Sapienza Rome University

School of Doctorate in Neuroscience

PhD Program in Psychobiology and Psychopharmacology

Corticolimbic catecholamines in stress

modelling the appraisal of controllability

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Academic Year 2011/2012

Stay Human.

V.A.

Acknowledgments

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Roma
December 27, 2011

The author has been supported by the EU-funded Integrated Projects
ICEA - Integrating Cognition, Emotion and Autonomy, contract no. FP6-IST-027819-IP.
IM-CLeVeR - Intrinsically Motivated Cumulative Learning Versatile Robots, contract no. FP7-IST-IP-231722.

This research has been carried out jointly with the "Laboratory of computational Embodied Neuroscience" within the "Istituto di scienze e tecnologie della cognizione" of the Consiglio Nazionale delle Ricerche.

Apart from my tutors Prof. Stefano Puglisi Allegra and Dr. Gianluca Baldassarre, I would like to thank Prof. Simona Cabib, Dr. Marco Mirolli and Dr. Franceco Mannella. Their help has been essential in grounding the generated models on solid computational and psychobiological hypotheses.

Special thanks go to Dr. Buscemi, Mr. Falciano, Mr. Zaius, Mr Hypochondria and -last, but not least- Ms Lucinda Vargas: I appreciate the patience and the efforts.

Contents

Abstract

According to appraisal theorists, coping strategies start after the evaluation of those internal and environmental conditions which are perceived, remembered or imagined as negatively affecting an agent. This evaluation phase characterises the appraisal (Lazarus, 1985), which eventually triggers the physiological and motor responses that belong to the subject's learned or selectively evolved repertoire as those that are most likely to help ending the stressing stimulus. Experiments dealing with long lasting inescapable stress conditions (e.g. restraint test, Porsolt test) perfectly illustrate the appraisal theory: as soon as the stressing stimulus is perceived, the naive subject (typically a rat) tries to perform the escape strategies of its repertoire. The active coping phase lasts several minutes (time varies according to the experimental paradigm) and it is characterised by high dopamine (DA) release in the Nucleus Accumbens (NAcc) and hyperactivity (when allowed). Nevertheless, if the stressing stimulus persists and any effort to escape from it is worthless, the subject eventually changes its behavioural strategy, thus starting the passive coping phase, which is characterised by accumbens DA release significantly below the basal level and immobility. We have decided to address the problem of investigating the neural mechanics underlying the appraisal of controllability using an anatomic and systemic approach (e.g. see Armony et al. 1997). That is to say, we have developed a neural mass model, using Matlab application, characterised by a) few network units simulating the activity of neural populations via standard leaky functions (Dayan and Abbott, 2001); b) an architecture wholly constrained on the basis of the known brain

anatomy.

The target data consist of microdialyses recorded by Pascucci et al. (2007) during a restraint experience lasting 240': this experiment has been selected because of its slow dynamics and the solid amount of data concerning catecholamine releases. Indeed, the recordings show the role of guidance played by the medial PreFrontal Cortex (mPFC) in establishing the amount of mesoaccumbens DA in three different conditions: sham and either DA or norepinephrine (NE) selective depletion in the mPFC.

The model is grounded on three key hypotheses: (*1*) vmPFC NE allows prelimbic cortex (PL) to guide active coping strategies and support the cost of these responses by enhancing NAcc DA levels; (*2*) vmPFC DA allows infralimbic cortex (IL) to block active coping attempts when these are unsuccessful by decreasing NAcc DA levels below baseline; (*3*) the learning process involving IL and PL leads to the transition from active to passive coping strategies. In conclusion, the model proved to be able to simulate and reproduce rather accurately all the target data, hence providing a good systemic representation of the mechanisms causing these dynamics in rats. Furthermore, the model provides several predictions resulting from the simulation of specific lesions, paving the way for new experiments that might either falsify or verify the model and its core hypotheses.

Chapter 1

Introduction

Emotions play a central role in the life of mammals, shaping the way these organisms perceive and understand the world, biasing their beliefs and affecting their behaviours as a response to external and internal conditions.

Despite their pivotal function and broad presence across species, emotions are an extremely elusive psychobiological phenomenon: subjective variances (considering timing, body/brain regulatory reactions, neural activity and behaviours) and low experimental control on the internal conditions of the organism make it difficult to establish univocal correlations leading to specific emotions starting from a causal chain of stimuli or conditions. Furthermore, emotions are a perfect example of emergent phenomena arising from the interaction among systems and therefore they are better represented as dynamic flows of activities taking place in heterogenous interconnected systems rather than static states (i.e. in classic functionalist perspective: Putnam 1967) realised by homogenous ”emotional centres” in the brain.

This heterogeneity is amplified by the vast amount of available tools deployed to measure and record activity and functioning of the systems involved. In the latest years, the field of neuroscience has seen a tremendous advancement of traditional investigation techniques and the emergence of several technologies (e.g., structural and functional brain imaging techniques, transcranial magnetic stimulation, anatomical tracing techniques, voltammetry and microdialysis, multielectrode array recording, genetic manipulation; Heuschkel et al., 2002; Lomber, 1999; Raichle and Mintun, 2006; Rothwell, 1997; Toga and Mazziotta, 1996): these techniques are producing data characterised by different time scales, different levels of granularity – from molecular levels to neural population levels, different involvement of time – synchronous data vs. time series, etc..

All things considered, any analysis of this complex psychobiological phenomenon has to deal with most, if not all, of the following: (A) variability, concerning time and amounts of recorded measurements; (B) performed or attempted behaviours; (C) broad regulation of both the body and the brain via hormones and catecholamine and (D) several neural systems interacting with one another, generating circular causal interactions.

The grain of analysis of bio-constrained models. Among the several possible approaches, the next chapters of this dissertation endorse an ”anatomic perspective” of the appraisal theory (Marsella et al., 2010). According to appraisal theorists, emotions result univocally from a sequence of evaluations taking into account a flow of information provided by the perceived world (i.e. its significance for the organism) and the general condition of the very organism performing the appraisal (Scherer et al., 2001). Depending on the available internal and external resources – and considering among the resources the agent's known repertoire of actions – the evaluation evokes a single emotive response (Lazarus, 1985, 1991; Lazarus and Folkman, 1984) characterised by the aforementioned features.

The appraisal theory shifts the attention from the elusive concept of emotion to a vague concept of evaluation: nonetheless, the latter is sufficient for the anatomic approach to pursue a concrete explanation of the processes realising it, focussing on the neural mechanism underlying emotional responses (Ledoux, 1996; Panksepp, 1998). This approach pushes forward the generation of artificial neural circuits characterised by architectures whose design matches as much as possible the structure of the biological neural system under analysis. These anatomically-constrained neural circuits (or anatomically-inspired, depending on the reliability of the final structures) are the core of the computational models which are asked to simulate and successfully replicate the brain functioning characterising both fast, automatic emotional responses and slower, differentiated ones (e.g. see Armony et al. 1997).

When it comes to generate models of emotions, the commitment introduced by any approach –such the anatomic one here described– is not sufficient *per se*

in determining a comprehensive procedure: any simplified representation –i.e. model– of reality relies on a set of theories bridging the representation to the real phenomenon it is representing. The predictions the model provides thanks to the analysis of its functioning must refer once again to the real phenomenon it is representing and they must be tested having in mind the exact ratio of the simplification employed. A model does not provide hints about data it is not representing and it cannot represent data at scales different from the one used to perform the starting simplification: both uses of the model eventually lead to meaningless results. Thus, the process of simplification is pivotal: the chosen grain of analysis must be kept constant when considering (A) the target data the model aims to replicate, (B) the functions ascribed to the systems that are represented in the model and finally (C) the predictions that will be tested.

The otherwise abstract problem of the grain of analysis becomes immediately clear when considering the wide range of possibilities, targets, processes and experiments than can be addressed in the field of neuroscience and emotions in particular (the issue is widely discussed in the field of philosophy of science: concerning psychological predicates and emotions, see Bechtel and Mundale 1999). Indeed, the anatomical approach may be used to create a wide variety of models in the continuum between a fine and a coarse grain: the resulting models will be focussing on representing accurately phenomena involving molecular reactions, dynamics of the activity of the receptors, features of single ion channels, spiking activity of each neuron in the network, micro architecture of each neural area involved, average activity of populations and macro structure allowing these areas to interact with one another, broad effects of the catecholamines on entire neural regions, abstract functions to ascribe to macro neural systems using a behavioural or evolutionary perspective and finally, the behaviour itself (including learning and higher cognition).

Incidentally, the vast amount of data that can be potentially addressed to using computational models pushes forward the use of the concept of bio-constrained (Mirolli et al., 2010) rather than the narrowed concept of anatomically-constrained models. Indeed, bio-constrained models built by *computational and systems neu-*

roscience (Churchland et al., 1993; Churchland and Sejnowski, 1994; Dayan and Abbott, 2001; Sejnowski, 1986), are constrained by a wider set of differentiated evidence coming from anatomy, as well as from physiology, neuropathology or behavioural analysis.

The validation problem and the choice of the ”mean field” grain. The choice of the grain of analysis always comes at a price: a finer grain virtually allows better explanations and more accurate predictions, but it actually leads the problem of setting the parameters and -as a consequence- it leads to weakening the chances to start a fruitful process of validation/falsification of the theories the model relies on.

The reasoning leading to this conclusion is not straightforward and requires a brief explanation. First of all, the finer the grain of analysis, the more it requires the model to incorporate data and constraints: each of these sets of data and constraints is characterised by its own spatial and temporal scale, requiring a number of assumptions, ad-hoc hypothesis and -what is more- increasing the number of variables and parameters to be tuned to allow the models to replicate and explain their target phenomena.

In literature, the problem of finding the appropriate set of parameters has been solved using powerful regression techniques such as the Genetic Algorithms (GAs; Chou and Voit, 2009; Moles et al., 2003; Ruppin, 2002), which have been used to tune the model described in this dissertation. GAs are widely known and used in tuning parameters with non-linear regressions (Kapanoglu et al., 2007; Vander Noot and Abrahams, 1998; Yao and Sethares, 1994; Zhengjun et al., 1995) and are generally considered as very effective because of their ”blind” search within the parameter space (via randomly generated solutions and selections *a posteriori*). In bio-constrained models, the recurrent connectivity characterising the neural architectures and the non-linear interactions between the system components result in a wide and complex multidimensional parameter space which the GAs explore looking for a set of parameters that might allow the model to fit any provided set of target data.

The GAs (as any other regression tool) may then fall into two different kinds of problems: first, the simulation of all the desired interactions may become computationally prohibitive when coupled with the highly complex neural architecture of a bio-constrained model. For instance, a neural network characterised by either a high number of units or an extremely detailed and complex simulation of the chemical interactions may easily result in a parameter space so vast that there is simply not enough time or computing power to explore even a small part of it. As a consequence no regression is possible and the hypotheses the model is grounded on cannot be validated or falsified.

A second, more subtle, problem relies on the use of a vast number of parameters: the regression tool may succeed in finding a satisfying set of values for the parameters, but this result might be once again useless in the process of either validation or falsification of the model. This is the case of underdetermined models, a problem which is firmly bound with the concept of constraints and available degrees of freedom: if the introduced constraints are insufficient to restrict the degree of freedom arising from the free parameters (i.e. if there are not enough data to keep each of the modeled functions, interactions or mechanisms), the solution to the problem of setting the parameters will be plausibly found, but it will not provide any evidence in support of the core hypotheses of the model and hence it will not provide any interesting explanation of the target phenomenon. In short, an underconstrained condition entails the parameters allow replicating so many different (and often conflicting) data, that the whole concept of the model becomes irrelevant.

To avoid falling in either problem, this dissertation thesis focusses on a model characterised by mean-field neural networks (Bojak et al., 2003), having as a core element of the neural system a standard leaky neural unit (Dayan and Abbott, 2001). The dynamics of each of these computational units represent the activity of a whole population of real neurons (e.g., measurable with a mean field potential recording) allowing a considerable simplification of the microarchitecture and dynamics pertaining the single cells and focussing on the systemic interactions among neural populations and their resulting functioning.

Mean field models come in many varieties and are becoming increasingly popular due to their versatility in capturing different features of the average activity of neural masses: a compromise between the mentioned fine grain and coarse grain analysis perfectly matching data coming from different experiments such as the limited spatial resolution of noninvasive neuroimaging techniques (Friston et al., 2003; Marreiros et al., 2010) or the invasive microdialysis used to record slow tonic catecholaminergic releases in long time scale, such as the ones addressed by the model here presented.

Structure of the dissertation thesis. The whole dissertation thesis is focussed on a bio-constrained model simulating the releases of catecholamines in rats subjected to long lasting inescapable stressful conditions: the restraint test (Cabib and Puglisi-Allegra, 1996; Pascucci et al., 2007). The model simulates the processes taking place in rats due to the huge amount of data that neuroanatomy, neurophysiology and psychophysiology have accumulated about the rat nervous system: these data represent a perfect repository of information to use whilst building the structure of the model, also providing a sufficient amount of constraints to offset the number of free parameters.

Chapter 2 deals with the theoretical problem of the appraisal of controllability, explaining in details how the neural model has been built and describing the core features of the method used to set the parameters, i.e. the GAs and the way this tool allows tuning both the free parameters and the very structure of the model. The focus of this chapter is on the way the appraisal of controllability changes depending on the number of exposures to the same stressful experience: the model successfully replicates all target data, pushing forward a series of predictions.

Chapter 3, which is a slightly modified version of an article recently submitted to the Journal of Neuroscience, deals with the brain mechanisms realising the specific dynamics of the neuromodulators during the restraint test. The chapter describes in details the functioning of the model, its biological constraints and the computational features used to simulate both mean field activities and the

effects and dynamics of the neuromodulators on the target areas.

The model successfully replicates the whole set of target data providing a description of the plausible neural activity during the experiment and pushing forward four predictions concerning lesions that are not only helpful in understanding the processes underlying stress coping, but in strengthening the process of falsifcation/validation of the model.

The appendix at the end of the dissertation thesis provides a table of the acronyms used throughout the dissertation and the table of the parameters evolved using the GAs and allowing the model to replicate all the data here presented.

Chapter 2

The appraisal of controllability

Abstract

According to appraisal theorists, emotional and behavioural responses any organism evokes in the attempt to cope with perceived stimuli follow the evaluation (appraisal) of both internal and external conditions characterising the subject.

We rely on a system-level bio-constrained neural model to provide an explanation of the neural mechanisms realising the evaluation of controllability: in particular, this chapter focusses on catecholaminergic data coming from rats subjected to restraint test (Cabib and Puglisi-Allegra, 1996; Pascucci et al., 2007). The model provides a description of the neural mechanism underlying the process of appraisal, showing the cause of the different coping strategies deployed by naive and repeatedly exposed subjects in presence of a long-lasting, inescapable stressor.

The high number of neural systems involved in the process and the required simulation of the effects of the neuromodulators result in increasing the number of variables and parameters to be set in the model. Genetic algorithms have been chosen as a tool to overcome this problem: this powerful non-linear regression tool has successfully managed in setting the free parameters of the model and in guiding the development of its neural architecture. This tuning process allowed the model to replicate all target data, providing several predictions.

2.1 Introduction

2.1.1 Appraisal

The emotional and behavioural response to stress conditions plays a fundamental role in the adaptation of organisms. Following the appraisal theory (Scherer et al., 2001), these responses are realised as a consequence of an evaluation process taking into account both internal and external conditions and biasing higher level cognitive processes. First, the evaluation establishes the nature of the event and its significance for the organism and secondly, it assesses the chances for the

organism to cope with the event, depending on the available resources. Therefore, in presence of a stressor and depending on the appraisal, the stressed organism can trigger several different responses resulting in the attempt to remove, escape or tolerate the stressor itself (Lazarus, 1985, 1991; Lazarus and Folkman, 1984).

The appraisal theory relies on two core assumptions (Scherer et al., 2001): first, there is a bijective relation between sequences of appraisals and the emotional and behavioural responses it elicits: appraisals precede emotions so that each single sequence always evokes the same physiological and behavioural response (giving an account of individual and temporal differences in emotional response to the same stimuli). Secondly, the appraisal system normally evokes those responses which are more likely to be efficacious in coping with the provided stimuli: inadequate understanding of the event or of the available resources (either internal or external) and a poor repertoire of actions lead to inappropriate appraisals, causing irrational emotive reactions and behaviours. This condition may be artificially induced interfering with the mechanics of the appraisal and it may become pathological when the appraisal is constantly unable to carry out its normal evaluation process.

The physiological changes taking place during the evaluation process and its produced sequence of appraisals have been widely investigated. In particular, the use of animal models (Hull, 1943; Tolman, 1932) and the ability of controlling neurophysiological variables (via neuromodulator agonists and antagonists, lesions, inactivation and microdialysis techniques) have been granting a solid and constant inflow of data which mainly concerns conditioning (either pavlovian/instrumental and positive/aversive), goal-oriented behaviours and, what is more important for the purpose of this paper, stress coping (Amat et al., 2005, 2008, 2006; Cabib and Puglisi-Allegra, 1994, 1996; Maier, 1984; Maier and Watkins, 2005; Pascucci et al., 2007). Nonetheless, this huge amount of data concerning neural and cathecolaminergic activities is not *per se* sufficient to give an account of the nature and functioning of the mechanism causing these regulations and the resulting behavioural responses, so that our understanding of the

brain mechanisms underlying the phenomena targeted by the data is not increasing at the same pace.

