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Review

Molecular Pharmacological Dissection of Short- and Long-Term Memory

Luciana A. Izquierdo,[1] Daniela M. Barros,[1] Monica R. M. Vianna,[1,3] Adriana Coitinho,[1] Tiago deDavid e Silva,[1] Humberto Choi,[1] Beatriz Moletta,[1] Jorge H. Medina,[2] and Ivan Izquierdo[1]

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SUMMARY

1. It has been discussed for over 100 years whether short-term memory (STM) is separate from, or just an early phase of, long-term memory (LTM). The only way to solve this dilemma is to find out at least one treatment that blocks STM while keeping LTM intact for the same task in the same animal.

2. The effect of a large number of treatments infused into the hippocampus, amygdala, and entorhinal, posterior parietal or prefrontal cortex on STM and LTM of a one-trial step-down inhibitory avoidance task was studied. The animals were tested at 1.5 h for STM, and again at 24 h for LTM. The treatments were given after training.

3. Eleven different treatments blocked STM without affecting LTM. Eighteen treatments affected the two memory types differentially, either blocking or enhancing LTM alone. Thus, STM is separate from, and parallel to the first hours of processing of, LTM of that task.

4. The mechanisms of STM are different from those of LTM. The former do not include gene expression or protein synthesis; the latter include a double peak of cAMP-dependent protein kinase activity, accompanied by the phosphorylation of CREB, and both gene expression and protein synthesis.

5. Possible cellular and molecular events that do not require mRNA or protein synthesis should account for STM. These might include a hyperactivation of glutamate AMPA receptors, ribosome changes, or the exocytosis of glycoproteins that participate in cell addition.

KEY WORDS: short-term memory; long-term memory; memory types.

[1] Centro de Memoria, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

[2] Instituto de Biología Celular y Neurociencia Eduardo de Robertis, Buenos Aires, Argentina.

[3] To whom correspondence should be addressed at Centro de Memoria, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Ramiro Barcellos 2600-anexo, 90035-003 Porto Alegre, RS, Brazil; e-mail: mrmvianna@yahoo.com.br.

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INTRODUCTION

Long-term memories are not established in their definitive form immediately after they are acquired. Their consolidation requires a number of precisely timed molecular processes in the neurons that make memories. These sequential processes are linked to one another in a complex way (Izquierdo and McGaugh, 2000; Izquierdo and Medina, 1997). They have been best studied in the CA1 subregion of the hippocampus, which is involved in many if not all forms of explicit or declarative memory in mammals (Izquierdo and McGaugh, 2000; Izquierdo and Medina, 1997; Squire, 1992; Taubenfeld *et al.*, 1999). The molecular processes that underlie the synaptic changes necessary for memory formation include two peaks of increased activity of cAMP-dependent protein kinase, the phosphorylation of the transcription factor $CREB_1$, gene activation, and protein synthesis (Bernabeu *et al.*, 1997; Cammarota *et al.*, 2000; Izquierdo and McGaugh, 2000; Izquierdo and Medina, 1997; McGaugh, 2000; O'Connell *et al.*, 2000; Taubenfeld *et al.*, 1999). The first of these peaks occurs within the first 5 min after training, and the second, which depends on the first, occurs 2–6 h later in the rat (Bernabeu *et al.*, 1997; Cammarota *et al.*, 2000; Taubenfeld *et al.*, 1999; Vianna *et al.*, 1999, 2000b), in the chick (Rose, 2001), and in the crab (Locatelli *et al.*, 2002). The dual-peak phenomenon was first described by Matthies and his group (Grecksch and Matthies, 1980; Matthies, 1982), and appears to be a very general phenomenon inherent to LTM consolidation across different tasks and species (see Izquierdo and McGaugh, 2000; Izquierdo and Medina, 1997).

Hours, days, or even years later other processes may also contribute to give memories their final shape. These processes include extinction, forgetting (Vianna *et al.*, 2001), relearning, the addition or redistribution of information, and mixtures with other memories. While some of these protracted events have also been interpreted as a form of consolidation (Squire, 1992), it is more customary to reserve this word to designate the events that underlie the processes that take place in the first few hours after acquisition (Izquierdo & McGaugh, 2000; McGaugh, 2000).

William James (1890) was the first to note that there must be a parallel, short-term system that takes charge of memory while the formation of its long-term form is not yet completed, i.e., in the first few hours after training. This concept was developed further by McGaugh (1966) and Gold and McGaugh (1975). A major question is that of whether short-term memory (STM) processes constitute an entity separable from long-term memory (LTM) formation, or is instead a mere phase of the latter (McGaugh, 1966). This is important conceptually (Gold and McGaugh, 1975), as well as from a biochemical and a clinical point of view.

The only way to demonstrate a separation of STM and LTM is to devise an experiment in which STM can be effectively blocked while leaving LTM of the same learning intact, in the same task and in the same animal (Izquierdo *et al.*, 1998a,b,c). In recent years this was accomplished by pharmacological means both for the STM and LTM of synaptic facilitation in *Aplysia* ganglion cells, which may be viewed as a model of memory of sensitization (Emptage and Carew, 1993), and for inhibitory avoidance learning in rats (Izquierdo *et al.*, 1998a,b,c), which is the most studied form of conditioned fear (see Vianna *et al.*, 2001) and whose retention is widely regarded as a form of explicit memory (Gold, 1986; Izquierdo and McGaugh, 2000; McGaugh, 2000).

In a classic paper, McGaugh (1966) identified "three memory trace systems: one for immediate memory . . . one for short-term memory (which develops in a few seconds or minutes and lasts for several hours); and one which consolidates slowly and is relatively permanent." Immediate memory (Jacobsen, 1936) superposes temporally with working memory (Goldman-Rakic, 1991), which is a nonarchival system that keeps information available during seconds or, at most, a few minutes (Barros *et al.*, 2001a,b; Goldman-Rakic, 1996; Izquierdo *et al.*, 1998c). STM and LTM are, instead, memory types of an archival nature that may differ in nature (Izquierdo *et al.*, 1998a) but not in contents. The terms STM and LTM are applied usually to explicit or declarative memories; they are seldom used in the literature on implicit or procedural memories, whose STM has not been studied in detail (Izquierdo *et al.*, 2000).

