Post-learning molecular reactivation underlies taste memory consolidation

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It is considered that memory consolidation is a progressive process that requires post-trial stabilization of the information. In this regard, it has been speculated that waves of receptors activation, expression of immediate early genes, and replenishment of receptor subunit pools occur to induce functional or morphological changes to maintain the information for longer periods. In this paper, we will review data related to neuronal changes in the post-acquisition stage of taste aversion learning that could be involved in further stabilization of the memory trace. In order to achieve such stabilization, evidence suggests that the functional integrity of the insular cortex (IC) and the amygdala (AMY) is required. Particularly the increase of extracellular levels of glutamate and activation of N-methyl-D-aspartate (NMDA) receptors within the IC shows a main role in the consolidation process. Additionally the modulatory actions of the dopaminergic system in the IC appear to be involved in the mechanisms that lead to taste aversion memory consolidation through the activation of pathways related to enhancement of protein synthesis such as the Protein Kinase A pathway. In summary, we suggest that post-acquisition molecular and neuronal changes underlying memory consolidation are dependent on the interactions between the AMY and the IC.

Keywords: conditioned taste aversion, glutamate, dopamine, molecular reactivation, memory consolidation

INTRODUCTION

Memories of aversive events are more likely to persist over time. The strong and long–lasting representation of these memory traces may depend on the molecular mechanisms required for the consolidation process. A good example of a long–lasting memory trace representation is the conditioned taste aversion (CTA), a learning model where animals associate a novel taste (conditioned stimulus, CS) with gastric malaise (unconditioned stimulus, US), decreasing the CS intake in further presentations. This kind of learning can be acquired with only one-trial and the CS can be separated from the US by many hours, which allows a temporal resolution of the mechanisms involved in the gustatory stimulus acquisition and the further association with the gastric malaise (Naor and Dudai, 1996; Yamamoto et al., 1998; Welzl et al., 2001). Since this task involves the recognition and avoidance of toxic and potentially deadly food, the efficient consolidation and storage of this information makes CTA a good model to study the molecular mechanisms through which memories are established. Overall, these mechanisms would have to promote synaptic plasticity in the structures that are related to acquisition and storage of the information; among these, the insular cortex (IC) and the amygdala (AMY) are key structures involved in acquisition and consolidation of CTA (Bermudez-Rattoni, 2004).

POST-ACQUISITION ACTIVATION AND TASTE MEMORY CONSOLIDATION

Formation of long-term memories is based on molecular and structural changes that allow neuronal networks to stabilize and support long-term storage. It has been proposed that this consolidation process relies on memory trace reactivation seen as neuronal post-learning activity in absence of sensory stimulation. Trace reactivation theory establishes that the expression of patterns of activity in neural ensembles during an experience should be spontaneously re-activated during subsequent periods of behavioral inactivity: even during post-training wakefulness, indicating that information can be maintained and processed concurrently within relevant cortical sites (Hoffman and McNaughton, 2002). For instance, these authors made simultaneous neural recordings in the macaque neocortex, i.e., posterior parietal cortex, motor cortex, somatosensory cortex, and dorsal prefrontal cortex after a sequential reaching behavior, the simultaneous analysis revealed that cells in all four areas exhibited similar firing related to the task, and interestingly those cells tended to be coactive afterward. According to this hypothesis, memory consolidation relies on reactivation and reorganization of newly acquired information. After the initial encoding of sensorimotor experience, a series of cellular, molecular, and systems-level alterations develop over time, engaging reactivation patterns in neocortical structures during awareness and sleep periods, stabilizing the initial memory representation, converting it into a long–lasting memory trace (Smith, 2001; Robertson et al., 2004; Walker and Stickgold, 2004; Stickgold, 2005; Ellenbogen et al., 2006; Gais et al., 2007; Rasch and Born, 2007). Particularly in CTA learning, there is evidence suggesting that such post-learning activities may be part of the trace consolidation mechanisms. For instance, single unit recording in the basolateral nucleus of the amygdala (BLA) showed an increase

of activity 30 min after CS–US pairing (Yamamoto and Fujimoto, 1991), and similar results were obtained from single unit recordings in the IC, where 20–30 min after CS–US pairing IC neurons had an increment of excitability (Yasoshima and Yamamoto, 1998). These reports suggest that the association between the CS and US could induce post-acquisition changes that create a long–lasting activation of these structures that may potentiate synaptic efficacy even after stimulation has ceased.