The difficulties in investigating appraisal are due to the several neural components (areas and modulators) it requires at different psychobiological level and the very nature of these interactions, which rely on a type of causality characterised by a high degree of circularity (Lewis, 2005). This paper proposes to address this complexity using a computational model, focussing in particular on the appraisal of stress controllability, targeting data concerning the specific evaluation processes determining how to cope with novel and previously experienced uncontrollable stressors. This specific type of evaluation has been chosen because of the wealthy amount of data (Amat et al., 2008, 2006; Bland et al., 2003; Cabib and Puglisi-Allegra, 1994, 1996; Maier and Watkins, 2005; Pascucci et al., 2007) and because the appraisal of controllability interestingly leads to completely different behaviours, even in presence of the same persistent stimulus: the complex relation established between constant external conditions and a pattern of differentiated coping strategies allows to simplify part of the evaluation process, blocking one of the fundamental variables of the evaluation (i.e. the external conditions) and focussing on the remaining one (i.e. the evaluation of internal condition).

2.1.2 Coping Strategies

Stress-coping strategies can be grouped into two broad categories (Lazarus, 1985, 1991; Lazarus and Folkman, 1984; Rosenstiel and Keefe, 1983): (a) 'problem-focused strategies' or 'active (proactive) coping', referring to responses directed to the external environment and aimed at removing or avoiding the source of stress; (b) 'emotionally-focused strategies' or 'passive coping', referring to 'internal responses' directed to reduce or sustain the impact of the stressor, both physically and psychologically, for example releasing endorphins to mitigate pain (Frew and Drummond, 2008; Tejedor-Real et al., 1995).

In this respect, dopamine (DA) presence in nucleus accumbens (NAcc), the terminal region of the mesoaccumbens dopamine system, plays a central role.

A wealth of ex vivo and in vivo studies demonstrates an elevation of mesoaccumbens DA release in response to unconditioned aversive stimuli such as foot shock, tail shock, tail pinch, and restraint (Horvitz, 2000). Mesoaccumbens DA release is also observed in species-typical stressful experiences such as in male rats or mice under attack of conspecifics (Miczek et al., 2008). Moreover, DA antagonists at doses that do not interfere with motor responses block the expression of species-typical defensive strategies towards aggressors whilst DA agonists stimulate the expression of these responses towards non aggressive conspecifics (Belzung et al., 1991; Filibeck et al., 1988; Puglisi-Allegra and Cabib, 1988). The use of high doses of DA antagonists in the NAcc is a critical cause of motor deficits: it impairs a number of different types of behaviour in different contexts, including aversive tasks involving for instance place avoidance and taste aversion (Huang and Hsiao, 2002; Salamone, 1994; Salamone and Correa, 2002).

An increased DA release in NAcc in stressing conditions could have the functions of energizing behavioral attempts to cope with the stressor, ascribing high incentive salience to goals of actions and favouring quick and durable learning of effective coping attempts (Berridge, 2007; Di Chiara and Bassareo, 2007; Niv et al., 2007; Salamone et al., 2007; Schultz, 2007). This idea is also supported by experiments showing that manipulating the amount of DA in the NAcc results in altering the disposition of the subjects to make and sustain any effort, independently of the knowledge about the possible results (Berridge and Robinson, 1998; Salamone and Correa, 2002). For example, a low amount of NAcc DA results in a decreased disposition to select actions which might lead to high rewards but require high efforts (Salamone et al., 2003).

Dopamine in NAcc also plays an important role in passive coping strategies. In fact, in (novel) unavoidable/uncontrollable prolonged stressing conditions after the aforementioned initial increase, NAcc DA falls below the baseline and this fall generally correlates with the rats inactivity: restrained rodents show this initial enhancement of DA release, followed by substantial decrement if the animals are not released from the stressful condition within 20 to 40 minutes (Cabib

et al., 2002; Imperato et al., 1993; Pascucci et al., 2007; Puglisi-Allegra et al., 1991). In the Porsolt's Forced Swimming Test (FST), in which animals experience an energy demanding condition (they are placed in a small water tank with no way out), the DA inhibition takes place within few minutes bringing forth inactivity (Rossetti et al., 1993).

The initial high release of NAcc DA during prolonged stress has been recorded in naive rats both in inescapable aversive conditions and in controllable ones. Nonetheless, if the rat is exposed to several trials of uncontrollable stress, the initial high response is inhibited, even if it is still possible to record the second below the baseline DA release in NAcc (Bland et al., 2003; Cabib and Puglisi-Allegra, 1994). Inhibition of mesoaccumbens DA release is also related to the reduction of the attempts to escape or remove the source of stress: such a behaviour, known as 'behavioral despair', is typically observed in FST (Porsolt et al., 1977). When first immersed in the water tank, naive animals show vigorous attempts to escape from the tank by swimming and struggling to climb its walls. These responses are soon replaced by episodes of immobility of increasing length. Independent studies have demonstrated that chronic antidepressant treatments capable of reducing FST-induced despair also prevent FST-induced accumbal DA decrease (Rossetti et al., 1993).

Summing up, a model of the appraisal of stress controllability has to deal with the brain mechanisms underlying the regulation of NAcc DA, giving an account of the different evaluation of uncontrollability showed by naive and repeatedly stress exposed rats. Increasing evidence indicates that the medial prefrontal cortex (mPFC) plays the key role role of guidance in this regulation (Amat et al., 2005; Pascucci et al., 2007; Spencer et al., 2004): this hypothesis is consistent with data coming from both anatomical analysis (Jankowski and Sesack, 2004) and records of neural activity and behaviours expressed in the presence of selective neural inhibition (Peyron et al., 1998).

The goal of this paper is to use a neural-network computational model (Gurney, 2007) providing detailed hypotheses on the brain mechanisms underlying the appraisal of controllability in the case of long lasting inescapable stress con-

ditions. The soundness of the model has been tested reproducing in detail the dynamics of the neuromodulators as recorded in rats engaged in novel and repeated restraint test Cabib and Puglisi-Allegra (1996); Pascucci et al. (2007).

2.2 Parameter Setting

The key feature of bio-constrained models is that they rely on neural architectures and functioning mechanisms that are constrained by empirical evidence on the anatomy, physiology, and neuropathology of the biological neural systems that are being modeled. These constraints can range from the features of single ion channels to the dynamics of neurotransmitters and neuromodulators, from the features of single neurons and the micro-architecture of local neural circuits to the macro-architecture of the whole brain. As a matter of fact, the increasing amount of addressed data and constraints incorporated into the models leads to a parallel increase in the number of variables and parameters of the models. This in turn leads to the increasingly difficult problem of finding the appropriate set of parameters that may allow the models to replicate and explain the target data: the recurrent connectivity, the use of heterogeneous time-scales (e.g., for functioning and for learning), and the highly non-linear dynamics of the interactions between the sub-parts of a model, make the relation between a certain parameter set and the functioning of the system with respect to the target data very indirect and difficult to understand and manage.

The difficulties to set the parameters imply that the whole validation process of the model becomes weak, uncertain and time consuming. The risk to generate ill-grounded falsifications is strongly increased because valuable hypotheses and model variants may be discarded because the researcher's failure in finding a suitable set of parameters to reproduce the target data can be mistakenly confused with the model's inability to reproduce these data. An efficacious solution to this problem comes from the use of Genetic Algorithms (GAs; Chou and Voit, 2009; Moles et al., 2003; Ruppin, 2002). In this section the technique relying on the use of GAs is described in detail, showing how they allow searching for

large parameters sets in bio-constrained neural models. The technique is first illustrated in general and then applied to the model addressing the problem of the appraisal of controllability.

2.2.1 Genetic Algorithms

GAs have been initially proposed as a model of Darwinian evolution, where increasingly complex organisms evolve due to the two principles of selection of the fittest and reproduction with variation (Goldberg, 1989; Holland, 1975; Mitchell, 1998). GAs are based on computational abstractions of the basic mechanisms that underly natural evolution, like different kinds of selection regimes, inheritance mechanisms, crossover techniques, and mutations processes.

In general, a GA works as follows. First, the *genome* of an *individual* (e.g., the parameters of a neural network that undergo the evolutionary process) is encoded in a suitable data structure (e.g., a vector of numbers encoding the connection weights of the network). Then an initial *population* of these genome is created (e.g., randomly), and each individual *phenotype* (e.g. the neural network corresponding to a genome) is tested with the task at hand to evaluate its *fitness* (performance). The fitness is then used to select the fittest genomes, which are reproduced with random variations so to generate a new population. If this process is iterated several times (*generations*), individuals with high fitness eventually emerge.

GAs have been used as a powerful technique for searching for solutions to the optimization problems involving large parameter spaces (Gulsen et al., 1995; Zheng and Lewis, 1994). GAs have been applied to several different research fields, including computer science (Fogel, 1998), machine intelligence (Fogel, 2005), electronics (Zebulum et al., 2002), biology (Unger and Moult, 1993), financial forecast (Chen, 2002), and economics (Allen and Karjalainen, 1999).

Furthremore, GAs have also been shown to be very effective when applied to find the parameters of non-linear regressions models (Kapanoglu et al., 2007; Vander Noot and Abrahams, 1998; Yao and Sethares, 1994; Zhengjun et al., 1995). In this case, GAs are used to find the parameters of mathematical non-

linear functions capturing the relations between the independent and dependent variables of a target phenomenon. Their strength stems from the fact that the GAs search within the parameter space in a "blind" fashion, that is to say, they generate solutions randomly and then select them *a posteriori*, so they do not need to rely upon gradient ascend or similar techniques that become analytically untractable when non-linearities overcome a certain level of complexity. This is the main reason making of the GAs a successful tool in the mentioned heterogenous environments: robust algorithms with respect to the shape of the function relating the variables of interest.

Two similar fields of application of GAs are very important for this research: Artificial Life (*ALife*) (Langton, 1997) and Evolutionary Robotics (*ER*) (Baldassarre and Nolfi, 2009; Baldassarre et al., 2007; Nolfi and Floreano, 2000). In these fields GAs are typically used to evolve the connection weights of neural-networks that control the behavior of simulated organisms or robots interacting with the environment in complex dynamical ways via noisy sensors and actuators (e.g. Schembri et al., 2007a,b). In this case the fitness that drives the evolutionary process is a quantitative measure of the behavioral performance of the artificial systems with respect to the task decided by the experimenter.

The application of GAs to ALife models poses problems that are shared with their application to bio-constrained models. First, ALife models often have a significant number of parameters. Second, in both types of models GAs search for parameters of systems characterised by complex, circular interactions: in ALife models, much of the complexity of the relations between the searched parameters and the target behaviour arises from the controller-body-environment circular interactions (Baldassarre, 2008); in bio-constrained models, the complexity stems from the highly structured architecture of the system, often incorporating recurrent connectivity and non-linear interactions between the system components. The successful application of GAs to ALife models suggests that this methodology might be successfully used for optimizing the parameters of bio-constrained neural systems so as to fit target empirical data related to behavioural and/or neural activity recordings (molecular, electrical, etc.).

However, there are also important differences between the two fields of application. The first one is the smaller emphasis of bio-constrained models on the brain-body-environment interactions. Indeed, even though desirable in principle, the simulation of these interactions is often computationally prohibitive if added to the internal complexity of bio-constrained models. Second, and most important, contrarily to the ALife approach the bio-constrained modeling approach gives a great importance to the consistency of the model's architecture and functioning with biological anatomical and physiological evidence: these constraints are indeed as important as those deriving from the target data. These differences in the kind of models and in their scientific goals generate different problems and require that the application of the GAs is adapted accordingly.

2.3 Methods: Parameter Search using Genetic Algorithms

This section describes in detail the methodology used to search the parameters in dynamic bio-constrained models. The first step consists in identifying a set of target data and building a model with an architecture and functioning consistent with known anatomical and physiological empirical evidence. Then, the parameters to be searched and the fitness function to measure the parameters quality must be defined in details.

The application of the GAs requires setting a number of features and meta-parameters for the GAs themselves, before the model can start using this powerful regression tool to search for the parameters that allow the model to best fit the target data (validation process). Once the correct parameters have been found (if they exist), the model is considered validated and it can be finally used to produce predictions on new functional or anatomical phenomena.

The whole process can then be iterated aiming to produce increasingly comprehensive models furnishing integrative explanations of multiple data and experiments.

2.3.1 Target Data

The target data are a first fundamental source of constraints of the model, and are used to guide the process of the model validation. Target data may consist of any kind of empirical data provided by the literature, including recordings of neural activity (of single units or whole neural populations), of neuromodulator dynamics, of behaviours exhibited by certain organisms, data related to different kinds of lesions, and so on.

There are two major types of data: synchronous data and time series. Synchronous data are represented by a set **d** of n integers, real numbers, or symbols d_j:

$$\mathbf{d} = \{d_1, d_2, ..., d_n\} \tag{2.1}$$

Time series are instead represented by temporally ordered (usually numerical) data, for example a vector **d** of n real numbers d_j:

$$\mathbf{d} = (d_1, d_2, ..., d_n) \tag{2.2}$$

The use of synchronous data poses only the problem of assigning to different weights to different data (see section 2.3.5 below). Instead, data consisting in time series pose further problems, as their single points are linked by time. Indeed, the shape of data in time is often considered by the biologist more important than their absolute values as it represents the time dynamics of various aspects of the studied phenomenon, often revealing important causal relations between them. This temporal features of time series can be indirectly captured by requiring that the model fits the absolute values of the data points without directly taking into account time information. Alternatively, one can ask the GA to search for parameters that reproduce the time derivatives of the time series, in alternative or in addition to the request of capturing their absolute values.

An important aspect of biological data is that different replications of the same experiments mostly produce different data, making it difficult to define the target data to validate the model. If the different outcomes do not differ substantially, a simple straightforward solution to the problem is to merge the data gathered in

the different replications by considering their average. In the case the data differ substantially, one can try to isolate those data that have a lower variance and that are more directly relevant for the theories of interest. In the case study we will see how it is possible to give a higher importance to these sub-sets of data (see also below the section on fitness).

2.3.2 Definition of the Architecture and Functioning of the Model

The definition of the architecture of the model and of the functioning mechanisms of its components is of course of the most importance, as these aspects of the model implement the biological assumptions and hypotheses that represent the main contribution of the whole modeling research itself.

Despite the overwhelming amount of data produced by neuroscience, the construction of models that can account for the available empirical data typically requires the formulation of new hypotheses related to the existence of particular architectural features or mechanisms that are not currently supported by neuroscientific evidence, or it may require to take a position with respect to conflicting theories. The hypotheses and assumptions that are implemented in a model are validated as far as the model is able to reproduce the available empirical findings, showing that those hypotheses are sufficient (even though not necessary) for explaining the data.

The definition of the architecture of the model is based on three main kinds of constrains: (1) biological constraints coming from the relevant neuroscientific knowledge already available in the literature; (2) computational constraints that must be considered for bulding a model that might allow to reproduce the targe data; (3) epistemological constraints that push the researcher to identify the minimum number of components and the simplest functioning mechanisms that, on one side, conform with the known empirical evidence and, on the other, are sufficient to reproduce and explain the target data.

2.3.3 Overview of a Genetic Algorithm

This section illustrates the general procedure to implement a standard GA. This procedure might in part change depending on the decisions made on the various aspects of the algorithm (see below). The pseudo-code of the procedure is shown in Figure 2.3.3.

```
INIT Genotypes with initial parameter values
WHILE stopping criterion is not reached
    Create Population of individual phenotypes from genotypes
    FOR each individual of the Population
        Compute the fitness of the individual according to the FitnessFunction
    END FOR
    Select the best individuals of the Population
    Reproduce selected individuals thus reating a nuew population of Genotypes
    Mutate the new Genotypes
END WHILE
```

Figure 2.1: *The pseudo-code of a genetic algorithm procedure.*

The application of a GA to find the parameters of a solution to an optimization problem (e.g. a model to reproduce certain data) involves few fundamental algorithmic steps, each of which has several variants. The first step consists in encoding the parameters to be optimised in a numerical vector (the *genotype*). These parameters might for example be the connection weights of a neural network, or its learning rates, or the time constants of different sub-sets of leaky neurons forming the network, etc. As a second step, a certain number (*population size*) of different genotypes of this type are generated, for example by drawing their values randomly within certain ranges. Then each genotype (i.e., the set of parameters it encodes) is used to generate an instance of the model (*individual*) so to have as many models as the genotypes (*population* of individuals). Next, each individual model is evaluated on the basis of the optimization problem at hand: the better the optimization, the better its score (*fitness*). The

fitness is then used to select a subset of individuals of the population that is used to create a second *generation* of genotypes with the same population size. The genotypes of the selected individuals are used to create the new population of genotypes by randomly changing some parameters chosen at random (*mutation*) and/or by mixing two or more selected individuals to form new ones (*cross-over*). The whole process of fitness computation, selection, and generation of a new population is then iterated a certain number of times (*number of generations*). By iteratively letting parameters sets (genotypes) reproduce on the basis of their ability to replicate target data (fitness), and adding random variations trhough mutations and re-combinations, the evolutionary process is eventually able to find optimal candidate solutions.

2.3.4 Selection of the Parameters to Evolve, their Encoding, and Ranges

An important aspect to decide before running the GA algorithm concerns the parameters on which the GA should work. The definition of the parameters involves three main decisions. The first decision is about the aspects of the model that will undergo the optimization by the GA. In this respect, the parameters to be selected to this purpose should have two features: (a) they should represent aspects of the model that it is not possible to set to particular values on the basis of known biological constraints; (b) one has good reasons to believe that they can substantially affect the behaviour of the model with respect to the target data.

