MECHANISMS OF AMYGDALA FACILITATED CORTICO-STRIATAL PLASTICITY

by

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A Dissertation submitted to the Graduate School-Newark Rutgers, The State University of New Jersey in partial fulfillment of the requirements for the degree of:

Doctor of Philosophy

Graduate Program in Integrative Neuroscience

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Newark, New Jersey

January, 2010

ABSTRACT OF THE DISSERTATION

Mechanisms of amygdala facilitated cortico-striatal plasticity

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The amygdala is known to mediate the enhancing effects of emotional arousal on learning and memory. The increased firing rate of neurons in the basolateral complex of the amygdala (BLA) is believed to facilitate memory storage in various target structures, such as the striatum. Changes in the efficacy of cortical inputs to the striatum are thought to underlie motor learning and habit formation, making this pathway a perfect model to test the effects of BLA activity on synaptic plasticity and learning. My thesis provides evidence that BLA synapses have an N-methyl-D-aspartate receptor – to α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid receptor ratio higher than that of cortical synapses onto the same striatal neurons (Chapter 3). This property allows BLA inputs to facilitate the induction of heterosynaptic long term potentiation *in vitro* (Chapters 4 and 5). I also show that temporal coupling of BLA and striatal neurons *in vivo* occurs during coherent bursts of gamma activity observed in the local field potentials. Changes in the coherence of BLA-striatal gamma paralleled learning of a striatal-dependent task (Chapter 6). Together, these findings point towards a new mechanism of amygdala-facilitated learning.

Acknowledgements

I thank my advisor, Denis Paré for his patient and diligent mentoring during my Ph.D. training. I also thank my committee: Bart Krekelberg, David M. Lovinger, Farzan Nadim, and James M. Tepper, for their support, critiques and advice that helped produce this thesis and shaped my thinking. The experiences and interactions we had will stay with me, and help me throughout my career. My thanks also extend to my wife, Carla, for her understanding and continuous help.

Table of Contents

List of illustrations

List of abbreviations

aCSF	artificial cortico-spinal fluid
AMPA	α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid
APs	action potentials
BL	basolateral nucleus of the amygdala
BLA	basolateral complex of the amygdala
BM	basomedial nucleus of the amygdala
CE	central nucleus of the amygdala
CICR	Ca^{2+}-induced Ca^{2+}-release
CS	conditioned stimulus
CTX	cortex
EPSP / C	excitatory postsynaptic potential / current
GP	globus pallidus
im	Intra-muscular
LFP	local field potential
LTD	long-term depression
LTP	long-term potentiation
MSNs	medium spiny neurons
NMDA	N-methyl-D-aspartate
OFC	orbitofrontal cortex
PEH	peri-event histogram
RI	rhythmicity index
sc	subcutaneous
SEM	standard error of the mean
SN	substantia nigra
STN	subthalamic nucleus
THL	thalamus
US	unconditioned stimulus
VTA	ventral tegmental area

CHAPTER 1

Introduction

1.1) Background Information

There are many factors contributing to whether a particular experience is remembered or forgotten. One of the most obvious and perhaps most studied, is the level of emotional arousal surrounding the event. Quite often, remembering an episode from the past evokes an emotional response similar to that associated with the original event, and in many cases the initial level of emotional arousal predicts whether a certain experience will be later remembered or not.

The fact that emotional events are generally better remembered than neutral ones is well documented. Most studies find a positive correlation between the self-reported degree of emotional arousal when confronted with a certain item (picture, movie, story, etc.), and the recall of that particular item at a later date (Cahill et al., 1996; Canli et al., 2000; McGaugh, 2000; Phelps, 2006). Moreover, it was shown that presenting scenes with high emotional content improves the recall of previously seen, neutral images (Anderson et al., 2006). This lack of specificity suggests a very broad, non-specific mechanism, in which the effects of the overall emotional arousal extend even to events that did not directly contribute to the current emotional state.

In a search for the neuronal substrate of emotions, early work pinpointed the temporal lobe as a possible site. In 1939, Heinrich Klüver and Paul Bucy found that monkeys with bilateral lesions of the temporal lobe exhibited significant changes in behavior, becoming tame, fearless, and their emotions flattened. However, the changes were not specific; they also included a mixture

of inappropriate behaviors such as placing inedible objects in the mouth, exhibiting unusual sexual behavior, etc. While most of the behavioral changes mentioned above turned out to be the result of surgically removing other regions of the temporal lobe, amygdala lesions proved to be responsible for the emotional changes described by Klüver and Bucy (Weiskrantz, 1956). Subsequent anatomical tracing, electrical stimulation, and lesion studies established the amygdala as one of the key sites responsible for processing emotional responses.

Many lines of evidence support this: the amygdala is connected to several regions involved in the somatic and visceral expression of emotions (such as the hypothalamus and various brainstem nuclei), and receives inputs from areas dealing with conscious perception (e.g. the cingulate and prefrontal cortices) (Young et al., 1994). Electrical stimulation of the amygdala in humans evokes a mixture of conscious feelings of fear, sadness, happiness and anxiety (self-reported), as well as autonomic responses associated with emotions (e.g. changes in heart rate and skin conductance) (Lanteaume et al., 2007). Consistently, patients affected by Urbach-Wiethe disease (characterized by the selective, bilateral calcification of the amygdala) perform poorly when asked to judge facial expressions of emotions or to remember previously seen pictures with a high emotional content (Siebert et al., 2003).

In a search for the physiological mechanisms underlying these effects, many animal studies found that immediate post-training manipulations that

increase or decrease the firing rate of neurons in the basolateral nucleus of the amygdala (BLA), respectively enhance or impair memory consolidation (McGaugh, 2000; Pare, 2003; McGaugh, 2004). Thus, the current model is that BLA activity during, and in the 1-2 hours following emotional events is critically involved in the consolidation of memories. During this time, an increased BLA neuronal firing rate is believed to facilitate plasticity in other structures.

Despite the fact that we have learned much about the role of the amygdala in the modulation of emotional memories, many questions remain. How exactly does the amygdala influence memory? What types of memory can be enhanced by the amygdala? Is the amygdala part of the neural circuit storing memories, or of a parallel modulatory system? Does it influence areas that store memories directly, or via other structures?

As detailed in the following chapters, there is substantial evidence suggesting that the amygdala has the ability to enhance many types of memory. Synaptic plasticity in different pathways (believed to underlie changes in behavior and thus memory) is usually enhanced by increased amygdala activity, and this facilitation depends (in some cases) on acetylcholine release. Thus, it appears that emotional stimuli could enhance memory by increasing the firing rate of amygdala projection neurons, which in turn would lead to acetylcholine release in various structures (via activation of the basal forebrain) and facilitated plasticity.

However, this mechanism doesn’t explain how the amygdala can facilitate memories that depend on structures devoid of extrinsic cholinergic inputs (such

as the striatum). One possibility is that the widespread efferent connectivity of the basolateral nucleus of the amygdala (BLA) allows it to directly influence and coordinate activity in different brain regions. This thesis provides evidence that BLA synapses are endowed with special properties that allow them to facilitate heterosynaptic plasticity, bypassing the need to activate other modulatory structures.

Chapter 3 tests the hypothesis that BLA synapses onto medium spiny neurons (MSNs) in the ventral striatum can facilitate heterosynaptic plasticity because they have a higher ratio of N-methyl-D-aspartate (NMDA) to α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid (AMPA) receptors, than that found at cortical synapses onto the same MSNs. I present evidence showing that the NMDA-to AMPA ratio at BLA synapses is two times higher than that of cortical synapses. The relevance of these results is considered in the context of NMDA receptors' role in many forms of synaptic plasticity, as well as learning (Kandel and Pittenger, 1999; Malenka and Bear, 2004).

Chapter 4 expands on these findings, directly testing the hypothesis that BLA inputs can facilitate induction of long-term plasticity (LTP) at cortico-striatal synapses. BLA stimuli increased both the incidence and amplitude of cortico-striatal LTP in an *in vitro* preparation. Selective blockade of NMDA receptors at BLA synapses on striatal neurons prevented these facilitatory effects. Here, I also describe the temporal rules governing the BLA-mediated enhancement of heterosynaptic plasticity.

Chapter 5 examines the molecular pathways involved in the facilitation of cortico-striatal plasticity by BLA activity. I show evidence that Ca^{2+} release from intracellular stores is required for these effects. I also test the involvement of dopaminergic D_1 receptors, and show that a basal level of D_1 activity is required for the BLA facilitation of cortico-striatal LTP.

Chapter 6 investigates the *in vivo* activity of BLA and striatal neurons during the acquisition of an appetitive stimulus-response task. Here, I identify coherent gamma oscillations as a physiological signature of BLA-striatal interactions, and use this measure to monitor neuronal changes that accompany learning.

Together, these results provide evidence converging on a new mechanism that enables the amygdala to directly facilitate plasticity in its targets. In no way conflicting with previous models of post-training amygdala-facilitated heterosynaptic plasticity, the mechanisms described here provide an alternative that might explain how BLA facilitates memory formation in emotionally arousing conditions.

1.2) Role of the amygdala in the facilitation of memory by emotions

Many studies support the notion that the amygdala mediates the effects of emotional arousal on memory. While some forms of learning (such as fear conditioning) seem to require the amygdala for storage and retrieval, many others do not use the amygdala as a storage site but are facilitated by an increased activity of amygdala neurons. The prevalent model suggests that emotions cause the release of stress hormones that increase the firing rate of neurons in the amygdala, which in turn facilitate plasticity in other structures. However, it is currently unknown how this increased amygdala activity facilitates plasticity in other structures.

1.2.1) Anatomical considerations

The amygdala is a collection of nuclei (approximately 13) grouped together based on functional, morphological, and anatomical criteria (Sah et al., 2003). The three main nuclear groups are the basolateral complex of the amygdala (BLA - comprised of the lateral, basal, and accessory basal nuclei), the central nucleus (CE), and a superficial group (with a cortex-like organization), each of them with several subdivisions (Price et al., 1987). Inputs to the amygdala arise mainly from the cerebral cortex (providing uni- and polymodal sensory inputs), thalamus (with primary sensory information), hypothalamus, as

well as various modulatory systems of the basal forebrain and brainstem (which provide information about the ongoing behavior and the autonomic system).

In turn, the amygdala has a wide range of projections(see (Sah et al., 2003). The BLA complex sends axons to structures involved in various forms of memory (the medial temporal lobe, prefrontal cortex, striatum) while the CE projects to regions that control the behavioral and autonomic expression of emotions (the hypothalamus, thalamus, midbrain, pons, basal forebrain, locus coeruleus).

The main output fiber systems are: **a)** the stria terminalis, which loops in the sagital plane before distributing axons to various targets; **b)** the amygdalofugal pathway, which contains axons that contact more anterior structures (such as the striatum and hypothalamus); **c)** the external capsule which carries amygdala fibers to the lateral aspect of the brain.

Most inputs to the amygdala enter laterally (at the level of BLA nuclei), after which the information is conveyed to more dorso-medial regions (the CE nuclei). This sequential transfer is paralleled by area specific information processing. For example, electrical stimulation of the CE produces fear-related responses even in naive animals, while stimulation of the BLA has variable effects, depending on the animal's training history (Kapp et al., 1982; Iwata et al., 1987). This suggests that the BLA can associate behaviorally relevant stimuli with fear-related responses.

1.2.2) The amygdala mediates the effects of emotions on memory consolidation

Because selective amygdala lesions rarely happen in humans, many turned to animal studies to address questions about the role of this structure in behavior. There are many subjective aspects of emotions, making them difficult to study in animals; however, "fear" turned out to bridge this gap, since its collection of (in)voluntary responses includes aspects which are easily recognized regardless of species (changes in blood pressure and heart rate, freezing, startle, etc.). Most of these manifestations are associated with increased levels of circulating “stress hormones”, which raises the possibility that the same hormones might also be responsible for the effects of emotions on memory.

Long-term memories do not form instantaneously, but consolidate over a variable period of time, during which they are particularly vulnerable. Different neuromodulatory systems (the adrenergic one in particular) have the ability to alter this consolidation phase and either enhance or reduce retention. For example, it was found that high levels of stress that activate the adrenergic system impair learning; however, when moderate, stress actually enhances retention. This results in an inverse U-shape when plotting performance as a function of stress level (Gold and van Buskirk, 1978b, a).

The same effects can be observed when animals are injected with adrenaline or cortisol, supporting the notion that these hormones are responsible

for modulating memory formation (McGaugh and Petrinovich, 1965). In agreement with this, blocking beta-adrenergic (Cahill et al., 1994) or corticosterone receptors (Cordero and Sandi, 1998) impairs the effects of stress on learning. Moreover, these two systems interact, such that attenuating the increase of cortisol produced by aversive stimulation blocks the effects of epinephrine injections (Roozendaal et al., 1996). At the same time, adrenalectomized animals can compensate within 10 days for the loss of adrenaline with increased levels of cortisol, performing as well as controls in an inhibitory avoidance task (Borrell et al., 1983).

Several studies provided evidence that these hormones act at the level of the amygdala to modulate memory consolidation. Inactivation or lesion of the BLA (but not CE) blocks the memory modulatory effects of both adrenalectomy (Roozendaal et al., 1998) and peripheral glucocorticoid administration (Roozendaal and McGaugh, 1996b). The same effects were demonstrated for stria terminalis lesions (Liang and McGaugh, 1983; Roozendaal and McGaugh, 1996a). Moreover, infusing beta-adrenergic antagonists in the BLA blocks the memory enhancements produced by post-training peripheral administration of epinephrine or glucocorticoids, as well as those of stress (Liang et al., 1986). Similarly, intra-BLA injections of drugs that increase (Dickinson-Anson et al., 1993) or decrease (Salinas et al., 1993; Coleman-Mesches and McGaugh, 1995) BLA firing rates enhance or reduce retention, respectively.

These findings suggest that stress hormones can facilitate memory

consolidation by increasing the firing rate of BLA neurons. Recently, it was shown that indeed, stress hormones such as glucocorticoids increase the excitability of BLA neurons (Duvarci and Pare, 2007), and that following an emotional event, BLA neuronal firing rates remain increased for several hours (Pelletier et al., 2005).

1.2.3) Role of the amygdala in storage vs. modulation of memory

Much of what we know about the role of the amygdala in learning and memory comes from studies on fear conditioning. This paradigm allows an initially neutral stimulus – the conditioned stimulus or CS (such as a light, sound, context), to gain a predictive value through repeated pairings with an unpleasant stimulus (the unconditioned stimulus – US) that produces a specific, measurable behavior (e.g. freezing). The association is considered learned when presentation of the CS alone elicits the same behavioral response as the US (could be startle, freezing, place preference, etc.).

Contrary to other types of classical conditioning in which a relatively high number of CS-US contingencies are required to achieve maximum learning, fear conditioning occurs after only a few CS-US pairings, delivered in one session. This relatively simple but powerful technique initiated a quest for the neuronal substrates of conditioned fear memories. Extrapolation of the findings to other species (including humans) seems reasonable, since fear responses and the fear

circuit are similar across species (Morris et al., 1998; Bechara et al., 2003).

According to the current model, information about both the CS and US (arriving through cortical and thalamic pathways) converge in the amygdala at the level of the BLA, which in turn activates the CE, producing the unconditioned responses. Thus, the CS-US temporal contingency is believed to cause a potentiation of the synapses conveying CS information to the BLA, such that subsequent presentations of the CS alone would suffice to elicit the same responses as the US (see (LeDoux, 2003) for a detailed description, and (Pare et al., 2004; Phelps and LeDoux, 2005) for updates on the model). Indeed, a large number of studies support the idea that changes in synaptic strength within the amygdala (synaptic plasticity in the form of long-term potentiation - LTP; or long-term depresion - LTD) occur, and are necessary for fear conditioning (LeDoux, 2000; Malkani and Rosen, 2000; Blair et al., 2001; Maren, 2001). Thus, it seems that for classical fear-conditioning, the amygdala is a memory storage site.

On the other hand, the ability of the amygdala to facilitate memory storage in other structures is just as well supported. For example, Packard and colleagues trained rats on one of two tasks: a hippocampal task (learning the location of a submerged platform based on external visual cues around the water maze), or a striatal dependent task (same task, except the visual cue was placed on the platform itself) (Packard et al., 1994). Local intra-BLA injections of amphetamine a few minutes after training improved performance in both tasks, when tested 24h later. However, temporarily blocking BLA activity with lidocaine

injections right before testing retention had no effect on performance, suggesting that the amygdala is not the memory storage site, and is not required for retrieving the memory (Packard et al., 1994). Inactivating the hippocampus right after training the rats on the submerged platform maze blocked the enhancing effects of intra-BLA amphetamine, while no change was observed in the visible platform task. Conversely, injecting lidocaine in striatum after training, did not affect performance in the hippocampal task, but reversed the facilitatory effects of amphetamine injections in the amygdala in the visible platform task (Packard and Teather, 1998).

Similarly, lesions or inactivation of the BLA in a variety of different tasks right before testing retention, have no effect on performance (Liang et al., 1982; Parent et al., 1995). This contrasts with the results of studies in which animals are trained in fear-conditioning paradigms. Indeed, lesioning the BLA even as late as 16 months after fear-conditioning, impairs recall (Gale et al., 2004). This apparent contradiction can only be resolved by the differences between the behavioral tasks used. Whether the underlying factor is the high salience of the US used for fear-conditioning or some other factor, remains to be seen. However, it is clear that depending on the task, the amygdala can facilitate the consolidation of many emotional memories, as well as store some component of Pavlovian fear memories.

1.2.4) Possible mechanisms of amygdala-facilitated memory consolidation

The data reviewed above suggests that the amygdala mediates the effects of emotional arousal on memory consolidation. According to the generally accepted model, stress hormones released during an emotional event increase the firing rate of BLA neurons. In turn, BLA activity would somehow facilitate synaptic plasticity in other brain regions. But how exactly does the increased firing rate of BLA neurons translate into facilitated synaptic plasticity in other structures?

To address this issue, several groups tested the effects of electrical BLA stimulation on LTP induction in various pathways. Both the perforant path and the thalamo-cortical pathway proved sensitive to this manipulation, exhibiting increased levels of LTP when BLA was co-stimulated, compared to control conditions (Ikegaya et al., 1995b; Akirav and Richter-Levin, 1999b, a; Frey et al., 2001; Dringenberg et al., 2004). Moreover, high frequency stimulation of the perforant path produces lower amounts of LTP in animals with ipsilateral BLA lesions (Ikegaya et al., 1994, 1995a). Also, BLA stimuli delivered prior to tetanus of the perforant pathway enhance LTP (Akirav and Richter-Levin, 1999b, a), while stimulating BLA after thalamo-cortical LTP induction stabilizes it (Dringenberg et al., 2004).

Since all experiments investigating the BLA facilitation of LTP were performed in vivo, two possible mechanisms might explain these results: 1. BLA stimulation activates a third structure that modulates plasticity; 2. BLA directly

influences (through its widespread projections) the regions in which facilitated LTP was observed (hippocampus and cortex).