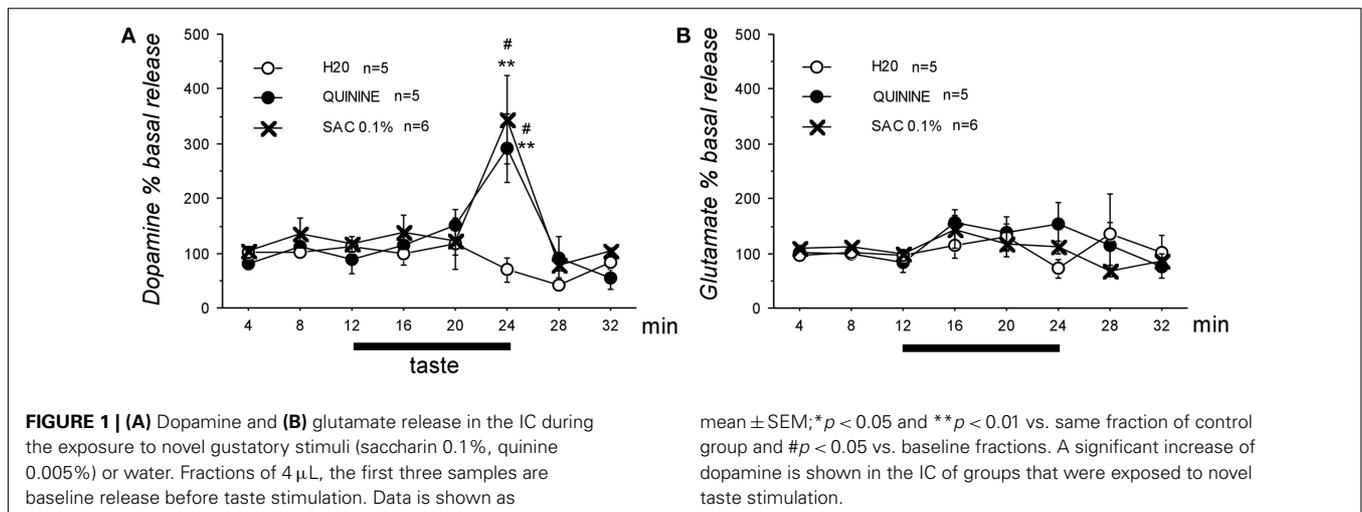## CTA CONSOLIDATION: INSULAR CORTEX AND AMYGDALA

