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The role of glycogen derived Sactate in cocaine-related memories.

Boury-Jamot Benjamin

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Centre de Neurosciences Psychiatriques, département de Psychiatrie

The role of glycogen derived lactate in cocaine-related memories.

Thèse de doctorat en Neurosciences (# 210)

présentée à la

Faculté de Biologie et de Médecine
de l'Université de Lausanne

par

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Jury

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Programme doctoral interuniversitaire en Neurosciences des Universités de Lausanne et Genève

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The role of glycogen derived lactate in cocaine-related memories

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pour Le Doyen
de la Faculté de Biologie et de Médecine

Prof. Jean-Pierre Hornung

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Abstract:

Drug memories that associate contextual cues with the effects of drugs are known to shape persistent drug seeking behaviors in rodents. In abstinent humans, drug cues are known to evoke salient, persistent and overwhelming memories of drug taking experiences, thereby inducing higher risks of craving and relapse.

Since the transfer of glycogen derived lactate from astrocytes to neurons is required for long-term memory, we explored the possibility that disrupting glycogenolysis in astrocyte could impair the acquisition and maintenance of positive affective memories associated with cocaine-associated cues. We have observed that rats treated with intra-basolateral amygdala infusions of the inhibitor of glycogen phosphorylase, 1,4-dideoxy1,4imino- D-arabinitol (DAB) could prevent the formation and the maintenance of cocaine-induced conditioned place preference. Then, we demonstrated that drug memory was rescued by L-Lactate/DAB co-administration through a mechanism requiring the synaptic plasticity related transcription factor Zif268, and extracellular signal-regulated kinase (ERK) signaling pathway. Interestingly, co-administration of DAB and L-Pyruvate failed to do so, demonstrating that L-Lactate played a non-metabolic role in this process. Moreover, L-Lactate co-administrated in BLA with dopamine 1 or beta-adrenergic receptors antagonists rescued the effects of these inhibitors, showing that dopaminergic and beta-adrenergic pathways seem to be linked to lactate metabolism during the formation of CPP behavior.

We then targeted the prefrontal cortex (PFC) and showed comparable results in both formation and maintenance of cocaine-induced CPP. In contrast, our observations suggested that glycogen derived lactate has no specific role on drug-related memory in brain regions involved in reward circuitry.

Taken together, these results give a pointer to a signaling role of astrocytic lactate in both acquisition and maintenance of cocaine-seeking behavior following a BLA PFC temporal pathway and open novel therapeutic avenues to reduce the long-lasting impact of drug cues on conditioned responses to cocaine.

Résumé:

Les mémoires liées aux drogues qui associent des indices contextuels aux effets des drogues sont connues pour façonner les comportements persistants de recherche de drogue chez les rongeurs. Chez les humains abstinents, ces indices contextuels sont connus pour évoquer des souvenirs saillants, persistants et irresistibles des expériences de prise de drogue, entraînant ainsi des risques plus élevés d'envie et de rechute.

Étant donné que le transfert du lactate dérivé du glycogène des astrocytes aux neurones est nécessaire pour la mémoire à long terme, nous avons exploré la possibilité que la perturbation de la glycogénolyse dans les astrocytes puisse compromettre l'acquisition et le maintien de souvenirs affectifs positifs associés à ces indices liés à la cocaïne. Nous avons observé que les rats traités avec des infusions de l'inhibiteur de glycogène phosphorylase, le 1,4-didésoxy-1,4-imino-D-arabinitol (DAB) dans l'amygdale basolatérale (BLA) pourraient empêcher la formation et la maintenance de la preference de place induite par la cocaïne. Ensuite, nous avons démontré que la mémoire liée à la drogue a été préservée par la co-administration de L-lactate/DAB par un mécanisme nécessitant le facteur de transcription Zif268 associé à la plasticité synaptique et la voie de signalisation de la kinase régulatrice de signal extracellulaire (ERK). Fait intéressant, la co-administration de DAB et de L-Pyruvate échoue à faire de même, démontrant que le L-Lactate a joué un rôle non métabolique dans ce processus. De plus, le L-lactate co-administré dans la BLA avec des antagonistes des récepteurs dopamine 1 ou bêta-adrénergiques a inversé les effets de ces inhibiteurs, montrant que les voies dopaminergiques et bêta-adrénergiques semblent être liées au métabolisme du lactate lors de la formation du comportement de CPP.

Nous avons ensuite ciblé le cortex préfrontal (PFC) et avons montré des résultats comparables dans la formation et le maintien du CPP induit par la cocaïne. En revanche, nos observations ont suggéré que le lactate dérivé du glycogène n'a aucun rôle spécifique sur la mémoire liée à la drogue dans les régions cérébrales impliquées dans les circuits de récompense.

Pris ensemble, ces résultats donnent un rôle de signalisation du lactate astrocytaire à la fois dans l'acquisition et la maintenance du comportement de recherche de cocaïne via à une voie temporelle BLA/PFC et ouvrent de nouvelles voies thérapeutiques dans le but de réduire l'impact durable des indices contextuels liés aux drogues sur les réponses conditionnées à cocaïne.

List of abbreviations:

5-HT:	5-hydroxytryptamine (serotonin)
AK:	adenylate kinase
AMPA:	alpha-amino-3-hydroxy-5-methyl-isoxazole propionic acid
ANLS:	astrocytes neurone lactate shuttle
BLA:	basolateral amygdala
cAMP:	cyclic adenosine monophosphate
CBF:	cerebral blood flow
CPP:	conditioned place preference
DAB:	1,4-didesoxy1,4imino-D-arabinitol
DSM:	diagnostic and Statistical Manual of Mental Disorders
EAAT1:	Excitatory Amino Acid Transporter 1
ERK1/2	extracellular signal–regulated kinase 1/2
Gbe1:	glycogen branching enzyme
Glast:	glutamate Aspartate Transporte
GLT-1:	glutamate transporter 1
Glut-1:	astrocytic glucose transporter 1
GP:	glycogen phosphorylase
GPR81:	G-coupled cell surface receptor 81
Gys1:	glycogen synthase 1
HDAC:	histone deacetylase
Hipp:	hippocampus
IEG:	immediate early genes
LDH:	lactate deshydrogenase
LTD:	long term depression
LTP:	long term potentiation
MCT:	monocarboxylate transporter
NAcc:	nucleus accumbens
NAD(H):	nicotinamide adenine dinucleotide (reduced)
NMDA:	N-methyl-d-aspartate
ODN:	oligonucleotides
PFC:	Prefrontal Cortex
PK:	phosphorylase kinase
PTG:	protein targeting glycogen
SERT:	serotonin transporter
TTX:	tetrodoxin
VTA:	ventral Tegmental Area
Zif268	zinc finger protein 225

1 Table of contents

1. INTRODUCTION

1.1. Cocaine Addiction

1.1.1. A major health concern

From the last decade, the number of drug-users in the world has increased by around 10%. In 2014, 247 millions of people aged between 15 and 64 years used at least one drug and 29 millions suffered from drug dependence which lead to more than 200 000 drug related deaths[1].The difficulty to exactly define drug addiction reflects the multiple causes and consequences of this disease. This complex definition of addiction is often subjected to debate [2,3] and sometimes mingled with the term « substance dependence » [4]. The multiple criteria defining the dependence and addiction (listed in Figure 1) are subjected to debate as well [5]. To simplify, in this thesis, I will only use the terms « drug addiction » and refer to the criteria published in the fifth and last edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM V), by the American Psychiatric Association in 2013.

	DSM-IV Abuse[a]		DSM-IV Dependence[b]		DSM-5 Substance Use Disorders[c]	
Hazardous use	X	≥1 criterion	–		X	≥2 criteria
Social/interpersonal problems related to use	X		–		X	
Neglected major roles to use	X		–		X	
Legal problems	X		–		–	
Withdrawal[d]	–		X	≥3 criteria	X	
Tolerance	–		X		X	
Used larger amounts/longer	–		X		X	
Repeated attempts to quit/control use	–		X		X	
Much time spent using	–		X		X	
Physical/psychological problems related to use	–		X		X	
Activities given up to use	–		X		X	
Craving	–		–		X	

[a] One or more abuse criteria within a 12-month period *and* no dependence diagnosis; applicable to all substances except nicotine, for which DSM-IV abuse criteria were not given.
[b] Three or more dependence criteria within a 12-month period.
[c] Two or more substance use disorder criteria within a 12-month period.
[d] Withdrawal not included for cannabis, inhalant, and hallucinogen disorders in DSM-IV. Cannabis withdrawal added in DSM-5.

Figure 1: Different criteria used by the American Psychiatric association between DSM-IV (1994) and DSM-V (2013). From Hasin et al, 2013 [5].

The DSM refers to different substances such as caffeine, alcohol, heroin, and cocaine and defines the substance use disorders when two or more criteria are observed for at least 12 months. The numbers of criteria (eleven) participates to the complexity of the definition of

drug addiction. However, a simple definition is often used, presenting addiction as a chronic brain disease characterized by a compulsive drug-seeking and drug-taking behavior despite negative consequences. This definition is in accordance with the criteria described in the DSM. For example, the « craving » is an increasing and compulsive drug taking behavior after a long period of abstinence and the criteria « activities given up to use » are the « negative consequences » in the simple definition. Indeed, drug addicted people become mostly focus on the obtention of their addictive substance, neglecting the social effect on their life (loss of work, social isolation, divorce…), economical and legal consequences (incarceration, lack of money…) or simply the effect on their health (AIDS, viral hepatitis…). Therefore, one of the differences between other chronic brain diseases (schizophrenia, Alzheimer disease…) and drug addiction is that the second one is perceived by the public more as a social problem than as a real brain disease. People abusing illegal drugs are often considered criminal or marginal, although they should also be considered as patients suffering from a chronic brain disorder [6]. More generally, drug addiction is not only a public health concern, it also impacts worldwide politic, economy, criminality, healthcare and ecology. In particular, cocaine trafficking combines all these characteristics and represents a major global threat in the world.

1.1.2. The impacts of coca production and cocaine use

The use of cocaine has evolved over the years and is often described as one of the most harmful drug for the users themselves but also for the surrounding people [7].

Cocaine is an alkaloid drug derived from coca leaf which is cultivated and used by the population living in the Andes mountains for more 1200 years[8]. The leaves, containing less than 1% of coca, are commonly chewed or drunken in coca tea and used for their stimulant effects by this population. Interestingly, the short term physiological effects of coca leaf chewing seem like coca powder. Although the use of coca leaf by millions of people for centuries seems not to produce aversive effects in South American, and that long-term consumption does not induce tolerance or craving after deprivation, it has been shown that chronic consumption could affect cognitive functions. Despite the use of coca leaves appears to be integrated in social and economic lives of the south American countries, the effects on cognition could reflect the role of coca leaf consumption in the unfavourable social position

of these consummers[9]. In occidental countries, the dramatic consequences of cocaine drug use may have started by the chemical isolation of the molecule. Indeed, the stimulant effects of coca leaf were discovered by the European conquistadors[10] but the chemical isolation of cocaine was made lately in 1860. Firstly used as medication like a local anaesthetic agent and notably promoted by Freud for its potential therapeutical applications[11], the danger of cocaine rapidly appeared and be replaced by safer molecules such as novocaine [12]. Then, cocaine was perceived as a good, chic and relatively expansive product, with strong stimulant and rewarding properties. This good perception of cocaine use was prolonged in the sixties despite the illegal state of cocaine since the beginning of the 20th century. The apparition of the free base of cocaine, crack, which is cheaper than cocaine powder increased the number of users. Nowadays, around 19 million of people use cocaine in 2014, an increase of more than 30% compared to 1998. Although the peak was in 2007, the number of cocaine users is increasing in 2014 and according to the last report of the United Nations Office on Drugs and Crime in 2016[1], the current projections for 2015 tend to confirm this trend. The increasing market lead also to an increase of coca leaves cultures. The government of the first coca producer, Columbia, has started coca eradication, some studies showed that coca cultivation continues to have a strong impact on population and environment. First, the herbicide used for coca eradication has been shown to impact health and environment. Second, the coca cultivation is moving in different territories, increasing the deforestation [13,14]. Lastly, coca production is affecting the environment of producer countries as well as the health care of consumer countries. The illegal trafficking from South America countries to Asia, USA and Europe through African continent plays also a major role in economy of these countries. This worldwide concern has multiple consequences and despite the preventive measures taken to limit the production, cocaine use is still increasing.

1.1.3. The lack of cocaine treatment

Because the production of coca appeared to be difficult to control, an efficient treatment to help the increasing number of patients is critical to find. Interestingly, even if the effects of cocaine are strength, between 5 and 12% of people consuming cocaine become addicted [15,16]. However, the destructive behaviours and loss of control for this addicted population is a major health problem. Cocaine and more specifically, the free-base form, crack, is known to be one of harmful illegal drug[7] and despite the high prevalence of drug addiction, only 1 in 6 people with drug use disorders is in treatment [1]. The neurosciences have made major achievements in the comprehension of molecular and cellular mechanisms of cocaine addiction but no effective treatment has been found. One of the promising treatment could be a vaccine against the effects of cocaine, but it is already subject to debate regarding the ethical implication of a vaccine [17] . The lack of effective treatment may be correlated by the lack of new theories on addiction. It appears that focusing on innovative mechanisms and theories could be a potential interesting way to treat addiction. For example, the research mainly focus on the role of the neurotransmitter dopamine in cocaine addiction for more than forty years, without finding an efficient treatment [18].

1.1.4. From maladaptive learning to compulsive drug seeking behavior

A central problem in the treatment of drug addiction is the high risk of relapse, often precipitated by re-exposure to the environment. This conditioned response can occur despite years of abstinence from drug use, and represents a major challenge for the treatment of addiction. Indeed, it is now well established that drug memories that associate contextual cues with the effects of drugs of abuse shape and maintain persistent drug seeking behaviors[19]. Preclinical observations have long evidenced that, through predictive association with the drug's effects, drug conditioned stimuli can precipitate the reinstatement of previously extinguished drug-seeking behaviours [20] [21]. In abstinent humans, drug cues are known to evoke salient, persistent, and overwhelming memories of drug-taking experiences, thereby inducing higher risks of craving and relapse [22] [23]. A current consensus suggests that persistence

of drug addiction would depend on the remodelling of synapses and circuits responsible for long-term associative memory. In other words, both clinical and laboratory observations have converged onto the hypothesis that addiction usurps neural processes that normally account for reward-related learning [24]. The initial pavlovian association between the drug (unconditioned stimulus) and the contextual stimulus (conditioned stimulus) lead through maladaptive conditioning to an instrumental memories leading seeking to drug taking and seeking behaviour as well as relapse [25]. Drug cues can spark intrusive and overwhelming memories of drug-taking experiences, thereby leading to overpowering motivational strength and decreased capacity to control the desire to consume drugs. Moreover, converging evidence has revealed that memory and addiction share both neural circuitry and molecular mechanisms [26] [27] [28]. Learning the significance of a predictive cue to trigger the appropriate behavioural response is thought to require the storage of specific patterns of information in the brain [19].

Some hypothesis argue that increasing and repetitive cocaine intakes contribute to a homeostatic dysregulation in brain [29,30]. Because the reward effects on brain decrease after each cocaine use, the subject may be motivated to increase doses, entering in a cycle of dysregulation of his brain reward system [30]. This neuro-adaptation could be responsible of the compulsive behaviour, as well as the impulsivity and lead to relapse. However, other studies showed that repetitive cocaine taking provokes a neuro-adaptation which regulated the attribution of incentive salience to the stimuli. These modifications of brain circuits and cells may lead to an incentive sensitization which become pathological [31,32]. Interestingly, authors describe the associative learning as part of the process of drug addiction which could be linked to incentive sensitization. The learning of the association between the stimulus and the response participates to the formation of habits, promoting compulsivity and drug seeking behaviour [33,34]. Studies have shown that the formation of habits in drug addiction involved several brain regions such as prefrontal cortex (PFC), hippocampus and basolateral amygdala (BLA) projecting to the nucleus accumbens (NAcc). More specifically, BLA is known to integrate associative informations and to translate them to the NAcc core. This interaction BLA-NAcc core participates to the formation of drug seeking behaviour from maladaptive learning. Hippocampus also integrates contextual association to the NAcc shell and PFC participates to the impairment in executive control through it role in reinstatement via glutamate projection

to the NAcc. In fact, formation of habits from maladaptive learning involve a transition of from prefrontal cortical to striatal regions as well as from ventral to dorsal striatal subregions[35] (Figure 2).

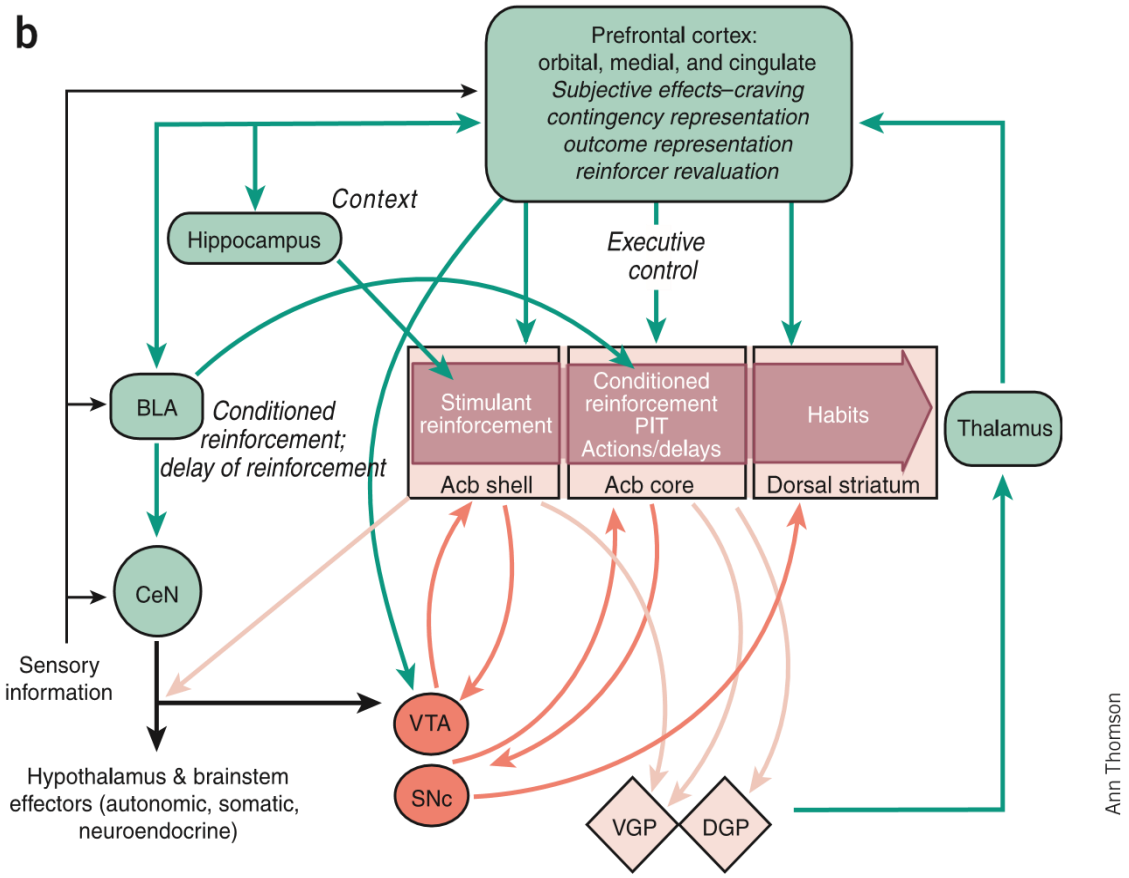

Figure 2: Reinforcing effects of drugs may engage stimulant, pavlovian-instrumental transfer and conditioned reinforcement processes in the nucleus accumbens shell and core and then engage stimulus-response habits that depend on dorsal striatum. Green/blue arrows, glutamatergic projections; orange arrows, dopaminergic projections; pink arrows, GABAergic projections; Acb, nucleus accumbens; BLA, basolateral amygdala; CeN, central nucleus of the amygdala; VTA, ventral tegmental area; SNc, substantia nigra pars compacta.[35]

Then, targeting the maintenance of cocaine-related memory could be an interesting therapeutic way to treat several memory related diseases and more specifically to treat drug-related memory disorders where environmental cues are linked to the reward effect of the drug. [36] [37] [38].

The persistence of the maintenance of high risk of relapse in addiction, even after many years, and the increasing difficulty to find a proper treatment could be explained by the nature of memory mechanisms. In addiction, these contextual-cues memories have been studied during

three different steps: the formation, the extinction and the maintenance (figure 3) [20 39 40 41 42]. The maintenance of this maladaptive learning has been shown to be dependant of protein synthesis and susceptible to disruption by protein synthesis inhibitors such as anisomycin [43 44]. Adrenergic receptors have also be a target of researches, the antagonists of beta and alpha adrenergic receptors showing a decrease in drug-seeking preference and in cue-induced preference [45–48].

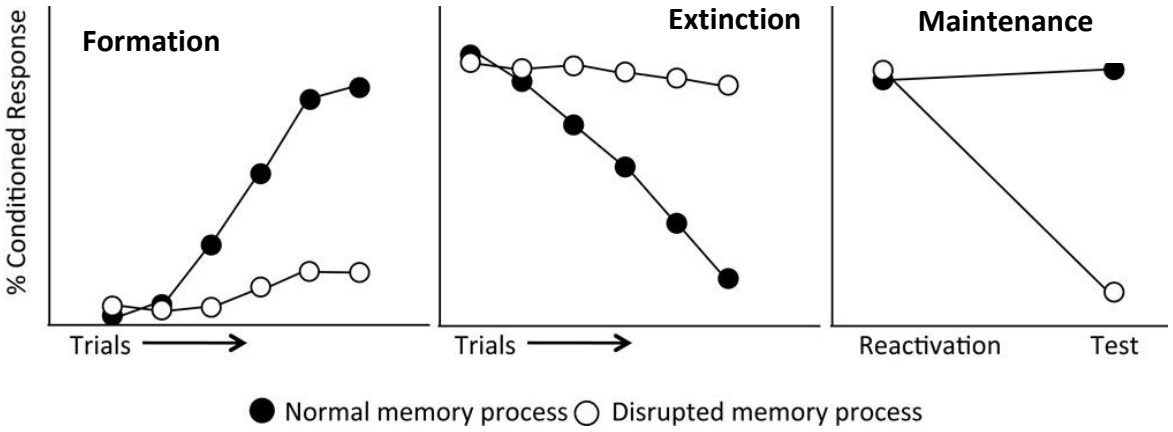

Figure 3: Cocaine-cues related memory can be prevented or disrupted in three different phases. Trials during training consolidate conditioned responses in normal (black circles) whereas a repeated exposure to the contextual stimuli without the unconditioned stimulus leads to a decrease of the responses. Finally, the reconsolidation of memory occurying during a single re-exposure to the contextual stimuli updates and reinforces the memory. Protein synthesis blockers can block each step of the conditioning. (adapted from Tronson and Taylor, 2013 [49])

Other studies have published in vivo results on memory formation prevention and memory maintenance disruption related to addiction [50 51 52]. As it will be developed in this introduction, some studies have already investigated and shown that propranolol, MAP kinase ERK inhibitor, Zif268 or ODN antisense are able to interfere with the maintenance of the memory and abolished the initial memory related to the reward effect of the drug[53,54] (further development at point 1.3).

1.1.5. Effect of cocaine in brain: a complex circuitry involving different brain regions and neurotransmitters

1.1.5.1. The dopamine hypothesis

Acute cocaine use produces feelings of euphoria, increases elevated mood and acts as an energy booster. These short-term effects encourage the consumer, which has also an increase in self-esteem, to repeat his cocaine use. However, this rewarding effect, which was first explained through dopamine release in the brain, is not sufficient to explain the addictive properties of cocaine. Indeed, such as high palatable food and strong natural rewards, the direct and short term effect of acute administration of cocaine is known to increase dopamine in limbic system and in different forebrain regions interacting together to drive both motivational and rewarding effects of the drug [55] [56] [57]. However, the increase in dopamine is higher with cocaine compared to high palatable food intake and the dopamine response does not increase with repeated exposure of high palatable food in contrast to cocaine[58]. Basically, dopamine neurotransmission is regulated by complementary fine tuning systems. Among other mechanisms, transporter expression can be up- or down-regulated, directly impacting the electrical signal by vacuuming the amount of dopamine in the synaptic cleft. At the molecular level, cocaine binds to the dopamine transporter (DAT) and acts as a reuptake inhibitor [59-60]. With a similar mechanism, cocaine alters the serotonin and noradrenaline neurotransmission as well, but a current consensus acknowledges the critical role of the DAT in the reinforcing properties of the drug [61,62,63]. More specifically, the dopamine VTA projections on NAcc are known to be required in the reward effect of cocaine [57,64]. But dopamine cannot be simply defined has a molecule driving the reward effect of a drug. It has been shown that dopamine burst in monkey could predict a reward. Therefore, dopamine signalling appeared to be more linked to a prediction of a reward than the effect of the reward itself [65]. Other studies have shown that rat model with a depletion of dopamine in neostriatum and nucleus accumbens still expressed a pleasure to consume sucrose showing that mesolimbic and neostriatal dopamine are not involved in the hedonic effect of a reward [66]. The authors conclude that "dopamine may be more important to *incentive salience*

attributions to the neural representations of reward-related stimuli". Other dopamine projections from VTA to PFC and amygdala have also been identified to play a role in cocaine neurocircuitry[67]. The PFC integrates the informations of pre-limbic brain regions and drive the behavioural and motor responses and prelimbic cortex has been shown to participate to cocaine reinstatements[68,69]. The role of PFC in cocaine induced reinstatement has been shown by the infusion of baclofen in prelimbic area[70] as well as tetrodoxin (TTX) administrations [68]. In contrast, the BLA in drug addiction has been associated to the formation and maintenance of cocaine associated memories [71] [72] [73] [74] and dopamine has been also identified to drive the reward-related learning[75,76].Moreover, BLA and the dopamine projections going to the PFC are also required for a cue-induced cocaine reinstatement but not for a cocaine reinstatement in rats (modulating more by the dopamine and glutamatergic system in VTA and NAcc), showing the role of dopamine projections in the memory component of drug reinstatement [77] [78] [79].

A single exposure to cocaine has been shown to induce Long Term Potentiation (LTP) of AMPA (alpha-amino-3-hydroxy-5-methyl-isoxazole propionic acid)-receptor-mediated currents in VTA up to five days [80]. NMDA (N-methyl-d-aspartate) blockers have been shown to prevent this AMPA receptor related LTP demonstrating that AMPA and NMDA receptors play a role in dopamine response under acute cocaine injection. In contrast, repeated exposure of cocaine in self administration paradigm showed a persistent potentiation of dopamine neurons in VTA, up to 3 months. This potentiation in only transient if cocaine is passively administrated or in presence of natural reward [81]. However, study using double and triple knock-out mice of dopamine and serotonin transporters have shown a lack of cocaine-conditioned place preference in DAT knockout mice with no or one copy of the SERT gene [82]. Optogenetic stimulations of DA neurons in VTA have also been described to induced a place preference or to stimulate self administration and addictive behaviours in rodent [83–85]. Even though dopamine singling plays a major role in cocaine reward pathway, it cannot fully explain all the aspects of cocaine taking by itself.

1.1.5.2. The glutamate hypothesis

Dopamine is not the only neurotransmitter involved in the reinforcing properties of cocaine. Glutamate seems to play a central role in the processes underlying the acquisition, the reinforcement, the craving and the reinstatement of cocaine seeking[86–88]. Glutamatergic receptors seemed to be required for cocaine reinstatement, the blockade of α-amino-3-hydroxy-5-methyl-4-isoazole propionic acid (AMPA)/kainate receptors in NAcc core blocking the cocaine seeking behavior [86] as well as in the NAcc Shell [87]. In rodent, relapse is lead by glutamatergic input from prefrontal cortex to the nucleus accumbens. Inhibiting the prefrontal cortical glutamatergic neurons projecting to the NAcc prevented the increase in glutamate occurring during cued-induced reinstatement [88,89]. On one side, glutamatergic transmission within the mesolimbic-accumbens system appears to be also increased by cocaine [90]. Glutamate has been also found to be increased transiently in basolateral amygdala (BLA) in reward seeking behavior [91] and in nucleus accumbens during cocaine induced reinstatement in self administration. On another side, yoked cocaine administration and self-administration by itself failed to increase glutamate level in NAcc. Moreover, repeated cocaine injections occurring during self-administration have been shown to lead to a glutamate decrease in the same brain region [92]. In fact, chronic administrations of cocaine deeply modify brain circuitry and metabolic homeostasis [93].

In particular, cocaine impacts astrocytic glutamate homeostasis notably by attening the glutamate glutamine cycle They play a critical role in glutamate replenishment through glutamate to glutamine metabolization[94 95 96]. Briefly, glutamate is converted into glutamine in the astrocytes and transferred to neurons to be metabolized into glutamate to refill the storage of neurotransmitters for presynaptic excitatory neurons. After cocaine administrations, it has been demonstrated that the astrocytic glutamate transporter 1 (GLT-1) is decreased in nucleus accumbens leading to a decrease of glutamate uptake. The accumulation of neurotransmitters in the synaptic cleft activates extras synaptic glutamate receptors such as mGLUR2/3 and mGLUR5. mGLUR2/3 inhibitory receptors, participate to the presynaptic regulation of glutamate release, whereas the activation of mGLUR5 receptors induces Long Term Depression (LTD). Taken together, cocaine intake seems to induce a glutamate homeostasis dysregulation over time, leading to a change in LTP and LTD.

Interestingly, the increase of glutamate transporter GLT-1 in NAcc core, responsible for more than 80% of glutamate clearance [97], decreased cue-induced cocaine seeking behavior. Finally, blockade of glutamate uptake by the two glutamate transporter inhibitors TBOA (DL-threo-beta-benzyloxyaspartate) and DHK (dihydrokainate) reversed the effect of GLT-1 increase on this behavior demonstrating glutamate uptake played a core role in cocaine seeking behavior maintenance.