DISSECTION OF STM FROM LTM

The procedure most used to differentiate STM from LTM has been the microinjection of pharmacological treatments whose effect is brief and reversible (Martin, 1991), shortly before or after training, into specific nervous structures (Emptage and Carew, 1993; Izquierdo *et al.*, 1998a,b,c, 1999; Vianna *et al.*, 1999, 2000a,b). It is simply not possible to attempt such a dissection by lesion studies, by the use of transgenic animals, or by biochemical measurements of molecular changes in brain structures (Izquierdo *et al.*, 1999; Izquierdo and Medina, 1998). The proactive effect of lesions or of permanently altered gene expression may in fact affect any or all of the neural or behavioral events that may take place in life, and offer no clue as to which of them may be primarily affected. Biochemical changes measured within the first few hours after training could in principle pertain to the processing of either the STM or LTM at that time (Vianna *et al.*, 1999, 2000a,b). For example, a sharp increase of cAMP-dependent protein kinase (PKA) is seen in the hippocampus of rats immediately after one-trial avoidance training (Bernabeu *et al.*, 1997; Vianna *et al.*, 2000a). A priori, it is impossible to say whether that increase is linked to STM or to LTM processing: Indeed, the intrahippocampal administration of PKA inhibitors at the time of that peak blocks both STM and LTM, which does not clarify the issue (Vianna *et al.*, 2000b). Administration of the inhibitors into the hippocampus 22–90 min after training, however, selectively hinders STM and leaves LTM unaffected (Vianna *et al.*, 2000a,b). Therefore, these pharmacological findings indicate that PKA activity is necessary for STM between 0 and 90 min after acquisition, and the early PKA peak, but not PKA activity in the minutes that follow, is necessary for LTM (Vianna *et al.*, 2000a). Similarly, there is an increase of calcium–calmodulin dependent protein kinase II (CaMKII) in the rat hippocampus after inhibitory avoidance training, coupled with increased phosphorylation of neighboring glutamate AMPA receptors and increased binding of AMPA to these receptors (Cammarota *et al.*, 1998). In principle, these events could underlie either STM and LTM formation, or both. However, immediate posttraining intrahippocampal infusion of a CaMKII inhibitor blocks LTM but not STM (Izquierdo *et al.*, 2000). Therefore, CaMKII is involved in LTM but not in the simultaneous formation of STM (Izquierdo *et al.*, 1999).

Many treatments have been studied for their influence on STM and LTM. The task most widely studied has been one-trial inhibitory avoidance in rats which,

because of its brevity, enables a clear separation between training and posttraining events and events related to retrieval (Gold, 1986; McGaugh, 1966, 2000). The treatments were given by posttraining bilateral infusion into restricted brain regions. As will be seen, some treatments influenced both STM and LTM simultaneously, others affected STM but not LTM, and others affected LTM but not STM.

The treatments whose effect on STM and LTM was studied simultaneously include a wide variety of substances, from receptor agonists and antagonists to enzyme inhibitors and stimulants, administered into different brain structures. The drugs used were the glutamate AMPA receptor blocker, CNQX (0.5 μg/side); the glutamate NMDA receptor antagonist, AP5 (5.0 μg/side); the glutamate metabotropic receptor generic antagonist, MCPG (2.5 μg/side); the $GABA_A$ receptor agonist, muscimol (0.5 μg/side); the muscarinic receptor agonist, oxotremorine (0.12 and 0.6 μg/side) and its antagonist, scopolamine (2.0 μg/side) (Barros *et al.*, 2001b); the dopamine D_1 receptor agonist, SKF38393 (7.5 μg/side); and the the D_1 antagonist, SCH23390 (2.5 μg/side); norepinephrine (0.3 μg/side); the β-adrenoceptor antagonist, timolol (0.3 μg/side); the 5HT1A receptor agonist, 8-HO-DPAT (2.5 μg/side); the blocker of that receptor, NAN-190 (2.5 μg/side); the CaMKII inhibitor, KN-62 (3.6 μg/side); the nonselective inhibitors of protein kinase C (PKC), staurosporin (2.5 μg/side), and Gö7874 (1.96 or 8 nM; the selective inhibitor of $PKC_{\alpha/\beta II}$, Gö 6974 (0.92or 4.6 nM) (Vianna *et al.*, 2000a); lavendustin A, which inhibits Tyr kinases at a low dose (0.1 μg/side) and the cGMP-dependent protein kinase (PKG) at a higher dose (0.5 μg/side); the specific Tyr kinase inhibitor, radicicol (1 and 5 0.5 μg/side); the inhibitor of phosphoinisitide-3-kinase, LY294002 (5 and 50 μM); the inhibitor of guanylyl cyclase,, LY83583 (2.0 μg/side); the specific PKG inhibitor, KT5823 (2.5 μg/side); the inhibitor of MAPK kinase, PD 098059 (0.05 μg/side); the cAMP analog, 8 Br-cAMP (1.25 μg/side); the selective PKA antagonists, KT5720 and Rp-cAMPs (0.1 and 0.5 μg/side); the PKA stimulant, Sp-cAMPs (0.1 and 0.5 μg/side); and the stimulant of adenylyl cyclase, forskolin (0.5 μg/side).

All these drugs, at the same doses, had been previously shown to affect LTM when given pre- or posttraining; this occurs regardless of whether the animals had been previously tested for STM or not (Izquierdo *et al.*, 1999; Izquierdo and McGaugh, 2000).

The effect on STM and LTM of bilateral infusions of these drugs immediately posttraining into the hippocampus is shown in Fig. 1. Their effect on STM and LTM when they were infused into the entorhinal cortex is shown on Fig 2. The effect of treatments infused bilaterally into other brain areas (posterior parietal cortex, anterolateral prefrontal cortex, and basolateral amygdala) is shown in Fig 3. In addition, the effect of several of these treatments on working memory (WM), when given 5 min prior to training into posterior parietal, anterolateral prefrontal, and entorhinal cortex, into the hippocampus or into the basolateral amygdala, is shown on Fig. 4. The effects of Gö6974 and Gö 7874 given into the hippocampus and of intra-amyfdala infusions of oxotremorine are not shown in the Figures; they can be found in Vianna *et al.* (2000b) and Barros *et al.* (2001a) respectively.