Regarding the first possibility, much evidence suggests that the basal forebrain cholinergic system mediates the effects of BLA stimulation on synaptic plasticity. Normal cholinergic activity seems to be required for LTP induction. Blocking muscarinic receptors reduces LTP (Kirkwood et al., 1999; Pesavento et al., 2000; Kuczewski et al., 2005; Origlia et al., 2006), while activating them enhances LTP (Brocher et al., 1992). Moreover, muscarinic antagonists (such as scopolamine) applied intraventricularly block the stabilizing effects of BLA stimulation on LTP, but not on the induction of LTP (Frey et al., 2001; Dringenberg et al., 2004).

However, the amygdala is reciprocally connected with the basal forebrain (Nagai et al., 1982), which raises concerns about the interpretation of these findings. Specifically, it is difficult to determine: a) whether the muscarinic antagonists act at the level of the amygdala, or in the tested pathway; and b) if electrically stimulating the amygdala activates the cholinergic system anterogradely, or by back-firing basal forebrain axons projecting to the BLA. While the first issue has been addressed by showing that intra-BLA infusion of scopolamine has no effect on amygdala-modulated plasticity (Ikegaya et al., 1997), the last point still remains a valid concern.

The second possible mechanism for the BLA-facilitation of plasticity remains un-investigated. The BLA also exerts its facilitatory effects on plasticity

in structures that do not receive projections from the basal forebrain, such as the striatum (Packard et al., 1994). Thus, it is possible that BLA axons directly modulate the properties of their postsynaptic targets, placing them in a state favorable for the induction of activity-dependent synaptic plasticity (for example, by lowering the activity threshold for LTP induction, enhancing the amplitude of LTP, or converting short-term into long-term plasticity). The amygdala has direct projections to structures such as hippocampus, striatum, prefrontal cortex, perirhinal and rhinal cortices, which are known to participate in learning, storage, and retrieval of memories. As such, it seems possible that the memory modulatory effects of the amygdala could, in some cases at least, be explained by a direct influence of BLA axons on these structures.

However, for this to be possible, the axons of BLA projection cells would have to be endowed with special properties allowing them to change the plasticity threshold of their targets. In order to exert their effects, BLA axons could either directly contact a large population of neurons by diffusely branching throughout their target structures, or they could contact specific subsets of interneurons able to locally distribute the effects of BLA activity.

So far, the possibility that BLA axons directly facilitate heterosynaptic plasticity in their targets has not been tested. If indeed true, this mechanism would allow the amygdala to quickly and efficiently influence memory consolidation in different ways than previously considered. My thesis focuses on some of these questions:

- Do BLA projections differ from others converging on the same neurons?
- Can they facilitate synaptic plasticity of other converging inputs?
- What are some of the molecular and temporal aspects that characterize these effects?
- How do these mechanisms come into play during learning, and how does the amygdala interact with its targets to achieve this?

To address these questions, I performed both *in vitro* and *in vivo* experiments. I used an *in vitro* preparation to record the activity of medium spiny striatal neurons in the ventral striatum, while electrically stimulating cortical and BLA axons converging onto these cells. This technique allowed the manipulation of precise variables such as the intracellular environment, or specific receptors activity. Another advantage is that both cortex and the BLA send axons to the striatum, but these projections are not reciprocated. Thus, electrical stimulation of the BLA and cortex produce striatal responses which are exclusively orthodromic.

To investigate the effects of amygdala activity on learning, I performed simultaneous multi-site recordings in the BLA, cortex, striatum and thalamus in animals undergoing learning of a striatal stimulus-response task. This allowed me to identify unique physiological signatures of interaction between these structures, and parallel behavioral performance with neurophysiological changes.

1.3) The role of the striatum in learning

Although initially described as a system purely involved in movement control, a considerable body of data established that the striatum plays a critical role in goal-directed behavior and learning. Due to its connectivity, the striatum is in a position to integrate events across different sensory modalities and to select a course of action between competing ones. Thus, synaptic plasticity in the striatum has important consequences, and is believed to underlie several forms of learning, including habit formation (DeLong, 2000; Mahon et al., 2004).

1.3.1) Structure and anatomical connections

The basal ganglia consist of four nuclei which can be easily identified in most vertebrate species: the striatum (comprised of the caudate, putamen and nucleus accumbens), globus pallidus (GP, with an external and internal segment), substantia nigra (SN, divided in pars compacta and pars reticulata), and the subthalamic nucleus.

Figure 1 summarizes the connectivity of the basal ganglia. The outputs consist of axons originating in substantia nigra pars reticulata and the internal segment of globus pallidus. They project to the thalamus, pedunculopontine nucleus, superior colliculus, and the lateral habenular nucleus.

The inputs originate in various cortical areas (almost all cortical regions

send projections to the basal ganglia) and intralaminar thalamic nuclei, both of which send excitatory glutamatergic projections to the striatum, the main input station of the basal ganglia. The output of the striatum is GABA-ergic, and generally confined to other nuclei of the basal ganglia: **a)** by means of a direct pathway, the striatum connects to the internal segment of globus pallidus and substantia nigra pars reticulata; **b)** an indirect pathway conveys striatal projections to the external segment of globus pallidus, which in turn projects to the substantia nigra, and is reciprocally connected with the subthalamic nucleus (Wilson, 1998; DeLong, 2000).

Although the striatum contains various types of neurons, ~95% are medium spiny neurons (MSNs). MSNs receive all major striatal inputs, and are also the projection neurons of the striatum (Wilson, 1998). According to *in vivo* studies, MSNs have a bistable membrane potential that is preferentially maintained between -85 and -90 mV (the ‘down state’), and occasionally shifts to a depolarized level of approximately -55 mV (the ‘up state’). MSNs never fire while in the down state. Thus, it is believed that convergence of several excitatory inputs is required to move MSNs into the up state and generate action potentials (Pennartz et al., 1994; Wilson, 1998).

Specific areas of the striatum are preferentially connected with specific areas of the cerebral cortex, thalamus, and limbic system, providing an anatomical isolation of functions. The traditional view that the basal ganglia perform a mere convergence of cortical inputs onto a limited number of targets

was challenged by evidence that information originating in different parts of the cortex remains segregated in parallel pathways that go, via the striatum, to the pallidum or substantia nigra (Heimer et al., 1982; Alexander et al., 1986). Moreover, these basal ganglia output regions send projections back to the same cortical and thalamic areas, creating feedback circuits believed to underlie functional loops (Wilson, 1993; Mengual et al., 1999).

Interestingly, lesions of specific striatal areas tend to produce deficits similar to those observed when the afferent cortex is lesioned (Mink, 2003); e.g. inferior caudate lesions mimic those of orbitofrontal cortex; lesions of the dorso-lateral caudate produce deficits similar to lesions of dorso-lateral prefrontal cortex.

The use of well-defined behavioral tasks, combined with lesions of circumscribed striatal territories, has revealed functional dissociations between different parts of the striatum (Koechlin et al., 2000; Mair et al., 2002). This has led to the proposal that the striatum is comprised of several functional circuits (DeLong, 2000) involved in executing various functions, such as:

- “executive functions”: dorsolateral prefrontal cortex → head of caudate nucleus → dorso-medial internal pallidal segment, rostral substantia nigra pars reticulata → ventral anterior and medial dorsal thalamus → dorsolateral prefrontal cortex

- “empathy and social behavior”: lateral orbitofrontal cortex → ventro-

medial caudate nucleus → (through the same targets mentioned before) → orbitofrontal cortex

- "motivated behavior": anterior cingulate cortex → ventral striatal regions → ventral and rostro-medial pallidum, rostro-dorsal substantia nigra pars reticulata → paramedian part of the medial dorsal thalamic nucleus → anterior cingulate cortex.

Both thalamic and cortical inputs to the caudate and putamen follow a certain "patchy" distribution (Kalil, 1978; Graybiel, 1984; Alexander and Crutcher, 1990). This heterogeneous innervation was later shown to reflect two histochemically distinct compartments within the striatum: the matrix (characterized by a strong acetylcholinesterase staining) and the striosomes (with a weak acetylcholinesterase staining). Although originally described using acetylcholinesterase staining, the two regions can now be identified with a large number of biochemical markers (Graybiel, 1984).

Acetylcholinesterase staining also separates nucleus accumbens into a central part (the core) that contains the same striosome / matrix pattern as caudate-putamen, and a peripheral part (the shell), poorly stained, that surrounds the core on all aspects, except caudally and dorsally (Voorn et al., 1989; Zahm and Brog, 1992). Similar to the striosome/matrix duality in the caudate and putamen, many enzymes and proteins show distinct levels of expression between the shell and the core regions (Zahm and Brog, 1992).

A different segregation of striatal inputs was described in the dorso-lateral to ventro-medial axis: while the dorsal striatum receives inputs mainly from the motor, premotor, and sensory cortices (Flaherty and Graybiel, 1991), the ventral striatum is connected to the limbic system: hippocampus, amygdala, entorhinal cortex (Lynd-Balta and Haber, 1994a).

The concept of ventral striatum was initially proposed by Heimer, who defined it as the striatal region receiving inputs from the allocortex. Based on this criterion, nucleus accumbens, the ventral part of caudate-putamen, and the olfactory tubercle were included in this group. In a rostro-caudal direction, the ventral striatum extends between the head of the caudate and the anterior comissure (Fudge and Haber, 2002; Fudge et al., 2002). From this thesis' perspective, the ventral striatum is a region of particular interest because it is the neostriatal region most densely innervated by the amygdala.

1.3.2) Role of the striatum in learning – lesion studies

Despite many years of research, there is still no unifying theory for striatal function. As illustrated above, different regions of the basal ganglia (due to their specific sets of inputs) can be recruited in different tasks, and thus play different roles in guiding behavior (DeLong, 2000).

Generally, learning a new procedure requires the full attention of the subject, but in time the behavioral response becomes almost automatic

((Anderson, 1982; VanLehn, 1996). One of the advantages is that resources can be distributed to other (new) tasks, while still allowing the subject to perform and improve previously learned behavior. Thus, it is believed that different neuronal substrates support different memory systems, and are involved at different stages of learning.

Two such memory systems have been recognized over the years: the declarative or explicit, requiring conscious recollection, and non-declarative or implicit, not requiring conscious recollection (Squire, 1987). The hippocampus with its affiliated cortices on one hand, and the striatum on the other were identified as critical for learning declarative and non-declarative tasks, respectively.

Early experimental research on the behavioral effects of pre-training caudate nucleus lesions found these animals to be impaired in learning delayed responses, alternation, and conditioned avoidance tasks (Battig et al., 1960; Chorover and Gross, 1963; Neill and Grossman, 1970; Oberg and Divac, 1975; Kirkby et al., 1981). Similar studies employing lesions or inactivation of the striatum highlighted its role in various stimulus-response tasks: two-way active avoidance (Kirkby and Kimble, 1968), simultaneous tactile discrimination (Colombo et al., 1989), straight-alley runway behavior (Kirkby et al., 1981), conditional visual discriminatory learning (Reading et al., 1991), auditory discrimination learning (Adams et al., 2001).

This raised the possibility that some types of learning (and perhaps even

memory storage) depend on striatal integrity. However, pre-training lesions can affect a number of parameters unrelated to memory itself (eg. motivation, sensory and/or motor functions) which nonetheless can impair acquisition and/or performance on memory tasks. One way to address this issue is to compare learning tasks that share the same motivational, sensory, and motor requirements, but rely on different learning strategies, and different neuronal networks. As such, specific impairment in one task but not the other provides strong evidence that the lesioned area is critical for the acquisition and/or expression of one particular type of memory.

In one study, for example, rats were trained on two water-maze tasks. The animals had to learn to swim either to a specific cue that indicated the location of a submerged escape platform – which would vary from trial to trial (a stimulus-response type of learning); or to a certain spatial location (always the same, indicated by external cues around the pool) where the platform could be found (a spatial navigation task). Animals with bilateral lesions of the striatum had difficulties learning the stimulus-response task, but performed as well as controls in the spatial task (Packard and McGaugh, 1992). This indicates that, while striatal lesions impair the acquisition of stimulus-response associations, the results are not due to the animal's inability to perceive the stimulus, or perform the expected behavioral response. Moreover, since lesions of the fornix (the main output of hippocampus) had opposite effects (the animals were able to acquire the stimulus-response, but not the spatial task), it became obvious that hippocampus and striatum underlie different types of learning.

Many studies employed similar techniques to illustrate the specific involvement of the striatum in various learning tasks (McDonald and White, 1994; Parkinson et al., 1999; Stratford and Kelley, 1999; Zahm, 1999; Corbit et al., 2001; Fenu et al., 2001; Reynolds and Berridge, 2002; Li and Fleming, 2003). These results provide evidence that the striatum is involved in learning, and possibly storing some forms of memory. As detailed in the following section, this idea is also confirmed by electrophysiological recordings.

1.3.3) Role of striatum in learning – electrophysiological studies

Consistent with their role in learning, striatal neurons change their firing rate and pattern during task-acquisition. Stimuli of all sensory modalities can induce phasic responses in striatal neurons (Schultz et al., 2003). In addition, their firing rates seem to be closely associated with different components of the movement necessary to obtain a reward. For example, neurons in the latero-dorsal caudate-putamen adapt their firing rate during learning of a T-maze task, with particular preference for the first and last part of the maze. These changes are stable throughout the experiment, lasting for several days (Jog et al., 1999). At the same time, striatal neurons begin to discriminate auditory stimuli (paired vs. un-paired with reward) as early as 15 min after the beginning of training; similarly, extinction reduces the discrimination within 10 min (Aosaki et al., 1994).

These neuronal behaviors suggest that the striatum performs several

functions, possibly underlying both learning and storage of some forms of memory. Transient changes in neuronal activity during learning (such as increased firing rates in relation to novel instruction and movement-triggering stimuli (Tremblay et al., 1998); or to novel visual stimuli (Brown et al., 1995)), coexist with long-term changes that parallel performance in the task (Tremblay et al., 1998). Also, some studies report neuronal responses that become predictive of movement-initiating stimuli rather than movement itself (Jog et al., 1999). For example, it was shown that various parts of the striatum are involved at different stages of learning, with a transfer of function from medial to lateral regions as learning progresses (Yin et al., 2009).

As previously mentioned, the ventral and dorsal striatum receive distinct sets of inputs. *In vivo* multi-unit recordings confirmed the dissociation between these two regions. Neuronal activity recorded in the dorsal striatum revealed a strong influence of tactile stimulation and movement, resulting in a topographical map of the body (Carelli and West, 1991). On the other hand, neurons in the ventral striatum (particularly nucleus accumbens), appear to code motor sequences leading to reward (Woodward et al., 1999).

Consistent with its connectivity, the ventral striatum was found to mediate learning and operant responses to natural and drug rewards (Robbins and Everitt, 1996; Parkinson et al., 1999; Breiter et al., 2001; Corbit et al., 2001; Mair et al., 2002). For example, recordings performed while rats learned to lever-press for intravenous cocaine administration show that 50% of nucleus accumbens

neurons change their firing rate in relation to drug administration and prior to movement sequences preceding or following reward administration (Chang et al., 1996).

Similar results were obtained for various types of rewards such as water (Carelli and Deadwyler, 1997), heroin (Chang et al., 1998), and ethanol (Janak et al., 1999). Despite these commonalities, the neuronal changes exhibit high levels of discrimination for different rewards (e.g. only 4-6% of the neurons recorded in nucleus accumbens responded both to cocaine and heroin self-administration) (Chang et al., 1998).

1.3.4) Cellular models of striatal memory storage

The evidence presented above suggests that the striatum is involved in various stages of learning, as well as recall. Although suggested, evidence that memories are also stored in the striatum is controversial.

The predominant view is that activity-dependent plasticity at various synapses is responsible for storage of various forms of memory. This idea stems from the theory formulated by Hebb, that neurons firing close in time to each other will strengthen their connection, such that subsequent activations of the presynaptic neuron will result in stronger responses in the postsynaptic neuron (Hebb, 1949). In 1973, Bliss and Lomo described a phenomenon (long-term potentiation - LTP) closely resembling the predictions made by Hebb (repetitive,

sustained activity of presynaptic fibers led to an increase of the responses evoked postsynaptically). Since they observed this phenomenon in the hippocampus (a structure previously known to support learning and memory), their findings raised the possibility that long lasting changes in synaptic strength underlie memory storage.

Subsequently, LTP was observed in various pathways relevant to learning. Moreover, an LTP-like increase in postsynaptic responses accompanies some forms of behavioral training (Rioult-Pedotti et al., 2000). This, combined with the fact that LTP is impaired by behavioral training and, in turn, its induction can impair learning of specific tasks (McNaughton et al., 1986; Castro et al., 1989; Barnes et al., 1994; Schroeder and Shinnick-Gallagher, 2005), strengthened the connection between synaptic plasticity and memory (Kandel and Pittenger, 1999; Martin et al., 2000; Malenka and Bear, 2004).

The most common form of LTP depends on NMDA receptors (Malenka and Bear, 2004). Briefly, Ca2+ entry through NMDA receptors is thought to activate a series of enzymes (mainly kinases), ultimately leading to increased postsynaptic AMPA responses. In the long term, this increase most likely occurs by incorporating more AMPA receptors into the postsynaptic membrane by means of protein transport, exocytosis and lateral diffusion (Bredt and Nicoll, 2003).

However, direct evidence that LTP and its counterpart, long-term depression (LTD), are the actual mechanisms for memory storage is still missing.

This is a valid criticism for any study trying to link synaptic plasticity to learning. Regardless, behavioral, electrophysiological, and computational findings agree that synaptic plasticity is the best candidate mechanism of memory storage.

With respect to the striatum, blocking NMDA receptors with intra caudate-putamen infusions of AP-5 (Packard and Teather, 1997b) or MK-801 (Packard and Teather, 1997a) prevents learning of striatal dependent tasks. LTP and LTD have been described in the cortico-striatal pathway using both *in vitro* and *in vivo* preparations (Calabresi et al., 1992b; Calabresi et al., 1992a; Lovinger et al., 1993; Charpier and Deniau, 1997; Partridge et al., 2000; Reynolds et al., 2001). Since sensory inputs reach the striatum mainly through the cortico-striatal pathway, plasticity at these synapses is believed to underlie motor learning and habit formation (Reynolds et al., 2001; Mahon et al., 2004). Changing the strength of specific cortical inputs to the striatum would thus result in the execution of specific behaviors, or the selection of a particular motor plan in response to the presentation of particular stimuli.

1.4) The amygdalo-striatal pathway

Although the striatal projections of amygdala neurons have been anatomically characterized, their function is poorly understood. A significant area of the striatum receives glutamatergic inputs form specific regions of the amygdala, and these inputs can influence the output of striatal neurons. Based

on the roles that the amygdala and striatum play in behavior, it is assumed that the amygdala may bring an emotional component to striatal information processing, and facilitate striatal-dependent memory storage. However, how exactly these two structures interact is still unknown.

