & interactions

Source	Mean Square	F	Sig.
Intercept	12736.080	2819.499	.000
treatment	38.720	8.572	.011
Error	4.517		

Table 3.6: Washout between-subjects effects

Figure 3.4. Daily weight comparison (in grams) among CORT and control groups (n=8). Days -5 to 0 are during habituation and days 1 to 14 are during CORT administration. Washout took place during days 18 to 46.



It is obvious that these 8 mice followed the same pattern displayed by the larger group during CORT administration and that this difference disappears by the end of washout. This is in complete agreement with Klug and colleagues who found that corticosterone administration resulted in reduced body weight compared to control, a result that disappeared after 2 weeks of washout (81). Other research groups have also found reduced weight gain in CORT treated mice but only for males (66). However, most research groups have found no significant difference in body weight after CORT administration (62, 79, 80, 84, 124 in rats), others have found the reverse phenomenon (53, 59, 76) while there are also groups that regrettably did not monitor the body weight (49).

3.1.2 Food and liquid consumption

Food and liquid consumption per cage was measured daily and then divided by the number of mice in the cage. Therefore, the comparison unit was the cage (n=6 for each group) and not the mouse (n=24 for each group), even though the results are expressed per mouse. For washout, there were only 2 cages (with 4 mice each) for each group so it was not possible to make a statistical comparison.

Food (2.97±0.30gr vs 2.71±0.15gr, ns) and liquid (7.74±3.03ml vs 5.04±1.36ml, ns) consumption were similar for cort and control (table 3.7) during treatment. However, there was a clear tendency of the CORT group to consume more that the control even though the average weight of the CORT group increased less than the control throughout the CORT administration. The non-significance of the difference could be due to the small sample size which resulted in large typical variation. However, the tendency was not a result of chance. It has been

documented that mice housed with nesting material were heavier than mice grouped in standard conditions, even though they consumed less food than the control group and this is considered a result of less stressful conditions (118, 122).

During washout, the average consumption was similar for both groups. The CORT group consumed more food during treatment than during washout while the control group consumed approximately the same amounts of food throughout the experiment (table 3.8). However, the sample size is too small to make a statistical comparison.

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
liquid.consumption	control	6	5.0396825	1.36498708	.55725364
	cort	6	7.7380952	3.02671402	1.23565082
food.consumption	control	6	2.7091270	.15563794	.06353892
	cort	6	2.9658730	.30031603	.12260351

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means	
	F	Sig.	t	Sig. (2-tailed)
liquid.consumption	3.303	.099	-1.991	.075
food.consumption	.991	.343	-1.859	.093

Table 3.7: Group Statistics and Independent samples t-test results for food (in grams) and liquid (in ml) consumption among CORT and control during treatment.

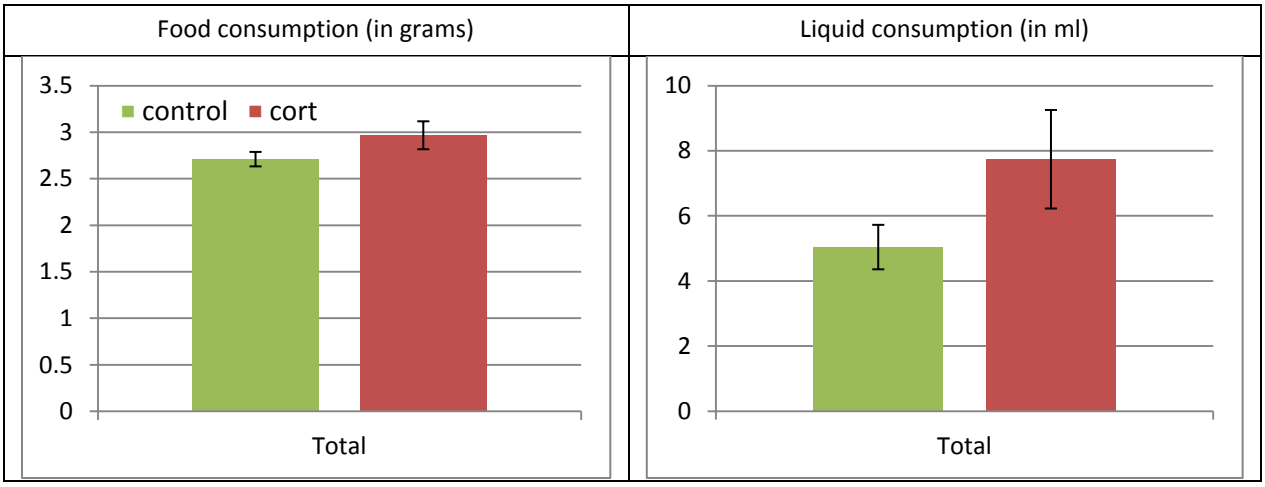


Figure 3.5: Food and liquid consumption during treatment

	control	cort
cort treatment	2.709126984	2.965873016
washout	2.878571	2.547321

Table 3.8: Mean Food consumption per day*mouse (in grams) during treatment

3.1.3. Adrenal glands

The adrenal glands' weight was computed for both glands of each mouse. It must be noted that due to the minor weight of adrenal glands the weighting procedure was subject to a large probability of error. Apart from the average adrenal weight we also computed the average normalized adrenal weight relative to the animal weight by dividing the adrenal weight to the body weight.

Group Statistics

	treatment	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
Relative weight	control	16	.0000673350	.00003078511	.00000769628
	cort	15	.0000475427	.00002482729	.00000641038
Adrenal weight	control	16	.001344	.0006175	.0001544
	cort	15	.000800	.0004106	.0001060

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means	
	F	Sig.	t	Sig. (2-tailed)
Relative weight	.003	.960	1.962	.059
Adrenal weight	.887	.354	2.866	.008

Table 3.9: Group Statistics and Independent samples t-test results for adrenal glands weight (in grams) and relative weight, among CORT and control after treatment.

Independent samples t-test showed that CORT group had significantly lower adrenal glands' weight than control (0.80±0.41mg vs 1.34±0.62mg, $p<0.01$). However, this difference became non-significant once adrenal glands were normalized for the mouse weight ($47.54x10^{-6}$ ±

$24.82x10^{-6}$ vs $67.34x10^{-6} \pm 30.79x10^{-6}$, ns; see table 3.9). After washout, independent samples t-test showed that no significant differences remained among groups (table 3.10).

In bibliography, in some cases adrenal weight has been found decreased in the CORT group (59, 62) while in others no significant difference was found (79, 124 in rats). It has also been found that CORT treatment induces a marked reduction of adrenal weight in male, but not in female mice (66).

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
Adrenal weight	cort	8	.001950	.0007091	.0002507
	control	8	.001500	.0005581	.0001973
Relative weight	cort	8	.0000860759	.00003377448	.00001194108
	control	8	.0000690337	.00002680011	.00000947527

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Adrenal weight	1.982	.181	1.410	14	.180
Relative weight	1.561	.232	1.118	14	.282

Table 3.10: Group Statistics and Independent samples t-test results for adrenal glands' weight (in grams) among CORT and control after washout.

3.1.4 Animal physiology and behaviour

Apart from the measured parameters mentioned, there were also some non-quantified observations made concerning the physiology and behaviour of mice. First of all, during treatment, the CORT group produced more fecal boli and urine than control. This was observed also at EPM, during which the CORT group defecated much more than control, exactly as indicated previously (76). It has been documented that higher defecation and urination behaviour of rodents is indicative of anxiety (97, 98). Furthermore, the pattern of defecation

was different among groups. Normally, mice prefer defecating in the front of the cage, under the feeder (121). In our experiment, the CORT group displayed the aforementioned behaviour, with a larger amount of feces deposited at the outer part of the cage (image 3.1) while the control group did not show any particular preference.

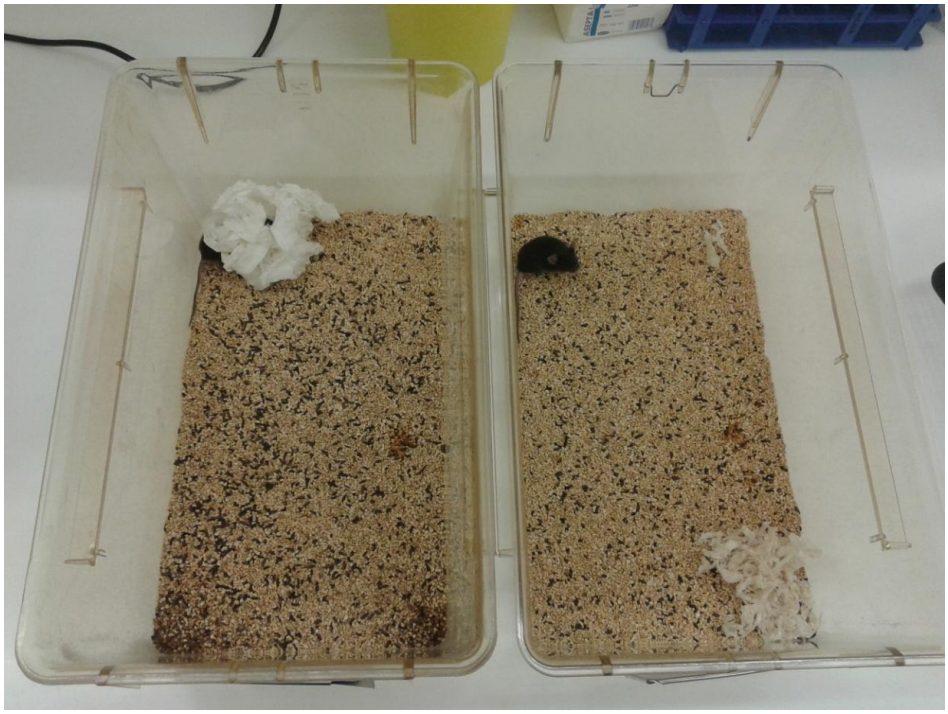


Image 3.1: In the CORT group cage (left), after 2 weeks, a high amount of feces is obvious, concentrated especially in the outer part of the cage. The tissue is almost intact in the inner part of the cage. In the control group (right) feces are obviously less, scattered equally in all the cage. The tissue is torn in multiple pieces to construct a better nest and left in the outer part of the cage.

Another difference among groups concerns the tissue that was left in the cage as nesting material. Mice use the tissue as shelter and therefore keep it at the part of cage they rest. In the IVC cages used in our experiment, cages are placed in shelves on the wall with a central lighting inside the room. As a result, the outer part of the cages is on the same side with the feeder and is more illuminated than the inner. Previous research has shown that mice partition

their cages and preferentially use as shelter the area under the feeder (120). Therefore, it was expected that the control group would use the illuminated, external part of the cage as shelter while the CORT group would prefer to sleep in the inner, darker part. Indeed, the two groups demonstrated exactly this behaviour in all cases (image 3.1).

Apart from the location at which the mice put the nest, another difference observed among groups was the amount of processing that each group devoted to their nest. Normally, mice chow and tear the tissue in multiple pieces rapidly in order to create a dome-like nest that can protect them from the cold (lab mice are housed at 20-24^{o}C but their lower natural temperature is 30^{o}C). The more processed the tissue, the better insulated will the nest be by trapping heat within and reducing overall heat loss to the environment (122). In our experiment, the control group displayed normal behaviour, tearing the tissue rapidly and creating a high-quality nest. The CORT group, on the other hand, kept the tissue almost intact throughout the treatment (image 3.1).

Also, there was another observation that highlighted the differential growth among groups. When CORT mice were sacrificed their tails were found to be much shorter and thinner than control (image 3.2). Unfortunately, this difference was not measured. However, bibliography suggests that reduced tail length is associated with lower body weight and tail length, in particular, is considered an index of skeletal growth. As such it has been supported that reduced tail length is related to growth hormone deficiency (99).

Lastly, another non-measured observation was the fact that the adipose tissue of CORT mice was increased compared to control, especially the one surrounding the adrenal glands. This is in

line with bibliography that supports that 4 weeks of corticosterone administration induce increased fat in adipose tissue (62).

Taken together, our results indicate that the CORT treatment resulted in an obvious stress phenotype, manifested by reduced weight gain, increased food consumption, lower adrenal weight, thicker adipose tissue, thinner and shorter tails, increased fecal boli and urine and worse quality nests.