The design of all these experiments was very simple. It consisted of training in the one-trial step-down inhibitory avoidance task, followed either immediately or

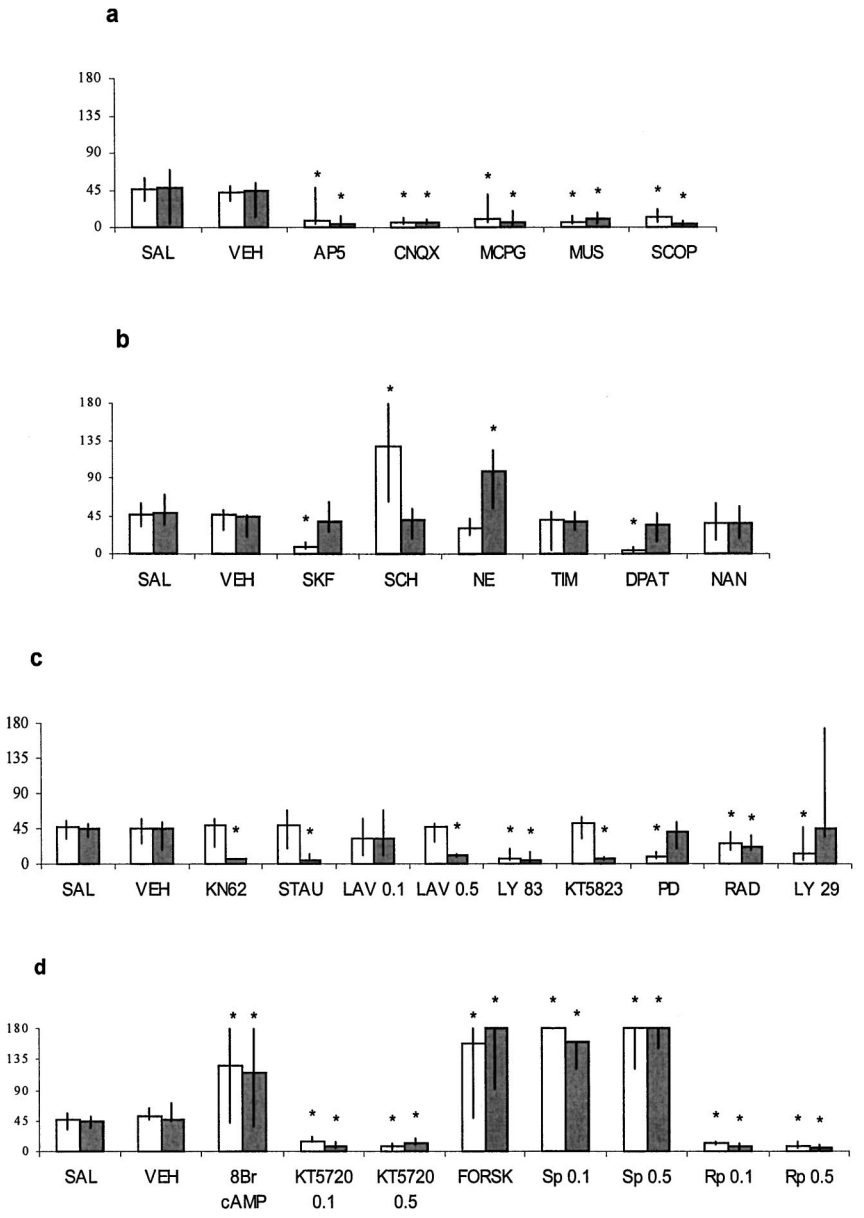

Fig. 1. Memory of one-trial step-down avoidance, expressed as median (interquartile range) test session step-down latency, in seconds, was measured 1.5 h after training (STM) and again 24 h after training (LTM). Data from Izquierdo *et al.* (1998a,b,c, 1999, 2000); Bianchin *et al.* (1999); Walz *et al.* (1999); Vianna *et al.* (1999, 2000a,b,c); Barros *et al.* (2001a,b); and Pereira *et al.* (2001a,b). In this as in the following two figures, $N = 10$ per group, except for the control groups in which $N = 20$ and were composed of randomly selected animals from the original papers. Infusion volume was 0.5 μL in all cases. The drugs used were dissolved in either saline (SAL) or vehicle (VEH, 2% DMSO in saline). Asterisks indicate significant differences from both control groups at a $p < 0.02$ level in Mann–Whitney U tests, two-tailed. The drugs used were applied here bilaterally into the CA1 region of the dorsal hippocampus, immediately posttraining. They were: (a) the glutamate AMPA receptor blocker, CNQX (0.5 μg/side);

several minutes after training by the bilateral infusion of the drugs into the desired brain region, and testing the animals twice in the avoidance task: the first time, 1.5 or 3 h after training, in order to measure STM; the second, 24 h after training, to test for LTM (Izquierdo *et al.*, 1998a,b,c). In the WM experiments, the drugs were given 5 min prior to training and the test session started 3 s after the training footshock; i.e., it measured immediate memory (Jacobsen, 1936), which is considered as synonymous with WM (Goldman-Rakic, 1991).

Avoidance training was as follows. Rats were placed on a 2.5 cm high, 7.0 cm wide platform at the left of a 50.0 × 25.0 × 25.0 cm box whose floor was a series of parallel 0.1 cm caliber stainless steel bars spaced 1.0 cm apart. The animals were placed on the platform facing its rear left corner. Latency to step down placing the four paws on the grid was measured with an automatic device. In the training session, immediately upon stepping down, the animals received a 0.5 mA, 1.0-s scrambled footshock (in some experiments the shock intensity was slightly less and its duration longer). In the test session, no footshock was given and step-down latency was cut off at 180 s; i.e., values equal to or higher than 180 s were counted as 180 s. Data were analyzed by nonparametric statistics (Kruskal–Wallis analysis of variance for each experiment, and individual Mann–Whitney *U* tests, two-tailed). The choice of these training parameters was due to the fact that they yield median retention test latencies of about 40 s (Barros *et al.*, 2001a,b, in press; Izquierdo *et al.*, 1998a,b,c, 2000, in press-a,b), which are always significantly different from training latencies and far below the 180 s ceiling. This permits an analysis of both stimulant and depressant agents.

