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Memory Retrieval and its Lasting Consequences

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Many, if not all psychiatric diseases are accompanied by memory disturbances, in particular, the dementias, schizophrenia, and, to an extent, mood disorders. Anxiety and stress, on the other hand, cause important alterations of memory, particularly its retrieval. Here we discuss several new findings on the basic mechanisms of consolidation, retrieval and extinction of a prototype form of episodic memory in the rat: conditioned fear. The findings point the way for investigations on the pathology of these aspects of memory in health and disease. Emphasis is placed on the parallel processing of retrieval in several cortical areas, on the links between retrieval and the onset of extinction, on the fact that extinction involves new learning requiring gene expression, and on the differences between the retrieval of recent or remote long-term memories.

Keywords: Memory consolidation; Memory retrieval; Memory extinction; Working memory mechanisms; Remote memory

INTRODUCTION: CONDITIONING, RETRIEVAL AND EXTINCTION

In the first two decades of the XX century, Pavlov described the principles through which stimuli become associated in order to produce memories. Pavlov (1956) reported that learning takes place when a stimulus or constellation of stimuli (conditioned stimulus, CS) is paired with another stimulus (unconditioned stimulus, US) that produces a clear-cut response. The CS is normally a neutral stimulus (light, tone, odour) or group of stimuli (an environment, a training apparatus) that elicits, at most, orienting or investigative responses. The US is what Pavlov used to call a "biologically significant" stimulus or group of stimuli: a foot-shock, food, a pleasant or unpleasant circumstance or situation. The response to the CS becomes changed by its pairing with the US; instead of a mere orienting response, the subject reacts to the CS with a pattern of movements related to the US, called a conditioned response (CR).

It is also known since Pavlov that repeated presentation of the CS without the US results in extinction of that response. Thus, a retrieval session may trigger extinction, if it is carried out without US. Extinction involves a new learning: the CS becomes dissociated from the US, and associated with a new consequence, i.e. the lack of reinforcement. Perception of this new association takes place in the first retrieval session (Vianna *et al.*, 2001; Szapiro *et al.*, 2002).

Upon retrieval, the animals may use the learned response (CR) instrumentally in order to obtain the US, if it is pleasant or rewarding, or to avoid it, if it is unpleasant or punitive. This is called instrumental conditioning, and is extinguished more slowly than classic Pavlovian conditioning (Konorski, 1948).

Retrieval is, of course, the only possible measure of memory (James, 1890), and the fact that it can initiate extinction is important. Our cognitive life consists mainly of memories that are more or less extinguished for the lack of reinforcement. Extinction is not forgetting: extinguished memories are rapidly revived if some kind of reminder stimulus is given, and very particularly, if the original learning situation is recreated (Riccio and Richardson, 2000), including the neurohumoral

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and hormonal conditions present at the time of the original training (Izquierdo, 1989). Extinction consists of a gradual active inhibition of retrieval, which, as will be seen, depends on gene expression, protein synthesis and the participation of several metabolic pathways. Forgetting is, instead, the passive loss of memories, resulting from the loss of neurons or synapses that occurs with the passage of time, or on the mere disuse of synapses (see Eccles, 1957). When the loss is very large and incapacitating, we call it an amnesic syndrome. But, short of that, across a rather indefinable border, there is a vast area of incomplete and missing memories in normal humans, which begins in early life and extends into senility. A 4-year old child does not remember any more where his mother used to keep the pacifiers. A 30-year old woman cannot revive the actual pain suffered during her last delivery. A normal 40- or 70-year old person often does not remember what movie (s)he saw on TV last night, or what was the exchange rate of his country's currency to the dollar 23 years ago. Some of these memories may have been lost; but others were just extinguished, and an appropriate cue might bring them back. Amnesic syndromes do not result from extinction, but from the effective loss of memory functions.

Some patients remember too vividly frightening memories that are often false; this constitutes what we call as phobic disorders (Freud, 1952). The accepted therapy is, exposure to the frightening memory until it extinguishes; often with some demonstration to the patient that the source of the phobia was not as bad as (s)he thinks, or can be circumvented.

The present paper reviews recent findings from our laboratories on memory retrieval, reactivation and extinction measured in rats in the one-trial contextual fear task called inhibitory or passive avoidance (Netto and Izquierdo, 1985; Izquierdo and Medina, 1997; Izquierdo and McGaugh, 2000). These findings may bear on the memory disturbances seen in almost all, if not all, psychiatric disorders. Anxiety, stress, depression, schizophrenia and the various dementias are accompanied by specific disturbances of consolidation and/or retrieval, accompanied by characteristic neurohumoral and/or structural alterations (Cahill and McGaugh, 1998; Danion *et al.*, 2001; Egan *et al.*, 2001). Anxiety, stress and depression are accompanied by altered peripheral and central monoamine levels and by hiperfunction of the pituitary-adrenal system. These affect memory formation and retrieval to various degrees. Schizophrenia and the various dementias are accompanied by distinct morphological changes in areas of the brain that are in charge of mnemonic functions, and specific memory alterations occur in these diseases.