Many studies have proven that two temporal lobe structures, the IC and the amygdala (AMY) are highly involved in taste memory formation. These studies have demonstrated either by lesions or by administration of several neurotransmitters antagonists before the CS presentation, that CTA is affected or impaired in one or both structures. Although, these effects could be evaluated performing a long-term memory (LTM) test, it is unclear if the affected stage was the acquisition or the consolidation of the memory trace. In order to evaluate this, a short-term memory (STM) test may clarify the role of some neurotransmitter systems in the different memory stages. For instance, the administration of scopolamine, a muscarinic antagonist, into the IC before CTA acquisition affected both STM and LTM indicating that the LTM effect is attributable to an impairment of the actual memory trace formation (Naor and Dudai, 1996; Ferreira et al., 2002). Conversely, blockade of the N-methyl-D-aspartate receptors (NMDAr) before CTA training impairs only LTM leaving STM intact, indicating that the activity of these receptors is required for memory consolidation (Ferreira et al., 2002; Bermudez-Rattoni, 2004). Another neurotransmitter system that has been involved in CTA memory consolidation is the dopaminergic system. Disruption of dopamine projections in the IC by the administration of a catecholaminergic toxin (6-hydroxydopamine) before CTA training, impairs acquisition of this task (Fernandez-Ruiz et al., 1993) and the blockade of the D1 type receptors before CTA training impairs LTM (Berman et al., 2000). Accordingly, we have seen by using *in vivo* microdialysis that the first presentation of taste stimuli, like saccharin or quinine, induces a significant increase of dopamine release but not glutamate within the IC, suggesting
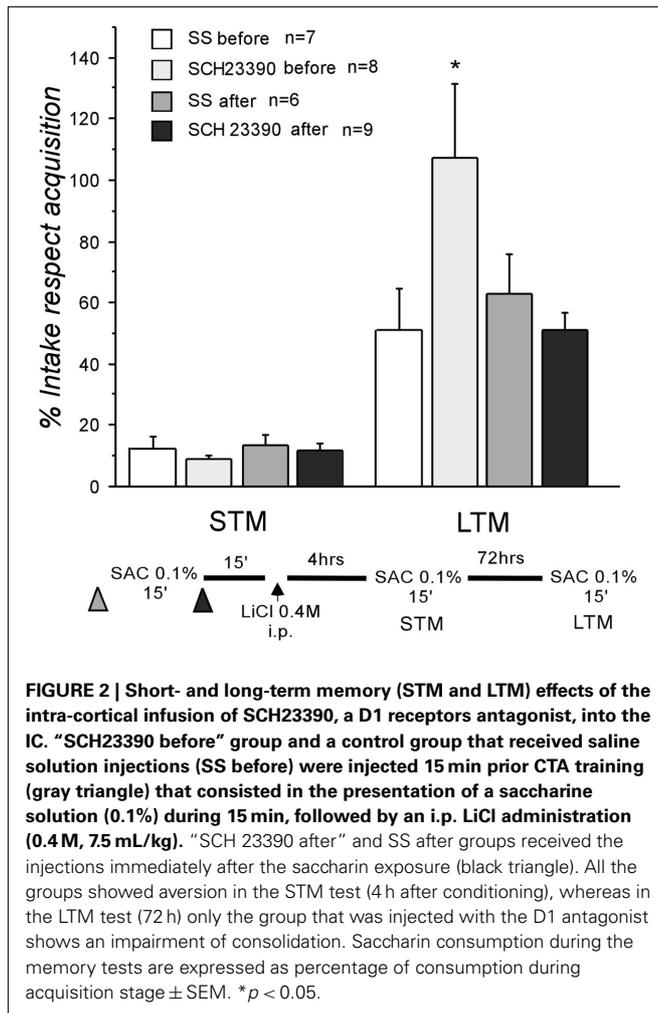
a differential role of these neurotransmitters in taste processing (**Figures 1A,B**). The dopaminergic increment is thought to be related to the novelty of the stimulus, since the presentation of water did not induce any significant changes in dopamine release and both saccharin (0.1% v/v, sweet) and quinine (0.005% v/v, bitter) solutions, being different taste modalities, yet novel stimuli, induced a dopamine increase (Guzman-Ramos et al., 2010). Some evidences show similar results in other structures receiving dopaminergic aferences such as nucleus accumbens (Bassareo and Di Chiara, 1997; Feenstra et al., 2000) and prefrontal cortex (Bassareo and Di Chiara, 1997; Feenstra et al., 2000; Rossetti and Carboni, 2005; De Leonibus et al., 2006) during the exposure to novel stimuli. In this regard, it has been considered that dopaminergic responses are not only related to the rewarding quality of the stimuli, but also to their salience (Ljungberg et al., 1992; Ungless, 2004). For instance, a salient novel gustatory stimulus is important for the animals, since it can produce either favorable or aversive consequences. Hence, dopamine increase may be a suitable signal that triggers the mechanisms to store relevant information. In CTA training, we have addressed whether the dopaminergic signal related to the CS presentation was involved in the acquisition or the consolidation of the memory trace. To do so, we blocked the D1 receptors before the CS–US exposure and performed STM and LTM tests. Interestingly, pre-trial treatment only impaired LTM leaving STM intact; and when the D1 receptors were blocked just after the CS presentation, neither STM nor LTM were impaired (**Figure 2**), indicating that the dopaminergic action within the IC during the CS processing is involved specifically on CTA memory consolidation (Guzman-Ramos et al., 2010).

From this evidence we could say that both STM and LTM storage mechanisms are triggered during training. Muscarinic receptors are involved in STM, whereas D1 and NMDA receptors are activated to further consolidate the memory trace.

PROTEIN SYNTHESIS INVOLVED IN POST-ACQUISITION ACTIVITY FOR TASTE MEMORY CONSOLIDATION

Among the molecular differences between STM and LTM mechanisms is the dependence on protein synthesis (Davis and Squire, 1984; Martin et al., 2000; Dudai, 2004). The consolidation process

FIGURE 1 | (A) Dopamine and **(B)** glutamate release in the IC during the exposure to novel gustatory stimuli (saccharin 0.1%, quinine 0.005%) or water. Fractions of 4 μL, the first three samples are baseline release before taste stimulation. Data is shown as

mean ± SEM; *$p < 0.05$ and **$p < 0.01$ vs. same fraction of control group and #$p < 0.05$ vs. baseline fractions. A significant increase of dopamine is shown in the IC of groups that were exposed to novel taste stimulation.