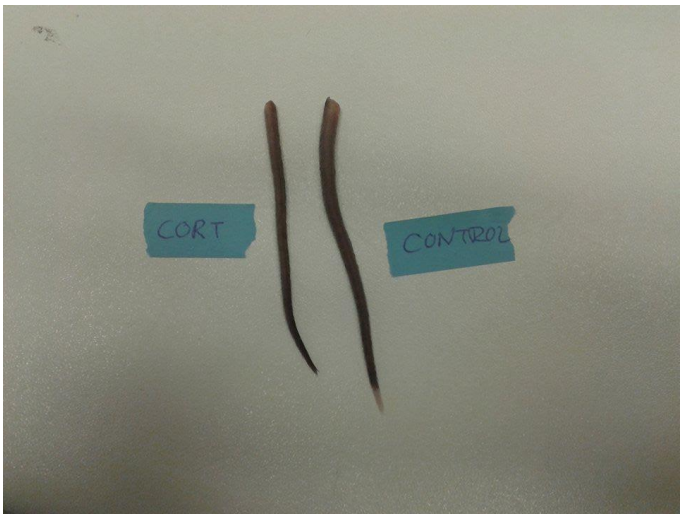


Image 3.2: Tails of sacrificed mice from the CORT (left) and control group (right).

3.2. Effects of CORT treatment on anxiety behaviour

In the Elevated Plus Maze, we measured the total distance covered, number of entrances (frequency) and time spent in open and closed arms. We also calculated M=$\frac{closed-open}{closed+open}$, where *closed* and *open* are the time spent in closed and open arms respectively. Normality tests showed that total distance covered, frequency and time spent in closed arms followed the normal distribution, while frequency and time spent in open arms and M index did not. Thus, the first three parameters were compared among groups with independent samples t-test and frequency and time spent in open arms, and the M index were compared with the non-parametric Mann-Whitney test. The results showed that CORT group covered significantly less

total distance than control (912.15±260.29cm vs 1126.97±209.38cm, $p<0.05$), meaning that control group was significantly more mobile than CORT. This fact, in turn, means that frequencies cannot be used as measures of anxiety. An independent samples t-test also showed that CORT mice spent significantly more time than control in closed arms (225.82±29.07sec vs 199.96±32.10sec, $p<0.05$).

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
Total distance	cort	17	912.15335	260.288002	63.129113
	control	18	1126.97422	209.382017	49.351815
Time in closed	cort	17	225.8165	29.07337	7.05133
	control	18	199.9600	32.09839	7.56566
Frequency in closed	cort	17	14.4118	5.07517	1.23091
	control	18	17.1667	4.27372	1.00733

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means	
	F	Sig.	t	Sig. (2-tailed)
Total distance	.920	.345	-2.698	.011
Time in closed	.002	.963	2.493	.018
Frequency in closed	.285	.597	-1.741	.091

Table 3.11: Group Statistics and Independent samples t-test results for Total distance (cm), time spent (sec) and frequency of entrances in closed arms among CORT and control after washout.

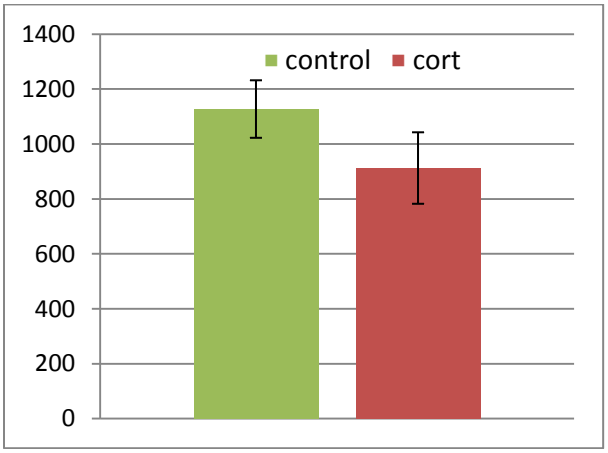


Figure 3.6a: Total distance covered (in cm)

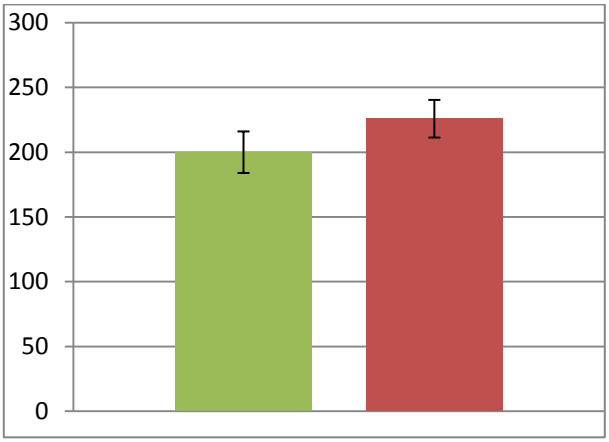


Figure 3.6b: Time spent in closed arms (in seconds)

Since total distance was significantly different among groups, we only took into account the time spent in open arms and the M index. Correcting for multiple comparisons we needed a specific significance (<0.0167) which was the case for all three measures. Therefore, we concluded that the CORT group displayed an increased anxiety-like behaviour compared to control since CORT mice spent significantly more % of the time in closed arms, entered less times in open arms and had a higher value in the M index.

Ranks

	treatment	N	Mean Rank
Time in open	control	18	22.17
	cort	17	13.59
Frequency in open	control	18	21.42
	cort	17	14.38
M	control	18	13.56
	cort	17	22.71

Test Statistics

	Time in open	Frequency in open	M
Mann-Whitney U	78.000	91.500	73.000
Asymp. Sig. (2-tailed)	.013	.040	.008

Table 3.12: Mann Whitney results for time and frequency in open arms and M.

Examining the behaviour of mice in the EPM after washout, we first checked whether the measures follow the normal distribution, as before. Comparisons showed that there was no significant difference among groups for any of the measures. Therefore, we can conclude that after CORT treatment, the CORT group displayed significantly more anxiety-like behaviour than control. This difference disappeared on the second EPM after washout.

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
Total distance (in cm)	control	8	1143.17575	239.545988	84.692296
	cort	8	1002.87138	364.986700	129.042285
Frequency in open	control	8	3.3750	3.50255	1.23834
	cort	8	2.2500	2.43487	.86086
Time in closed (in sec)	control	8	233.0300	24.69928	8.73251
	cort	8	218.5800	54.81418	19.37974
Frequency in closed	control	8	14.8750	4.45413	1.57477
	cort	8	14.2500	7.02546	2.48388

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
Total distance (in cm)	Equal variances not assumed	4.781	.046	.909	12.087	.381
Frequency in open	Equal variances assumed	2.014	.178	.746	14	.468
Time in closed (in sec)	Equal variances assumed	2.682	.124	.680	14	.508
Frequency in closed	Equal variances not assumed	5.732	.031	.213	11.845	.835

Test Statistics[a]

	time.in.open	M
Mann-Whitney U	30.500	30.000
Exact Sig. (2-tailed)	.900	.878

Table 3.13: Comparisons after washout

3.3. Effects of CORT treatment on endogenous cortical activity

As described in the Methods section, each LFP event was manually characterized as Upstate, biphasic or multiphasic. Comparisons among groups were made for each type of event (upstate / biphasic / multiphasic) and a variety of parameters were examined as potential comparison indices. These included duration of event, event occurrence, rectified area, network index as

well as the absolute and relative power of events in distinct frequency bands: delta (1–4Hz), theta (4–8Hz), alpha (8–12Hz), beta (12–30Hz) and gamma (30–100Hz). Duration and occurrence are the measures mostly used to identify differences among groups in bibliography.

Normality tests showed that for upstates and multiphasics duration and rectified area followed the normal distribution. No other parameter for any category of events followed the normal distribution. We compared the duration and rectified area for upstates and multiphasics with independent samples t-tests. The only parameter that displayed a significant difference was the upstates' duration where the CORT group displayed smaller duration than control. However, after correcting for multiple comparisons, this difference did not reach significance.

Group Statistics

	treatment.code	N	Mean	Std. Deviation	Std. Error Mean
duration.upstates	control	19	1.84626	.436481	.100136
	cort	11	1.53955	.269145	.081150
rectified.area.upstates	control	19	.270737	.1098948	.0252116
	cort	11	.222909	.0955609	.0288127
duration.multiphasics	control	19	1.21079	.330097	.075729
	cort	11	1.18309	.196559	.059265
rectified.area.multiphasics	control	19	.12768	.050010	.011473
	cort	11	.14845	.067604	.020383

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
duration.upstates	Equal variances assumed	2.540	.122	2.102	28	.045
rectified.area. upstates	Equal variances assumed	.147	.705	1.202	28	.239
duration. multiphasics	Equal variances assumed	1.869	.182	.252	28	.803
rectified.area. multiphasics	Equal variances assumed	1.996	.169	-.963	28	.344

Table 3.14: Comparison results for upstates and multiphasics after treatment.

We also compared the occurrence of upstates with Mann Whitney test and there was also no significant difference.

In order to investigate whether one of the groups or one of the events' categories was affected in a different way from the rest, we calculated the distributions of all the categories (upstates, biphasics, multiphasics) for each group (CORT, control) and every electrophysiology measure. However, no clear difference was observed among any groups or events' categories (see Appendix I).

For washout, the same analysis was done as previously and no significant difference was found among groups for any of the parameters.

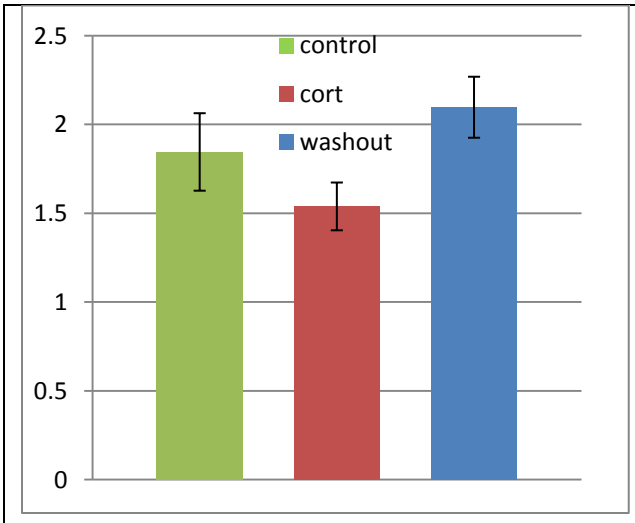


Figure 3.7a: Upstates' duration (in secs) for control and CORT after treatment and CORT after washout.