THE CHOICE OF A TASK WHOSE CONSOLIDATION IS WELL STUDIED

Contextual fear is a paradigm much used in rodents that results in a CR that resembles phobia to a degree. In contextual fear, conditioning the CS is the context of a training apparatus and the US is a footshock. Animals learn that a certain region of the apparatus is associated with the shock. Retrieval is tested by presenting the CS alone: i.e. by exposing the animal to the apparatus without the shock. Depending on the conditions of training, the CR is either general immobility (Corcoran and Maren, 2001) or the specific inhibition of some movements (Netto and Izquierdo, 1985). The retrieval test also initiates extinction of the CR, that is, *new* learning, that the CS no longer predicts the US (Konorski, 1948; Pavlov, 1956; Vianna *et al.*, 2001).

Here, we used a form of contextual fear called one-trial step-down inhibitory (passive) avoidance. Rats are placed on a platform (CS) and received a footshock (US) when they step down from it onto a grid. Animals learn to remain longer on the platform than they do on the training session (CR). The name "avoidance" should not obscure the fact that this is not really an instrumental task: it is acquired through a *single* CS (context)-US (footshock) pairing, which implies an impossibility for the animal to actually use the CR (to stay in a safe platform or compartment) and instrumentally avoid the US. When the animals are tested, they are exposed to the CS alone, which is the method of choice for initiating extinction. In fact, the long-term memory of one-trial avoidance is extinguished by repeated testing at 24 h intervals (Izquierdo *et al.*, 1999b).

The one-trial paradigm is a task of choice for memory studies (Gold, 1986), for three reasons. First, it is acquired in a few seconds, so the training session can be easily separated from the ensuing consolidation, and stages of the consolidation process can be easily discriminated from each other (Izquierdo and Medina, 1997). Second, it permits a clear-cut distinction between learning, retrieval and extinction (Vianna *et al.*, 2000b; 2001). Third, the consolidation of this task is now very well known to rely on the CA1 region of the hippocampus, in association with major hippocampal connexions: the entorhinal, parietal and cingulate cortex, plus the medial septum and the basolateral amygdala (Izquierdo *et al.*, 1992; Izquierdo and Medina, 1997). It is impossible to differentiate learning from consolidation, retrieval and extinction in multi-trial tasks; in successive trials these processes becomes hopelessly mixed.

The series of events leading to long-term memory consolidation of the one-trial avoidance task in the hippocampus involves: (a) initially, an activation of glutamate AMPA, NMDA, and metabotropic

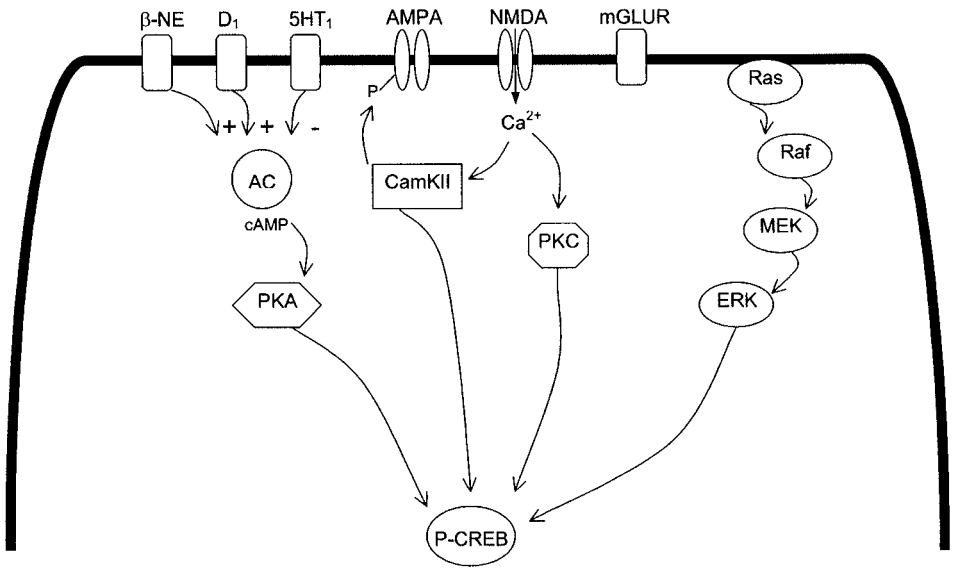

FIGURE 1 The main receptors, enzyme pathways and substrates that need to be activated in CA1 pyramidal cells in order for memory of a contextual fear task (inhibitory avoidance) to be formed. Glutamate AMPA, NMDA and mGLUR (metabotropic) receptors are necessary. Activation of the NMDA receptors and of Ca^{2+} channels increase intracellular Ca^{2+} levels, which stimulates CaMKII and PKC. CaMKII phosphorylates the receptive subunit of the AMPA receptor. β-NE, dopamine D_1 and $5HT_{1A}$ receptors modulate adenylyl cyclase (AC), the enzyme that synthesizes cAMP from ATP. CAMP binds to the regulatory subunit of PKA and activates the enzyme. Meanwhile, the MAPK pathway (or Ras-Raf-MEK-ERK pathway, see Walz *et al.*, 1999 and Szapiro *et al.*, 2000) becomes activated in parallel, perhaps as a consequence of stress. PKA, MAPK, CaMKII and PKC all may phosphorylate and thereby activate the nuclear constitutive factor CREB, which triggers transcription and in consequence protein synthesis. In physiological conditions, probably PKA is the main signaling pathway involved in the phosphorylation of CREB.