MAIN RESULTS

Overall considered, the main result of the findings summarized here is 11 of the treatments blocked STM without affecting LTM in the same animals for the avoidance task. Since none of the treatments affected LTM when the STM test was omitted (Izquierdo *et al.*, 1999), and none of them affected retrieval when given 1.5 h prior to a test session (Izquierdo *et al.*, 1998a,b,c, 1999), the results can safely be interpreted as

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the glutamate NMDA receptor antagonist, AP5 (5.0 μg/side); the generic glutamate metabotropic antagonist, MCPG (2.5 μg/side); the $GABA_A$ receptor agonist, muscimol (MUS, 0.5 μg/side); the muscarinic receptor antagonist, scopolamine (2.0 μg/side); (b) the dopamine D_1 receptor agonist, SKF38393 (7.5 μg/side); and the D_1 antagonist, SCH23390 (2.5 μg/side); norepinephrine (0.3 μg/side); the β-adrenoceptor antagonist, timolol (0.3 μg/side); the 5HT1A receptor agonist, 8-HO-DPAT (2.5 μg/side); the blocker of that receptor, NAN-190 (2.5 μg/side); (c) the CaMKII inhibitor, KN-62 (3.6 μg/side); the nonselective inhibitors of protein kinase C (PKC), staurosporin (2.5 μg/side), and Gö7874 (1.96 or 8 nM; the selective inhibitor of $PKC_{\alpha/\beta II}$, (0.92or 4.6 nM) ; (d) lavendustin A, which inhibits Tyr kinases at a low dose (0.1 μg/side) and the cGMP-dependent protein kinase (PKG) at a higher dose (0.5 μg/side); the specific Tyr kinase inhibitor, radicicol (RAD, 5.0 μg/side); the inhibitor of phosphoinisitide-3-kinase, LY294002 (LY29, 5 and 50 μM); the inhibitor of guanylyl cyclase, LY83583 (LY83, 2.0 μg/side); the specific PKG inhibitor, KT5823 (2.5 μg/side); the inhibitor of MAPK kinase, PD098059 (PD,0.05 μg/side); the cAMP analog, 8 Br-cAMP (1.25 μg/side); the selective PKA antagonists, KT5720 and Rp-cAMPs (Rp) both at 0.1 and 0.5 μg/side; the PKA stimulant, Sp-cAMPs (Sp, 0.1 and 0.5 μg/side); and the stimulant of adenylyl cyclase, forskolin (FORSK, 0.5 μg/side). Note that four of the treatments (SKF, DPAT, PD, LY29) inhibited STM while leaving LTM unaltered. Eleven treatments blocked both STM and LTM (AP5, CNQX, MCPG, MUS, SCOP, LY83583, KT5720 (0.1 and 0.5 μg/side); Rp-cAMPs (0.1 and 0.5 μg/side); and RAD. Four treatments (both doses Sp, 8-Br-cAMP, FORSK and both doses of Sp) enhanced both memory types. SCH enhanced STM while leaving LTM intact. Four treatments (KN62, STAU, LAV 0.5, and KT5823) depressed LTM but not STM.

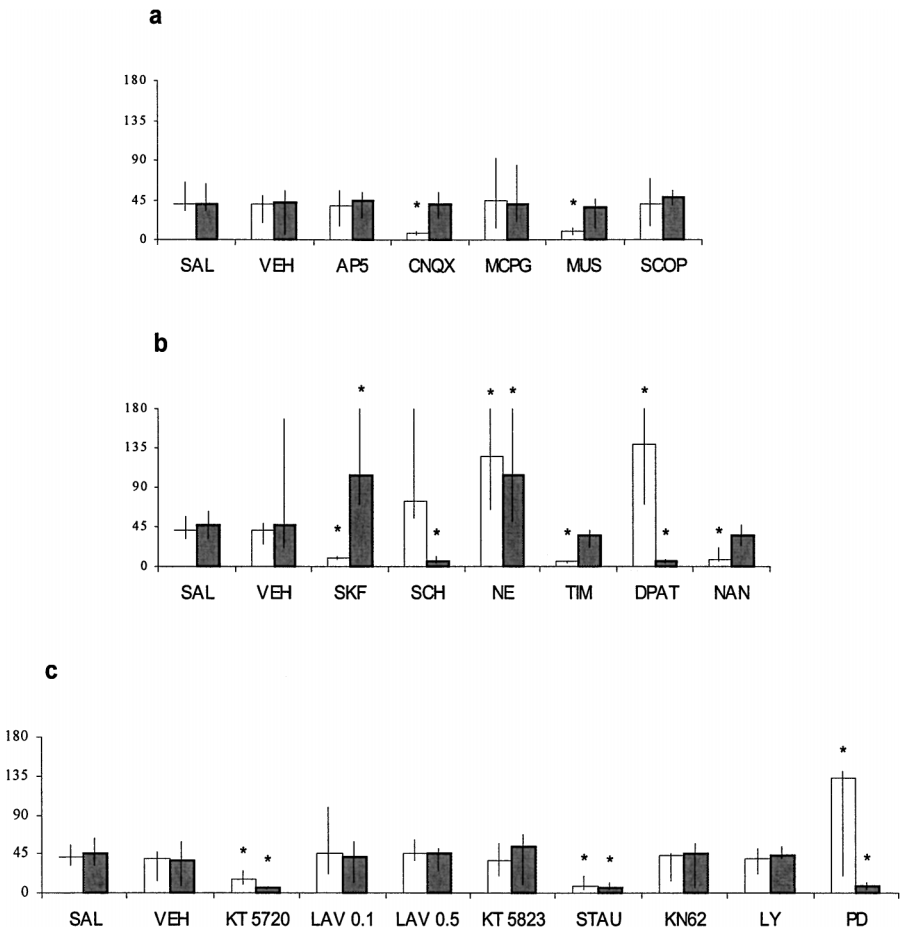

Fig. 2. Same as Fig. 1, but for treatments infused bilaterally in the entorhinal cortex. LY here represents LY83583, the guanylyl ciclase inhibitor; the other LY compound was not used into the entorhinal cortex. Only the higher dose of KT5720 (0.5 μg/side) was used here. Five treatments (CNQX., MUS, SKF, NAN, TIM) depressed STM but not LTM. Two treatments blocked both STM and LTM (KT5720 and STAU); and NE enhanced both memory types. SCH enhanced STM and depressed LTM intact. DPAT and PD enhanced STM and depressed LTM. depressed LTM but not STM.

showing that STM can be blocked independently of LTM formation, and is therefore a separate process, not necessarily related to LTM consolidation. They amply satisfy the criterion expressed in the Introduction concerning the need for *at least one* experiment showing a blockade of STM without affecting (or, in some cases even enhancing) LTM (Emptage and Carew, 1993; Izquierdo *et al.*, 1998a,b,c, 1999). (Note that several treatments suppressed STM and at the same time actually enhanced LTM, and that several depressed LTM selectively, but did not affect STM; see Figs. 1–3).