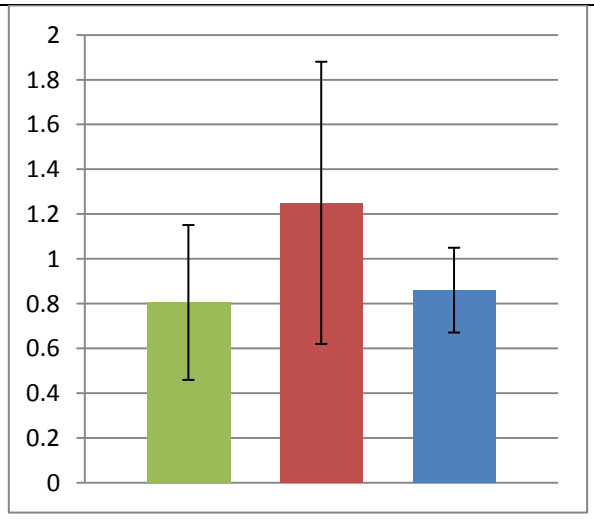
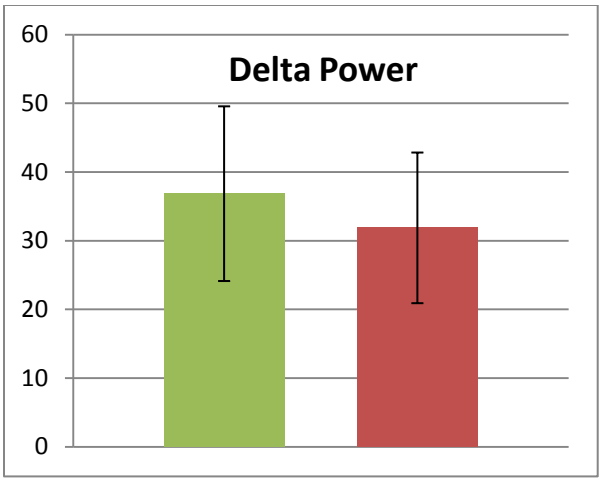
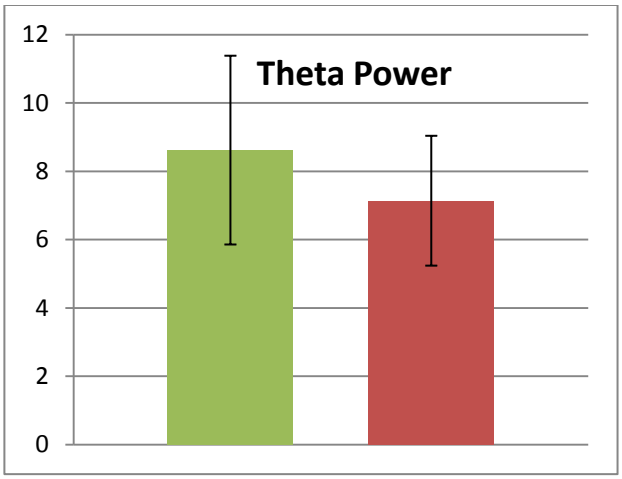
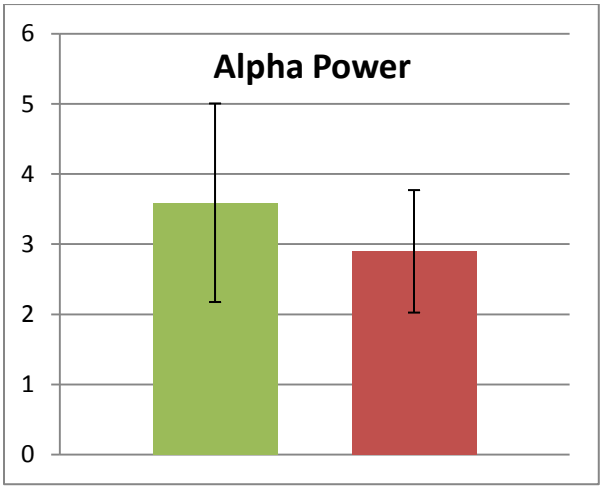
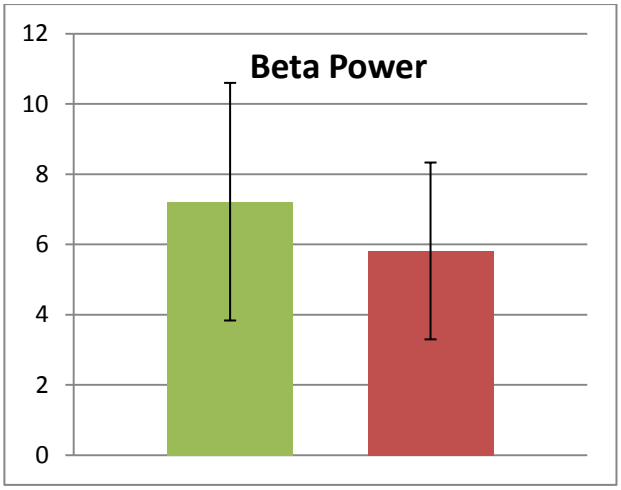


Figure 3.7b: Upstates' occurrence for control and CORT after treatment and CORT after washout.









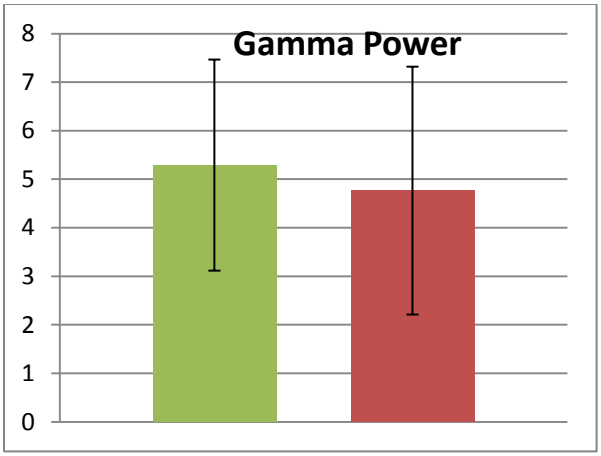


Figure 3.8:
Power Spectra for delta (1–4Hz), theta (4–8Hz), alpha (8–12Hz), beta (12–30Hz) and gamma (30–100Hz)

In conclusion, even though CORT treatment resulted in significant changes in the anatomy and behaviour of treated mice compared to control, it was not able to cause significant effects in the cortical network activity modeled by Upstates. This result indicates that Up and Down

states are a robust phenomenon, not particularly susceptible to increased levels of corticosterone during puberty. On the other hand, since we worked with a relatively small sample we might have missed differences that would have been masked by the large standard deviation of our sample. For this reason we examined the degree of correlation between variables that indicate the animals' stress level (i.e. body weight, open arms frequency and open arms duration) with the main parameters of Upstate activity (ie. occurrence, duration, network index)". This analysis did not show any significant correlations (see Appendix II).

4. Discussion

4.1. Anatomy and Physiology

Based on previous research, we expected that the CORT treated mice would present some or all symptoms of metabolic syndrome (53, 59, 62, 131) and demonstrate depression-like behaviour (76, 79, 81, 129, 136). In our experiment, all anatomical and physiological differences observed among groups were in line with previous research by Lee and colleagues, showing that 4 weeks of corticosterone administration induce adrenal hypertrophy, increased adipose tissue and reduced body weight (62). Furthermore, exactly as in (62), these differences were reversed after washout. Klug and colleagues have also found reduced body weight gain after 3 weeks of CORT treatment (81) while another group has found reduced body weight after chronic injections of corticosterone (135). However, other researchers have found different results concerning body weight. Some researchers observed no significant difference among groups (62, 80, 84, 124 in rats, 129) while others have noted that long-term corticosterone administration (either through injection or through drinking water) lead to an increased body weight compared to control (53, 59, 76, 130, 136). More importantly, the Karatsoreos lab found dose-dependent difference in body weight with high doses of CORT increasing body weight more that low doses (53, 59, 131) compared to control. There are many crucial factors that could be responsible for the different results: mice age / gender / strain, CORT concentration / duration / administration method or living conditions (cage, food, temperature, humidity, handling). However, the discrepancy between the Karatsoreos' results and ours is harder to

interpret because we followed the same CORT protocol and mice were in both cases all male, of the same strain and age.

In our experiment, the CORT group demonstrated reduced weight gain while consuming the same amount of food and liquid with the control group. To be accurate, the CORT group consumed more than the control but the difference did not reach significance due to the small sample size (number of cages). Previous research has found Increased food consumption (59, 136) or in some cases similar consumption (129). Since the CORT mice ate and drank slightly more than the control, reduced appetite cannot explain the difference in body weight. However, as proposed by researchers in the field of animal behaviour and well-being, reduced weight in combination with increased food consumption can be explained by heat loss due to poor nest quality (122). Indeed, it was observed that the nests of CORT mice were of poor quality, which might have resulted in greater heat loss and, eventually, reduced weight gain. Therefore, we can hypothesize that CORT administration resulted in mice becoming more passive and neglecting to take care of their nest, which in turn resulted in greater heat loss and more weight loss. However, this hypothesis does not explain why this was not the case in the Karatsoreos experiment (53).

Even though there is a lot of controversy on the effect of corticosterone administration on body weight, the results concerning the adrenal glands and the surrounding adipose tissue are not so contradicting. In most cases, after chronic corticosterone administration CORT mice exhibit either lower (59, 62, 66) or similar (79, 124 in rats) adrenal weight compared to controls. In all previous reports where adipose tissue was measured, it was increased in the CORT group (59, 79). In our experiment, adrenal glands' weight was lower for the CORT group compared to

control (but this difference became non-significant when the glands' weights were normalized to the animals' total body weight). This result comes as no surprise. Since in this paradigm the needs of the organism for corticosterone are covered exogenously, endogenous secretion is inhibited. As a result, the adrenal glands become hypoactive and grow less in size than normal. On the contrary, when stress is induced behaviourally (through restraint, immobilization, maternal separation, food / liquid restriction, cold, social defeat, sleep deprivation etc) the experimental group presents adrenal hypertrophy compared to the control (26, 58, 123, 128) as a result of endogenous hypersecretion of corticosterone. Adipose tissue was not measured in our experiment but it was observed that it was clearly more in the CORT group compared to control.

4.2. Behaviour

In the Elevated Plus Maze, mice belonging to the CORT group covered less total distance than controls, spent more time in the dark arms of the maze, made fewer entries in the open arms and defecated more during testing (and generally). Once again, this result was identical to Lee and colleagues (62) who found increased time spent in closed arms by the CORT group after 4 weeks of treatment. This difference disappeared after 4 weeks of washout both in our experiment and theirs. In a large scale test battery that included 1671 mice tested in an Open-Field arena, a Mirror-Chamber Box, an EPM and an Elevated Square Maze, increased autonomic functions like defecation (and urination) were associated with reduced locomotion, longer latencies to enter new test areas and less time spent in anxiogenic areas, a pattern that is consistent with an interpretation of a behavior pattern reflecting fear or anxiety (98). Taken

together, the results of our experiments are all in accordance with the conclusions of this investigation and suggest that the CORT mice displayed anxiety-like behaviour, as anticipated.

In apparent contrast to our results, Gourley and colleagues had found increased locomotor activity in the CORT group compared to control and similar open arm entries among groups (79). However, Gourley's protocol and ours have many differences. In our experiment, mice at the time of testing were 6 weeks old while in Gourley's experiment they were at least 12 weeks old. Also, the preparation of the CORT solution was different. In addition, Gourley included a habituation ('weaning') period for mice after CORT treatment in order to allow for normal recovery of endogenous CORT secretion. However, the most important parameter that needs to be taken into consideration concerning the behavioural testing is the time between the end of the CORT treatment and the EPM. In our case mice were tested in the EPM immediately after 2 weeks of CORT whereas in the experiment by Gourley mice were tested 2 weeks after the termination of the treatment. As we observed after our 4-week washout phase, mice did not display any difference in the EPM (or in physiological measurements) indicating that all effects of corticosterone administration had been negated. It is probable that this reversal may have happened even earlier than 4 weeks. Therefore, we believe there is no contradiction between our result and the one from Gourley.

Other groups using the CORT protocol have chosen different behavioural tests in order to examine for anxiety or depression-like effects (Elevated Zero Maze, 76; Forced Swimming Test, 76, 79, 80, 129, 135, 136; Open Field Test, 76, 84, 136; Novel Object Recognition, 84; Y-Maze, 66, 81; Tail Suspension Test, 78, 129, 135; Light-Dark Box, 132) and therefore our results are not

comparable to theirs. Regrettably, the Karatsoreos lab has not included any behavioural testing in their experiments whatsoever (49, 53, 59, 130). This is unfortunate because we have followed the CORT administration protocol that they have proposed and therefore comparisons with this group's results would have been more informative.

As described previously in the results section, apart from the EPM test, we made some significant observations concerning defecation and urination, nest state and quality, tail thickness and length that were not quantified. In general, few researchers have made any similar observations in relation to corticosterone administration. Firstly, Van Donkelaar and colleagues (76) noted that during a 20 min open field session the CORT group demonstrated significantly increased defecation and they interpreted this fact as a result of disturbed emotionality instead of fearfulness or anxiety. As has been reviewed in the past (137), emotionality is a particularly complex and vague notion, which is difficult to define in animals. Furthermore, the association between defecation and emotionality requires the relation of defecation with heart rate, avoidance learning and other factors. More importantly, as noted by Henderson and colleagues (98), increased defecation and urination, when combined with decreased locomotion (especially in anxiogenic locations like illuminated or predator scented spots), suppressed rearing and late exploration of novel areas, is a clear indication of anxiety. In our experiment, CORT treated mice displayed 4 out of 5 QTLs (Quantitative Trait Loci). We can therefore conclude that the behaviour of CORT mice was characteristic of anxiety. However, it is regrettable that most researchers in the field of CORT administration do not include a thorough defecation index because it is clear that it is one of the safest measures of anxiety in rodents, it is widely used in stress research (98, 138).

Even though we noticed that the tails of CORT mice on the day of sacrifice were thinner and shorter than control, we found no mention of tail length or thickness in any research paper related to CORT administration. It has been proposed that tail length can be used as a measure of rodent growth (99) and, based on our observations, it might be a useful index of development.