(mGluR) glutamate receptors (Izquierdo and Medina, 1997); (b) immediately after, an activation of calcium/calmodulin kinase II (CaMKII), which phosphorylates and activates the AMPA receptor (Cammarota and Bernabeu, 1998); (c) a dual activation of the cAMP-dependent protein kinase A (PKA), first immediately after training, and then again 3–6 h later (Bernabeu *et al.*, 1997; Vianna *et al.*, 2000b); (d) an increase of the phosphorylated state of the constitutive transcription factor CREB (cAMP response element binding protein) in the nucleus of CA1 cells, simultaneously with the PKA peaks (Bernabeu *et al.*, 1997; Taubenfeld *et al.*, 1999); (e) a late (3 h) increase of sensitivity in the amnestic effect of inhibitors of the mitogen-activated protein kinase (MAPK) given into CA1 (Walz *et al.*, 1999); and (f) a dual peak of sensitivity to inhibitors of transcription (Fig. 1) or of protein synthesis (Quevedo *et al.*, 1999), coincident in time with the two PKA/P-CREB peaks. Steps (c) through (d) depend on the previous activation of NMDA receptors in CA1: they do not take place if the NMDA antagonist, AP5, is infused into that region immediately after training (Cammarota *et al.*, 2000).

A CAVEAT ON THE BIOCHEMISTRY OF MEMORY

It must be pointed out that the biochemical events that follow training, themselves, do not carry the information pertinent to the memory that is formed. Long-term memories rely on synaptic changes, both morphological and functional, which underlie retrieval when the appropriate stimuli are presented in test sessions (Ramón y Cajal, 1893; Kandel and Squire, 2000; Geinisman, 2002). The code of memories is synaptic, and not metabolic.

There is reason to believe that the synaptic changes that underlie memories depend on the late phase of gene transcription and the resulting protein synthesis (Kandel and Squire, 2000; Muller *et al.*, 2002; Regan, 2002). The molecular events that take place between receptor stimulation and protein synthesis are mere boosters of the signals that are needed in order for this transduction process to take place. They are, however, necessary boosters; if they are blocked, consolidation does not take place and memories are not formed.

WORKING MEMORY

Working memory, as given in Baddeley (1997), is defined as a non-archival system located mainly in the prefrontal cortex that operates on-line or at the most, for a few seconds or minutes after the training experience (Goldman-Rakic, 1996; Izquierdo *et al.*, 1998c). Working memory is in charge of the sensorimotor gating that is essential for an adequate perception of reality and its comparison with

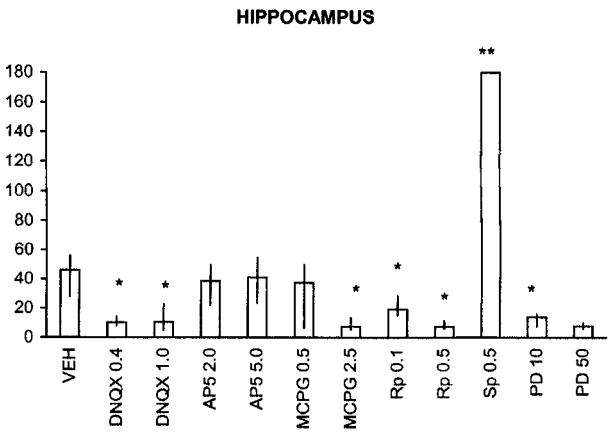

FIGURE 2 Molecular mechanisms of retrieval in CA1 region of the hippocampus. The following drugs were infused bilaterally into each of these structures 10 min prior to a test session of the one-trial avoidance task, carried out 24 h after training: the AMPA receptor blocker, DNQX (0.4 or 1.0 μg/side); the NMDA receptor blocker, AP5 (2.0 or 5.0 μg/side); the mGluR antagonist, MCPG (0.5 or 2.5 μg/side); the inhibitor of PKA, Rp-cAMPs (Rp, 0.1 or 0.5 μg/side); the stimulant of PKA, Sp-cAMPs (Sp, 0.5 μg/side); and the MAPKK inhibitor, PD098059 (PD, 10 or 50 μM on each side). Retrieval was inhibited by DNQX, MCPG, Sp and PD, enhanced by Sp, and not affected by AP5. Thus, retrieval requires intact AMPA receptors and mGluRs, PKA and MAPK.

preexisting memories (Geyer *et al.*, 2001). This is deficient in schizophrenia, and constitutes one of the cardinal symptoms of this disease (Artiges *et al.*, 2000). It is distinct from short- and long-term memory (see below), and leaves no detectable biochemical trace.

Working memory is regulated by dopamine D1 and cholinergic muscarinic receptors in the antero-lateral prefrontal cortex (Izquierdo *et al.*, 1998) and by muscarinic cholinergic mechanisms in the basolateral amygdala (Beninger *et al.*, 2001; Izquierdo *et al.*, 2002).

Schizophrenics usually report that they perceive reality as complex and often menacing ("I see everything at the same time", "all that surrounds me is coming at me"). This reveals a failure of sensorimotor gating, which results from an insufficient degree of prepulse inhibition, i.e. by the mechanism by which stimuli are able to reduce perception of others that follow (Geyer *et al.*, 2001). The result of this is a deficiency of working memory functions (Artiges *et al.*, 2000).