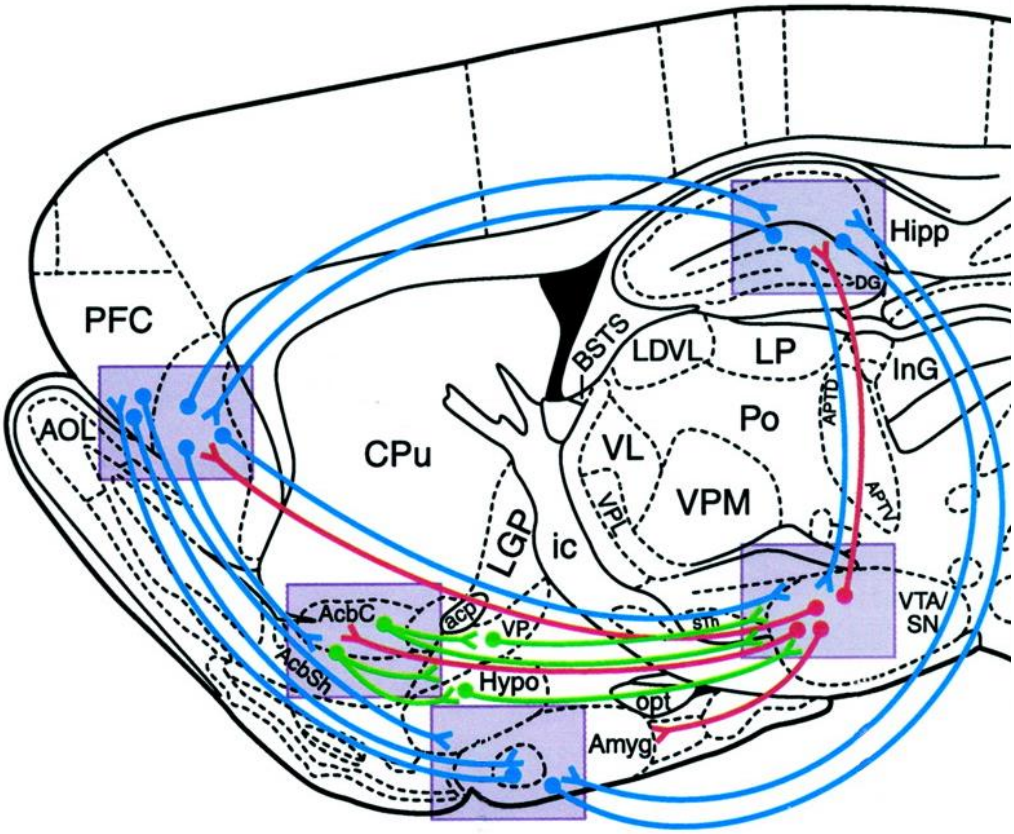

Figure 4: schematic representation of the cortico mesolimbic system and the projections between different brain regions are involved in drug addiction and reward-related learning. Blue lines represent glutamatergic pathways between prefrontal cortex (PFC), amygdala (Amyg), hippocampus (Hipp), nucleus accumbens core and shell (respectively AcbC and AcbSh), and ventral tegmental area (VTA). Red lines represent dopamine systems and green lines GABAergic pathways. AcbC, Accumbens core; Acb shell, accumbens shell; Cpu, caudate–putamen; VP, ventral pallidum;Hypo, hypothalamus; SN, substantia nigra. Other abbreviations can be found in Paxinos and Watson (1998). Base on Kelley et al, 2002 [98].

Summary:

In the first part of this introduction, we have seen that cocaine addiction is a major health concern, which can be characterized as a maladaptive learning. This memory component of cocaine addiction leads to habits and then compulsive behaviour. Recent evidences have highlighted an active role of metabolism and more specifically of astrocyte neurons interaction in memory processing. The second part of the introduction will focus on the possible link between metabolism and cocaine related memory and how metabolism could help to target cocaine related memory to treat addiction disease.

1.2. Astrocyte Neuron interaction in memory processing

1.2.1. The astrocytes: key players in tripartite synapse

Dopamine and glutamate, involved in the reinforcing properties of cocaine are also known to be closely related to astrocytes. Glial cells play a central role in synaptic transmission but their role was for a long time limited to a physical support, simply described as a sort of glue for the neurons. Nevertheless, for the last decades, the role of glial cells has been well described and they are actually involved in the brain homeostasis, metabolism, as well as in several diseases or brain disorders [99-100]. The glia is composed of two different subtypes, macroglia and microglia in which the astrocytes are the most complex and abundant glia cell subtypes. The astrocytes are divided into five different subtypes, reflecting their complexity and diversity of roles [101 102].

Despite a large consensus focusing on spike-time dependent synaptic plasticity [103,104], converging evidence now acknowledge that the role of astrocytes extends by far a supportive function for neurons, and rather contributes to information processing, signal transmission, regulation of neuronal excitability, and synaptic plasticity [105 106 107]. In the current view of the brain, astrocytes are localized near blood vessels and facilitate the distribution of nutriments and molecules to neurons. The astrocyte network unsheathes millions of billions synapses permitting an uninterrupted supply of energy substrates [108 102], not only providing structural support, but also regulation of neuronal activity and synaptic plasticity. Pioneering work has established that glia contributes to short-term plasticity by modulating neurotransmitter release from nearby presynaptic elements, and by activating postsynaptic glutamate

receptors. Afterwards, compelling evidence has fuelled the emerging concept that the synapse is a three-sided (tripartite) organization, in which astrocytes are essential partners of the chemical synapse [102 109 110 111]. In this perspective, glial cells sense synaptic activity through a broad variety of ion channels, transporters, and receptors expressed on their surface. Besides a role in the clearing of neurotransmitters (notably glutamate), thereby regulating cleft concentration and limiting diffusion to neighbouring synapses, sensors at the glial membranes can trigger the activation of a broad range of intracellular messengers, including calcium waves [112 113]. In turn, the release of active substances from glial cells, the so called glio transmitter, modulates the synaptic strength, notably by promoting the insertion of AMPA receptors at the surface of post-synaptic neurons. How this astrocyte-dependent control of synaptic strength and metabolic coupling underlies cognitive functioning and pathological adaptations responsible for brain pathologies and psychiatric diseases remains an open debate [114 100].

Recent studies highlighted the role of astrocytes in learning, memory and cocaine related memory [115–118]. Moreover, the active coupling between astrocytes and neurons has been shown to play a role in many psychiatric diseases such as Alzheimer, Parkinson or several mood disorders involving astrocyte dysfunctions [119 120 121]. Growing evidence suggest that a specific molecule, L-lactate, which has been considered as a waste product for a long time, could play a central role in memory and reward seeking behaviour. The release of lactate by the astrocyte and its transfer to the neuron has been described as the Astrocyte Neurone Lactate Shuttle (ANLS) model and has been linked to active learning and memory processing and synaptic activity.

1.2.2. The astrocytes neuron lactate shuttle model (ANLS)

The astrocyte network, known to form highly organized anatomical domains that are interconnected through gap junctions, contacts up to hundreds of thousands of synapses permitting an uninterrupted supply of energy substrates [122]. In particular, the metabolic coupling between astrocytes and neurons posits that glycogenolysis-dependent lactate is released from astrocytes [123–125] and imported into neurons [126,127]. This astrocyte neuron lactate shuttle was first described by Pellerin and Magistretti [128] in an in vitro model. They

showed that lactate instead of glucose seemed to be the preferred energy source of neurons in a subtends neuronal activity. These finding have been confirmed in vivo using two-photo microscopy. Under basal condition, glucose seems to be uptake at the same rate in neurons and astrocytes, but during an intense neuronal activity, through a sensory stimulation, astrocytes uptake more glucose than the neurons [129]. This glucose uptake has been described to be the result of glutamate uptake by the astrocyte in ANLS model. As it has been written earlier in cocaine addiction, glutamate release during synaptic activity by the presynaptic neuron has been shown to be uptake by the astrocytes through glutamate transporter GLAST (EEAT1) and GLT-1 (EEAT2) [130]. Recent time lapse imaging revealed the dynamic remodelling of GLT-1 transporter in developing astrocytes through spine-like structures. Astrocytes dynamically shape their cell surfaces to be close to the synapse, reinforcing the role of astrocyte's dynamic in the tripartite synapse. Their adaptation and localization near excitatory synapse participate to glutamate clearance and synaptic activity regulation [131] [132]. These finding have been recently confirmed by Murphy-Royal and colleagues using high-resolution live imaging techniques. They have shown that glutamate uptake by the astrocytes is also regulated by neuronal transmission and that GLT-1 transporters are dynamically mobile near the activated synapse. Glutamate transporters increased their diffusion to the glial cell surface under active condition but also rapidly reduced this diffusion under low active conditions [133]. The transport of glutamate into the astrocytes has many consequences on astrocyte metabolism. Glutamate uptake into the astrocyte uses a co-transport with sodium ion, increasing Na+ concentration in astrocyte intracellular milieu [134]. To equilibrate sodium concentrations, Na(+)/K(+)-ATPase pump appeared to be required in glutamate transport and uptake. To support this assumption, It has been demonstrated that inhibition of the Na(+)/K (+)-ATPase pump decreased glutamate uptake into astrocytes[135]. Interestingly, the co-localization of glutamate transporters and Na(+)/K(+)-ATPase in human has been recently described [136] reinforcing the evidence of this tight coupling. Then, the activation of the Na(+)/K(+)-ATPase pump to equilibrate Na(+) concentrations during this co-transport requires energy and lead to a decrease of ATP concentration in the astrocyte. To provide sufficient energy to cellular mechanisms, glucose is uptake by the astrocyte from blood vessels. In order to regulate this uptake, astrocytes participate actively into cerebral blood flow regulation[137] [138] by increasing it through different mechanisms. A first hypothesis is that ATP consumption

due to Na (+)/K (+)-ATPase utilization could produce a metabolic signal to increase cerebral blood flow. Another mechanism involved mGLUR receptors on astrocyte cell surface via potassium release and [K+] extracellular concentration elevation. An alternative pathway involving also mGLUR receptor on astrocytes cell surface could influence the smooth muscle around arterioles via production and release of metabolites of arachidonic acid[17]. Nevertheless, all these finding suggested a facilitation of glucose intake from blood vessel by the astrocytes. Once glucose is in the cell, it is metabolized into pyruvate via glycolysis which produces ATP as energy for the cell. The elevation in glucose concentration into the astrocytes has been shown to increase lactate production and release.

1.2.3. Lactate transfer from astrocyte to neuron

The final product of glycolysis, pyruvate, is catalyzed by the lactate dehydrogenase (LDH) to be metabolized into lactate. The different isoforms of the LDH are known to differ in their affinity with their different substrates, lactate and pyruvate. The LDH isoform LDH5 promotes the transformation from pyruvate to lactate and LDH1 promotes the opposite reaction. In brain, the repartition of these isoforms appears to be cell specific, neurons expressing the LDH1 form and astrocytes the LDH5 form. This repartition promotes lactate production by the astrocyte and lactate utilization by the neuron, supporting the ANLS model. Lactate transfer from the astrocytes to neuron has been described in vitro, showing lactate is transported through monocarboxylate transporters (MCT) with a cell specific repartition in brain. In rodent, MCT1 transporter is expressed on the cell surface of the astrocytes and brain endothelial cells. It facilitates the export of lactate into the extracellular space and is responsible for the entrance of lactate from the blood vessels into the astrocytes. Astrocytes express another MCT on their cell surface, the MCT4 which are exclusively found on astrocytes and responsible for the release of lactate in the extracellular space. On contrary, on the neuron cell surface, the MCT2 are mainly expressed and facilitate the entrance of lactate into the neuron. More precisely, it has been suggested that MCT2 are mainly localized on post synaptic neurons, supporting the role of lactate in synaptic activity[18].

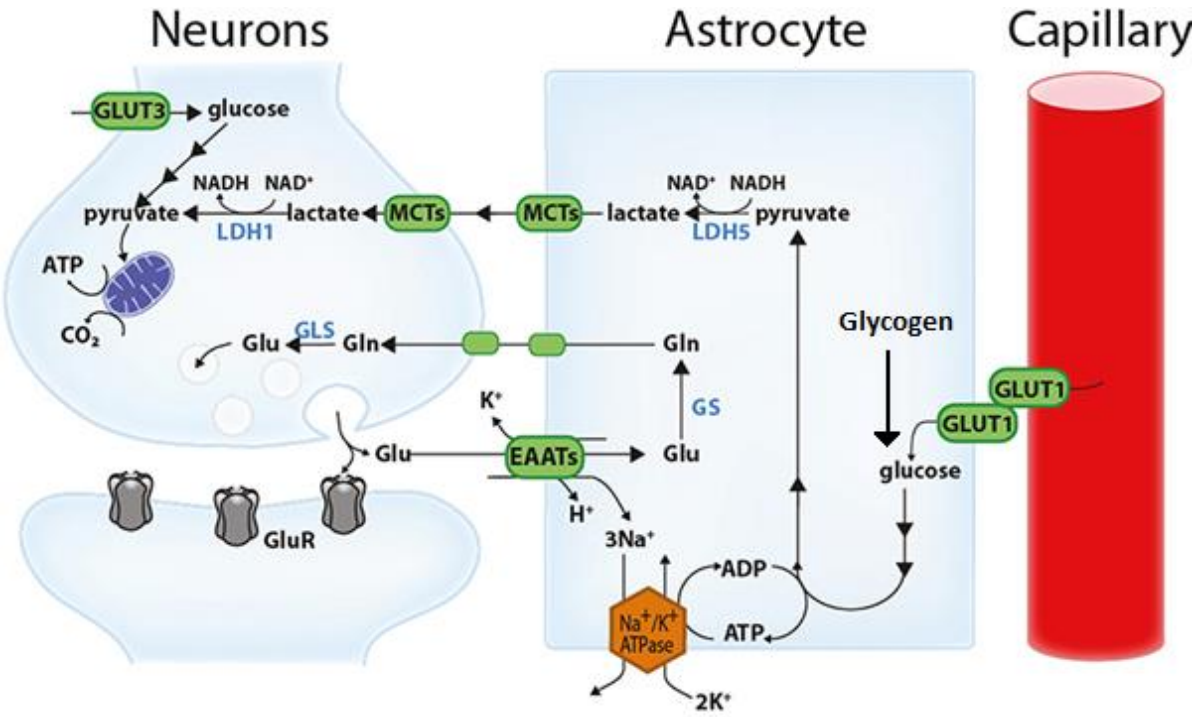

Figure 5: Astrocyte neuron lactate shuttle model.

Glycogen or glucose is the two-main sources of astrocytic lactate which use specific transporters (MCTs) to be transferred from the astrocyte to neuron. Glutamate release during synaptic activity is uptake by the astrocyte and induces lactate production. The recycling of glutamate into glutamine is also a part of the ANLS. Modified from Bélanger, Allamand and Magistretti, 2011 [122].

The affinity for the transporter is also different and stereospecific, L-Lactate being more transported than the non-metabolized form of lactate, D-lactate. MCT2 transporter has a high affinity for L-lactate (km around 0.7 mM) compare to MCT1 (Km= 3.5mM) and MCT4 (Km 35mM). This transfer of lactate from astrocytes to neuron has been recently confirmed in an in vivo study using genetically encoded lactate sensor. They also revealed that pyruvate injection into blood vessels increased lactate release by the astrocyte and accumulation in the neurons, according to the ANLS model [139]. The findings about the dysregulation of glutamate uptake after cocaine administrations and the role of astrocyte in cocaine addiction may suggest an implication of lactate release in this disorder. ANLS described a utilization of glucose during synaptic activity to produced lactate but another “form” of glucose is also able to produce lactate: the glycogen. Interestingly, glycogen mobilization is linked to glutamate uptake by the astrocyte, dopamine and noradrenalin.

1.2.4. From glycogen to lactate in brain

1.2.4.1. Glycogen distribution sustains neuronal activity and ANLS model

In mammals, glycogen represents the main storage of glucose [140]. Although only 1% of total body glycogen is stored into the brain, its role has been revealed to be a supportive source of energy in brain. It is mainly stored into astrocytes[141] and could be rapidly metabolized into glucose to provide additional energy to the cell under synaptic activity. Because the global reserve of glycogen in brain is low (in human and in rat), it has been demonstrated that glycogen in brain plays a role of buffer, rather than of a storage of glucose. Swanson and colleagues have shown in 1992, that glycogen could be rapidly metabolized during synaptic activity, showing its ability to provide suitable energy in short time delay [142]. Glycogen distribution in brain correlates also with high synaptic density. Recent study showed, using immunochemistry on microwaves-fixed mouse brain, that glycogen could be substantially found in hippocampus, striatum, and cortex[143,144] with a higher concentration of glycogen in the hippocampus. However, some brain regions contain also notable concentration of glycogen, such as hypothalamus or the amygdala[144]. These authors also confirmed that glycogen is mainly stored in astrocyte rather than neuron. More precisely, they have located the presence of glycogen in the processes of the astrocytes, and only a low concentration in the somata and a dispatched distribution of glycogen in aged mice (2 years old). The total amount of glycogen is comparable with young mice, but the average molecule size is decreased. Other recent imaging techniques showed that glycogen seems to be stored near pre-synaptic button than dendritic spines and are associated with monoaminergic varicosities [145].

To deliver energy during synaptic activity, glycogen breakdown in body is performed by glycogen phosphorylase (GP) which exists into three different isoforms, discovered a long time ago : muscle (GPM), brain (GPB) and liver (GPL) [146]. Except for the liver form, the other isoforms of glycogen phosphorylase are not tissue specific. For example, GPB is the mostly form found in brain, but it is also expressed in the cardiac muscle cells. In addition, a recent in vitro study suggested that glycogen degradation into astrocytes could be catalyzed by both astrocytic isoforms of glycogen phosphorylase GPM et GPB [147]. GP are activated directly by

Ca^{2+} and indirectly by the cyclic adenosine monophosphate (cAMP) through a phosphorylase kinase (PK). This PK could be activated by another kinase, the protein kinase A, stimulated by cAMP or by the Ca2+ fixation on its calmodulin subunit. GP could be also activated by the lack of nutrient, when glucose concentration is low, or in total absence of glucose. In these cases, adenylate kinase (AK) tries to restore the level of ATP from ADP. The production of ATP also promotes AMP which will activate GP to stimulate ATP production through glycogenolysis. Finally, another mechanism involving the increase of extracellular K^+ has been identified to promote glycogen breakdown. The potassium is uptake by the astrocytes via Na(+)/K(+)-ATPase pump and by the Na^+–K^+–Cl^- co-transporter 1 which is expressed on astrocyte cell surface. Potassium entry into the cell produces an elevation of Ca^{2+} which trigger GP activation [148]. Sotelo-Hitschfeld and colleagues demonstrated that K^+ stimulation also increased NADH/Nad+ radio, intracellular lactate and pyruvate concentrations and decreased glucose intracellular concentration. The authors suggested that lactate release could be mediated by a depolarization of the astrocytes by extracellular K^+ [149].

1.2.4.2. Glycogen metabolism and memory: the critical role of lactate

For the last decades, the role of glycogen derived lactate in neuron-astrocyte coupling was essentially to provide an energetic source supporting the neuronal activity. Several recent studies described that lactate is involved in working memory in rodent [116] and in aversive long term memory formation [117]. Both studies used a glucose analog as a glycogen phosphorylase inhibitor (1,4-Dideoxy-1,4-imino-D-arabinitol hydrochloride, DAB) to decrease glycogen-derived lactate release occurring during synaptic activity. Newmann and colleagues measured the increase of lactate level release in hippocampus during working memory behavioral test and showed that a glycogen breakdown blockade disrupted this increase as well as working memory itself. They proved also that lactate administrations into the hippocampus rescued the disruption induced by the phosphorylase inhibitor DAB. Interestingly, they also showed that glucose and DAB co-administration fully rescued the effects of DAB. In the same way, Suzuki et al, measured the increase of lactate release induced by aversive learning and they blocked this elevation with the glycogen phosphorylase inhibitor DAB in the hippocampus.

They showed that lactate is also involved in aversive long term memory formation but not in short term memory. On contrary to Newmann experiment, the co-administration of glucose and DAB did not fully rescue the amnesia, supporting the hypothesis that lactate is required for long-term aversive memory formation. Interestingly, they demonstrated that blocking lactate release prevent the learning-induced upregulation of the immediate early genes such as ARC and Zif268 [150] [151] involved in long term memory formation and conditioned place preference consolidation as well. Lactate positively modulated the phosphorylation of proteins – cofilin and the transcription factor CREB respectively involved in skeleton formation and upregulation of synaptic plasticity. Transfer from astrocytes to neuron has been also shown to be required for aversive long term memory formation. The blockade of the neuronal MCT2 by antisense oligonucleotides (ODN) impaired the memory formation, and exogenous lactate injection failed to restore it. On contrary, the impairment caused by MCT1 or MCT4 ODN was totally recovered by lactate injection. These experiments demonstrated that the critical transfer of lactate into the neuron is required for learning processing. In mice, the transport of lactate and the metabolism of glycogen have been shown to be modulated by aversive learning [152]. The expression of genes related to the ANLS, such as MTC, LDH, alpha2 subunit of the Na(+)/K(+)-ATPase and to the glycogen synthesis such as the protein targeting glycogen (PTG) and glycogen branching enzyme (Gbe1) and glycogen synthase (Gys1) were increased following training session in an aversive memory paradigm. Glucose uptake in hippocampus and amygdala was also increased during the retention test as well as the astrocytic glucose transporter 1 (Glut-1) suggesting a glycolysis production of lactate during test.

Gene expression involved in memory is modulated by lactate in vitro. Yang and colleagues demonstrate in neurons and astrocytes co-cultures that lactate promoted the expression of C-fos, ARC and Zif268 mRNA levels[153]. This increase has been shown to be MCT dependent and to potentiate NMDAR signaling. The expression of Zif268 was totally abolished after the application of antagonist of NMDA receptors, MK801 and more importantly by the glutamate binding site selective competitive inhibitor D-(-)-2-amino-5-phosphonopentanoic acid. Same observations were reported using a selective antagonist of the glycine site (L-689.60) showing L-lactate potentiated NMDA receptors already activated. NADH has also been implicated in this activation. Its application increased Zif268 and ARC expression which was inhibited by the

presence of MK801. The expression of these genes was confirmed in vivo, in the sensory-motor cortex of anesthetized mice following lactate administration. In addition to the blockade of lactate transfer into the neuron, the non-metabolized analog of L-Lactate, D-lactate, did not modulate gene expression. Taken together these experiments demonstrated that the G-coupled cell surface receptor GPR81, which is activated by both D- and L-Lactate seems not be implicated. However, Bozzo and colleagues argued that this GPR81 receptor could be involved in cortical neuron activity [154]. Neither pyruvate nor glucose reproduced the same effects as lactate application on gene expression, sustaining a non-energetic role of lactate. This property of lactate was also confirmed by in vivo experiments where lactate transfer into neuron seemed to be required for memory formation and maintenance. In addition, as it has been shown in vivo, pyruvate application did not reproduce the effect of lactate on memory formation, reinforcing the proposed mechanisms involving NADH action on NMDAR. Latham and colleagues recently published another role of D- and L- lactate on gene expression, showing lactate inhibited the histone deacetylase (HDAC) and increase gene expression [155]. They highlighted a new role of lactate in transcription as an epigenetic level like others HDAC inhibitors, trichostatin A and butyrate. Another mechanism of lactate on signaling has been proposed in the locus coeruleus by Tang and colleagues [156]. They demonstrated that lactate triggered noradrenaline (NE) released, and was inhibited by DAB or by the blockade of glutamate transport.

1.2.4.3. The crosstalk between glycogen regulation and cocaine-related pathways.

1.2.4.3.1. Glycogen synthesis and degradation are regulated by glutamate

According to the original ANLS model, glutamate uptake into the astrocytes results in glucose utilization and lactate production by the astrocytes. Moreover, the role of glutamate uptake on glycogen modulation had been already suggested in astrocytes culture by Swanson et al [157]. They found that the incubation of glutamate and aspartate increased glycogen content in the astrocytes through glucose utilization. These findings were being completed by Hamai and colleagues in 1999 [158], demonstrating that glutamate also increased glycogen synthesis through glucose utilization. They showed that glutamate uptake into the astrocytes

increased glucose uptake but did not increase glycogen synthase activation on contrary to the action of insulin. Further, insulin increased glucose uptake into the astrocyte significatively less than glutamate application, suggesting different mechanisms in glycogen synthesis. Glutamate has been also involved in Ca^{2+} elevation in the astrocytes [159] involving several glutamate metabotropic, purinergic and muscarinic acetyl choline receptors[113]. To support the coupling of glycogen and glutamate uptake, Genda and colleagues described the co-localization of the glycogen phosphorylase and the glutamate transporter GLT-1 in the brain [160]. This finding suggested that glutamate uptake plays a role in glycogen degradation instead of its synthesis. In addition, the inhibition of glycogen phosphorylase blocking D-aspartate uptake into astrocytes, this uptake was also described to be dependent to glycogen breakdown. Recent evidence in human stem cells also suggested that glutamate stimulation in neurons and astrocytes co-culture produced glycogen degradation, and more importantly lactate release. Neuronal activity has also been linked to a rapid glycogen turnover. Indeed, the blockade of glutamate transport into the astrocytes during electrical stimulation completely disrupted lactate release and glycogen utilization [161]. Taken together, these studies showed that glutamate uptake is glycogen dependent into a bi-directional way. The uptake of glutamate required glycogen degradation, and glycogen synthesis required glutamate uptake. Recently, Gibbs discussed the role of glycogen in glutamate synthesis and recycling into astrocytes during memory processing, probably involving a high turnover of glycogen content. In old chick, glycogen breakdown blockade by DAB blocked the increase of glutamate induced by training [162].

Past studies have already described an increase in glycogen utilization in muscle but not in liver, and a correlation with an increase of blood glucose and lactate after cocaine utilization [163] [164]. However, the glycogen metabolism in cocaine addiction is poorly studied. As discussed above, glutamate into synaptic cleft could be responsible for glycogen modulation and then glucose uptake leading to lactate release. These findings may suggest a dysregulation in glycogen metabolism and lactate released in an advanced state of drug addiction.

1.2.4.3.2. A possible role of dopamine in glycogen utilization

Glucose and lactate have also been linked in vivo to dopamine signaling. In vivo micro dialysis study has revealed that dopamine receptors agonist and antagonist in NAcc are able to modulate glucose and lactate concentrations [165]. Glucose extracellular concentration appeared to be increased after D2R antagonist application (bromorphine) and both extracellular glucose and lactate levels increased by D1R agonists. The authors speculated that the stimulation of post synaptic D1 receptors increased cerebral blood flow and glutamate release. First action would participate to the transfer of glucose from blood vessels into the extracellular space, and second one would promote glycogen breakdown increasing lactate production and release in extracellular space, as it has been discussed before. These results support the hypothesis that cocaine administration could be link to glycogen utilization and then lactate release. Dopamine receptor have been found to be expressed on astrocyte suggesting a pharmacological role in the modulation of dopamine response to cocaine through cyclic AMP (AMPc) and Ca2+ intracellular changes[166].

1.2.4.3.3. Noradrenaline receptors regulate glycogen metabolism

Cocaine is known to increase NA[60] in brain which participates to the glycogen metabolism by activating the adrenergic receptors (AR) expressed on astrocytes cell surface. The different isoforms of AR, β_1-AR and the β_2-AR, participates to glycogen breakdown through distinct mechanisms. The first one acts via the elevation of intracellular cAMP whereas the second one via an increase of intracellular Ca^{2+} concentration [167,168]. On contrary, the α_2-AR seems to participate to the inhibition of glycogen breakdown and to its resynthesize in the astrocytes[169]. In vivo, Alberini's lab has highlighted the role of astrocytic beta AR in the hippocampus on aversive memory formation[170]. An interesting study on the link between adrenergic pathway and glycogen metabolism has shown that DAB, the glycogen phosphorylase inhibitor, blocked the glycogen breakdown normally induced by zinterol, a β_2-AR selective agonist. More importantly, memory formation promotes by zinterol is prevented by DAB administration[162,171]. These results demonstrated that memory formation related to noradrenalin appears to be glycogen dependant. But the authors also proved that DAB was ineffective to prevent the increase of memory formation induced by a specific agonist of β_1-

AR, CL316243. Although memory formation is promoted by both of the beta-adrenergic receptors, their molecular downstream pathways seem to differentially involved glycogen utilization. However, in this process, glycogen appeared to be critical for memory formation and these experiments revealed a complex regulation of glycogen synthesis and breakdown by NE. Moreover, their role in reward associated pavlovian conditioning has been already investigated. In conditioned place preference, propranolol I.P injections have been shown to block the drug related memory maintenance[45,172]. Another study published in 2014 has shown that propranolol administered into BLA but not in NAcc blocked morphine CPP reconsolidation[48]. However, these studies did not focus on glycogen metabolism during CPP memory formation and maintenance. But taken together, studies on memory involving adrenergic pathways suggested that glycogen utilization induced by NA on astrocytes could play a role in drug-related memory reconsolidation but it involved different receptors and brain regions.

1.2.4.3.4. Glycogen breakdown is linked to serotonin pathway

Cocaine administration also increases extracellular serotonin (5-HT) [173] by blocking their reuptakes through 5-HT transporter. 5-HT acts on different brain regions (for example NAcc, VTA and hippocampus) through multiple receptors (7 different classes and 16 subtypes) on drug reward pathway [174] which are also expressed on astrocytes surfaces and participate to the modulation of intracellular Ca^{2+} elevation leading to glycogen utilization [175]. The link between glycogen utilization and serotonin has been made under an intense exercise. Lactate increased induce by exercise is correlated with a glycogen degradation and an increase of serotonin turnover in rat hippocampus[176]. Serotonin has been also implicated in cerebral blood flow (CBF) regulation, involving again astrocytes which could modulate CBF depending on the brain region [177]. Interestingly, it has been demonstrated that serotonin enhanced memory consolidation involving glycogen utilization. 5HT increased the performance in a discrimination memory task, which has been disrupted by DAB administration [178].

1.3. Memory is labile and susceptible to disruption

The different state of memory and its mechanism have been long studied in the giant marine snail, aplysia. The gill-withdrawal reflex of aplysia is a defensive process which has provided an identification of several mechanism of memory: sensitization, habituation, and classical conditioning. Sensitization is characterized by the adaptation of an aversive stimulus. In aplysia, it has been investigated by a shock on the tail to provoking this defensive reflex. Interestingly, a single shock on the tail is responsible for the formation of a short-term memory lasting few minutes and independent of protein synthesis. In contrast, multiple repeated and spaced shocks promote a long-term memory lasting few days in a protein synthesis dependant manner[179]. Rapidly after the shock, serotonin receptor activation leads to a cAMP increase on presynaptic neurons. This elevation of cAMP participates to the synaptic changes occurring during short term memory [180]. In contrast, repeated stimulations of the tail contribute to serotonin elevation which leads to an increase of cAMP in intracellular milieu for several minutes. This longer cAMP increase has been found to activate Protein Kinase A (PKA) which lead to neurotransmitters release participating to shape synaptic changes in long-term memory formation [181].

1.3.1. Memory Consolidation

1.3.1.1. General aspect

The period right after a conditioning session, when the memory starts to be formed is often called consolidation. In other words, consolidation is the period when fresh memory become stabilized allowing its permanently storage in brain but also referred to the strengthening of memory already established occurring after a post training session. CREB plays a central role in consolidation and is involved in a cascade of immediate-early genes (IEGs) expression activation, which is critical for the first phase of memory consolidation. Other IEGs, cell adhesion molecules, and enzymes that control the degradation of intracellular or extracellular proteins are also induced by CREB. They will later regulate the expression of late genes which participate in a second delayed phase of consolidation depending of the brain region [182–184].