FIGURE 2 | Short- and long-term memory (STM and LTM) effects of the intra-cortical infusion of SCH23390, a D1 receptors antagonist, into the IC. "SCH23390 before" group and a control group that received saline solution injections (SS before) were injected 15 min prior CTA training (gray triangle) that consisted in the presentation of a saccharine solution (0.1%) during 15 min, followed by an i.p. LiCl administration (0.4 M, 7.5 mL/kg). "SCH 23390 after" and SS after groups received the injections immediately after the saccharin exposure (black triangle). All the groups showed aversion in the STM test (4 h after conditioning), whereas in the LTM test (72 h) only the group that was injected with the D1 antagonist shows an impairment of consolidation. Saccharin consumption during the memory tests are expressed as percentage of consumption during acquisition stage ± SEM. *$p < 0.05$.
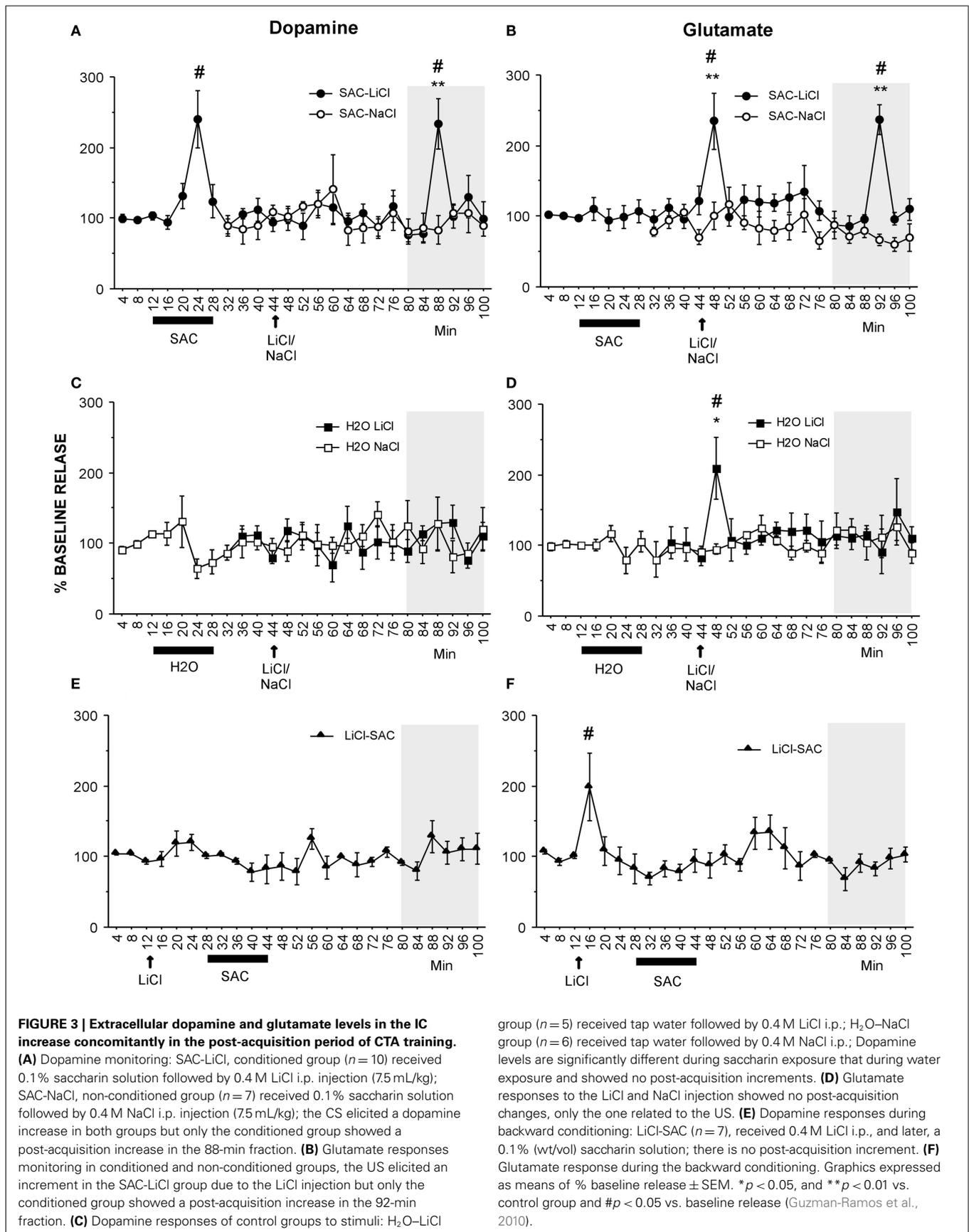
is thought to be a progressive stabilization that requires time and involves synaptic plasticity based on the synthesis of new proteins that allow neuronal changes underlying the memory trace storage. Hence, one of the most popular approaches to study the role of any brain structure in memory consolidation has been for many years the administration of protein synthesis inhibitors. Administration of anisomycin in the IC before and after the CS presentation has an effect on LTM (Rosenblum et al., 1993), similarly, the administration of this drug into the central subnucleus of the amygdala (CeA), but not in the BLA affects LTM for CTA (Bahar et al., 2003; De la Cruz et al., 2008; Garcia-DeLaTorre et al., 2009).