1.4.1) Description of the amygdalo-striatal projections

The amygdalo-striatal pathway was first mentioned in a study performed by Krettek and Price in 1978. Using small injections of radioactive amino acids in different regions of the amygdala, they found a specific distribution of projections to the striatum. Only injections in the basolateral and basomedial nuclei labeled fibers in the striatum, mostly in the ventral putamen, nucleus accumbens and olfactory tubercle – the ventral striatum (Krettek and Price, 1978).

It was later shown that the amygdala axons projecting to the striatum have a topographical distribution: rostral amygdala injections label fibers in the lateral part of the putamen, nucleus accumbens and olfactory tubercle, while projections from the caudal part of the amygdala are found in the medial parts of striatum. Also, caudal regions of the basolateral nucleus of the amygdala (BLA) project to the rostral part of striatum, while progressively more rostral BLA areas send fibers to the caudal striatum (Russchen and Price, 1984). Amygdala axons make “en passant” synapses with striatal neurons, suggesting a significant divergence in the amygdalo-striatal pathway (Russchen and Price, 1984).

Russchen and collaborators provided evidence that the ventral part of the caudate nucleus is also targeted by the amygdala (Russchen et al., 1985). They noted that in many of these areas, the distribution of fibers follows a pattern of patches, in which specific zones are avoided while others are densely innervated. Subsequently, it was shown that amygdala fibers projecting to the caudate nucleus aggregate in the striosomes (Ragsdale and Graybiel, 1988).

Nucleus accumbens has a similar topographical distribution of amygdala axons (Wright et al., 1996). The caudal part of the BLA projects to the dorso-medial shell, and to the striosomes in the core. The rostral part of the BLA sends fibers to the lateral accumbens shell and to the patches of the lateral nucleus accumbens core, while fibers from the mid-rostrocaudal part of the BLA synapse in the ventral region of the shell, as well as the core (Wright et al., 1996).

Most studies report that all amygdala projections to the striatum originate in the basolateral and basomedial nuclei. One study, however, reports that injections of anterograde tracers in the lateral nucleus label a small area of the ventromedial caudate (Fudge et al., 2002). The result though, could be due to a large injection site that encroached upon the basolateral nucleus (see Fig. 8 in (Fudge et al., 2002)). Anterograde tracers injected in CE do not label the striatum, suggesting that the amygdalo-striatal pathway is composed solely of axons originating in the basal nuclei of the amygdala and ending in the ventral striatum.

Projection neurons of the BLA are excitatory (Carlsen, 1988; Smith and

Pare, 1994). BLA axon terminals found in cortex are enriched in glutamate and form asymmetric synapses (Smith and Pare, 1994). Consistent with this, ultrastructural features of the synapses formed by amygdala axons in the striatum suggest they are excitatory. Amygdala terminals form asymmetric synapses onto spines, and the presynaptic elements have clear, round vesicles (Kita and Kitai, 1990).

Around 90-95% of the synapses formed by amygdala axons in the ventral striatum contact spines (usually en passant), and 8-12% of those make synapses with more than one spine (Johnson et al., 1994). This pattern suggests a large divergence, raising the possibility that BLA activation has global effects on striatal activity. This is consistent with the idea that BLA activity might facilitate striatal-dependent learning by directly influencing the properties of MSNs (see section III in this chapter). In order to understand how this might happen, I will consider several studies on the interaction between the amygdala and other striatal inputs.

1.4.2) Overlap and interactions between the amygdala and other striatal inputs

Many projections to the striatum seem to follow a pattern of local connectivity that respects the striosomal borders (Kalil, 1978). It is thus conceivable that specific interactions between various inputs occur at the level of MSNs, ultimately determining the firing rate of these output neurons.

Tracing studies revealed specific patterns of segregation and convergence in the striatum (Brog et al., 1993; Wright and Groenewegen, 1995; Wright et al., 1996; Wright and Groenewegen, 1996; Fudge and Haber, 2002). Afferents coming from the cortex, hippocampus, thalamus and amygdala overlap, forming identifiable topographical maps: a particular region of cortex projects to a restricted area of the striatum, where it overlaps with fibers coming from precise areas of the amygdala, hippocampus and thalamus.

Moreover, striatal outputs seem to be segregated in a similar fashion. Small injections of tracers in the targets of nucleus accumbens showed that retrogradely labeled MSNs are organized in clusters with similar outputs (Berendse et al., 1992; Groenewegen et al., 1999). Combining anterograde tracer injections in areas projecting to the nucleus accumbens with retrograde tracer injections in accumbens targets revealed that specific clusters of output neurons receive specific inputs (Groenewegen et al., 1999).

These findings raised the question: what kind of information processing is being performed at the level of striatum, and what is the functional significance of these neuronal clusters? A proposal still awaiting experimental confirmation is that the clusters represent ensembles of neurons that are synchronously activated by specific sets of inputs. The combined activity of these MSNs would then be able to regulate downstream targets (Pennartz et al., 1994).

Then, how do various striatal inputs interact, and how does this affect targeted structures? In this respect, the dopaminergic system received

considerable attention. Two main regions provide dopaminergic inputs to the central nervous system: the substantia nigra pars compacta (SNc), and the ventral tegmental area (VTA). SNc and VTA axons reach higher structures through several pathways (DeLong, 2000; Mink, 2003):

- Mesolimbic pathway – originates in the VTA and projects to the limbic system, including the amygdala and ventral striatum (has been implicated in addiction and the positive symptoms of schizophrenia)

- Mesocortical pathway – originates in VTA and projects to the cortex, especially the frontal lobe (believed to be involved in motivation, and the negative symptoms of schizophrenia)

- Nigrostriatal pathway – originates in SNc and provides the main dopaminergic input to the dorsal striatum (loss of which causes motor dysfunctions such as Parkinson's disease)

A detailed description of the dopaminergic projections to the striatum (Zahm, 1992) led to elaborated functional models (Smith and Bolam, 1990). These inputs were shown to form synapses on dendritic shafts and on the neck of spines, in a key position to modulate other incoming signals (Meredith, 1999).

For example, electrical stimulation of the VTA decreases the number of spikes generated in nucleus accumbens neurons by thalamic stimuli (Akaike et al., 1981). Similarly, responses to amygdala stimulation recorded in the nucleus accumbens were decreased 100 ms after VTA stimulations, while depletion of

dopamine abolished this reduction (Yim and Mogenson, 1982). In a different study, ventral pallidum activity was recorded while delivering electrical stimuli in the amygdala. The latency of the inhibitory responses showed a bimodal distribution (presumed to reflect a direct and an indirect pathway), and 54% of the late-responses were attenuated by injection of procaine or D-amphetamine into the nucleus accumbens, or by stimulating the VTA (Yim and Mogenson, 1982).

These studies suggest that the amygdala can influence nucleus accumbens' targets, and this is gated by local dopamine release in the striatum. A more recent study suggests that the modulation is in fact reciprocal (Floresco et al., 1998). BLA electrical stimulation increases the level of dopamine in nucleus accumbens. Blocking glutamatergic transmission in nucleus accumbens reduced the dopaminergic response in a dose-specific manner. The authors interpreted these results as proof that axons from BLA presynaptically modulate dopaminergic release in nucleus accumbens (Floresco et al., 1998).

However, the majority of BLA fibers form synapses on the spines of MSNs, so it is likely that the main effect of BLA activation occurs post-synaptically. As mentioned before, during the down-state, MSNs have highly negative membrane potentials and never fire action potentials (APs). It is thus believed that synchronous activity of several sets of inputs is required to move them in the up-state and induce APs. This raises the possibility that some striatal inputs might be gated, such that they would only have access to their

downstream targets if MSNs are brought to an up-state by another input.

Thus, a neuronal structure able to switch MSNs between up and down-states would have the ability to impose a certain temporal pattern of striatal activity, selectively allowing some inputs to generate spikes in these neurons. It was proposed that projections from the hippocampus could perform such a function, since lesions of, or lidocaine injections into the fornix induce a preference for the down-state of the intracellularly recorded MSNs. Under these conditions prefrontal cortex or amygdala stimulation fails to elicit action potentials in MSNs (O'Donnell and Grace, 1995).

Fibers coming from the BLA and hippocampus overlap in an area of nucleus accumbens corresponding to the medial region of the shell. Using a “paired-pulse” paradigm it was found that preceding hippocampal stimuli with amygdala pulses resulted in enhanced responses of ventral striatal neurons to hippocampal stimuli (Mulder et al., 1998). In contrast, delivering the first pulse to the hippocampus, significantly reduced the following responses to the amygdala stimulus (Mulder et al., 1998). This was interpreted as evidence that hippocampal axons generate long-lasting inhibitory responses in ventral striatal neurons (probably $GABA_B$ dependent), during which stimuli delivered to the amygdala result in smaller responses. Conversely, when the amygdala was stimulated first, it could have increased the excitability of striatal neurons, enhancing responses to the second, hippocampal stimulus (Mulder et al., 1998).

The studies presented above shed some light on the interaction between

the amygdala and other striatal inputs. However, interpreting these results should be done with caution. In general, combining a whole-brain preparation with local electrical stimulations carries several risks, including: activating fibers of passage; inducing non-specific effects by recruiting polysynaptic pathways. Thus, it becomes obvious that the level of current knowledge is limited and more work is necessary to explain the influence of the amygdala on striatal information processing.

1.4.3) Role of the amygdalo-striatal pathway in learning

Although the computations performed by the striatum are poorly understood, its role in learning is well established (see previous sections). Since in some forms of learning, both the striatum and the amygdala are recruited, it is possible that an interaction between the two is required in order to achieve a new behavior.

The first direct evidence of such interplay came from a study by Packard and collaborators. The authors used a Morris spatial water maze task (hippocampus dependent), and a modified version of the same task, in which the location of the escape platform was identified by a local visual cue (striatum dependent). Retention of both tasks could be enhanced by increasing amygdala excitability. Indeed, animals that received intra-amygdala injections of D-amphetamine immediately post-training performed better than controls when

tested 24h later, long after the effects of the drug had vanished (Packard et al., 1994). Similar results were observed when D-amphetamine was injected in the hippocampus for the spatial task only, or caudate nucleus for the cued task only. Since inactivating the amygdala during the retention test did not affect recall, the authors concluded that the interaction between the amygdala and striatum, or amygdala and hippocampus is required only for learning, and not for performance of the task (Packard et al., 1994).

Indeed, temporarily inactivating the hippocampus immediately after the training session with lidocaine injections blocked the intra-amygdala amphetamine effects in the spatial task, and temporary inactivation of the striatum prevented learning of the cued task (Packard and Teather, 1998). This suggests that communication between the amygdala and its targets during the immediate post-training period is required for the facilitatory effects on learning.

Other groups showed that the striatum plays an important role in tasks originally considered amygdala-dependent (Burns et al., 1996; Pothuizen et al., 2005):

- The startle reflex can be attenuated by preceding the stimulus with one of smaller amplitude (prepulse inhibition). The intensity of the pre-pulse stimulus determines the reduction of the startle reflex; however, temporary inactivation of the core (but not shell) region of nucleus accumbens before testing, blocks this effect (Pothuizen et al., 2005).

- Morphine injections in nucleus accumbens impair the acquisition of context fear conditioning, but spare the actual CS-US association for auditory fear conditioning (Westbrook et al., 1997).

- Intra-striatal infusion of D-amphetamine was shown to facilitate the acquisition of a second order conditioning, in which a light previously associated with water delivery becomes a reinforcer, leading the rats to press a lever even in the absence of reward. Lesions of the amygdala prevented this form of learning (Cador et al., 1989).

- Intra-amygdala injections of a D_3 receptor agonist enhanced the association between CS (light) and intra-accumbens administration of D-amphetamine, perceived as a reward in itself (Hitchcott and Phillips, 1998).

All these studies support the idea that an interaction between the striatum and amygdala is essential for the acquisition, and sometimes expression of learned behaviors. It also seems that, a hierarchical organization does exist, in the sense that amygdala brings an emotional component to the information being processed by the striatum. If plasticity at cortico-striatal synapses underlies some form of memory (as supported by several studies described in the previous section), then the amygdala could be in a key position to modulate learning by directly influencing the ability of the cortico-striatal pathway to undergo LTP and LTD - like changes.

Indeed, cortico-striatal synapses can express moderate levels of LTP

(Calabresi et al., 1992b; Calabresi et al., 1992a; Lovinger et al., 1993; Charpier and Deniau, 1997; Partridge et al., 2000). However, manipulations that removed the Mg^{2+} block of NMDA receptors (such as post-synaptic depolarization, or excluded Mg^{2+} from the extracellular environment) were found to facilitate LTP induction (Calabresi et al., 1992a). It is thus possible that *in vivo*, the amygdala (and specifically the BLA) can substitute for these non-physiological manipulations, enhancing the incidence and amplitude of cortico-striatal LTP.

Here, I test the hypothesis that BLA synapses on MSNs are endowed with specific properties that allow them to directly facilitate cortico-striatal plasticity. Specifically, Chapter 3 tests the hypothesis that the contribution of NMDA receptors to post-synaptic responses is higher at BLA than that at cortical synapses on MSNs. Chapter 4 addresses the question of whether amygdala inputs can facilitate the induction of cortico-striatal LTP. I also test the hypothesis that the high NMDA-to-AMPA ratio identified in Chapter 3 underlies this effect, and I investigate the temporal rules governing this heterosynaptic facilitation. Chapter 5 identifies some of the intracellular mechanisms responsible for the amygdala-facilitated cortico-striatal plasticity. In particular, I test the hypotheses that Ca^{2+}–induced Ca^{2+}–waves mediate the effect of BLA stimulation on cortico-striatal LTP induction, and that BLA stimulations act solely as facilitators (they do not compensate for dopaminergic D_1 or muscarinic receptors lack of activity). Chapter 6 tests the hypothesis that the amygdala and striatum coordinate their activity at a cellular level *in vivo,* and that this interaction parallels behavioral performance during learning of a striatal-dependent task.

CHAPTER 2

General Methods

2.1) *In vitro* techniques

2.1.1) Preparation of brain slices

Guinea pigs (200 – 250 g) were deeply anesthetized with a mixture of ketamine (80 mg/kg), xylazine (12 mg/kg) and pentobarbital (60 mg/kg). The brain was then extracted in ice-cold artificial cortico-spinal fluid (aCSF – see below) and the two hemispheres sectioned in 400 µm coronal slices. The slices were then kept at room temperature for at least 1 h prior to recordings. In all experiments (unless otherwise stated), the aCSF contained (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose (pH 7.3, 300 mOsm). One slice was transferred at a time to a recording chamber, continuously perfused with oxygenated aCSF (4 ml/min), and maintained at a temperature of 32°C.

2.1.2) Recordings

Recordings were performed using the whole-cell patch technique. Glass microelectrodes (4-6 MΩ) were pulled from borosilicate glass capillaries and filled with a solution containing (in mM):130 K-gluconate, 10 N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid, 10 KCl, 2 $MgCl_2$, 2 ATP-Mg, and 0.2 GTP-Tris(hydroxy-methyl)aminomethane (pH 7.2, 280 mOsm). The data were digitized at 10 kHz and analyzed off-line with custom made software in Igor Pro 3.0, and MatLab 7.0.

Neurons were patched in an area of the striatum corresponding to the ventro-lateral aspect of caudate-putamen. Pairs of tungsten stimulating electrodes (epoxy coated, exposed tip 25 µm thick) were placed, 100 µm apart, in the BLA as well as cortex (lateral to the external capsule, in the associative auditory area – Fig. 2).

The stimulation intensity at cortical and BLA sites was adjusted to evoke postsynaptic responses of approximately 10 mV (0.1 – 0.3 ms pulses of 200 – 300 µA), after which we monitored changes induced by various drug applications, or other manipulations.

2.1.3) Occlusion test

We performed an occlusion test whenever we used two or more stimulation sites, to confirm that electrically stimulating each location activates different sets of fibers (Fig. 3B). For two stimulation sites, we recorded individual, as well as combined responses. The rationale behind this procedure is that the responses recorded at the soma are a sum of individual synaptic currents produced by the stimulation of a finite number of axons. Given two electrodes able to activate all fibers when stimulated together, and with a fraction *F* of overlap between them, an algebraic summation of the responses evoked by their individual stimulation would predict an amplitude bigger than the one physiologically possible (in a theoretical situation, the difference would be

precisely *F* * the response of all fibers activated together).

In contrast, if the two sets of stimulating electrodes activate non-overlapping inputs, responses to the paired stimuli should have an amplitude close to the algebraic sum of the individual ones (Fig. 3B). In our experiments, we considered the two sites to be overlapping if the response to the paired stimulation was smaller than the predicted one by more than 5%. These neurons were eliminated from the analysis. It should be noted that these tests were performed in voltage clamp, in order to minimize the involvement of voltage-gated channels, which would alter the linearity of the summation.

2.1.4) LTP protocol 1 (cortical-BLA pairing)

The LTP experiments began with the acquisition of a 10 min baseline period, during which cortical and BLA stimuli were applied one second apart, every minute. To increase our level of confidence that we only analyzed monosynaptic responses, the rising phase of EPSPs was considered, and the slope of its first half used as a measure of synaptic strength (Fig. 4A). Since a change in input resistance would confound our results, we monitored the response to constant current pulses (0.02 – 0.05 nA, for 0.5 s) throughout the whole recording session. Cells with fluctuations larger than 10% of the average (or showing a drift in resistance values) were excluded from the analysis.

All our recordings were carried out in current clamp, at a constant

membrane potential of -90 mV, to minimize time-dependent variations in driving force. Since most of the recorded cells had a resting potential close to -90 mV, little if any current had to be injected.

To induce LTP, we used a spike timing-dependent protocol during which we simultaneously stimulated two sites (either cortex and BLA, or two non-overlapping cortical sites), and paired them with an AP induced by somatic current injection (Fig. 4B). Since both cortex and amygdala responses showed similar latencies to electrical stimulations (between 3 and 6 ms), the APs were generated 7 ms post-stimulation (set to occur on the rising phase of the summated EPSPs). We repeated this protocol 60 times, at a frequency of 2 Hz (Fig. 4C) in order to mimic the *in vivo* firing rate of BLA projection neurons (Pare and Gaudreau, 1996; Pelletier et al., 2005).

Subsequently, we monitored the responses and input resistance every minute (same paradigm as the one used to record baseline) for at least 30 min. A neuron was considered to exhibit LTP or LTD if a significant change (compared to baseline) occurred in the slope of responses recorded 20 – 30 min after the pairing protocol (a Student's T-test was used for analysis). Before pulling the data together for between group-analysis, we normalized each neuron's responses to its own baseline mean value. Group results are presented as average ± the standard error of the mean at each time point.