The mechanisms underlying memory formation and consolidation are brain region and time specific. Hippocampus is one of the structures identified a long time ago as a key region of learning and consolidation of memory, especially contextual and spatial memory. LTP has been characterized in CA1 to be mediated through NMDA receptor and glutamate. Morris and colleagues have demonstrated that an antagonist of NMDA receptors blocked the LTP as well as the memory formation [185]. LTP is divided into different steps, an early and a late LTP. The early LTP is produced by a single train of stimuli and does not require protein synthesis nor translational processes and lasts for 1 to 3 hours. On contrary, the late phase of LTP which is produced by multiple trains of stimuli requires protein synthesis. Because the stimulation is longer and stronger, LTP lasts more than early LTP, for at least one day. This late LTP involves translation as well as transcription mechanisms requiring CREB, PKA and MAPK activation and leading to critical synaptic changes [186] [187]. Because long-term memory formation is protein dependant, many ways have been used to prevent it. Anisomycin, a protein synthesis inhibitor has been shown to block memory formation when injected into hippocampus before contextual memory dependant paradigm and more than 90% of protein synthesis has been shown to be required to efficiently block memory formation [188]. Many studies have found that memory formation could be blocked using anisomycin after training session in rodents as well. But memory consolidation required different protein synthesis waves which are separated in time. In cocaine-related memory, Li and colleagues have demonstrated that consolidation in BLA in a cocaine CPP can be blocked with the infusion of protein kinase cyclin-dependent kinase 5 inhibitor immediately after training but not 6 hours after [189]. Brain metabolism is also involved in consolidation. Microdialysis studies showed that elevation of glutamate, glucose and lactate have been measured in BLA during the acquisition and also during retrieval in an inhibitory avoidance task and same elevations have been observed in hippocampus as well [190] [117].

Moreover, whereas an interfering new learning, the electroconvulsive shocks or the use of lactate transport blockers can prevent memory formation, dopamine, noradrenergic or NMDA receptors modulators can also enhance it [191] [192] [193].

1.3.1.2. Associative memory consolidation

BLA is also a core brain region in memory formation, mainly in memory related to contextual and emotional cues. In 2006, Fuchs and colleagues have shown that a pre-training excitoxic lesion of basolateral amygdala prevented conditioned place preference formation showing that BLA is critical for cocaine-cues related memory[194]. Four years later, it has been demonstrated that sodium channels in the BLA mediate the consolidation of cocaine associated learning [195]. The same year, Rozeendal and colleagues showed that BLA is involved in fear conditioning but also in object recognition through noradrenalin modulation. They have respectively injected atonolol and propranolol, two beta-adrenergic receptors blockers, after training session to block memory consolidation [196,197]. BLA, and more specifically astrocytes in BLA, play a critical role in fear memory consolidation. Stehberg et al have demonstrated that the blocking of connexin cx-43, a connexin hemi channel involved in gliotransmission and astrocytes intercommunication prevented fear memory formation. Co-administration of several gliotransmetter, including lactate, rescuing the effects of the connexin blockers [198].

Interestingly, infusions into medial prefrontal cortex also prevented memory formation but not in dorsal hippocampus even though hippocampus is a core brain region involved in contextual memory. BLA is not the brain region where emotional memory is stored but it modulates the informations stored in other brain region. For example, PFC and BLA are known to interact each other during memory consolidation through the dopaminergic projections from BLA to the mPFC.

The inactivation of BLA disrupts the transfer of information from BLA to mPFC during consolidation. Then, the activation of BLA seemed to drive decision making involving the mPFC as well as neuronal activity in mPFC [199 200].

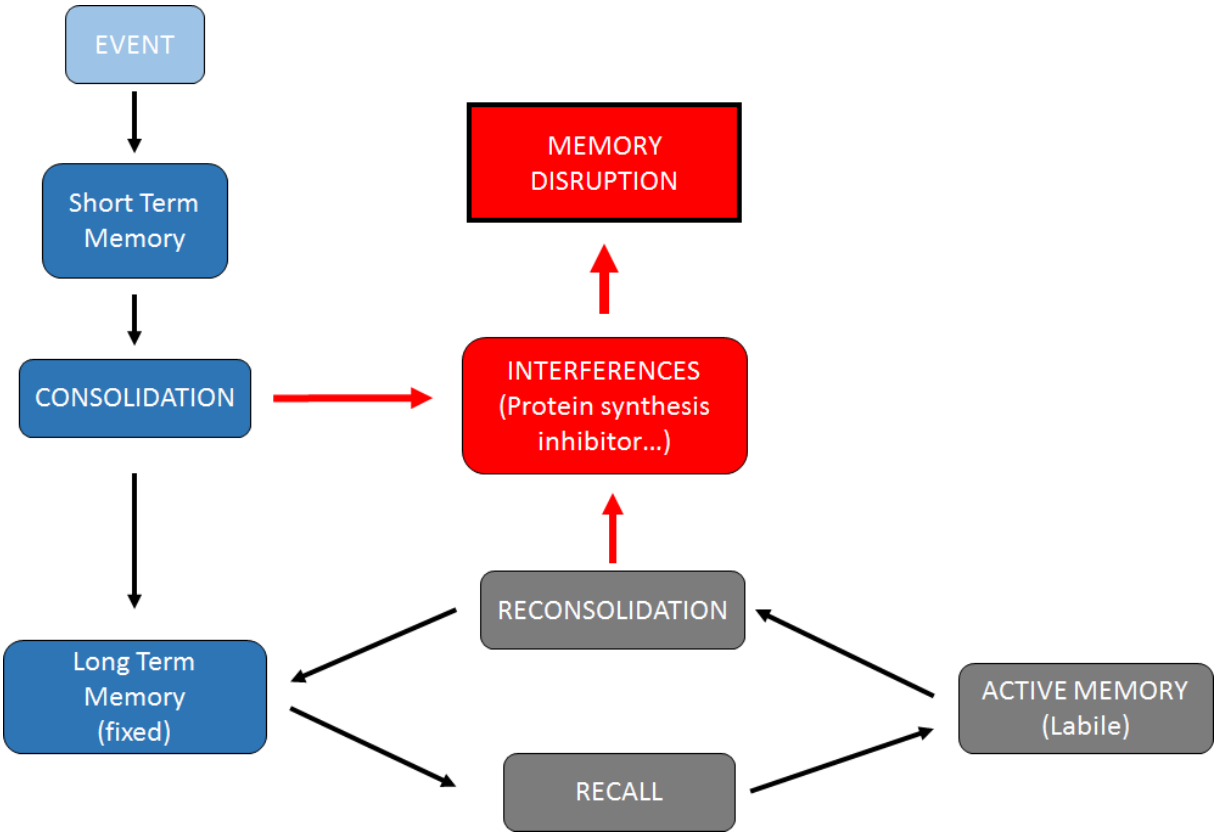

Figure 6: Schematic representation of memory process. Memory consolidation is a process allowing the encoding of an event from short term memory to long term memory. The fixed memory can be recalled and become labile during the reconsolidation process. Consolidation and reconsolidation require protein synthesis and are susceptible to disruption leading to the erasure of the stored memory.

In conclusion, memory formation and consolidation are driven by complex mechanisms that can be potentially destabilized or disrupted. Consolidation occurs from minutes to hours after the initial event which initializes the storage of fresh memory.

1.3.2. Reconsolidation updates already established memories

As well as memory formation, the maintenance of memory is not a static state and is susceptible of interferences leading to its destabilization and its disruption. Indeed, the memory previously stored can be recalled, consolidated but also forgotten. Another state is initialized after the recall and update of a memory: the reconsolidation phase. For example,

when a subject is only re-exposed to the contextual environmental stimuli which were present during the memory formation, the specific memory coding for the association is reactivated and then the reconsolidation phase starts [201] [202] [203]. During retrieval memory become labile to integrate new informations (as an update of the existing memory) but also to reinforce the memory [204] [205].As well as memory formation, anisomycin, protein synthesis blockers, a new learning and lactate transport blockers have been used to destabilized this labile state and to disrupt the memory previously stored [206] [207] [208]. Memory reconsolidation is a process involved in different types of memories. It has been well described in aversive paradigm using fear conditioning or inhibitory avoidance [209] [210] but also in spatial and contextual memory involving hippocampus [211] [212] and in appetitive learning [53] [213]. The fact that memory consolidation and reconsolidation are often blocked with the same pharmacological agents, or methods often lead to a misunderstanding of the distinction of these two processes. However, temporal and regional different mechanisms have been found, as well as in this thesis, and different molecular pathways seem to be involved in one or the other phase. The memory maintenance is characterized by the strength of the initial engram and the time window of the retrieval. It has been shown by Suzuki and colleagues in contextual fear conditioning, that a longer conditioning session lead to a stronger memory which become more resistant to disruption using anisomycin in rat. Interestingly, the same authors showed that a longer re-exposure to the contextual cues was necessary to disrupt this stronger memory [214]. In a more complex way, Inda and colleagues have tested the interaction between time and repetition of retrieval test. They have demonstrated that time participates to increase initial memory storage up to 20 days as well as the repetition of three retrieval sessions just after the training session. They also showed that multiple retrieval sessions a long time after training (more than 50 days) lead to extinction. In conclusion for the authors, memory reconsolidation is a part of memory consolidation and participate to the strengthen of memory [210].Moreover, these findings have also been showed in fear conditioning paradigm. Mice tested twenty days after the re-exposure combined with anisomycin injections still froze and expressed a fear conditioned behavior. Interestingly, this study used multiple injections to block protein synthesis during 8 hours, in order to disrupt the different protein synthesis waves occurring during memory reconsolidation [215]

Although reconsolidation most likely contributes to updating memories [216] [217] [218], its disruption may reduce the impact of aberrant memories on behavior[37] [39] [54-219]. As several aspects of addiction depend on mnemonic processes induced by drug experience, disrupting drug-related memories represents a promising approach to help reducing relapse propensity on subsequent exposure to drug-paired stimuli and thereby may encourage abstinence [52].
In conclusion, memory is one of the key components of addiction and presents an interesting therapeutic way to decrease the relapse among addicted patient. However, current therapeutic identified targets on NMDA receptors or on adrenergic receptors are difficult to translate to human medicine or already presented several weakness [220] [221] and need further experiments and new discoveries.

1.4. Aim of the projet

Cocaine addiction is a major health concern, and treatments are limited. A theory about drug addiction linking memories associating the contextual cues with the effects of drugs of abuse present a promising interest. These cocaine related memories are known to shape and maintain persistent drug-seeking behaviors in rodents[19] and promote relapse in humans. Many questions remain to be answered about the mechanisms by which long-term memories for drug-paired cues resist to extinction and contribute to the formation of habits and drug-seeking behavior. Interestingly, once consolidated, memories can again become transiently labile and sensitive to protein synthesis inhibitors if reactivated [203,222,223]. These findings may offer a critical contribution to clinical practice as they suggest that protein synthesis blockade after reactivation may selectively reduce or even eliminate long-lasting memories, including those linked to drug addiction [224]. Although metabolic coupling has long been considered a key mechanism through which astrocytes and neurons actively interact in response of neuronal activity [225,226], only recent evidence revealed that interference with lactate transfer from astrocytes to neurons impairs long-term memory formation [116,117,171,227].
In this study, we want to assess the role of glycogen derived lactate in the formation and the maintenance of cocaine-cues related memory. To target glycogen breakdown, the administration of the glycogen phosphorylase inhibitor DAB, already used in aversive memory formation, was chosen. Because conditioned place preference assesses the impact of

pharmaceutical agents on the association between a specific context and the reward effect of cocaine, we decided to first target the metabolism of glycogen in BLA involved in formation and maintenance of cocaine CPP. In a second part, we explored two major questions to develop our finding in BLA. First, what could be the upstream regulations of glycogen utilization in BLA during CPP formation? And second, is glycogen involved in cocaine-cues related memory in decision making and reward circuitry related brain regions? By these experiments, we wanted to show that astrocytes and more importantly, lactate release from glycogen breakdown is involved in a brain-region and time-scale dependant manner in cocaine-cues related memory and could represent a promising and innovative therapeutic way.

2. RESULTS

2.1. Article 1: Disrupting astrocyte-neuron lactate transfer persistently reduces conditioned responses to cocaine.

Original article.

Contribution: I have participated to the design and the optimization of the different experiments. I have performed all the brain surgeries and behavioural experiments, and a part of the molecular analysis in collaboration with Anthony Carrard. I have collected all the data and performed the statistical analysis.

I have also participated to the redaction of the manuscript.

See end of the document

2.2. Article 2: Lactate release from astrocytes to neurons contributes to cocaine memory formation.

Review article.

Contribution: I have written the first draft of this review and participated to the revisions of the manuscript. I have also participated to the conception of the schema resulting of our proposed model of the tripartite synapse.

See end of the document

2.3. Supplementary results

2.3.1. Potential role of adrenergic and dopaminergic pathways in lactate release

We have demonstrated that glycogen breakdown inhibition appeared to be critical for CPP formation and maintenance in the basolateral amygdala. DAB, injected 15 minutes before each conditioning session, successfully blocked memory formation, without apparent aversive or permanent effect and modulated the expression of genes involved in cocaine induced CPP. How cocaine and/or CPP could modulate glycogen degradation and thus lactate production was an open question after these observations. To explore it, we decided to block CPP formation with other products related to glycogen and cocaine and assess the role of lactate in these inhibitions.

The first pharmacological agent was propranolol. As described in the introduction, propranolol has been used in CPP and other memory disorders and successfully has prevented the formation or the maintenance of these memories. Propranolol is a non-specific beta receptors blockers. Beta receptors are known to promote glycogen breakdown as well as its resynthesize. We hypothesized that the effect of propranolol in CPP formation involved glycogen degradation and thus lactate production. We replicated the CPP conditioning experiment with a batch of rats treated with intra-BLA administrations of propranolol 15 minutes before each cocaine session. As shown in figure 7, propranolol successfully prevented CPP formation. Interestingly, a co-administration of propranolol and lactate rescued the effect of propranolol itself. This experiment showed that lactate seemed to be linked to the blockade of CPP formation induced by propranolol.

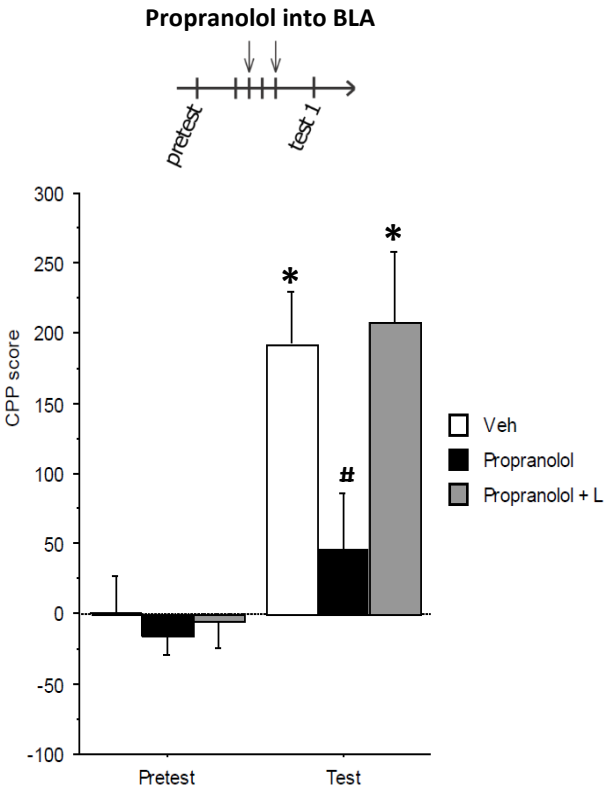

Figure 7. Astrocyte-derived lactate is linked to beta adrenergic receptor signaling for the acquisition of a cocaine-induced conditioned place preference (CPP). Experimental timeline is shown above the graphic. Data represent CPP mean score (± s.e.m.) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. A two-way repeated-measures analysis of variance revealed a significant session × treatment interaction ($F_{3,44} = 4.467$, $P<0.05$), and post hoc analyses demonstrated a significant preference for the compartment previously paired with cocaine injections in vehicle and propranolol +L-Lactate-treated animals (*p<0.05, ***p<0.001 compared with pretest score, n = 15 and 11, respectively), while propranolol did not exhibit any preference (###p<0.001, n = 13 and 11, respectively) compared with respective pretest conditions. BLA, basolateral amygdala.

Because, the release of lactate could also be modulated in vivo by D1 receptor agonists [165] and cocaine acts as a blocker of dopamine reuptake, we would like to know if antagonist of D1 receptor administrated during CPP conditioning could be linked to lactate. A last batch of animals was then administrated with SCH22290, a specific D1 receptor antagonist known to prevent CPP Formation. As expected, this group of animal did not exhibit a preference for cocaine paired side (figure 8). Exactly as our previous experiments, another group of animals received a co-administration of the inhibitor and lactate. Again, animals receiving the co-administration had a clear-cut preference.

As the beta-adrenergic receptors, D1 receptors seem to be link to lactate action in appetitive memory formation. At this point, we hypothesized that role of D1 and beta adrenergic receptor during cocaine-induced CPP formation involved lactate release and perhaps through glycogen degradation in the BLA.

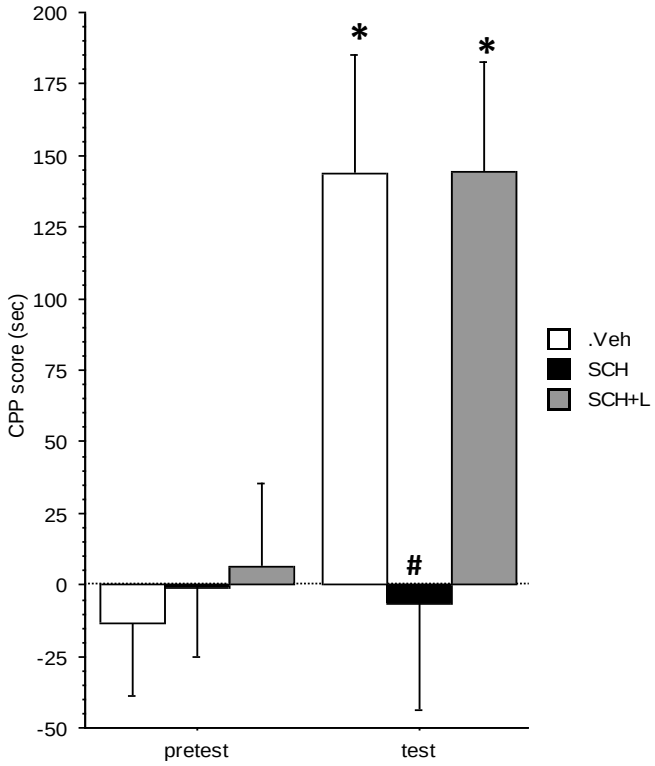

Figure 8. Astrocyte-derived lactate is linked to dopamine 1 receptor signaling for the acquisition of a cocaine-induced conditioned place preference (CPP). Experimental timeline is shown above the graphic. Data represent CPP mean score (± s.e.m.) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. A two-way repeated-measures analysis of variance revealed a significant session × treatment interaction ($F_{3,44} = 4.467$, $P<0.05$), and post hoc analyses demonstrated a significant preference for the compartment previously paired with cocaine injections in vehicle and SCH22290 + L-Lactate-treated animals (*p<0.05, ***p<0.001 compared with pretest score, n = 15 and 11, respectively), while SCH22290 did not exhibit any preference (###p<0.001, n = 13 and 11, respectively) compared with respective pretest conditions. BLA, basolateral amygdala.

2.3.2. Role of glycogen and lactate in cocaine-related memories in other brain regions involved in reward pathway

2.3.2.1. In CPP formation

After showing that glycogen and lactate in BLA seemed to play a key role in cocaine induced CPP, we were interested to know if glycogen breakdown could be involved in other brain regions related to CPP formation. We were interested in the mPFC known to be involved in decision making and finally in VTA and NAcc Core known to be involved in motivational aspect and reward effects of cocaine. Three groups of animals performed a CPP training, receiving DAB infusions respectively in the PFC, VTA or NAcc Core (Figure 9)**.** Interestingly**,** in all these brain regions, DAB treated animals had a clear-cut preference for side previously

paired with cocaine after conditioning sessions. Glycogen breakdown did not prevent the formation of CPP behavior in contrast to the effect of DAB into BLA.

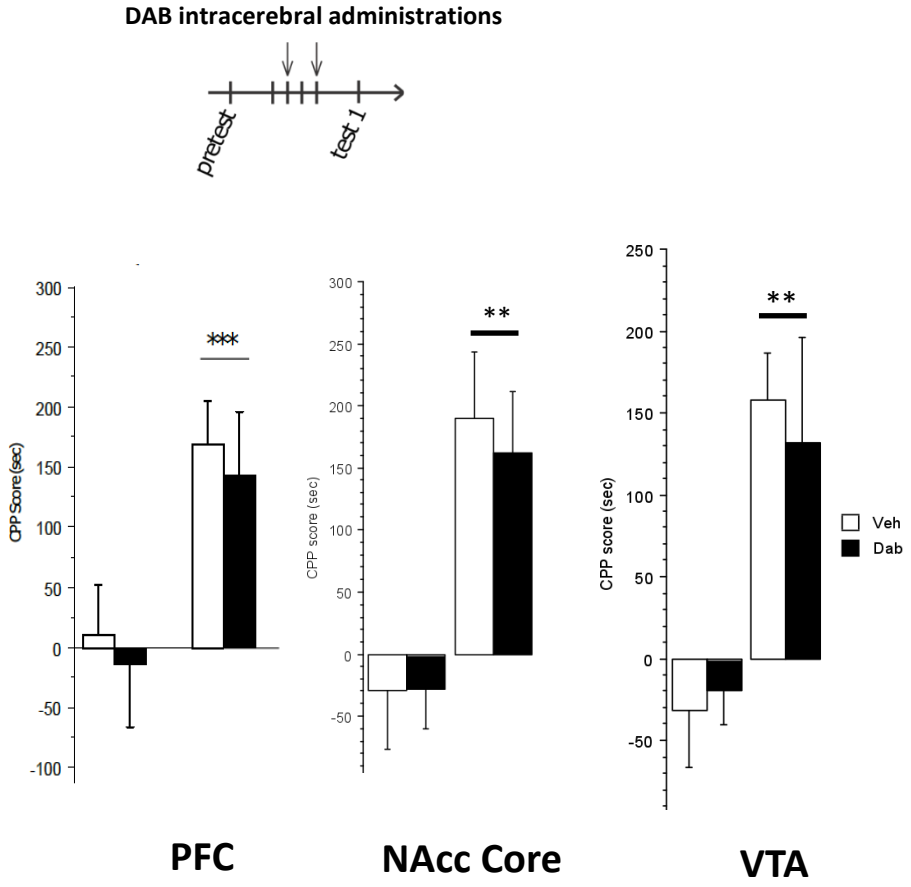

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Figure 9: DAB administration in other brain regions failed to prevent CPP formation.

General timeline of the four experiments is shown above the graphics. Data represent CPP mean score (± s.e.m.) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. DAB treated animals exhibited a clear cut preference for cocaine-paired side in the four brains regions. A Two-way repeated-measures analysis of variance followed by bonferonni post hoc tests revealed a significant session effect for PFC, nucleus accumbens core (NAcc Core) and Ventral Tegmental Area (VTA) (Respectively: $F(1,13) = 21.715$, n=7-8, $F(1,16)=13,346$ n=8-10, $F(1,17)=10,817$, n=9-10. ***p<0.001 **p<0.01)

2.3.2.2. **In the maintenance of CPP: involvement of PFC**

Whereas DAB administrations in the previous brain regions failed to prevent memory formation, the role of glycogen in the maintenance of memory remains unknown. We first tested to replicate our observation in BLA by injecting DAB 15minutes and 5hours after the re-exposure to the contextual cues in PFC, NAcc or VTA (Figure 10). A significant effect of treatment appeared for one experiment (NAcc at test2) due to a lower CPP score in vehicle treated animals compared to historical data. However, in these three brain regions DAB administrations failed to disrupt memory already established.

Interestingly, past research showed a time-dependant consolidation phase in PFC in morphine CPP[182]. By injecting an inhibitor of protein synthesis, anisomycin, at different time points, they have demonstrated that protein synthesis in consolidation in BLA occurs right after the re-exposure. This inhibitory effect was still present until the animals were administrated with the protein synthesis inhibitor 6 hours after the re-exposure. On contrary, in PFC, blocking protein synthesis right after or 6 hours after the contextual re-exposure failed to block consolidation. They showed that an injection of anisomycin had to be delayed up to 12 hours after the retrieval to block the consolidation. The transfer of information during memory process seems to follow a BLA to PFC axis in a time dependant manner. We decided to explore this hypothesis on glycogen metabolism in our protocol and to inject another group of animals with DAB 12 hours after the re-exposure. Thus, this group of animals received DAB in PFC twice, 15 minutes before and 12 hours after the contextual cues re-exposure (figure 11). In contrast with the -15min /5 hours delay injections, the double delayed injections of DAB successfully disrupted memory already established. The preference for side previously paired with cocaine was disrupted for DAB treated animal, up to two weeks (Figure 11, test 3 at one week and test 4 at two weeks) even if the animals received a priming injection of cocaine right before a final test (test 5). To assess the role of lactate in PFC, a group of rats received a co-administration of DAB + L-Lactate. This group had a clear-cut preference for side previously paired to cocaine, demonstrating that lactate and not only glycogen was responsible of the maintenance of cocaine CPP in PFC.

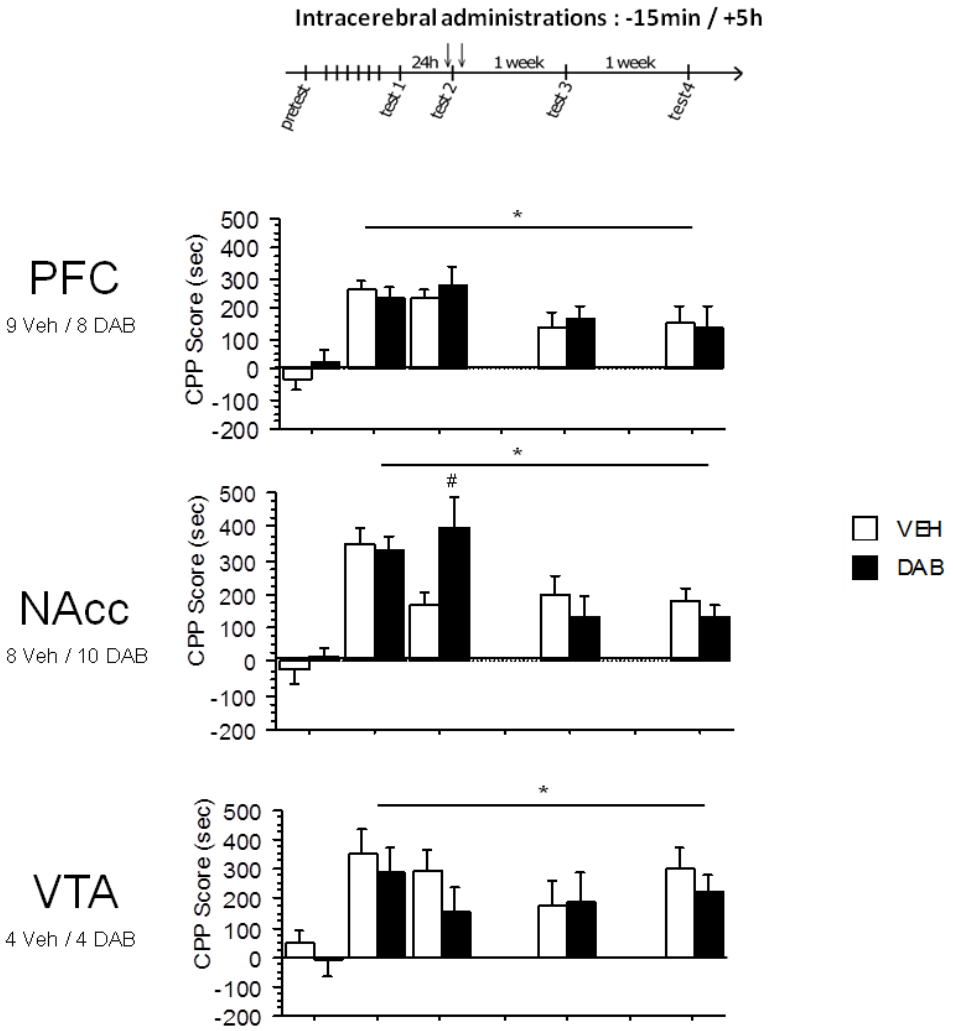


Figure 10: **A double administration of DAB into PFC, NAcc and VTA 15minutes before and 5 hours after re-exposure does not disrupt an established cocaine-induced conditioned place preference.** Experimental timeline is shown above the graphic. Data represent CPP mean score (± SEM) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. A Two-way repeated measures ANOVA followed by Bonferroni post hoc tests revealed a preference for the compartment previously paired with cocaine in all groups up to two weeks. Post-hoc analysis also revealed a significant difference in test 2 in NAcc experiment.