As an example of protein expression in the IC and the amygdala related to the CTA consolidation process is the protein c-fos. This protein is an immediate early gene product that regulates the transcription of "late response genes" contributing to long-term neuronal changes (Herdegen and Leah, 1998; Walton et al., 1999). In CTA training, the CS and US association elicits an increase in c-fos expression in the CeA and in the IC (Yamamoto et al., 1997; Wilkins and Bernstein, 2006). Furthermore, the local administration of an antisense oligonucleotide (ASO) in the AMY or in the IC impaired CTA LTM seen in the 24-h test (Yasoshima et al., 2006). Consolidation of CTA is also related to the synthesis of

brain-derived neurotrophic factor (BDNF), a neuronal growth factor that has been involved in plasticity-related events such as long-term potentiation (LTP; Messaoudi et al., 2002; Bramham and Messaoudi, 2005) and memory formation of several tasks (Mizuno et al., 2000; Bekinschtein et al., 2008; Ma et al., 2011). Taste aversion learning induces an increase of BDNF expression in the CeA and the IC whereas the inhibition of this expression by local administration of BDNF ASO affects LTM but not STM (Ma et al., 2011). Another protein involved in CTA consolidation is CREB, it has been reported that the administration of ASO of CREB into the AMY before CTA training produced significant deficits on LTM measured 3–5 days after conditioning, however, STM remained intact (Lamprecht et al., 1997). These particular examples provide evidence that some proteins engaged in synaptic plasticity and memory consolidation are mainly related to the consolidation of CTA CS–US association and not only to CS or US exposure, indicating a role in the stabilization of the memory trace formed by the stimuli pairing.

NEUROTRANSMITTERS INVOLVED IN POST-ACQUISITION ACTIVITY FOR TASTE MEMORY CONSOLIDATION

It has been proposed that long-term stabilization of memory may need reactivation of the biochemical pathways that were initially active during training in order to sustain the levels of proteins required for the ongoing consolidation process. This hypothesis is supported by evidence showing that NMDA receptor synthesis and activity is required for memory consolidation. In this regard, Cui et al. (2005) demonstrated by using a specific inducible knockout mouse that CTA impairments are evident when the NR1 subunit of the NMDAr in the forebrain regions was decreased from 1 up to 3 weeks after CTA training,. These results suggest that a prolonged glutamatergic/NMDAr activity is engaged in CTA LTM formation (Shimizu et al., 2000; Cui et al., 2005). Similarly, memory impairments for taste aversion memory have been demonstrated by the blockade of NMDAr activity at 30, 60, or 120 min after CTA acquisition (Gutierrez et al., 2003). Altogether these results are in accordance with the cellular consolidation theory stating that molecular changes that underlie consolidation might occur within hours or even days after the post-trial stage. Within these molecular changes neurotransmitters release has been scarcely studied. Recently, we have reported by using *in vivo* microdialysis that the CS–US pairing in CTA training induces a significant concomitant increase of glutamate and dopamine within the IC. As we can see in **Figure 3**, while monitoring CTA training, saccharin exposure induces a dopamine increase and the LiCl administration induces a glutamate increase in the IC. Interestingly, about 40 min after the association of both stimuli, dopamine and glutamate extracellular levels showed again a significant and transient increase. Nonetheless, this phenomenon was not related to the CS or US presentation alone, since saccharin followed by NaCl administration, or water followed by LiCl did not produce any of the post-acquisition neurotransmitters release as observed after the saccharin–LiCl association. Furthermore, the backward conditioning, which involves the same stimuli that were associated, failed to induce such post-learning changes in dopamine and glutamate. Altogether, these results indicated that only the forward association of the stimuli induced post-trial increments of