In our experiment, the nest building behaviour of CORT mice during treatment was completely different from the control group. CORT mice left the tissue almost intact and did not create a good quality nest. In contrast, control animals tore and chewed the paper rapidly in order to build their nest. Nest quality is an important parameter of anxiety in animals (119, 122) and this is obvious from the fact that a) there is a specific early-life stress protocol that consists of dam and pups being housed in cages with limited nesting / bedding material (139, 140) and b) one of the simplest and most popular methods of environmental enrichment is enhancing the nesting material (118, 134). In addition, nest building by mice has been quantified and can be easily assessed with a simple and quick test (68). Even so, no research paper was found to mention the effect of CORT to the quality of nests. After our observation, we propose that nest building should be included in experiments as a measure of anxiety-like behaviour induced by corticosterone administration.

To conclude, in our experiment, corticosterone treatment induced various anatomical, physiological and behavioural effects, which were all reversed after 4 weeks of washout. This reversal is in accordance with previous studies showing that daily oral corticosterone treatment for 21 days causes temporary dendritic retraction in CA3 and dentate gyrus (26) as well as in

mPFC (125) but that these effects are fully reversible. This indicates that exogenous corticosterone treatment is an effective protocol for the induction of stress-like symptoms, which can be reversed within weeks. It could be interesting to examine whether longer-term exposure to cort may eventually lead to non-reversible effects.

One additional thought concerning the various behavioural parameters discussed has to do with the stress experienced by lab animals due to standard housing conditions. It is well documented (118, 119, 120, 122, 140) that the housing conditions for experimental mice are by themselves stressful. Mice housed under a slightly more enriched environment consume less food while gaining more weight (118), are less aggressive (119), are more active and display a much more natural and rich repertoire of behaviours (120). In standard conditions, mice are housed at 20-24^{o}C, much lower than their lower critical temperature (~30^{o}C) of the thermoneutral zone (the temperature range along which animals are able to maintain their normal body temperature without expending energy). This means that mice could be permanently under a certain amount of stress due to cold (122). They are also housed in a cage that has limited (one tissue by default) or no nesting material at all. This also stresses mice because they are unable to build a satisfactory nest, especially in the case of dam and pups (140). Therefore, one interesting question is whether our control groups consist of normal or stressed animals. This is not only a matter of ethics but also one of research quality (120). If control animals are already stressed, then a large part of research that showed no significant results should be reevaluated. Furthermore, we should also try to make the housing conditions less stressful for mice by increasing the heat closer to 30^{o}C and providing more tissues as nesting material (77).

4.3. Electrophysiology

In our experiment, we recorded spontaneous network activity from brain slices kept in oxygenated aCSF, as described in the methods section. Having divided the LFP events in three categories (Upstates, Biphasics, Multiphasics), we computed the averages of various parameters (duration, occurrence etc) for each group. We found no difference among groups for any parameter of the Biphasics and Multiphasics, while the only parameter significantly different for the Upstates was the event duration, which lost significance after multiple comparisons correction. We believe that the absence of significant differences can be interpreted as an indication that the Upstates / Downstates activity pattern is a robust phenomenon that withstands hormonal alterations in the organism. In this case, the CORT administration did not manage to change cortical network dynamics, even though it resulted in all the aforementioned anatomical, physiological and behavioural changes. It should be noted that after the cort treatment, duration and occurrence of Upstates were neither significantly different nor completely identical. On the contrary, after washout both measures were virtually identical for both groups and equal to the values of control in the initial phase. This could be interpreted as a phenomenon that was robust enough to avoid the effects of CORT and return to normal during washout.

Potential limitations:

Of course, it is possible that differences indeed existed but we were not able to capture them. This could have happened for various reasons.

1. Attribution of LFP events in 3 separate categories.

As discussed in the methods section, events were manually categorized in one of three types of activity based on their appearance and spectral power content. Hence it is theoretically possible that under a different classification scheme, the results might have been different. However, we believe the classification is both legitimate and useful. Concerning the allocation of events in the first two groups (Upstates / Biphasics) previous work in the lab has confirmed the categorization by cluster analysis. This was based on 3 non-overlapping parameters (event duration, normalized beta power and negative peak amplitude) and showed nearly perfect agreement with expert user categorization. In this prior work, there remained a number of events which did not comply with the criteria for either event category and were excluded from further analysis. In this thesis, such events were introduced as a third distinct category (multiphasics). In order to investigate the usefulness of the categorization, we calculated the total variation of all the events for a number of event parameters (duration, occurrence, rectified area and different frequencies) and the total variation of each category for the same measures. To calculate the total variation explained for each measure, we first calculated the variation before categorization through the formula: $Vt = (X - AV)^2$,

where ***X*** is the value of the measure for each recording and ***AV*** is the average for all recordings.

Then we calculated the variation after categorization for each category through the formulas: Vu = $\frac{(X-AVu)^2 * u}{n}$, Vb = $\frac{(x-AVb)^2 * b}{n}$, Vm = $\frac{(x-AVm)^2 * m}{n}$,

where for each measure: AVu, AVb and AVm are the averages of Upstates, Biphasics, Multiphasics for each recording, u, b, m are the number of Upstates, Biphasics and Multiphasics in each recording and n is the total number of events for each recording. The sum of the total variation of the three categories was smaller than the total variation of all the events together, indicating that the categorization explained a large part of the variation for most of the measures. We therefore believe that the classification we have chosen is appropriate as it is able to provide a useful separation of events based on their specific properties.

Total variation calculation

	control		cort	
	Vt	Vu+Vb+Vm	Vt	Vu+Vb+Vm
occurrence	61.496	34.614	40.43	12.858
duration	3.717	1.710	0.716	0.080
rectified area	0.159	0.008	0.051456562	0.038
absolute delta	6281.974929	6212.033	2475.177246	2408.711
absolute theta	256.233155	243.431	83.55232698	58.765
absolute alpha	51.61726297	55.789	16.51541014	13.275
absolute beta	246.8076234	290.036	106.8617012	113.746
absolute gamma	111.2144829	122.215	107.9534041	129.089

At this point, we do not know what is the biological substrate of the different event types and what kind of difference exists underneath. Even though all of these events are known to be manifestations of local network activity, it is not clear what factors account for the different event morphologies we observe. It could be proven in the future that all of these events belong to the same category of local neuronal activity and that their difference is just quantitative. For instance, one possibility is that when a few neurons synchronize they produce a biphasic event but when a large number of neurons become excited at the same

time the result is an upstate. On the other hand, it could be that it's not the number of synchronizing neurons that varies but the degree of synchronization. For example it could be that neurons that become completely synchronized produce biphasics while neurons that synchronize less produce upstates (or vice versa). Further investigations will undoubtedly shed light to the biological substrate of these local network events.

2. Response variability

Another factor that may have masked cort-induced differences is the large variability inherent in the recordings. Spontaneous Up state activity is known to be highly variable, reflecting the properties of the local network (41, 42). This variability could be due to somewhat different neuronal populations participating in the activity measured in each recording. Indeed, even though we always target the barrel field of the primary somatosensory cortex, the precise location onto which the electrode is attached is not exactly the same. Also, it is not always possible to record from the same slice for all mice. There is a chance that the phenomenon we are recording through LFP is of a very local nature. In this case, targeting the exact same neuronal population at the same slice for all mice of both groups could be the route to follow in future research.

Another factor that needs to be addressed is the acuity and precision of the procedure. Even though the slices are always coronal and cut at exactly 400μm, the initiation of the slice cutting depends on the specific experiment conditions and the mouse anatomy. Specifically, the way the brain is glued to the cutting platform (angle, coronal depth) might not be completely identical in each experiment, especially since the procedure must be

done very quickly in order to keep the tissue alive. Finally, it is obvious that one mouse could have different brain anatomy from another. All these factors could be of some significance to the comparison results, but we believe that since they apply to both groups their contribution should be similar. Therefore, the only way these effects could have an effect to the comparison would be by increasing the typical variation and hence possibly reducing the significance of the difference. As suggested previously, these concerns might also be addressed in future research by targeting the same neuronal population in the same slice for all mice.

3. Regionally-specific vulnerability

Our study is the first to investigate the effect of chronic corticosterone administration on spontaneous network dynamics in vitro. We recorded from somatosensory cortex because this is where spontaneous activity has been found to be more robust and has been most extensively studied (41). However, the somatosensory cortex has not been associated with corticosterone or stress protocols as strongly as other brain areas like the prefrontal cortex, hippocampus and amygdala (37, 70, 125, 126, 133). Therefore, a next step should be to record from these brain areas that are related to stress. In order to do so, brain slices should be cut in a different angle, possibly like the ones described by Krugers and colleagues (133). Since the PFC, hippocampus and amygdala are also implicated in learning and memory, the investigation should include behavioural tests that examine these cognitive functions. **Comparison with previous results** As discussed in the introduction, Jöels and her colleagues (123) found stress-induced enhancement of functional connectivity

between the somatosensory, visual cortex and the DMN. At first sight, this may appear to contradict our results. However, there are some important differences between the two experiments. Firstly, in our experiments the recording was performed on brain slices kept in oxygenated aCSF some hours after the sacrifice. In the case of the Jöels lab, the animal was alive under anesthesia. This introduces a big difference between the two experimental procedures. However, the most important differentiating factor is the recording methodology. We recorded spontaneous electrical activity from a specific area in the somatosensory cortex through LFP while the Jöels lab used fMRI on the whole brain. As discussed in the introduction, the fMRI is an indirect way of tracing brain activity whereas LFP recordings are definite events of neuronal - synaptic activity. Also, fMRI cannot reach the local level of description that LFP does. Thus, we believe that the events we are recording are of a more local nature than the network activity measured in the Jöels lab. This means that our results (no difference among groups) are not contradictory to the ones by Jöels (increased connectivity in stress group). A possible way to investigate both results would be to use an array of electrodes that would cover the mentioned brain regions and record LFP simultaneously from all related areas. This way we should be able to investigate the relationship between the two levels of description.

Future research

Apart from ideas and suggestions discussed in the previous sections, there are some further factors that should be investigated in order to achieve a deeper understanding of the effects of stress on spontaneous cortical activity in mice. Since exogenous corticosterone administration

does not grasp the whole stress response and effect, a different direction could be to investigate the effect of chronic stress on cortical network dynamics through a well-documented protocol like restraint stress (50) or maternal separation (65). Also, further research both in relation to CORT treatment and LFP recordings should be done on female mice, which show differential responses to stress protocols (58, 51) and corticosterone treatment (66) and also have differential spontaneous network activity patterns (40).

To conclude, we should also give some perspective on the possible mechanisms that are responsible for the effects of corticosterone administration on the mice anatomy and physiology. One of the most interesting explanatory schemes concerning the relation between stress and depression is the neuroplasticity hypothesis. Specifically, it has been proposed that chronic stress alters neuroplasticity in amygdala, prefrontal cortex and hippocampus, eventually leading to depression (67, 69). Indeed it has been shown that after chronic stress (64) apical dendrites of hippocampal CA3 neurons retract while after 5 days of corticosterone administration on male mice (66), hippocampal cell proliferation was reduced. Additionally, chronic restraint stress has been found to impair neurogenesis in the hippocampus while also significantly decreasing the hippocampal volume (126). These detrimental effects of chronic stress to the neuroplasticity of important brain areas are similar to some of the neurological complications of diabetes, which is a chronic metabolic stressor (87, 131) and therefore also has physiological effects that resemble Cushing's syndrome. This relationship among metabolic deficiencies, altered neuroplasticity and depressive behaviour could be the key to understanding the effect of stress on neuropsychological disorders. It must be noted however that most of the studies mentioned have been done on rats and not mice, therefore, we should

be careful in comparing these results to findings that refer to mice. Secondly, the majority of research in the field of stress is done on male rats or mice. The effects of stress protocols on female mice are different than in male mice (54) and this should be taken into account when trying to draw associations.

Concluding statements:

The results of the present study indicate that two weeks of oral corticosterone treatment to adolescent mice have significant detrimental effects to the anatomy, physiology and behaviour of the treated animals but do not result in significant changes to the spontaneous local cortical activity, suggesting that the upstate / downstate activity is a robust network phenomenon that resists the effects of exogenous corticosterone administration.

5. References

[1] Levitis, D.A., Lidicker, W.Z., & Freund, G., 2009. Behavioural biologists do not agree on what constitutes behaviour. *Animal Behaviour 78*, 103-110.