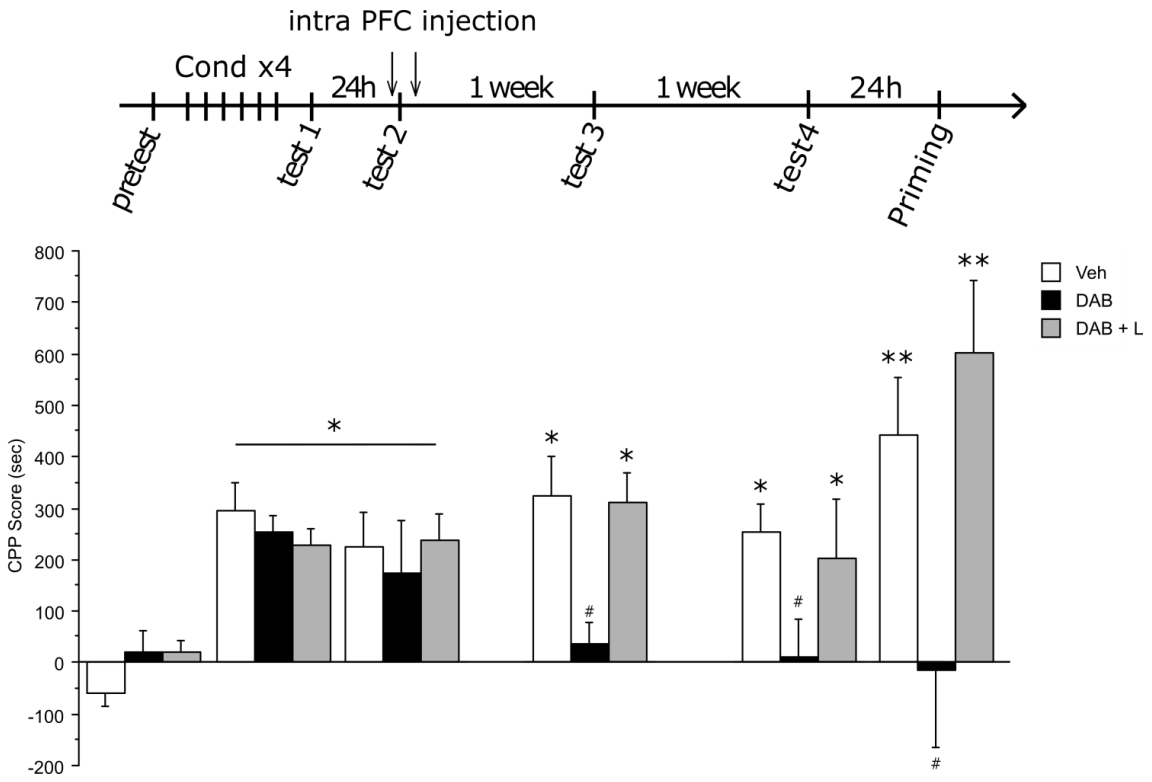


Figure 11**: A double administration of DAB into PFC 15minutes before and 12hours after re-exposure disrupted an established cocaine-induced conditioned place preference.** Experimental timeline is shown above the graphic. Data represent CPP mean score (± SEM) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. A Two-way repeated measures ANOVA followed by Bonferroni post hoc tests revealed a preference for the compartment previously paired with cocaine in Veh and DAB-Lactate treated animals group up to two weeks. DAB injections into the PFC 15 min prior to test 2 failed to block the preference for the cocaine-paired compartment compared with vehicle-treated animals, during retrieval. A second bilateral administration of DAB into the PFC 12h after test 2 induced a difference for the cocaine compartment for up to 2 weeks, whereas rats that received a second (DAB+L-Lactate) administration exhibited a significant cocaine-seeking behavior (test 3, #P<0.01 compared with vehicle animals, *P<0.05 compared with pretest conditions) Veh n=8 and in DAB group, n=7, DAB+Lactate n=8

3. DISCUSSION

3.1. Glycogen derived lactate is involved in cocaine-cues related memories

3.1.1. Lactate plays a critical role in BLA on cocaine-related memories

Cocaine addiction could be characterized as a brain disease of a maladaptive learning. Past researches have shown that memory can be prevented during the formation but can also be disrupted after its establishment during a recall. Protein synthesis inhibitor, beta-AR blockers, dopamine or glutamate receptors inhibitors have been shown to affect memory formation or maintenance during consolidation and reconsolidation [228] [229] [230] [207]. Despite decades of research on addiction, no efficient treatment has been discovered. In the present thesis, we wanted to propose an alternative way to understand the memory component of addiction. Our results show that astrocytes play a role in cocaine related memories in line with previous studies demonstrating their other active roles, including information processing, signal transmission and regulation of neural and synaptic plasticity [231] [232] [110] . In particular, a recent study revealed that glycogen derived lactate transfer from astrocytes into neurons was essential for the induction of the molecular changes required for long-term aversive memory formation [117]. Although their participation on cocaine seeking behavior has also been demonstrated, enhancing their role in the synaptic process [233] [234] [93] we demonstrated that glycogen metabolism and more specifically ANLS hypothesis plays a critical role in this process. We focused, in the major part of this work, on conditioned place preference, a commonly used paradigm to test pharmaceutical agent in drug related memory [235] [236] and showed that glycogen breakdown disruption, using a glycogen phosphorylase inhibitor in the BLA impairs the acquisition, the retrieval and the long-term maintenance of positive affective memories associated with cocaine-paired cues. These effects of DAB seem to be transient, a second training without intracranial administration of the inhibitor in the BLA leading to a clear-cut preference for all animals. Another lab had also demonstrated this transient effect of DAB and whether lactate transport would play a similar role in drug-paired memories [208] . At first, these authors reported, in the basolateral amygdala of conditioned rats, a significant increase in lactate dialysate levels measured over 50 minutes after exposure to cocaine cues. These

dialysate measurements confirmed also the transient increase of lactate in hippocampus after an inhibitory avoidance training found by Alberini's lab [117]. To confirm the hypothesis that lactate is critical for memory formation, we showed that the inhibitory effect of DAB administration is rescued by L-Lactate. The rescuing effects of lactate on memory formation loss could be first explained as an energy supply which is critical for memory and neuronal activity. However, DAB and L-Pyruvate co-administration failed to rescue the effect of the inhibitor alone. Our demonstration supports the hypothesis that lactate does not only play an energetic role in memory formation. Because of the specific distribution of the neuronal MCT2 and the astrocytic MCT1 and their respective higher and lower affinity for pyruvate, we can speculate that injected pyruvate has entered directly into neurons rather than in astrocytes. Thus, we concluded that the prevention of memory formation was not due to an energy deficit. Pyruvate was chosen instead of glucose to demonstrate the critical role of the metabolization of lactate into pyruvate in neurons. Recently evidence in the Magistretti's lab demonstrated that lactate, in astrocytes cell cultures, could be a positive modulator of already activated NMDA receptors [153]. In this study, L-Lactate increased the neuronal expression of synaptic plasticity-related genes, including Arc, c-Fos, Zif268 and brain-derived neurotrophic factor (Bdnf), through a mechanism involving N-methyl-D-aspartate receptor (NMDAR) activity and its downstream signalling cascade extracellular signal–regulated kinase 1/2 (ERK1/2). Indeed, the metabolization of lactate into pyruvate produces NADH which is required to induce gene expression related to long term memory. This NADH seemed to influence the redox state of neurons and possibly redox-sensitive NMDA receptor subunits. Furthermore, MK801, a NMDA receptor antagonist, have been shown to block this gene expression, demonstrating that memory related gene expression, induced by lactate, is NMDA receptor dependant. Interestingly, these genes are also mainly studied in cocaine-related memory. For example, it is well known that the immediate early gene Zif268 modulates the synaptic morphology and plasticity underlying the learning processes that strengthen conditioned responses to cocaine [237] [238] and is involved in memory and synaptic plasticity, promoting cell growth factor and proliferation [239]. Interestingly, Valjent and colleagues have shown that *Zif268*-deficient mice were unable to develop a cocaine CPP behaviour but acquired a clear cut preference for side previously paired with food reward [238]. Moreover, other paradigm such a as operant conditioning in rats showed that cocaine conditioned

stimulus induced Zif268 expression in basolateral amygdala, prefrontal cortex, nucleus accumbens but not in the hippocampus nor in prelimbic area of the medial prefrontal cortex or the amygdala central nucleus [240]. Taken together, these results demonstrated a critical role of Zif268 in cocaine responses involving different brain regions but its expression differs depending of the paradigm chosen.

Our results confirm that cocaine CPP and lactate also mediates intracellular responses for cell signalling and regulation of gene expression required for long-term positive memory formation [117 122 127 241]. BNDF is induced by several physiological processes such as whisker stimulation in barrel cortex [242] , learning and contextual memory [243 244], in depression [245] and more importantly cocaine seeking behaviour[246 247 248]. Graham and colleagues have demonstrated that acute injection of cocaine increase BDNF gene expression in NAcc in self-administration paradigm. The behavioural expression of cocaine seeking was also disrupt by the administration in NAcc of BDNF antibodies [249]. In the VTA, it has also been shown that BDNF administration increase relapse response in self- administration [246] whereas administrations in NAcc increase cocaine conditioned place preference [250]. Our results revealed increased levels of BNDF gene expression in BLA after cocaine CPP suggesting an active role of BNDF in appetitive memory formation in BLA. By blocking glycogen utilization, we also showed that the action of BNDF is glycogen dependant and could explain the memory prevention. However, L-Lactate rescued the expression of Zif268 but not that of Bdnf, suggesting that lactate mediated rescuing of cocaine-associated memories during cocaine conditioning may not depend on Bdnf in the BLA. The fact our behavioral observations did not correlate with the absence of recue of BNDF mRNA expression by Lactate could be surprising but have several explanations.

First, a previous study has shown in fear conditioning paradigm that Bdnf was specifically required for consolidation of the initial contextual cues, whereas Zif268 was required for strengthening the souvenir [251,252]. Assuming that L-Lactate co-administration compensated the effect of DAB and permitted the stabilization of a novel memory following the first cocaine conditioning (consolidation phase), one can speculate that L-Lactate co-

administration prior to the second cocaine conditioning permitted the reconsolidation phase that strengthens the association between the drug effects and the context [253] [254]. Because we sacrificed the animals only after the second conditioning session, we were not able to measure the potential rescue of DAB/Lactate co administration on BNDF level during the consolidation. This study used the same time point to sacrifice the animal, 2 hours after the last session. In this thesis, we choosed two different time points, 1 and 3 hours, assuming that immediate early genes are expressed before BNDF. However, considering that the increase of lactate seems to last one hour after conditioning[117,208], it is still possible that lactate does not participate to BDNF rescue at all. To exactly assess the role of BNDF and Zif268 and more importantly, the role of lactate in their expressions, a complete analysis of Bdnf and Zif268 expression in rats treated with DAB and L-Lactate following one cocaine injection would be necessary to confirm this double dissociation assumption [251]. However, one unique cocaine administration remains a weak conditioning, and the acquisition of a CPP would be uncertain even if a single paired session in cocaine CPP has been already investigated in mice [255].

Second, we know that cocaine induces an increase in BDNF gene expression and its protein [256], it would be interesting to look at protein levels in the BLA. Indeed, previous studies have shown that lactate could have a role in epigenetic regulation by inhibiting histone deacetylases [155] [257]. We can speculate that inhibiting HDAC, lactate could upregulate gene expression such as BNDF. Taken together, these hypotheses reinforce the need to look at protein levels to complete the molecular impacts of lactate. Regarding ARC mRNA expression, ours findings are in line with previous study showing that Arc is more driven by contextual exploration and Zif268 more involved in associative learning[258]. This assumption could explain the reason why we did not see any change in Arc mRNA expression in BLA and could confirm that CPP exploration was sufficient to promote its expression. DAB seemed to have mainly targeted the associative learning.

Nevertheless, our striking observation suggested that if L-Lactate, unlike L-Pyruvate, mediates intracellular responses for cell signalling and regulation of gene expression required for long-term positive memory formation, this is probably the result of increasing intracellular levels of NADH, thereby influencing the redox state of neurons and possibly redox-sensitive NMDA receptor subunits [153]. Suzuki's work on aversive long term memory formation showed

that the blockade of lactate entry into the neuron prevents memory formation. Disruption of the astrocytic (MCT4) and neuronal (MCT2) lactate transporters was shown to prevent the retention of an inhibitory avoidance task. However, L-Lactate administration successfully rescued the amnesia after MCT4 disruption only, suggesting that lactate import into neurons (via MCT2) is essential for long-term aversive memory formation. This observation is in line with our findings, suggesting that lactate into neurons participates to long term appetitive memory formation. Furthermore, another group working on the same hypothesis, confirmed our assumptions by blocking lactate transport in BLA during a cocaine CPP [118]. Confirming the physiological role of lactate transport in cocaine memories, they revealed that the expression of cocaine-induced CPP correlated with an increased activity of MCT1 and MCT2 (not MCT4) in the BLA (not in the central amygdala), within two hours of exposure to the cocaine-paired context. They next dissected the respective roles of MCT1 and MCT2, confirming that, in contrast to the disruption of MCT1 expression in the BLA, which impaired the cocaine-induced CPP but could be rescued by L-Lactate co-administration, the effect of antisense-mediated knock-down of the neuronal lactate transporter MCT2 on cocaine memory was not rescued. These observations discard the hypothesis of an activation of the lactate receptor, GPR81 aka hydroxycarboxylic acid receptor (HCAR1), present in neurons cell surface [259,260].

Not surprisingly, several studies have reported that NMDAR antagonists administered into the basolateral amygdala (BLA) impaired retention of appetitive [261] and drug memories [262], while activation of ERK pathway [263] [44] and Zif268 [264] was shown to be necessary for the reconsolidation of addictive drug memories. These observations suggest that long-term memory formation requires high metabolic demands within the underlying active neuronal network and call for a better understanding of the molecular mechanisms involved in the complex reciprocal exchanges of metabolic intermediates between neurons and glia.

The second demonstration of this study is that disruption of glycogen metabolism not only transiently impairs acquisition of cocaine-induced CPP but also persistently disrupts an established conditioning. Indeed, rats with a strong cocaine preference showed transient amnesia after inhibition of glycogen phosphorylase prior to re-exposure to the drug context.

Unexpectedly, rats exhibited a spontaneous recovery a week after treatment. One possible explanation is that blocking glycogen phosphorylase prior to the re-exposure trial most likely impaired the retrieval process and thus contributed to delay but did not persistently disrupt the reconsolidation process. This phenomenon of spontaneous recovery has been observed in several studies [265–267]. The duration of contextual cues exposition during the recall had also been shown to be critical for the resilience of the initial memory. It has been demonstrated in inhibitory avoidance paradigm that animals, after receiving a treatment that blocks reconsolidation, have their memory transiently disrupted. Six days after the administration of anisomycin right after a retrieval test, rats were retested in inhibitory avoidance task. Despite they received a protein synthesis blocker after the re-exposure of contextual cues, they still had an intact memory and did not enter the dark compartment. This spontaneous recovery could be the reflection of a temporally impairment of memory retrieval or an enhancement of the extinction of memory [268].

Memory consolidation has been shown to involve different protein synthesis waves in time. Around 3 to 6 hours after conditioning, de novo protein are synthesized and required for the storage of the memory [269–271]. To test the hypothesis that glycogenolysis is necessary for reconsolidating contextual appetitive memories, we blocked glycogen breakdown twice, prior and after the memory retrieval window. Hence, we showed a long-lasting amnesia for cocaine associated memory after prolonged disruption of glycogen metabolism in the BLA. Furthermore, the unresponsiveness to cocaine cues persisted even after cocaine priming injection, suggesting a permanent disruption of the contextual appetitive memories. The ERK pathway is stimulated by drugs of abuse in striatal neurons through coincident activation of dopamine D1 and glutamate NMDA receptors and is considered to be critical for drug-induced long-lasting behavioral effects [26]. Further, converging evidence has shown that ERK phosphorylation and immediate-early gene expression are stimulated by drug-associated cues in the absence of drugs and have a key role in long-lasting drug-seeking behaviors [168 272 273]. In line with these observations, we showed that not only the long-lasting indifference for the cocaine compartment following inhibition of glycogenolysis in the BLA was associated with decreased phosphorylated ERK1/2 protein levels but we also demonstrated that lactate-induced recovery of contextual appetitive memories correlated with restored levels of phosphorylated ERK1/2 in the BLA. In line with recent in vitro observations from ours [161] and

the demonstration that application of the NMDAR-agonist, D-cycloserine, potentiates the reconsolidation of appetitive memories [51], our data strongly support the idea that L-Lactate stimulates the neuronal expression of synaptic plasticity-related gene through a mechanism involving NMDAR activity and its downstream signalling cascade ERK1/2 [161]. However, upstream of the protein synthesis required for reconsolidation, there may be an initial destabilization process, named deconsolidation [274], which most likely requires NR2B-containing NMDA receptors[91 92 97] and protein degradation[275]. Hence, further studies are required to assess whether lactate may supply activated neurons with NADH, stimulating redox-sensitive NMDAR subunits possibly triggering destabilization of appetitive memories. Lactate may also supply activated neurons with sufficient energy required for synaptic protein degradation. In a second step, lactate may also contribute to the subsequent phase of the plasticity process by providing sufficient energy required for activation of signalling pathways and protein synthesis underlying long-term memory formation.

3.1.2. Possible upstream regulations of lactate release from glycogen

We mainly focused on glycogen inhibition in this study. Interestingly, glycogen distribution in brain correlates with high synaptic density, notably in the hippocampus, striatum, and cortex [144,276]. Recent imaging techniques revealed that glycogen seems to be stored near pre-synaptic bouton, rather than dendritic spines, and are associated with monoaminergic varicosities[277]. Further, glycogen phosphorylase (GP), which is responsible for glycogen breakdown in astrocytes, can be activated via phosphorylation. The latter results from a signaling cascade involving Ca2+ and cyclic adenosine monophosphate (cAMP), ultimately activating the GP-phosphorylating enzyme phosphorylase kinase (PK) [278,279]. Past observations have long reported a role for noradrenaline [168,280], dopamine [281,282], serotonin [162,178], and endocannabinoid [283,284] in glycogenolysis. For example, adrenergic signaling can exert opposing influences on astrocytic glycogen metabolism. It can either stimulate glycogen degradation by increasing cytosolic Ca2+ through a1-Gq-coupled receptors or by stimulating production of cAMP through b1/2-Gs-coupled receptors. Or can it inhibit glycogen degradation through a 2-Gi-coupled receptors [278]. Surprisingly, despite accumulating evidence showing how different signaling pathways may elicit glycogen degradation in astrocytes, little

is known about the upstream crosstalk that may converge onto lactate release, ultimately leading to long-term memory formation. A recent report though established the critical role of astrocytic beta 2-AR in hippocampal long-term memory consolidation [170]. Because glycogen breakdown in the BLA prevents CPP related memory formation, we wanted to assess the link between adrenergic and dopamine pathways. We showed that beta-adrenergic and D1/D2 antagonists injected into BLA prevented CPP memory formation but more importantly, the co-administrations of DAB and Lactate with the respective antagonists rescued the memory formation prevention. Our results demonstrate the role of lactate in dopamine and adrenergic pathways modulated by cocaine administration and involved in cocaine-cues related memory.

The measure of lactate concentrations as well as glycogen content measurements after antagonist administrations and CPP conditioning could give us the demonstration that dopamine and adrenergic receptor are upstream to glycogen degradation leading to lactate production. Indeed, in vivo measurements of extra cellular lactate and glucose in NAcc after antagonist of D1 receptors [165] revealed an increase for both of them but the utilization of glycogen during cocaine CPP remains unclear. It is well known that noradrenaline [45,285,286], dopamine [72,287,288], serotonin [174,289] neurotransmissions regulate conditioned reward, synaptic strengthening [290,291], and extracellular lactate release [165,292]. However, the question remains whether a beta adrenergic, dopamine D1, 5HT 2B/2C, known to disrupt cocaine memories, might all converge onto the same lactate-dependent signaling pathway and onto a same glycogen phosphorylase activation.

3.2. The challenge to treat cocaine addiction by targeting lactate metabolism

3.2.1. The late phase of reconsolidation in PFC

Our findings on memory reconsolidation in BLA were promising to assess the role of lactate in cocaine related memory. Our hypothesis was confirmed by DAB administrations in PFC. However, we noticed that the same schedule of administration failed to disrupted

memory already established. Indeed, by injecting DAB in PFC fifteen minutes before and five hours after the re-exposure to the contextual cues associated with cocaine, we failed to disrupt the preference. Contrary to BLA injection, we also demonstrated that DAB failed to block the retrieval of CPP immediately after the first administration. Even if a memory is permanantly and well stored in the brain, the expression of the souvenir by itself is also subjected to disruption. For example, the recall of the memory trace occurring during the re-exposure, can also been disturbed by chemical compounds. Li et al have shown that anisomycin injected in BLA 30 minutes before retrieval blocked the expression of the preference in a cocaine CPP [189]. Our results in BLA confirmed the role of BLA in the retrieval. However, this retrieval and the expression of memory are independent from the reconsolidation of this memory. Indeed, memory reconsolidation remains intact even if the retrieval is disturbed [293]. Thus, the double administrations of DAB may have a double effect on memory retrieval and memory reconsolidation. In object recognition, it appeared that retrieval could be AMPA receptors dependant, whereas reconsolidation is more NMDA receptors dependant. However, PFC has been shown to be involved in recall of spatial memories [294]. We can speculate that the mechanisms of lactate are different in these two memories processes. Co-injecting DAB with glucose before memory retrieval in BLA could confirm this hypothesis. Indeed, it has been shown that the effect of blocking glycogen breakdown could be rescued by co-administration of DAB and glucose. In the Alberini lab, glycogen breakdown preventing aversive long term memory formation was partially rescued by DAB and glucose co-administration. These studies suggested that different forms of memories could more or less depend on pure energetic demand. We could then speculate that energetic demand and memory retrieval are differentially modulated, maybe through activation of glycogen phosphorylase, in PFC and BLA but did not influence on the memory reconsolidation. Different ways to activate the multiples isoforms of GP could reveal different functions and causes of glycogen breakdown. In fact, GPB and GPM have been showed to have different sensitivity of activation. GPB seems to be preferentially stimulated through allosteric activation by AMP, and GPM through phosphorylation. It has been hypothesized that this first way of activation could be linked to an intracellular energy demand and the second way linked to extracellular signals [295] [296].

As in morphine CPP consolidation, we noticed that a DAB delayed administration into PFC, 12hours after the re-exposure, disrupted memory reconsolidation. Interestingly, co administration of lactate and DAB rescued the effect of the phosphorylase inhibitor DAB. In the study showing an early phase of consolidation in BLA and a late phase in PFC, the authors described an early phase of consolidation involving the map Kinase ERK and a late phase independent of the kinase but calcium-calmodulin-dependent (CaMKII) dependant. CAMKs are known to be involved in glutamatergic transmission[297],memory and addiction [298] [299]. Moreover, CAMKs are also linked to lactate metabolism through the modulation of lactate deshydrogenase[300] and to cofilin regulation[301] known to be modulated by lactate in vivo[117,208]. Because we observed a modulation of ERK phosphorylation in the BLA, it would not be surprising to observe a modulation of CAMKs in PFC in cocaine CPP under DAB administrations. Taken together, these results on the delayed injection experiment highlighted the complexity of consolidation and reconsolidation processes and the necessity to identify the right time point of inhibitors injections depending of the brain region. These considerations are well known and appeared in several behavioral paradigms and molecular pathways such as consolidation involving adrenergic receptors[183] or protein synthesis inhibitors[269] [302]. In our case, although higher concentrations of DAB did not require multiple injections[118], we also demonstrated that DAB administrations have a transient effect highlighting the difficulty to translate our observations into a therapeutic treatment. Glycogen and lactate metabolism appear to be region and time specific, which may require a more efficient glycogen phosphorylase inhibitor, higher concentration, or multiple administrations. At last, our hypothesis on the role of lactate on cocaine related memory and its role on aversive memory[117] suggest to explore the effects of DAB administration in hippocampus. Again, because the consolidation[303] and reconsolidation[304] processes are known to involved multiple protein synthesis waves in hippocampus, it is possible that DAB administration need to be delayed and then increases a little more the difficulty to provide a therapeutic protocol.

3.2.2. Glycogen derived lactate may only be implicated in brain regions involved in cocaine related memory

Our supplementary data on NAcc and VTA are in line with the previous conclusions. By showing that glycogen derived lactate seemed not be involved in CPP formation and maintenance in these reward related brain regions, we raised two questionable points. First, although these brain regions are involved in CPP formation and maintenance [305], the blocking of glycogen breakdown failed to assess the potential role of lactate in this behavior formation. One possible explanation could be the delay between the conditioning and testing sessions. As explained in the introduction, this delay can modulate the memory retrieval, a longer delay could promote memory strengthening. For example, studies have shown that animals tested one week after ERK inhibitor intrahippocampal injections had a long term memory impaired [306]. Another factor could be the time of injection in brain by itself. We know that long term memory storage of salient stimuli and novelty is dependant of a hippocampus-VTA loop. Dopaminergic connections between these two brain regions participate to the storage of recent memory into a long-term form[307]. Moreover, it has been shown that consolidation involving dopamine pathway in hippocampus occurs up to 17 hours after the cocaine place association [308] and a recent study demonstrated that hippocampus is involved more in stronger aversive situation[309]. Our CPP protocol is composed of only two conditioned sessions which could be a weaker form of learning which may be hippocampus independent. Thus, we can speculate that our specific protocol did not allowed to assess the role of reward related brain regions and their interaction with brains regions involved in contextual memory due to a shorter conditioning. Furthermore, it is still possible that glycogen is not involved in these brain regions at all. Because DAB specifically blocks glycogen breakdown, it is still possible that lactate was released during conditioning from glucose uptake. Interestingly, past research has demonstrated that acute administration of cocaine produced a rapid elevation of glucose concentrations in NAcc. Authors have first observed an increase of glucose followed by a larger and more prolonged second increase (Respectively 5-8 seconds and 15 minutes) [310]. Additionally, the injection of a peripherally active cocaine analog, cocaine-methiodide, did not produce the second elevation of glucose, nor increased locomotor activity. This second

elevation of glucose has also been found to be specifically blocked by D1 and D2 receptor antagonists [311]. These finding suggested a differential modulation in glucose utilization under cocaine administration. Moreover, study has shown an in vivo increase in glucose and lactate concentration in NAcc after the application of dopamine receptors agonists[165].These findings strongly suggested that glucose and lactate metabolism play a role in cocaine circuitry. The fact that we have only targeted glycogen and not glucose could explain the absence of potential effect of lactate in our results. Blocking lactate transport may help to validate or discard the role of lactate in reward related brain regions. However, in absence of prevention of CPP formation, the role of lactate receptor on neuron cell surface cannot be rejected and would need further experiment.

3.2.3. Is disruption of associative learning sufficient to erase drug?

The complexity of memory formation and maintenance, as well as the intricacy of cocaine addiction, raises the question: is memory disruption sufficient to decrease drug seeking behaviors, in particular drug craving and the vulnerability to relapse after a period of protracted abstinence? Indeed, our result mainly targeted an associative memory during a pavlovian conditioning. Although cocaine CPP is commonly used in pharmacological studies, it does not assess the role of glycogen derived lactate in cocaine seeking behaviors in general. Instrumental memories have been underinvestigated in the present thesis and the role of lactate remains elusive in such conditioning. Moreover, due to technical reasons, we decided to limit the numbers of pairings in our cocaine CPP procedure. Consequently, our protocol may have conducted to a weaker memory, more susceptible to lactate signalling disruption. Bourtchouladze and colleagues have shown that a weak contextual fear conditioned training required a single injection of anicomysin despite a stronger training session required multiple injections, right after and fours after the training session [312]. This observation calls for an alternative compound that could cross the blood brain barrier in order to apply a chronic treatment in longer protocol.

We have tested the preference for the cocaine-paired side two weeks after the treatment, however it is known that the age of memory is a critical factor acting on the strength of the engram. More the retrieval is delayed after the storage of the memory and more this memory is resistant to disruption [228]. Disrupting these memories could for example require a longer re-exposure to the context. The reconsolidation and the extended time window of the retrieval has also been showed to involve epigenetic modulations, which are induced also by cocaine[313–316] and could explain the resistance of remote memories [317] [318]. A complete epigenetic analysis could explain the observations in the different brain regions. Furthermore, CPP did not assess the role of withdrawal, incubation craving nor instrumental conditioning. However, Zhang and colleagues showed that DAB administration into BLA decreased cocaine self administration [208]. The main difference with our self-administration protocol is that the injection of DAB occurs after a reminder conditioning session whereas we wanted to know the effect of DAB on cocaine intake. Our preliminary results on cocaine self administration reveal the challenge to decrease drug seeking behavior and relapse after a prolonged access to cocaine. Studies have shown that extensive access to cocaine induce several changes in reward circuitry[29] such as a modification in dopamine signalling in the BLA[319] or a serotonin and glutamate dysregulation in NAcc[320] [321]. Moreover, at a molecular level, the acute administration of cocaine in rats showed a increase of Zif268 RNA in rat dorsal striatum [322] and nucleus accumbens [323]. After acute administration of cocaine, Zif268 gene expression is also upregulated in the basolateral and central amygdala. In contrast, after chronic administration of cocaine, the IEG is upregulated only in the central amygdala. ERK is known to be involved in long lasting cocaine [263] [324], induced by D1 and NMDA pathways [325] [326]. The increase in BLA appeared to be ERK dependant whereas the increase in central amygdala was ERK independent [327]. It is also involved in the expression of Zif268 through Elk-1 and CREB activation [328]. Finally, chronic exposure to cocaine may modulate ERK which could explain the change in expression of Zif268 in amygdala. It is also possible that chronic exposure to cocaine change the distribution of MCT in brain. Indeed, a research has shown that MCT1 transporters can be expressed on neurons cell surface following cerebral ischemia[136]. This finding may suggest a different distribution of MCT on brain region involved in reward circuitry after a long exposure of cocaine. Neuro-adaptation occurring in brain during cocaine addiction may also impacts cerebral metabolism. We have seen that dopamine transporters and glutamate

release appeared to be dysregulated after chronic exposure to cocaine. We may then speculate that targeting memory by metabolism modulation involved different metabolic pathways depending of the state of the addiction.

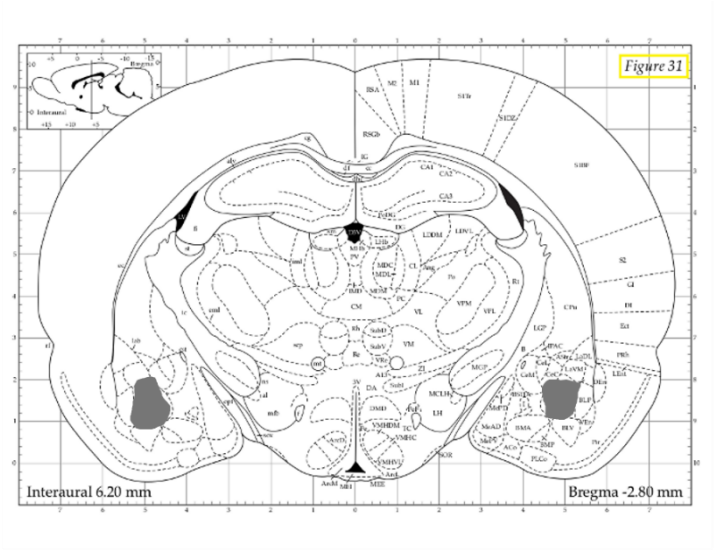
The transition from a goal-directed behavior, during the association between the stimuli and the response, to the habits involved different brain regions and may need an adaptation of the treatment. Indeed, manipulation on dopamine circuitry have been shown to be ineffective after the shift from ventral to striatal brain regions [329,330]. Our findings on BLA and PFC could be irrelevant in a self administration paradigm involving withdrawal and craving aspects of addiction. The relevance to target reconsolidation of memory to treat habits has been recently addressed by Vousden and Milton[331]. Authors pointed out the fact that reconsolidation of stimulus-responses itself could be dependant of prediction-error(PE)[332] and dopaminergic pathways[333]. PE is also responsible of the destabilization of associative learning[334,335] and by extension could help to destabilize habits memories. At last, a study has shown that well-learn instrumental behavior can reconsolidate and is susceptible to disruption through NMDA receptors antagonists [336] suggesting that targeting memory reconsolidation could be an effective therapeutic way.