The second decision concerns the data structures conceived to encode the selected parameters. Based on theoretical reasons and simplicity arguments, the initial proposals of GAs suggested to encode any type of parameter with binary codes (Holland, 1975). The following research, however, has shown that GAs work, or work even better, on data structures more similar to the features of the phenotype (often because it helps in compacting the search space). For example, the most common case is to use parameters represented in a vector $\mathbf{p}$ of n real numbers p_i (for example to encode the connection weights or other quantitative

aspects of a neural network; see Mitchell, 1998):

$$\mathbf{p} = \{p_1, p_2, ..., p_n\} \tag{2.3}$$

Alternatively, one might use discrete or even symbolic parameters to determine qualitative aspects of the model, for example the particular features of the Hebbian learning rule used by the system or its architectural aspects (Floreano and Urzelai, 2000; Vonk et al., 2002).

The third decision involves the imposition of limits to the range of values that each parameter can assume. In principle, it is possible to establish parameters with free ranges. However, often the nature of the encoded parameters (e.g., learning rates, neural unit decays, neuromodulator efficacy, learning rule types, etc.) impose biological constraints to the ranges of the evolved parameters. For example, if some parameters encode the strength of glutamatergic or GABAergic connections, they should be constrained to assume respectively positive and negative values. As we will see in the present model of appraisal, other considerations might also lead to bound the ranges of evolving parameters also leading to the advantage of reducing the size of the parameter space boosting the evolutionary process and reducing the risk that the search falls in local-optima (i.e., non-optimal solutions surrounded by worse solutions).

2.3.5 The Fitness Function

The fitness function is the means through which a particular parameter solution is evaluated. In our case, it measures the distance between the data produced by the model and the target data according to some matrix. A matrix that can be suitably used in our case, borrowed from linear and non-linear statistical regression approaches, is the mean square error, MSE, which takes the average of the square differences between the target data elements d_j and their equivalent b_j provided by each phenotype m:

$$SMN_m = \frac{\sum_j^n \left[(d_j - b_{m,j})^2 \right]}{n} \tag{2.4}$$

After the fitness function is applied, the resulting fitness might be scaled before being used to select the best individuals. The scaled fitness is a number obtained on the basis of a particular function of the fitness value. For example, the scaled fitness of an individual might be the rank of its fitness within the population (i.e., a number between 1 and P, where P is the size of the population). The scaling allows tuning the relation between the performance of individuals and their probability of selection (see below).

A last very important aspects related to the fitness and its application to bio-constrained models regards the fact that the target data do not have all the same importance. For example, within the points of a time series one might give a high importance to a particular aspect of the curve with respect to another portion of it. For example, one might want that the parameters found by the GA allow the model to closely match some data points (e.g., a certain neuromodulator being precisely at the baseline level in a certain period of time) while tolerating some inaccuracy for some other data points (e.g., the neuromodulator level being at some positive values in another period of time). An effective solution to this problem is to assign a different weight to the error of different data points:

$$SMN_m = \frac{\sum_j^n \left[w_j \cdot (d_j - b_{m,j})^2 \right]}{n} \tag{2.5}$$

where w_j represents the weights assigned to each data point. This solution is rather important as it allows to quantify and operationalise the fact that one gives high importance to particular qualitative aspects of the target data.

2.3.6 Setting Other Features of the Algorithm and the related Metaparameters

This section briefly describes the other aspects of a genetic algorithm that have to be set before running it. These aspects are not specific to the application of a GA to finding the parameters of a bio-constrained neural model, but they are important because they affect the success and efficiency of the algorithm.

Initial population. There are various ways of defining the values of the initial population of genomes. The simplest one is to generate them randomly on the basis of a uniform probability distribution. A more effective way is to select the initial genotypes so that they are (approximately) uniformly distributed in the genotype space. This choice avoids generating too similar genotypes, and reduces the possibility that the search falls in a local optimum.

Selection scheme. Once all individuals of one generation have been tested, the selection scheme determines which individuals are selected for reproduction according to their fitness. A number of possible selection schemes have been developed, among which the most popular are the *roulette wheel*, the *rank*, and the *tournament* selection schemes.

In the roulette wheel selection scheme individuals are represented as slices in a wheel such that the size of each slice is proportional to fitness of the corresponding individual. The wheel marker then spins for a random time and the individual selected for reproduction is the one corresponding to the slice where the marker stops. In this way, the probability of reproduction for each individual corresponds to the fitness of the individual divided by the total fitness of the population. This selection scheme has the problem of premature convergence: at the beginning of the simulation the variance in the fitness is usually very high so the fittest individuals will tend to spread in the population very fast; then, when the population has converged –meaning that all individuals in the population are very similar to each other– all the individuals will tend to have approximately the same fitness and hence the selection probabilities, so individuals will be selected at random and this will prevent further improvements.

The rank selection scheme consists in selecting individuals with a probability which is not proportional to their fitness, but which depends on the ranking of the individuals in the population. In the most simple and common rank method, one sorts the individuals according to their fitness and then selects n best individuals for reproduction. This method avoids that the population converges too quickly by both preventing that the fittest individuals reproduce too quickly at

the beginning of evolution, when the fitness variance is high, and keeping high selection pressure afterwards, when the fitness variance is low.

The tournament method can be described as follows: choose two individual randomly from the population, select the fittest for reproduction, return the two individuals to the population so that they can be chosen again and repeat the procedure until you have selected the right number of individuals. This procedure produces a selective pressure similar to that produced by the rank method, but is usually less computationally expensive than it as it avoids sorting the entire population, which can be very time-consuming.

Reproduction. Reproduction can be either ”sexual” or ”a-sexual”. The difference between these two reproduction schemes lies in the fact that the former includes the application of cross-over between the genomes of two parents, while the latter is based on the cloning of single genomes. The simplest kind of cross-over is the single-point one: take two selected individuals; choose randomly one point for dividing the genomes of the two in two parts; generate one new individual by taking the first part of the genome from the first parent and the second part from the second parent and another by taking the first part from the second parent and second part from the first parent. One problem in the use of single-point cross-over is that it treats different points in the genome differently: in particular, the end-points of the genome strings are treated differently from the central ones in that they will always be exchanged. One solution is to adopt a double-point cross-over: two points are randomly selected and the segments which are exchanged are the two between those two points. Applying double-point cross-over is like treating the genome as a circle, so that there is no difference in the probability of cross-over between the centre and the periphery of the genome. An extreme variant of this solution is to take each parameter of the genotype of the new individual from either one of the two parents with the same probability.

Mutations. After having produced the right number of genomes by either cloning individuals or by applying cross-over between pairs of parents, mutations are ap-

plied to those genomes with a certain probability. There are various ways of mutating the parameters of genomes, which depend on the genetic enconding that have been chosen. If the genetic encoding is binary, mutations consist in flipping the binary value of each parameter with a certain probability. If the parameters are real numbers, one can either replace the mutating gene with a randomly chosen value or change the current value by adding to it a random value chosen within a certain range on the basis of a certain probability distribution, for example flat or Gaussian.

Elitism. Whatever the selection and reproduction schemes chosen, one can prevent that good solutions are lost by not being selected or being destroyed by cross-over and mutation by retaining the best n individuals (one or more) and assuring that they are included into the next generation without any modification. This can substantially improve the effectiveness of the evolutionary research.

Stopping criterion. Another thing to be decided is the criterion for stopping the iterations of the algorithm. The simplest stopping criterion consists in stopping the research when a certain number of generations is achieved. This is very easy to implement and ensures that the algorithm stops after a fixed amount of time. On the other hand, a lot of time might be wasted either because the fitness of the best individual of each generation has reached a plateau well before the end of the simulation, or, even worst, because the evolutionary process has not completed when the last generation has been reached and so a good solution has not been found due to the early stopping of the algorithm.

Another possible stopping criterion is based on the fitness of the best individual of a given generation: the algorithm stops whenever the best individual has reached a fitness that overcomes a given threshold. This criterion ensures that the process stops only when a good set of parameters has been found, but has the problem that if a good set of parameters can not be found (or even if the evolving population has converged on a local maximum) the algorithm can go on forever. For this reason, this criterion is typically used in conjunction with the

previous one, so that the algorithm stops either when the fitness has overcome the threshold or after a certain number of generations have been accomplished. An effective variant stops the research when the improvement of fitness of the best individual in the last n generations is below a certain threshold.

Another possibility is to stop the research when the variance of the genomes of a generation is below a certain threshold. The rationale is that a very low genetic variability means that the algorithm has converged on a good solution (either local or global), and so a further improvement in fitness is unlikely to occur. One shortcoming of this method is that it might be difficult to set the appropriate threshold. Another one is that calculating genetic variance might be time-consuming.

Replications. When evolutionary experiments are repeated several times by using different starting conditions, often slightly or substantially different solutions are obtained. This happens in particular when the genotype space is large and/or when the fitness test is stochastic and involves complex processes (e.g., as those used in ALife, in particular when one evolves systems that learn during the test). In this cases it is important to run the algorithm several times so that the parameters space is explored effectively.

Meta parameters. Most of the above specified methods have their own (meta-)parameters that must be set for running the genetic algorithm, for example: number of individuals in the population, number of best individuals to be selected (if one uses the ranking method of selection), cross-over and mutation probabilities, number of best individuals for elitism, number of generations and/or fitness or genetic variance thresholds, number of replications of the whole evolutionary process.

All the decisions about the meta-parameters and the methods must be taken on heuristic grounds, because the best combination of methods and parameters crucially depends on the evolutionary research problem at hand. In fact, although there is an entire community of researchers devoted to study which kinds

of methods work bests for which kind of problem (Evolutionary Computation), no clear rule has been found so far, and whereas many useful new methods are being developed and a lot of knowledge on the pros and cons of different evolutionary set-ups is being produced, the actual decisions on which methods to use and on the meta-parameters must be taken by combining expert-knowledge with common sense and trial-and error. Nonetheless, it is important to clarify that whereas it might be difficult to find the combination of methods and metaparameters that works best in any specific case, it is not difficult to find a combination that is satisfying for our purposes, i.e. that can reliably be expected to find a set of model parameters that is able to reproduce our target data in case it exists (provided that the evolutionary process is replicated several times).

2.3.7 Falsification vs Validation

Once the search for the parameters of a bio-constrained model is run, one can obtain different levels of fit of the target data. One can set a certain threshold of quantitative and qualitative level of such fit above which the model is considered to have passed the test, and below which the model is considered to have failed it. The case of failure can be considered a form of weak falsification. Indeed, as bio-constrained models tend to have several parameters and to be very complex, the failure might depend on the fact that the GA felt in a local minima and not on the fact that the model is not sufficient to reproduce the target data. Notwithstanding this limit, it is important to consider that such falsification is however much stronger than, for example, a falsification based on a manual search for the parameters.

In the case the model passes the target-data test, this validation should be never considered a definitive confirmation. In fact, as mentioned above, bio-constrained models often contain some hypothesis and mechanisms that computationally are capable of producing certain needed functions but that are not supported by direct biological evidence. Moreover, bio-constrained models often contain a large number of parameters. On one side this is justified by the fact that they are required to satisfy many constraints, namely not only the request to

fit target data but also the constraints imposed on its architecture and functioning. On the other side, however, this increases the possibility that an excessive number of parameters are introduced so that the number of degrees of freedom of the model exceeds the number of constraints imposed on it.

One way to further corroborate the model is of course to increase the number of constraints imposed on it, both in the form of the requirement to reproduce and account for further target data (e.g., from other experiments), or to impose further biological constraints on its architecture and functioning mechanisms. The other, even more important, way is to derive from the model new predictions and test them with new empirical experiments.

2.4 The case study: modelling the appraisal of controllability

2.4.1 Target Data Analysis

The model is required to provide an explanation of the mechanism resulting in the releases of DA and norepinephrine (NE) in the ventro-medial prefrontal cortex (vmPFC) and the release of DA in nucleus accumbens (NAcc) during restraint tests run with rats (Pascucci et al., 2007). The releases in the vmPFC have been recorded on naive rats only, whereas DA release in the NAcc has been recorded both on naive rats and on rats repeatedly subjected to restraint (Cabib and Puglisi-Allegra, 1996): see figures 2.2 and 2.3 respectively.

The test consists in placing each rat in a restraint box, keeping it immobile by leaving only the head outside the box (more details in Pascucci et al., 2007): the microdialysis samples in naive rats (labelled as ”day 1” in the graphs) were collected every 20 minutes for the whole duration of the experiment -240 minutes- generating 13 samples (first sample collected at time 0), whereas in the case of the rats repeatedly exposed to the stressor (labelled as ”day 6” in the graphs), the 13 samples have been collected in 120 min. using a 10 min. interval, in rats exposed to restraint once per day, 6 days in a row. Using the perspective of the GA the target data consist of 4 time series (two for the recordings of the two neuromodulators in the vmPFC and two for the recordings of DA in NAcc in the

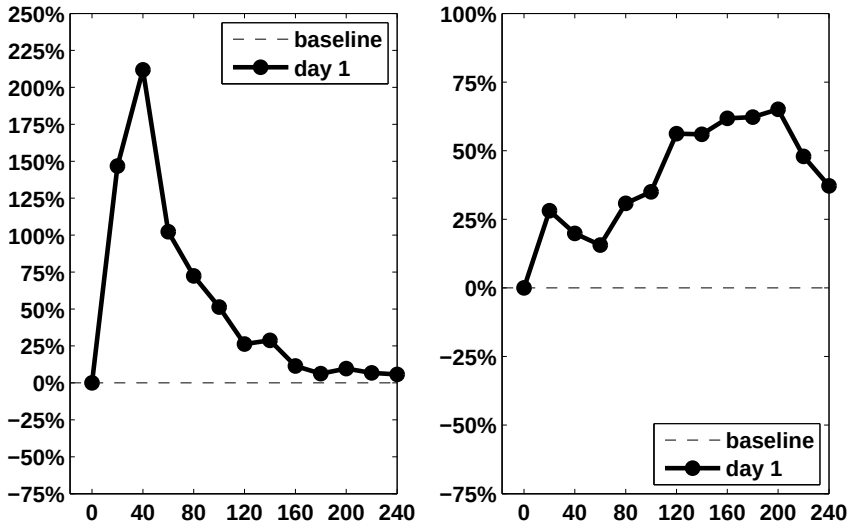

Figure 2.2: *NE (left) and DA (right) releases in vmPFC recorded with microdialysis in rats subjected to restraint (modified from: Pascucci et al., 2007).*

two different conditions), each encoded in a vector of 13 real numbers, for a total of 52 data points.

An analysis of these target data gives an example of the reason why it is not useful to use the GA to pursue only a quantitative match and that qualitative goals should be taken into consideration as the main objective. For instance, the pictures in figure 2.3 (modified from Cabib and Puglisi-Allegra 1996; Pascucci et al. 2007) show data recorded during the same type of experiment and performed in the same laboratory, on different rats: e.g. the graphs point out that the highest peak of DA in NAcc is reached after 20 minutes in both cases, but the single value is significantly different (+75% vs +50% of the basal level), so that the vector of the time series results in quantitative differences. Yet, the dynamics and the slopes characterising the two curves are significantly similar: the presence of DA in NAcc shows a fast increment and a single peak after 20 mins, before starting a constant decrease which lasts for the whole experiment. These considerations imply that the timing of the NAcc DA peak produced by the model has a major importance if compared with the actual value which may tolerate variances. The problem described for the DA recordings is not unique: all the recorded data belonging to the target time series are characterised by sim-

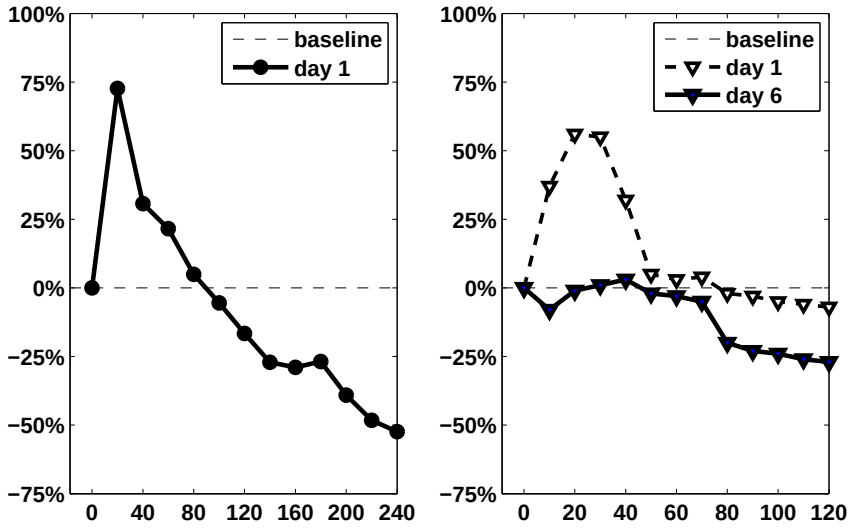

Figure 2.3: *Meso-accumbens DA releases recorded with microdialysis in two rats, both subjected to restraint (modified from: Pascucci et al., 2007 and Cabib and Puglisi-Allegra, 1996, respectively). Since test and recording procedure can be considered identical, it is possible to compare the first 120 minutes of the left figure with the whole day-1 dynamics showed in the right figure: note the quantitative difference and the qualitative similarities between the two curves.*

ilar differences.