2.1.5) LTP protocol 2 (temporal constraints)

To test whether the BLA facilitation of cortico-striatal LTP requires co-activation of the two sets of inputs, we used a second LTP inducing protocol. Cortical stimulation was delayed relative to the BLA, while still pairing each electrical stimulus with a postsynaptic AP (intracellular current injections with a 7 ms delay, 2 ms duration, similar to the previously described protocol I.4). Pairing all stimuli with APs was necessary to induce cortico-striatal LTP. In a small subset of neurons we tested this, separating BLA and cortex stimuli by 80 ms, and pairing only one of the two sites with APs; no cortico-striatal LTP was induced (data not shown).

To allow the evaluation of a wider range of inter-stimulation intervals (250 ms, 500 ms, and 800 ms), we repeated the pairings with a frequency of 1 Hz instead of 2 Hz (Fig. 14a1). The same number of stimuli as before (60) was delivered to each site, after which we assessed the change in the slope of cortically-evoked EPSPs.

2.1.6) Minimal stimulation experiments

Minimal stimulation recordings were performed in voltage-clamp mode, using a modified intracellular solution (in mM): 125 Cs-sulfate, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 2 ATP-Mg, and 0.2 GTP-tris(hydroxy-methyl) aminomethane (pH 7.2, 280 mOsm). Cells were voltage-

clamped at -50 mV. Then we gradually increased the stimulation intensity at each site (cortex and BLA) until both EPSCs and failures were recorded. The EPSCs were separated from failures off-line, using the histogram of amplitude of responses. The values were binned in 0.1pA steps, and fitted with a mixed two-Gaussian distribution using the expectation-maximization (EM) algorithm implemented in Matlab R2009a. The intersection of the two Gaussians was used as a cutoff to separate EPSCs from failures (considered noise). To compare the amplitude of responses (AMPA+NMDA or non-NMDA), only instances when EPSCs occurred were used to obtain the average for each cell, at each site (cortex and BLA).

2.2) *In vivo* techniques

2.2.1) Surgery

Procedures were approved by the Institutional Animal Care and Use Committee of Rutgers State University, in compliance with the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services). Six adult male cats were pre-anesthetized with a mixture of ketamine and xylazine (15 and 2 mg / kg, intra-muscular – im.) and artificially ventilated with a mixture of ambient air, oxygen, and isoflurane. Atropine (0.05 mg / kg, im.) was administered to prevent secretions. The end-tidal CO_2 concentration was maintained at 3.7 ± 0.2 %, and the body temperature at 37-38^{o}C using a heating

pad. Bupivacaine (1 ml, subcutaneous – sc.) was administered in the region to be incised 15 min prior to the first incision. In sterile conditions, an incision was performed on the midline of the scalp and the skull muscles were retracted. A reference screw was inserted in the skull overlying the cerebellum and silver-ball electrodes were placed in the supraorbital cavity to monitor eye movements. In addition, four screws were cemented to the skull to later fix the cat's head without pain or pressure. Finally, after trepanation and opening of the dura mater, an array of high-impedance tungsten microelectrodes (10-12 MΩ; Frederic Haer Co., Bowdoin, ME) was stereotaxically lowered to the regions of interest (see below).

Finally, the animals were administered penicillin (20,000 UI / kg, im.) and an analgesic (Ketophen, 2 mg / kg, sc., daily for 3 days). Recording sessions began eight days after the surgery.

2.2.2) Recording sites and construction of microelectrode array

The microelectrode array included six electrodes aimed to the basal amygdala nuclei, eight electrodes aimed to the ventral striatum, eight electrodes aimed to primary or associative auditory cortical areas, and five electrodes aimed to rostral or posterior thalamic intralaminar nuclei. To construct the microelectrode array, a computer controlled milling machine was used to drill small holes in a Teflon block, at stereotaxically defined relative positions. Then,

microelectrodes were inserted in the holes, adjusting the length of each microelectrode such that recordings could be obtained simultaneously from the various recording sites. After cementing the electrodes, the Teflon block was inserted in a tightly fitting Delrin sleeve, which was cemented to the skull. During the recording sessions, the electrodes could be lowered as a group by means of a micrometric screw.

2.2.3) Histology

At the end of the experiments, the animals were given an overdose of sodium pentobarbital (50 mg/kg, i.v.). Recording sites were marked with electrolytic lesions (0.5 mA, 5-10 s), after which the brains were extracted and fixed with a solution containing 2% paraformaldehyde and 1% glutaraldehide in 0.1 M phosphate buffer saline. The brains were later sectioned on a vibrating microtome (at 100 μm) and stained with cresyl violet to verify the position of recording electrodes. Microelectrode tracks were reconstructed by combining micrometer readings with the histology (Fig. 16).

2.2.4) Recordings

During the experiments, neuronal activity was sampled at progressively more ventral locations, in 100 μm intervals. To insure mechanical stability, the

microelectrodes were moved only once a day, 30 min prior to beginning data acquisition. The signals picked up by the electrodes (0.1 Hz to 20 kHz) were observed on an oscilloscope, digitized (Multi Channel Systems, Reutlingen Germany), and stored on a hard disk. Spike sorting and analysis of local field potentials (LFPs) was performed off-line, with custom software written in Matlab 2009a.

2.2.5) Muscimol injections

To assess the contribution of BLA activity to striatal LFPs, we compared the effects of saline vs. muscimol infusions in the BLA on striatal LFP power. To this end, under isoflurane anesthesia and sterile conditions, two cats were implanted bilaterally with stainless steel guide cannulas aimed at the rostro-caudal center of the BLA, under stereotaxic guidance. The cannulas were positioned at the dorsal limit of the BLA, allowing full dorsoventral access for drug infusions. In these cats, we also placed tungsten microelectrodes in the striatum, as described above.

One week after the surgery, the animals were gradually adapted to head restraint. During this period, they had restricted access to food, as for the subjects participating in the learning task (see below) and were only fed while in the recording room. Once adapted to head restraint, recording sessions began with a 15 min baseline recording period, after which a total volume of 1 µl of

saline or muscimol (4 nM in saline) per hemisphere was infused in the BLA at a rate of 0.08 µL/min. To this end, a microsyringe with a 25-gauge needle was lowered through the guide cannula and the solution was pressure-injected at ten equidistant sites (0.2 mm spacing) centered on the inner 2 mm of the BLA. The procedure was repeated for the contralateral side, and the recording continued for an additional 30 min. Two to three saline, and two to three muscimol infusions were performed on alternating days.

2.2.6) Behavior

Four cats were trained on a stimulus-response task where the termination of one of two tones (CS^+) coincided with the presentation of a liquid food reward (Gerber's pureed baby food "Sweet potatoes and turkey"; 2 ml/trial). The food was available for only 1 s, and the animals quickly learned to lick during this interval, consuming the food in more than 90% of CS^+ presentations (even during the first training session). The CS^+ and CS^- tones lasted 3 seconds and were presented in a random order with 20–40 s inter-tone intervals. The identity of the CS^+ and CS^- (3 and 12 kHz) was varied systematically across cats and had no effect on learning progression. Each daily training session, around 60 CS^+ and 60 CS^- trials were performed. Licking behavior was detected when the cats' tongues interrupted an infrared beam. The animals were only fed during the recording sessions. As a result, they were aroused and remained awake at all times. The cats' weight was monitored daily to maintain it within 10% below the

initial value.

After five consecutive training sessions, the CS-reward contingencies were reversed. That is, the initial CS^+ became CS^-, and vice-versa. Three such reversal sessions were recorded.

2.2.7) Data analysis

Data was analyzed offline with custom software written in Matlab 7.1 (The MathWorks Inc., Natick, MA). Spike-sorting was performed on digitally filtered data (high pass filter > 150 Hz), using a supervised k-means clustering algorithm. To analyze LFP interactions in specific frequency bands, the raw data was filtered with a 10 Hz bandpass filter, centered on the required frequency (e.g. results presented for 40 Hz correspond to data filtered in a 35–45 Hz band). For comparison purposes, all 2D and 3D histograms were normalized to the total number of events used, emphasizing their relative distribution rather than the absolute numbers. Statistical analyses consisted of repeated measures ANOVAs followed by Bonferonni-corrected t-tests. All values are reported as average ± SEM. To study learning-related fluctuations in BLA-striatal coherence, power in the particular frequency band under consideration was calculated in one-second windows (sliding in 100 ms steps) around the onset of the two tones for the two recording sites. Coherence was estimated by computing the product of the powers for the two recoding sites for each one-second time window.

Learning was assessed by monitoring the proportion of CS^+ and CS^- presentations during which anticipatory licking occurred. In addition, using the 3 sec windows preceding tone onsets, we computed the proportion of trials with spontaneous licking for comparison with tone-evoked behavior.

CHAPTER 3

Aim 1

Expression of ionotropic glutamate receptors at amygdala and cortical inputs on MSNs

3.1) Background

Increased amygdala activity can facilitate plasticity in other structures such as visual cortex or hippocampus (Ikegaya et al., 1994, 1995b; Dringenberg et al., 2004), and this effect is believed to depend on the activity of cholinergic neurons in the basal forebrain (Frey et al., 2001; Dringenberg et al., 2004). However, the amygdala can also modulate striatal-dependent learning (Packard et al., 1994; Packard and Teather, 1998), even though striatum receives little, if any, cholinergic projections from the basal forebrain (Mesulam et al., 1992). Since the source of striatal acetylcholine is intrinsic, a different mechanism could be responsible for the facilitation of striatal-dependent learning by the BLA.

BLA neurons project to the ventral striatum, and are thus in a position to directly modulate neuronal activity at this level. In this section, I test the hypothesis that BLA synapses onto MSNs (the principal cells of striatum) are endowed with special properties that might allow them to facilitate heterosynaptic activity-dependent plasticity. It has been proposed that a high ratio of NMDA to AMPA receptors might allow a non-specific and persistent postsynaptic activity (Fuster and Alexander, 1971), which would, at the same time, allow the target neurons to increase their temporal summation capabilities, and perhaps favor NMDA-dependent synaptic plasticity. Although previously proposed as a cellular basis for the facilitation of learning by the amygdala (Pare, 2003), this mechanism has not been investigated so far.

To directly address the question of whether BLA inputs to MSNs of the

ventral striatum have a different NMDA-to-AMPA ratio than cortical inputs to the same cells, we performed *in vitro* experiments using brain slices obtained from guinea pigs. The advantage of this system is that projections from both BLA and cortex to the striatum are unidirectional, and they can be preserved in coronal slices. Moreover, these projections overlap in a relatively large area of the ventral caudate-putamen. This allows us to characterize, in the same MSN, responses to electrical BLA and cortex stimulations, while being confident that the excitatory postsynaptic potentials (EPSPs) do not result from activating pluri-synaptic pathways (a strategic advantage over *in vivo* studies).

3.2) Methods

Brain slices were prepared as described in section 2.1.1, and we recorded MSNs using whole-cell patching (section 2.1.2). Under normal conditions, at rest (around -90 mV for MSNs), NMDA receptors are blocked by Mg^{2+}, and ionic flow through these receptors occurs only when Mg^{2+} is removed by depolarization. One common way to reveal this current is to keep neurons at a positive potential in voltage-clamp mode, thus releasing Mg^{2+} from its binding site. MSNs, however, are not very good candidates for voltage-clamp experiments, due to their complex dendritic arbor, and very low input resistance. Indeed, the effects of current injection at the soma drastically diminish with increasing distance from the patching pipette, a phenomenon known as lack of spatial clamp. To avoid this problem, we recorded synaptic responses at rest, and instead removed the Mg^{2+}

ions from the aCSF to reveal the NMDA component. Thus, in all experiments in which we measured the NMDA-mediated responses, a Mg^{2+} – free aCSF was used for brain extraction, slicing and recording.

3.3) Results

3.3.1) Properties of MSNs

Whole-cell *in vitro* recordings of MSNs were obtained under visual guidance. MSNs were identified by their characteristic electrophysiological properties (Kawaguchi et al., 1989) including low input resistance (69.8 ± 3.1 MΩ), extremely negative resting potential (-90.0 ± 0.5 mV), and inward rectification in the hyperpolarizing direction (Fig. 5A; (Kawaguchi et al., 1989; Nisenbaum and Wilson, 1995; Tepper et al., 1998)). In a subset of experiments, we could verify that these physiological features characterized MSNs, by adding neurobiotin to the intracellular solution for post-hoc morphological identification of recorded cells. All recovered cells that had been classified as MSNs on the basis of these electrophysiological criteria (n = 8) had the typical morphology of principal striatal neurons including multiple primary dendrites that branch extensively and bear a high density of spines (Fig. 5B).

Electrical stimulation of BLA and cortex elicited synaptic responses in all recorded neurons. In some cases however, the amplitude was smaller than our required threshold (10 mV); these MSNs were not included in the analysis. The

EPSP onset latency for BLA stimuli was 5.3 ± 0.1 ms, and for cortex 4.3 ± 0.1 ms, consistent with monosynaptic EPSPs.

3.3.2) NMDA-to-AMPA ratio

MSNs were patched in a Mg^{2+}-free aCSF, and responses to electrical stimulation of BLA and cortex recorded (BLA and cortical stimuli were delivered 2s apart, at 0.1 Hz). To measure the NMDA-to-AMPA ratio, we consecutively added picrotoxin (100 μM, blocking inhibitory responses), CNQX (20 μM, to block AMPA responses) and AP-5 (100 μM, to ascertain the the remaining response was mediated by NMDA receptors) to the Mg2+ -free aCSF. Consistent with the idea that projections from both BLA and cortex are glutamatergic, adding CNQX and AP-5 in the presence of picrotoxin, abolished BLA and cortical responses in all recorded neurons (n = 15).

The NMDA-to-AMPA ratio was calculated by dividing the amplitude of the isolated NMDA responses (recorded in the presence of picrotoxin and CNQX), to the AMPA component (amplitude of EPSPs recorded in picrotoxin only, minus the NMDA component; see Fig. 6A). As shown in figure 6B, we consistently found higher NMDA-to-AMPA ratios at BLA synapses compared to cortical inputs (n = 15, P < 0.0001 paired-T test). On average, the ratio was 0.38 ± 0.04 for BLA responses, compared to 0.22 ± 0.03 for cortical ones (Fig. 7C, P = 0.009, Student's T-test).

3.3.3) NR_{2A}/NR_{2B} subunit composition

The above results are consistent with several possibilities: 1) the number of NMDA receptors relative to AMPA receptors is higher at BLA synapses than at cortical synapses; 2) the number of receptors is similar, but the NMDA receptors at BLA synapses have an overall higher conductance; 3) a combination of the two. The second possibility could be explained by a different subunit composition of the NMDA receptors between the two sites.

Previous studies have shown that activation of NMDA receptors has different postsynaptic effects, depending on their subunit composition. With respect to synaptic plasticity, it appears that NMDA receptors expressing NR_1/NR_{2A} or NR_1/NR_{2B} are differentially required for LTP and LTD induction (Liu et al., 2004; Massey et al., 2004). This could be due to a differential link to intracellular second messengers of NR_{2A} and NR_{2B} containing receptors (Sala et al., 2000; Hardingham et al., 2002).

We therefore tested the possibility that NMDA receptors at BLA synapses on MSNs have a different subunit composition than receptors at cortical synapses. Responses to electrical stimulation of the two structures were recorded in striatal MSNs, in aCSF containing 0 Mg^{2+}, 100 µM picrotoxin and 20 µM CNQX (insuring that the measured responses were selectively NMDA-mediated). Next, we bath-applied 5 µM ifenprodil to specifically block the NR_{2B}–

containing receptors, and compared the effect on cortical vs. BLA responses. At the end of each experiment, to confirm that the measured EPSPs were NMDA-mediated, we added 100 μM AP-5 (which completely blocked the responses in all neurons; n = 9). For comparison, the EPSP amplitudes of each neuron were normalized to their respective NMDA responses, recorded immediately prior to ifenprodil application.

Ifenprodil decreased the amplitude of NMDA-mediated EPSPs evoked from both sites, suggesting that NR_1/NR_{2B} receptors contribute similarly at both sets of synapses (Fig. 7). We observed no significant difference in the response to ifenprodil between the two groups (cortical responses 0.55 ± 0.07, BLA responses 0.67 ± 0.08; P = 0.21, paired-T test; n = 9), consistent with the idea that BLA and cortical synapses onto MSNs have a similar NR_{2A} and NR_{2B} subunit composition.

3.3.4) Minimal stimulation

Since the subunit composition of NMDA receptors seems to be similar for BLA and cortical synapses on MSNs, it is likely that the different NMDA-to-AMPA ratio observed in 3.3.2 is due to the expression of different numbers of AMPA and/or NMDA receptors at each synapse. To test this hypothesis, we recorded the responses in MSNs (kept at -50 mV in voltage-clamp) to BLA and cortical minimal stimulations. We gradually increased the intensity of electrical

stimulation at each site until both EPSCs and failures were recorded. This method has been used to monitor the activity of individual synapses (Isaac et al., 1996), giving us the opportunity to directly compare the amplitude of AMPA, NMDA and combined responses.

Intracellular CS^{2+} was used to make MSNs more electrically compact (see Methods, 2.1.6). After finding the minimum stimulation intensities at which both EPSCs and failures could be observed, we recorded BLA and cortical responses for 20 minutes (approximately 200 stimuli applied every 6 sec). The presence of both EPSCs and misses leads to a bimodal distribution for the amplitude of responses, in which the peak centered on zero corresponds to failures (Fig. 8A). This distribution can be fitted with a 2-Gaussian mixture, using the expectation-maximization algorithm. We used the intersection of the two curves as a cutoff to separate the EPSCs from noise (Fig 8B). Using this procedure, we were able to measure the amplitude of responses at a synaptic level.

While the amplitude of responses was more variable at cortical than at BLA synapses, we found no significant difference in the total (AMPA + NMDA) amplitude of EPSCs: BLA 4.02 ± 2.01 pA; cortex 8.87 ± 5.4 pA; T-test P = 0.4, n=7. The probability of release was also similar at the two sites: BLA 0.53 ± 0.05; cortex 0.44 ± 0.09; T-test P = 0.43, n=7.

Next we applied AP-5 to the solution, in order to isolate the non-NMDA component (Fig. 8C), and again recorded the cortical and BLA responses to minimal stimulation. Consistent with our previous findings (3.3.2), cortical EPSCs

showed a smaller NMDA component than BLA EPSCs. There was a significant difference between the amplitude of BLA and cortical responses after AP-5 application: BLA 2.82 ± 1.91 pA; cortex 5.17 ± 2.81 pA; paired T-test P = 0.028, n=7.

Overall, these findings suggest that BLA synapses on MSNs have more NMDA, and less AMPA receptors than cortical synapses. This property in turn, might give BLA synapses preferential access to intracellular second messenger systems as a consequence of larger Ca^{2+} influx. Since several forms of synaptic plasticity (cortico-striatal LTP included) require activation of NMDA receptors (Calabresi et al., 1992a), it seems possible that the higher NMDA-to-AMPA ratio at BLA inputs might facilitate plasticity in the cortico-striatal pathway. This possibility is tested in Chapter 4.