Glutamate, cholinergic muscarinic, β-noradrenergic, dopaminergic D_1, and 5HT1A receptors, adenylyl cyclase, PKA, phosphoinositide-3-kinase, Tyr-kinases, guanylyl cyclase, guanylyl cyclase, PKG, the Erk pathways and various isoforms of

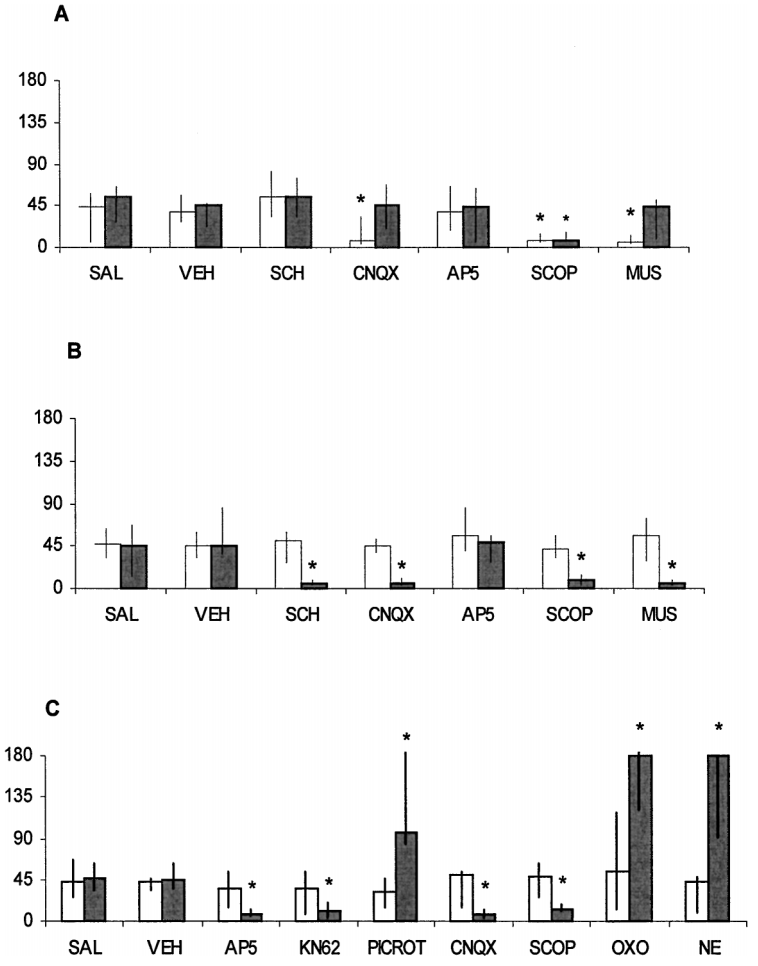

Fig. 3. Same as preceding figures, but for treatments infused into the posterior parietal and anterolateral prefrontal cortex, and basolateral amygdala. Intraparietal CNQX and MUS depressed STM selectively; intra-amygdala PICROT and NE enhanced LTM selectively. Infusion of SCH, CNQX, SCOP or MUS into the prefrontal cortex, or of AP5, KN62, CNQX or SCOP into the amygdala depressed LTM but not STM. OXO given into the amygdala enhanced LTM.

PKC participate in the consolidation of LTM. These results have been amply discussed elsewhere (see Izquierdo and McGaugh, 2000; Izquierdo and Medina, 1997; McGaugh, 2000; Routtenberg, 2001; Vianna *et al.*, 1999, 2000a,b), and need not be commented here, except for the fact that the participation of most of these systems is sequential, time-dependent, and interdependent. For example, the activation of ionotropic glutamate receptors in CA1 very early on is necessary for the activation of all the other systems (Cammarota *et al.*, 2000); also in CA1, the participation of PKC_{γ}, which is predominantly presynaptic, precedes that of $PKC_{\alpha/\beta II}$, which is predominantly postsynaptic (Routtenberg, 2001; Vianna *et al.*, 2000a), The increase of

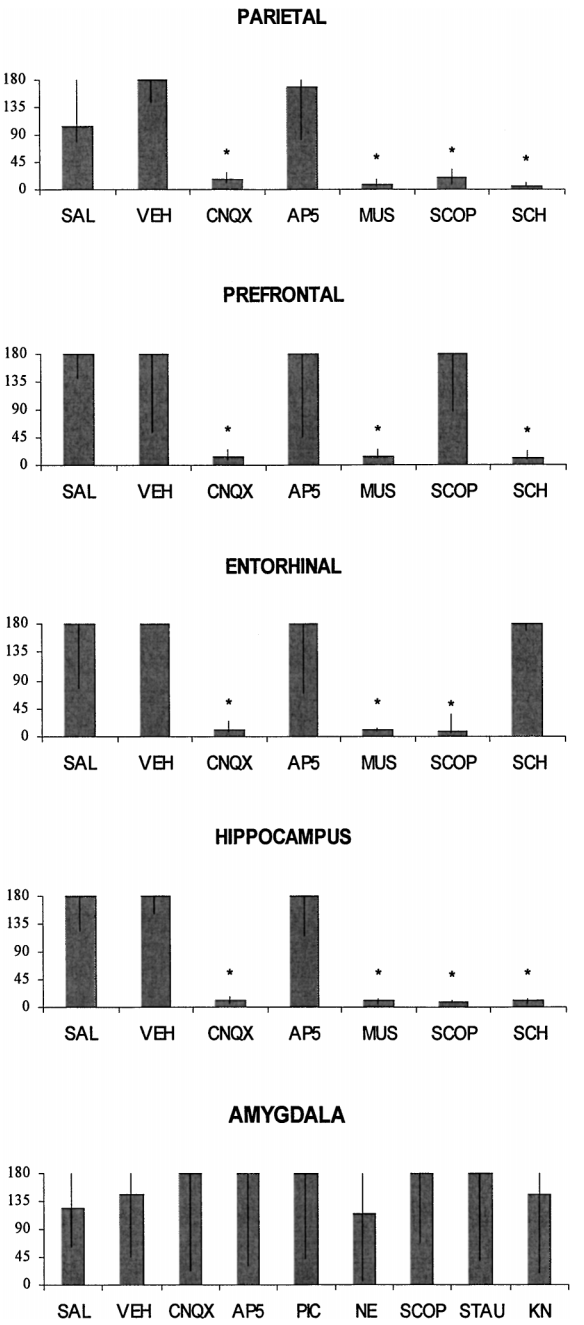

Fig. 4. Immediate memory (median [interquartile range] step-down test latency, in seconds) measured 3 s after the completion of inhibitory avoidance training. Immediate memory represents WM (Goldman-Rakic, 1991). Treatments were given by infusion into the desired structures 5 min before

CaMKII activity is linked to the phosphorylation and enhanced binding properties of AMPA receptors (Cammarota *et al.*, 1998). The activation of PKA in CA1 precedes that of the same enzyme in entorhinal cortex (Pereira *et al.*, 2001a,b) and, in the hippocampus, correlates with phosphorylation of the transcription factor $CREB_1$, and occurs in two peaks, the first in the first 5 min posttraining and the second, 2–6 h later (Bernabeu *et al.*, 1997; Vianna *et al.*, 2000c). These two peaks occur at periods in which LTM consolidation is sensitive to inhibitors of gene expression and protein synthesis (Izquierdo *et al.*, in press-a,b). The first peak is probably involved in the tagging of synapses activated by the training experience (see Frey and Morris, 1998) and is necessary for the second (Vianna *et al.*, 2000c), which is in addition coincident with a period of sensitivity of LTM to MAPK inhibitors (Izquierdo and McGaugh, 2000; see Izquierdo and Medina, 1998; Walz *et al.*, 1999). Both peaks of PKA, P-CREB, need of gene expression and protein synthesis are necessary for the consolidation of LTM (Bernabeu *et al.*, 1997; Izquierdo and McGaugh, 2000; Vianna *et al.*, 2000c).