[2] Neuner, I., Arrubla, J., Werner, C. J., Hitz, K., Boers, F., Kawohl, W., & Shah, N. J. (2014). The default mode network and EEG regional spectral power: A simultaneous fMRI-EEG study. *PLoS ONE*, *9*(2).

[3] Jerbi, K., Vidal, J. R., Ossandon, T., Dalal, S. S., Jung, J., Hoffmann, D., … Lachaux, J.-P. (2010). Exploring the electrophysiological correlates of the default-mode network with intracerebral EEG. *Frontiers in Systems Neuroscience*, *4*(June), 27.

[4] Greicius, M. D., Srivastava, G., Reiss, A. L., & Menon, V. (2004). Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(13), 4637–42.

[5] Assaf, M., Jagannathan, K., Calhoun, V. D., Miller, L., Stevens, M. C., Sahl, R., … Pearlson, G. D. (2010). Abnormal functional connectivity of default mode sub-networks in autism spectrum disorder patients. *NeuroImage*, *53*(1), 247–256.

[6] Washington, S. D., Gordon, E. M., Brar, J., Warburton, S., Sawyer, A. T., Wolfe, A., … Vanmeter, J. W. (2014). Dysmaturation of the default mode network in autism. *Human Brain Mapping*, *35*(4), 1284–1296.

[7] Haneef, Z., Lenartowicz, A., Yeh, H. J., Engel, J., & Stern, J. M. (2012). Effect of lateralized temporal lobe epilepsy on the default mode network. *Epilepsy & Behavior : E&B*, *25*(3), 350–7.

[8] McGill, M. L., Devinsky, O., Kelly, C., Milham, M., Castellanos, F. X., Quinn, B. T., … Thesen, T. (2012). Default mode network abnormalities in idiopathic generalized epilepsy. *Epilepsy & Behavior : E&B*, *23*(3), 353–9.

[9] Lanius, R. A., Bluhm, R. L., Coupland, N. J., Hegadoren, K. M., Rowe, B., Théberge, J., … Brimson, M. (2010). Default mode network connectivity as a predictor of post-traumatic stress disorder symptom severity in acutely traumatized subjects. *Acta Psychiatrica Scandinavica*, *121*(1), 33–40.

[10] Sheline, Y. I., Barch, D. M., Price, J. L., Rundle, M. M., Vaishnavi, S. N., Snyder, A. Z., … Raichle, M. E. (2009). The default mode network and self-referential processes in depression. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(6), 1942–7.

[11] Bluhm, R., Williamson, P., Lanius, R., Théberge, J., Densmore, M., Bartha, R., … Osuch, E. (2009). Resting state default-mode network connectivity in early depression using a seed region-of-interest analysis: Decreased connectivity with caudate nucleus. *Psychiatry and Clinical Neurosciences*, *63*(6), 754–761.

[12] Öngür, D., Lundy, M., Greenhouse, I., Shinn, A. K., Menon, V., Cohen, B. M., & Renshaw, P. F. (2010). Default mode network abnormalities in bipolar disorder and schizophrenia. *Psychiatry Research - Neuroimaging*, *183(1),* 59–68.

[13] Horovitz, S. G., Braun, A. R., Carr, W. S., Picchioni, D., Balkin, T. J., Fukunaga, M., & Duyn, J. H. (2009). Decoupling of the brain's default mode network during deep sleep. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(27), 11376–11381.

[14] van Eimeren, T., Monchi, O., Ballanger, B., Strafella, A. P. (2011). Dysfunction of the Default Mode Network in Parkinson Disease, *Arch Neurol. 66(7):*877-883.

[15] Ma, N., Liu, Y., Fu, X. M., Li, N., Wang, C. X., Zhang, H., … Zhang, D. R. (2011). Abnormal brain default-mode network functional connectivity in drug addicts. *PLoS ONE*, *6*(1).

[16] Spreng, R. N., & Grady, C. L. (2010). Patterns of brain activity supporting autobiographical memory, prospection, and theory of mind, and their relationship to the default mode network. *Journal of Cognitive Neuroscience*, *22*, 1112–1123.

[17] Sämann, P. G., Wehrle, R., Hoehn, D., Spoormaker, V. I., Peters, H., Tully, C., … Czisch, M. (2011). Development of the brain's default mode network from wakefulness to slow wave sleep. *Cerebral Cortex*, *21*(9), 2082–2093.

[18] Greicius, M. D., Kiviniemi, V., Tervonen, O., Vainionpää, V., Reiss, A. L., & Menon, V. (2008). Persistent Default-Mode Network Connectivity During Light Sedation. *Human Brain Mapping*, *29*(7), 839–847.

[19] Norton, L., Hutchison, R., Young, G., Lee, D., Sharpe, M., & Mirsattari, S. (2012). Disruptions of functional connectivity in the default mode network of comatosepatients. *Neurology*, *53*(9), 1689–1699.

[20] Lu, H., Zou, Q., Gu, H., Raichle, M. E., Stein, E. a, & Yang, Y. (2012). Rat brains also have a default mode network. *Proc. Natl. Acad. Sci. U. S. A.*, *109*(10), 3979–84.

[21] Vincent, J. L., Patel, G. H., Fox, M. D., Snyder, A. Z., Baker, J. T., Van Essen, D. C., … Raichle, M. E. (2007). Intrinsic functional architecture in the anaesthetized monkey brain. *Nature*, *447*(7140), 83–86.

[22] Metherate, R., Cox, C. L., & Ashe, J. H. (1992). Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *12*(12), 4701–4711.

[23] Compte, A., Reig, R., Descalzo, V. F., Harvey, M. A., Puccini, G. D., & Sanchez-Vives, M. V. (2008). Spontaneous High-Frequency (10-80 Hz) Oscillations during Up States in the Cerebral Cortex In Vitro. *Journal of Neuroscience*, *28*(51), 13828–13844.

[24] Sanchez-Vives, M. V, & McCormick, D. a. (2000). Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nature Neuroscience*, *3*(10), 1027–1034.

[25] McEwen, B. S., & Sapolsky, R. M. (1995). Stress and cognitive function. *Current Opinion in Neurobiology*, *5*(2), 205–216.

[26] McEwen, B. S. (2000). The neurobiology of stress: From serendipity to clinical relevance. *Brain Research*, *886*(1–2), 172–189.

[27] Joëls, M., & Baram, T. Z. (2009). The neurosymphony of stress. *Nature Reviews Neuroscience*, *10*(6), 459–466.

[28] Chrousos, G.P., & Gold, P.W. (1992). The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA 267*, 1244–1252.

[29] de Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience*, *6*(6), 463–475.

[30] Selye, H. (1975) Confusion and Controversy in the Stress Field. *Journal of Human Stress*, 1:2, 37-44.

[31] Nicolaides, N. C., Kyratzi, E., Lamprokostopoulou, A., Chrousos, G. P., & Charmandari, E. (2015). Stress, the stress system and the role of glucocorticoids. *Neuroimmunomodulation*, *22*(1–2), 6–19.

[32] McEwen, B. S. (2007). Physiology and Neurobiology of Stress and Adaptation: Central Role of the Brain. *Physiological Reviews*, *87*(1), 873–904.

[33] Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nature Reviews. Endocrinology*, *5*(7), 374–81.

[34] Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic – pituitary – adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research, 53,* 865–871.

[35] Gunnar, M. R., & Quevedo, K. (2007). The neurobiology of stress and development. *Annual Review of Psychology*, *58*, 145–173.

[36] Maria, E., Reiche, V., Odebrecht, S., Nunes, V., & Morimoto, H. K. (2004). Stress, depression, the immune system and cancer. *The Lancet, 5(10),* 617–625.

[37] Joëls, M. (2009). Stress, the hippocampus, and epilepsy. *Epilepsia*, *50*(4), 586–597.

[38] McEwen, B.S., Bowles, N.P., Gray, J.D., Hill, M.N., Hunter, R.G., Karatsoreos, I. N., & Nasca, C. (2015). Mechanisms of stress in the brain. *Nature Neuroscience, 18(10),* 1353–1363.

[39] McEwen, B. S. (2008). Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *European Journal of Pharmacology*, *583*(2–3), 174–185.

[40] Sigalas, C., Konsolaki, E., & Skaliora, I. (2017). Sex differences in endogenous cortical network activity: spontaneously recurring Up/Down states. *Biology of Sex Differences*, *8*(1), 21.

[41] Rigas, P., Adamos, D.A., Sigalas, C., Tsakanikas, P., Laskaris, N.A. & Skaliora, I. (2015). Spontaneous Up states in vitro: a single-metric index of the functional maturation and regional differentiation of the cerebral cortex. *Front. Neural Circuits, 9:59*, 1-20.

[42] Sigalas, C., Rigas, P., Tsakanikas, P., & Skaliora, I. (2015). High-Affinity Nicotinic Receptors Modulate Spontaneous Cortical Up States In Vitro. *Journal of Neuroscience*, *35*(32), 11196–11208.

[43] Rovira, V., & Geijo-barrientos, E. (2016). Intra- and Interhemispheric Propagation of Electrophysiological Synchronous Activity and Its Modulation by Serotonin in the Cingulate Cortex of Juvenile Mice, 1–19.

[44] Watson, B. O., MacLean, J. N., & Yuste, R. (2008). UP States protect ongoing cortical activity from thalamic inputs. *PLoS ONE*, *3*(12).

[45] Mohajerani, M. H., Mcvea, D. A., Fingas, M., & Murphy, T. H. (2010). Mirrored Bilateral Slow-Wave Cortical Activity within Local Circuits Revealed by Fast Bihemispheric Voltage-Sensitive Dye Imaging in Anesthetized and Awake Mice, *30*(10), 3745–3751.

[46] Alfarez, D. N., Wiegert, O., Joëls, M., & Krugers, H. J. (2002). Corticosterone and stress reduce synaptic potentiation in mouse hippocampal slices with mild stimulation. *Neuroscience*, *115*(4), 1119–1126.

[47] Belzung, C., & Griebel, G. (2001). Measuring normal and pathological anxiety-like behaviour in mice: A review. *Behavioural Brain Research*, *125*(1–2), 141–149.

[48] Brydges, N. M. (2016). Pre-pubertal stress and brain development in rodents. *Current Opinion in Behavioral Sciences*, *7*, 8–14.

[49] Buret, L., & Buuse, M. Van Den. (2014). Corticosterone treatment during adolescence induces down-regulation of reelin and NMDA receptor subunit GLUN2C expression only in male mice: implications for schizophrenia. *International Journal of Neuropsychopharmacology*, *17*, 1221–1232.

[50] Buynitsky, T., & Mostofsky, D.I. (2009). Restraint stress in biobehavioral research: Recent developments. *Neuroscience and Biobehavioral Reviews*, *33*(7), 1089–1098.

[51] Kitraki, E., Kremmyda, O., Youlatos, D., Alexis, M. N., & Kittas, C. (2004). Gender-dependent alterations in corticosteroid receptor status and spatial performance following 21 days of restraint stress. *Neuroscience*, *125*(1), 47–55.

[52] Campos, A.C., Fogaca, M.V., Aguiar, D.C., & Guimaraes, F.S. (2013). Animal models of anxiety disorders and stress. *Revista Brasileira de Psiquiatria*, *35*(SUPPL.2), 101–111.

[53] Cassano, A. E., White, J. R., Penraat, K. A., Wilson, C. D., Rasmussen, S., & Karatsoreos, I. N. (2012). Anatomic, hematologic, and biochemical features of C57BL/6NCrl mice maintained on chronic oral corticosterone. *Comparative Medicine*, *62*(5), 348–360.

[54] Dalla, C., Pitychoutis, P. M., Kokras, N., & Papadopoulou-Daifoti, Z. (2010). Sex differences in animal models of depression and antidepressant response. *Basic & Clinical Pharmacology & Toxicology*, *106*(3), 226–233.

[55] Espejo, E.F. (1997). Structure of the mouse behaviour on the elevated plus-maze test of anxiety. *Behavioural Brain Research*, *86*(1), 105–112.

[56] File, S.E. (2001). Factors controlling measures of anxiety and responses to novelty in the mouse. *Behavioural Brain Research*, *125*(1–2), 151–157.

[57] Holmes, A. (2001). Targeted gene mutation approaches to the study of anxiety-like behavior in mice. *Neuroscience & Biobehavioral Reviews*, *25*(3), 261–273.

[58] Joëls, M., Karst, H., Krugers, H. J., & Lucassen, P. J. (2007). Chronic stress: Implications for neuronal morphology, function and neurogenesis. *Frontiers in Neuroendocrinology*, *28*(2–3), 72–96.