CHAPTER 4

Aim 2

Activating amygdala inputs enhances cortico-striatal LTP

4.1) Background

Several groups have investigated the mechanisms of BLA facilitated synaptic plasticity using *in vivo* preparations (Ikegaya et al., 1994, 1995b; Frey et al., 2001; Dringenberg et al., 2004). Unfortunately, this method allows only a limited number of experimental manipulations and interpretations, since BLA stimuli are likely to activate a number of structures, any of which might be the mediator of the facilitatory effects. In fact, it was shown that the cholinergic basal forebrain mediates the effects of BLA stimulation on cortical or hippocampal LTP (Brocher et al., 1992; Ikegaya et al., 1997; Kirkwood et al., 1999; Pesavento et al., 2000; Dringenberg et al., 2004; Kuczewski et al., 2005; Origlia et al., 2006). However, striatum does not receive cholinergic inputs from the basal forebrain, leaving open the question of whether BLA stimuli can directly affect striatal plasticity, and what would the underlying mechanism be.

To address the question of whether amygdala axons can directly modulate synaptic plasticity in the striatal MSNs, we tested the effects of electrical BLA stimulation on cortico-striatal LTP induction. LTP at cortical synapses on MSNs seems to be preferentially induced by manipulations that enhance the activity of NMDA receptors (postsynaptic depolarization, removal of Mg^{2+} from the aCSF; (Calabresi et al., 1992b; Calabresi et al., 1992a; Lovinger et al., 1993; Charpier and Deniau, 1997; Partridge et al., 2000). We hypothesized that because of their relatively high NMDA-to-AMPA ratio, activating BLA inputs to the striatum would facilitate the induction of cortico-striatal LTP, without the need for

pharmacological manipulations.

4.2) Methods

Brain slices were prepared as described in section 2.1.1, and we recorded MSNs using whole-cell patching (section 2.1.2). Pairs of stimulating electrodes were placed at two different cortical locations, and one BLA site (Fig. 6). We used the occlusion test (section 2.1.3) to determine the amount of overlap between various stimulation sites. Cortico-striatal LTP was induced using a modified spike timing-dependent protocol, as described in section 2.1.4 and section 2.1.5.

4.3) Results

4.3.1) BLA stimulation facilitates cortico-striatal LTP induction

In a first set of experiments, two non-overlapping cortical sites were paired with postsynaptic APs. The occlusion test confirmed that the cortical responses originated from independent sets of axons (amplitude of paired responses was 100 ± 1.4 % of the summed EPSCs; $P > 0.05$, paired T-test). Consistent with previous findings regarding cortico-striatal LTP induction (Calabresi et al., 1992a), our LTP-protocol (see section 2.1.4) elicited a mixture of LTP (44 % of

tested cells), LTD (37 % of cells), and no change (19 % of cells, Fig. 9C). Averaging all recordings (n = 16), we observed a small, but significant increase from baseline (119.9 ± 8.0 % of baseline, P = 0.023, n = 16, Fig. 9A).

Next, we tested the effects of BLA stimulation on cortico-striatal LTP induction. The occlusion test confirmed that there was no overlap between axons stimulated by electrodes placed in the BLA and those in cortex (amplitude of summed responses was 96.6 ± 2 % of the predicted one; P > 0.05, paired T-test). Pairing BLA and cortex with somatic APs, produced cortico-striatal LTP in almost all recorded cells (91 % of neurons, n = 11, Fig. 9C), while no LTD was observed. The incidence of LTP was significantly different from that obtained by pairing two cortical sites with APs (χ^2 test, P = 0.04).

Similarly, the amplitude of cortico-striatal LTP was higher when BLA was stimulated. The group average showed a 60.9 ± 13.2 % increase from baseline (P = 0.0012, n = 11, Fig. 9B), which was significantly larger than the 19.9 ± 8.0% increase observed with two cortical sites (P = 0.016, Fig. 9A, and D).

To determine whether this facilitation of cortico-striatal LTP by BLA stimulation is input specific, we used the same LTP induction protocol, and monitored the responses of a second, un-paired cortical site. As shown in figure 8B, responses to the stimulation of this cortical site (kept silent during the induction protocol) did not change (98.9 ± 9.5 % of baseline responses, P = 0.918, n = 5, Fig. 9D), suggesting that only synapses active during the pairing protocol are potentiated.

By contrast, the BLA synapses activated during the pairing protocol showed much smaller changes from baseline than the one observed at cortical inputs (122.2 ± 8.6 % of baseline, P = 0.02, n = 11, Fig. 10). This is intriguing, considering the higher ratio of NMDA-to-AMPA present at BLA synapses.

4.3.2) NMDA-dependence of cortico-striatal LTP

Many, but not all forms of LTP require NMDA receptor activation during the induction phase (Brown et al., 1988; Bear and Kirkwood, 1993; Malenka and Bear, 2004). In the cortico-striatal pathway, LTP induction critically depends on Ca^{2+} entry through NMDA receptors (Calabresi et al., 1992a). Consistent with these observations, we found that the addition of AP-5 (100 µM) to the aCSF right before and during the BLA-cortex-AP pairings prevented LTP induction. The amplitude of post-pairing responses under these conditions was 114.6 ± 11.2% of baseline (P = 0.27, n = 5, Fig. 9D). Similarly, when MSNs were dialyzed with the Ca^{2+} chelator BAPTA, no LTP was induced (100% of cells, n = 5, Fig. 11).

Bath-application of AP-5 blocks NMDA receptors both pre, and postsynaptically, preventing us from determining the critical site of action. Indeed, sometimes the NMDA receptors required for LTP-induction are located presynaptically (Humeau et al., 2003; Samson and Pare, 2005). In our case however, dialyzing MSNs with 1 mM MK-801 (intracellular) prevented LTP induction (95.5 ± 4.1 %, P = 0.2, n = 5, Fig. 9D). This suggests that Ca^{2+} entry

through postsynaptic NMDA receptors is required for the facilitatory effects of BLA on cortico-striatal LTP.

These findings support a model in which cortico-striatal LTP is facilitated by BLA activity, and requires activation of postsynaptic NMDA receptors. As shown in Chapter 3, BLA synapses on MSNs have a higher ratio of NMDA-to-AMPA than cortical ones. But which NMDA receptors are involved in the induction of LTP at cortico-striatal synapses: the ones postsynaptic to BLA axon terminals, cortical inputs, or both?

To address this question we modified a protocol previously used to block only synaptic NMDA receptors (Massey et al., 2004). This protocol takes advantage of MK-801 properties: a noncompetitive, irreversible open NMDA-channel blocker. By selecting the subset of inputs that are activated in the presence of MK-801, we were able to specifically block NMDA receptors at only one set of synapses: cortex, or BLA.

First, we tested whether we could selectively block NMDA receptors at BLA synapses using this method. To this end, we bathed brain slices in 5 μM MK-801, and stimulated the BLA site for 15 min at 1 Hz, after which the slice was washed with regular aCSF for 1 h, to remove the unbound MK-801. At this stage, a MSN was patched, and the NMDA-to-AMPA ratio measured, as previously described in Chapter 3.

Following this protocol, we observed a clear and selective reduction of the

NMDA component at BLA synapses. The NMDA-to-AMPA ratio for BLA inputs after the NMDA-blocking procedure (0.13 ± 0.03, n = 5) was significantly smaller ($P < 0.001$) than the values obtained in control conditions (0.38 ± 0.04, n = 15, Fig. 12). This dramatic change contrasts with the unchanged ratios measured at cortical inputs to MSNs, in the same experiment (0.19 ± 0.05, n = 5 after blocking BLA-NMDA receptors, vs 0.22 ± 0.03, n = 15 under control conditions; $P = 0.67$).

Having established the selectivity of this protocol, we tested whether blocking NMDA receptors at BLA or cortical inputs to the striatum interferes with cortico-striatal LTP induction. After selective blockade of NMDA receptors at BLA synapses, BLA-cortical pairings produced only a slight increase in the slope of cortically-evoked responses (126.6 ± 14.5 %, $P = 0.12$; n = 6; Fig. 13A). This was comparable in magnitude to that seen with the pairing of two cortical sites, in control aCSF (119.9 ± 8.0 %, $P = 0.699$, Fig. 9). When the same protocol was used to selectively block NMDA receptors at cortical synapses, BLA-cortex pairings failed to induce LTP at cortical inputs (102.6 ± 3.6 %, $P = 0.526$, n = 4; Fig. 13B).

Taken together, these results suggest a functional dissociation of NMDA receptors located at cortical vs. BLA synapses on MSNs. Although NMDA receptors at cortical synapses are essential for LTP induction, they are only responsible for a modest (about 20%) increase from baseline. The induction of high levels of LTP is dependent on NMDA receptors located at BLA inputs.

4.3.3) Temporal requirements for BLA-facilitated cotico-striatal LTP

Various temporal rules have been described for the induction of LTP. Spike timing-dependent protocols, similar to the one used here, emphasize the importance of the precise timing between the paired EPSP and AP. LTP usually occurs when APs follow the evoked responses in a temporal window of 0 – 40 ms (Bi and Poo, 1998; Dan and Poo, 2004), while delivering APs prior to EPSPs usually results in LTD.

In our protocol, we used a 7 ms delay between the stimulation of two sites (cortex + cortex / BLA) and somatic APs. Since the latency of the responses varied between 3 and 6 ms, the delay between EPSPs and APs was 1 – 4 ms, falling well within the window for inducing LTP.

The facilitatory effects of BLA stimulation however, are not necessarily constrained by these temporal rules. Since the activation of NMDA receptors at BLA synapses mediates these effects, cortico-striatal LTP might be enhanced even if we separate BLA and cortical stimuli by 100ms. As shown in figure 7A (Chapter 3), NMDA currents last for more than 200 ms at both BLA and cortical synapses, and thus can overlap for a significant amount of time.

So how temporally lax is the effect of amygdala stimulation on cortico-striatal LTP? This question is particularly important, since precise and persistent temporal coincidence between the activation of BLA and other striatal inputs might be achieved rarely *in vivo*. Specifically, the firing rates of BLA projection

neurons are much lower than those of cortical pyramidal cells (Softky and Koch, 1993; Cowan and Wilson, 1994; Niida et al., 1997; Pelletier et al., 2005). So how much variation can be tolerated in this system? We tested the hypothesis that precise temporal coincidence of BLA and cortical stimuli is not required for the BLA facilitation of cortico-striatal LTP. If this is true, then separating the BLA and cortical stimuli by increasing intervals (while still pairing each of them with APs), should still facilitate LTP induction at cortico-striatal synapses, but result in progressively lower amounts.

First, we wanted to rule out the possibility that the lower pairing frequency required for these experiments modifies the magnitude of LTP. To this end, we compared levels of cortico-striatal LTP induced by simultaneous BLA-cortex stimulations paired with somatic APs at 1 Hz rather than 2 Hz (LTP protocols 2.1.4 and 2.1.5). These two induction protocols produced nearly identical amounts of cortico-striatal LTP (1 Hz, 164.3 ± 15.5 %, n = 4, Fig. 14B, data-point at 0 s; 2 Hz, 160.9 ± 13.2 %, n = 11, Fig. 9D; T-test, P = 0.88).

Next, using a stimulation frequency of 1 Hz, we tested various BLA-cortex intervals (0.25, 0.5, or 0.8 s) while still pairing each of them with an action potential, and keeping unchanged the number of stimuli delivered at each site (Fig. 14A). Despite the lack of coincidence between BLA and cortical stimuli, separating the two inputs by as much as 500 ms produced a significant facilitation of cortico-striatal LTP (500 ms, 141.7 ± 10.6 % of baseline, P = 0.017; n = 5, Fig. 14B), whereas no significant LTP was observed with longer inter-

stimulus intervals (800 ms, 98.8 ± 16.25 %, n = 7, Fig. 14C).

The results of these experiments show that BLA inputs to the striatum have the ability to enhance cortico-striatal LTP. This effect extended only to cortical synapses that were activated within 500 ms following BLA stimuli, and required NMDA receptor activation at both BLA and cortical synapses on MSNs. Indeed, decreasing the NMDA-to-AMPA ratio at BLA synapses on MSNs blocked the facilitatory effects of BLA stimulation. This suggests that the NMDA receptors at BLA synapses activate one or more intracellular messengers, which are able to facilitate cortico-striatal LTP. The effect of the messenger(s) decays with time, but it is maintained for a period of at least 500 ms. Cortical inputs activated within this time-window find MSNs in a state favorable for LTP induction, and are thus potentiated. In Chapter 5, I investigate the role of Ca^{2+} release from intracellular stores as a potential mediator of the effects of BLA stimuli on cortico-striatal LTP.

CHAPTER 5

Aim 3

Ca^{2+}-induced-Ca^{2+}-release is required for
the facilitation of cortico-striatal LTP by BLA activation

5.1) Background

The results presented in the previous chapters raise a very important question: how is BLA activity translated into facilitated heterosynaptic plasticity? Do BLA axons contact MSN dendrites in a position favorable to modulate other inputs? Ultrastructural studies suggest that this is not the case (Kita and Kitai, 1990; Johnson et al., 1994): amygdala projections to the striatum form “en passant” connections with striatal spine heads, similar to cortical inputs, and do not have any specific morphological marker (such as synapsing on spine necks, or shafts). One possibility is that the Ca^{2+} entering through NMDA receptors at BLA synapses might passively diffuse, thus slightly increasing the concentration at cortical synapses and promoting LTP induction. However, the Ca^{2+} influx resulting from synaptic events in MSNs seems to be mostly restricted to the spine head generating it, and does not lead to significant changes in the adjacent dendritic shaft (Yuste and Denk, 1995; Carter and Sabatini, 2004). Therefore, the heterosynaptic effects of BLA stimulation are probably conveyed by an intracellular second messenger.

Is there an interaction between BLA inputs to the striatum and local dopamine activity? As detailed in the introduction (section 1.4.2), *in vivo* studies suggest that this is indeed the case, raising the possibility that dopaminergic activity might play a role in the effects of BLA stimuli on cortico-striatal LTP. Many studies demonstrated an important role of dopamine in modulating cortico-striatal plasticity (Reynolds and Berridge, 2002; Costa, 2007; Wickens et al.,

2007). In particular, blocking dopamine D_1 receptors was found to disrupt cortico-striatal LTP (Kerr and Wickens, 2001; Centonze et al., 2003; Pawlak and Kerr, 2008; Schotanus and Chergui, 2008). In this chapter, I test the hypothesis that BLA stimulation can bypass the requirement of D_1 receptor activity for cortico-striatal LTP induction. To this end, I attempt cortico-striatal LTP induction by BLA-cortex pairing in the presence of $D_{1/5}$ receptor antagonist SCH-23390.

The experiments described in this chapter, also address a different, but related question: how can we explain the timing of the BLA-stimuli effects on cortico-striatal LTP? The results of the previous chapter (Chapter 4, 4.3.3) show that these stimuli can affect cortical synapses activated even 500 ms later (Fig. 12). Thus, the intracellular messenger mediating the effects must be able to span the distance between the two sets of synapses, and last for at least 500 ms. One possibility is that intracellular waves of Ca^{2+}-induced Ca^{2+}-release (CICR) are generated by BLA stimuli. These events depend on intracellular Ca^{2+} stores (such as smooth endoplasmic reticulum), and can raise the Ca^{2+} concentration at distant locations, well beyond the limits of passive diffusion (Barbara, 2002). Moreover, CICR has a slow decaying time, which fits well with our requirements.

CICR can be triggered by calcium influx through NMDA receptors, and have a significant impact on synaptic homeostasis (Bardo et al., 2006). It is thus possible that the larger Ca^{2+} influx produced by the activation of NMDA receptors at BLA synapses triggers CICR. In the present chapter, I describe experiments suggesting that CICR is necessary for the induction of cortico-striatal LTP by

BLA-cortex pairing.

5.2) Methods

Brain slices were prepared as described in section 2.1.1, and we recorded MSNs using whole-cell patching (section 2.1.2). Pairs of stimulating electrodes were placed at one cortical location, and one BLA site. Induction of cortico-striatal LTP was attempted with the protocol described in section 2.1.4. BLA and cortical sites were co-stimulated, and paired with postsynaptic APs 60 times, with a frequency of 2 Hz. To test the involvement of CICR events, we placed ruthenium red (50 μM), or cyclopiazonic acid (CPA, 15 μM) in the intracellular recording solution. Conversely, the role of dopaminergic $D_{1/5}$ receptors was assessed by bathing the slices with 10 μM SCH-23390 for 5 min prior, and during the LTP-induction period.

5.3) Results

CICR can be induced by an initial, local increase in the intracellular Ca^{2+} concentration as a result of Ca^{2+} influx through NMDA receptors (Bardo et al., 2006). The ryanodine receptors located on smooth endoplasmic reticulum are activated by increased [Ca^{2+}], and subsequently release more calcium from these intracellular stores. Ca^{2+} moves away from the release site by passive

diffusion, activating more ryanodine channels and propagating a wave of intracellular Ca^{2+} away from its initiation site. Since the facilitation of cortico-striatal LTP by BLA stimuli requires Ca^{2+} entry through BLA-NMDA receptors (as described in the previous chapters), it is possible that CICR is a critical mediator of the BLA signal, by amplifying it, and increasing its spatial extent.

As mentioned earlier, repeatedly pairing BLA and cortical stimulations with postsynaptic APs leads to a significant enhancement of cortical responses in MSNs. We tested the involvement of CICR by blocking the ryanodine receptors (with 50 μM intracellular ruthenium red), and repeating the same LTP-inducing protocol in a subset of MSNs. Under these circumstances, the coincident pairing of CTX and BLA with APs did not result in LTP. Cortical responses were almost identical to the pre-pairing values (98.8 ± 4.7 %, $p = 0.736$, $n = 5$; Fig. 15A).

Similar results were observed when we depleted the intracellular Ca^{2+} stores with 15 μM CPA (a Ca^{2+}-ATPase inhibitor). Responses to cortical stimuli following the BLA-cortical pairings were 98 ± 2.17 % of baseline ($p = 0.4$, $n = 6$; Fig. 15B). As illustrated in figure 13, our pairing protocol in the presence of CICR blockers led to a small decrease of cortically evoked responses. However, these changes were transient, lasting less than 15 minutes.

These experiments provide a novel mechanism by which cortico-striatal LTP can be modulated: **1.** Increased BLA activity would activate postsynaptic NMDA receptors located on MSNs; **2.** The subsequent Ca^{2+} influx, acting on ryanodine receptors located on endoplasmic reticulum initiates CICR; **3.** The

ensuing propagating Ca^{2+} waves increase [Ca^{2+}] at cortical synapses, favoring the induction of cortico-striatal LTP.

But how can this pathway be integrated with the currently known mechanisms of cortico-striatal LTP? A substantial body of data indicates that striatal plasticity requires dopaminergic activity. In many studies, blocking dopaminergic D_1 receptors disrupted cortico-striatal LTP (Kerr and Wickens, 2001; Centonze et al., 2003; Pawlak and Kerr, 2008; Schotanus and Chergui, 2008).