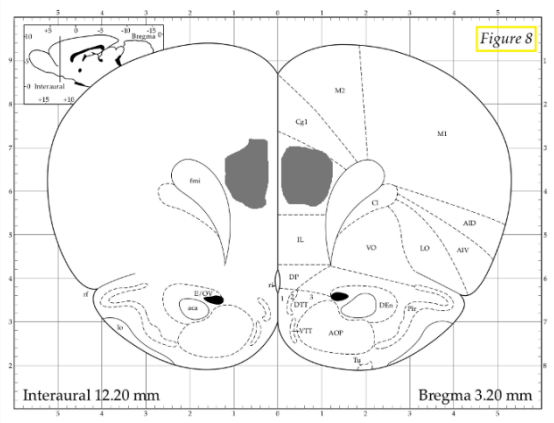
4. CONCLUSION :

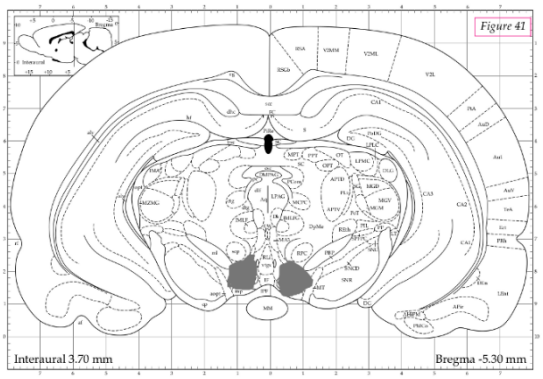
Cocaine addiction is a global health concern without efficient treatment. Discovering treatments to fight efficiently against drug abuse and dependence is a challenge for both medicine and basic science. One theory of addiction associates the formation of drug seeking behavior and habits as a maladaptive learning. The recent evidences showing that memory is not a static state and is susceptible to disruption open a way to treat this maladaptive learning. Despite several effective researches on animal model, there is no currently approved medication for the treatment of cocaine dependence, which calls for the development of novel therapeutic agents. Targeting associative memory to disrupt drug seeking behavior in human represents a challenge and require a comprehension of the mechanism occurring during the different states of addiction. In the present thesis, we have shown that astrocyte-neuron lactate transfer could play a critical role in the formation and the maintenance of appetitive memory formation. By showing that a molecule, long considered as a waste

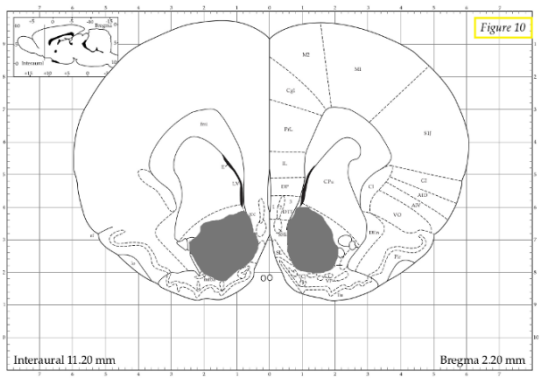
product, could act on gene expression and behavior related to memory, we have open a novel therapeutic way which could target in the future the habits memories. Although the understanding on glycogen and more specifically lactate metabolism remains unclear, it appeared to be linked to multiple cocaine related pathways such as serotonin, adrenaline dopamine and glutamate and offered promising hypothesis to understand the missing element driving the switch between initial associative learning to cocaine addiction.

ANNEXE : SUPPLEMENTARY FIGURES









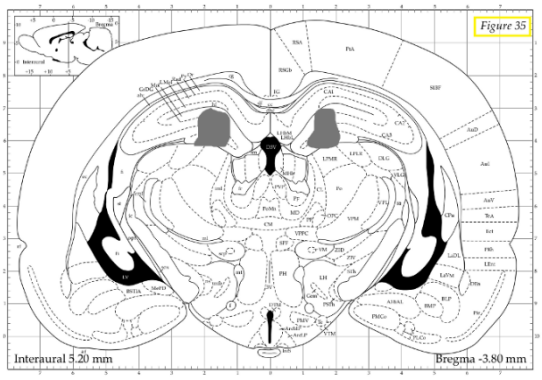


Figure S1: **Photomicrographs of representative cannula placements and schematic representations of injection sites in the BLA, PFC, NAcc Core and Hippocampus.**

(A) PFC (B) BLA, (C) Nucleus Accumbens Core (D) Hippocampus

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ORIGINAL ARTICLE

Disrupting astrocyte–neuron lactate transfer persistently reduces conditioned responses to cocaine

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A central problem in the treatment of drug addiction is the high risk of relapse often precipitated by drug-associated cues. The transfer of glycogen-derived lactate from astrocytes to neurons is required for long-term memory. Whereas blockade of drug memory reconsolidation represents a potential therapeutic strategy, the role of astrocyte–neuron lactate transport in long-term conditioning has received little attention. By infusing an inhibitor of glycogen phosphorylase into the basolateral amygdala of rats, we report that disruption of astrocyte-derived lactate not only transiently impaired the acquisition of a cocaine-induced conditioned place preference but also persistently disrupted an established conditioning. The drug memory was rescued by L-Lactate co-administration through a mechanism requiring the synaptic plasticity-related transcription factor *Zif268* and extracellular signal-regulated kinase (ERK) signalling pathway but not the brain-derived neurotrophic factor (*Bdnf*). The long-term amnesia induced by glycogenolysis inhibition and the concomitant decreased expression of phospho-ERK were both restored with L-Lactate co-administration. These findings reveal a critical role for astrocyte-derived lactate in positive memory formation and highlight a novel amygdala-dependent reconsolidation process, whose disruption may offer a novel therapeutic target to reduce the long-lasting conditioned responses to cocaine.

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INTRODUCTION

Drug memories that associate contextual cues with the effects of drugs of abuse are known to shape and maintain persistent drug-seeking behaviours in rodents.[1] In abstinent humans, drug cues are known to evoke salient, persistent and overwhelming memories of drug-taking experiences, thereby inducing higher risks of craving and relapse.[2,3] Preclinical observations have long reported that, through predictive association with the drug's effects, drug-conditioned stimuli can precipitate the reinstatement of previously extinguished drug-seeking behaviours.[4–6] However, many questions remain to be answered about the mechanisms by which long-term memories for drug-paired cues resist extinction and contribute to the enhanced drive to take drugs. A large body of evidence suggests that persistence of drug addiction depends on the remodelling of synapses and circuits that are thought to be characteristic of long-term associative memory.[7]

Over the past decade, converging evidence has revealed that memory and addiction share both neural circuitry and molecular mechanisms.[8–13] It has been suggested that learning the significance of a predictive cue to trigger the appropriate behavioural response requires the storage of specific patterns of information in the brain.[1] As disrupting protein synthesis immediately after learning has been shown to prevent memory formation,[14] a current consensus considers that the stabilization of a new memory occurs through a process known as consolidation and requires gene expression. Importantly, once consolidated, memories can again become transiently labile and sensitive to protein synthesis inhibitors if reactivated.[15–18] These findings may offer a critical contribution to clinical practice as they suggest that protein synthesis blockade after reactivation may selectively reduce or even eliminate long-lasting memories, including those linked to drug addiction.[19] Although reconsolidation most likely contributes to updating memories,[20–22] its disruption may reduce the impact of intrusive or aberrant memories on behaviour.[23–31] As several aspects of addiction depend on mnemonic processes induced by drug experience, disrupting drug-related memories represents a promising approach to help reducing relapse propensity on subsequent exposure to drug-paired stimuli and thereby may encourage abstinence.[32]

Although metabolic coupling has long been considered a key mechanism through which astrocytes and neurons actively interact in response of neuronal activity,[33,34] only recent evidence revealed that interference with lactate transfer from astrocytes to neurons impairs long-term memory formation.[35–38] The astrocyte network, known to form highly organized anatomical domains that are interconnected through gap junctions, contact up to hundreds of thousands of synapses permitting an uninterrupted supply of energy substrates.[39] Both glucose and lactate can be transported to neurons as metabolic substrates but astrocytic storage of glycogen has been considered as a supplemental energy reserve available to neurons when demand is high. In particular, the metabolic coupling between astrocytes and neurons posits that glycogenolysis-dependent lactate is released from astrocytes[40–42] and imported into neurons.[43,44] Of particular

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interest, recent evidence demonstrated that learning resulted in lactate release in the hippocampus and that lactate transfer from astrocytes into neurons was critical for the induction of the molecular changes required for long-term memory formation.[38] Disruption of the astrocytic (MCT4) and neuronal (MCT2) lactate transporters was shown to prevent the retention of an inhibitory avoidance task. However, L-Lactate administration successfully rescued the amnesia after MCT4 disruption only, suggesting that lactate import into neurons (via MCT2) is essential for long-term memory formation.[38] One possible mechanism is that lactate may supply activated neurons with sufficient energy required for activation of signalling pathways underlying long-term memory formation.[38] An alternative explanation would suggest that lactate may directly act as a signalling molecule for plasticity mechanisms.[45–48]

In line with this latter assumption, recent evidence has shown that L-Lactate increases the neuronal expression of synaptic plasticity-related genes, including *Arc*, *c-Fos*, *Zif268* and brain-derived neurotrophic factor (*Bdnf*), through a mechanism involving *N*-methyl-D-aspartate receptor (NMDAR) activity and its downstream signalling cascade extracellular signal–regulated kinase 1/2 (ERK1/2).[45] This observation is of particular relevance given the key role played by NMDAR to initiate reconsolidation, which destabilizes memories and promotes a transient labile state following retrieval, and subsequently leads to long-term memory storage.[49–52] Not surprisingly, several studies have reported that NMDAR antagonists administered into the basolateral amygdala (BLA) impaired retention of appetitive[53] and drug[54] memories, while activation of ERK pathway[31] and *Zif268* (ref. 27) was shown to be necessary for the reconsolidation of addictive drug memories.

These observations suggest that long-term memory formation requires high metabolic demands within the underlying active neuronal network and call for a better understanding of the molecular mechanisms involved in the complex reciprocal exchanges of metabolic intermediates between neurons and glia. Therefore, we investigated whether disrupting the glycogen-derived L-Lactate release from astrocytes by administering an inhibitor of glycogen phosphorylase (1,4-dideoxy-1,4-imino-D-arabinitol (DAB)) into the BLA of rats was sufficient to impair the acquisition and/or the maintenance of a cocaine-induced conditioned place preference (CPP). Our findings show that storage and retrieval of addictive drug memories require the astrocyte–neuron lactate transfer, whose disruption may offer a novel therapeutic potential to reduce the long-lasting debilitating impact of drug cues on conditioned responses to cocaine.

MATERIALS AND METHODS

Animals and surgery

All experiments were performed in accordance with the Swiss Federal Act on Animal Protection and the Swiss Animal Ordinance and were approved by the cantonal veterinary office (authorization 1999 to BB).

Rats were anaesthetized by inhalation of 1–3% isoflurane in oxygen and implanted bilaterally with cannula guides (home made from 22 G syringes, Terumo, Eschborn, Germany). Initial experiments were aimed at targeting the lateral ventricle (anterior–posterior A/P −0.6; mediolateral ML +/−1.9; dorsoventral D/V −3.2 mm from the skull surface). However, rats receiving intracerebroventricular administrations of the inhibitor of glycogen phosphorylase DAB still exhibited a preference for the compartment previously paired with cocaine administration (Supplementary Figure S1A). Using a similar approach, bilateral infusions of DAB into the prefrontal cortex (A/P: 3.2; ML +/−0.8; D/V −4 mm) also failed at blocking the cocaine-induced place preference (Supplementary Figure S1B). Hence, we targeted the BLA by using the following coordinates for a bilateral implantation of cannula guides (A/P −2.8 mm, ML +/−5 mm, D/V −7.5 mm; Supplementary Figure S2). Dental cement was used to anchor the cannula to the brain skull and cannulas were kept patent by insertion of a stylet to prevent obstruction. Injectors (homemade from 27 G syringes, Terumo) were placed 1 mm above the cannula guide to prevent brain tissue damage. Animals received a 0.1 mg kg^{-1} intraperitoneal injection of buprenorphine (Temgesic, Reckitt Benckiser, Wallisellen, Switzerland) before surgery and recovered for at least 7 days before starting the behavioural tests.

Drugs

Cocaine (Macfarlan Smith, Edinburgh, UK) was dissolved in sterile saline. Animals received 15 mg kg^{-1} doses, at a dose volume of 1 ml kg^{-1}. Vehicle solution (NaCl 0.9%) was injected at a dose volume of 1 ml kg^{-1}. All injections were given intraperitoneally.

Drugs were dissolved in sterile saline (NaCl 0.9%). DAB was administered at 150 pmol per side and L-Lactate and L-Pyruvate at 100 nmol per side (Sigma-Aldrich, Buchs, Switzerland).[38] Drugs were injected using a 5 µl Hamilton syringe (Harvard Apparatus, Les Ulis, France) at a rate of 250 nl min^{-1} over 2 min. After infusion, the injectors were kept in place for an additional 60 s.

Conditioned place preference

The apparatus consisted of three arenas divided into two distinct chambers ($45 \times 45 \times 30$ cm^3), separated by a corridor ($40 \times 15 \times 30$ cm^3), whose access was closed on demand with two guillotine doors. Chambers had different floors (perforated plastic plates versus Lego base plate) and different walls (white dots versus white stripes). Time spent in each compartment was monitored with a video tracking system (Ethovision Pro 3.16 Noldus, Wageningen, The Netherlands).

Statistical analysis

Data are shown as mean ± s.e.m. For most of the behavioural studies, data sets were subjected to analysis of variance, followed by Bonferroni and Tukey *post hoc* tests to confirm intra-session and intra-group differences, respectively. Statistical analyses were performed with Statview 5.0 (SAS Institute, Cary, NC, USA), using an α level of 0.05.

Further details about Material and methods, including statistics, are described in Supplementary Information.

RESULTS

Inhibition of glycogen metabolism in the BLA impairs the acquisition of cocaine-induced CPP, while L-Lactate co-administration restores the appetitive memory through a mechanism requiring *Zif*268

After having explored the CPP apparatus during the pretest session, rats implanted bilaterally with chronic indwelling cannulas targeting the BLA were infused either with vehicle or with the inhibitor of glycogen phosphorylase DAB, 15 min prior to being injected with cocaine (15 mg kg^{-1}, intraperitoneal), and confined in one chamber for 20 min. Animals received two cocaine and two saline injections on alternative days (see Material and methods in Supplementary Information). On the test day, DAB-treated rats, unlike vehicle-treated animals, did not show any preference for the compartment previously paired with cocaine administration (Figure 1). Co-administration of DAB and L-Lactate rescued the preference for the cocaine-paired compartment. In contrast, co-administration of DAB and L-Pyruvate (an energetic equivalent to L-Lactate) failed to rescue this preference. These data provide evidence for a critical role of glycogen-derived lactate in cocaine-induced CPP. The reduced preference for the previously cocaine paired compartment was not the consequence of undesirable or aversive side effects as bilateral administrations of DAB into the BLA did not reduce the locomotor activity in an open field apparatus; it also did not induce any place aversion (Supplementary Figure S3). The reduced preference for the cocaine side was not the consequence of damaged brain tissue owing to local injections as rats, transiently unresponsive to cocaine cues after DAB treatments, exhibited a marked preference for the cocaine compartment after another conditioning 1 week later (Supplementary Figure S4).

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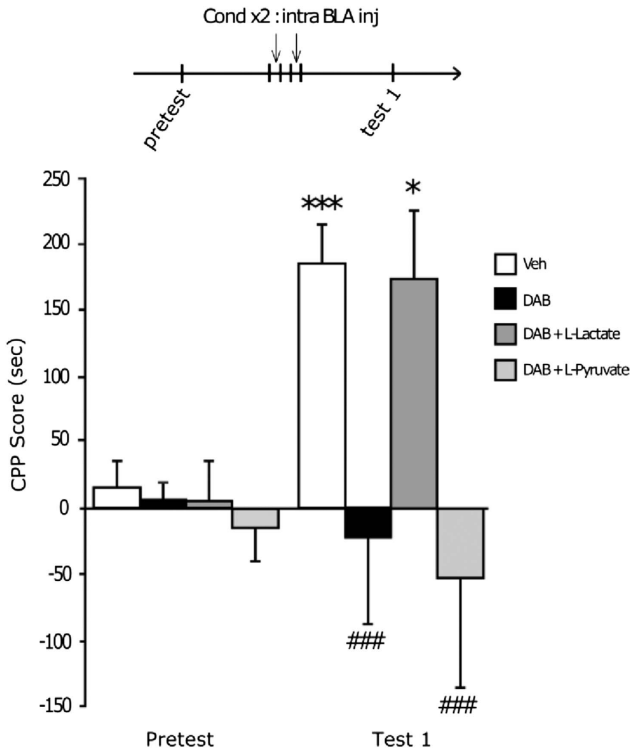

Figure 1. Astrocyte-derived lactate is required for the acquisition of a cocaine-induced conditioned place preference (CPP). Experimental timeline is shown above the graphic. Data represent CPP mean score (± s.e.m.) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. A two-way repeated-measures analysis of variance revealed a significant session × treatment interaction ($F_{3,44} = 4.467$, $P < 0.05$), and *post hoc* analyses demonstrated a significant preference for the compartment previously paired with cocaine injections in vehicle and 1,4-dideoxy-1,4-imino-D-arabinitol (DAB)+L-Lactate-treated animals (*$P < 0.05$, ***$P < 0.001$ compared with pretest score, $n = 15$ and 11, respectively), while DAB- and DAB+L-Pyruvate-treated rats did not exhibit any preference (###$P < 0.001$, $n = 13$ and 11, respectively) compared with respective pretest conditions. BLA, basolateral amygdala.

We then examined whether disrupting astrocytic glycogen mobilization influenced the expression levels of molecular markers of memory consolidation. We replicated the conditioning with four groups of rats: three groups were conditioned with cocaine (vehicle/cocaine; DAB/cocaine; DAB+L-Lactate/cocaine) and the last rats received vehicle treatments during the CPP procedure (vehicle/vehicle). *Arc* and *Zif268* expression was assessed 1 h after the last cocaine conditioning,[55] and *Bdnf* expression was measured 3 h after.[56]

Although recent evidence implicated *Arc* in the consolidation of explicit and implicit forms of memory, and in maladaptive plasticity associated with drug addiction,[57,58] *Arc* expression remained unchanged in the BLA following cocaine CPP (Figure 2), whereas cocaine conditioning effectively increased Arc expression in the nucleus accumbens (Supplementary Figure S5). Meanwhile, data revealed that rats conditioned with cocaine administrations and injected with vehicle showed a significant increase in *Zif268* and *Bdnf* expression in the BLA compared with unconditioned rats (Figure 2). Not only the increased expression of *Zif268* and *Bdnf* was abolished by DAB treatment but also L-Lactate treatment rescued the inhibitory effect of DAB on *Zif268* expression without, however, any effect on *Bdnf* mRNA levels (Figure 2).

Blocking glycogen metabolism in the BLA before and after contextual re-exposure persistently disrupts cocaine CPP

We then investigated whether acquired cocaine CPP was sensitive to glycogen blocking in the BLA. As shown in Figure 3, two groups of rats were conditioned with cocaine injections for 3 days. Twenty-four hours after the end of conditioning, rats were tested for CPP (test 1) to assess their preference for the compartment previously paired with cocaine administration. Twenty-four hours after test 1, one group received vehicle infusions and the other received DAB infusions into the BLA prior being tested once again in the CPP arena (test 2). Animals were tested again twice, at 24-h intervals (tests 3 and 4), and finally, their preference for the cocaine side was assessed 1 week later (test 5).

In contrast to a single injection performed 5 h after test 2 that had no effect (Supplementary Figure S6), DAB administered into the BLA 15 min prior test 2 immediately blocked place preference expression. This effect was prolonged for up to 2 days (tests 3 and 4), but 1 week after treatment, both groups expressed a marked preference for the compartment previously paired with cocaine administration (test 5). Hence, inhibition of glycogen phosphorylase immediately prior to re-exposure to the context transiently impaired the cocaine-induced CPP. One possible explanation is that each cocaine conditioning session activated glycogen metabolism, therefore blocking glycogen phosporylase prior the re-exposure trial only delayed but did not persistently disrupt the reconsolidation process. Interestingly, previous reports have established that a consolidated memory can become transiently labile and sensitive to protein synthesis inhibitors if reactivated.[15–18] To test the hypothesis that glycogen breakdown is necessary for reconsolidation of contextual appetitive memories, DAB was administered into the BLA before and after the re-exposure. As shown in Figure 4, two groups of rats were conditioned for 3 days. Twenty-four hours after the end of conditioning, rats were tested for CPP (test 1) and tested again 24 h later (test 2), which served as a re-exposure trial to reactivate the memory. Rats received intra-BLA administrations of vehicle or DAB just prior and 5 h after test 2. Animals were tested again twice, at 7-day intervals (tests 3 and 4), and finally, their preference for the cocaine side was challenged with a cocaine priming injection 24 h later (test 5). In line with the above-mentioned observations, DAB injections into the BLA 15 min prior to test 2 blocked place preference expression. Of critical importance, the second DAB administration into BLA extended the amnesia for up to 2 weeks (tests 3 and 4), and the indifference to cocaine compartment persisted even after a cocaine priming injection (test 5), suggesting a permanent disruption of the contextual appetitive memories. However, the same procedure replicated in rats conditioned with highly palatable chocolate flavoured food pellets failed to block the CPP (Supplementary Figure S7), suggesting that glycogen breakdown may be necessary for reconsolidation of contextual appetitive memories specific to cocaine.

Importantly, disruption of cocaine memory was found to be critically dependent upon re-exposure of the conditioning context as conditioned rats injected with DAB and returned to their home cages (not in the CPP apparatus) still exhibited a strong preference for the compartment previously paired with cocaine administrations when returned in the CPP apparatus 24 h later (Supplementary Figure S8). These data strongly support the key role of glycogen breakdown in the formation of the predictive association between the context and the drug effects.

The preference for the cocaine compartment is impaired following disruption of glycogen metabolism in the BLA but is rescued by L-Lactate

To demonstrate the key role of glycogen-derived lactate in the reconsolidation of memories for drug-associated cues during

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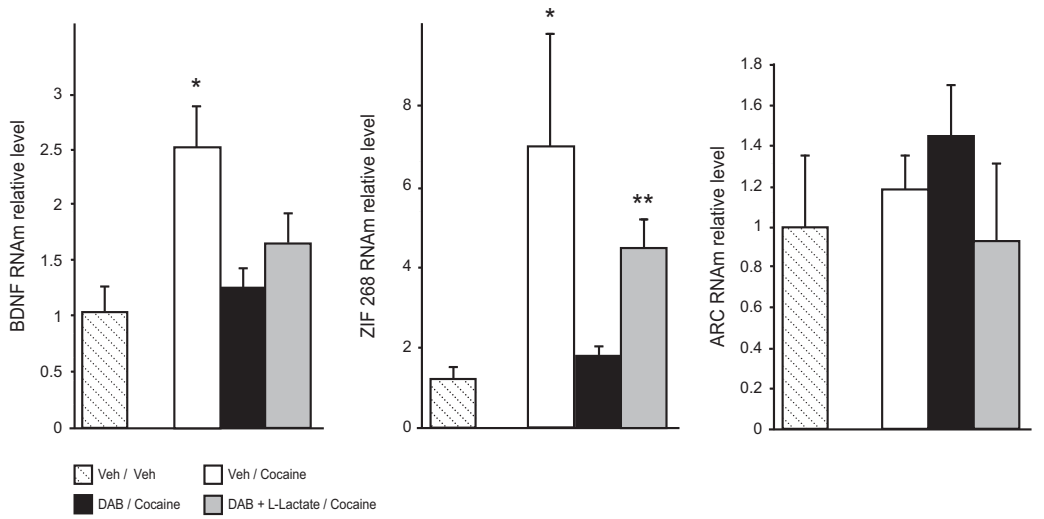

Figure 2. Astrocyte-derived lactate modulates gene expression involved in conditioned responses to cocaine. mRNA levels are expressed relative to control animals. Cocaine-induced place preference correlated with increased *Bdnf* and *Zif268* mRNA expression (*$P < 0.05$, compared with Veh/Veh). This increase was abolished after 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) treatment ($P > 0.05$ compared with Veh/Veh animals). Co-administration of DAB and L-Lactate rescued the expression of *Zif268* mRNA (**$P < 0.01$, compared with Veh/Veh) but not that of *Bdnf* ($P > 0.05$, compared with Veh/Veh). Cocaine conditioning or intra-basolateral amygdala treatments did not alter *Arc* mRNA expression ($P > 0.05$, compared with Veh/Veh). *Bdnf* measures, Veh/Veh $n = 5$, Veh/cocaine $n = 5$, DAB/cocaine $n = 8$, DAB+L-Lactate/cocaine $n = 10$; *Zif268* measures, $n = 4$, 4, 5 and 8, respectively; Arc measures, $n = 4$, 7, 6 and 5, respectively.

cocaine CPP, we examined the effect of DAB+L-Lactate co-administration.

As shown in Figure 5a, three groups of rats were conditioned with cocaine for 3 days. Twenty-four hours after the end of conditioning, preference for the cocaine compartment was assessed (test 1). Twenty-four hours later, rats were administered twice into the BLA, 15 min prior test 2 and again 5 h later, with either vehicle, DAB or DAB+L-Lactate. Confirming our former observations, all rats exhibited a preference for the cocaine compartment after 1 week, with the exception of those previously treated with DAB (Figure 5a, test 3).

As ERK activity has a key role in the long-term alterations in synaptic plasticity that result from repeated cocaine exposure,[59] we examined ERK phosphorylation in the BLA after test 3 (Figure 5b). Although DAB administration did not influence the phosphorylation of ERK in naive rats (Supplementary Figure S9), our data show reduced p-ERK protein levels in cocaine-conditioned rats receiving DAB treatments compared with animals receiving vehicle or DAB+L-Lactate. This observation strongly supports that glycogen breakdown triggers ERK activation that correlates with long-lasting cocaine-induced CPP.

DISCUSSION

Although astrocytes have long been considered to have mainly a supportive function for neurons, a growing body of evidence suggests that they fulfil other active roles, including information processing, signal transmission and regulation of neural and synaptic plasticity.[46–48] In particular, a recent study revealed that lactate transfer from astrocytes into neurons was essential for the induction of the molecular changes required for long-term aversive memory formation.[38] In the present study, we extend these findings by revealing that disrupting glycogen breakdown in the BLA impairs the acquisition, retrieval and long-term maintenance of positive affective memories associated with cocaine-paired cues. Importantly, we show that L-Lactate, but not L-Pyruvate, administered into the BLA rescued the preference for the cocaine compartment during conditioning. In addition, our results confirm that lactate also mediates intracellular responses for cell signalling and regulation of gene expression required for long-term positive memory formation.[38,39,45,60] Of particular relevance, we demonstrate that DAB-induced disruption of glycogenolysis in the BLA impairs the expression of *Bdnf* and *Zif268*, known to modulate the synaptic morphology and plasticity underlying the learning processes that strengthen conditioned responses to cocaine.[61,62] Interestingly, L-Lactate rescued the expression of *Zif268* but not that of *Bdnf*, suggesting that lactate-mediated rescuing of cocaine-associated memories during cocaine conditioning may not depend on *Bdnf* in the BLA. A similar observation reported a double dissociation in the hippocampus, where *Bdnf* was specifically required for consolidation, whereas *Zif268* was required for strengthening contextual fear conditioning.[63,64] Assuming that L-Lactate co-administration compensated the effect of DAB and permitted the stabilization of a novel memory following the first cocaine conditioning (consolidation phase), one can speculate that L-Lactate co-administration prior to the second cocaine conditioning permitted the reconsolidation phase that strengthens the association between the drug effects and the context.[26–28] Hence, the preference for the cocaine compartment coincided with an increased expression of *Zif268* but not of *Bdnf*. Analysis of *Bdnf* and *Zif268* expression in rats treated with DAB and L-Lactate following one cocaine injection would be necessary to confirm this double dissociation assumption.[64] However, one unique cocaine administration remains a weak conditioning, and the acquisition of a CPP would be uncertain. Nevertheless, our striking observation suggests that if L-Lactate, unlike L-Pyruvate, mediates intracellular responses for cell signalling and regulation of gene expression required for long-term positive memory formation, this is probably the result of increasing intracellular levels of NADH, thereby influencing the redox state of neurons and possibly redox-sensitive NMDA receptor subunits.[45]

In contrast to these results collected with a CPP paradigm, the conditioned responses to cocaine in rats trained for self-administering intravenous cocaine remained unaffected after bilateral administration of DAB into the BLA (Supplementary

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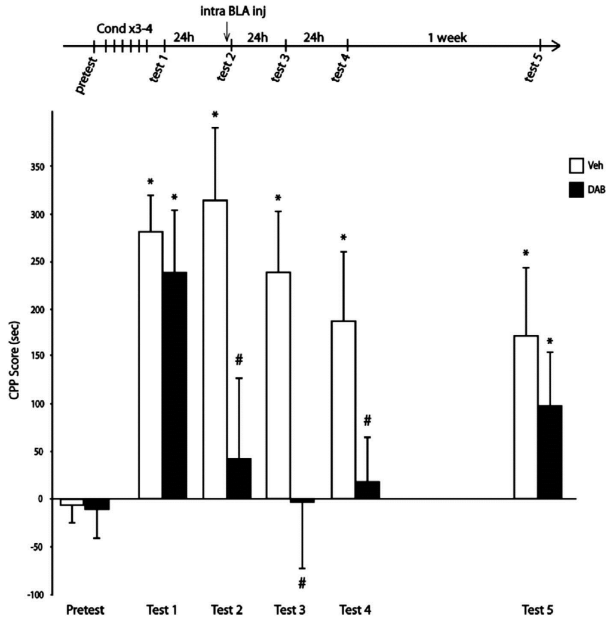

Figure 3. A single injection of 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) into the basolateral amygdala (BLA) transiently disrupts already established cocaine-induced conditioned place preference (CPP). Experimental timeline is shown above the graphic. Data represent CPP mean score (±s.e.m.) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. A two-way repeated-measures analysis of variance revealed a significant session × treatment interaction and *post hoc* analysis revealed a preference for the compartment previously paired with cocaine administration in all rats ($F_{5,19} = 3.301$, $^{*}P < 0.05$ compared with pretest score, at test 1). DAB injections into the BLA 15 min prior test 2 prevented the expression of the place preference. This effect was prolonged for up to 2 days (on tests 3 and 4, $\#P < 0.05$ compared with vehicle-treated animals, $^{*}P < 0.05$ compared with pretest conditions). One week after treatment, both groups expressed a clear-cut preference for the compartment previously paired with cocaine administration ($^{*}P < 0.05$ compared with pretest conditions, Veh, $n = 10$; DAB, $n = 11$).