An important decision about the target data is whether to consider all the points of the time series as equally important or to require different degrees of accuracy for different sub-sets of the data. The example here proposed addresses the psychobiological problem of finding the mechanism that regulates the release of dopamine in the NAcc in the two conditions of naive and overtrained rats: Pascucci et al. (2007) found that both below- and above-baseline releases of DA in NAcc are determined by the activity in the vmPFC (and its modulation performed by DA and NE), entailing the role of guidance of the vmPFC in generating the target effects in the two conditions (more details in chapter 3 of this thesis).

Thus, the optimization process should produce a model that generates time series with these features: the presence of DA in NAcc must show a first peak at the beginning of the experiment in the naive condition, but not in the repeatedly exposed one. This peak must be temporally tuned with both first peaks of DA

and NE recorded in the vmPFC. On the contrary, the two conditions must not show significant differences in the second part of the experiment, when limbic DA decreases showing a release below the baseline. Finally, the accuracy of the dynamics characterising DA in NAcc must be given a higher priority if compared with the one characterising the two releases recorded in the vmPFC, which are nonetheless going to be considered as successfully simulated if they will show a substantial qualitative match with the target data.

2.4.2 Definition of the architecture of the model

The core neural systems of the model relies on the two areas that are responsible for the release of DA and NE (the Ventral Tegmental Area, VTA, and the Locus Coeruleus, NE, respectively), and the ventro-medial Prefrontal Cortex (vmPFC), which is considered to be responsible for the role of guidance of the dynamics of DA in NAcc: for a detailed description of the architecture of the model, its functioning and its computational features, see chapter 3, sec.3.2, of this thesis.

In rats, vmPFC is mainly composed of the PL and IL cortices. The present model is based on the hypothesis that during prolonged and inescapable stressful conditions the IL learns to inhibit the PL, which is assumed to control goal-directed behaviors (Yin and Knowlton, 2006). As a consequence, an anti-Hebbian learning rule is implemented between PL and IL, which is assumed to play a pivotal role in explaining the complex dynamics that characterises the target data.

Given the particular connectivity bridging the vmPFC and the VTA (Carr and Sesack, 2000; Jackson et al., 2001) and considering the important role the Amygdala (Amg) plays in any emotionally-driven behaviour and in controlling the activity of both LC (Pitkänen et al., 2000) and VTA (Ahn and Phillips, 2002; Fudge and Haber, 2000), this neural system has also been added to the model. Finally, all the connections bridging the various neural components of the model, corresponding to either glutamatergic or gabaergic connections, have been selected on the basis of the anatomical empirical evidence existing in literature (see figure 2.4).

This brief description of the procedure followed to design the architecture of

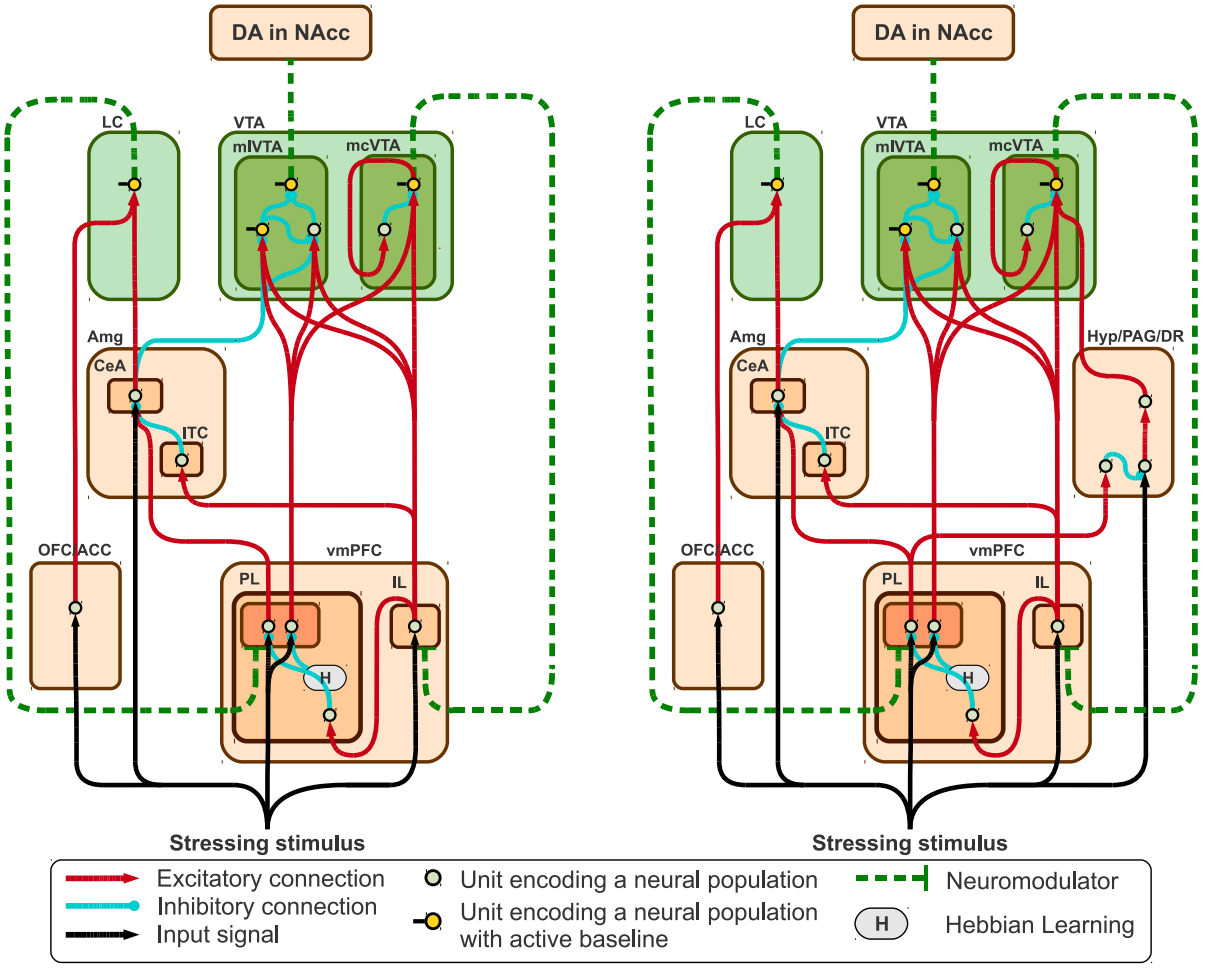

Figure 2.4: *Evolution of the neural architecture of the two model. The left picture represents the first developed version, which the GA showed to be unable to replicate all target data (falsification). The right picture represents the model that succeeded in reproducing all target data (validation). Continuous red and blue lines represent, respectively, glutamatergic and GABAergic connections, whereas black lines represent the input. Dashed lines represent neuromodulatory connections.*

the model, based on the incorporation of biological constraints, exemplifies how easily the complexity of the agent's neural network increases and hence how easily the problem of setting the values of all its parameters may become unfeasible. Indeed, it is be possible to design architectures capable of reproducing the same target data reproduced here while not incorporating the biological constraints related to the investigated phenomenon: these architectures would have a much simpler architecture and functioning and by far fewer parameters. However, these architectures would not be useful to accomplish the overall goal of this research as they would not allow to understand the detailed brain mechanisms

underlying the investigated phenomena. Moreover, in the long run they would miss the important goal of accounting for an increasing number of experiments while keeping intact the core principles of the model.

2.4.3 Selection of the parameters

The architecture has about 30 connection weights, several baselines assigned to the dopaminergic and noradrenergic areas (which simulate the activities these populations have independently of external input), decay rates characterising leaky integrators, reuptake and depletion rates for each neuromodulator, a Hebbian learning rate, and several coefficients used to simulate additive and multiplicative effects of the neuromodulators on target areas.

Each of these parameters may be incorporated in the vector representing a genotype of the GA. However, in this case the parameter space would be huge and, consequently, the search for an optimal set of parameters by the GA would be very time consuming and subject to falling in local optima. For these reasons, it was necessary to work on the list of the possible parameters to select those to be optimised by the GA and those that could be set by hand: some parameters were excluded on the basis of biological constraints (e.g., the leaky decays of the neural units had to be consistent with the actual decays of the neural population they simulated), some were set to 1 as the rest of the system was assumed to be able to compensate a possible wrong choice using the other free parameters (e.g., the value of 1 was used for most weights bridging the input signal to their targets).

Section 2.3.4 briefly described the problems arising from the selection of the ranges of each parameter. In this case, most of the weights assigned to the connections vary from a minimum of 0.3 to a maximum of 5 (and the equivalent negative for inhibitory connections): this choice is caused by the attempt to avoid disproportional connection weights. The two exceptions (concerning PL-VTA connectivity) were introduced as the GA tended to find values close to the lower limit. As a consequence, this limit was lowered but not set to 0 as biology provides a clear constraint concerning the existence and importance of those specific

connections (these connections might be necessary to implement other important functions, so we wanted to see whether the system could be capable of tolerating their presence when simulating the target data). This criterion of the change of the range limits depending on the resultsf of the GA was also useful for setting the ranges of the parameters that had minor or no constraints: for instance, the values corresponding to the levels of DA and NE in cortex in correspondence of a depletion.

Finally, the additive coefficients of the neuromodulators were forced to evolve low values, $0.1 < value < 0.4$, in order to prevent the GA from finding high values of the additive component with respect to the multiplicative components, as this would have made the function of neuromodulators similar to that of neurotransmitters. Final values are showed in table A.2.

2.4.4 GA Fitness function and meta parameters

The model was implemented in Matlab© using the Matlab© GA toolbox for the genetic algorithm. We briefly present here our most important choices for the meta-parameters and the main features of the chosen functions, but it is important to note that most of the GA options were left to the default values.

The population consisted of 200 individuals and the evolution was run for 200 generations (fixed number). The parameters were normalised in $0 < value < 1$ to make the mutation ranges homogenous for all parameters. The initial values of the genotypes of the population were chosen to be approximately uniformly distributed on the whole space of the parameters (see appendix, tab.A.2) based on the function ”feasible”.

The scaling function chosen was ”Rank” (default option) that encodes the fitness values into the numerical ranks of individuals. With respect to elitism, a single individual characterised by the best fitness was chosen to be replicated without any cross-over and mutation (elite = 1). The other individuals of the new population were created in two ways: 80% with both crossover and mutation, and the residual 20% with mutation only (default options).

The function chosen to perform the selection of individuals was ”stochastic

uniform" (default option) which for each individual generates a number of offspring proportional to the ratio between its fitness and the total fitness of the population. The function chosen to perform the crossover was "scattered" (default option) which produces each individual based on a random selection of each parameter from either parent. The mutations were performed using the function "adaptive feasible". This function performs random mutations biased toward or against the direction of the last mutation depending on the fact that it increased or decreased the fitness (the size of the mutation diminishes when a limit of a range is approached).

Finally, a weighted mean squared error was used as fitness, assigning a weight of 10 to the square error related to the curves describing the DA release in NAcc, in the naive condition. All other point data were assigned a weight of 1.

2.4.5 Tuning the architecture using Genetic Algorithm

The first model investigated was unsuccessful as the GA showed to be unable to find the parameters that produced a satisfactory fit of the target data. In particular, the simulated DA dynamics in vmPFC and NAcc during the second part of the experiment showed substantial qualitative and quantitative difference with respect to the target data (see figure 2.5).

An analysis of the best individual performance made it clear that during the second part of the experiment the system required a positive input reaching the mesocortical module of the VTA and a negative one reaching the mesolimbic module of the same system. The timing of these two missing signals is the same and – taken for granted the biological constraint that the cortex has the role of guidance of the described dynamics during stress coping – both signals must directly or indirectly originate from the change of activity characterising the vmPFC (see figure 3.5c,d).

The literature provides evidence that this new neural system may be represented by a channel of information starting from the Hypothalamus (Hyp) and periaqueductal gray (PAG) and reaching the VTA via Dorsal Raphe (DR): the latter system is controlled by the activity of the vmPFC (Peyron et al., 1998;

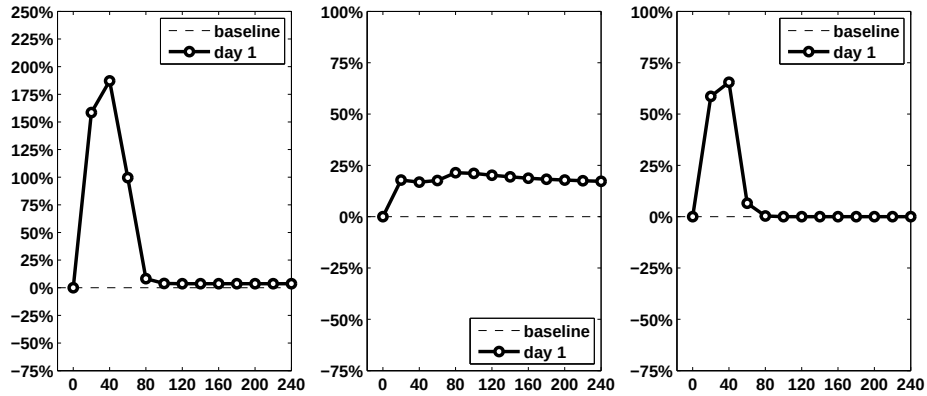

Figure 2.5: *Simulation of the releases of neuromodulators (NE in vmPFC, left; DA in vmPFC, centre; DA in NAcc, right) recorded during a test performed on "naive" agents using the early version of the model (which does not have the Hyp/PAG/DR component). Note the differences in the right parts of the graphs, when comparing them with the target data (figure 2.3 and 2.2).*

Radley et al., 2009) and it is known to have an important role in experiments concerning the lack of control of aversive stimuli (Amat et al., 2005). For the purpose of this model, these systems can be considered as if they were a single channel (Hyp/PAG/DR) because they both concur to the activity of the VTA with the same dynamics and timing (Bandler et al., 2000; Geisler et al., 2007). The neural architecture of the model was then modified accordingly and the GA could be run again with few additional parameters.

This time the GA proved to be successful (see fig. 2.6, validating the hypotheses underlying the model, and providing a useful tool for producing new predictions to be empirically tested. The fitness (mean square error) reached by the best individuals of the two models are slightly different: the first model (without the Hyp/PAG/DR component) reached a fitness of 25.2576, whereas the second one reached a fitness of 22.2543 (with a difference of about 13.45%).

Still, considering the high variances found in the biological microdialyses, the rough information provided by the fitness value is not sufficient to establish a strong preference between the two models concerning their biological accuracy. However, the qualitative comparison between the two model clearly speaks in favor of the second model and against the first one: the target dynamics and the slopes which have been considered as important by the biologists are clearly

replicated by the second model but not by the first one (see in particular the dynamics characterising the second part of the experiment -passive coping phase- in figures 2.3 and 2.2).

This whole process of weak falsification and validation implies that the assumptions underlying the second model, in contrast to those of the first one, are sufficient for explaining the available empirical data, and can thus be plausibly considered as representing the mechanisms that take place in the brains of real rats in conditions of long-lasting, inescapable stressful conditions. Hence, the model can now be used for producing new predictions to be tested in novel empirical experiments.

2.5 Results and predictions

Once the model manages to successfully replicate the target data, the causal processes taking place in the simulated agent can be considered a reliable simplification of the actual functioning taking place in real rats during the target experiment. Thus, the established analogy between the model and the actual brain of the rat allows drawing several important conclusions about the causal processes realising the appraisal.

The model pushes forward the hypothesis that the evaluation of the stressor controllability is a consequence of the interaction between IL and PL, which allows detecting discrepancies between actual and expected outcomes associated to performed or attempted actions. Initially, restrained rats execute actions belonging to their repertoire to try to actively cope with the new stressing condition, pursuing the goal to put an end to it. During this phase, the expected action-outcome association is provided by the PL, whilst IL provides the ”mismatch control” being activated by the presence of significant differences between the actual result of the attempted action and the expected one (Balleine and Dickinson, 1998; Coutureau and Killcross, 2003; Killcross and Coutureau, 2003).

This control is performed via a direct inhibitory effect IL has on PL and via a broad opposing influence these cortices have on various sub-cortical areas: the

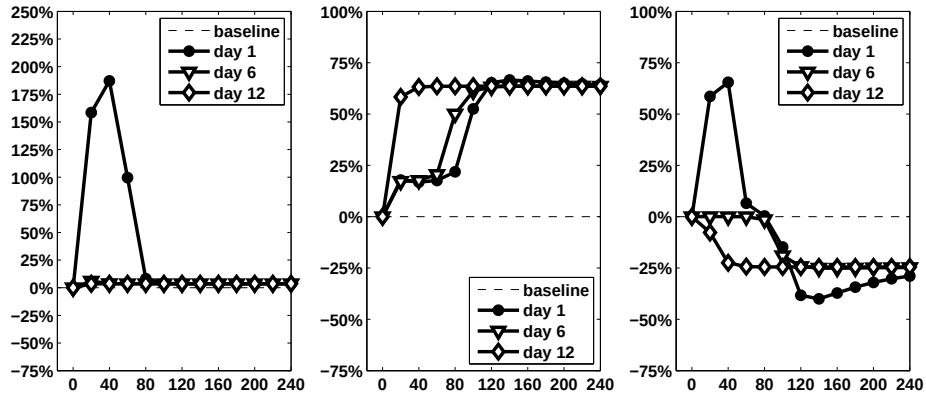

Figure 2.6: *Simulation of the releases of neuromodulators (NE in vmPFC, left; DA in vmPFC, centre; DA in NAcc, right) recorded during a test performed on "naive" and "repeatedly stress exposed" agents using the final version of the model (characterised by the presence of the Hyp/PAG/DR neural area). Note all "day 1" dynamics, which almost perfectly match the target data and "day 6" DA in NAcc dynamic, which matches its target and establishes a prediction (time series of target data lasts 120 minutes, which is half of the simulated time). All other simulated releases (day 6 and day 12) represent the model prediction concerning releases of the neuromodulators.*

more the action-outcome associations fail, the more IL increases its inhibition (in the model, via anti-Hebbian learning): the progressive suppression of PL output is the neural correlate signalling the fact that every action belonging the rat repertoire is failing in removing the stressor. When PL is completely inhibited, the desired outcome is no longer pursued because it is finally evaluated as beyond the possibilities of the agent: therefore, in the present experimental conditions, the stressor is perceived as uncontrollable.