We tested the hypothesis that the BLA facilitation of cortico-striatal LTP is not dependent on dopaminergic activity by blocking $D_{1/5}$ receptors with bath applications of 10 μM SCH-23390 (a selective $D_{1/5}$ receptor antagonist) for 5 min prior, and during the LTP-induction period. As shown in figure 15C, under these manipulations no cortico-striatal LTP was observed following BLA-cortex pairings (change from baseline: 107.5 ± 5.8 %). This suggests that, even in brain-slice preparations, there is a physiologically significant amount of dopaminergic activity that is required for cortico-striatal LTP induction. The recruitment of postsynaptic CICR waves following BLA stimuli cannot bypass this mechanism, and specifically blocking dopamine $D_{1/5}$-receptor activity prevents LTP induction.

CHAPTER 6

Aim 4

In vivo evidence of BLA – striatal interaction during learning

6.1) Background

The experiments presented in the previous chapters support the notion that during emotional arousal, the amygdala can directly enhance the plasticity of its targets, facilitating synaptic changes in pathways involved in relevant behavior. These experiments however, required coincident stimulation, and large intracellular current injections to induce postsynaptic APs. Thus, the question of how coordination of the amygdala and striatum might occur *in vivo* still remains.

Any model trying to explain amygdala facilitated learning would have to incorporate several aspects such as the interaction between various structures that are necessary for processing information, learning, storage and expression of the learned or modified behavior. There is, however, a general lack of knowledge in this regard. Most studies have focused on learning-induced changes in various systems, one structure at a time. How these alterations ultimately affect behavior is usually inferred from the connectivity within the system. In fact, there is very little data on how different regions interact to produce coherent behavior, and how these interactions change with learning.

For example, many neurons in both the amygdala and orbitofrontal cortex (OFC) encode outcome expectancy during learning (Holland and Gallagher, 2004), and amygdala lesions decrease the proportion of such neurons in the OFC (Schoenbaum et al., 2003). This suggests that information about expectancy is transferred from the amygdala to the OFC; however, similar

lesions of the OFC reduce the number of expectancy neurons in the amygdala (Saddoris et al., 2005), reflecting interdependence rather than a hierarchical transfer from one region to another.

The power of such studies (along with their interpretation) is limited however, since they can't provide accurate information about physiological interactions as they occur during learning. We decided to directly address these points, and to find out how the *in vitro* properties of the BLA-striatum pathway identified in Chapters 3-5 might come into play *in vivo*. We performed single unit and local field potential (LFP) recordings in cats learning an auditory, appetitive stimulus-response task. Neuronal activity was recorded simultaneously in several brain regions likely involved in learning this form of behavior: auditory cortex, BLA, striatum, and thalamus.

In this chapter, I characterize the interaction between BLA and the striatum, with an emphasis on how emotionally relevant information might be transferred from the former to the latter. I also test the hypothesis that this interaction changes with learning, reflecting the improvements in behavioral performance.

6.2) Methods

Six cats were implanted with an array of tungsten microelectrodes, for recording single unit and LFP activity in the BLA, auditory cortex, the putamen

region of the striatum, and intralaminar thalamic nuclei (sections 2.2.1-4).One week later, the animals were trained on an auditory appetitive stimulus-response task (section 2.2.6), and neuronal activity was recorded for off-line analysis (section 2.2.7). To determine the contribution of BLA activity to striatal LFP power, two different cats were implanted with cannulas aimed at the BLA for saline or muscimol infusion, and tungsten electrodes aimed at the putamen. The LFP activity was recorded in putamen, and compared before vs. after muscimol or saline infusion (section 2.2.5).

6.3) Results

6.3.1) Database

Unit activity and LFPs were recorded in six cats using high-impedance tungsten microelectrodes. All striatal recordings were obtained in a ventral sector of the putamen adjacent to the amygdala, where neostriatal projections of the cat BLA are densest (Pare et al., 1995). Histological controls (Fig. 16) revealed that our sample of extracellularly recorded cells included 139 putamen, 159 cortical, 55 thalamic intralaminar, and 152 BLA neurons. Analyses of firing rates and spike durations (see Fig. 17) revealed that within each structure, ≥ 70% of the neurons fell in one dominant cell class.

These neurons presumably correspond to principal striatal (medium spiny), cortical (pyramidal or stellate), thalamic (relay), or BLA (pyramidal)

neurons. The following analyses are restricted to these dominant classes of neurons.

The firing pattern of most neurons recorded in the putamen was similar to that of medium spiny neurons (Fig. 18). As previously described (Hori et al., 2009) these neurons were mostly silent, with brief periods of increased firing rate (around 10 – 40 Hz, Fig. 18B4 and 18C4) resulting in an overall low spiking frequency (< 5 Hz, Fig. 17B). This pattern is apparent in the histograms of inter-spike intervals (Fig. 18B3 and 18C3) and that of firing rates (Fig. 18B4 and 18C4). A small proportion of cells (8%) had higher firing rates (> 5 Hz), shorter spike duration, but similar spiking patterns (Fig. 18A). These cells, however, were not included in the analysis.

6.3.2) Correlated amygdalo-striatal activity in the waking state

In search of a possible physiological signature of BLA-striatal interactions, we first analyzed the coherence spectra of simultaneously recorded BLA and putamen LFPs during epochs of spontaneous waking (Fig. 19). This analysis revealed that, with the exception of frequencies below 3 Hz, coherence of BLA and putamen LFPs was maximal in the gamma range (35-45 Hz). To test if this property also applied to other major striatal inputs, we then carried out the same analysis between putamen and cortical sites (Fig. 19B) or between putamen and thalamic sites (Fig. 19C). Whereas all combinations of recording sites showed

coherent low frequency activity, the coherence of gamma oscillations was clearly much higher between putamen and BLA LFPs ($F(2,3819) = 1255.64$, $p < 0.0001$). In contrast, the coherence of BLA and thalamic or cortical gamma was low (Fig. 20).

Importantly, the same weak relationship was seen between striatal gamma vs. cortical or thalamic gamma whether we separately considered primary or associative auditory areas as well as posterior or anterior intralaminar thalamic nuclei. For instance, figure 15d shows digitally filtered LFPs (35-45 Hz) for two cortical (primary, CTX-1; associative, CTX-2), thalamic (anterior, THL-1; posterior, THL-2) putamen (anterior, STR-1; posterior STR-2), and BLA (lateral, BLA-1; medial BLA-2) sites.

Visual inspection of these signals confirmed the preferential coupling of striatal gamma to BLA activity, relative to thalamic and cortical fast oscillations. One possible explanation for this result could be that BLA recording sites are physically closer to the putamen than thalamic or cortical recording sites. At odds with this possibility however, the same results were obtained when we separately considered BLA, cortical, and thalamic recording sites that were equidistant from the putamen (Fig. 21).

Next, we sought to obtain quantitative estimates of the phase relationship between striatal gamma vs. cortical, thalamic, or BLA gamma (Fig. 22). To this end, striatal gamma cycles of high amplitude (> 2.5 SD of average) were identified and the interval between the peak of the striatal gamma cycles vs. the

other sites was measured. Figure 18 plots these phase lags (x-axis) against the amplitude of the corresponding gamma cycles (left y-axis), normalized to the amplitude of striatal gamma.

Observed phase lags covered the whole spectrum (-180 – +180 degrees), however they were much more variable at cortical and thalamic sites (Fig. 22 A-B, D-E) than in the BLA (Fig. 22 C, F). We tested this by computing an ANOVA on the cycle-to-cycle deviations from the average phase lag for the three combinations of recording sites (cortex and putamen; thalamus and putamen; BLA and putamen; $F(2,7317)=1526.5$, $p < 0.0001$).

A similarly variable phase relationship was seen between BLA and cortical or thalamic gamma (Fig. 22 G and H). On average, there was a 40 ± 14 degrees phase shift between BLA and striatal gamma, corresponding to an interval of 22 ms. We observed no relationship between the distance separating the recording sites and phase lags ($r = 0.001$; Fig. 23).

6.3.3) Contribution of BLA to striatal gamma power

To determine whether the coherent gamma activity seen in the BLA and putamen resulted from the influence of a common input or if the BLA contributed to the generation of gamma activity in the striatum, we compared the effects of bilateral intra-BLA infusions of 1 µl (per hemisphere) saline ($n = 4$) or muscimol ($n = 5$; 4 nM in saline) on putamen LFP power.

Here, it should be noted that the volume of the cat BLA is much higher than in rats, with the cat basolateral nucleus spanning > 4 mm in the dorsoventral axis, compared to 1 mm in rats. Thus, to expose as many BLA neurons to muscimol as possible while minimizing mechanical distortion of the tissue and muscimol diffusion outside BLA, the total injected volume (1 µl / hemisphere) was divided in 10 infusion sites (0.1 µl each) separated by 0.2 mm along a single dorsoventral microsyringe track that spanned the central 2 mm of the BLA. In each subject, this process was repeated sequentially in both hemispheres, resulting in total infusion times of 25 minutes (see methods in section 2.2.5).

Whereas intra-BLA saline infusions had no effect on putamen LFP activity (Fig. 24 A, D, black trace in C; post-hoc paired T-test, $p = 0.2$), muscimol infusions caused a statistically significant reduction of striatal LFP power that was mostly restricted to gamma activity (Fig. 24 A, D, red line in C; post-hoc paired T-test, $p = 0.0005$).

6.3.4) BLA – striatum unit coupling

To examine the relationship between gamma oscillations and unit activity in the BLA and striatum, we constructed peri-event histograms (PEHs) of unit activity around the positive peaks of gamma oscillations recorded by the same microelectrode as the unit activity.

To this end, we first isolated gamma by digitally filtering the LFPs (35-

45Hz bandpass) and then identified gamma cycles with positive peak exceeding the 2.5 SD of the overall filtered signal (Fig. 25A). Then, we computed histograms of spike firing for each cell around these reference times. Figure 25 shows examples of such PEHs for BLA (Fig. 25B), and putamen neurons (Fig. 25C). Although these PEHs give the impression that BLA and putamen cells fired repetitively in relation to gamma, this was not usually the case. In fact, although gamma activity typically occurred in short bursts comprised of 2-6 consecutive high amplitude cycles, BLA and striatal cells fired infrequently during each burst (BLA 0.16 ± 0.02 spikes per burst; putamen, 0.18 ± 0.04 spikes per burst).

To determine whether the gamma modulations of unit activity seen in the PEHs were statistically significant, we computed a rhythmicity index (RI) for each cell. The RI was obtained by averaging the difference in spike counts between the three center peaks and troughs of the PEHs and dividing the result by the average of the entire histogram to normalize for variations in firing rates. Statistical significance of the RI was tested by recomputing the PEHs after shuffling the spike times, and repeating this process 1000 times. The actual RI was considered significant if it was higher than 95% of the randomly generated RIs. Using this approach, it was determined that 49% of BLA and 28% of putamen cells were significantly modulated by gamma activity.

Next, we examined when BLA and striatal cells fired with respect to gamma activity. To this end, for each cell with a significant RI, we located the bin with the highest counts in the PEHs and computed a frequency distribution of

peak times. As shown in figure 25D-E, this revealed a similar entrainment of BLA and putamen unit activity by local gamma with both cell types firing preferentially during the rising phase of positive gamma cycles.

To test whether gamma activity affected functional coupling between BLA and striatal neurons, we next examined how LFP power in different frequency bands affected the correlation between putamen and BLA unit activity. To this end, we first crosscorrelated unit activity in 362 pairs of simultaneously recorded BLA and putamen neurons. With rare exceptions, these crosscorrelograms were flat (Fig. 26A).

However, when we excluded putamen spikes occurring during periods of low (< 2.5 SD) gamma power, many crosscorrelograms exhibited significant coupling (Fig. 26B). Significance of the correlograms was assessed by shuffling each of the BLA spike trains 1000 times, recomputing the correlogram each time, and comparing them to the actual correlogram. A correlogram was considered significant when the sum of its central bins (± 5ms from origin) was higher than that seen in 95% of the randomly generated correlograms.

To quantify how the incidence of significant BLA-striatal correlations changed in relation to gamma activity, each cell couple was assigned a correlation index (by comparing the sum of the central ± 5 ms bins of the correlograms to that of the correlograms generated after shuffling of the BLA spike trains). The correlation indexes were expressed as percentiles (Fig. 26C).

Figure 26C shows the frequency distribution of these correlations indexes for both types of correlograms (those including all spikes vs. those computed during periods of high amplitude striatal gamma). The proportion of correlograms with central bins ≥ 2.5 SD was 15% during high amplitude gamma, compared to 8% when all spikes were considered. To determine whether this was statistically significant, we performed a X^2-test analysis, comparing the incidence of cell couples with central crosscorrelograms peaks higher and lower than 2.5 SD when all spikes were included vs. only those occurring during periods of high amplitude striatal gamma. The difference was found to be significant ($p = 0.0042$).

To test whether this enhancement of BLA-striatal unit correlation was selective to gamma related activity, we repeated this analysis in various frequency bands by digitally filtering LFPs in sliding windows of 5 Hz. Using this approach, we observed that significant increases in BLA-putamen unit correlation preferentially occurred in relation to gamma activity (Fig. 26D). Moreover, when the same analysis was repeated for all available pairs of simultaneously recorded cortical and putamen cells (n = 298; Fig. 26E), or thalamic and putamen (n = 82; Fig. 26F) neurons, this enhancement of unit correlations by gamma activity was found to be selective to BLA-striatal activity (X^2-tests: cortico-striatal, $p = 0.88$; thalamo-striatal, $p = 0.29$). Carrying out the same analysis for cortical and BLA or thalamic and BLA cell couples failed to reveal an increase in unit correlation by gamma activity (Fig. 27).

To determine whether the increased BLA-striatal correlation revealed in the above analysis was in fact due to increased discharge rates, we compared baseline firing rates to those seen during periods of high gamma power (>2.5 SD). However, increases in gamma power were not associated with changes in the firing rates of BLA (% change from low to high gamma of 3.2 ± 8.7%; paired t-test, $p = 0.14$) or putamen (14.4 ± 19.6 %; paired t-test, $p = 0.41$) neurons.

Overall, the above suggests that BLA neurons impose gamma activity on the putamen and that functional correlation between BLA and putamen cells is highest during periods of high amplitude gamma. Thus, coherent striatal and BLA gamma LFP activity is a physiological signature of amygdalo-striatal interactions. Importantly, this increased coupling of BLA and striatal activity by gamma is not associated with changes in firing rates.

6.3.5) Coordination of amygdalo-striatal interactions by gamma during learning

To investigate whether gamma oscillations also coordinate amygdalo-striatal interactions during learning, cats were trained on a stimulus-response task. Prior work has revealed that memory formation on such tasks is striatal-dependent (Grahn et al., 2008).

In our case (Fig. 28), cats that had restricted access to food (kept at 90% of their initial bodyweight) gradually learned that the termination of one of two

tones (CS^+, 3 sec) coincided with the brief availability (1 sec) of a liquid food reward. The animals quickly adapted to the food restriction (they were only fed during the recording sessions), remaining aroused and awake at all times.

Each daily training session, the cats received around 60 randomly interspersed presentations of the CS^+ and CS^- with variable inter-trial intervals (range 20 to 40 sec). Five consecutive daily training sessions occurred with the same CS^+ prior to switching reinforcement contingencies. Learning progression was assessed by measuring the rate of anticipatory licking during the CS^+, prior to reward delivery.

Figure 28B illustrates licking rate (y-axis) around the CS^+ (black) and CS^- (red) for trials obtained during the first (dashed lines) and last (continuous lines) two training sessions (averaged across all cats). Although the rate of anticipatory licking during the CS^+ or CS^- (shaded area) did not differ significantly at early stages of training (Sessions 1-2, $F(1,1431) = 0.252$, $p = 0.615$), the difference became significant at late training stages (Sessions 4-5, $F(1,967) = 9.8$, $p = 0.0018$). Plotting the proportion of CS^+ and CS^- trials with anticipatory licking as a function of daily training sessions (Fig. 28C) revealed that the difference between the CS^+, CS^- and spontaneous licking reached statistical significance at the third training session ($F(2,890) = 11.6$, $p < 0.0001$).

Previous studies using stimulus-response tasks have revealed that BLA unit activity encodes expected outcomes (Schoenbaum et al., 1998, 1999; Saddoris et al., 2005; Paton et al., 2006). In support of the idea that this

information is used during learning, it was reported that BLA-lesioned animals are impaired in associating reward values to specific stimuli in a similar stimulus-response task (Balleine et al., 2003). These results suggest that BLA might bring an emotional or motivational component to striatal processing during learning.

Consistent with this, we observed a strong parallel between improvements in behavioral performance and correlated amygdalo-striatal gamma during the acquisition of this task (Fig. 29; $F(1,10) = 6.072$, $p = 0.0334$). Early in training (Fig. 29 A,B), when the rates of anticipatory licking during the CS^+, CS^- and chance were statistically indistinguishable, the CS^+ (black) and unrewarded tone (red) elicited a modest but significant increase in coherent BLA-striatal gamma (paired t-test, $p = 0.005$). However, at this initial stage of training, the increase in coherent BLA-striatal gamma elicited by the CS^+ and CS^- were statistically indistinguishable (paired t-test, days 1-2, $p = 0.72$). As learning progressed, the CS-related BLA-striatal gamma coupling augmented and became significantly more important in response to the CS^+ (Fig. 29C-E; paired t-test, days 1-2 vs. days 3-5, $p = 0.002$; t-test, CS+ vs. CS- on days 3-5, $p = 0.0001$).

Importantly, the first training session where the CS^+ elicited significantly larger increases in coherent amygdalo-striatal gamma activity than the CS^- (Fig. 29C, Day 3) coincided with the training session where behavioral evidence of discrimination between the CS^+ and CS^- became maximal (Fig. 28C, Day 3). Moreover, when the reinforcement contingencies were reversed (Fig. 29F-H), the identity of the CS eliciting larger increases in coherent BLA-striatal gamma

switched and the time course of this effect again paralleled the evolution of behavioral improvements (compare Fig. 29I and 28C).

Further supporting the notion that increases in coherent BLA-striatal gamma are closely related to learning on this task, a significant positive correlation was found between the proportion of trials with anticipatory licking in a given session vs. the difference in coherent BLA-striatal gamma elicited by the CS+ and CS- in the same session ($r = 0.55$, $p < 0.01$; Fig. 30A). Yet, the increase in coherent BLA-striatal gamma was not a simple motor correlate since trials with vs. without anticipatory licking did not differ in this respect (Fig. 30B).

Importantly, learning-related changes in correlated gamma were only seen between BLA and putamen recording sites, not between putamen and cortical or putamen and thalamic recording sites (Fig. 31A-C). Moreover, examination of learning related fluctuations in other frequency bands shown in figure 26D-F to increase coupling between putamen and cortical or thalamic units failed to reveal significant changes (Fig. 31D-E).