FIGURE 3 | Extracellular dopamine and glutamate levels in the IC increase concomitantly in the post-acquisition period of CTA training. **(A)** Dopamine monitoring: SAC-LiCl, conditioned group ($n = 10$) received 0.1% saccharin solution followed by 0.4 M LiCl i.p. injection (7.5 mL/kg); SAC-NaCl, non-conditioned group ($n = 7$) received 0.1% saccharin solution followed by 0.4 M NaCl i.p. injection (7.5 mL/kg); the CS elicited a dopamine increase in both groups but only the conditioned group showed a post-acquisition increase in the 88-min fraction. **(B)** Glutamate responses monitoring in conditioned and non-conditioned groups, the US elicited an increment in the SAC-LiCl group due to the LiCl injection but only the conditioned group showed a post-acquisition increase in the 92-min fraction. **(C)** Dopamine responses of control groups to stimuli: H_2O–LiCl

group ($n = 5$) received tap water followed by 0.4 M LiCl i.p.; H_2O–NaCl group ($n = 6$) received tap water followed by 0.4 M NaCl i.p.; Dopamine levels are significantly different during saccharin exposure that during water exposure and showed no post-acquisition increments. **(D)** Glutamate responses to the LiCl and NaCl injection showed no post-acquisition changes, only the one related to the US. **(E)** Dopamine responses during backward conditioning: LiCl-SAC ($n = 7$), received 0.4 M LiCl i.p., and later, a 0.1% (wt/vol) saccharin solution; there is no post-acquisition increment. **(F)** Glutamate response during the backward conditioning. Graphics expressed as means of % baseline release $\pm$ SEM. *$p < 0.05$, and **$p < 0.01$ vs. control group and #$p < 0.05$ vs. baseline release (Guzman-Ramos et al., 2010).

glutamate and dopamine. These neurochemical signals appear to be related specifically to the consolidation process, since blockade of the NMDAr in the post-acquisition stage impairs only long, but not STM and the simultaneous blockade of the dopaminergic D1 receptors and the NMDA induces a greater impairment in CTA consolidation, suggesting a synergic role of these neurotransmitter systems (Guzman-Ramos et al., 2010).

A considerable amount of evidence indicates that D1 receptors activity can modify the strength of synaptic transmission, potentiating NMDAr conductance by means of NR1 subunit phosphorylation through PKA, enhancing the excitability of neurons and leading to a cooperative action that may strengthen the memory trace formation (Cepeda et al., 1998; Wang and O'Donnell, 2001; Jay, 2003; Tseng and O'Donnell, 2003, 2004; Hallett et al., 2006). The role of the dopaminergic system in synaptic plasticity and memory consolidation has been proven, particularly through the activation of cAMP/PKA/CREB pathway involving protein synthesis induction (see Jay, 2003). Similarly, dopamine has also been related to the persistence of the LTM trace, since the intrahippocampal infusion of a D1 agonist 9 h after training in a one-step inhibitory avoidance task makes a weak training last longer, indicating that dopaminergic signal can modulate the maintenance of LTM storage through a post-acquisition activity (Rossato et al., 2009).

Further mechanisms are involved in the long-term maintenance of the CTA memory trace, for instance, the cortical activity of PKM ζ, a Protein Kinase C (PKC) isoform that is persistently active after the consolidation period, is necessary for persistence of memory for several weeks and even after 3 months from the CTA training; the blockade of this enzyme in the IC impairs LTM in an apparently irreversible way (Shema et al., 2007, 2009). Moreover, the over expression of PKM ζ in this brain structure about 2 weeks after training, enhances LTM performance in the CTA tests (Shema et al., 2011). On the other hand, the post-acquisition blockade of PKM ζ in the BLA has no effect on LTM maintenance, but PKM ζ inactivation 15 min after CS presentation, in the CS–US interval, produces a significant effect on taste aversion tested 2 days after conditioning (Gamiz and Gallo, 2011). This implies that CTA memory trace maintenance is dependent on the activity of this enzyme within the IC, and its role on the AMY may be related to the acquisition stage.

AMYGDALA AND INSULAR CORTEX INTERACTION ON CTA LONG-TERM STORAGE

The amygdala and the IC have reciprocal projections (Pitkanen, 2000; Price, 2003); and some data suggests that the BLA projection to the gustatory cortex is important for the taste aversion memory stabilization. For instance, it has been demonstrated that tetanic stimulation of BLA induces LTP in the IC, increasing the neuronal response to low frequency stimulation (Escobar et al., 1998b; Jones et al., 1999). Thus, LTP induction in the BLA–IC projection before CTA acquisition enhances this task retention by making the extinction process slower (Escobar and Bermudez-Rattoni, 2000). In agreement with the role of NMDAr on memory consolidation, it was demonstrated that such potentiation depends on the NMDAr activity in the IC, since intra-cortical administration of antagonists of this receptors impair both CTA and