Biochemical Mechanisms Involved in the Retrieval of Long-term Memory

Both retrieval (Barros *et al.*, 2000; Eldridge *et al.*, 2000) and extinction (Corcoran and Maren, 2001) are processed by the hippocampus. In particular, it has been recently shown that there is a selective activation of CA1 neurons during the recall of contextual memories (Hall *et al.*, 2001). However, as will be seen, recent findings indicate that several other cortical structures participate in retrieval along with the hippocampus.

The effect on retrieval of the bilateral infusion of the antagonist of NMDA glutamate receptors, AP5 (D-2-amino-5-phosphonovalerate, 2.0 or 5.0 μg/side), the antagonist of AMPA receptors, DNQX (0.4 or 1.0 μg/side), the generic glutamate metabotropic receptor antagonist, MCPG (0.5 or 2.5 μg/side), the PKA inhibitor, Rp-cAMPs (0.1 or 0.5 μg/side), the PKA stimulant, Sp-cAMPs (0.5 μg/side), and the MAPK inhibitor PD098059 (10 or 50 μM) was studied in the CA1 region of the rat hippocampus, as reported elsewhere (L.A. Izquierdo *et al.*, 2000) (Fig. 2). Here, we show the effect of the same treatments when given bilaterally 10 min before retention testing into the entorhinal cortex (Fig. 3a), posterior parietal cortex (Fig. 3b), anterior cingulate cortex (Fig. 3c) and basolateral amygdala (Fig. 3d). Control animals received the vehicle in which the drugs were dissolved (2% DMSO in saline). Infusion volume was 0.5 μl per side in all cases in this and all following experiments using localized drug administrations. Infusions were 10 min prior to retrieval. Retrieval was measured 24 h after training. Retrieval is immediately followed by an increase in the activity of two enzymes of the MAPK pathway, p42 and p44 in CA1 (Szapiro *et al.*, 2000).

As can be seen, in all the cortical structures, MCPG, Rp-cAMPs and PD098059 depressed, and Sp-cAMPs enhanced retrieval performance. DNQX depressed retrieval when given into CA1, entorhinal or parietal cortex, but not when given into the anterior cingulate cortex. AP5 depressed retrieval when given into the parietal or anterior cingulate cortex but not when given into CA1 or entorhinal cortex. The mGluR antagonist, MCPG, was effective when given into any of the cortical structures studied. Therefore, the enzyme requirements for retrieval (PKA, MAPK) were similar in all the cortical structures studied, but the type of glutamate receptors required was different in each case: mGluRs were required in all cortical areas, AMPA receptors were required in all areas except the cingulate cortex, and NMDA receptors are required for retrieval in parietal and cingulate cortex (Barros *et al.*, 2000)

It may be added here that, additionally, we have shown that the bilateral infusion into CA1 of a specific inhibitor of the protein kinase C (PKC) α/βII isoform(s) (Vianna *et al.*, 2000a), or of an inhibitor of tyr-protein kinases (Pereira *et al.*, 2001) inhibits retrieval. This suggests an additional involvement of PKC α/βII and of tyr-kinases in CA1 in retrieval. On the other hand, the CaMKII inhibitor, KN-62 has no effect on retrieval when given into CA1 suggesting that this enzyme is not involved in this process (Izquierdo *et al.*, 2000).