The end of the process resulting in the appraisal of controllability coincides with the switch of coping in naive rats: the active coping, characterised by high DA in NAcc e high NE in cortex, is triggered by high neural activity in PL. As soon as PL is inhibited by IL, a cascade effect involving Amg and Hyp/PAG/DR eventually affect DA releases, causing the dynamics described for the passive coping.

It has been mentioned that after six repetitions of the restraint test (one test per day) sham rats exhibit a NAcc DA level which never goes above the baseline in the first phase and decreases below baseline after 60-80 mins (figure 2.3). At the same time the rats show no initial behavioural attempt to actively cope

with the stressing condition. The model supports the hypothesis that repeated experiences of the stressing test make rats learn that the stressing condition it is experiencing is not controllable: the appraisal of controllability is then easier because the cortical interaction between IL and PL has already partially or completely inhibited the association between actions and desired outcome in the recognised restrained conditions.

The model provides a specific explanation for the described phenomenon, ascribing the dynamic of DA in NAcc to the presence of NE in cortex: after 6 exposures, the partial inhibition of PL results in diminishing the input reaching the LC (directly from the cortex and indirectly via the Amg). As a consequence, LC is unable to release the initial high large amount of NE back in the cortex (figure 2.6, left), resulting in a furthered diminished activity in PL and -consequently- in Central Amg (CeA) and therefore, in the absence of the initial peak of DA generated by the mesolimbic area of the VTA.

This prediction is consistent with the known positive causal relationship between NE in vmPFC and DA in NAcc, which has been described using selective cortical depletion (Pascucci et al., 2007) and is part of the successfully simulated data gained using the present model (see chapter 3, sec. 3.3).

The simulation of the repeated experience has been secured strengthening the initial weight of the synapses bridging IL to PL: this choice simulates the fact that there has been already a learning process taking place between the two cortices, followed by a spontaneous partial recovery. Interestingly, the starting point is rather arbitrary and once found the parameter allowing the simulation of the data characterising 6 repeated exposition, it is possible to double that amount to have a hint of the results of a stressful experience repeated several more times (the values of 0, 1 or 2 have been used respectively for the day 1, day 6 and day 12 simulated test).

Simulating the case of day 12 of repeated exposures to the same stressor: the model predicts that the rats would show an immediate decrease of NAcc DA below baseline, starting the passive coping strategy as soon as the agent perceives the presence of the stressor. The mechanism realising this dynamic relies once

again on the inability of PL to activate the response of the LC (and the initial high release of NE), but this time activity in PL is not even sufficient to inhibit Hyp/PAG/DR which consequently almost immediately enhance the activity of the circuit involving the mesocortical DA (which reaches its maximum earlier than in any other test) and IL: this circuit indirectly inhibits the dopaminergic area of the mesolimbic VTA, thus determining the immediate passage to the second phase (corresponding to an immediate behavioural despair).

This complex interaction among several neural areas is consistent with both unpublished material concerning DA release in NAcc (experiments carried out by the same group providing the target data for this model) and with published data concerning serotoninergic release (related to DR activity) in a different set of experiment relying on uncontrollable stressors (Amat et al., 2005; Maier and Watkins, 2005, 2010).

Submitted to *The Journal of Neuroscience*. Authors: Vincenzo G. Fiore, Francesco Mannella, Simona Cabib, Marco Mirolli, Stefano Puglisi-Allegra, Gianluca Baldassarre

Chapter 3

Corticolimbic catecholamines in stress: A computational model

Abstract

The brain determines what is stressful and on this basis regulates physiological and behavioural adaptive responses. Converging evidence ascribes a major role to catecholamines in these processes: in particular, data show that tonic norepinephrine (NE) and dopamine (DA) outflows in the ventromedial prefrontal cortex (vmPFC) regulate DA outflow in the nucleus accumbens (NAcc). As frontal cortical areas are involved in the appraisal of environmental challenges, and DA transmission in the NAcc is involved in active and passive coping, the interplay between cortical NE and DA in the cortex and subcortical DA could translate the appraisal of the stressful experience into the motivational state required to deal with it adaptively. This paper proposes a computational system-level model of the brain mechanisms underlying these processes, grounding it on three key hypotheses: (a) vmPFC NE allows prelimbic cortex (PL) to guide active coping strategies and energizes these responses by enhancing NAcc DA outflow; (b) vmPFC DA allows infralimbic cortex (IL) to block active coping attempts, when these are unsuccessful, by decreasing NAcc DA levels below the baseline; (c) learning processes involving IL and PL lead to the transition between coping strategies. The model, whose architecture relies on known functional and structural connectivity of the brain areas involved, is validated by reproducing the fluctuations of target catecholamines measured in three conditions: sham, vmPFC NE depletion, and vmPFC DA depletion. The model represents the first integrated operational explanation of the investigated phenomena and produces predictions that can be tested in future empirical experiments.

3.1 Introduction

Stressful events (*stressors*) are experiences that an organism appraises as difficult to control or avoid by relying on its current repertoire or physiological, behavioural, and psychological reactions (Anisman and Matheson, 2005; Folkman et al., 1986; Huether et al., 1999; Lazarus, 1993; Ursin and Eriksen, 2004).

After a first primary appraisal, which leads to an event as being classified as challenging, the organism implements an active coping strategy based on suitable responses (Ganzel et al., 2010; McEwen, 2007). Through a secondary appraisal the organism establishes whether the stressor is controllable/avoidable through active coping strategies, or uncontrollable/unavoidable, thus requiring a shift toward a passive coping strategy aimed at saving energy and resources. Converging evidence suggests that stress appraisal processes involve the frontal cortices (Amat et al., 2005; Maier and Watkins, 2010; Ohira et al., 2008; Phan et al., 2004; Salomons et al., 2007; Wager et al., 2008).

The dynamics of cortical and limbic tonic amines play a key role in the brain reaction to stress (Amat et al., 2005; Bland et al., 2003; Cabib and Puglisi-Allegra, 1994; Cabib et al., 2002; Inglis and Moghaddam, 1999; Maier and Watkins, 2005; Pascucci et al., 2007; Puglisi-Allegra et al., 1991). During stressful experiences, increased tonic dopamine (DA) levels within nucleus accumbens (NAcc) are associated with the expression of active coping strategies aimed at removing or escaping the stressor (Cabib and Puglisi-Allegra, 1994; Cabib et al., 2002; Grappi et al., 2003; Mangiavacchi et al., 2001; Rada et al., 1998; Scornaiencki et al., 2009). Instead, decreased levels of tonic DA in NAcc are associated with the implementation of passive coping strategies (Imperato et al., 1993; Mangiavacchi et al., 2001; Pascucci et al., 2007; Pothos et al., 1995; Puglisi-Allegra et al., 1991; Rossetti et al., 1993). These observations are consistent with a widely shared view according to which tonic mesoaccumbens DA supports response vigor in pursuing costly goals (Cagniard et al., 2006; Floresco et al., 2008; Niv et al., 2007; Salamone et al., 2003).

In this paper we propose a system-level model that explains the slow dynamics of tonic catecholamines involved in the appraisal and coping of long-lasting, inescapable stressful situations, focussing on the brain mechanisms through which NE and DA levels in vmPFC regulate DA levels in NAcc. The main hypotheses pushed forward by the model are: (a) high NE in vmPFC allows prelimbic cortex (PL) to contribute to performing goal-directed behaviour and to increasing NAcc DA via its control over sub-cortical regions such as the amygdala; (b) high

DA in vmPFC allows infralimbic cortex (IL) to drive NAcc DA levels below baseline both via its connections to VTA and to various sub-cortical areas; (c) learning mechanisms leading IL to progressively inhibit PL cause the transition from active to passive coping strategies. The model represents the first integrated operational explanation of the investigated phenomena and can be used as a framework to produce predictions to be tested in empirical experiments and to build more detailed models, e.g. for investigating human pathologies such as depression.

3.2 Methods

3.2.1 The target data: microdialysis experiments in sham and vmPFC DA/NE depleted rats

The target experiments tested the causal relationship between stress-induced changes in NE and DA outflow in vmPFC on DA in NAcc (evaluated by intracerebral microdialysis) by means of a selective depletion of each catecholamine in the vmPFC through local infusion of a neurotoxin 6-hydroxydopamine (6-OHDA), following selective protection of NE or DA by peripheral administration of desipramine or GBR 12909 respectively (see Pascucci et al. 2007, for methodological details). Throughout the experiments rats were subjected to restraint, a common psychogenic stressor (Figueiredo et al., 2003).

Sham-depleted (Sham) animals showed the same pattern of cortical and subcortical stress responses as observed in non-manipulated rats (Pascucci et al., 2007). The immediate impact of the novel stressful experience promoted an increase of NE and DA in dialysate from the vmPFC that peaked between 20-40 min from stress onset and then declined. However, the increase in NE levels was much larger than the DA increase (35-40%) and once having declined to pre-stress (baseline) levels it stabilized and remained unchanged throughout the stressful experience. Instead, DA levels showed a second peak and stabilized to a plateau of 70% of baseline levels. Changes in DA levels in dialysate samples collected from NAcc were characterized by a peak increase within 20 minutes

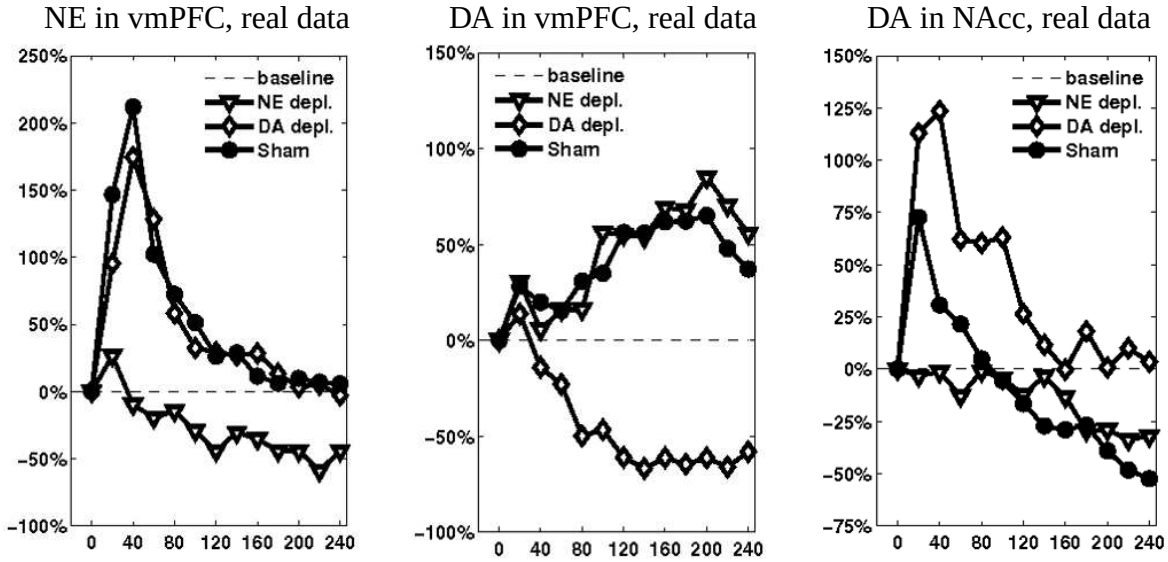

Figure 3.1: *Levels of NE (a) and DA (b) measured in the vmPFC, and DA measured in NAcc (c), during four hours of restraint experiment run in three different conditions: sham, depletion of vmPFC NE, and depletion of vmPFC DA. Modified from Pascucci et al. 2007*

from stress onset followed by a decline below basal levels that reached a plateau (-40% of baseline) by the end (240 minutes) of the stressful experience (figures 3.1a-c).

The depletion procedure did not influence baseline levels of catecholamine measured following 3 days of recovery from the surgery. Instead, depletion of each catecholamine in the vmPFC had specific effects on the cortical and sub-cortical stress responses. NE depletion selectively prevented stress-induced NE outflow in vmPFC and DA outflow in NAcc, whereas DA depletion selectively prevented the later large increase in cortical DA and the reduction of NAcc DA below baseline levels (Pascucci et al. 2007 and figures 3.1a-c).The experiments targeted with the model (Pascucci et al., 2007) involved rats that were restrained for four hours, a condition that is known to be highly stressful (Figueiredo et al., 2003). Figures 3.1a-c show the levels of NE in vmPFC, and of DA in vmPFC and Nacc - measured during the experiment through microdialysis - as percent changes with respect to the mean of three samples collected prior to the stressor.

These data demonstrate a positive causal relationship between NE in vmPFC and DA in NAcc and a negative causal relationship between DA in vmPFC and

DA in Nacc, in agreement with other results obtained with different stressors and methods (Cabib et al., 2002; Deutch et al., 1990; Doherty and Gratton, 1996; Scornaiencki et al., 2009; Stevenson and Gratton, 2003; Ventura et al., 2002).

Table 3.1: ***List of key references supporting the connectivity of the model***

DA targets in Nacc	(Carr and Sesack, 2000)
DA targets in vmPFC	(Briand et al., 2007) (Margolis et al., 2006) (Lammel et al., 2008)
NE targets in vmPFC	(Glavin, 1985) (Aston-Jones et al., 1999) (Briand et al., 2007) (Radley et al., 2008)
IL-PL relation	(Coutureau and Killcross, 2003) (Vertes, 2006)
vmPFC regulation of the VTA	(Carr and Sesack, 2000)
vmPFC control over the Hyp/PAG/DR	(Radley et al., 2009) (Vertes, 2006)
vmPFC differential control over CeA and ITC in the Amg	(Vidal-Gonzalez et al., 2006) (Vertes, 2006) (Peters et al., 2009)
the Hyp-DR channel towards the mescortical VTA	(Geisler et al., 2007)
CeA control over the mesolimbic VTA	(Wallace et al., 1992) (Ahn and Phillips, 2002) (Floresco et al., 2003) (Grace et al., 2007)
OFC-ACC regulation of the LC	(Aston Jones and Cohen, 2005)
CeA regulation of the LC	(Berridge and Waterhouse, 2003) (Curtis et al., 2002)

3.2.2 The biology behind the model

The explanation of the phenomena and target data presented in the previous section called for the design and implementation of a system-level model involving a rather large number of neural systems and two neuromodulators. Indeed, the initial analysis of the relevant neuroscientific literature suggested that the dynamics

of NAcc DA and vmPFC NE/DA in stressing situations arise out of the interaction among several different brain areas rather than from specific processes occurring in isolated areas.

Figure 3.2 shows the functional components of the model and the main relationships among them. The detailed circuits of the model are shown in figure 3.3.

The appraisal of a stressful situation is based on the available information about the external environment and the organism's physiological and psychological state (Folkman et al., 1986; Lazarus, 1993). Information about the stressful condition has four different targets in the model. The first is the orbitofrontal cortex (OFC) and the anterior cingulate cortex (ACC), involved in emotional appraisal and stress perception (Pruessner et al., 2008). The second is the vmPFC, involved in the modulation of classic stress responses (Diorio et al., 1993; Radley et al., 2006; Sullivan and Gratton, 2002; Tavares et al., 2009), based in particular on its role in the performance of goal-directed behaviour and habitual response regulation (Balleine and Dickinson, 1998; Coutureau and Killcross, 2003; Killcross and Coutureau, 2003). The third is the central nucleus of amygdala (CeA), which is involved in emotional and behavioural stress responses (Koob, 2009) and is also responsible for the regulation of various neuromodulatory systems in stressful conditions (Davis and Whalen, 2001). The fourth and last is a group of brain areas classically associated with physiological and behavioural (especially innate) responses to stressors, namely the hypothalamus (Hyp), periaqueductal gray (PAG), and dorsal raphe nucleus (DR; Herman et al. 2005; Keay and Bandler 2001; Maier and Watkins 2005).