CHAPTER 7

Discussion

Several decades of research have identified the amygdala as a major player in processing emotions. The BLA nucleus in particular, is critically involved in mediating the effects of emotional arousal on learning and memory. Injection of amphetamines in the BLA just after training facilitates learning of both striatal and hippocampal dependent tasks, yet intra-amygdala injections of lidocaine just before testing retention have no effect on performance (Liang et al., 1982; Packard et al., 1994; Parent et al., 1995). This indicates that the amygdala is not the storage site of the facilitated memories.

The studies presented in this thesis were undertaken to answer the following question: how does increased BLA activity facilitate learning and memory storage in other neuronal structures? We predicted that amygdala projections could directly influence plasticity in their targets, and focused on BLA-striatum interactions. First, we used an *in vitro* model to show that BLA synapses on MSNs in the striatum are endowed with special properties that allow them to facilitate heterosynaptic plasticity. We studied some of the molecular and temporal requirements underlying this effect, leading us to suggest that intracellular waves of CICR mediate the facilitatory effects of BLA on cortico-striatal LTP induction.

Second, we investigated the interaction between the striatum and BLA *in vivo*. We found that coherent high power gamma oscillations (probably originating in the BLA) entrain, and correlate the firing of neurons in the BLA and striatum, thus providing a physiological signature of communication between

these two structures. Using this measurement, we observed a strong parallel between BLA-striatum interactions, and performance in an appetitive stimulus-response task. Several points of interest are discussed bellow.

7.1) BLA inputs facilitate the induction of cortico-striatal LTP

The *in vitro* studies were undertaken to test whether BLA inputs can facilitate activity-dependent plasticity at cortical synapses onto principal striatal neurons. The interest in this issue stems from pharmaco-behavioral studies implicating BLA activity in the facilitation of striatal-dependent memories by emotional arousal. In keeping with this finding, our results indicate that BLA activity can facilitate induction of LTP at converging cortico-striatal inputs. This effect was unusual in that it required NMDA receptor activation at both inputs, yet it could be seen even when BLA and cortex were activated 0.5 s apart during LTP induction.

Activity dependent modifications in the efficacy of cortico-striatal synapses are believed to contribute to motor learning (Mahon et al., 2004). In keeping with this view, post-training AP5 injections in the striatum impair memory formation in striatal-dependent tasks (Packard and Teather, 1997b). Moreover, the cortico-striatal pathway exhibits various forms of activity-dependent plasticity (Calabresi et al., 1992b; Calabresi et al., 1992a; Lovinger et al., 1993; Charpier and Deniau,

1997; Partridge et al., 2000; Kerr and Wickens, 2001; Ronesi and Lovinger, 2005).

Another line of investigation indicates that, in emotionally arousing conditions, post-learning BLA activity facilitates striatal-dependent memories. Indeed, injection of amphetamines in the BLA just after training increases striatal-dependent storage of response information (Packard et al., 1994). Yet, intra-amygdala injections of lidocaine just before testing retention have no effect on performance (Packard et al., 1994). Together, these findings suggest that the BLA enhances the formation of striatal-dependent memories by facilitating NMDA-induced cortico-striatal plasticity.

Our results support this view. First, we found that BLA inputs express a higher NMDA-to-AMPA ratio than cortical synapses. Second, pairing the activation of cortical and BLA inputs with somatic APs enhanced the incidence and amplitude of cortico-striatal LTP. This facilitation depended on NMDA receptors located at BLA synapses and was input-specific, occurring only at cortical synapses that were activated within 500 ms of BLA inputs. Moreover, blocking the release of Ca^{2+} from intracellular stores impaired cortico-striatal LTP. Considered together, these findings suggest that the facilitating effects of amygdala activity on cortico-striatal LTP result from the ability of BLA inputs to raise, via NMDA receptors and intracellular stores, the postsynaptic Ca^{2+} concentration beyond levels critical for LTP induction.

7.2) Lax timing requirements for the BLA-facilitated cortico-striatal LTP

Despite the NMDA dependence of the BLA effect, the LTP facilitation did not require precise co-activation of BLA and cortical inputs, provided that both inputs were paired to postsynaptic APs. In fact, the LTP facilitation was seen even when BLA and cortical stimuli were separated by as much as 0.5 s during induction, which contrasts with earlier reports on spike timing-dependent plasticity, where near simultaneous pre- and post-synaptic activation was required for LTP induction (Dan and Poo, 2004). Thus, our findings imply that strong BLA inputs trigger an intracellular signal that can affect the fate of cortical synapses activated within 0.5 s of the BLA input.

Given the NMDA dependence of the BLA effect, it is possible that the Ca^{2+} influx caused by BLA stimuli decays slowly enough for cortical inputs occurring 0.5 s later to be significantly affected. Another possibility is that Ca^{2+} entry through NMDA receptors triggers one or more downstream signaling cascade(s). Our results are consistent with this second option, and support a model in which Ca^{2+} entry through NMDA receptors at BLA synapses onto MSNs triggers propagating waves of CICR, thereby facilitating the induction of LTP at striatal synapses.

On the surface, the loose temporal coordination required for BLA inputs to facilitate cortico-striatal LTP might seem to represent an obstacle for the

selective enhancement of particular sets of cortico-striatal synapses during learning. However, this problem might be circumvented if the BLA effect required that multiple pairings with cortical inputs occurred over time before a significant potentiation is achieved. Although 60 such pairings were sufficient to induce LTP in our conditions, the minimum number of coincident cortical–BLA firings might be different *in vivo* where cortico-striatal neurons exhibit sustained spontaneous activity (Cowan and Wilson, 1994; Niida et al., 1997). In these conditions, BLA inputs might only facilitate cortical synapses that consistently and repeatedly exhibit phasic increases in activity around BLA spikes.

7.3) Relation to previous work on the BLA-facilitation of synaptic plasticity.

Although many studies have examined how the BLA facilitates synaptic plasticity in its targets, their relevance to cortico-striatal plasticity is unclear. For instance, one model centers on the ability of the BLA to recruit cortico-petal basal forebrain cholinergic neurons (Weinberger, 2004). This BLA-driven release of acetylcholine would facilitate plasticity in cortical networks.

Indeed, it was found that muscarinic receptor blockade interferes with the stabilizing and facilitating effects of BLA stimulation on LTP of thalamo-cortical (Dringenberg et al., 2004) and perforant path (Frey et al., 2001) synapses. However, because the cholinergic innervation of the striatum has an exclusively

intrinsic origin (Mesulam et al., 1992) and BLA axons do not appear to target striatal cholinergic interneurons (Kita and Kitai, 1990), such a mechanism is unlikely to explain our results.

Rather, these findings along with our data suggest that BLA axons may influence synaptic plasticity through several parallel mechanisms. These data are further supported by differences in the connectivity of these various networks. Indeed, although BLA directly projects to the striatum, it has no direct projections to the dentate gyrus or visual cortex in subprimate species (Pitkanen et al., 2000). Yet, BLA stimulation after LTP induction can enhance and stabilize LTP of perforant path and geniculocortical synapses. In further contrast with our findings, the BLA enhancement of the perforant path LTP is independent of NMDA receptors (Frey et al., 2001). Thus, it seems likely that in this case, the BLA-mediated facilitation of synaptic plasticity was entirely indirect, via the cholinergic basal forebrain. A challenge for future studies will be to examine the effect of BLA inputs on activity-dependent plasticity in the rhinal cortices where the BLA has direct as well as indirect (via the cholinergic basal forebrain) projections.

7.4) Relevance to BLA modulation of striatal-dependent learning.

Much evidence indicates that different parts of the striatum participate in different forms of memory (Packard and Knowlton, 2002; Yin and Knowlton, 2006; Grahn et al., 2008). The dorso-lateral striatum seems to be required for habit formation, where fixed stimulus-response associations are acquired gradually. In contrast, the ventral striatum, the region investigated here, is believed to be involved in flexible place learning and goal-directed behavior. Although BLA inputs to the striatum are densest in its ventral part, significant BLA projections reach more dorsal striatal sectors (Ragsdale and Graybiel, 1988). In addition, pharmaco-behavioral studies indicate that the BLA can facilitate the formation of stimulus–response associations (Packard et al., 1994).

In light of these data, the mechanisms evidenced here may well apply to both dorsal and ventral striatal memory functions. Our *in vivo* results support this notion. Here, we recorded the putamen and found a strong BLA-striatal interaction that paralleled learning. Depending on the criteria used, this region is sometimes considered dorsal striatum while in other cases it is considered as ventral striatum (Lynd-Balta and Haber, 1994b, a; Fudge and Haber, 2002; Fudge et al., 2002; Grahn et al., 2008).

7.5) Dopamine and BLA activity requirements in cortico-striatal plasticity

A substantial body of data has demonstrated the role of dopamine in striatal plasticity. In particular, dopamine is an essential modulator of cortico-striatal plasticity (Reynolds and Wickens, 2002; Costa, 2007; Wickens et al., 2007). For example, blocking dopaminergic $D_{1/5}$ receptors disrupts cortico-striatal LTP (Kerr and Wickens, 2001; Centonze et al., 2003; Pawlak and Kerr, 2008; Schotanus and Chergui, 2008). Consistent with this, we found that in the presence of the $D_{1/5}$ antagonist SCH-23390, BLA-cortex pairings with postsynaptic APs failed to induce cortico-striatal LTP. This result indicates that the BLA-mediated facilitation of cortico-striatal LTP requires a "background" level of dopaminergic activity.

This data may seem surprising, especially since the source of striatal dopamine is mainly extrinsic (VTA, and SNc) and not present in slice preparations; however, it is consistent with previous findings. For example, Schotanus and Chergui also found that SCH-23390 impairs cortico-striatal LTP induction in slices that were **not** pre-treated in any way (Schotanus and Chergui, 2008). Together, these results suggest that striatal slices retain a basal level of dopaminergic activity that plays an essential role in cortico-striatal LTP induction *in vitro*.

How might D_1 receptors modulate LTP induction? D_1 receptor activation

was shown to enhance NMDA currents (Cepeda et al., 1993; Wirkner et al., 2004; Zhang et al., 2005; Kruse et al., 2009). In our case, this might explain why blocking D_1 receptors impairs cortico-striatal LTP induction, possibly in a way similar to AP-5 or MK-801 application. Future studies could directly test this hypothesis, by measuring BLA- and cortically-evoked NMDA currents in MSNs, in control conditions vs. after application of SCH-23390.

7.6) Gamma oscillations as a substrate of amygdalo-striatal interactions *in vivo*

In order to propose a model of how the amygdala facilitates learning we have to address the issue at several levels. This includes identifying the interactions between BLA and its various targets that are necessary for processing information, learning, storage and expression of the learned or modified behavior. There is, however, a general lack of knowledge in this regard. Most studies have focused on learning-induced changes in various systems, one structure at a time. How these alterations ultimately affect behavior is usually inferred from the connectivity within the system. In fact, there is very little data on how various regions interact to produce coherent behavior, and how these interactions change with learning.

The results of our *in vivo* study shed light on this question, by showing that BLA neurons generate periods of gamma activity in the striatum. Indeed, the fact

that intra-BLA muscimol infusions caused a selective decrease in striatal gamma constitutes strong evidence that it was generated in the BLA and not by a common input to the BLA and putamen. Moreover, coupling between BLA and putamen unit activity was selectively increased during periods of high striatal gamma. The depolarization of MSNs that was produced by the arrival of BLA inputs at the gamma frequency probably contributes to enhance NMDA receptor activation and calcium influx in MSNs, thereby facilitating induction of heterosynaptic activity-dependent plasticity, as described in Chapter 4.

Consistent with this, we also found a close temporal relationship between fluctuations in coherent BLA-striatal gamma and striatal-dependent learning. The emergence of tone-specific coherent amygdalo-striatal gamma activity occurred during the same training session as when behavioral evidence of discrimination first reached significance. Interestingly, upon reversal of tone-reward contingencies, the new CS^- induced the same high level of BLA-striatal gamma coherence as in the previous sessions when it was paired with food delivery. The new CS^+ however, slowly gained the ability to generate gamma coherence values above this level. This observation is consistent with several hypotheses, none of which can be addressed using the available data.

First, it is possible that continuing the experiment beyond the 3 days of reversal might reveal a reduction in the gamma-coherence levels induced by the new CS^-, maybe even back to baseline values.

Second, continuously presenting a former CS^+ without reinforcement is

known to produce extinction of conditioned behavior (in our case anticipatory licking, which decreases after reversal). However, this does not necessarily mean that the animal lost the tone-reward association, but rather that it is suppressing the learned behavior. If this is the case, the high levels of BLA-striatal gamma coherence would only signal the presence of a behaviorally important stimulus (still recognized even without the reward), and not the imminent occurrence of a learned behavior (licking). This is supported by our observation that CS^+ presentations induce the same levels of gamma coherence, whether they contain anticipatory licks or not.

Third, it is possible that the gamma coherence is a measure of general arousal, and that during the reversal sessions the animal is generalizing the "meaning" of tone, while still maintaining a level of discrimination between CS^+ and CS^-. While at a behavioral level this does not seem to be the case (the cats had very few anticipatory licks during the reversal CS^-), we did not have a pure CS^- to test the idea of generalization (a third tone that is not paired with food at any point during the recordings). Future experiments are needed to address these points.

Notably, evidence that gamma activity generated in the BLA contributes to the induction of learning-related synaptic plasticity was also obtained in other targets of the BLA. For example, it was reported that during the acquisition of an appetitive trace-conditioning task, which is thought to be dependent on the hippocampus, the CS gradually acquired the ability to evoke coherent gamma

oscillations in the BLA and rhinal cortices (Bauer et al., 2007) and that this effect contributed to enhance rhinal transfer of neocortical inputs to the hippocampus (Paz et al., 2006; Paz et al., 2007). Together with these earlier studies, our data suggest that the amygdala-mediated facilitation of memory depends on the ability of the BLA to generate gamma oscillations that facilitate the induction of activity-dependent synaptic plasticity in target neurons.

It is, however, unclear how amygdala imposes gamma to the striatum. A higher number of NMDA receptors at BLA synapses on MSNs (as shown in chapter 3.3.2) would actually interfere with the generation of gamma, since they produce a long lasting depolarization, incompatible with high firing rates. However, BLA cells do not fire at gamma frequencies, but they follow gamma oscillations with a certain phase preference. The same is true for many putamen neurons. Thus it seems likely that the gamma oscillations present in the amygdala and putamen serve only to couple the firing of the two structures. While our experiments support the idea that gamma generated in the BLA is then transferred to the striatum, the mechanism underlying this phenomenon remains unknown.

7.7) Future directions

In Chapter 5, I presented evidence that tonic dopaminergic activity is necessary, even in slice preparations, for the induction of cortico-striatal LTP.

This requirement could not be circumvented by BLA stimuli delivered during the pairing protocol, and blocking dopaminergic D_1 receptors with bath-applied SCH-23390 prevented the BLA-enhancement of cortico-striatal LTP. A possible explanation for these results lies in the ability of D_1 receptors to enhance NMDA-mediated currents (Wirkner et al., 2004; Zhang et al., 2005; Kruse et al., 2009). Thus, it is possible that **blocking "tonicaly" active D_1 receptors reduces the NMDA component of (probably) both cortical and BLA responses in MSNs, and impairs LTP induction**. This hypothesis can be directly tested by measuring the NMDA component of BLA and cortically-evoked responses in MSNs, before and after application of D_1 antagonists, such as SCH-23390. Due to the higher NMDA-to-AMPA ratio at BLA synapses, it is likely that BLA responses will be predominantly affected.

The *in vivo* experiments presented in Chapter 6 were undertaken to shed light on the BLA-striatal interaction that occurs during learning, and to see how the model of BLA-facilitated cortico-striatal plasticity (presented in Chapters 3-5) might come into play *in vivo*. While we made significant progress in this direction, several questions still remain:

Does BLA activity change cortico-striatal interactions? The experiments in Chapter 6 focused on the BLA-striatal pathway and how these two structures interact during the acquisition of an appetitive, striatal-dependent task. It is commonly believed, however, that plastic changes at cortico-striatal synapses underlie learning (Reynolds et al., 2001; Mahon et al., 2004), a point

that we did not address. From this perspective, the BLA-striatal interaction should play a modulatory role, allowing specific cortical synapses onto MSNs to be altered. Our sample of simultaneously recorded neurons in the three regions (BLA, striatum and cortex) did not allow us to test this possibility. However, it is conceivable that with a larger sample size, we would be able to test the hypothesis that BLA modulates the cortico-striatal interaction at a cellular level, and to investigate the rules governing this effect. A larger database of neurons would also allow us to monitor **how the BLA-striatum interaction (seen at a cellular level) changes with learning**. So far we were able to observe preferential neuronal coupling between BLA and striatal neurons during gamma power bursts, by combining all pairs of simultaneously recorded cells in the two structures. This method, however, ignores between-sessions variability that could otherwise reveal more about learning-related mechanisms.

What is the significance of coherent BLA-striatal gamma activity in the absence of external stimuli? The *in vitro* results of Chapter 4 (section 4.3.3) suggest that cortical inputs are facilitated only if they become active after BLA stimuli (see Fig. 14). Yet, several behavioral studies showed that post-training BLA activity enhances learning (Packard et al., 1994; Packard and Teather, 1998; McGaugh, 2004). This apparent contradiction can be resolved if, during the critical time in which BLA activity is increased and affects memory consolidation (1-3 hours post-training), cortex and striatum replay earlier activity patterns (Ribeiro et al., 2004). This phenomenon has been described in hippocampus and cortex (Wilson and McNaughton, 1994; Louie and Wilson,

2001; Ji and Wilson, 2007). However, it is currently unknown whether the amygdala is involved in this process. With respect to our data, it is possible that the bursts of coherent gamma activity in BLA and striatum represent periods of activity-replay. This hypothesis could be tested by looking at the level of "similarity" of neuronal activity during coherent BLA-striatal gamma.

Does BLA interact in a similar way with other targets? Whether the BLA-putamen interaction revealed by our *in vivo* and *in vitro* experiments is unique to these two structures, or it extends to others, is an important question. Using intra-BLA muscimol injections we showed that the amygdala imposes gamma activity in the striatum. This makes it plausible that other amygdala targets also receive significant gamma modulation from BLA-activity. The abundant projection from the amygdala to the nucleus accumbens (Fudge et al., 2002) makes this region a particularly interesting site for performing *in vivo* recordings. One could then compare the interactions between nucleus accumbens and BLA to the results presented in Chapter 6. Also, previous studies found that the amygdala and rhinal cortices become coherent in the gamma frequency band, preferentially around behaviorally-relevant stimuli (Bauer et al., 2007). Thus, it is plausible that BLA activity has similar effects in all its targets, and coherent gamma activity is a physiological signature of emotional arousal. During these periods, BLA could facilitate plasticity in its targets (by a mechanism similar to the one suggested by our *in vitro* results). Technical difficulties prevent us, at least for now, from testing the effects of BLA stimuli on rhinal cortex plasticity *in vitro*. Previous *in vivo* studies emphasized the role of acetylcholine as

a mediator of BLA stimulations on plasticity in the hippocampus and visual cortex (Frey et al., 2001; Dringenberg et al., 2004). Thus, dissecting the specific contribution of BLA inputs vs. cholinergic ones to these regions remains a challenge for the future.