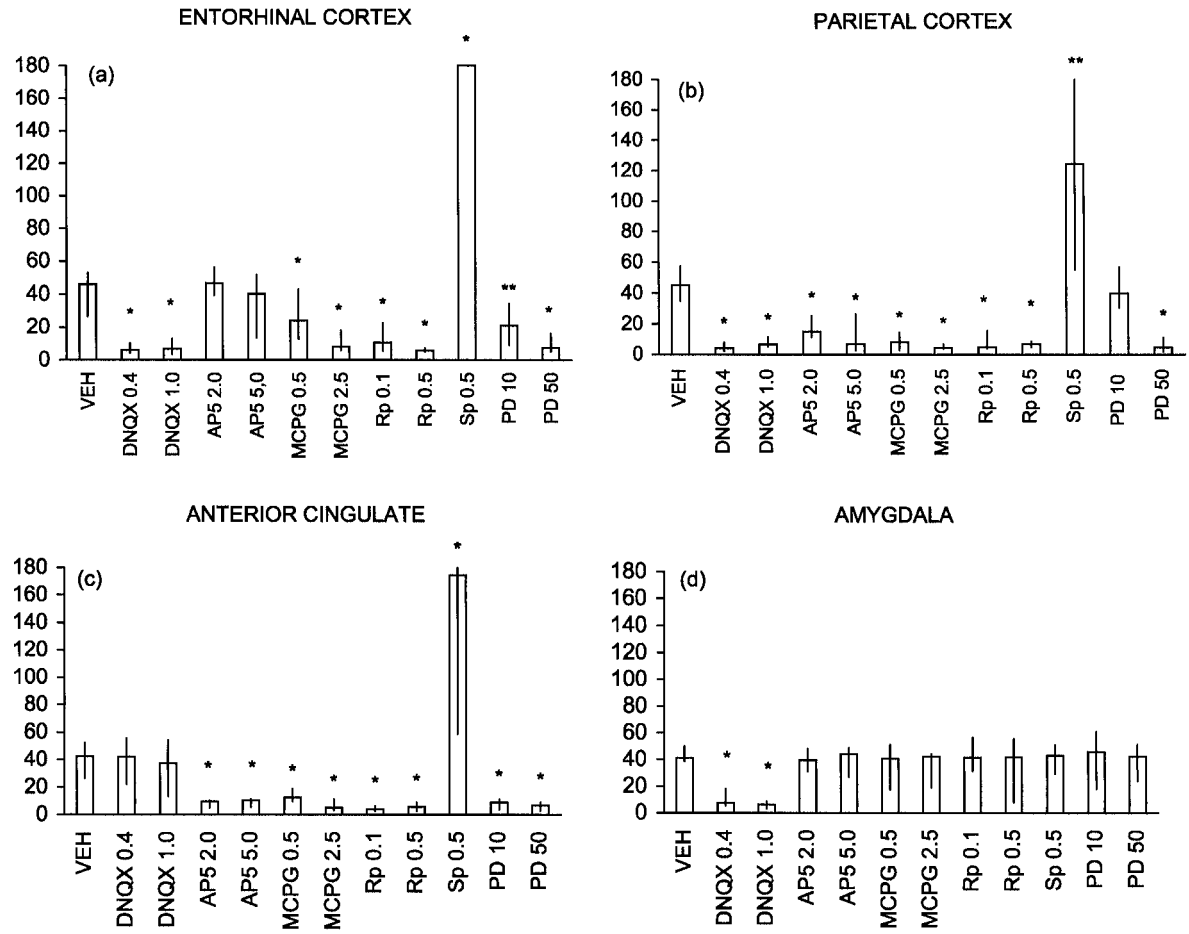

FIGURE 3 Same as Fig. 2, but with bilateral infusions of the drugs into: a, entorhinal cortex; b, posterior parietal cortex; c, anterior cingulate cortex; d, basolateral nuclear complex of the amygdala. DNQX inhibited retrieval in all structures except the cingulate cortex. AP5 inhibited retrieval only when given into the parietal or anterior cingulate cortex. MCPG, Rp and PD inhibited retrieval, and Sp stimulated retrieval, in all structures except the basolateral amygdala (Barros *et al.*, 2000). Therefore, different types of glutamate receptors and PKA and MAPK are required for retrieval in hippocampus, entorhinal cortex, parietal cortex and anterior cingulate cortex. In the basolateral amygdala the only requirement for retrieval appears to be intact AMPA receptors.

Thus, retrieval of long-term memory of the one-trial task involves the simultaneous operation of a rather complex molecular machinery in at least four different cortical areas of the brain; the only differences among areas reside in the differential involvement of glutamate receptor types in each region.

With the exception of DNQX, none of the other substances had any effect when given bilaterally into the basolateral amygdala (Fig. 3d). This does not detract from the importance of this structure in retrieval (e.g. de Quervain *et al.*, 1998); it merely shows that it participates using regular glutamatergic transmission without a necessary involvement of signaling pathways. It is possible that the role of the basolateral amygdala in the retrieval of one-trial avoidance may be modulatory, as it is in the consolidation of that task (Izquierdo *et al.*, 1992; Cahill *et al.*, 1999; Izquierdo and McGaugh, 2000).

In all cases, behavioral controls were carried out studying the effect of these substances, given at the same doses into the same structures on open-field and plus-maze behavior, in order to rule out possible influences of the treatments on locomotion, exploration or conflict behavior. None of the treatments had any such effect; therefore, their influence on retention testing can be interpreted in terms of actual retrieval and not of non-specific actions on motility (Izquierdo *et al.*, 2000; Barros *et al.*, 2002).

The Modulation of Retrieval by Monoamines and Acetylcholine

Dopaminergic D_1, β-noradrenergic, serotonergic 1A and muscarinic cholinergic receptors in the hippocampus and other areas of the brain modulate the consolidation of short- and long-term memory (Izquierdo *et al.*, 1999a; Izquierdo and McGaugh, 2000). We decided to find out whether they are also involved in the modulation of retrieval of the one-trial task in the areas in which we had studied the molecular mechanisms of retrieval (see preceding section). Dopaminergic D_1, β-noradrenergic, serotonergic 1A and muscarinic cholinergic receptors