Convergent empirical evidence supports the idea that PL and IL cortices play a key role in stress coping. First, it has been demonstrated that PL activation constrains, whereas IL activation facilitates, classic physiological stress responses (Diorio et al., 1993; Radley et al., 2006; Sullivan and Gratton, 2002; Tavares et al., 2009). Second, PL and IL play opposite roles in fear reactions, with PL enhancing and IL inhibiting them (Peters et al., 2009; Sotres-Bayon and Quirk, 2010; Vidal-Gonzalez et al., 2006). Third, PL is involved in action-

outcome learning and goal-directed behaviour expression whereas IL is involved in switching to a stimulus-response behavioural mode (Balleine and Dickinson, 1998; Coutureau and Killcross, 2003; Killcross and Coutureau, 2003). Finally, IL and PL are richly interconnected and most of their opposing influences on behavioural and physiological responses involve these connections (Vertes, 2004, 2006). The model is based on the following hypotheses: PL plays a key role in the expression of goal-directed behaviour after the primary appraisal and controls input processing in various sub-cortical areas; PL-IL interplay contributes in implementing the second appraisal which leads to the second phase; IL control over PL and various sub-cortical areas during the second phase is responsible for the shift to passive coping.

Stress-induced changes in DA levels within vmPFC and NAcc are mainly caused by the ventral tegmental area (VTA) projecting cells (Abercrombie et al., 1989; Barrot et al., 1999, 2000; Inglis and Moghaddam, 1999; Kalivas and Duffy, 1995). vmPFC and NAcc receive DA afferents from different populations of VTA DA cells and these are controlled by different and largely independent circuits (Briand et al., 2007; Carr and Sesack, 2000; Lammel et al., 2008; Margolis et al., 2006). In the model, these two different VTA populations (respectively called mesocortical VTA -mcVTA- and mesolimbic VTA -mlVTA-, see figure 3.2), play a key role in the decoupled dynamics of vmPFC DA and NAcc DA levels measured in the target experiments (see below). Stress-induced changes of NAcc DA levels are slow and detectable by intracerebral microdialysis (Cabib and Puglisi-Allegra 2011 for review), which suggests that they depend on tonic or population firing of VTA dopaminergic neurons (Floresco et al., 2003; Grace et al., 2007). VTA also receives afferents from the central nucleus of amygdala (CeA): the inhibition of CeA, and hence of its inhibitory input to VTA, leads to an increase of NAcc DA (Ahn and Phillips, 2003), suggesting that this input is part of a double inhibition mechanism (cf. also Floresco et al. 2003; Grace et al. 2007). For these reasons, the model hypothesises that stressors increase NAcc DA levels via a double inhibition mechanism involving CeA and driven by both direct stimuli from the environment and a strong modulation from vmPFC. These

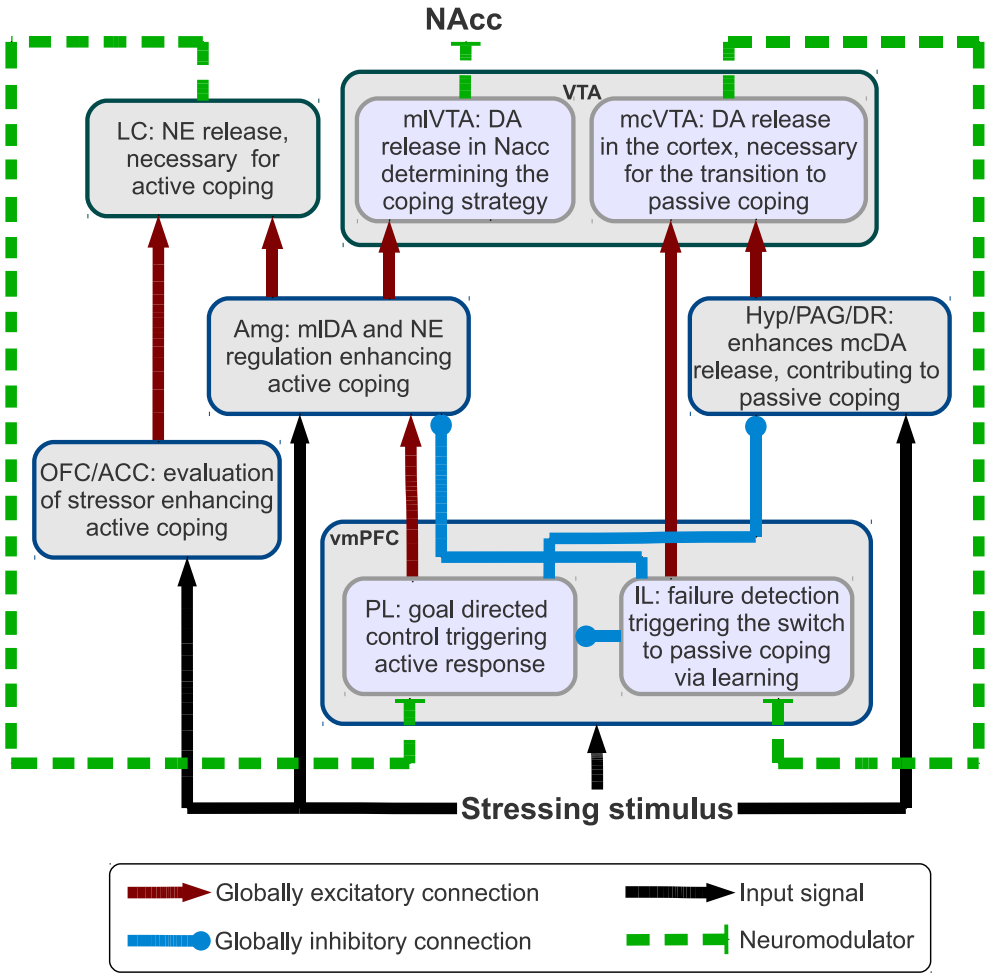

Figure 3.2: *Functional representation of the architecture of the model. This simplified representation shows the net excitatory/inhibitory influence that each component has on the target components, and not the nature of the specific neural pathways connecting them. The text in the boxes indicates the main functional role that the components contribute to implementing in the model with respect to the appraisal of stimuli and the consequent stress responses.*

processes are functionally summarised in figure 3.2, which shows a global excitatory effect of Amg activation on mlVTA activation and an excitatory effect exerted by vmPFC on Amg (the weak effect of PL on VTA is omitted from figure 3.2 for clarity; see figure 3.3 for the detailed circuits).

Stress promotes an increase in tonic NE levels in the vmPFC: this increment is due to the vastly diffused efferences originating from the relatively small group of cells of locus coeruleus (LC; Aston-Jones et al. 1999; Berridge and Waterhouse 2003; Glavin 1985; Valentino and Van Bockstaele 2001). LC receives strong convergent projections from the OFC and the ACC, which have been sug-

gested to drive transitions between phasic and tonic modes in NE neurons to fit the behavioral/cognitive states with environmental conditions (Aston Jones and Cohen, 2005). LC activity is also modulated by CeA (Curtis et al., 2002) via a significant innervation of the pericoerulear region (Berridge and Waterhouse, 2003) and through the excitatory corticotropin-releasing hormone (CRH; Bouret et al. 2003; Jedema and Grace 2004; Van Bockstaele et al. 2001).

Tonic neuromodulator releases involve receptors that are differentially located among the layers of the cortex, so that the same neuromodulator may differently affect its target subregions depending on the receptors it activates. For example, the NE has different effects on target cortical areas depending on its concentration and on the distribution of alpha1 and alpha2 receptors (Arnsten, 2009; Briand et al., 2007). Based on these possible differential effects that NE can cause in different target areas, the model hypothesises that NE increases the activation of the PL neural population connected to Amg and Hyp/PAG/DR (thus increasing DA release in NAcc) and inhibits the activation of the PL neural population connected to VTA (see figure 3.3).

Cortical processes also influence how CeA contributes to regulating the activation of VTA dopaminergic neurons (Everitt et al., 2000, 1999; Jalabert et al., 2009; Wallace et al., 1992). Specific connections between the PL and the IL, and their different targets within CeA, support opposite modulation of CeA output neurons by vmPFC: in particular, PL activation excites CeA output neurons, whereas IL activation inhibits them through the activation of GABAergic-neuron intercalated nuclei (ITC) of Amg (Peters et al., 2009; Vidal-Gonzalez et al., 2006). Finally, PL and IL show significant differences in their efferent connections towards Hyp, PAG, and DR: in the model these areas have been considered together both for lack of data concerning their activity (in this specific kind of long lasting inescapable stress experiments) and also because they tend to react with coherent timing (Keay and Bandler, 2001), also resulting in similar effects on VTA dopaminergic release (Geisler et al., 2007). PL has a predominantly inhibitory effect on this combined area Hyp/PAG/DR (Radley et al., 2009) diminishing its capacity to react to stressors and therefore the likelihood

of affecting DA release. The model assumes that Hyp/PAG/DR affect the system during the second phase, when the inhibitory effect of the PL ceases: in particular, according to the model Hyp/PAG/DR target the mesocortical VTA system and are responsible for the large increase in DA levels in vmPFC during the second phase of the experiments (passive coping).

3.2.3 The dynamics of stress responses

The functioning of the model, in particular during the two phases of the target experiments, is illustrated in figure 3.3. In the initial phase of the experiment (figure 3.3a), the stressor leads to a strong activation of the PL and this putatively corresponds to the implementation of an active coping/problem solving behavioural strategy. PL activation fosters high tonic cortical NE levels through excitation of CeA inputs to LC, resulting in a general enhancement of arousal and the processes supporting problem-solving and goal-directed behaviour (OFC/ACC contribute to activate LC as they evaluate the situation as stressful). The activation of the PL also constrains the levels of tonic cortical DA that would be caused by Hyp/PAG/DR responses to stress via their influence on mcVTA. Via CeA, the self-feeding circuit involving PL-Amg-LC is able to offset the endogenous activity of GABAergic neurons within the mlVTA and their activation by vmPFC. Eventually, this circuit results in the removal of the inhibition of a population of mesoaccumbens DA neurons that leads to a high efflux of DA into Nacc, which in turn is thought to energise the active coping response to stress.

The persistent input from the stressor, due to the failure of active coping attempts (uncontrollability), in the model triggers a learning process which strengthens the inhibitory connections between the PL inter-neuron population, activated by the IL, and PL output neurons (see figure 3.3). This process is assumed to correspond to the progressive inhibition, by IL, of all active behaviours that fail to produce the desired outcome, i.e. the removal of stress. As a result of this learning mechanism, the activity of PL output neurons slowly decreases, triggering a cascade of processes that start the transition to passive-coping (second phase).

In particular, the progressive inhibition of PL by IL (figure 3.3b) reduces PL excitation of CeA and hence causes a return of vmPFC NE to pre-stress levels. Moreover, it removes PL inhibition of Hyp/PAG/DR and these areas consequently start to excite mcVTA, thus causing a significant increase in tonic vmPFC DA. The enhanced activity of IL resulting from increased DA levels speeds up the learning process within the vmPFC; moreover, it increases the activity of the ITC, thereby further suppressing the activity of CeA. Furthermore, inputs from IL excite GABAergic interneuron populations within the mlVTA, which are no longer inhibited by the CeA. For this reason mesoaccumbens DA neurons are strongly inhibited, which causes Nacc DA levels to drop below baseline (a condition which is known to correlate with a passive coping strategy).

3.2.4 The computational mechanisms used to implement the model

The need to build a system-level model, and at the same time to keep the explanation of the target phenomena at a reasonably simple level, led us to simplify and abstract as many details as possible when not central for the explanation of the phenomena of interest.

The model was constrained at three main levels: its macro-architecture, the functioning of its components and its overall functioning. The macro-architecture of the model was fully constrained using relevant data from neuro-anatomy (see table 3.1). The functioning of the model components and the effects produced on them by the neuromodulators was constrained on the basis of biologically-plausible dynamical equations; whereas the representation of the inescapable stressor was simplified with a single input signal entering the model at the beginning of the simulated experiment (i.e. after 20 minutes without any input) and remaining stable for the rest of the time. With respect to its overall functioning, the model was thus constrained by requiring it to reproduce the dynamics of DA and NE in vmPFC, and the dynamic of DA in the Nacc, in the three different conditions reported in Pascucci et al. (2007).

Due to the slow dynamics of the target data and the importance of neural population dynamics, standard leaky neural units (Dayan and Abbott, 2001) were

used as building blocks, so that the dynamics of each unit of the model represent the activity of a whole population of real neurons (e.g., measurable with a mean field potential recording, Bojak et al. 2003, rather than with a single cell recording):

$$\begin{aligned} \tau_j \cdot \dot{u_j} =& - u_j + b_j + \sum_i [w_{ji} \cdot a_i] \\ a_j =& [tanh[u_j]]^+ \end{aligned} \tag{3.1}$$

where τ_j is the time constant of the unit *j*, u_j and a_j are respectively the action potential and the activation of unit *j*, b_j is the baseline activation of the unit, w_{ji} is the synaptic strength of the connection between unit *i* and unit *j* (this can be either excitatory or inhibitory), $\dot{u}$ is the derivative of *u* in time, $[x]^+$ is a function returning its argument if this is positive and zero if it is negative, and tanh[x] is the hyperbolic tangent function.

An important feature of the model is the simulation of the slow accumulation and reuptake of the neuromodulators in the extrasynaptic space of target areas, and the multiplicative/additive effects they have on such areas. The accumulation and reuptake mechanisms of neuromodulators are simulated through the following equation (one for each different target area):

$$\tau_{nk} \cdot l_{nk} = - (th_{nk} \cdot tanh[l_{nk}]) + ((1 - d_{nk}) \cdot w_{nk} \cdot a_n) \tag{3.2}$$

where l_{nk} represents the level of the neuromodulator *n* in the extrasynaptic space of the target area *k*, τ_{nk} is a time constant regulating the speed of the dynamics of this neuromodulator, w_{nk} is the strength of the neuromodulatory connections linking the unit a_n, which produces the neuromodulator *n*, to the target area *k*; th_{nk} is the *n* neuromodulator reuptake capacity of the target area *k*: this implies that when the level of the neuromodulator l_{nk} drops below a threshold representing the overall reuptake capacity of the system, the injection of the neuromodulator $(w_{nk} \cdot a_n)$ and its reuptake $(-th_{nk} \cdot tanh[l_{nk}])$ compensate and l_{nk}

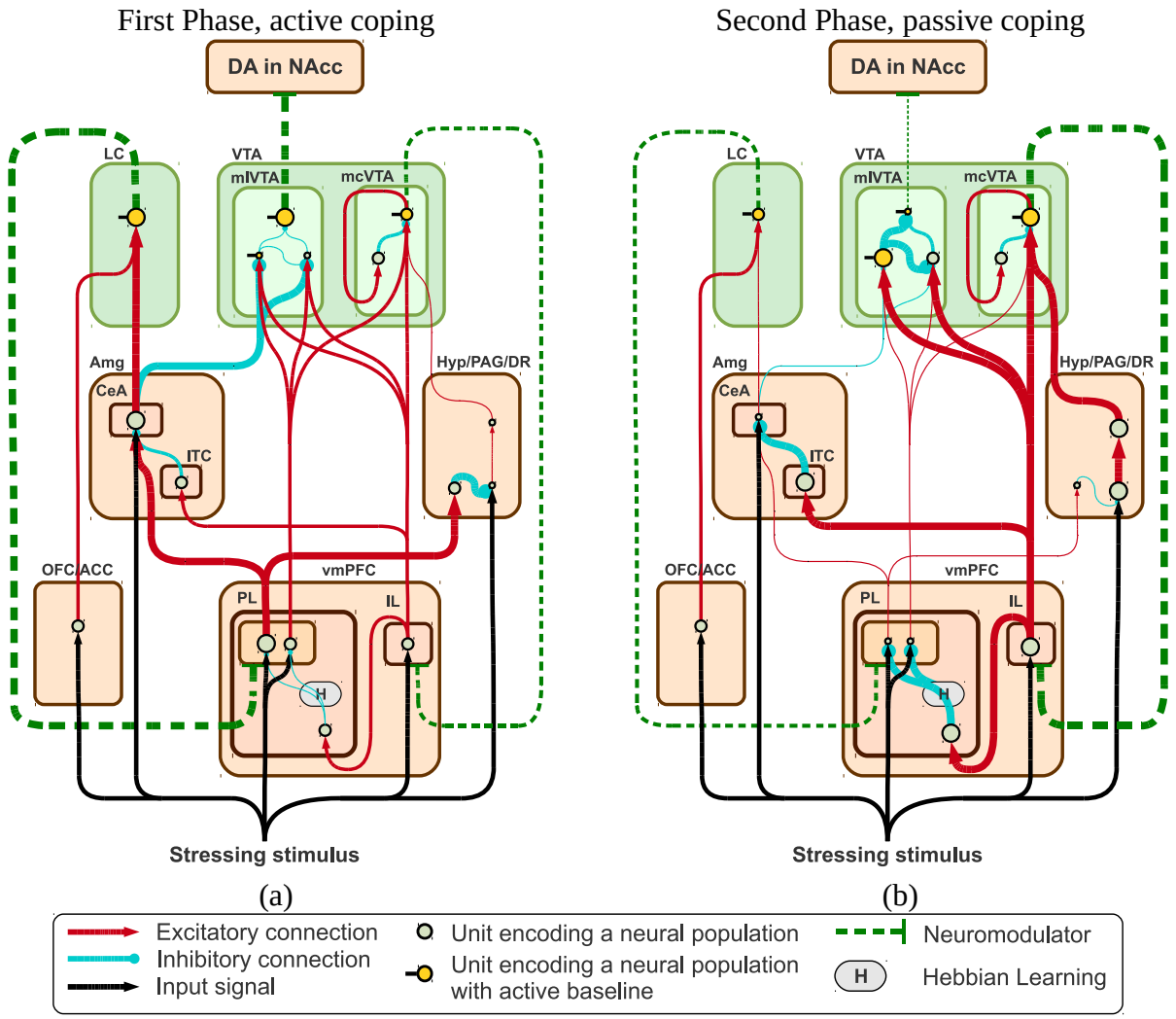

Figure 3.3: *Neural architecture of the model showing its components and sub-components (rounded square areas), their neural assemblies (circles), and their connections (links). The size of circles and links respectively encode the level of activity of neural assemblies and the strength of the signals transmitted between them during the first phase (a) and second phase (b) of the experiment in the sham condition.*

reaches an equilibrium (the higher the injection rate, the higher this equilibrium); conversely, when it exceeds this threshold the level of the neuromodulator starts to increase progressively (see Fellous and Linster 1998 for alternative ways of modelling these phenomena).