[59] Karatsoreos, I. N., Bhagat, S. M., Bowles, N. P., Weil, Z. M., Pfaff, D. W., & McEwen, B. S. (2010). Endocrine and physiological changes in response to chronic corticosterone: A potential model of the metabolic syndrome in mouse. *Endocrinology*, *151*(5), 2117–2127.

[**60**] Kumar, V., Bhat, Z. A., & Kumar, D. (2013). Animal models of anxiety: A comprehensive review. *Journal of Pharmacological and Toxicological Methods*, *68*(2), 175–183.

[61] Lalonde, R., & Strazielle, C. (2008). Relations between open-field, elevated plus-maze, and emergence tests as displayed by C57/BL6J and BALB/c mice. *Journal of Neuroscience Methods*, *171*(1), 48–52.

[62] Lee, R. S., Tamashiro, K. L. K., Yang, X., Purcell, R. H., Harvey, A., Willour, V. L., … Potash, J. B. (2010). Chronic corticosterone exposure increases expression and decreases deoxyribonucleic acid methylation of Fkbp5 in mice. *Endocrinology*, *151*(9), 4332–4343.

[63] Lupien, S. J., Maheu, F., Tu, M., Fiocco, A., & Schramek, T. E. (2007). The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. *Brain and Cognition*, *65*(3), 209–237.

[64] Magarinos, A. M., & McEwen, B. S. (1995). Stress-induced atrophy of apical dendrites of hippocampal CA3 neurons: Comparison of stressors. *Neuroscience*, *69*(1), 83–88.

[65] Millstein, R. A., Ralph, R. J., Yang, R. J., & Holmes, A. (2006). Effects of repeated maternal separation on prepulse inhibition of startle across inbred mouse strains. *Genes, Brain and Behavior*, *5*(4), 346–354.

[66] Mo, C., Pang, T. Y., Ransome, M. I., Hill, R. A., Renoir, T., & Hannan, A. J. (2014). High stress hormone levels accelerate the onset of memory deficits in male Huntington's disease mice. *Neurobiology of Disease*, *69*, 248–262.

[67] Nacher, J., Pham, K., Gil-Fernandez, V., & McEwen, B. S. (2004). Chronic restraint stress and chronic corticosterone treatment modulate differentially the expression of molecules related to structural plasticity in the adult rat piriform cortex. *Neuroscience*, *126*(2), 503–509.

[68] Deacon, R. M. (2006). Assessing nest building in mice. *Nature Protocols*, *1*(3), 1117–1119.

[69] Pittenger, C., & Duman, R. S. (2008). Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *33*(1), 88–109.

[70] Rademacher, D. J., Meier, S. E., Shi, L., Vanessa Ho, W. S., Jarrahian, A., & Hillard, C. J. (2008). Effects of acute and repeated restraint stress on endocannabinoid content in the amygdala, ventral striatum, and medial prefrontal cortex in mice. *Neuropharmacology*, *54*(1), 108–116.

[71] Rodgers, R. J., Cao, B. J., Dalvi, A., & Holmes, A. (1997). Animal models of anxiety: An ethological perspective. *Brazilian Journal of Medical and Biological Research*, *30*(3), 289–304.

[72] Sheridan, J. F., Padgett, D. A., Avitsur, R., & Marucha, P. T. (2004). Experimental Models of Stress and Wound Healing. *World Journal of Surgery*, *28*(3), 327–330.

[73] Sheridan, J. F., Feng, N. G., Bonneau, R. H., Allen, C. M., Huneycutt, B. S., & Glaser, R. (1991). Restraint stress differentially affects anti-viral cellular and humoral immune responses in mice. *Journal of Neuroimmunology*, *31*(3), 245–255.

[74] Hogg, S. (1996). A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacology Biochemistry and Behavior*, *54*(1), 21–30.

[75] Ulman, E. A., Compton, D., & Kochanek, J. (2008). Measuring Food and Water Intake in Rats and Mice. *ALN Magazine*, 17–20.

[76] Van Donkelaar, E. L., Vaessen, K. R. D., Pawluski, J. L., Sierksma, A. S., Blokland, A., Canete, R., & Steinbusch, H. W. M. (2014). Long-term corticosterone exposure decreases insulin sensitivity and induces depressive-like behaviour in the C57BL/6NCrl mouse. *PLoS ONE*, *9*(10), 1–10.

[77] Short, A. K., Fennell, K. A., Perreau, V. M., Fox, A., O'Bryan, M. K., Kim, J. H., … Hannan, A. J. (2016). Elevated paternal glucocorticoid exposure alters the small noncoding RNA profile in sperm and modifies anxiety and depressive phenotypes in the offspring. *Translational Psychiatry*, *6*(6), e837.

[78] Gourley, S. L., Wu, F. J., Kiraly, D. D., Ploski, J. E., Alexia, T., Duman, R. S., & Taylor, J. R. (2008). Regionally specific regulation of pERK1/2 MAP KINASE in a Model of Antidepressant-Sensitive Chronic Depression. Biol. Psychiatry, 63(4), 353–359.

[79] Gourley, S. L., & Taylor, J. R. (2009). Recapitulation and reversal of a persistent depression-like syndrome in rodents. *Current Protocols in Neuroscience*, (SUPPL.49), 1–11.

[80] Gourley, S. L., Kiraly, D. D., Howell, J. L., Olausson, P., & Taylor, J. R. (2008). Acute Hippocampal Brain-Derived Neurotrophic Factor Restores Motivational and Forced Swim Performance After Corticosterone. *Biological Psychiatry*, *64*(10), 884–890.

[81] Klug, M., Hill, R. A., Ho, K., Choy, C., Kyrios, M., Hannan, A. J., & Buuse, M. Van Den. (2012). Long-term behavioral and NMDA receptor effects of young-adult corticosterone treatment in BDNF heterozygous mice.

[82] Walf, A.A., & Frye, C.A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc*, *2*(2), 322–328.

[83] Schneider, P., Ho, Y.-J., Spanagel, R., & Pawlak, C. R. (2011). A novel elevated plus-maze procedure to avoid the one-trial tolerance problem. *Frontiers in Behavioral Neuroscience*, *5*, 1–8.

[84] Dobarro, M., Orejana, L., Aguirre, N. & Ramirez, M.J. (2013). Propranolol reduces cognitive deficits , amyloid b levels , tau phosphorylation and insulin resistance in response to chronic

corticosterone administration. *International Journal of Neuropsychopharmacology*, *16*, 1351–1360.

[85] Hájos, N., Ellender, T. J., Zemankovics, R., Mann, E. O., Exley, R., Cragg, S. J., Freund, Tamas F, & Paulsen, O.. (2009). Maintaining network activity in submerged hippocampal slices: importance of oxygen supply. *European Journal of Neuroscience*. 29, 319–327.

[86] Ivanov A, & Zilberter, Y. (2011). Critical state of energy metabolism in brain slices: the principal role of oxygen delivery and energy substrates in shaping neuronal activity. Frontiers in Neuroenergetics 3:9, 1-13.

[87] Reagan, L. P. (2013). Diabetes as a chronic metabolic stressor: causes, consequences and clinical complications. *Experimental Neurology*, *233*(1), 68–78.

[88] Hájos, N., & Mody, I. (2009). Establishing a physiological environment for visualized in vitro brain slice recordings by increasing oxygen supply and modifying aCSF content. *Journal of Neuroscience Methods*, *183*(2), 107–113.

[89] Harris, R. J., Wieloch, T., Symon, L., & Siesjo, B. K. (1984). Cerebral Extracellular Calcium Activity in Severe Hypoglycemia : Relation to Extracellular Potassium and Energy State. *Journal of Cerebral Blood Flow and Metabolism*, *4*(1), 187–193.

[90] Rigas, P., & Castro-Alamancos, M. A. (2007). Thalamocortical Up States: Differential Effects of Intrinsic and Extrinsic Cortical Inputs on Persistent Activity. *Journal of Neuroscience*, *27*(16), 4261–4272.

[91] Rigas, P., & Castro-Alamancos, M. A. (2009). Impact of Persistent Cortical Activity (Up States) on Intracortical and Thalamocortical Synaptic Inputs. *Journal of Neurophysiology*, *102*(1), 119–131.

[92] Maclean, J. N., Watson, B. O., Aaron, G. B., & Yuste, R. (2005). Internal Dynamics Determine the Cortical Response to Thalamic Stimulation. *Neuron*, *48*, 811–823.

[93] Maclean, J. N., Watson, B. O., Aaron, G. B., & Yuste, R. (2005). Internal Dynamics Determine the Cortical Response to Thalamic Stimulation. *Neuron*, *48*(layer 4), 811–823.

[94] Oppenheim, A. V., Schafer, R. W., and Buck, J. R. (1999). Discrete-Time Signal Processing, 2nd Edn. Upper Saddle River, NJ: Prentice-Hall.

[95] vanDrongelen, W., Lee, H.C., Stevens, R.L., & Hereld, M. (2007). Propagation of seizure-like activity in a model of neocortex. J. Clin. Neurophysiol. 24, 182–188.

[96] Recio-Spinoso, A., Fan, Y., & Ruggero, M. A. (2011). Basilar-Membrane Responses to Broadband Noise Modeled Using Linear Filters With Rational Transfer Functions. *IEEE Transactions on Biomedical Engineering*. *58*(5), 1456–1465.

[97] Crumeyrolle-Arias, M., Jaglin, M., Bruneau, A., Vancassel, S., Cardona, A., Daugé, V., … Rabot, S. (2014). Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology*, *42*, 207–217.

[98] Henderson, N. D., Turri, M. G., DeFries, J. C., & Flint, J. (2004). QTL Analysis of Multiple Behavioral Measures of Anxiety in Mice. *Behavior Genetics*, *34*(3), 267–293.

[99] Donahue, L. R., & Beamer, W. G. (1993). Growth hormone deficiency in “little” mice results in aberrant body composition, reduced insulin-like growth factor-I and insulin-like growth factor-binding protein-3 (IGFBP-3), but does not affect IGFBP-2, -1, or -4. *Journal of Endocrinology*, *136*(1), 91–104.

[100] Purves, D., Augustine, G. J., Fitzpatrick, D., Hall, W. C., Lamantia, A.-S., Mcnamara, J. O., & Williams, S. M. (2004). *Neuroscience*. *Sunderland*. 3rd edition.

[101] Hämäläinen, M. S., Hari, R., Ilmoniemi, R. J., Knuutila, J., & Lounasmaa, O. V. (1993). Magnetoencephalography - theory, instrumentation, and applications to noninvasivee studies of the working human brain. *Reviews of Modern Physics*.

[102] Goldenholz, D. M., Ahlfors, S. P., Hämäläinen, M. S., Sharon, D., Ishitobi, M., Vaina, L. M., & Stufflebeam, S. M. (2009). Mapping the signal-to-noise-ratios of cortical sources in magnetoencephalography and electroencephalography. *Human Brain Mapping*, *30*(4), 1077–1086.

[103] Gavaret, M., Maillard, L., & Jung, J. (2015). High-resolution EEG (HR-EEG) and magnetoencephalography (MEG). *Neurophysiologie Clinique*, *45*(1), 105–111.

[104] Brookes, M. J., Woolrich, M., Luckhoo, H., Price, D., Hale, J. R., Stephenson, M. C., … Morris, P. G. (2011). Investigating the electrophysiological basis of resting state networks using magnetoencephalography. *Proceedings of the National Academy of Sciences*, *108*(40), 16783–16788.

[105] Buzsáki, G., Anastassiou, C. A., & Koch, C. (2012). The origin of extracellular fields and currents — EEG, ECoG, LFP and spikes. *Nature Reviews Neuroscience*, *13*(6), 407–420.

[106] Horovitz, S. G., Fukunaga, M., de Zwart, J. A., van Gelderen, P., Fulton, S. C., Balkin, T. J., & Duyn, J. H. (2008). Low frequency BOLD fluctuations during resting wakefulness and light sleep: A simultaneous EEG-fMRI study. *Human Brain Mapping*, *29*(6), 671–682.

[107] Freeman, W. J., Ahlfors, S. P., & Menon, V. (2002). Combining fMRI with EEG and MEG in order to relate patterns of brain activity to cognition. *International Journal of Psychophysiology*, *73*(1), 43–52.

[108] Ritter, P., & Villringer, A. (2006). Simultaneous EEG–fMRI. *Neuroscience & Biobehavioral Reviews*, *30*(6), 823–838.