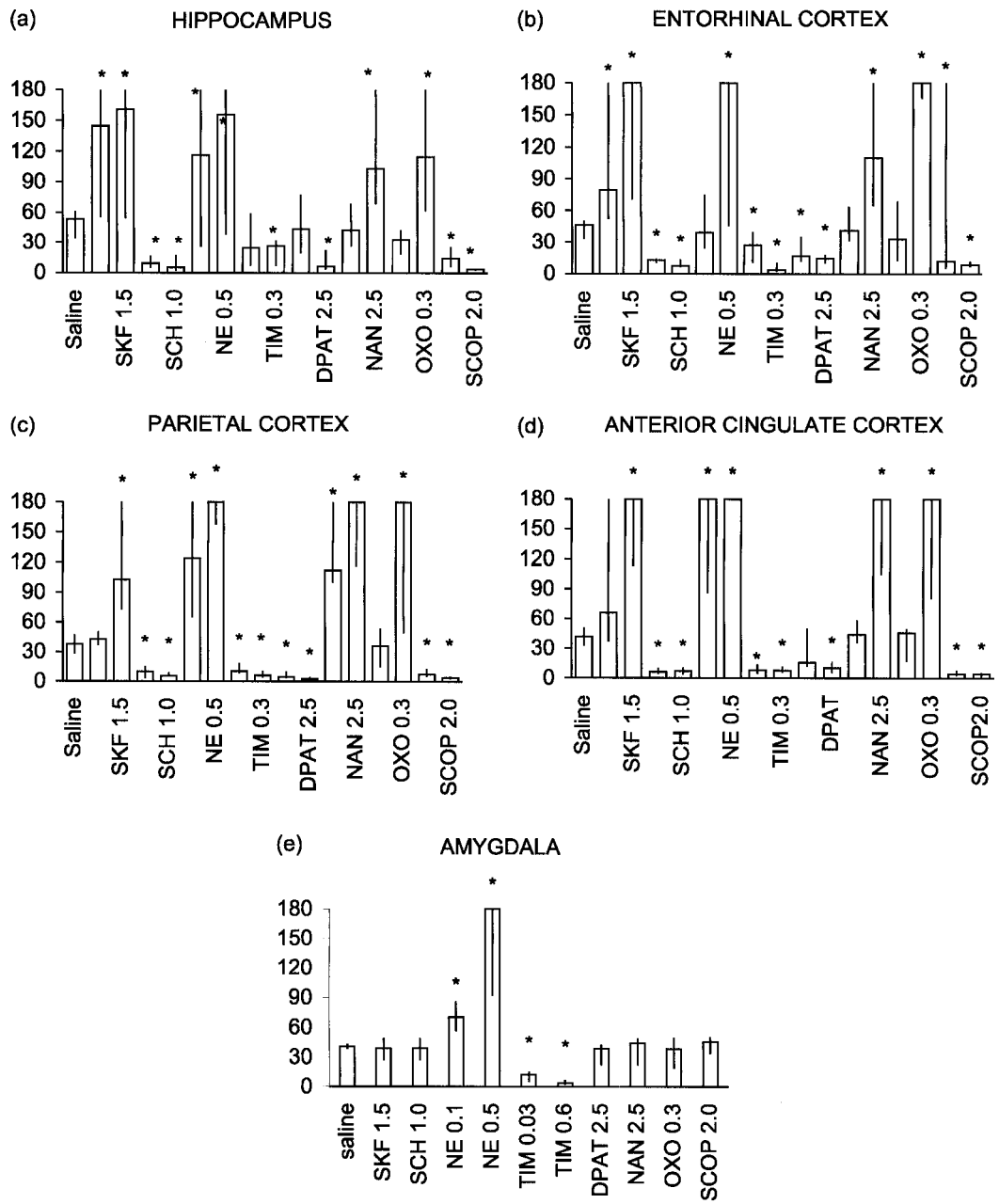

FIGURE 4 Modulation of retrieval in a, CA1 region of the hippocampus; b, entorhinal cortex; c, posterior parietal cortex; d, anterior cingulate cortex. The following drugs were infused bilaterally into each of these structures 10 min prior to a test session of the on-trial avoidance task: the Dopamine D1 agonist, SKF38393 (SKF, 0.3 or 1.5 μg/side); the D1 antagonist, SCH23390 (SCH, 0.2 or 1.0 μg/side); norepinephrine (NE, 0.1 or 0.5 μg/side); the β-blocker, timolol (TIM, 0.05 or 0.3 μg/side); the 5HT1A agonist, 8-HO-DPAT (DPAT, 0.5 or 2.5 μg/side); the 5HT1A antagonist, NAN-190 (NAN, 0.5 or 2.5 μg/side); the cholinergic muscarinic stimulant, oxotremorine (OXO, 0.05 or 0.03 μg/side); and the muscarinic receptor antagonist, scopolamine (SCOP, 0.4 or 2.0 μg/side). In all structures, retrieval was enhanced by SKF, NE, NAN and OXO, and depressed by SCH, TIM, DPAT and SCOP. In the basolateral amygdala, thus, dopaminergic D1, β-noradrenergic, 5HT1A and cholinergic muscarinic receptors modulate retrieval simultaneously in CA1, entorhinal cortex, posterior parietal cortex and anterior cingulate cortex. D1, β and muscarinic receptors facilitate, and 5HT1A receptors inhibit retrieval. In the basolateral amygdala (E), modulation is restricted to a stimulant role of β-noradrenergic receptors: only two of the drugs had an effect: NE, which enhanced retrieval, and TIM, which depressed retrieval (see Barros *et al.*, 2001).

are widely believed to respond to levels of alertness and anxiety, and to be involved in the perception and reaction to anxiety, stress and mood changes (Izquierdo and McGaugh, 2000).