To perform the simulated depletions, it is important to reproduce the slow dynamics exhibited by the target neuromodulator observed in real experiments (figure 3.1a-c). Therefore, we introduced a slow dynamics affecting the variable d_{nk} in formula 3.2 (*n* is the neuromodulator, *k* is area targeted by the neuromodu-

lator). In simulated sham rats d_{nk} was set to zero, so it did not alter the dynamics of the neuromodulator. Instead, in experiments simulating the depletions of either DA or NE in vmPFC this variable was set to a value in the range [0, 1] so that the injection of the neuromodulator into the target area was suitably lowered. In particular, d_{nk} was regulated progressively towards the desired level d'_{nk} (set to 1 at the moment of the depletion) according to the following equation:

$$\tau_{d_{nt}} \cdot d_{nt} = - d_{nt} + d'_{nt} \tag{3.3}$$

The model also simulates the *additive* and *multiplicative* effects that the neuromodulators produce on the target neuron populations (effects respectively based on the passive channels K+, Na+, and Ca++, and the active channels AMPAR and NDMAR: Fellous and Linster 1998). In this respect, the equation of formula 3.1 relative to the computation of the activation potential u_j of the model units was modified as follows to reproduce the NE and DA effects:

$$\begin{aligned} \tau_j \cdot \dot{u}_j = -u_j + \frac{1 + \sum[\mu_{elk} \cdot l_k]}{1 + \sum[\mu_{dlk} \cdot l_k]} \cdot (b_j + \sum_i [w_{ji} \cdot a_i]) + \\ + \sum[\alpha_{elk} \cdot l_k] - \sum[\alpha_{dlk} \cdot l_k] \end{aligned} \tag{3.4}$$

where the coefficients μ_{elk} and α_{elk} respectively regulate the *multiplicative excitatory* and *additive excitatory* effects of the neuromodulator *l* on target area *k*, whereas the coefficients μ_{dlk} and α_{dlk} respectively regulate the *multiplicative inhibitory* and *additive inhibitory* effects of the neuromodulator *l* on the same area. Note that both the multiplicative and additive effects of the neuromodulators leave the signals unaltered if the level of the neuromodulators is zero. Moreover, the multiplicative effects depend on the size of the local glutammaergic/GABAergic signals, whereas the additive ones are independent of them. It should also be noted that the same neuromodulator may have either excitatory or depressive effects on different target areas depending on the distribution of its specific receptors: for instance, in the model NE is assumed

to have a multiplicative and additive excitatory effect on the vmPFC population of neurons connected to the Amg ($\mu_{eNEvmPFC} > 0$, $\alpha_{eNEvmPFC} > 0$, $\mu_{dNEvmPFC} = 0$, $\alpha_{dNEvmPFC} = 0$) and a multiplicative and additive depressive effect on the vmPFC population of neurons connected to VTA ($\mu_{eNEvmPFC} = 0$, $\alpha_{eNEvmPFC} = 0$, $\mu_{dNEvmPFC} > 0$, $\alpha_{dNEvmPFC} < 0$).

Finally, the Hebbian learning processes leading to the increase in the strength of internal connections of vmPFC are implemented using the following learning rule:

$$w_{ji}[t] = w_{ji}[t-1] + \eta \cdot [a_j - th_j]^+ \cdot [a_i - th_i]^+ \tag{3.5}$$

where w_{ji} is the connection weight between unit *i* and unit *j*, η is a learning rate, and th_j and th_i are the thresholds that the activations of a_j and a_i of the two units have to overcome in order to trigger the learning process.

The model has been implemented in MatlabTM and the equations of the model were integrated with the Euler method with a time step of 10 secs. This long time step afforded fast simulations and at the same time results that were still accurate given the very slow dynamics of the target phenomena.

The parameters of the model were found using a non-linear regression method where the data to fit were those collected by microdialysis and reported in figure 3.1a-c. The regression method used is based on a genetic algorithm that searches the parameters to minimise the average quadratic error between these data and those reproduced by the model. Genetic algorithms represent powerful non-linear regression methods that can be used with very complex non-linear models, such as the one used here, where it is difficult or even impossible to analytically derive the parameters of the model from the target data (Gulsen et al., 1995; Kapanoglu et al., 2007; Vander Noot and Abrahams, 1998). An important decision regarding the target data is whether to consider all the points of the time series as equally important or to require different degrees of accuracy for different sub-sets of data. In our case, the error related to the curves describing the DA release in NAcc was considered particularly important, so we assigned a weight

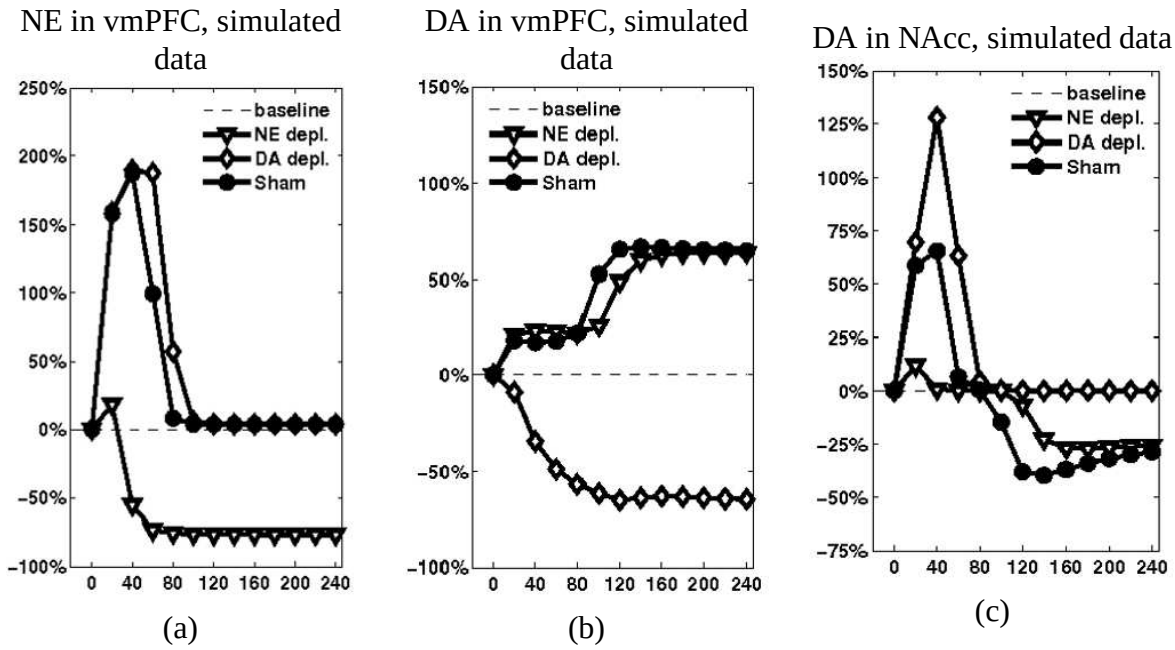

Figure 3.4: *Simulations of the releases of the neuromodulators -cortical NE (a), cortical DA (b) and mesolimbic DA (c)- recorded in the three conditions. Note the stressing stimulus is presented to the system after 20 minutes of time simulation, in order to reach a starting equilibrium point, given the basal activity of the neuromodulators.*

of 10 to the error in them whereas all other data points were assigned a weight of 1.

3.3 Results

3.3.1 Simulation of target data from microdialysis measurements

Figures 3.4a-c presents the dynamics of the neuromodulators produced by the model with the parameters found by the genetic algorithm (shown in table A.2) when it was used to simulate the sham, NE depleted, and DA depleted conditions of the target experiment.

The comparison between real (figure 3.1a-c) and simulated (figure 3.4a-c) data shows a substantial match, indicating that the assumptions and hypotheses implemented in the model are computationally sound and sufficient to reproduce the target phenomena. First, the model reproduces the main catecholamine dynamics in the sham condition: an initial high level of vmPFC NE followed by a return to baseline; an initial moderate increase in vmPFC DA followed by an

even higher increase; a consequent NAcc DA level that first rises above baseline and then drops below it. Moreover, the model reproduces the main features of the neuromodulator dynamics in the case of both NE or DA depletion in vmPFC: in particular, the fact that cortical NE is necessary for the initial high level of NAcc DA whereas cortical DA is necessary for NAcc DA to fall below baseline during the second phase of the experiement.

Figures 3.5a-d show the activation of four units of the model (representing neural populations) during the simulations of the three conditions of the experiment: PL output population directed to Amg/VTA; CeA output population; IL output population; and Hyp/PAG/DR output population. These activations help to understand how, in the model, the brain areas corresponding to these components act in concert to produce the catecholamine dynamics described above. The sham condition shows that PL and Amg (figures 3.5c and 3.5a, respectively) are mainly activated during the first phase of the test to support goal-directed/problem solving processes underlying active coping. Even IL (figure 3.5d) has a relatively high activation during this phase. These processes cause, and are supported by, a high level of vmPFC NE. The final outcome of all these processes is the increase in NAcc DA. During the second phase IL activity further increases, which results in the inhibition of PL and CeA and in the activation of Hyp/PAG/DR (figure 3.5b). These processes cause, and are supported by, a high level of vmPFC DA. The final outcome of all these processes is the decrease of DA NAcc below baseline.

The vmPFC NE depletion causes a loss of about 10% in the peak response of PL in the first phase, and an anticipation of its decrease of about 20 mins (figure 3.5c). This lower activity propagates to CeA (figure 3.5a), which is no longer able to offset the inhibitory effects in the mlVTA caused by GABAergic interneuron populations. This is the main reason why, in the model, NE depletion in vmPFC prevents NAcc DA from increasing during the first phase. At the same time, PL lower activation slows down the IL-PL Hebbian learning processes resulting in a slightly delayed increase in the activations of IL (figure 3.5d) and of Hyp/PAG/DR (figure 3.5b), which in turn result in a slightly delayed decrease

below baseline of DA levels in NAcc.

The vmPFC DA depletion causes a major lower activation of the IL during the whole test (figure 3.5d). This slows down the IL-PL learning processes and the consequent decrease in PL output activation (figure 3.5c). The stronger and more persistent activity of the PL supports a higher activation of CeA (figure 3.5a) and again delays (even more than in the NE depletion condition) the activation of Hyp/PAG/DR (figure 3.5b). The lower IL activity due to the depletion of vmPFC DA prevents IL from having its inhibitory effect on mlVTA, and hence prevents NAcc DA from dropping below baseline.

3.3.2 Predictions

The main hypothesis implemented by the model is that the fluctuations of catecholamines in vmPFC and their role in the modulation of DA levels in NAcc critically depend on the interactions between the PL and the IL. Our model, which implements this hypothesis and which has been validated by reproducing the fluctuations of catecholamines observed in normal and lesioned animals, can be used to derive a number of empirical predictions. In particular, we simulated lesions to four different connections by setting those connections to zero (while leaving all other parameters of the model unchanged, i.e. as in table A.2): PL-Amg, PL-VTA, IL-Amg, and IL-VTA. The resulting dynamics of the NAcc DA, which represent the predictions of the model, are reported in figures 3.6a-d, where they are compared with the dynamics of NAcc DA in the sham condition.

The simulation of the lesion of PL-VTA connections reveals the importance of the globally inhibitory effect that PL exerts on NAcc DA levels: when these connections are removed, these levels are higher during both the first and the second phase, although the above/below -baseline features of the NAcc DA does not qualitatively change with respect to sham rats (figure 3.6a). The lesion of PL-Amg connections produces more interesting effects: first, NAcc DA remains at baseline during the first phase, and then decreases below baseline but after a delay with respect to the sham rats (figure 3.6b). These dynamics, similar to those obtained with the vmPFC NE depletion (figure 3.6c), reveal the significant

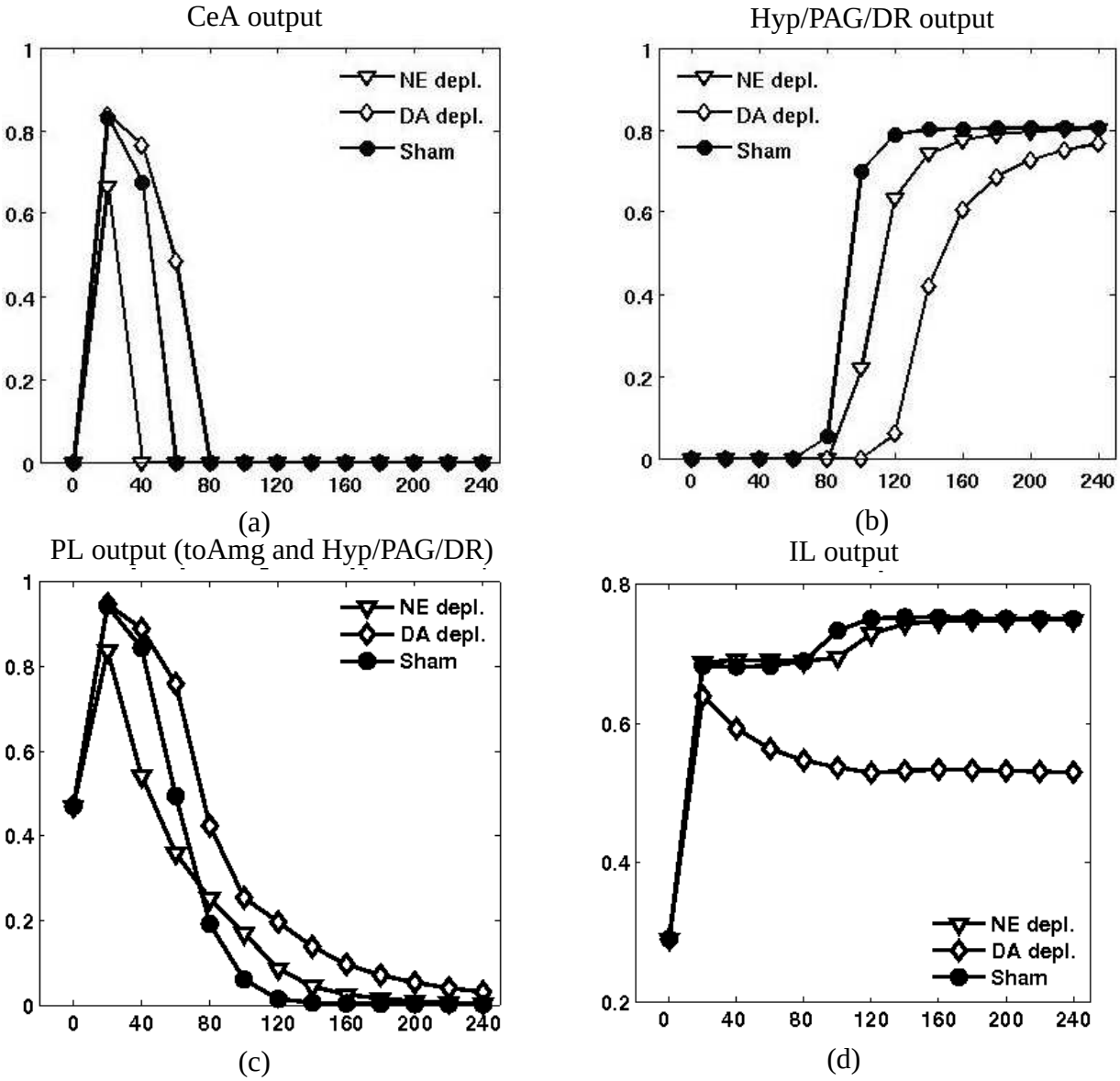

Figure 3.5: *Activities recorded in four different units of the model simulating the mean field neural activity of the corresponding neural populations of the rat brain. Note that the basal release of DA and NE affects the activation status of the cortices -graphs (c) and (d)- even before the stressing stimulus is presented (time 0), determining the starting equilibrium which is then affected by the stressor.*

influence that PL exerts on mlVTA via Amg, with the support of NE frontal levels. The lesion of IL-VTA connections produces a significant increase in NAcc DA during the first phase and a baseline level in the second part of the simulation, versus the below-baseline level of sham rats (figure 3.6c). These dynamics, similar to those recorded after the vmPFC DA depletion (figure 3.6c), reveal the