Do pathological or extreme conditions change the interaction between BLA and its targets? Probably the most studied paradigm involving the amygdala is fear conditioning (see section 1.2.3 for a description). In fact, for a long time it was believed that the amygdala only processes negative stimuli, probably because it is easier to find neuronal changes under extreme conditions. Many studies since, have shown that the amygdala is also involved in processing positive stimuli. Regardless, integrating information about, and producing adequate responses to potentially harmful stimuli remains an important part of the amygdala function. Fear conditioning, and other stressful situations are believed to induce plasticity in the amygdala and change the way in which relevant stimuli are processed. In some extreme cases this leads to pathological conditions (such as post traumatic stress disorder, or other anxiety disorders), with severe consequences on behavior. This altered behavior is likely paralleled by changes in the way neuronal structures process information and interact. Thus, a future direction of research is to investigate these changes, in the hope of better understanding the system, and identifying new forms of treatment.

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Figure legends

Figure 1. Schematic representation of the basal ganglia connectivity. Components of the basal ganglia are presented in green: GPe – globus pallidus external segment; GPi – globus pallidus internal segment; SN – substantia nigra (pc – pars compacta; pr – pars reticulata); STN – suthalamic nucleus

Figure 2. Diagram illustrating the experimental approach. Pairs of stimulating electrodes were placed in cortex (CTX) and amygdala (BLA). MSNs in the ventral striatum were recorded using the whole-cell patch technique. D – dorsal; L – lateral; M – medial; V – ventral.

Figure 3. Electrode placement and occlusion test for LTP experiments. **A**. Pairs of stimulating electrodes were placed in cortex (CTX 1 and CTX 2) and BLA. MSNs were patched in the ventral striatum. **B**. To ascertain that the electrodes stimulated different sets of fibers, we performed an occlusion test (the example illustrates the test performed at two cortical sites): we compared the amplitude of responses when both sites were simultaneously stimulated, with the algebraic sum of responses to the individual stimulation of CTX 1 and CTX 2. Note that the predicted amplitude is almost identical to the one recorded.

Figure 4. LTP induction and recording protocol. **A.** Cortical EPSPs were recorded before and after LTP induction, and analyzed off-line. The beginning and the point of peak amplitude were detected (dotted lines), and the slope of the first half (red line) used as a measure of synaptic strength. **B.** Two sites (BLA – B and cortex – C in this case) are simultaneously stimulated and paired with APs generated by intracellular current pulses (2 ms duration, at 2 nA). The inset illustrates the temporal relation between the summated EPSP, and the AP generated 7 ms later. **C.** The protocol is repeated 60 times, at 2 Hz (A1). One of these pairings (framed by the thin black line) is the example presented in **B**.

Figure 5. Properties of MSNs. **A**. Voltage responses to current pulses of gradually increasing amplitudes. Inset shows the current-voltage relationship (note the deviation of voltage responses from the linear prediction – red dotted line). **B**. Morphological properties of a MSN as revealed by intracellular injection of neurobiotin. Arrowheads point to local axon collaterals.

Figure 6. NMDA-to-AMPA ratio at cortical and BLA synapses on MSNs. **A**. Responses to the electrical stimulation of cortex and BLA were recorded in 0 Mg2+ aCSF, while adding picrotoxin, CNQX, and AP-5. Red dotted lines show the specific AMPA and NMDA components of the BLA – EPSP. **B**. The NMDA-to-AMPA ratio of cortical (CTX, left column) and BLA (right column) responses is presented for all recorded MSNs (n = 15). **C**. Group average of the data presented in B. The NMDA-to-AMPA ratio is significantly higher at BLA synapses than at cortical synapses (P value is the result of a Student's T-test).

Figure 7. The effect of bath-applied ifenprodil on BLA and cortical (CTX) responses. Graph displays the amplitude of CTX and BLA responses in the same MSNs (n = 9) during various drug applications. For comparison, amplitudes were normalized to those recorded in 0 mM Mg^{2+}, 100 μm picrotoxin (Picro) and 20 μm CNQX (the NMDA component). Ifenprodil (IF) application affected BLA and CTX responses equally. Bars represent averages ± S.E.M.

Figure 8. Minimal stimulation experiments. **A**. Example histogram of EPSCs amplitude recorded in the same MSN using BLA (top) and cortical (CTX, bottom) minimal stimulation. The histograms are fitted with a 2-Gaussion mixture distribution; pR – probability of release. **B**. BLA (top) and cortical (bottom) responses recorded during a 10 min period. Upper part of graphs shows superimposed recorded traces: red – EPSCs, black – failures. Lower part plots amplitude of responses (y-axis) vs. time (x-axis). The red horizontal line shows the cutoff used to separate EPSCs from failures. **C**. Average shape of identified EPSCs for BLA (top) and cortical (bottom) minimal stimulation. Black traces represent the recordings performed in 0 Mg^{2+} (AMPA + NMDA components); red traces illustrate the effect of AP-5 application (the non-NMDA component).

Figure 9. BLA stimuli facilitate cortico-striatal LTP. **A**. Pairing of two cortical sites (CTX) produces significant, but modest LTP. Normalized slope of cortically-evoked EPSPs (y-axis) plotted as a function of time (x-axis). Top shows average of 10 consecutive EPSPs before (dotted line) and after (solid line) the pairing protocol. **B**. Pairing of BLA and CTX produces a large increase of responses evoked by the paired cortical stimulus (upper black lines) but not of those evoked from an unpaired cortical stimulation site (upper red lines). Normalized slope of cortically-evoked EPSPs (y-axis) plotted as a function of time (x-axis). **C**. Incidence of cortico-striatal LTP and LTD, after pairing two cortical sites (grey bars) or one cortical and one BLA site (black bars) with postsynaptic action potentials (APs). Data is presented as percent of total number of cells in each group. **D**. Levels of cortico-striatal LTP (normalized to pre-pairing values) observed in various experimental conditions. Pairing BLA and CTX with APs (C+B) increases the slope of cortically evoked EPSPs. In the presence of extracellular AP-5 or intracellular MK-801 this enhancement is not observed. Significantly less LTP occurs when two cortical sites are paired (C+C), and no LTP is observed at un-paired cortical sites (uC).

Figure 10. Pairing of BLA and cortical stimuli induces small changes at BLA synapses. Normalized slope of BLA-evoked EPSPs (y-axis) plotted as a function of time (x-axis). Data is presented as average ± S.E.M. at each recorded time-point around the pairing protocol (C+B). Note the small change in the slope of EPSPs compared to that of cortical responses of the same neurons (Fig. 9B)

Figure 11. BAPTA impairs cortico-striatal LTP induction. Intracellular BAPTA prevents the induction of cortico-striatal LTP following the pairing of BLA and cortical stimuli (C + B). Normalized slope of cortically-evoked EPSPs (y-axis) plotted as a function of time (x-axis).

Figure 12. Blocking of NMDA receptors at BLA synapses on MSNs. The ratio of NMDA-to-AMPA is reduced for BLA responses after blocking NMDA receptors at BLA synapses on MSNs (red lines and bars), compared to control conditions (black lines and bars). No change in the NMDA-to-AMPA ratio occurred at cortical synapses (CTX). Left panel – pair-wise comparison of NMDA/AMPA for CTX and BLA responses. The group average is presented in the right panel.

Figure 13. Functional dissociation of NMDA receptors based on synaptic location. **A**. Blocking BLA-NMDA receptors reduces the amplitude of cortico-striatal LTP to levels similar to those seen with the pairing of two cortical sites. **B**. CTX-NMDA blockade prevents the induction of LTP.

Figure 14. Temporal window for the BLA-facilitation of cortico-striatal LTP. **A**. An example of stimulation paradigm. Cortical (CTX) stimuli follow BLA stimuli by500-ms. Both sites are stimulated 60 times at a frequency of 1 Hz and paired with somatic APs. **B.** Cortico-striatal LTP produced when BLA and cortical stimuli are separated by 0.5 s. Results are shown as normalized slope of cortically evoked EPSPs (y axis) as a function of time (x axis). Representative examples of cortically-evoked EPSPs (average of 10 consecutive responses) before (red lines) and after (black lines) the induction protocol are provided at the bottom of the graph. Average of five experiments. **C.** Amount of cortico-striatal LTP as a function of the BLA–cortex inter-stimulus interval. A linear fit to the data points is shown in red. **D.** Cortico-striatal LTP produced when BLA stimuli preceded cortical ones by 0.8 s. Results are shown as normalized slope of cortically-evoked EPSPs (y axis) as a function of time (x axis). Average of 7 experiments.

Figure 15. CICR and D1 receptors are required for LTP. **A, B.** Blocking CICR-events with ryanodine receptor antagonists (ruthenium red; **A**), or depleting intracellular Ca^{2+} stores (CPA; **B**) impairs cortico-striatal LTP induction by cortex-BLA pairings. **C.** SCH-23390 (a dopaminergic D_1-receptor antagonist) blocks cortico-striatal LTP.

Figure 16. Histological verification of recording sites. Coronal sections stained with cresyl violet. Arrows and arrowheads point to electrolytic lesions performed at the end of the experiments to mark the last recording sites in the BLA (**A, D**), primary and secondary auditory cortices (**B, E**), rostral and caudal intralaminar nuclei (**C, F**), as well as putamen (**D, E**). Scale bar in **E** also valid for panels **A** and **D**. Abbreviations: BL, basolateral amygdaloid nucleus; BM, basomedial amygdaloid nucleus; CL, central lateral thalamic nucleus; CM, central medial thalamic nucleus; CP, cerebral peduncle; GP, globus pallidus; HA, habenula; HF, hippocampal formation; LA, lateral nucleus of the amygdala; LG, lateral geniculate nucleus of the thalamus; LP, lateroposterior thalamic nucleus; OT, optic tract; PC, paracentral thalamic nucleus; PU, putamen; R, rhinal sulcus; RE, reticular thalamic nucleus; V, ventricle; VP, ventroposterior thalamic nucleus.

Figure 17. Firing rates and spike durations. Baseline firing rate and average spike duration of each recorded unit is presented for all recorded structures (**A**–**D**). Frequency distribution of firing rates in cortical (**A**, n = 159), striatal (**B** , n = 139), BLA (**C**, n = 152), and thalamic (**D**, n = 55) neurons. Insets in **A**–**D** plot spike duration (y-axis) as a function of firing rate (x-axis). As shown in **E**, spike duration was defined as the interval between the onset of the negative component of the spike to the peak of the subsequent positivity. Frequency distributions of firing rates revealed evidence of heterogeneity in our samples. Therefore, in an attempt to restrict our analyses to the main cell type in each structure (red bars), we ignored neurons whose firing rates clearly fell outside the peak of the distributions (black bars).

Figure 18. Firing pattern of putamen neurons. Example of a fast firing putamen neuron (**A**), and predominant, typical low firing neurons (**B** and **C**). Left column illustrates the recorded spikes in black, and the average spike shapes superimposed in red (**A1**, **B1**, **C1**). Autocorrelograms of unit activity are presented in **A2**, **B2** and **C2,** followed by histograms of inter-spike intervals (**A3**, **B3**, **C3**). The firing rate of these neurons was calculated in one second intervals, and presented in **A4**, **B4** and **C4**, as histograms.

Figure 19. Coherent LFP activity in the BLA and striatum. The graphs in **A**-**C** plot coherence (y-axis) vs. frequency (x-axis) for all available pairs of simultaneous LFP recordings in the BLA and striatum (STR; **A**; n = 696), auditory cortex (CTX) and striatum (**B**; n = 572) as well as intralaminar thalamus (THL) and striatum (**C**; n = 304). **D, E**. LFPs simultaneously recorded at various sites (see main text) and digitally filtered to isolate gamma (35-45 Hz) at a slow (**D**) and fast (**E**) time base.

Figure 20. Frequency dependence of the coherence between LFPs simultaneously recorded in the BLA and thalamus (**A**) or BLA and cortex (**B**). Plots of coherence (y-axis; average ± SEM) vs. frequency (Hz). Dashed lines indicate SEM.

Figure 21 Impact of distance between recording sites on gamma coherence. Gamma coherence (y-axis; average ± SEM) plotted as a function of distance between recording sites (x-axis) for various combinations of recorded structures, as indicated in the legend shown in the upper right. BLA-striatal gamma coherence (solid red circles) is higher than seen with all other combinations of recorded structures, even when only considering pairs of

recording sites separated by the same distance.

Figure 22. Phase offsets in the gamma frequency between structures. **A**-**C.** Brief periods of high amplitude striatal gamma (thin line) superimposed with cortical (**A**), thalamic (**B**) and BLA (**C**) gamma oscillations (thick lines). Panels **D**-**H** plot normalized frequency distributions of phase lags (x-axis) between striatal vs. cortical (**D**), striatal vs. thalamic (**E**), striatal vs. BLA (**F**), BLA vs. cortical (**G**) and BLA vs. thalamic (**H**) gamma as a function of normalized gamma amplitude (y-axis). The lines overlaid on these graphs represent the average gamma cycles seen at the corresponding sites.

Figure 23. Distance – gamma offset relationship between recording sites. Angle of arrows indicates average phase relation (see polar plot for legend). Origin of arrows indicates distance between recording sites (refer to x-axis). Length of arrows is inversely proportional to standard deviation of phase offset (the shorter the arrows, the higher the variability). The length was computed using conventional vector averaging techniques, using equal weights for each cycle-to-cycle offset.

Figure 24. Intra-BLA muscimol infusions reduce striatal gamma power. **A**, **B**. Striatal LFP power (color-coded) for different frequencies (y-axis) plotted as a function of time (x-axis) in experiments where either saline (**A**) or muscimol (**B**) was slowly infused in the BLA, over a period of 25 min. **C**. Gamma power (y-axis) ± SEM (dashed lines) as a function of time (x-axis) when either saline (black) or muscimol (red) was infused in the BLA. In **A**-**C**, the thick black lines indicate infusion periods. **D**, **E.** Power spectrum of striatal LFPs during the pre- (black) and post-infusion (red) periods in experiments where either saline (**D**) or muscimol (**E**) was infused in the BLA. Dashed lines indicate SEM.

Figure 25. Striatal and BLA neurons are entrained by gamma oscillations. High amplitude gamma cycles were detected in the digitally filtered LFPs (**A**, upper trace). PEHs of unit activity recorded on the same electrode (**A** lower trace) were constructed around them. **B**-**C**. Examples of PEHs of BLA (**B**) and striatal (**C**) unit activity around the positive peaks of high amplitude gamma cycles (black traces). The average of the corresponding LFP is superimposed (red traces). To facilitate comparison between PEHs, we normalized the data to the average bin value in each PEH. **D**-**E**. Frequency distributions of firing peak times for BLA (**D**) and striatal (**E**) neurons in

relation to gamma.

Figure 26. Gamma oscillations couple BLA and striatal neurons. **A.** Example of a typical crosscorrelogram that included all spikes generated by a simultaneously recorded pair of BLA and striatal neurons. **B.** Crosscorrelogram of unit activity for the same cell couple after excluding striatal spikes occurring during periods of low amplitude gamma. **C.** Frequency distribution of correlation indices for all BLA–striatal crosscorrelograms. Black, all spikes; Red, analysis restricted to striatal spikes occurring during high amplitude striatal gamma. **D**-**F.** Color-coded frequency distributions of correlation indices (x-axis) plotted as a function of the frequency of striatal LFPs (y-axis) used to select spikes included in the crosscorrelograms. The bottom x-axis expresses the correlation indexes in percentiles. The correspondence in Z-scores can be found in the top x-axis. This was done for all simultaneously recorded couples of BLA and striatal (**D**), cortical and striatal (**E**), or thalamic and striatal (**F**) neurons.

Figure 27. Same analysis as in figure 22, but for cortical and BLA (**A**) or thalamic and BLA (**B**) neurons. Color-coded frequency distributions of correlation indices (x-axis) plotted as a function the frequency of BLA LFPs (y-axis) used to select spikes included in the crosscorrelograms. The bottom x-axis expresses the correlation indexes in percentiles. The correspondence in Z-scores can be found in the top x-axis

Figure 28. Progression of licking behavior during training. **A.** The animals were presented with two tones, one CS^- (red) and one CS^+ (black), in random order and with variable inter-tone intervals (20-40 s). The termination of the CS^+ coincided with the delivery of a liquid reward (R). **B.** Normalized licking frequency (y-axis) plotted as a function of time (x-axis) around the tone presentations (gray shading) at early (dashed line) and late (solid line) stages of learning (CS^+, black; CS^-, red). **C.** Percent trials with anticipatory licks (y-axis) during the CS^+ (black) and CS^- (red) plotted as a function of daily training sessions (x-axis; sessions 1-5). Starting from session r1 (gray shaded area), the tone-reward contingencies were reversed (CS^+ red; CS^- black). Gray line indicates proportion of trials with spontaneous licking ± SEM.

Figure 29. Learning-related changes in correlated amygdalo-striatal gamma. **A**-**H.** Coherence (y-axis) vs. time (x-axis) around onset of CS^+ (black lines **A**-**E**;

red lines **F**-**H**) and CS^- (red lines **A**-**E**; black lines **F**-**H**) ± SEM (dashed lines). Two tone-reward contingences were used: the first between training Days 1-5 (**A**-**E**) and the second, where the identities of the CS^+ and CS^- were reversed, between Days r1-r3 (**F**-**H**). **I.** Difference in gamma coherence between the two tones (y-axis) as a function of recording sessions (x-axis).

Figure 30. Behavioral performance and BLA-striatal gamma coherence. **A.** Changes in BLA-striatal gamma coherence parallel behavioral performance. The difference between BLA-striatal gamma coherence induced by CS^+ vs. CS^- (y-axis) is plotted against the proportion of CS^+ trials with anticipatory licking (x-axis). Each data point represents a training session. A significant correlation was found (red line, $r = 0.55$, $p < 0.01$). The correlation remains significant ($r = 0.37$, $p < 0.05$) even when the two top-most data points are excluded from the analysis. **B.** Percent change in BLA-striatal gamma coherence during trials without (left) or with (right) anticipatory licking during the CS+. The difference was found not significant (t-test, $p = 0.58$).

Figure 31. Learning-related changes in coherence between structures. Only coherence of gamma between BLA and striatum (**A**) follows behavioral changes. For all panels, the y axis plots the difference in coherence elicited by the two tones (y-axis) as a function of recording sessions (x-axis). Panels **A**-**C** examine fluctuations in gamma coherence between striatal and BLA (**A**), striatal and cortical (**B**), or striatal and thalamic (**C**) recording sites. **D**-**E.** These panels present learning-related fluctuations in coherence of 70 Hz between cortical and striatal LFP activity (**D**), or 50 Hz between thalamic and striatal recording sites (**E**). In all cases dashed gray lines indicate SEM.

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