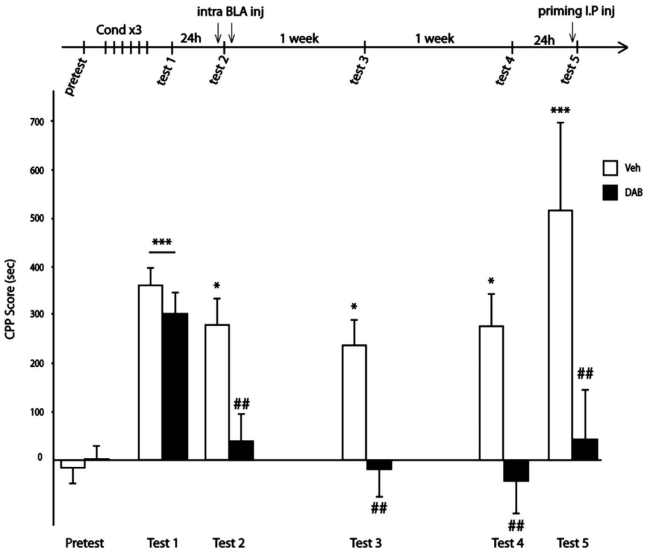

Figure 4. A double injection of 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) into the basolateral amygdala (BLA) permanently disrupt an already established cocaine-induced conditioned place preference (CPP). Experimental timeline is shown above the graphic. Data represent CPP mean score (±s.e.m.) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. A two-way repeated-measures analysis of variance revealed a significant session × treatment interaction and *post hoc* analysis revealed a preference for the compartment previously paired with cocaine in all rats ($F_{5,10} = 3.126$, $^{***}P < 0.001$ compared with pretest score, at test 1). DAB injections into the BLA 15 min prior to test 2 immediately blocked the preference for the cocaine-paired compartment compared with vehicle-treated animals. A second bilateral administration of DAB into the BLA 5 h after test 2 prolonged the effect for up to 2 weeks (on tests 3 and 4, $\#\#P < 0.01$ compared with vehicle animals, $^{*}P < 0.05$ compared with pretest conditions). Twenty-four hours after test 4, all rats were challenged with a cocaine priming injection (15 mg kg^{-1} intraperitoneal (IP)). Whereas rats formerly treated with vehicle still exhibited a clear-cut preference for the compartment previously paired with cocaine administration, DAB-treated animals still did not exhibit any preference for the cocaine-paired compartment (test 5, $\#\#P < 0.01$ compared with vehicle animals, $^{***}P < 0.001$ compared with pretest conditions, Veh, $n = 11$; DAB, $n = 11$).

Figure S10), an observation that has already been reported using NMDA antagonism.[65]

The second demonstration of this study is that disruption of glycogen metabolism not only transiently impairs acquisition of cocaine-induced CPP but also persistently disrupts an established conditioning. Indeed, rats with a strong cocaine preference showed transient amnesia after inhibition of glycogen phosphorylase prior to re-exposure to the drug context. Unexpectedly, rats exhibited a spontaneous recovery a week after treatment. One possible explanation is that blocking glycogen phosphorylase prior to the re-exposure trial most likely impaired the retrieval process and thus contributed to delay but did not persistently disrupt the reconsolidation process.

To test the hypothesis that glycogenolysis is necessary for reconsolidating contextual appetitive memories, we blocked glycogen breakdown twice, prior and after the memory retrieval window. Hence, we showed a long-lasting amnesia for cocaine-associated memory after prolonged disruption of glycogen metabolism in the BLA. Furthermore, the unresponsiveness to cocaine cues persisted even after cocaine priming injection, suggesting a permanent disruption of the contextual appetitive memories.

The ERK pathway is stimulated by drugs of abuse in striatal neurons through coincident activation of dopamine D1 and glutamate NMDA receptors and is considered to be critical for drug-induced long-lasting behavioural effects.[8] Further, converging evidence has shown that ERK phosphorylation and immediate-early gene expression are stimulated by drug-associated cues in the absence of drugs and have a key role in long-lasting drug-seeking behaviours.[59,66,67] In line with these observations, we showed that not only the long-lasting indifference for the cocaine compartment following inhibition of glycogenolysis in the BLA was associated with decreased phosphorylated ERK1/2 protein levels but we also demonstrated that lactate-induced recovery of contextual appetitive memories correlated with restored levels of phosphorylated ERK1/2 in the BLA. In line with recent *in vitro* observations from ours[45] and the demonstration that application of the NMDAR-agonist, D-cycloserine, potentiates the reconsolidation of appetitive memories,[68] our data strongly support the idea that L-Lactate stimulates the neuronal expression of synaptic plasticity-related gene through a mechanism involving NMDAR activity and its downstream signalling cascade ERK1/2.[45]

However, upstream of the protein synthesis required for reconsolidation, there may be an initial destabilization

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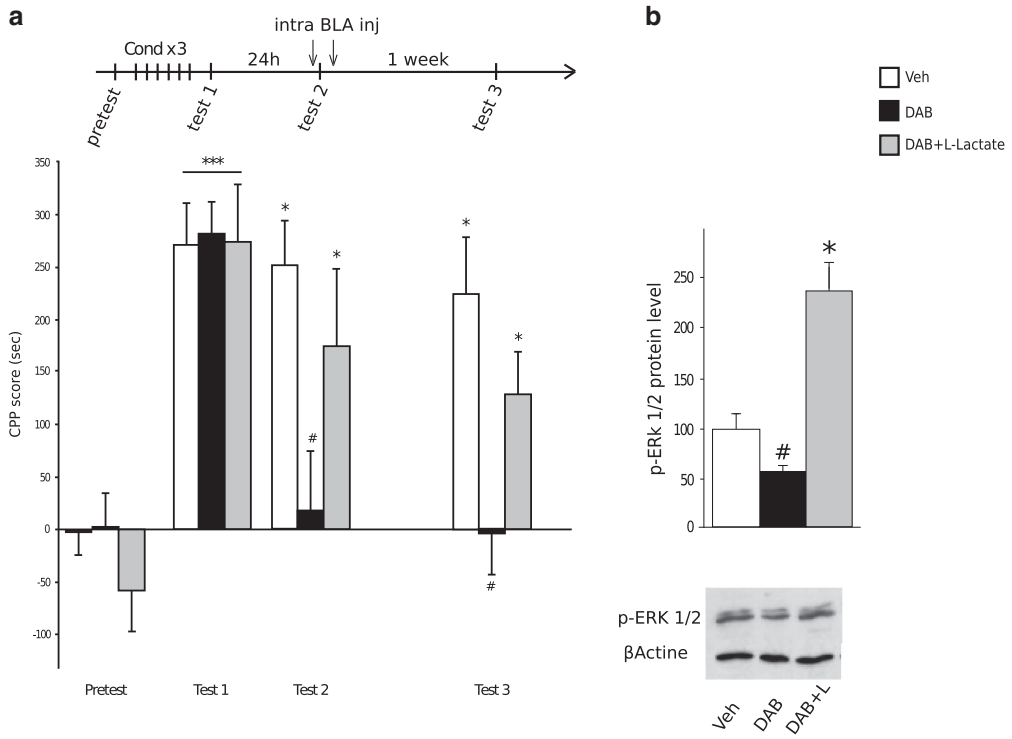

Figure 5. L-Lactate co-administration abolishes the 1,4-dideoxy-1,4-imino-D-arabinitol (DAB)-induced disruption of conditioned responses to cocaine and restores the expression of p-ERK (phosphorylated extracellular signal–regulated kinase) in the basolateral amygdala (BLA). (**a**) Experimental timeline is shown above the graphic. Data represent conditioned place preference (CPP) mean score (±s.e.m.) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. A two-way repeated-measures analysis of variance revealed a significant session × treatment interaction and *post hoc* analysis revealed a preference for the compartment previously paired with cocaine in all rats ($F_{2,36} = 3.820$, $^{***}P < 0.001$ compared with pretest score at test 1). DAB injections into the BLA 15 min prior to test 2 immediately blocked the preference for the cocaine-paired compartment compared with vehicle-treated animals, whereas co-administration of DAB and L-Lactate permitted the expression of the cocaine-induced place preference ($^{*}P < 0.05$ compared with pretest conditions). A second bilateral administration of DAB into the BLA 5 h after test 2 prolonged the indifference for the cocaine compartment for up to 1 week, whereas rats that received a second (DAB+L-Lactate) administration exhibited a significant cocaine-seeking behaviour (test 3, $\#P < 0.01$ compared with vehicle animals, $^{*}P < 0.05$ compared with pretest conditions). (**b**) ERK protein phosphorylation (measured 15 min after test 3) was significantly decreased after the double administration of DAB into the BLA ($\#P < 0.05$, compared with the Veh group), while the double co-administration of (DAB+L-Lactate) restored the expression of p-ERK ($^{*}P < 0.05$, compared with the Veh group. Veh, $n = 7$; DAB, $n = 8$; DAB +L-Lactate, $n = 9$).

process, named deconsolidation,[69] which most likely requires NR2B-containing NMDA receptors[49–51] and protein degradation.[70] Hence, further studies are required to assess whether lactate may supply activated neurons with NADH, stimulating redox-sensitive NMDAR subunits possibly triggering destabilization of appetitive memories. Lactate may also supply activated neurons with sufficient energy required for synaptic protein degradation. In a second step, lactate may also contribute to the subsequent phase of the plasticity process by providing sufficient energy required for activation of signalling pathways and protein synthesis underlying long-term memory formation.

Overall, we show that glycogenolysis-dependent lactate release is essential for mediating intracellular responses underlying long-term regulation of gene expression required for cocaine-induced long-lasting behavioural effects. Our results confirm the importance of astrocyte–neuron metabolic interactions in cognitive functions and, for the first time, demonstrate the key role of the astrocyte–neuron metabolic coupling in positive affective memory storage and retrieval. These findings open novel therapeutic avenues to reduce the long-lasting impact of drug cues on conditioned responses to cocaine.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Disrupting astrocyte-neuron lactate transfer persistently reduces conditioned responses to cocaine

SUPPLEMENTARY INFORMATION

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SUPPLEMENTARY INTRODUCTION

Although astrocytes have long been considered to mainly have a supportive function for neurons, a growing body of evidence suggests that they display other active roles including information processing, signal transmission and regulation of neural and synaptic plasticity (Halassa et al., 2010; Henneberger et al., 2010; Perea et al., 2009; Magistretti and Allaman 2015). Further, recent evidence demonstrated a role for astrocytes in extinction/reinstatement procedures of cocaine self-administration (Kalivas, 2009). Interestingly, if glucose is an important source of energy for the brain, mainly astrocytes, and almost not neurons, store glycogen (Brown et al., 2005; Magistretti, 2006; Vilchez et al., 2007), which can be rapidly converted to pyruvate/lactate and metabolized in the tricarboxylic acid cycle or used for biosynthesis of glutamate (Wiesinger et al., 1997). Hence, brain energy utilization may also influence glutamate homeostasis and participate to glutamate signalling between neurons. Since a recent assumption suggests that failure to control drug use may be linked to an enduring imbalance between synaptic and non-synaptic glutamate (Kalivas, 2009), excessive metabolic coupling between astrocytes and neurons may contribute to uncontrolled drug seeking behaviour. Noteworthy, glutamate uptake protects ionotropic glutamate receptors in the synaptic cleft from exposure to non-synaptically released glutamate (Kalivas, 2009). Recent evidence demonstrated that most of the basal extracellular glutamate is derived from

constitutive cysteine-glutamate exchange (Baker et al., 2002), and further observations reported that cocaine-induced changes in cysteine-glutamate exchange might increase glutamatergic neurotransmission and thereby, facilitate drug-seeking behaviours (Kalivas, 2004; Kalivas et al., 2005).

Overall, glutamate homeostasis is defined as the regulation of extracellular glutamate levels in the synaptic and perisynaptic area, which has been shown to closely depend on the balance between glial and synaptic glutamate release and elimination. By controlling glutamate access to ionotropic and metabotropic glutamate receptors, brain energy utilization may regulate glutamate homeostasis, and as such, may represent a key regulator of synaptic activity and plasticity. However, results reported in this article point to a key role of lactate produced from glycogen as a signalling molecule for mediating enhanced retention of cocaine-related associative memories. Thus it would appear that the formation of lactate as a signalling molecule rather than an effect on glutamate homeostasis is involved in the astrocytic glycogen metabolism-dependent effects described here.

Noteworthy, the nutritional status is important when considering the astrocytic glycogen metabolism since food restriction may exhaust glycogen stores hence leaving DAB without effect. In these conditions, and not only because food restriction may have induced a stress response possibly interfering with cocaine conditioning, rats had to be fed *ad libitum* to prevent any confounding effects of DAB on glycogen-derived lactate memory consolidation.

Meanwhile, despite glycogen breakdown inhibition, one could consider that astrocytes may use extracellular glucose-derived glucose-6-phosphate for glycolysis. However, it has been shown that local injection of glucose, unlike lactate, only marginally and considerably more slowly rescues the DAB-evoked inhibition of memory consolidation (Suzuki et al, 2011).

SUPPLEMENTARY METHODS

Male Wistar Han rats (200-250g at the beginning of the experiment, Charles River, France) were individually housed after surgery and kept under a reversed light dark cycle (darkness from 8am to 8pm). Rats were allowed *ad libitum* access to food and water, and testing sessions were performed during the dark phase of the light/dark cycle.

Estimation of the expected size of effect was based on preliminary studies assessing the effect of DAB on cocaine-induced conditioned place preference rats, in which control rats displayed a mean CPP score of 250 sec, and DAB-treated rats a mean CPP score of 50 sec. The required sample sizes to detect a difference with an alpha level 0.05 and a desired power of 0.8 is estimated is approximatively 8 rats, meaning 4 rats are required in each arm of the study (Whitley and ball, 2002).

Study A : acquisition of cocaine CPP

All CPP procedure followed an unbiased counterbalanced protocol. At day1, animals performed a pretest session allowing a freely exploration of both compartment during twenty minutes. Animals with an initial preference (difference in time spent in each compartment >180sec) were excluded. Animals were then divided into four groups (Vehicle, DAB+Vehicle, DAB+L-Lactate, and DAB+L-Pyruvate) following a counterbalanced unbiased repartition. The rats underwent fours conditioning sessions (day 2 to day 5). On day 2, animals received ip injections of saline (Nacl 0.9%, 1ml/kg) and were immediately confined in one compartment of the arena for twenty minutes. Twenty-four hours later, rats received intra-BLA administrations of DAB+Vehicle, DAB+L-Lactate, or DAB+L-Pyruvate using a micro pump (Harvard Apparatus, France) connected to a Hamilton syringe (5µL, Harvard Apparatus). A total volume of 500nL was delivered each side over a two-minute period. The injectors were kept in place for additional 60 sec. Fifteen minutes after, rats received ip injections of cocaine (15mg/kg, 1ml/kg) and were immediately confined in the alternate compartment for twenty consecutive minutes. These two pairing sessions were repeated twice. Twenty-four hours after the last cocaine session, animals performed a post-test (post-test 1) session during which they were allowed to freely explore the entire arena for 20 minutes.

Study B : maintenance of cocaine CPP

In contrast to study A, during which 2 cocaine conditionings only were performed to minimize risks of brain tissue damage following local infusions into the BLA, rats underwent here 3 cocaine pairing sessions to maximize the strength of conditioning. A first post-test was performed to confirm the preference for the side previously paired with cocaine administrations. Only rats with a preference for the cocaine-paired compartment above 100 sec were included in the study. Animals were then divided into two groups (DAB and vehicle) following a counterbalanced unbiased repartition. A first batch of these animals performed a second post-test 24h after the first one and received, 15 minutes before, an intra-BLA injection of treatment (see above). They performed two additional post-test, 48h and 72h after post-test 1 without any treatment. Finally, a last post-test was made, 1 week after post-test 2.

The other group of animals performed the same conditioning (3 pairing sessions), a post-test 1 to assess the preference but receive a double intra-BLA administration of treatment, 15 minutes before and 5 hours post-test 2. They also performed two additional post-test (one week and two weeks after treatment). Finally, a last post-test was performed at day 15 with priming injection of cocaine (15mg/kg ip, 1ml/kg).

A similar approach was replicated to assess the effect of glycogen breakdown in the BLA on rats conditioned with a natural reinforcer. Rats were first given 2 highly palatable food pellets (5g

each) in their home cage (5TCY Test Diets chocolate flavored pellets, IPS Ltd, London, GB; see Rossetti et al., 2014) while being mildly food restricted otherwise (14g chow pellet per day), so that their body weight remained stable over 3 days. On the first conditioning session, food restricted rats were given 3 chocolate flavored pellets (a total of 15g) in the rewarding compartment and nothing in the other compartment. On the second, the third and fourth conditioning sessions, rats were not food restricted any longer and were given 2 chocolate flavored food pellets in the rewarding compartment. Therefore, 4 pairing sessions were performed to maximize the conditioned place preference induced by the natural reinforcer. Otherwise, DAB administration was identical as that presented above.

Open field

The apparatus was composed of a plastic circle arena (135 cm diameter). The animals were allowed to freely explore the arena for 20 minutes. During the last 10 minutes, a green plastic bottle was positioned in the middle of the arena. Rats behavior was assessed by monitoring both the time spent in the center of the arena (a 20cm-diameter circle) and the total distance travelled in the arena using a video tracking system (Ethovision Pro 3.16 Noldus, The Netherlands). DAB (150pm per side) or vehicle was administered into BLA fifteen minutes before the experiment.

Histology for cannula placement

At the end of behavioral experiment, animals were sacrificed by decapitation after a short anesthetization with isoflurane. Brains were removed and post fixed overnight with 4% paraformalehyde solution. Coronal sections of 80uM were made through the targeted area to confirm cannula placement. Histological assessment revealed that twelve rats had infusion sites cannulas that extended beyond the BLA. These animals were excluded from the analyses.

Molecular study

Animals were briefly sedated with isoflurane before rapid decapitation. Measures of *Zif268* and *Arc* were made in rats sacrificed one hour post-conditioning (Guzowski et al., 2001), and *Bdnf* was measured in rats sacrificed three hours post-conditioning (Tao et al., 1998) (Figure 2). For assessing ERK phosphorylation, rats were sacrificed 15 minutes after the post-test performed one week after the DAB infusion (Figure 5, test 3). Measures of ERK phosphorylation were also made in naïve rats sacrificed twenty minutes after DAB infusion (Miller and Marshall, 2005) (Figure S9). Brains were placed into a matrix and then cut into slices of 2 mm. Brain samples were immediately placed on dry ice and stored at -80°C until use.

ARN extraction/RT-PCR

The total RNA was isolated from basolateral amygdala micro punches using RNeasy Plus Mini kit (Qiagen), according to the manufacturer's instructions. 100ng of total RNA was used for reverse transcription into first-strand cDNA with TaqMan reverse transcription reagents (Applied Biosystems). The resulting cDNA was then amplified by real-time quantitative PCR using Power SYBR Green PCR Master Mix (Applied Biosystems) and a 7500 Real-Time PCR System (Applied Biosystems). Forward and reverse primers pairs were used for specific mRNA amplification: 5'-GCAATGCCGAACTACCCAAT-3' and 5'-GAACCGCCAGCCAATTCTC-3' for BDNF; 5'-TTTCTCTGCCTTGAAAGTGTC-3' and 5'-GAACGACACCAGGTCTCAAC-3' for Arc; 5'-TCTGAATAACGAGAAGGCCGTGGT-3' and 5'-ACAAGGCCACTGACTAGGCTGAAA-3' for Zif268; 5'-ACCGTGAATCTTGGCTGTAAAC-3' and 5'-CGCAGTTGTTCGTGGCTCTC-3' for TBP; 5'-CACACCCGCCACCAGTTCG- 3' and 5'-CTAGGGCGGCCCACGATGGA-3' for β-actine. The analysis of relative mRNA levels was performed using a delta-CT (ΔΔCt) relative quantification model (Livak and Schmittgen, 2001) with β-actine and TBP as reference genes. Each value is expressed as fold change relative to the mRNA level of the control value.

Western Blot Analysis

Western blot analysis was performed with rabbit anti-phospho-Thr202/Tyr204 p44/42 MAPK (1:1000; Cell Signaling Technology) and anti-β-actine (1:10000; Abcam) antibodies. ECL horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibody (1:10000; Amersham Biosciences) was used as a secondary antibody. Results were quantified with ImageJ software (National Institute of Health), and densitometric values were normalized to corresponding β-actine levels.

For immunodetection of phosphorylated MAPK, blots were blocked in 50 mM Tris-HCl, pH7.5, 150mM NaCl, 0.1% Tween-20 complemented with 5% skim milk for 1 hr at room temperature. The blots were incubated overnight at 4°C in 50mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween-20 with 5% skim milk in the presence of a polyclonal rabbit anti-Phospho-p44/42 MAPK antibody (Cell Signaling Technology, diluted at 1:1000). Differences in protein gel loading and blotting were determined by reprobing the blots with a monoclonal mouse anti-β-actine antibody (Abcam) at a dilution of 1:1000. ECL horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibody (1:10000; Amersham Biosciences) was used as a secondary antibody. Results were quantified with ImageJ software (National Institute of Health), and densitometric values were normalized to corresponding β-actine levels.

Jugular vein catheterization

An indwelling catheter was implanted into the right external jugular vein under oxygen/isoflurane vapor anesthesia (Boutrel et al., 2005). Briefly, a 14 cm length of silastic tubing (0.3 mm inner diameter, 0.6 mm outer diameter, Silastic, Dow Corning Corporation, Midland, MI, USA) was fitted to a 22-gauge steel cannula (PlasticsOne, Roanoke, VA, USA) that was bent at a right angle and then embedded in a cement disk (Dentalon Plus, Heraeus Kulzer, Germany) with an underlying nylon mesh. The catheter tubing was inserted 2.5 cm into the right jugular vein and delicately anchored to the vein. The catheter ran subcutaneously to the base located above the midscapular region. All incisions were sutured and coated with antibiotic ointment (Bepanthen® Plus, Bayer AG, Zurich, Switzerland). Animals received a 0.1mg/kg/ip injection of buprenorphine (Temgesic®, Reckitt Benckiser, Wallisellen, Switzerland) before surgery. After surgery, rats were allowed 7 days to recover prior to initiation of self-administration sessions, during which 0.25 ml of 0.9% saline containing heparin (30 USP units/ml) was infused daily through the catheter to forestall clotting. Catheter patency was confirmed after completion of the experiment by the infusion of 0.02 to 0.03 ml of Etomidat-®Lipuro (B. Braun, Melsungen, Switzerland). Loss of muscle tone and clear signs of anesthesia within 2 sec of infusion indicated catheter patency. Only rats with a patent catheter were included in the final results.

Cocaine self-administration, extinction and reinstatement

The experiments were conducted in rat operant chambers (Med Associates, St Albans, VT, USA). Rats were trained to self-administer intravenous cocaine (obtained from Hänseler AG, Herisau, Switzerland) in the presence of an olfactory cue (thyme sprinkled in the sawdust underneath the grid) under a fixed ratio 1, timeout 20-s (FR1 TO20-s) schedule of reinforcement during daily 2-h sessions 7 days per week. A single pressing on the active lever was paired with the illumination of a light cue located above the lever and the delivery of the reinforcer (2.5 mg/ml, 0.1 ml over 4 sec) through the tubing into the intravenous catheter by a Razel syringe pump. Responses on the active lever during the TO period and responses on the inactive lever were recorded but were without scheduled consequence. After rats established stable responding (≤ 25% variation of the mean responses for three consecutive sessions), they underwent 2-h extinction sessions with saline infusion in absence of both the olfactory cue and the illuminated lever until reaching the extinction criterion. The criterion was achieved when responses on the active hole were <30% of the mean responses obtained during the 3 days achieving the acquisition criteria across 3 consecutive extinction sessions. Tests for reinstatement were conducted under the same conditions used in the cocaine self-administration phase (olfactory cue and illuminated lever) but saline replaced cocaine

Statistics

For each experiment, the normal distribution of the data was assessed using the Shapiro–Wilk tests, and homogeneity of variances was assessed using a Bartlett's test.

Given the high reinforcing properties of cocaine, we reasoned that the cocaine-induced conditioned place preference would be clear-cut, while the effect of DAB was expected to significantly decrease place preference since it was shown to drastically disrupt aversive memory (Suzuki et al., 2011). In these conditions, we applied a Bonferroni correction to the post hoc analyses. However, while designing the experiment on natural reinforcers, we expected a reduced conditioned place preference, notably a week after conditioning. For this reason, data were subjected to a two-factor repeated measures analysis of variance (ANOVA), followed by Fisher's post hoc tests.

Regarding Figure S4, when rats were exposed to a second cocaine conditioning a week after DAB infusion, the Shapiro-Wilk test revealed that data were not normally distributed. A non-parametric Friedman analysis was conducted followed by Wilcoxon tests to compare the effect of session and Mann-Whitney tests to assess intra-group differences. Data from open field experiments (total distance and time spent in centre) were analysed using Student unpaired t-tests.

For molecular studies, data were not normally distributed. Non-parametric Kruskal Wallis tests were performed followed by Mann-Whitney tests to assess intra-group differences.

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1: Administration of DAB into the lateral ventricle or into the prefrontal cortex failed to disrupt the acquisition of a cocaine-induced place preference. Experimental timeline is shown above the graphic. Data represent CPP mean score (± SEM) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. A, a two-way repeated measures ANOVA followed by Bonferroni post-hoc analysis revealed a significant preference for the compartment previously paired with cocaine administrations in all rats ($F(1,7)=69.035$, ***$p<0.001$ compared to pretest conditions) but no effect of treatment ($p>0.05$; $F(1,7)=0.326$) and no interaction ($p>0.05$; $F(1,7)=0.44$) in Veh group, n=4 and in DAB group, n=5. B, a two-way repeated measures ANOVA followed by Bonferroni post-hoc analysis revealed a significant preference for the compartment previously paired with cocaine administrations in all rats $F(1,14)=29.889$, ***$p<0.001$ compared to pretest conditions) but no effect of treatment ($p>0.05$; $F(1,14)=0.217$) and no interaction ($p>0.05$; $F(1,14)=0.007$) in Veh group, n=8 and in DAB group, n=8.

Figure S2: Photomicrographs of representative cannula placements and schematic representations of injection sites in the BLA. (A) The arrow points the ventral point of the cannula guide. (B) Schematic representations of the injector tips. Due to the overlap between the

infusion needle tips, this representation does not reflect the total number of animal used in the experience.

Figure S3: Intra-BLA administration of DAB did not reduce the locomotor activity nor induced any aversive-like effect. Rats were infused bilaterally with Vehicle or DAB (150pm per side) 15 minutes prior the experiment. A. Data represent the mean time (± SEM) spent in the center of the open field arena (with and without object) and the mean distance (± SEM) travelled by rats. All rats exhibited the same behavior in response to local infusions ($p>0.05$, t-test, Veh n=4, DAB n=5). B. Data represent mean time (± SEM) spent in the DAB-paired compartment expressed in percent of total time. A two-way repeated measures ANOVA revealed no effect of DAB treatment ($p>0.05$, $F(1,10)=0.719$, in Veh group, n=6 and in DAB group, n=6).

Figure S4: A cocaine-induced place preference was restored in rats previously treated with DAB. Experimental timeline is shown above the graphic. Rats transiently unresponsive to cocaine cues after DAB treatments were again conditioned with cocaine a week after DAB treatments. Data represent CPP mean score (± SEM) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. DAB injections (150pm per side) into the BLA during the first conditioning sessions prevented the expression of a cocaine seeking behavior during test 1 (#$p<0.05$, compared to Veh), while Veh treated animals exhibited a strong preference for the compartment previously paired with cocaine administrations (*$p<0.05$ compared to pretest conditions). Following a second cocaine conditioning (with no intra-BLA treatment), all rats displayed a clear-cut preference for the previously paired cocaine compartment (*$p<0.05$ compared to pretest conditions; Veh n=12, DAB n=11).

Figure S5: Cocaine administrations during conditioning induced a significant increase in *Arc* and *Zif*268 mRNA levels in the ventral striatum. mRNA levels are expressed relative to control animals (Veh/Veh). All rats that received cocaine injections exhibited an increased expression of *Arc* and *Zif*268 mRNA in the ventral striatum, whatever the treatment they received into the BLA (t-test *$p<0.05$, compared to the Veh/Veh group, n=5 in each group).

Figure S6: A single injection of DAB into BLA 5 hours after re-exposure does not disrupt an established cocaine-induced conditioned place preference. Experimental timeline is shown above the graphic. Data represent CPP mean score (± SEM) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. A Two-way repeated measures ANOVA followed by Bonferroni post hoc tests revealed a preference for the compartment previously paired with cocaine in both group up to two weeks ($F(4,28)=7.111$

***p<0.001, **p<0.01 compared to pretest score) and no effect of treatment (F(1,28)=0.15. p>0.05) in Veh group, n=5 and in DAB group, n=4.

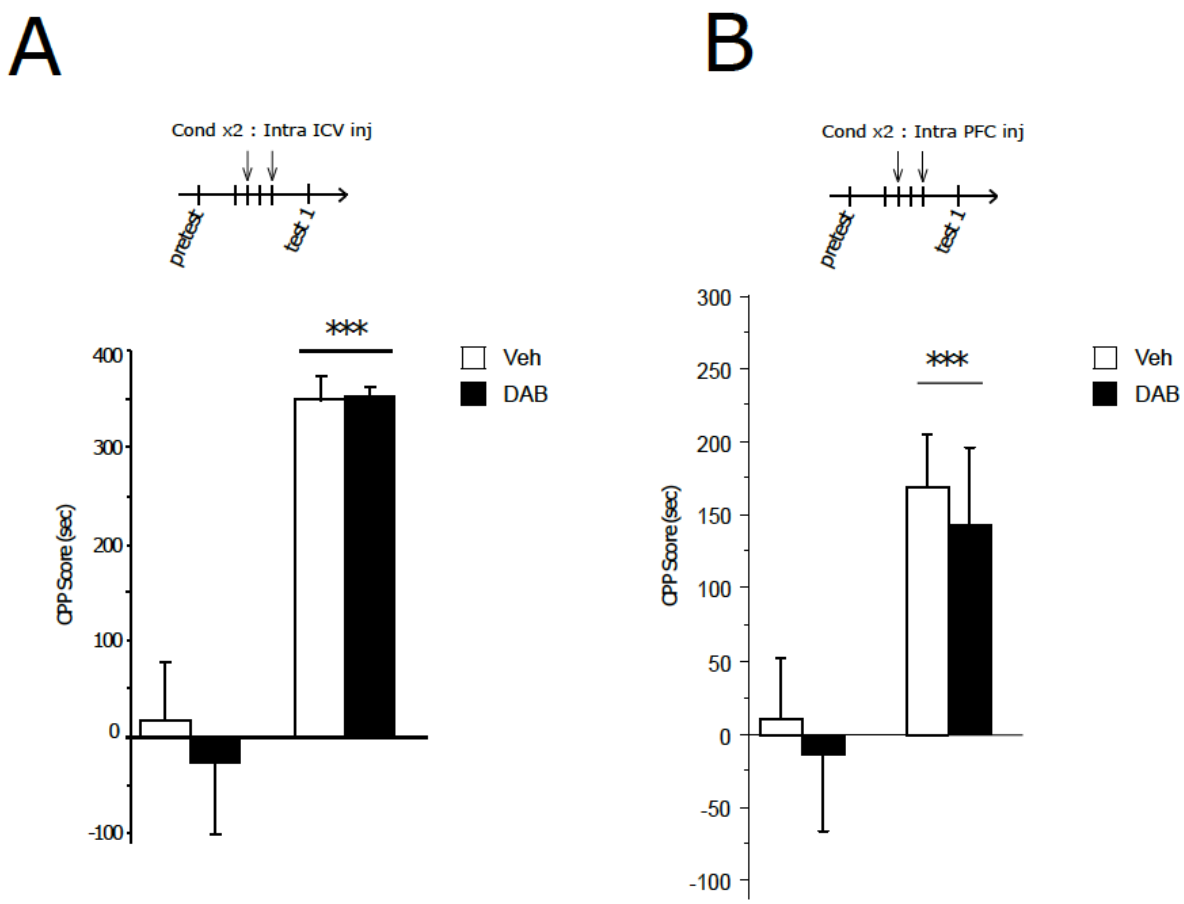
Figure S7: A double injections of DAB into the BLA does not disrupt the established preference for the compartment previously paired with highly palatable food. Experimental timeline is shown above the graphic. Data represent CPP mean score (± SEM) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. A two-way repeated measures ANOVA followed by Fischer post hoc tests revealed a long-lasting preference for the compartment previously paired with food in both groups. (* p<0.05 compare to pretest conditions, F(3,28)=5.017, in Veh group, n=7 and in DAB group, n=5).