Animals received bilateral infusions in the CA1 area of the hippocampus (Fig. 4a), or the entorhinal (Fig. 4b), posterior parietal (Fig. 4c) or anterior cingulate cortex (Fig. 4d) of the following drugs: the dopamine D_1 agonist, SKF38393 (0.3 or 1.5 μg/side), the D_1 antagonist, SCH23390 (0.2 or 1.0 μg/side), norepinephrine (0.1 or 0.5 μg/side), the β-adrenoceptor antagonist, timolol (0.03 or 0.06 μg/side), the 5HT1A agonist, 8-HO-DPAT (0.5 or 2.5 μg/side), the 5HT1A antagonist NAN-190 (0.5 or 2.5 μg/side),

the cholinergic muscarinic stimulant, oxotremorine (0.06 or 0.3 μg/side), or the muscarinic receptor antagonist, scopolamine HBr (0.4 or 2.0 μg/side). Control animals received saline; drugs were dissolved in saline. Infusion volume was 0.5 μl in all cases. Infusions were 10 min prior to retrieval, 24 h after training.

The effect of the various treatments was almost identical in all four brain areas: CA1 (Fig. 4a), entorhinal cortex (Fig. 4b), posterior parietal cortex (Fig. 4c) and anterior cingulate cortex (Fig. 4d). In all cases, SKF38393, norepinephrine, NAN-190 and oxotremorine enhanced, and SCH23390, timolol, 8-HO-DPAT and scopolamine depressed retrieval performance. Therefore, it can be concluded that through simultaneous actions upon all the cortical regions studied, dopamine D1, beta-noradrenergic and muscarinic cholinergic receptors enhance, and 5HT1A receptors depress, long-term memory retrieval. It seems likely that these modulatory influences underlie the changes in retrieval seen in circumstances of anxiety, stress or alterations of mood; such as the flashbacks or "blanks" that occur in such conditions (Barros *et al.*, 2001).

Again, as in the previous experiment, here none of the treatments was found to alter open-field or plus-maze behavior (Barros *et al.*, 2000; 2001), so their influence on retention testing may be genuinely interpreted as due to an effect on retrieval.

Thus, in addition to relying on a relatively complex molecular machinery acting simultaneously in four different cortical areas, retrieval can be strongly modulated by drugs that act on the main receptors that are known to be activated by alertness, mood, emotion and affect, in those same brain areas. The receptor agonists and antagonists used here are known to influence the PKA and PKC signaling pathways indirectly: the monoamines by influences on adenylyl cyclase, and the muscarinic agents by influences on PKC or other pathways (see Izquierdo and Medina, 1997; Barros *et al.*, 2001). It is conceivable that their action on retrieval is mediated by such influences.

This and the preceding experiment show that retrieval of a memory as simple as that of one-trial avoidance does not involve exclusively the hippocampus. It is, instead, dependent on strongly modulated parallel processing involving a variety of receptors and signaling pathways at four different cortical regions.

Extinction of Long-term Memory

As commented above, retrieval performed in the absence of a US initiates extinction (Pavlov, 1956). Extinction is perhaps the most visible consequence of retrieval. A memory whose retrieval is reiterated often enough, without the US, will fade; and this has important adaptive consequences. A brain clogged with memories that never fade, will be, for most purposes, a rather useless brain, as Borges's famous story "Funes the Memorious" showed. In that story, the central character, Funes, is condemned to remember everything all the time; for example, a whole day of his life. But in order to retrieve an entire day of his life he needs another entire day of his life, so he is unable to move on. As Borges states, one needs to extinguish (or forget) memories in order to be able to make generalizations.

Another consequence of retrieval is that it may be entangled with other memories that are being formed or retrieved at the same time, thus forming complex and often false memories. This is found frequently in the elderly, who mix events or facts about one person (for example, the son) with those about another (for example, a brother).

Extinction of the long-term retrieval of the one-trial fear task has been shown to occur upon repeated testing over several days (Izquierdo *et al.*, 1999b). Berman and Dudai (2001) recently reported that the extinction of conditioned taste aversion is prevented by the infusion of anisomycin into the insular lobe at the time of the first retention test of a series. This finding suggested that extinction requires protein synthesis. The insula is crucial for the consolidation of conditioned taste aversion (Dudai *et al.*, 1995).

Intrahippocampal infusion of the transcription blocker, DRB did not affect retrieval measured 15 min later, but blocked extinction measured on three successive test sessions carried out 24, 48 and 72 h after the first test (Fig. 5). The effect is similar to that reported previously for the protein synthesis inhibitor, anisomycin (Vianna *et al.*, 2001). These results indicate that the initiation of extinction requires, like consolidation, new mRNA synthesis.

Intrahippocampal administration of muscimol, a $GABA_A$ receptor agonist, blocks context-specific extinction of a fear conditioning task related to one-trial avoidance (Corcoran and Maren, 2001). The indirect $GABA_A$ antagonist, picrotoxin, enhances extinction of one-trial avoidance when given systemically (McGaugh *et al.*, 1990). AP5 given into the basolateral amygdala hinders extinction of fear-potentiated startle (Falls *et al.*, 1992).

We decided to find out whether the infusion into CA1 of the NMDA receptor antagonist, AP5 (5.0 μg/side), the PKA inhibitor, Rp-cAMPs (0.5 μg/side), the CaMKII inhibitor, KN-62 (0.1 μg/side) and the MAPK inhibitor, PD098059 (50 μM), given 15 min prior to a retrieval test 24 h after training, affected extinction measured by the retrieval performance in further retention tests.