The data on STM are summarized in Figs. 1 (hippocampus), 2 (entorhinal cortex), and 3 (posterior parietal cortex, anterolateral prefrontal cortex, and basolateral amygdala). The findings show that the following molecular systems are necessary for STM formation: (1) glutamate AMPA but not NMDA receptors in the hippocampus, entorhinal, and parietal cortex; cholinergic muscarinic receptors in prefrontal cortex and hippocampus, (2) β-noradrenergic, and 5HT1A receptors in hippocampus and entorhinal cortex, dopaminergic D_1 receptors in prefrontal cortex, hippocampus, and entorhinal cortex; (3) adenylyl cyclase, PKA, phosphoinositide-3-kinase, Tyr-kinases, guanylyl cyclase, PKG, $PKC_{\alpha/\beta II}$ but not other isoforms of PKC in hippocampus; (4) the MAPK pathways in entorhinal cortex but not hippocampus (Walz *et al.*, 1999). Hippocampal CaMKII (Izquierdo *et al.*, 2000). Gene expression and protein synthesis in CA1 are not required for STM (Izquierdo *et al.*, in press-a,b), as is the case with short-term synaptic potentiation in CA1 (see Frey and Morris, 1998 and Izquierdo and McGaugh, 2000 for references). Indeed, several of our findings on the molecular pharmacology of STM in CA1 are similar to those that have been reported for short-term potentiation in the same structure; in contrast, those on LTM consolidation are similar, but not identical, to those that have been reported for long-term potentiation (Izquierdo and McGaugh, 2000; Izquierdo and Medina, 1997).

Unlike LTM consolidation, in which the mechanisms mentioned participate sequentially and some are prerequisite for others (see above) most of the mechanisms that participate in STM formation appear to be necessary in the first few minutes after training (Izquierdo *et al.*, 1999), with one important exception: PKA activity, which is needed for STM during at least 1 h after training, and is in this case not linked to $CREB_1$ phosphorylation (Vianna *et al.*, 2000c).

← training. Immediate or working memory depressed by CNQX, MUS, SCOP, and SCH given into the posterior parietal cortex, by CNQX, MUS, and SCH given into the anterolateral prefrontal cortex, by CNQX, MUS, and SCOP given into the entorhinal cortex, and by CNQX, MUS, SCOP, and SCH given into hippocampus. It was not affected by any of the treatments shown here given into the basolateral amygdala. It is, however, enhanced by oxotremorine and depressed by scopolamine given into the basolateral amygdala (Barros *et al.*, 2001a).

Note that in the preceding lines we have referred to LTM consolidation and to STM formation, not consolidation. It is perhaps inappropriate to speak about the consolidation of STM, since STM vanishes quickly: it lasts 4.5 h or less (Izquierdo *et al.*, 1998a; Medina *et al.*, 1999).

DISSECTION OF WM FROM STM

Much of the earlier literature, specially the clinical literature, confuses STM with WM. More recent accounts differentiate clearly between the two. WM is a transient, nonarchival system that relies basically on the prefrontal cortex (Goldman-Rakic, 1996) and operates within a time frame of seconds. STM involves instead other brain areas (see above and Figs. 1–3), and holds *the same memory* that then becomes LTM for several hours (Izquierdo *et al.*, 1999; McGaugh, 1966, 2000), a topic that will be dealt with below. The data shown in Fig. 4 and elsewhere (Barros *et al.*, 20001a,b; Bianchin *et al.*, 1999; Izquierdo *et al.*, 1998c) shows the effect of several treatments on WM. They given by infusion into the desired structures 5 min before training. Immediate or working memory was depressed by CNQX, MUS, SCOP, and SCH given into the posterior parietal cortex, by CNQX, MUS, and SCH given into the anterolateral prefrontal cortex, by CNQX, MUS, and SCOP given into the entorhinal cortex, and by CNQX, MUS, SCOP, and SCH given into hippocampus. It was not affected by any of the treatments shown in Fig. 4 into the basolateral amygdala. It is, however, enhanced by oxotremorine and depressed by scopolamine given into the basolateral amygdala (Barros *et al.*, 2001a).

These findings (Izquierdo *et al.*, 1998b) show that in addition to dopamine D_1 receptors in the anterolateral prefrontal cortex, as studied by Goldman-Rakic (1996), many other receptor systems in at least five other regions of the brain regulate, or are in charge of, WM.

Several of the treatments shown here affect WM and STM simultaneously in the hippocampus, and others affect WM and LTM simultaneously in the prefrontal cortex and the amygdala. These findings suggest links between AMPA glutamatergic, D_1 and cholinergic receptor mediated processes between the three different memory types, and these links involve different brain areas. Figure 4 omits reference to a recently discovered cholinergic muscarinic mechanism in the basolateral amydala (Beninger *et al.*, 2001) that affects WM both for spatial learning and for the one-trial avoidance task. In the latter case, it regulates WM and LTM (Barros *et al.*, 2001a).

A CASE FOR INTERMEDIATE MEMORY?

At one time or other, several authors who have assumed for no good reason that STM is not separate from LTM but merely a stage of it have proposed that there is an "intermediate" form of memory (ITM) that links both. The sheer existence of ITM has been contested recently (see Markowitsch, 1997) simply on grounds that there is no real evidence of anything like that in laboratory animals or humans.

However, very recent experiments have convincingly described a form of memory intermediate between STM and LTM that may indeed by called "intermediate-type memory" both for serotonin induced facilitation and for sensitization in the mollusk *Aplysia* (Sutton *et al.*, 2001, 2002). These two paradigms are related and the former is often used as a model for the latter. It may be remembered here that the first data suggesting a dichotomy between STM and LTM were obtained using the serotonin-induced facilitation model of electrophysiological plasticity in *Aplysia* (Emptage and Carew, 1993). Further, an ITM-like process triggered by massed trial learning has been described in a form of habituation learning in the crab (Hermitte *et al.*, 1999).