Figure S8: Rats injected in their home cages still exhibited a strong preference for the compartment previously paired with cocaine administrations when returned in the CPP apparatus. Experimental timeline is shown above the graphic. Data represent CPP mean score (± SEM) expressed in seconds as time spent in cocaine compartment minus time spent in saline compartment. A two-way repeated measures ANOVA and Bonferroni post-hoc analysis revealed an effect of session (F(2,7)=6,802 *p<0.001 compared to pretest conditions) but no effect of treatment (p>0.05 ; F(1,7)=0.504) and no interaction (p>0.05 ; F(2,7)=1.028) in Vehicle (n=5) and DAB treated rats (150pm per side, n=4).

Figure S9: DAB administration into the BLA does not modulate ERK phosphorylation. Rat were sacrificed twenty minutes after intra-BLA injections. (p>0.05, t-test. Veh n=7,DAB n=8).

Figure S10: DAB injections into BLA have no effect on cocaine intake. Experimental timeline is shown above the graphic. Data represent mean (± SEM) number of lever presses during cocaine acquisition (average of the last three sessions), treatment session (15 min before which rats received intra-BLA finfusions), extinction (average of the last three sessions) and cue-induced reinstatement. A two-way repeated measures ANOVA followed by Bonferroni post hoc test revealed a session effect for both groups (F(3,21)=9.586) but no effect of treatment (F(1,21)=1.812).(*p<0.05, ** p<0.01 compare to extinction session, in Veh group, n=5 and in DAB group, n=4).

Figure S1


A
Cond x2 : Intra ICV inj
pretest
test 1
***
Veh
DAB
CPP Score (sec)
400
300
200
100
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-100
B
Cond x2 : Intra PFC inj
pretest
test 1
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CPP Score (sec)
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Figure S2

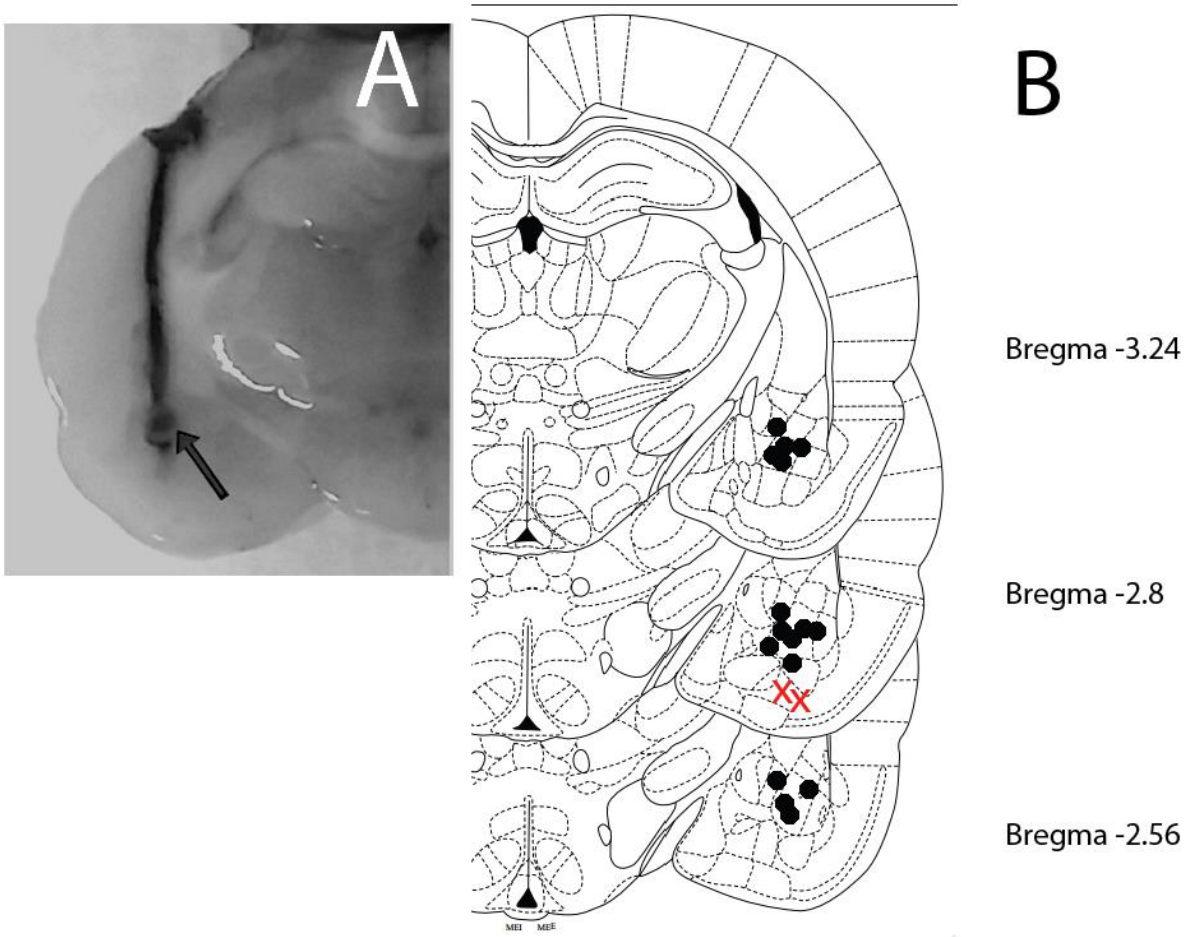


Figure S3

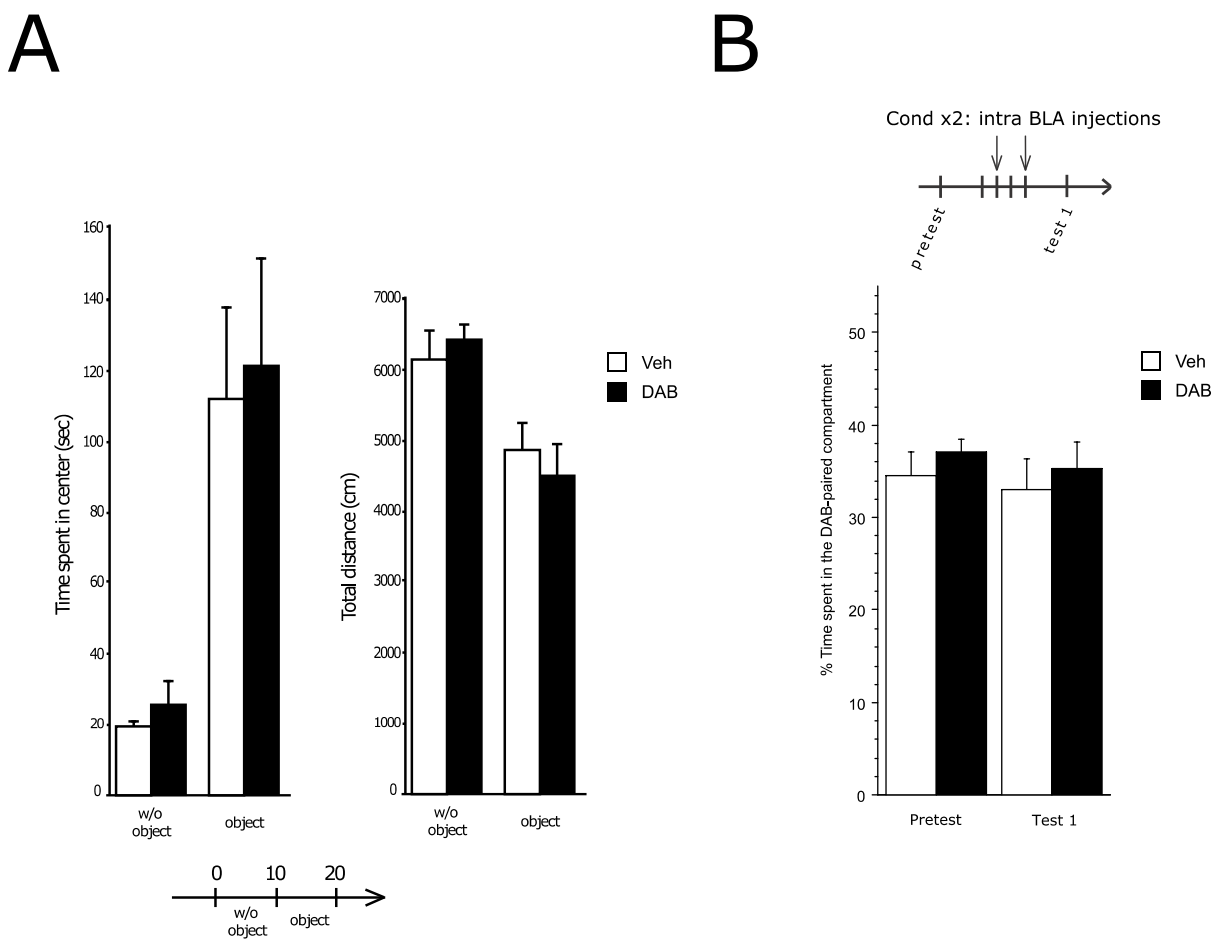
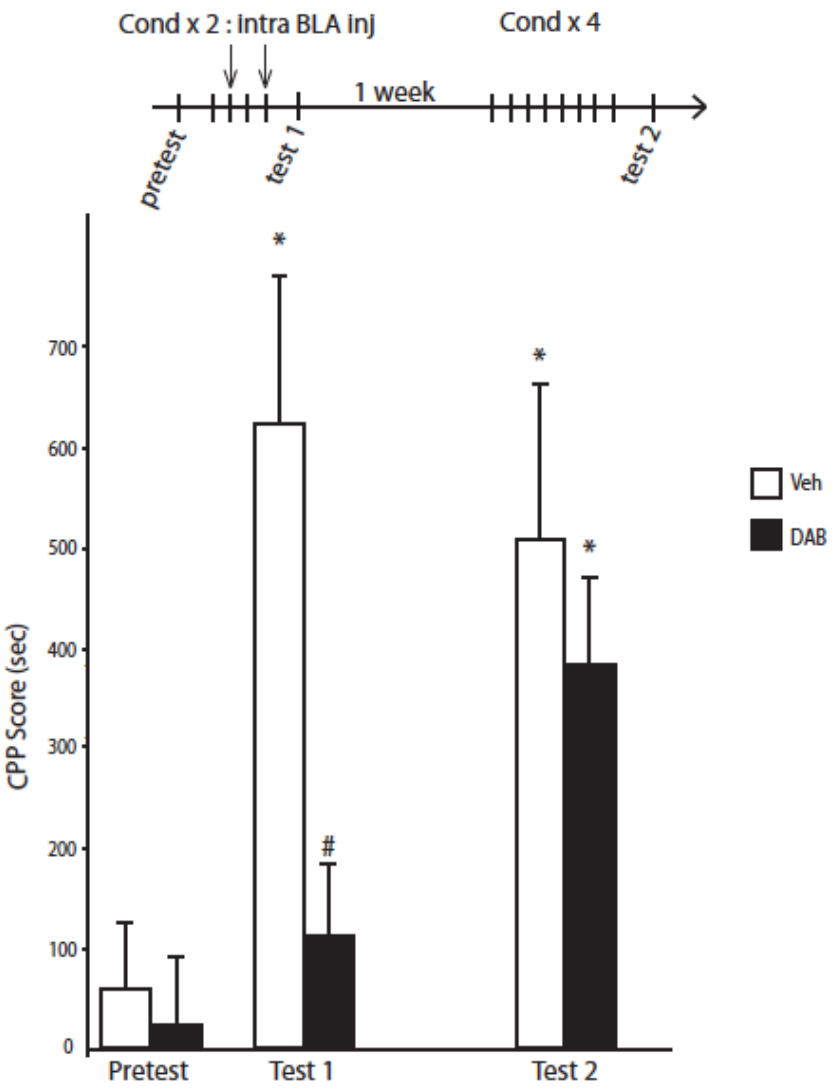

A
B
Time spent in center (sec)
Total distance (cm)
Veh
DAB
w/o object
object
0
10
20
Cond x2: intra BLA injections
pretest
test 1
% Time spent in the DAB-paired compartment
Pretest
Test 1

Figure S4


Cond x 2 : Intra BLA inj
Cond x 4
1 week
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test 1
test 2
CPP Score (sec)
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*
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#
Pretest
Test 1
Test 2
Veh
DAB

Figure S5

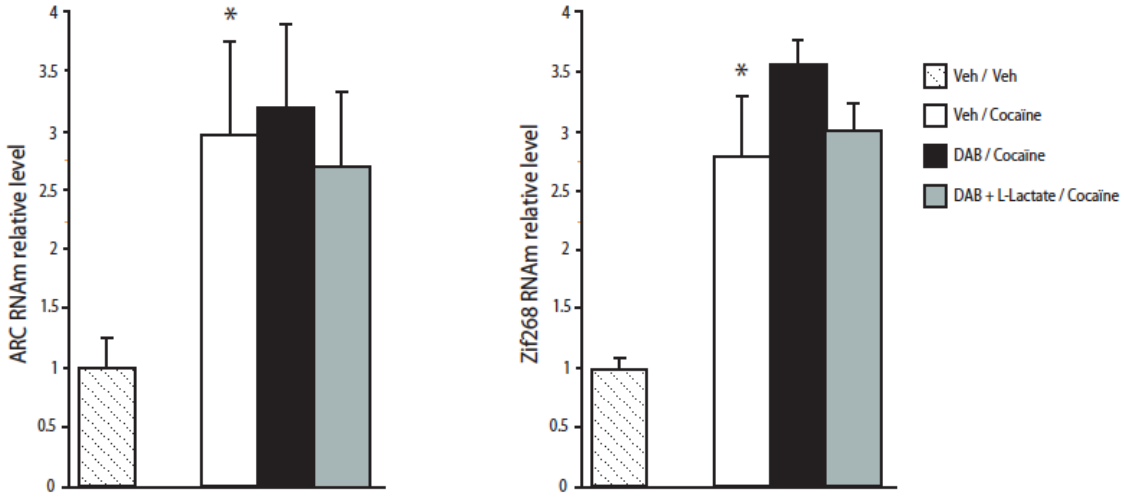
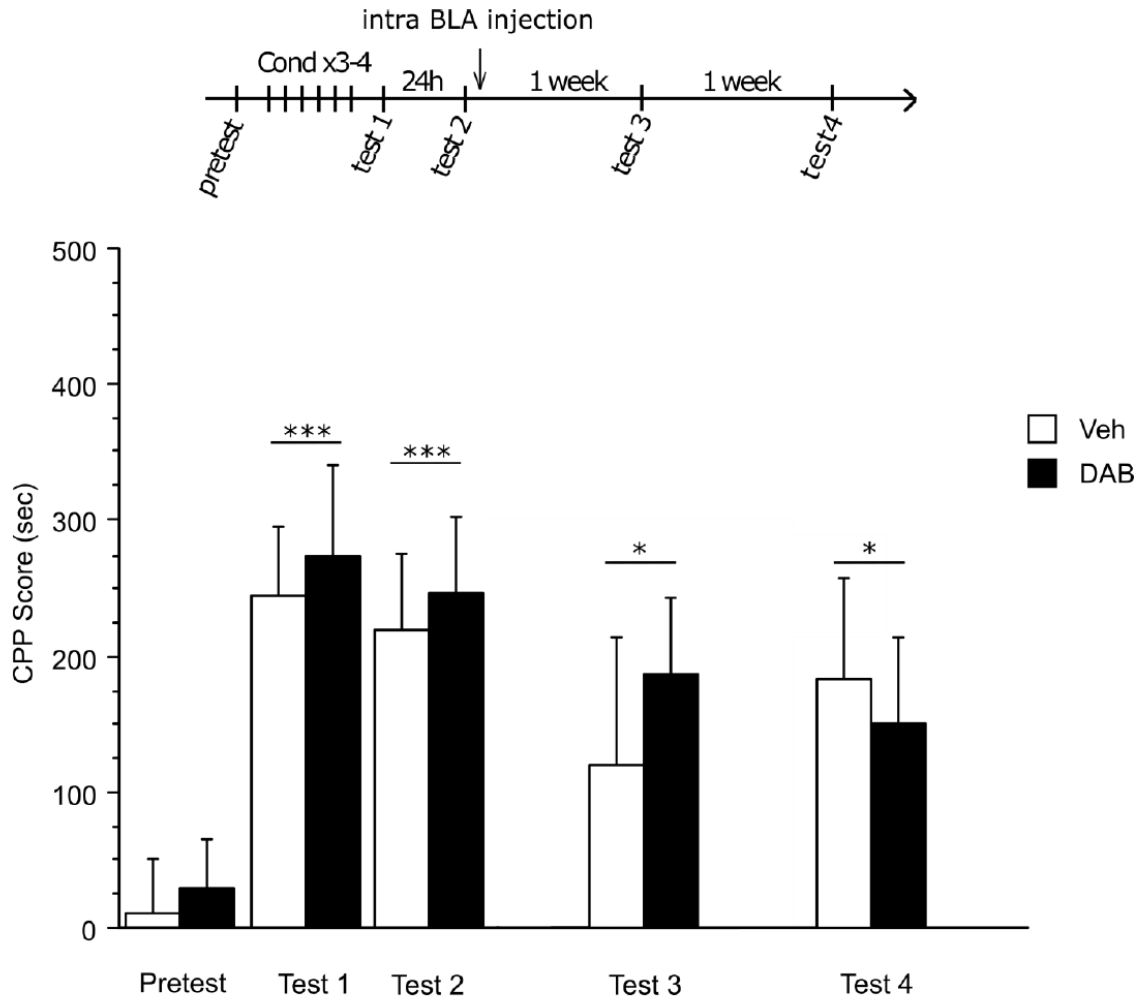

ARC RNAm relative level
Zif268 RNAm relative level
*
*
Veh / Veh
Veh / Cocaïne
DAB / Cocaïne
DAB + L-Lactate / Cocaïne

Figure S6


intra BLA injection
Cond x3-4
24h
1 week
1 week
pretest
test 1
test 2
test 3
test4
CPP Score (sec)
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400
300
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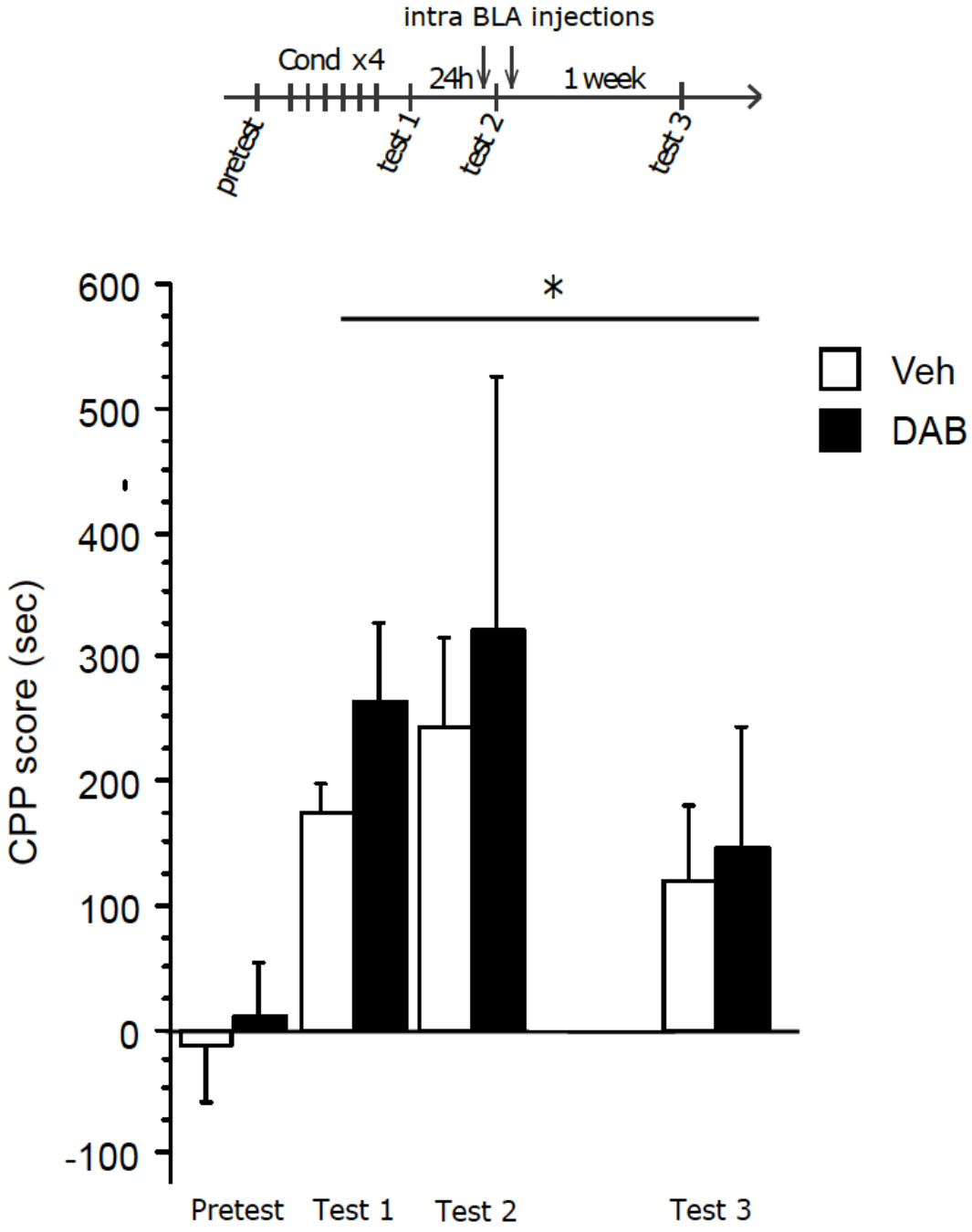
***
*
*
Veh
DAB
Pretest
Test 1
Test 2
Test 3
Test 4

Figure S7


intra BLA injections
Cond x4
24h
1 week
pretest
test 1
test 2
test 3
*
Veh
DAB
CPP score (sec)
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500
400
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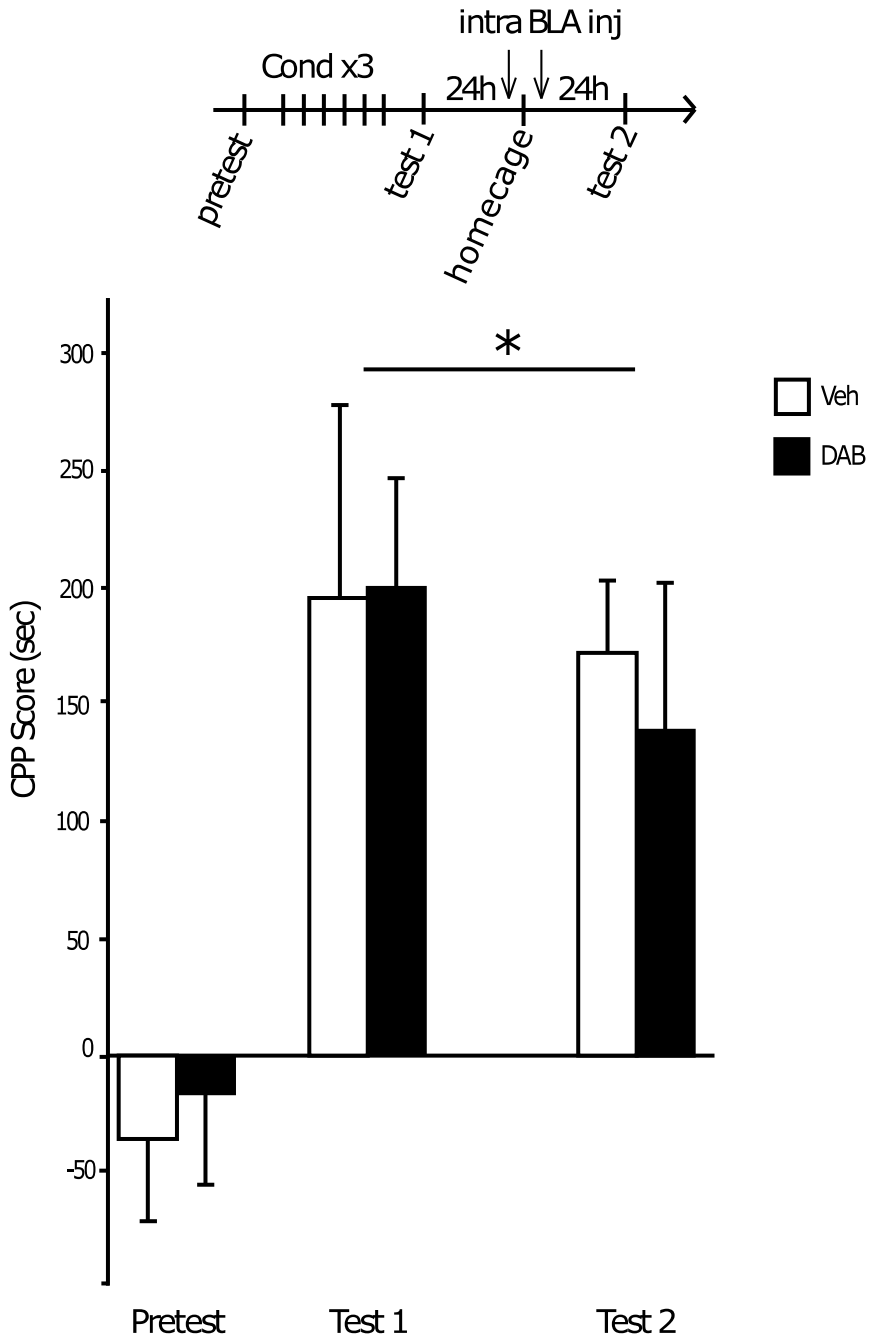
Pretest
Test 1
Test 2
Test 3

Figure S8


intra BLA inj
Cond x3
24h
24h
pretest
test 1
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test 2
*
Veh
DAB
CPP Score (sec)
300
250
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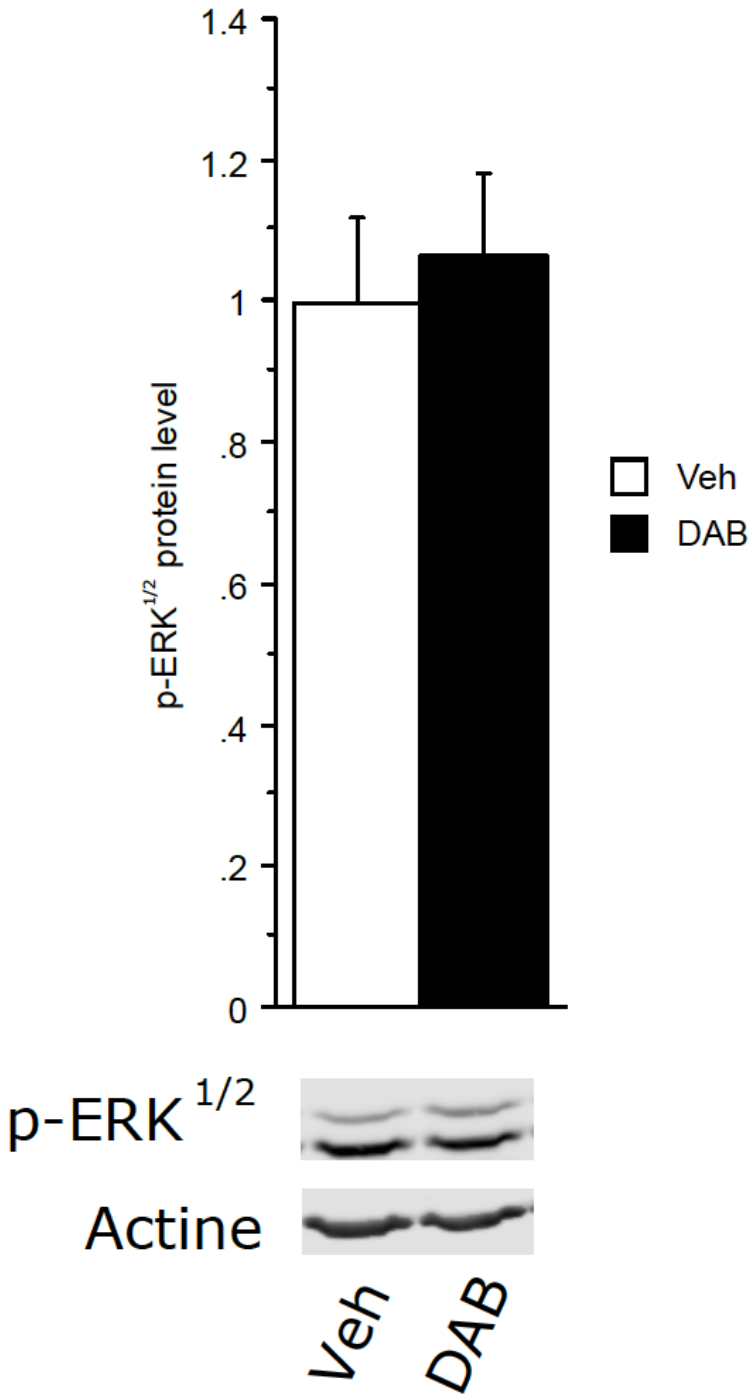
Pretest
Test 1
Test 2

Figure S9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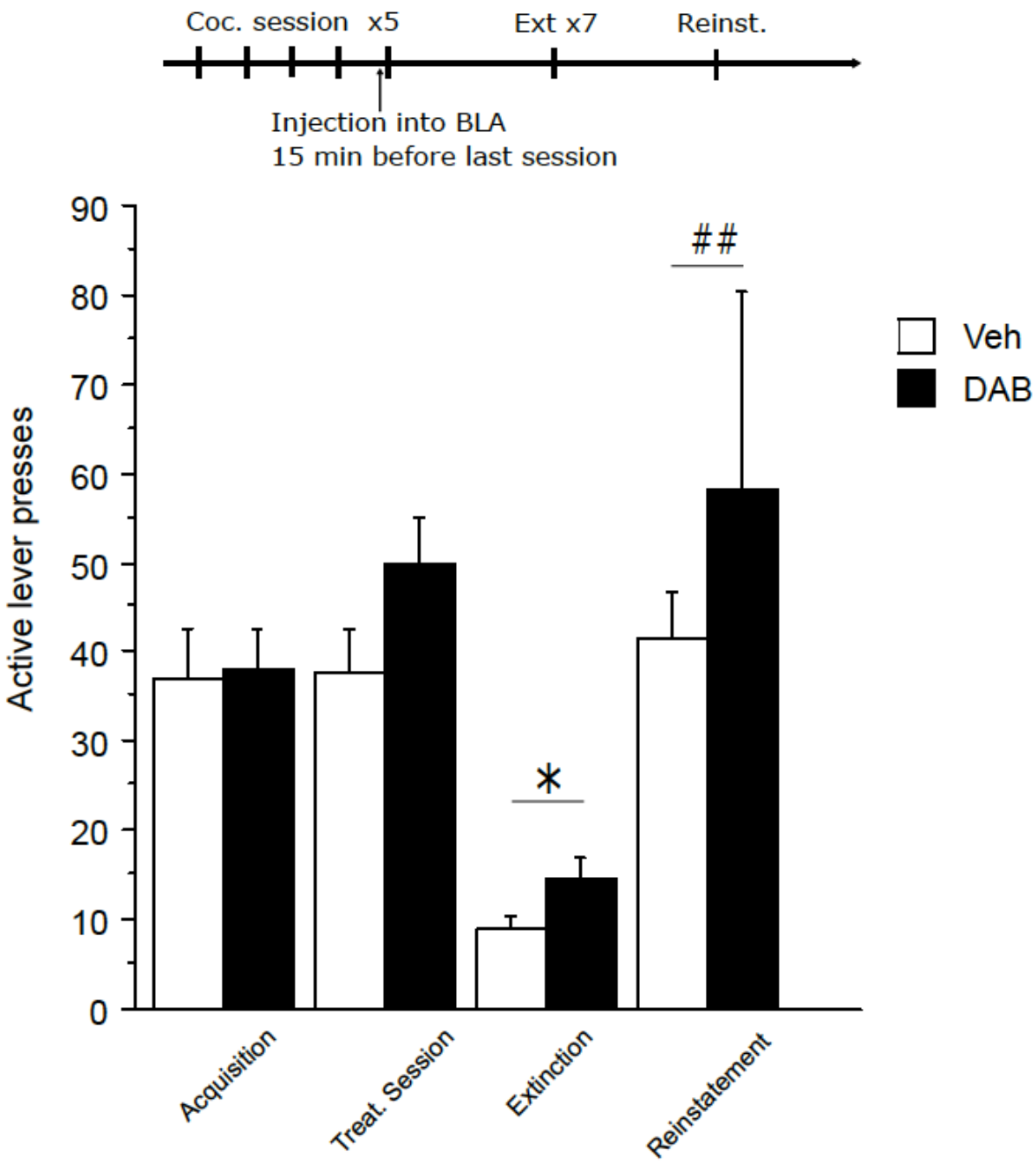
1.2
1
.8
.6
.4
.2
0
p-ERK1/2 protein level
Veh
DAB
p-ERK 1/2
Actine
Veh
DAB

Figure S10


Coc. session x5
Ext x7
Reinst.
Injection into BLA
15 min before last session
Active lever presses
90
80
70
60
50
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30
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10
0
##
*
Veh
DAB
Acquisition
Treat. Session
Extinction
Reinstatement

Lactate release from astrocytes to neurons contributes to cocaine memory formation

*Benjamin Boury-Jamot[1)2)], Olivier Halfon[3)], Pierre J. Magistretti[1)2)4)] and Benjamin Boutrel[1)3)]**

The identification of neural substrates underlying the long lasting debilitating impact of drug cues is critical for developing novel therapeutic tools. Metabolic coupling has long been considered a key mechanism through which astrocytes and neurons actively interact in response of neuronal activity, but recent findings suggested that disrupting metabolic coupling may represent an innovative approach to prevent memory formation, in particular drug-related memories. Here, we review converging evidence illustrating how memory and addiction share neural circuitry and molecular mechanisms implicating lactate-mediated metabolic coupling between astrocytes and neurons. With several aspects of addiction depending on mnemonic processes elicited by drug experience, disrupting lactate transport involved in the formation of a pathological learning, linking the incentive, and motivational effects of drugs with drug-conditioned stimuli represent a promising approach to encourage abstinence.