LTP induction in the BLA–IC pathway (Escobar et al., 1998a,b). Conversely, the possibility that amygdala NMDA activation could improve taste aversive memory has been demonstrated. Thus, BLA administration of glutamate before the gastric malaise induction during CTA training enhances aversive taste memory formation, and local administration of an NMDAr antagonist in the IC 1 h after conditioning impaired taste aversion memory enhancement (Ferreira et al., 2005). This suggests that the interaction among amygdala and IC through the glutamatergic system could contribute to the CTA memory trace establishment and consolidation even in post-acquisition stages. In this regard, amygdala post-acquisition activity seems to be required for CTA consolidation since the reversible inactivation of this structure by tetrodotoxin (TTX, a voltage-sensitive sodium channel blocker) 15 min and up to 1.5 h after CS–US pairing attenuates CTA memory, such attenuation is inversely proportional to the time interval between the acquisition and the intra-amygdalar injection of TTX (Roldan and Bures, 1994).

The amygdala functional integrity is also required for aforementioned post-acquisition neurochemical changes seen in the IC related to previous CS–US association; thus reversible post-acquisition blockade of the amygdala with bilateral TTX infusion hindered post-acquisition glutamate and dopamine increments in the IC and impaired CTA consolidation (Guzman-Ramos et al., 2010). Accordingly, these results indicate that amygdala activation is associated to IC post-acquisition activity. In addition, it would be possible that the amygdala needs to be re-activated after the acquisition of the task to outline the memory trace, which is consistent with the evidence that post-training brain activity is related to previous learning experience (Peigneux et al., 2006; Eschenko and Sara, 2008; Lansink et al., 2008; Marrone et al., 2008). Particularly in the amygdala, single unit recordings of spontaneous activity revealed that the firing rate of BLA neurons increased gradually after inhibitory avoidance training (tone paired with foot-shock), peaking at 30–50 min post-shock (Pelletier et al., 2005), a time frame that goes in accordance with the neurochemical reactivation herein described and with the effect of amygdalar post-acquisition manipulations that enhance memory. There is ample literature showing that emotionally arousing experiences have been related to increase in stress hormones such as glucocorticoids, exerting their central effect in the amygdala through the activation of β-adrenergic receptors (Ferry et al., 1999; Ferry and McGaugh, 2000; Roozendaal, 2000; McGaugh and Roozendaal, 2002). In this regard, CTA memory retention is enhanced after post-acquisition administration of corticosterone into the BLA and the IC (Miranda et al., 2008). Taken together, these results support the idea that keeping emotional experience in the long-term requires amygdala activity not only during the acquisition period through stimuli signaling, but through post-learning stages.

The post-acquisition engagement of the amygdalar activity has been related to the idea of spontaneous oscillatory activity in this structure that is generated by emotionally arousing conditions. Thus, neuronal recordings in freely moving animals have revealed that during these kind of experiences the firing rate of the BLA neurons increases and it is synchronized trough a theta frequency (4–7 Hz; Pelletier and Pare, 2004). Since theta activity dominates

during the learning period (Pare and Collins, 2000; Seidenbecher et al., 2003) the main consequence of the amygdalar oscillations is to produce temporal windows of neuronal discharging that facilitates the interaction among the structures that synchronized during the acquisition period (Pare et al., 2002), as could be the case of IC and AMY during CTA. This way, theta frequency activity would enhance the depolarization of afferent structures generating a neurochemical reactivation and promoting synaptic plasticity. For instance, it has been reported that CTA training produces an increment in BLA–IC functional connectivity seen as an increased correlation in the activity of simultaneously recorded neurons of these structures (Grossman et al., 2008). Therefore, plastic changes underlying memory trace consolidation, may need reactivation of particular biochemical pathways to sustain the levels of proteins that are required for the consolidation of memory (Wang et al., 2006).