As happened with the division between STM and LTM, where the first inkling was given by data from *Aplysia* (Emptage and Carew, 1993) and were then confirmed by studies on mammalian behavior, it may well turn out that there is an ITM-like process in mammalian memory too. Indeed, there are some indirect indications of that in studies on the amnesic effect of posttraining exposure to a novel environment (Medina *et al.*, 1999) on STM and LTM: in the first 3 h after training, STM measured in the first 3 h after training was insensitive to that procedure. It became partially sensitive to novelty-induced retrograde amnesia between 3 and 6 h after training. At 6 or more h, when memory became for all practical purposes "pure" LTM, it became fully sensitive to that effect (Medina *et al.*, 1999). This suggests that memories of different durations (3 h or less, 3–6 h, 6 h or more) are differentially affected by one given posttraining treatment (exposure to novelty) and may thus be regarded as being of three different types. Certainly other treatments should be studied in order to ascertain this.

A problem inherent to the comparison between findings on synaptic facilitation or sensitization in *Aplysia* (Emptage and Carew, 1993; Sutton *et al.*, 2001, 2002) and memory in rats (Izquierdo *et al.*, 1999) is that the duration of the various memory types is very different. STM lasts at least 3 h in the rat; short-term serotonin-induced facilitation in *Aplysia* lasts at most a few minutes (Emptage and Carew, 1993), and the STM of sensitization lasts less than 30 min (Sutton *et al.*, 2002). The ITM of sensitization lasts around 90 min in *Aplysia*, and its LTM has seldom been measured beyond 24 h (Sutton *et al.*, 2001, 2002). The phase that may be likened to ITM in the rat occurs between 3 and 6 h after training (Medina *et al.*, 1999); LTM of inhibitory avoidance in the rat lasts at least nearly 2 years (Barros *et al.*, in press). Further research will determine whether there is a real type of memory in mammals that may be compared what Carew and his coworkers have convincingly described as ITM in the mollusk. Should there be such a memory type in mammals, its demonstration will enhance the understanding of memory as a whole as a composite of various systems acting in parallel in the same nerve cells, and functionally linked to one another. Perhaps the detailed study of the memory of multitrial tasks in laboratory mammals, as has been done by Sutton *et al.* (2001, 2002) in the mollusk *Aplysia* or by Maldonado and his group in the crab *Chasmagnathus* (Hermitte *et al.*, 1999; Locatelli *et al.*, 2002) may lead to the detection of an ITM in mammals. This, as discussed above, has not been yet possible in rats or humans (Markowitsch, 1997). An experiment should be devised that selectively disrupts ITM in mammals without hindering either STM or LTM.

LINKS BETWEEN STM AND LTM

As said above, the *cognitive content* of STM and LTM is, by definition (James, 1890; McGaugh, 1966), the same. The same constellation of stimuli lead to basically the same response both shortly after training (STM) or many hours or days after training (LTM). Rats refrain from stepping down from the training platform both when they are requested to use their STM and when they are requested to use their LTM. Indeed, test step-down latencies are similar in control animals when these are tested 1.5, 3.0, or 24 h after training (Figs. 1–3).

The fact that many treatments can simultaneously affect both memory types when given immediately after training suggests that the same synaptic networks must be involved in STM and LTM. This must indeed be so (Izquierdo *et al.*, 1998a, 1999; James, 1890) lest the subjects experience a gap between both. Functionally, from the point of view of retrieval efficiency, STM must fade while LTM builds up in order that the mnemonic function remains functional from the moment of acquisition on (Medina *et al.*, 1999). Thus, in spite of their separate nature, which is dictated by the fact that different molecular systems are in charge of STM and LTM, there must be links between these two memory types, and these links must involve directly or indirectly the same synapses.

The fact that many treatments affect STM and LTM in the same way (Figs. 1–3) suggests links between the two (Barros *et al.*, 2001a,b; Izquierdo *et al.*, 1998b,c; Pereira *et al.*, 2001a,b). For example, AMPA, NMDA, and metabotropic receptors in hippocampus (Fig. 1), AMPA receptors in both entorhinal and parietal cortex (Figs. 2 and 3), PKA early after training in hippocampus (Fig. 1), cholinergic muscarinic mechanisms in all regions studied (Figs. 1–3), PKC and guanylyl cyclase in entorhinal cortex (Fig. 2) but not hippocampus (Fig. 1), and BDNF-triggered events in the CA1 region (Alonso *et al.*, in press) act possibly at the same time on both memory types. The latter may be related to MAPK-dependent processes (see paper by Alonso *et al.* in this issue).

Interestingly, MAPK and dopamine D_1 receptors appear to exert opposite roles in hippocampus and entorhinal cortex concerning STM and LTM regulation (Figs. 1 and 2). While the MAPK in hippocampus appears initially necessary for STM but not for LTM, the inhibition of this pathway by PD098059, or the stimulation of D_1 receptors by SKF38393 actually enhances STM in hippocampus while inhibiting LTM in the entorhinal cortex (see Izquierdo *et al.*, 1998c and Walz *et al.*, 1999).

FACTS AND SPECULATIONS ABOUT THE MECHANISMS OF STM

At this stage it is premature to hypothesize on which of the events listed above as involved in the formation of STM are prerequisites of the others. Contrarily to the process of LTM consolidation in which some events precede and are necessary for others, the molecular events involved in, and necessary for, STM occur very shortly after training. It is simply not possible to discriminate among them by the use of infusion procedures; let alone by biochemical analysis. The infusion procedure we used takes about 3 min (Izquierdo *et al.*, 1999, 2000, in press-a,b) and the infusates

remain in the attained region for several minutes, with a presumable time constant of diffusion away from it of around 20 min (Martin, 1991). The only treatments studied that affect STM for more than 20 min are PKA inhibitors (Vianna *et al.*, 1999, 2000b) and PKC inhibitors (Vianna *et al.*, 2000a), which block STM when given up to 1 h after training.

The mix of molecular systems that underlie or are involved in STM do not include the activation of constitutive transcription factors, gene expression or protein synthesis (see above); these are, instead, absolute requisites for LTM consolidation (Izquierdo *et al.*, in press-a,b).