Keywords:
astrocyte; cocaine; conditioning; lactate; memory

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Introduction

A central problem in the treatment of drug addiction is the high risk of relapse, often precipitated by exposure to the environment. This conditioned response can occur despite years of abstinence from drug use, and represents a major challenge for the treatment of addiction. Indeed, it is now well established that drug memories that associate contextual cues with the effects of drugs of abuse shape and maintain persistent drug seeking behaviors [1]. Preclinical observations have long evidenced that, through predictive association with the drug's effects, drug conditioned stimuli can precipitate the reinstatement of previously extinguished drug-seeking behaviors [2, 3]. In abstinent humans, drug cues are known to evoke salient, persistent, and overwhelming memories of drug-taking experiences, thereby inducing higher risks of craving and relapse [4, 5]. A current consensus suggests that persistence of drug addiction would depend on the remodeling of synapses and circuits responsible for long-term associative memory. In other words, both clinical and laboratory observations have converged onto the hypothesis that addiction usurps neural processes that normally account for reward-related learning [6]. Consequently, whereas survival necessitates constant adaptation of the behavioral repertoire, in which learning the significance of a predictive cue serves to select the most appropriate response, drug addiction triggers neuroplastic changes that ultimately restrain decision-making processes and free will. In particular, drug cues can spark intrusive and overwhelming memories of drug-taking experiences, thereby leading to overpowering motivational strength and decreased capacity to control the desire to consume drugs.

Over the last decade, converging evidence has revealed that memory and addiction share both neural circuitry and molecular mechanisms [7–9]. Learning the significance of a predictive cue to trigger the appropriate behavioral response is thought to require the storage of specific patterns of information in the brain [1]. Because disrupting protein

synthesis immediately after learning prevents memory formation [10], a current consensus considers that the stabilization of a new memory occurs through a process known as consolidation, and requires gene expression. Importantly, once consolidated, memories can again become transiently labile and sensitive to protein synthesis inhibitors if reactivated [11–15]. These findings suggest that protein synthesis blockade after reactivation may selectively reduce or even eliminate long-lasting memories, including those linked to drug addiction [16]. While reconsolidation most likely contributes to update memories [17–19], its disruption may reduce the impact of intrusive or aberrant memories on behavior [20–27]. Several aspects of addiction depend on mnemonic processes induced by drug experience. Hence, disrupting drug-related memories represents a promising approach to help reducing relapse propensity and thereby encouraging abstinence [28].

Learning processes have long been considered to require the storage of specific patterns of information in the brain, in particular through mechanisms requiring a dialogue within the neuron, between genes and synapses [29], and for which Long-Term Potentiation (LTP) has been considered crucial for the genesis and maintenance of dendritic arborization [30]. More recently though, another candidate was suggested to shape synaptic plasticity: the astrocyte [31], and the idea that astrocytes, like neurons, might have diverse, and region-specific, roles to play in development and function of the central nervous system is therefore slowly, but surely, gaining recognition.

Astrocytes contribute to synaptic plasticity

Despite a large consensus long focusing on spike-time-dependent synaptic plasticity [32, 33], converging evidence now acknowledges that astrocytes' role extends by far a supportive function for neurons, and rather contributes to information processing, signal transmission, regulation of neuronal excitability, and synaptic plasticity. In the current view, astrocytes are localized near blood vessels and facilitate the distribution of nutriments and molecules to neurons. The astrocyte network ensheathes millions of billions synapses permitting an uninterrupted supply of energy substrates [34, 35], not only providing structural support, but also regulation of neuronal activity and synaptic plasticity (Fig. 1). Pioneering work has established that glia contributes to short-term plasticity by modulating neurotransmitter release from nearby presynaptic elements, and by activating postsynaptic glutamate receptors. Afterwards, compelling evidence has fuelled the emerging concept that the synapse is a three-sided (tripartite) organization, in which astrocytes are essential partners of the chemical synapse (see Neuron 1, Neuron 2, and Astrocyte on Fig. 1) [31, 36–39]. In this perspective, glial cells sense synaptic activity through a broad variety of ion channels, transporters, and receptors expressed on their surface. Besides a role in the clearing of neurotransmitters (notably glutamate), thereby regulating cleft concentration and limiting diffusion to neighboring synapses, sensors at the glial membranes can trigger the activation of a broad range of intracellular messengers, including calcium waves [40, 41]. In turn, the release of active substances from glial cells modulates the synaptic strength, notably by promoting the insertion of AMPA receptors at the surface of post-synaptic

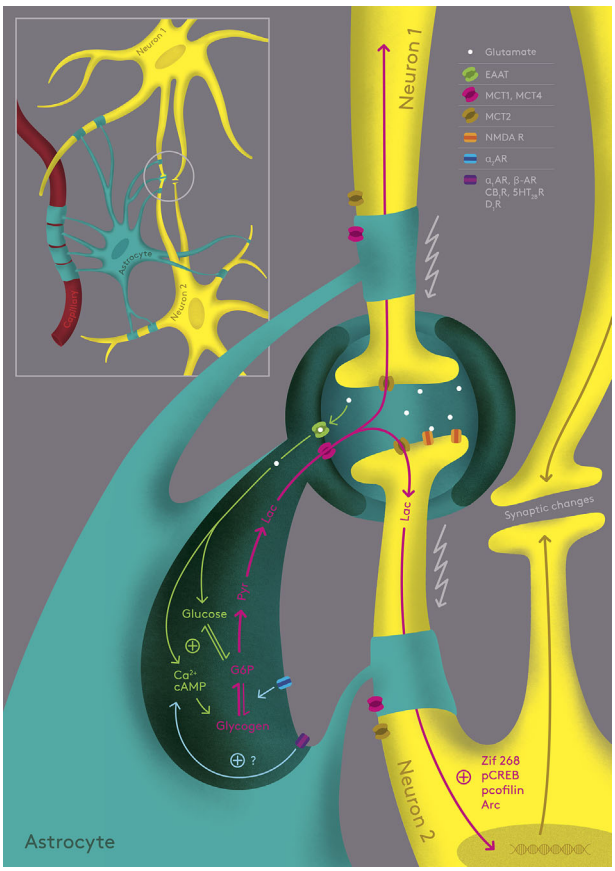

Figure 1. Glycogenolysis-dependent lactate release is essential for mediating intracellular responses underlying long-term regulation of gene expression required for long-lasting conditioned responses to cocaine cues. This conditioning requires both glutamatergic synaptic activity in neurons (via NMDA receptors) and glycogen-breakdown-dependent lactate release in astrocytes. Glutamate is imported into astrocytes via Excitatory Amino Acid Transporters (EAAT), where it triggers the activation of a broad range of intracellular messengers, including calcium waves, and cAMP, which secondarily activates glycogen metabolism. Glutamate also stimulates glucose import from blood vessels by activating glucose transformation into Glucose-6-phosphate (G6P). G6P is then metabolized into pyruvate (Pyr), which produces ATP and lactate (Lac). Different types of monoamines receptors are thought to regulate glycogen metabolism, and ultimately lactate release from astrocytes, among which α_1 and β adrenergic, serotonin 5-HT_{2B}, and Dopamine D_1 receptors, as well as cannabinoid CB_1 receptors. Lactate is exported from astrocytes via the monocarboxylate transporters MCT1 or MCT4 and imported into neurons via MCT2. Lactate import is necessary for activation of CREB-dependent gene expression, and the translation, at activated synapses, of the immediate early genes Arc and Zif268, and of pcofilin, which promotes cytoskeleton assembly and regulation of spine morphology, resulting in structural synaptic changes, and possibly formation of new synapses. Insert: the tripartite synapse: astrocytes, localized near blood vessels, not only facilitate the distribution of nutriments to neurons, but also contribute to information processing, signal transmission, regulation of neuronal excitability and synaptic plasticity.

neurons [42–44]. Supporting this view, a recent report showed that calcium-dependent release of D-serine from an astrocyte controls NMDAR-dependent plasticity in many thousands of excitatory synapses nearby [45], confirming previous observations claiming that activation of multiple neurons by astrocytic glutamate is a common feature of the astrocyte-neuron crosstalk [46]. Therefore, by synchronizing the neuronal activity, astrocytes bridge and influence neural circuits, and participate to the formation of complex neural ensembles that orchestrate plastic changes that ensure the stability and function of circuits [47, 48].

Astrocytes dynamically shape their cell surface while ensheathing synapses, participating to glutamate clearance, and synaptic activity regulation. Murphy-Royal et al. [49] revealed that glutamate release by presynaptic neuron and its uptake by the astrocytes through glutamate transporter GLAST (aka Excitatory Amino Acid Transporter, EAAT 1) and GLT-1 (aka EAAT2) dynamically depends on neuronal transmission. Indeed, glutamate transporters are dynamically mobile near the activated synapse, meaning they increase their diffusion to the glial cell surface under active condition but also rapidly reduce this diffusion under low active conditions [49]. Glutamate uptake into the astrocyte uses a co-transport with sodium (Na^+) ions, hence increasing sodium concentration inside the astrocyte. Most likely, Na^+/K^+-ATPase pumps ensure sodium export from the astrocyte since inhibition of Na^+/K^+-ATPase pumps has been demonstrated to decrease glutamate uptake into astrocytes [50]. This tight coupling has recently been supported with the demonstration that glutamate transporters and Na^+/K^+-ATPase co-localize in the human brain [51]. Since glutamate uptake is energy-dependent, the astrocyte network facilitates cerebral blood flow to ensure sufficient glucose uptake from blood vessels (for review, see [52]). Once inside, glucose is metabolized into pyruvate, which produces ATP (glycolysis), pyruvate is then metabolized into lactate through a lactate dehydrogenase (LDH) dependent process (Fig. 1). The LDH isoform LDH5, abundant in astrocytes, promotes the transformation from pyruvate to lactate and the LDH1 isoform, abundant in neurons, promotes the opposite reaction. Hence, lactate is preferentially produced in astrocytes and consumed in neurons. Lactate transfer from the astrocytes to neuron depends on monocarboxylate transporters (MCT), among which MCT1 transporters are expressed on the surface of endothelial cells and astrocytes, where they respectively facilitate the import of lactate from the blood vessels and its export from astrocytes into the extracellular space. MCT4 are exclusively found on astrocytes, where they ensure the release of lactate in the extracellular space, and MCT2 are found on neuron membranes, where they facilitate the entrance of lactate. Although, MCT2 have been reported mainly on post-synaptic neurons (for review, see [53]), the distribution of MCTs in the brain has been shown to adapt according to sudden metabolic needs. In particular, following an ischemic episode, neurons not only display enhanced MCT2 expression, but also start to exhibit MCT1 and MCT4 expression [54].

How this astrocyte-dependent control of synaptic strength and metabolic coupling underlies cognitive functioning and pathological adaptations responsible for brain pathologies and psychiatric diseases remains an open debate [48, 55]. However, these findings call for a better comprehension of the astrocyte-neuron crosstalk, in particular in the light of recent observations linking lactate signaling to long-term memory formation [56]. L-lactate (the active isomer hereafter referred as to lactate), released by astrocytes into the extracellular space, has recently been demonstrated to enter into neurons and promote the activation of signaling pathways underlying long-term memory formation.

Long-term memory formation requires astrocyte-neuron lactate transfer

Each astrocyte ensheathes tens of thousands (rodents) to millions (human beings) of synapses, permitting an uninterrupted supply of energy substrates. Both glucose and lactate can be transported to neurons as metabolic substrates, but astrocytic storage of glycogen has been considered a supplemental energy reserve available to neurons when demand is high. In particular, the metabolic coupling between astrocytes and neurons posits that glycogenolysis-dependent lactate is released from astrocytes, and imported into neurons [57–62]. However, the hypothesis of an astrocyte-neuron lactate shuttle has long been a matter of debate [63, 64]. First, since neurons are themselves extremely efficient in uptake and catabolism of glucose [65], a few authors have long questioned why consumption of lactate, produced by astrocytes, should be beneficial for neurons when glucose levels are not scarce. A partial explanation has recently been suggested with compelling evidence. This reveals that, besides its energetic role, lactate is also an important regulator of antioxidant defense mechanisms and energy metabolism, and therefore crucial for neuron survival [61, 62, 66, 67]. Second, given that all known mechanisms of lactate movement are passive, the astrocyte-neuron lactate transfer had to depend on a downward gradient, which required a proof of concept. The demonstration was established very recently with a report highlighting a significantly lower baseline lactate level in neurons in comparison to astrocytes, hence supporting the concept of compartmentalized lactate pools with a lactate flux from astrocytes to neurons [68].

Besides this energetic role, significant contributions recently emphasized that lactate transport from astrocytes into neurons was critical for memory consolidation [69–71]. Of particular interest, recent evidence demonstrated that learning resulted in lactate release in the hippocampus, and that lactate transfer from astrocytes into neurons was critical for the induction of the molecular changes required for long-term memory formation [72]. Further, Suzuki et al. demonstrated that the antisense mediated knockdown of the astrocytic (MCT1, MCT4) and neuronal (MCT2) lactate transporters prevented the retention of an inhibitory avoidance task. Further dissecting the cellular mechanism involved, lactate administration was shown to only rescue MCT1- or MCT4-knock-down-dependent amnesia; therefore demonstrating that lactate import into neurons (via MCT2) was essential for long-term memory formation.

To summarize, not only spatial learning and working memory in rats have been shown to increase the number of

astrocytes [73], but when activity-dependent changes involving high-energy demands occur – notably those required for episodic long-term memory consolidation – astrocyte-derived lactate seems to be a necessary step in promoting the molecular and morphological adaptations underlying memory storage [56]. These findings raise the intriguing question of whether lactate, released by astrocytes into the extracellular space – where it enters into neurons and promote further downstream processes (Fig. 1) – might play a key role in the regulation of glutamate-dependent synaptic strength [74], such as other gliotransmitters, including glutamate, ATP, GABA, D-serine, taurine, and the cytokine tumor necrosis factor alpha [43, 44, 48, 75].

Lactate: The missing link underlying maladaptive plasticity associated with drug addiction?

Following the seminal work reported by Suzuki et al. [72], another group tested whether lactate transport would play a similar role in the reconsolidation of drug-paired memories [76, 77]. At first, these authors reported, in the basolateral amygdala (BLA) of conditioned rats, a significant increase in lactate dialysate levels measured over 50 minutes after exposure to cocaine cues [76]. They then found that microinjection of the inhibitor of glycogen phosphorylase (1,4-dideoxy-1,4-imino-D-arabinitol, DAB) into the BLA not only abolished the expression of cocaine induced conditioned place preference for up to 14 days (even after a cocaine priming injection), but also reduced cue-induced drug seeking behaviors in rats previously trained to self-administer cocaine. Confirming the physiological role of lactate transport in the reconsolidation of cocaine memories, they revealed that the expression of cocaine-induced CPP correlated with an increased activity of MCT1 and MCT2 (not MCT4) in the BLA (not in the central amygdala), within 2 hours of exposure to the cocaine-paired context. They next dissected the respective roles of MCT1 and MCT2, confirming that, in contrast to the disruption of MCT1 expression in the BLA, which impaired the cocaine-induced CPP but could be rescued by L-Lactate co-administration, the effect of antisense-mediated knock-down of the neuronal lactate transporter MCT2 on cocaine memory was not rescued [76]. Ultimately, Zhang et al. established that inhibition of glycogenolysis, and the concomitant reduced lactate release, decreased the expression of downstream factors accounting for synaptic plasticity and memory reconsolidation, among which pCREB, pERK, and pcofilin (for review see [78]).

With these data suggesting that inhibiting lactate release disrupts existing drug-related memories, the questions remains whether interfering with lactate transport is sufficient to prevent the formation of such memories. We addressed this question by demonstrating that DAB treatment prior to sessions of cocaine conditioning not only impaired the acquisition of the place preference, but also the expression of Bdnf and Zif268, known to modulate the synaptic morphology and plasticity underlying the learning processes that strengthen conditioned responses to cocaine [98]. Further, it seemed important to assess that the reduced preference for the cocaine compartment was not the consequence of undesirable or aversive side effects. We therefore demonstrated that bilateral administrations of DAB into the BLA did not reduce the locomotor activity in an open field apparatus, nor did it induce any place aversion. How specific to cocaine lactate signaling is was also a key question to address. The same procedure replicated in rats conditioned with highly palatable chocolate flavored food pellets failed to block the conditioned place preference, suggesting that glycogen breakdown may be necessary for reconsolidation of contextual appetitive memories specific to cocaine, but not for those related to food reward [79]. However, further studies are needed to evaluate whether lactate signaling plays a more general role in drug-induced learning, in particular with opiates and alcohol.

Noteworthy, Zhang et al. [76] claimed that MCT-knock-down in conditioned rats, 1 hour before re-exposure to the cocaine context, had no effect on the retrieval or expression of cocaine-induced CPP. However, they acknowledged that administration of antisense oligodeoxynucleotides 1 hour before retrieval might not have been long enough to achieve reasonable MCT knock-down efficiency. We administered DAB, the inhibitor of glycogen phosphorylase, just before contextual re-exposure and reported a transient impairment of the cocaine-induced place preference (Fig. 2) [79]. This effect only persisted for 2 days, and 1 week after treatment, rats re-expressed a marked preference for the compartment previously paired with cocaine administration. A persistent disruption of cocaine-conditioned responses similar to that reported by Zhang et al. [76] required, in our hands, two injections: 15 minutes prior to and 5 hours after contextual re-exposure. Overall, these observations supported a key role for the astrocyte-neuron metabolic coupling in positive affective memory storage and retrieval, but further investigations were required to dissect the cellular and molecular mechanisms underlying this effect.

Therefore, we first considered the extracellular signal-regulated kinase (ERK) pathway as a relevant target to explore because it is considered critical for drug-induced long-lasting behavioral effects [7, 27], and for precipitating drug-seeking behaviors following presentation of drug associated cues [80–82]. Consequently, we demonstrated that not only the long-lasting indifference for the cocaine compartment following inhibition of glycogenolysis in the BLA was associated with decreased phosphorylated ERK ½ protein levels, but we also revealed that lactate-induced recovery of contextual appetitive memories correlated with restored levels of phosphorylated ERK ½ in the BLA. In line with recent in vitro observations [74] and the demonstration that application of the NMDAR-agonist, d-cycloserine, potentiates the reconsolidation of appetitive memories [83], our data strongly support the idea that lactate stimulates the neuronal expression of synaptic plasticity-related gene through a mechanism involving NMDAR activity and its downstream signaling cascade ERK ½ [74].

Overall, we showed that glycogenolysis-dependent lactate release is essential for mediating intracellular responses underlying long-term regulation of gene expression required for cocaine-induced long-lasting behavioral effects. Our

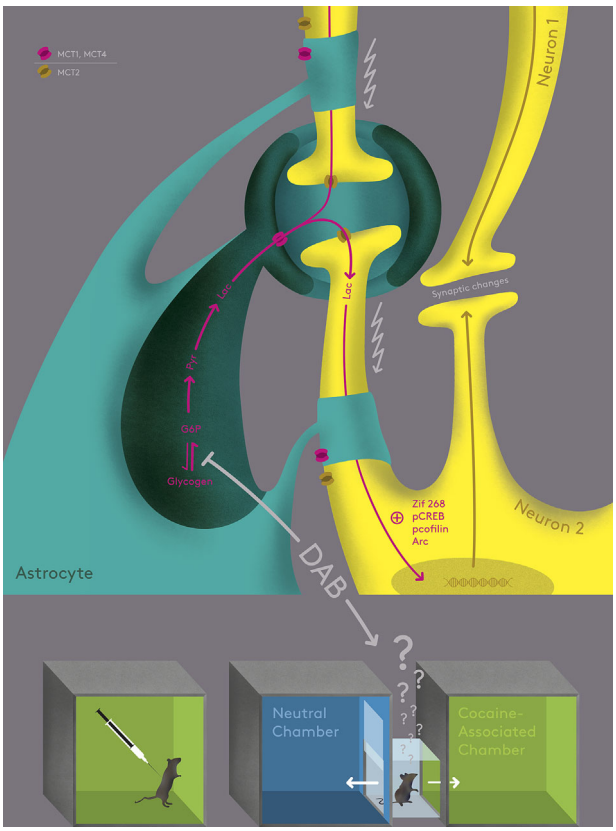

Figure 2. DAB administration into the basolateral amygdala disrupts glycogenolysis and concomitantly reduce lactate release. The cellular consequences include a decreased expression of downstream factors accounting for synaptic plasticity and memory reconsolidation (pCREB, pERK, and pcofilin). The behavioral consequence is the prevention or the disruption of already acquired cocaine-induced Conditioned Place Preference (for supplementary information on the CPP procedure, see [117].

results confirm the importance of astrocyte-neuron metabolic interactions in cognitive functions and, for the first time, demonstrate the key role of the astrocyte-neuron metabolic coupling in positive affective memory storage and retrieval. If these findings are promising, they first and foremost call for a further dissection of the brain networks whereby the reciprocal exchange of metabolic intermediates between neurons and glia underlies long-lasting conditioned responses to drug associated cues.

Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons

While converging reports highlighted the role of metabolic coupling in drug memory formation, a relevant study recently confirmed in vitro that lactate promotes the expression of c-fos, ARC, and Zif268 mRNA levels, in a MCT- and NMDAR-signaling-dependent manner [74]. Accordingly, the expression of Zif268 was totally abolished by antagonism of NMDA receptors, with MK801, or after treatment with the glutamate binding site selective competitive inhibitor D-APV. Similar observations were reported using the selective antagonist of the glycine site (L-689.60), showing that lactate potentiated NMDA receptors already activated. Interestingly, lactate increased intracellular levels of NADH, thereby modulating the redox state of neurons. Because NADH mimics all of the effects of lactate on NMDA signaling, the authors suggested that the rise in NADH levels might be a primary mediator of lactate effects. The induction of the synaptic plasticity-related genes Arc, c-Fos, and Zif268 was confirmed in vivo, in the sensory-motor cortex of anesthetized mice following lactate administration. Neither the administration of pyruvate, nor that of glucose, reproduced these effects, hence strongly suggesting a non-energetic role of lactate. Of note, administration of the inactive isomer D-lactate did not modulate any gene expression, suggesting that the G-coupled cell surface receptor GPR81 (activated by both L- and D-lactate) is most likely not implicated in this process. In line with these observations, Latham et al. [84] reported that lactate is an endogenous histone deacetylase (HDAC) inhibitor, deregulating transcription in an HDAC-dependent manner and most likely fine-tuning transcription at active genes. These authors conclude by suggesting that lactate may be an important transcriptional regulator, linking the metabolic state of the cell to gene transcription [84].

Upstream regulation of lactate release and strengthened cocaine related memories

While short-term memories require post-translational modifications, the consolidation, or stabilization of long-term memories depends upon the activation of a gene cascade and downstream modifications in neurons that store the acquired information [33]. Among the best characterized and widely proven gene expression mechanisms known to underlie memory consolidation are the activation of CREB (cAMP response element binding protein)-dependent gene expression [85], and the translation, at activated synapses, of the immediate early gene Arc (activity-regulated cytoskeletal protein). The Arc is believed to play a key role in actin cytoskeletal dynamics and regulate the membrane expression of AMPA receptors [78, 86]. Long-term synaptic plasticity and memory are accompanied by synaptic structural changes, which involve actin polymerization [87, 88] associated with the phosphorylation of the p21-activated kinase-cofilin cascade. Phosphorylated cofilin is one of the major regulators of F-actin dynamics in spines, promotes cytoskeleton assembly, and regulates spine morphology [88]. We, and others, have gathered substantial evidence showing that lactate, but not pyruvate or glucose, induces plasticity-related genes like Arc, pCREB, pcofilin Zif 268, and BDNF (Fig. 2), and demonstrated that lactate modulation of long-lasting conditioned responses to drug associated cues depends on a mechanism involving the Erk1/2 kinase.

Interestingly, glycogen distribution in brain correlates with high synaptic density, notably in the hippocampus, striatum, and cortex [89]. Recent imaging techniques revealed that glycogen seems to be stored near pre-synaptic bouton,

rather than dendritic spines, and are associated with monoaminergic varicosities [90]. Further, glycogen phosphorylase (GP), which is responsible for glycogen breakdown in astrocytes, can be activated via phosphorylation. The latter results from a signaling cascade involving Ca^{2+} and cyclic adenosine monophosphate (cAMP), ultimately activating the GP-phosphorylating enzyme phosphorylase kinase (PK) [91, 92]. Pioneer observations have long reported a role for noradrenaline [93, 94], dopamine [95, 96], serotonin [97, 98], and endocannabinoid [99, 100] in glycogenolysis. For example, adrenergic signaling can exert opposing influences on astrocytic glycogen metabolism. It can either stimulate glycogen degradation by increasing cytosolic Ca^{2+} through α_1-Gq-coupled receptors or by stimulating production of cAMP through $\beta_{1/2}$-Gs-coupled receptors. Or can it inhibit glycogen degradation through α_2-Gi-coupled receptors [91].

Surprisingly, despite accumulating evidence showing how different signaling pathways may elicit glycogen degradation in astrocytes, little is known about the upstream crosstalk that may converge onto lactate release, ultimately leading to long-term memory formation. A recent report though established the critical role of astrocytic β_2-AR in hippocampal long-term memory consolidation [101], but further studies are needed to establish whether glycogenolytic transmitters may regulate conditioned responses to cocaine through a lactate-dependent signaling pathway. While noradrenaline [102, 103], dopamine [104–106], serotonin [107], and cannabinoid [108–112] neurotransmission has been shown to regulate conditioned reward, synaptic strengthening [42, 113], and extracellular lactate release [114, 115], the question remains whether α_1/β adrenergic-, dopamine D_1-, $5HT_{2B/2C}$, and cannabinoid CB_1-antagonisms (Fig. 1), known to disrupt cocaine memories, might all converge onto the same lactate-dependent signaling pathway.

Conclusions and outlook

Discovering treatments to fight efficiently against drug abuse and dependence is a challenge for both medicine and basic science. Concurrent basic and clinical findings have suggested that addiction may be a disease of learning and memory [1], and that the desire or need to obtain a drug that can overwhelm an addict years after the last incidence of drug use is among the most debilitating long-term effects of drug abuse. Appreciation of the cyclical nature of addiction has led to the emergence of relapse prevention as a major clinical target for the long-term management of drug abuse [1].

There is no currently approved medication for the treatment of cocaine dependence [116], which calls for the development of novel therapeutic agents. Therefore, unraveling the functional organization of the astrocyte-neuron metabolic and signaling cooperation might give us a novel insight into the neural bases underlying the long-lasting debilitating impact of drug cues on conditioned responses to cocaine. The astrocyte-derived lactate may represent a unique target in the brain that links metabolic needs to the remodeling of synapses and circuits that shape long-term associative memories. Our findings may pave the way for the development of novel therapeutic approaches targeting neuron-astrocyte metabolic interactions, with a particular emphasis on the intercellular transfer of lactate.

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