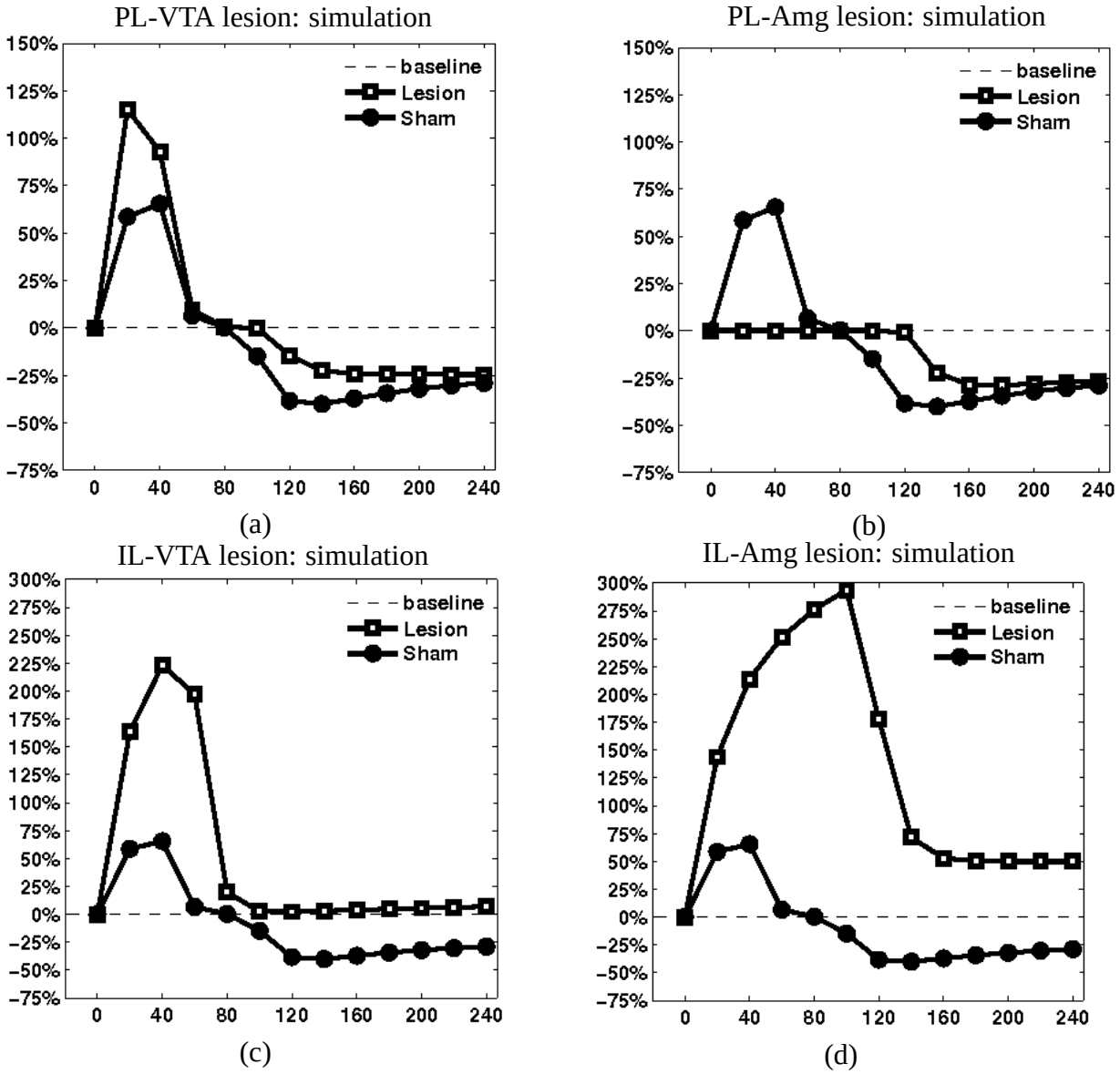

Figure 3.6: *Four predictions suggested by the model. The mesolimbic DA release was recorded after lesioning the model (blank square lines) and is compared with the known data characterising sham rats (filled circle lines). The lesions affect efferent projections of either PL or IL and their targeted area in the VTA and the Amg.*

key role that IL plays in controlling NAcc DA, with the support of frontal DA levels. The lesion of IL-Amg connections causes NAcc DA to have the greatest level of NAcc DA in the first phase, plus an above-baseline level even in the second phase (figure 3.6d). This clarifies the key role that IL inhibition of Amg plays in causing low levels of NAcc DA during passive coping.

These predictions may be falsified using combined contralateral lesions: for

practical reasons, this technique aims at isolating two neural regions via asymmetrical lesions of the areas, rather than directly involving the synapses bridging the areas themselves. Further, the final result is widely considered as similar and hence it can be used to test the present simulations, which have been carried out setting to 0 the connections involved (e.g. see Coutureau et al. 2009 for a PL-Amg lesion).

Chapter 4

Conclusions

This dissertation thesis presents a bio-constrained system-level model that reproduces and explains data obtained from experiments investigating catecholamine releases in rats forced to cope with long-lasting, unavoidable/inescapable stress, focussing on (1) how DA and NE levels in vmPFC control tonic levels of NAcc DA (Pascucci et al., 2007) and (2) the cause for the different NAcc DA release when the animal is repeatedly exposed to restraint (Cabib and Puglisi-Allegra, 1996). The experiment showed that the initial response to the stressor is characterized by an imbalance favouring NE over DA in vmPFC associated with high tonic levels of DA in NAcc. This phase is followed by a shift of the imbalance in favour of DA accompanied by a reduction of tonic NAcc DA below pre-stress levels. The experiment also supports the existence of a causal relationship between the cortical and the subcortical catecholaminergic concentrations as selective depletion of cortical NE or DA respectively eliminates the first-phase increase and the second phase decrease of NAcc DA.

The model proposes an explanation of the way in which the fluctuations in tonic levels of brain catecholamines support stress appraisal (primary and secondary) and hence, putatively, the motivational states suitable for supporting effective coping of novel stressors. In particular, the initial primary appraisal, marked by high NE levels in vmPFC, is directed toward recognising and evaluating the threat posed by the stressor (with the involvement of OFC/ACC), addressing it on the basis of reactive and goal-directed active-coping strategies (with the involvement of vmPFC, in particular PL, and Amg). This is in agreement with the general involvement of vmPFC with the control of hormonal and behavioural stress responses based on the catecholaminergic regulations occurring inside it (Cabib et al., 2002; Maier and Watkins, 2010; Scornaiencki et al.,

2009). In this respect, the high levels of cortical tonic NE may drive not only the general arousal of the system required to face the urgency of the situation (Aston-Jones et al., 1999; Berridge and Waterhouse, 2003), but also the shift to an exploratory/problem solving mode of operation (Aston Jones and Cohen, 2005) eventually triggered by the failure of the initial attempts to terminate/escape the stressing condition. At the same time, slightly above-baseline mesocortical dopamine levels in PFC might play an important adaptive role by preventing excessive behavioural and physiological stress reactivity (Sullivan, 2004). The high levels of NAcc DA resulting from the high activation of mlVTA, in turn caused by the activation of PL and Amg involved in the elaboration of the active coping strategy, possibly have the function of energising the preparation and implementation of actions as suggested by experiments showing that high levels of NAcc DA can support strenous and risky goal-directed responses (Salamone et al. 2007 see also Niv et al. 2007, for a model).

A key hypothesis of the model is that the persistence of the stressor might lead the IL to detect the failure of the active coping attempts and to progressively (learn to) inhibit them through the inhibition of PL and Amg. This hypothesis is consistent with the view that the extinction of no longer adaptive appetitive or aversive behaviours involves learning processes that lead to actively inhibiting, rather than forgetting, such behaviours (Quirk, 2002). Furthermore, it is consistent with data ascribing to IL a key role in these inhibitory processes and with the close neural interplay existing between IL and PL (Lebrón et al., 2004; Radley et al., 2006, 2008; Rhodes and Killcross, 2004; Van Aerde et al., 2008). These processes are suggested to lead to a shift to passive coping strategies based on a broad readjustment of the catecholamine levels and brain activation distribution. First, NE in vmPFC returns to pre-stress levels thus possibly diminishing arousal, attention to external events, goal-directed behaviours, and exploration of new solutions. Second, high levels of DA in PFC might enhance the processing of internal information vs. external stimuli and strengthen cognitive perseverance and internal focus (Cohen et al., 2002). Lastly, NAcc DA low levels possibly promote a decreased overt activity, in agreement with the experiments in

which inhibition of NAcc DA release can block this activity when the stressor is appraised as uncontrollable/unavoidable (Baldo and Kelley, 2007; Phillips et al., 2007; Ventura et al., 2002).

Genetic Algorithms have been used as regression tool to set the parameters of this complex bio-constrained neural model. The first chapter describes why the use of the genetic algorithm for searching model parameters can help the researcher not only in finding the set of parameters that optimise the correspondence between the model behavior and the target data, but also in defining the model architecture itself, hence defining the core hypotheses concerning the function of the model.

The model included at least three different neural mechanisms working at different time scales: electrical (activity of single units), chemical (dynamics of the neuromodulators), and pertaining to long term potentiation (learning in the vmPFC); the presence of these different types of neural interactions did not prevent the GA from finding the optimal parameters that were able to replicate the target data, it only resulted in an increase in the the time required for running the evolutionary search.

It is important to stress that the overall methodology is very flexible, in that it can be used for models that target any kind of empirical data. In the described case study the target data were time series of data representing the concentrations of different neuromodulators in different brain areas as measured by microdialyses in different conditions, but the same procedure (regression towards target data using weighted fitness) can be employed using any kind of quantitative target data, be they chemical, neural, or behavioral.

One important weakness of the proposed method is that the GAs requires choosing between several different sub-methods (mutation, crossover, selection etc.), each of which has its own meta-parameters to be set (population, generations, ranges etc.). Since no clear and accepted rule is available for making these decisions, the choice on the details of the genetic algorithm is rather arbitrary. This might seems to result in a switch of the problem of finding the correct parameters from the ones of the model to the ones of the GA. In practice the sit-

uation is much better than it appears. Indeed, even though it might be difficult to find the most *efficient* combination of methods and meta-parameters of the GA in each specific case, it is quite easy to find a combination that is satisfyingly *effective*: indeed the main problem in this cases is that good solutions take longer to be found. In fact, as the case study shown here has demonstrated, even using several default options of already-available software, like the Matlab© GA tool used here, can be enough for giving useful results.

Interestingly, the GAs have been used to set the parameters of the model so that it could replicate the specific set of data described in Pascucci et al. (2007), but the model also successfully replicates a different set coming from repeated exposure to restraint (Cabib and Puglisi-Allegra, 1996). This time, the recording only interested the DA release in the NAcc for 120 minutes, but the model managed to simulate all the three standard dynamics (NE and DA in vmPFC and DA in NAcc) for the whole 4 hours routine. This result is grounded on the assumption that each time the agent is subjected to the stressor, it spontaneously recovers at a certain pace: the daily repetition of the experiments results in accumulating the learned inhibition within the vmPFC and therefore in blocking the initial response. Eventually the model predict the repetition may also cause an immediate switch to the described second phase of tonic release, characterised by a depressive release of NAcc DA.

The model also produced four more predictions on the possible effects that lesions of PL and IL efferents reaching VTA and Amg would cause on the NAcc DA dynamics during restraint tests. In particular, a lesion of the PL-Amg connections would prevent NAcc DA from going above baseline levels in the first phase of the experiment; instead, a lesion of the IL-VTA connections would prevent NAcc DA from dropping below baseline levels during the second phase of the experiment. These predictions can be tested in experiments with real rats and the results would either support or falsify the two core hypotheses of the model for which (a) the stress-induced changes in tonic levels of vmPFC NE and DA that drive the NAcc DA accumulation preferentially involve PL and IL respectively, and (b) the main features of the dynamics itself is ultimately caused by

the strengthening of the IL inhibition towards PL and Amg.

Another possible empirical investigation that could help solve some unresolved aspects of the model concerns the involvement of Hyp/PAG/DR in driving the activation of mcVTA neurons during the passive coping phase. The relations between these and the other areas of the model are still not fully clear as there is little empirical evidence to narrow the targeted area in the VTA (i.e. there is evidence concerning DR-VTA connections, see Geisler et al. 2007, but not DR-mcVTA connections). In fact, it was the failure of the GA in finding optimal parameters for an early version of the model that led to the development of a second model, highlighting the necessity of relying on this specific connectivity bridging DR and mcVTA.

The comparison between the two models described in the case study exemplifies the process of (weak) falsification and validation of bio-constrained models made possible by the use of the GAs. Despite the fact that the GA does not search for the parameters through the whole parameter space, the guided search performed is still helpful in understanding the computational capabilities and limits characterising different models.

The fact that the first model lacks a specific signal reaching the VTA with the correct timing depends on the architecture of the neural system rather than on its parameters. Such conclusion might of course have been reached also by setting parameters through hand-tuning, but this would have required a much longer amount of time and the conclusion that an appropriate set of parameters does not exist would have be much less certain. Indeed, the latter conclusion can be demonstrated only after an exhaustive search in the parameter space which is practically unfeasible with many parameters as the required computational time grows exponentially with the number of parameters.

A possible way to shed more light on these issues, pursuing an in vivo process of validation/falsification, is to measure the levels of cortical serotonin (5-HT) during a restraint experiment: the increase of 5-HT during the second phase of the experiment would be consistent with an active involvement of DR in the DA regulations as hypothesised by the model. This outcome could also suggest the

existence of possible interesting relations between the mechanisms underlying stress coping and those underlying the outcomes of the experiments on learned helplessness, closely linked to the 5-HT system (Amat et al., 2005; Maier and Watkins, 2005, 2010).

The proposed model, which is the first computational integrated account of the target phenomena, could be developed in various directions in future work. First, it might be useful to investigate in greater detail the specific effects the catecholamines have on the several parts and layers of the vmPFC, bringing the model closer to the complexity of widespread projections of the real catecholamine systems. This would allow the important interactions existing between multiple neuromodulators targeting the same areas to be studied, in particular the frontal cortex (Briand et al., 2007). Second, it might be interesting to investigate the role played by the opioid system in stress coping as opioids have been shown to be involved in VTA regulation of vmPFC DA levels (Svingos et al., 2001). Finally, the hypotheses regarding the putative functional adaptive role of the various components, neuromodulators and processes of the model could be investigated in greater depth by implementing the details of the neural processes taking place in PL, IL, Amg, OFC-ACC, Hyp/PAG/DR. For example, the effects of NE and DA on goal-directed behaviour could be studied by implementing neural decision making mechanisms in PL (Gurney et al., 2001), the progressive inhibition of IL on PL might be further specified by implementing a neural action-failure detection mechanism within IL (Alexander and Brown, 2010), and the role of NAcc DA in energizing behaviour might be studied in greater depth by implementing a NAcc actually contributing to perform a simulated behaviour. An ”embodied” set-up, cf. Caligiore et al. 2010; Niv et al. 2007, could target experiments involving overt behaviour, e.g. a forced swimming test (Porsolt et al., 1977), possibly refining the model so as to produce more accurate predictions regarding the role played by NE, DA (and possibly 5-HT) in vmPFC and DA in NAcc and their correlation with the expression of overt and covert reactions to stress.

Appendix A

Tables

Table A.1: *All the acronyms used in this dissertation.*

Neuromodulators			
DA			Dopamine
GABA			Gamma-aminobutyric acid
Glu			Glutamate
NE			Noradrenaline/norepinephrine
5-HT			serotonin
Brain Areas			
Amg			Amygdala
	CeA		Central nucleus of amygdala
	ITC		Intercalated amygdaloid nuclei
DR			Dorsal raphe nucleus
Hyp			Hypothalamus
LC			Locus coeruleus
NAcc			Nucleus accumbens
OFC			Orbitofrontal cortex
PAG			Periaqueductal gray
PFC			Prefrontal cortex
	vmPFC		Ventromedial prefrontal cortex
		IL	Infralimbic cortex
		PL	Prelimbic Cortex
VTA			Ventral tegmental area
	mcVTA		meso-cortical ventral tegmental area
	mlVTA		meso-limbic ventral tegmental area

Table A.2: *Values found using GAs: parameters not specified have been set to 1; "inp", "int" and "out" respectively stand for input, interneuron and output population of a neural area.*

GLU (+) and GABA (-) connections		
Efferent	**Afferent**	**Value**
CeA	LC	0.3042
CeA	mlVTA-int1	-5
CeA	mlVTA-int2	-4.9267
Hyp/PAG/DR	mcVTA	0.6976
Hyp/PAG/DR-inp	Hyp/PAG/DR-out	1.4694
Hyp/PAG/DR-int	Hyp/PAG/DR-inp	-4.8477
IL	ITC	4.4910
IL	mcVTA	0.3541
IL	mlVTA-int1	3.9190
IL	mlVTA-int2	3.2051
Input	IL	0.4859
Input	OFC/ACC	0.5
ITC	CeA	-3.4307
mcVTA-int	mcVTA-output	-3.3512
mcVTA-output	mcVTA-input	1.6193
mlVTA-int1	mlVTA-output	-1.2221
mlVTA-int1	MlVTA-int2	-2.3243
mlVTA-int2	mlVTA-output	-1.5046
PL	CeA	3.8170
PL	mcVTA	0.2
PL	mlVTA-int1	2.6646
PL	mlVTA-int2	0.2
Neuromodulators: additive effects		
DA in PFC		0.3439
NE in PFC		0.3339
Neuromodulators: multiplicative effects		
DA in PFC		1
NE in PFC		1
Neuromodulators: baselines		
mcVTA-output baseline		1.9634
mlVTA-output baseline		1.7
mlVTA-int1 baseline		2
Leaky decays		
Neural units		30000
Neuromodulator accumulation		300000
Depletion decays		
Depletion decay of DA		3633600
Depletion decay of NE		1196480
Other parameters		
LC threshold		0.5
Learning rate in vmPFC		0.0064

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