Certainly it is logical to think that the events that involve glutamatergic receptors must precede all the other metabolic events, and that the protracted need for hippocampal PKA activity (Vianna *et al.*, 2000b,c) must rely on some of the other events, such as the up-regulation of adenylyl cyclase by dopamine D_1 or β-adrenoceptors in hippocampus.

Since STM (and WM) do not require on-going protein synthesis, their underlying molecular basis must depend on posttranscriptional events. Among these, the three best candidates at this stage are: (a) a sustained enhancement of AMPA receptor binding properties; (b) the exocytosis of cell adhesion-related glycoproteins; and (c) events dependent on the formation of polyribosomal aggregates. The three types of events have been described following behavioral training (Izquierdo *et al.*, in press a,b), and the first and the third have also been described following the induction of long-term potentiation in the CA1 region of the hippcampus (Sergueeva *et al.*, 1993; see Murphy and Regan, 1998 for references).

The increase in synaptic strength that follows one-trial avoidance training in rats by enhanced sensitivity of AMPA receptors (Cammarota *et al.*, 1996) was interpreted early on as CaMKII-dependent (Cammarota *et al.*, 1998). However, it may well depend on receptor phosphorylation by PKA or PKC (see Izquierdo, *et al.*, in press, b). Increased AMPA receptor function may by itself explain a potentiated state of synapses that have been recently activated as a result of the stimuli involved in training (see Cammarota *et al.*, 1996, 1998).

Glycoprotein synthesis has been much better studied than exocytosis so far, and exocytosis is much more likely to be involved in STM than synthesis: STM is insensitive to inhibitors of gene expression or protein synthesis (Izquierdo *et al.*, in press-a,b). The synthesis of glycoproteins following inhibitory avoidance training occurs, in the hippocampus of rats and in the chick brain, in at least three peaks: one starting at the time of training or immediately after (Murphy and Regan, 1998; Rose, 2001), another 5–7 h after training (O'Connell *et al.*, 1997, 2000; Rose, 2001), and ill others later on (O'Malley *et al.*, 1998). The glycoproteins whose synthesis and release is triggered by training experiences include cell adhesion molecules. Conceivably, synaptic transmission can remain enhanced for long times as a result of increased cell adhesion. Early exocytosis of some of these proteins may thus keep recently stimulated synapses "hot" for a long while, while subsequent processes underlying first synthesis of these molecules, and then protein-synthesis dependent structural changes are slowly built up in the same synapses that had been kept "hot" by enhanced cell adhesion (Murphy and Regan, 1998; O'Malley *et al.*, 1998, 2000).

Still another cellular event that could well be at the root of STM genesis and maintenance are the very rapid (<1–2 min), glutamate metabotropic receptor-mediated increase of postsynaptic polyribosome aggregates and ^{35}S-methionine incorporation into polypeptides described seven years ago by Weiler *et al.* (1995).

The tagging of synapses by rapid changes following stimulation has been described by Frey and Morris (1998) and proposed to be at the root of the synaptic specific changes that lead to the establishment of long-term potentiation lasting more than several hours. There is wide agreement that without some kind of tagging, lasting plasticity restricted to the synapses that have been recently stimulated is impossible. Synaptic tagging as described "in vitro" by Frey and Morris (1998) apparently depends on on-going protein synthesis. However, it may also, or primarily, involve posttranscriptional changes as may result from PKA- or PKC-mediated phosphorylation (Frey and Morris, 1998; see izquierdo and McGaugh, 2000). Any of these could in principle underlie STM.

The late peaks of glycoprotein synthesis (>5 h) are probably related to LTM (or eventually ITM). They involve changes that can be visible in standard histological preparations of the hippocampal region, particularly the gyrus dentatus (O'Connell *et al.*, 1997, 2000), as well as in ultrastructural studies (Kleim *et al.*, 1998; O'Malley *et al.*, 1998). Ramón y Cajal (1893) was the first to formally postulate effective morphological changes in synapses as the basis of lasting memories. After several decades in which it was fashionable among neurobiologists to ignore that postulation, careful histological and electron microscope studies showed that morphological changes effectively occur in brain areas that have been activated by particular behavioral experiences (Jones *et al.*, 1997; Kleim *et al.*, 1998; Klintsova and Greenough, 1999; O'Connell *et al.*, 1997, 2000; Weiler *et al.*, 1995) or even by repeated sessions of long-term potentiation (Geinisman, 2002). The structural changes observed include growth or multiplication of presynaptic terminals and dendritic spines, and are coincident with (O'Connell *et al.*, 2000) or occur slightly after (Bernabeu *et al.*, 1997; Cammarota *et al.*, 2000) the peaks of CREB phosphorylation that follow training. Some of the glycoprotein changes begin very early after training; others occur several hours later (Murphy and Regan, 1998; Ni Dhuill *et al.*, 1999; O'Malley *et al.*, 1998).

CONCLUSIONS

STM must rely basically on some of the nonstructural, biochemical changes that occur early on after training in the synapses activated by that training. The mechanisms of STM should involve processes keep recently activated synapses "hot," while LTM builds up. The phase in which STM mechanisms begin to fade away and those of LTM build up could well correspond to an intermediate type (or phase) of memory, which has been so far not yet demonstrated in mammals.

This postulation is not really original; it merely results from a blend of the much earlier predictions of Ramón y Cajal, and the hypotheses raised by the experiments of Geinisman, Greenough, Regan, and their collaborators.

The intrinsic biochemical steps of STM include the activation of glutamate NMDA, AMPA and metabotropic receptors in CA1, and by AMPA but not NMDA

receptors in entorhinal cortex. In addition, hippocampal PKA, $PKC_{\alpha/\beta II}$, PI-3K, and MAPK activity (the first two during at least 1 h posttraining) and entorhinal PKA and PKC are also necessary for STM formation. CaMKII appears to be excluded from this process in CA1 and entorhinal cortex; and entorhinal MAPK appears to play a normally inhibitory role in STM, opposite to the one it has in the hippocampus. Modulatory cholinergic muscarinic, dopaminergic D_1, β-noradrenergic and 5HT1A systems act differentially in the three brain structures mentioned in order to affect STM. In CA1 and entorhinal cortex the effects of the MAPK inhibitor, PD098059 and of the D_1 agonist, 8-HO-DPAT are surprisingly similar, suggesting an interaction between the two systems. The basolateral amygdala and the anterior prefrontal cortex are not involved in STM.

In addition, here we also review findings indicating that the brain areas and the receptor systems involved in WM regulation (Barros *et al.*, 2001a; Izquierdo *et al.*, 1998c) are far more complex than has hitherto been assumed (see Goldman-Rakic, 1991, 1996).

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