[109] Debener, S., Ullsperger, M., Siegel, M., & Engel, A. K. (2006). Single-trial EEG–fMRI reveals the dynamics of cognitive function. *Trends in Cognitive Sciences*, *10*(12), 558–563.

[110] Mulert, C., Jäger, L., Schmitt, R., Bussfeld, P., Pogarell, O., Möller, H.-J., … Hegerl, U. (2004). Integration of fMRI and simultaneous EEG: towards a comprehensive understanding of localization and time-course of brain activity in target detection. *NeuroImage*, *22*(1), 83–94.

[111] Ojemann, G., Ojemann, J., & Ramsey, N. F. (2013). Relation between functional magnetic resonance imaging (fMRI) and single neuron, local field potential (LFP) and electrocorticography (ECoG) activity in human cortex. *Frontiers in Human Neuroscience*, *7*(February), 34.

[112] Logothetis, N. K. (2008). What we can do and what we cannot do with fMRI. *Nature*, *453*(7197), 869–878.

[113] Ekstrom, A. (2010). How and when the fMRI BOLD signal relates to underlying neural activity: The danger in dissociation. *Brain Research Reviews*, *62*(2), 233–244.

[114] Logothetis, N. K., Pauls, J., Augath, M., Trinath, T., & Oeltermann, a. (2001). Neurophysiological investigation of the basis of the fMRI signal. *Nature*, *412*(6843), 150–7.

[115] Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*(6), 434–445.

[116] Jeong, Y. H., Park, C. H., Yoo, J., Shin, K. Y., Ahn, S.-M., Kim, H.-S., … Suh, Y.-H. (2006). Chronic stress accelerates learning and memory impairments and increases amyloid deposition in APPV717I-CT100 transgenic mice, an Alzheimer's disease model. *The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *20*, 729–731.

[117] Lanfumey, L., Mongeau, R., Cohen-Salmon, C., & Hamon, M. (2008). Corticosteroid-serotonin interactions in the neurobiological mechanisms of stress-related disorders. *Neuroscience and Biobehavioral Reviews*, *32*(6), 1174–1184.

[118] Van De Weerd, H. A., Van Loo, P. L. P., Van Zutphen, L. F. M., Koolhaas, J. M., & Baumans, V. (1997). Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. *Physiology & Behavior*, *62*(5), 1019–1028.

[119] Van Loo, P. L. P., Kruitwagen, C. L. J. J., Koolhaas, J. M., Van de Weerd, H. A., Van Zutphen, L. F. M., & Baumans, V. (2002). Influence of cage enrichment on aggressive behaviour and physiological parameters in male mice. *Applied Animal Behaviour Science*, *76*, 65–81.

[120] Olsson, I. A. S., & Dahlborn, K. (2002). Improving housing conditions for laboratory mice: a review of 'environmental enrichment'. *Laboratory Animals*, *36*, 243–270.

[121] Sherwin, C. M. (1996). Preferences of laboratory mice for characteristics of soiling sites. *Animal Welfare, 5(3),*283-286.

[122] Gaskill, B. N., Gordon, C. J., Pajor, E. A., Lucas, J. R., Davis, J. K., & Garner, J. P. (2013). Impact of nesting material on mouse body temperature and physiology. *Physiology & Behavior, 110-111*, 87-95.

[123] Henckens, M. J. A. G., Van der Marel, K., Van der Toorn, A., Pillai, A. G., Fernández, G., Dijkhuizen, R. M., & Joëls, M. (2015). Stress-induced alterations in large-scale functional networks of the rodent brain. *NeuroImage*, *105*, 312–322.

[124] Gourley, S. L., Kedves, A. T., Olausson, P., & Taylor, J. R. (2009). A history of corticosterone exposure regulates fear extinction and cortical NR2B, GluR2/3, and BDNF. *Neuropsychopharmacology*, *34*(3), 707–716.

[125] McEwen, B., & Morrison, J. (2013). The Brain on Stress: Vulnerability and Plasticity of the Prefrontal Cortex over the Life Course. *Neuron*, *79*(1), 16–29.

[126] Yun, J., Koike, H., Ibi, D., Toth, E., Mizoguchi, H., Nitta, A., … Yamada, K. (2010). Chronic restraint stress impairs neurogenesis and hippocampus-dependent fear memory in mice: Possible involvement of a brain-specific transcription factor Npas4. *Journal of Neurochemistry*, *114*(6), 1840–1851.

[127] Sanchez-vives, M. V, Massimini, M., & Mattia, M. (2017). Shaping the Default Activity Pattern of the Cortical Network. *Neuron, 94*, 993–1001.

[128] Koolhaas, J. M., Bartolomucci, A., Buwalda, B., de Boer, S. F., Flügge, G., Korte, S. M., … Fuchs, E. (2011). Stress revisited: A critical evaluation of the stress concept. *Neuroscience and Biobehavioral Reviews*, *35*(5), 1291–1301.

[129] Xu, Z., Zhang, Y., Hou, B., Gao, Y., Wu, Y., & Zhang, C. (2011). Chronic corticosterone administration from adolescence through early adulthood attenuates depression-like behaviors in mice. *Journal of Affective Disorders*, *131*(1–3), 128–135.

[130] Bowles, N. P., Hill, M. N., Bhagat, S. M., Karatsoreos, I. N., Hillard, C. J., & McEwen, B. S. (2012). Chronic, noninvasive glucocorticoid administration suppresses limbic endocannabinoid signaling in mice. *Neuroscience*, *204*, 83–89.

[131] Tamashiro, K. L., Sakai, R. R., Shively, C. A., Karatsoreos, I. N., & Reagan, L. P. (2011). Chronic stress, metabolism, and metabolic syndrome. *Stress*, *14*(5), 468–474.

[132] Ardayfio, P., & Kim, K.-S. (2006). Anxiogenic-like effect of chronic corticosterone in the light-dark emergence task in mice. *Behavioral Neuroscience*, *120*(2), 249–256.

[133] Wiegert, O., Joëls, M., & Krugers, H. (2006). Timing is essential for rapid effects of corticosterone on synaptic potentiation in the mouse hippocampus. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *13*(2), 110–113.

[134] Van De Weerd, H. A., Van Loo, P. L. P., Van Zutphen, L. F. M., Koolhaas, J. M., & Baumans, V. (1997). Preferences for nesting material as environmental enrichment for laboratory mice, 133–143.

[135] Zhao, Y., Ma, R., Shen, J., Su, H., Xing, D., & Du, L. (2008). A mouse model of depression induced by repeated corticosterone injections. *European Journal of Pharmacology*, *581*(1–2), 113–120.

[136] Crupi, R., Mazzon, E., Marino, A., La Spada, G., Bramanti, P., Cuzzocrea, S., & Spina, E. (2010). Melatonin treatment mimics the antidepressant action in chronic corticosterone-treated mice. *Journal of Pineal Research*, *49*(2), 123–129.

[137] Archer, J. (1973). Tests for emotionality in rats and mice: A review. *Animal Behaviour*, *21*(2), 205–235.

[138] Pare, W. P., & Glavin, G. B. (1986). Restraint Stress in Biomedical Research : A Review. *Neuroscience & Biobehavioral Reviews*, *10*, 339–370.

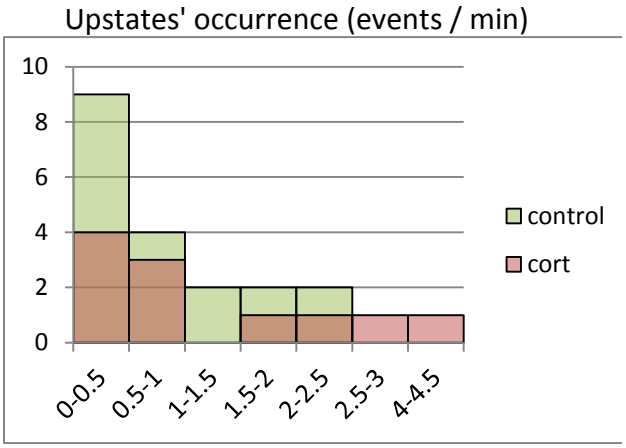
[139] Brunson, K. L., Kramár, E., Lin, B., Chen, Y., Colgin, L. L., Theodore Yanagihara, K., … Baram, T. Z. (2005). Mechanisms of Late-Onset Cognitive Decline after Early-Life Stress. *Journal of Neuroscience*, *25*(41), 9328–9338.

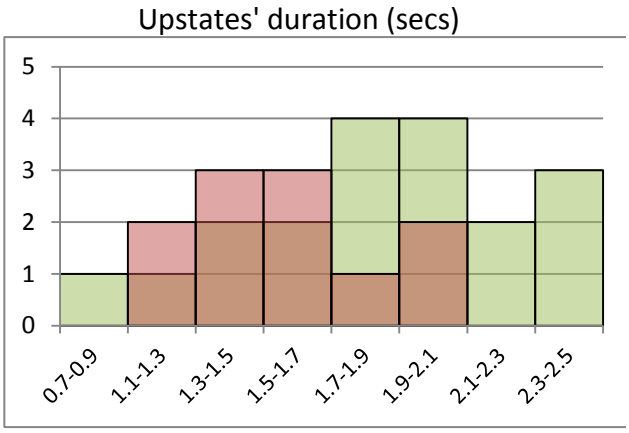
[140] Fenoglio, K. A., Brunson, K. L., & Baram, T. Z. (2006). Hippocampal neuroplasticity induced by early-life stress: Functional and molecular aspects. *Frontiers in Neuroendocrinology*, *27*(2), 180–192.

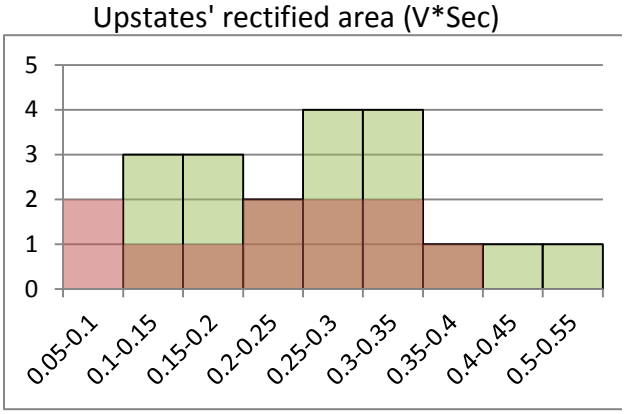
APPENDIX

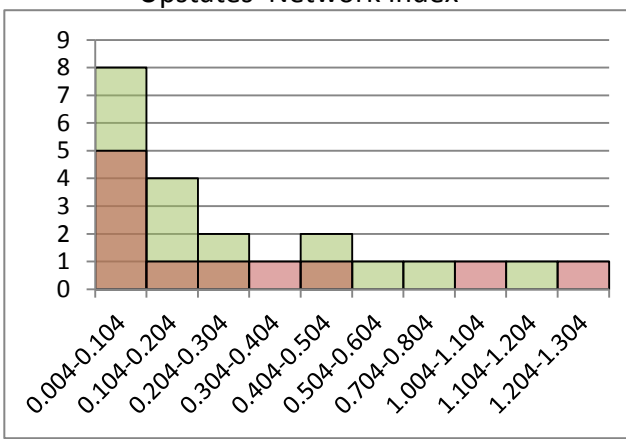
I. Electrophysiology Distributions

As mentioned in the results' section, we calculated the distributions of both groups (control vs cort) for all events' categories (Upstates, Biphasics and Multiphasics) and for all electrophysiology measures. From the distributions' histograms it was obvious that no significant changes existed among groups. Here we show the distributions' histograms for the four most important measures (duration, occurrence, rectified area and network index) of the Upstates category for both groups.