POST-TRAINING MOLECULAR CHANGES INVOLVED IN MEMORY CONSOLIDATION

In order to consolidate a memory trace the activation of several intracellular pathways must be triggered modulating protein synthesis and synaptic plasticity. For instance, the activation of the extracellular responsive kinase 1–2 (ERK1–2) in the IC is required for long- but not STM of CTA (Berman et al., 1998). However the implications of this kinase in post-acquisition stages of this task have not been assessed. Interestingly, a fear conditioning task induces two waves of ERK1–2 activation in the lateral amygdala and the BLA, the fist 60 min after conditioning and the second one about 6 h post-acquisition (Trifilieff et al., 2006). Similarly, a 60-min increase in ERK1–2 activity after paired presentation of tone and shock has been reported, and this effect is absent with the presentation of the CS or the US alone, or an unpaired tone-shock presentation (Schafe et al., 1999); which implies that the learning experience generates delayed reactivations that could be involved in the consolidation process. In a similar way, PKA activity is required for long-term stabilization of CTA memory, the inhibition of PKA in the IC during the post-acquisition stage impairs long- but not STM (Guzman-Ramos et al., 2010), in accordance with the dopamine and glutamate reactivations that are shown in this structure. Hence, dopaminergic modulation may facilitate the reactivation of PKA in aversive associative tasks, for instance the blockade of D1 receptors in the hippocampus after 3 or 6 h impair one-step inhibitory avoidance consolidation, and that the inhibition of PKA on the same temporal patterns renders the same effect (Bernabeu et al., 1997). As mentioned, the activation of kinases has a modulatory effect on protein synthesis and particularly expression of IEG like c-fos. In this regard, there is evidence of IEG expression after post-acquisition reactivation related to previous learning; that is, rats trained in the odor discrimination task had more c-fos expression than unpaired control rats in their prelimbic cortex, ventrolateral orbital cortex, and BLA (Tronel and Sara, 2002). Similarly, in a one-trial learning paradigm in which mice learned to enter a dark compartment to escape from an aversively illuminated area showed more Arg 3.1/Arc mRNA expression 15 min and 4.5 h post-training detected specifically in the learning group when compared to the control or the retrieval groups (Montag-Sallaz and Montag, 2003).

This kind of monitoring of protein expression after learning should provide patterns about the timeline of the required neuronal changes that underlie memory stabilization in the long-term. In this regard, CTA consolidation and some related protein expression waves has been reported, the IEG HZF-3 increases in the BLA at 1 and 3 h after CTA conditioning and this up-regulation is not present with the presentation of the flavor or the malaise induction only, supporting the idea of a specific role in the associative learning (Ge et al., 2003). Recent reports showed an interesting temporal dichotomy in the expression of BDNF in the IC and nuclei of the amygdala. Thus, from 2 to 6 h after CTA conditioning there was a significant BDNF increase within the CeA, and from 4 to 6 h an increase was observed in the IC and in the BLA, these up-regulations were related to the association of the stimuli, since the CS or the US alone and a delayed paring of CS–US did not induce BDNF increases (Ma et al., 2011).

CONCLUSION

As we have seen, many of the post-acquisition molecular changes in the amygdala or in the IC overlap shortly after conditioning, form 45 min to 1 h, and other waves of activity in at least 6 h, suggesting that there is a time frame where neurochemical changes trigger receptors and kinases activation leading to increased expression of proteins required for memory consolidation. Whether such reactivations are occurring repeatedly is still unclear, but several reports have indicated reactivation activity within a time frame that goes in accordance with cellular consolidation theory (Dudai, 2004). Thus, the neuronal changes caused by up-regulation of protein synthesis within the learning-engaged structures may occur within few hours or even days, as the Cui et al. (2005) have suggested. Such protein synthesis induction appears to be related to spontaneous activity after the exposure to the information (i.e., CS–US association). These kinds of mechanisms involved in the progressive stabilization of the information may be related to the salience of such information, or what we have been calling "emotional memories," which may be of life-saving importance for the animal. Such is the case of CTA learning where a specific flavor may be toxic and could have deadly consequences in the future. The relevance of the information may induce mechanisms that reinforce the memory trace in an efficient way to prompt retrieval and adequate behavioral change. We propose that this could be achieved by post-acquisition reactivation signals during post-training wakefulness. As mentioned, simultaneous neural recordings in the macaque neocortex, revealed that cells in all four areas exhibited firing related to the task (sequential reaching behavior), and those cells tended to be coactive afterward (Hoffman and McNaughton, 2002). Another example is the sequential replay in hippocampal place cells, where population activity in the hippocampus was recorded while rats ran back and forth on a linear track for a water reward at each end. During the run, each neuron's firing was tuned to a particular location along the track, which was stable from lap to lap. These locations define a temporal sequence of place-cell firing on the timescale of seconds. During awake period immediately after the spatial task, the same neurons fired again on the timescale of hundreds of milliseconds, but in the reverse temporal order, which may serve to propagate information from the rewarded location backward along incoming

trajectories (Foster and Wilson, 2006; Diba and Buzsaki, 2007). It is suggested that such replay might constitute a general mechanism of learning and memory because this is more readily observable in a new environment than a familiar one (Foster and Wilson, 2006). We propose that in CTA memory the post-acquisition activity involves amygdalar spontaneous reactivation that triggers neuronal changes within the IC since it can engage into oscillatory activity related to emotional learning promoting the facilitation of the neuronal interactions strengthening the memory trace.

ACKNOWLEDGMENTS

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