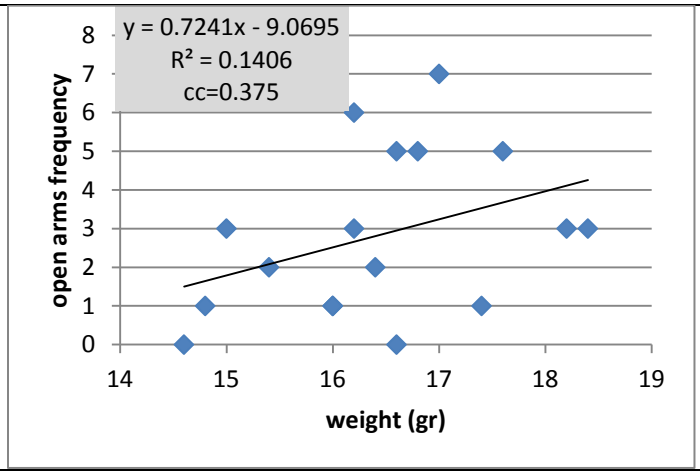


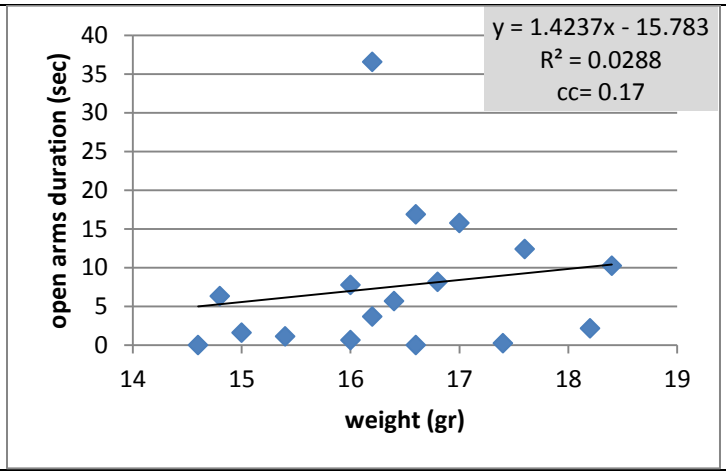


II. Correlations

In order to examine if there existed correlations that could explain the variation of the various measures for the CORT group, we calculated the r-squared value of all the possible combinations of measures. We used weight and EPM measures as independent variables while adrenal weight, EPM and electrophysiological measures served as dependent variables. The gradient of the correlation lines hint to some mild but intuitively valid correlations.

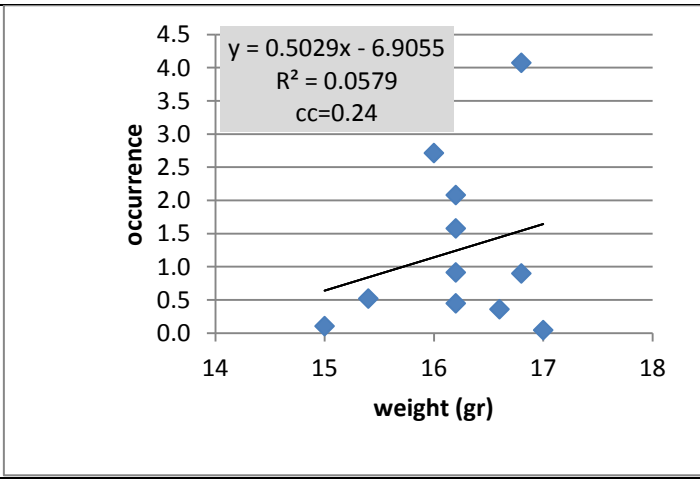
A. Weight - Behaviour

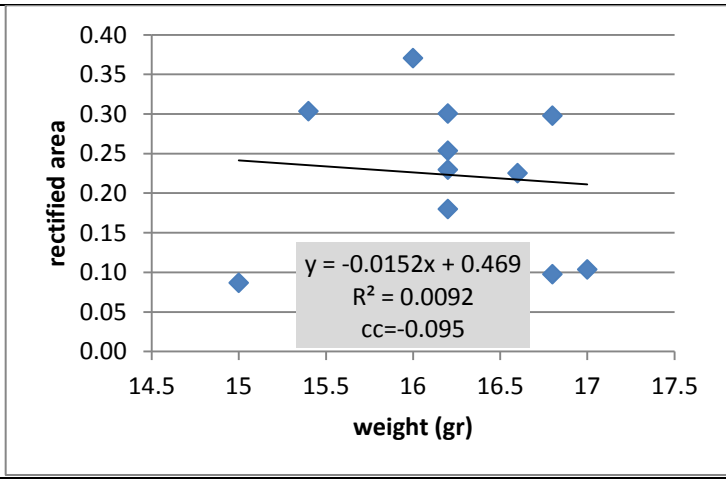


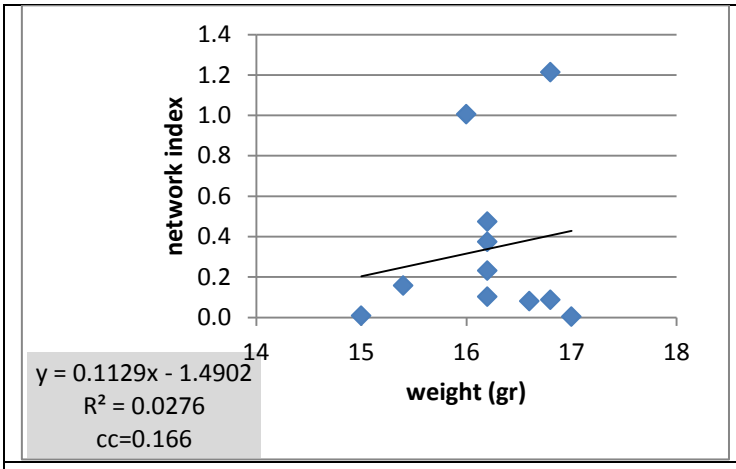


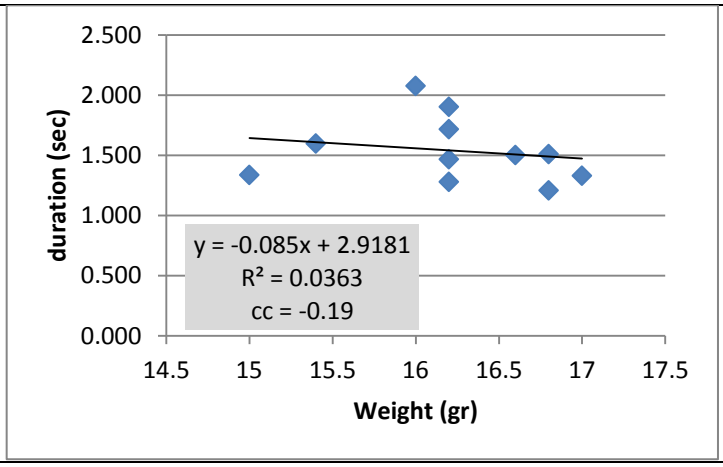
Tentative Conclusion 1:
This pattern is consistent with the idea that the heaviest animals are probably the least stressed ones

B. Weight - Electrophysiology



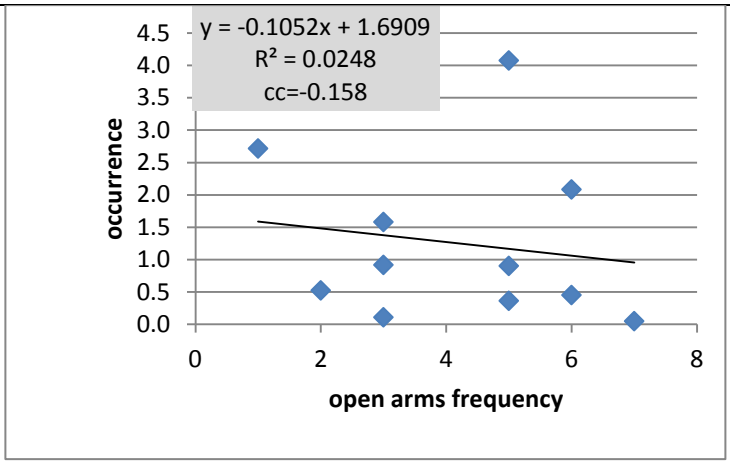


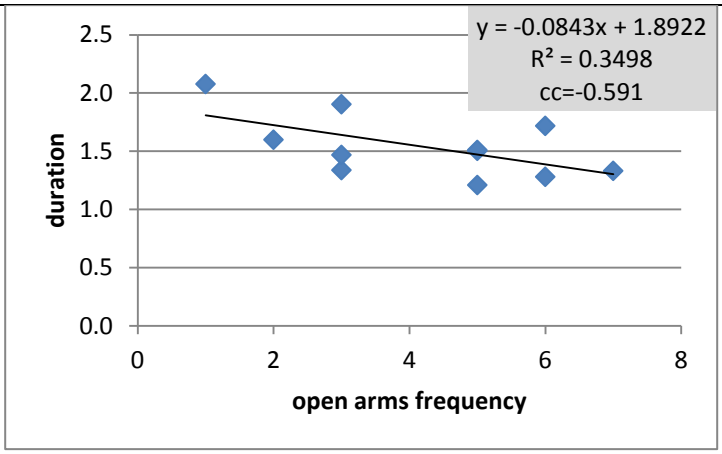


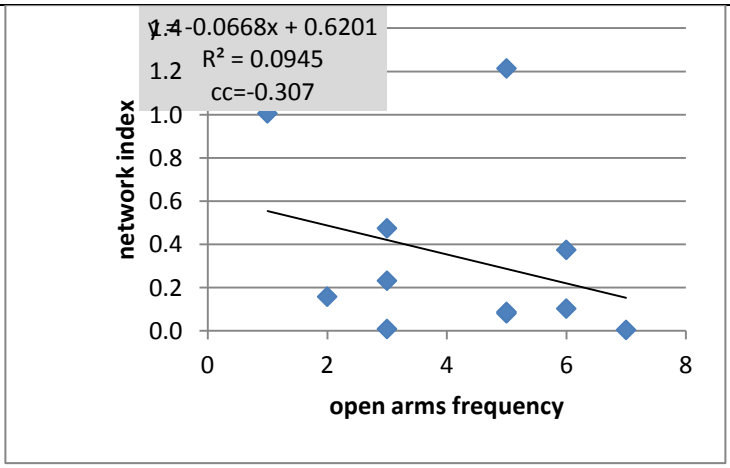


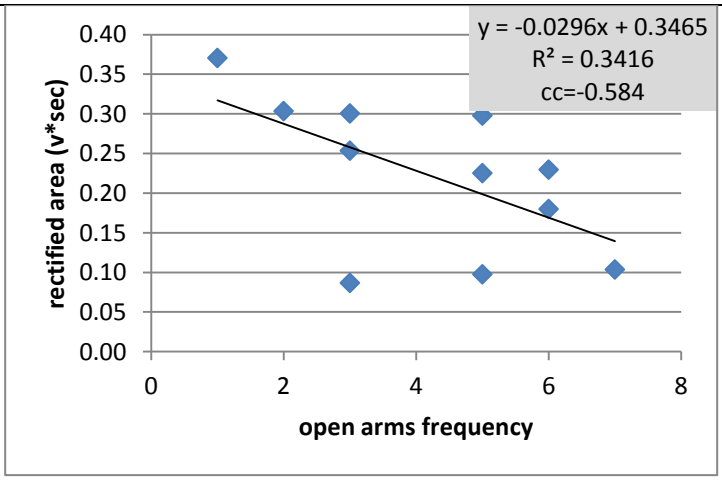
Tentative Conclusion 2:
The heaviest animals (ie the least stressed, if above conclusion is correct) tend to have more Upstates.

C. Behaviour - Electrophysiology









Tentative Conclusion 3:
The least stressed animals (more open arm entries) tend to have shorter duration Upstates, and a smaller network index.

It must be noted that the r-squared value in all cases was too low to consider that a specific correlation was significant. Of all the correlations examined, the biggest r-squared value calculated was 0.38 for open arms frequency - absolute delta power, meaning that open arms frequency in the Elevated Plus Maze could explain 38% of the variation of the upstates' absolute delta power. The only other correlations surpassing $r^2 = 0.1$ were the following:

- Open arms frequency - normalized alpha power = 0.36
- Open arms frequency - duration = 0.35
- Open arms frequency - negative peak = 0.35
- Open arms frequency - rectified area = 0.34
- Closed arms frequency - normalized theta power = 0.26
- Closed arms frequency - normalized delta power = 0.19
- Open arms frequency - absolute beta power = 0.19
- Closed arms frequency - normalized alpha power = 0.18
- Body weight - adrenal weight = 0.16
- Open arms frequency - normalized theta power = 0.16
- Body weight - open arms frequency = 0.14
- Open arms frequency - absolute alpha power = 0.12
- Open arms frequency - absolute theta power = 0.11

However, a low r-squared value does not necessarily mean that the correlation is non-existent or insignificant. A larger sample could possibly identify the real relations among the different measures.