The effect of bilateral infusions into CA1 of AP5, KN-62, Rp-cAMPs and PD098059 given prior to retrieval on further retrieval sessions carried out one

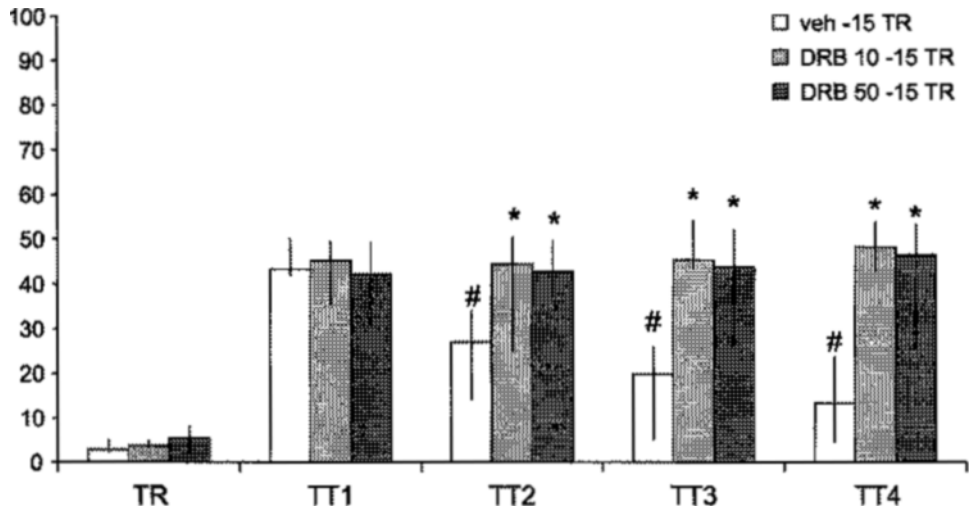

FIGURE 5 Effect of DRB (10 or 50 μM) given bilaterally into CA1 15 min prior to the first of four retention tests on retrieval of the one-trial inhibitory avoidance task. Testing was 24, 48, 72 and 96 h after training, respectively. Control animals received 0.5 μl of saline per side. The drug clearly inhibited the extinction of the task (Muller *et al.*, 2002). This is similar to what has been reported for the protein synthesis inhibitor, anisomycin (Vianna *et al.*, 2001). Thus, extinction is a new learning requiring, as the original task, gene transcription and protein synthesis at the time of the first retention test.

or more days later is shown in Fig. 6. Animals treated with Rp-cAMPs and PD098059, but not AP5 or KN-62, prior to the first test, showed impaired retrieval in that test, but not in the subsequent ones. As shown elsewhere (Barros *et al.*, 2000), here we found that the two former drugs impaired retrieval. There were no signs of extinction in any of the drug-treated groups: retrieval performance did not decline between the first and the fourth test. Therefore, the present data show that NMDA receptors, CaMKII, PKA and MAPK at the time of the first test are all necessary for the initiation of extinction. Further, they show that the actual level of retrieval performance in the first test is not crucial for extinction; probably the essential element is merely the detection of the CS-"no shock" association (Corcoran and Maren, 2001).

Thus, the main consequence of retention testing is to initiate extinction, which develops after that gradually over repeated tests. Extinction is a new learning that results from the association of the CS with the lack of the previous US (Konorski, 1948; Pavlov, 1956) and requires gene transcription (Fig. 5) and protein synthesis in CA1 (Vianna *et al.*, 2001) at the time of its inception. Its molecular pharmacology is remindful of, but not identical with, that of the memory consolidation of the avoidance task. It requires intact, NMDA receptors, CaMKII, PKA and MAPK activity in CA1 (Fig. 6). The big difference with consolidation is that extinction requires all these events plus transcription and protein synthesis at about the same time, whereas in consolidation, the NMDA activation precedes that of the enzymatic pathways, and these occur sequentially over several hours, culminating by a late peak of P-CREB elevation, gene transcription and protein synthesis 3–6 h after training (Izquierdo and Medina, 1997; Cammarota *et al.*, 1999, 2000; Kandel and Squire, 2000) (see also Fig. 1).

The molecular mechanisms that generate extinction are initiated at the time of retrieval in the CA1 region of the hippocampus, and include some of those that are involved in retrieval. Therefore, it may be said that retrieval "plants" the seeds of its own extinction in CA1. Some of the "seeds" (the MAPK and PKA signaling pathways) are required for retrieval itself; the others (NMDA receptors and CaMKII) are not.

Retrieval and Extinction of Short-term Memory

In a series of papers published between 1998 and 2000 (see Izquierdo *et al.*, 1998a,b; 1999b; Walz *et al.*, 1999), we showed that short- and long-term memory are separate and parallel processes. Short-term memory was defined as the archival system that maintains memories during the 3–6 h that it takes for long-term memory to become consolidated. Both forms of memory are distinct from working memory. The discovery that short- and long-term memory are separate stemmed from previous observations by McGaugh (1966) and Emptage and Carew (1983) and was based on the observation that a variety of pharmacological treatments given into CA1, the entorhinal or the parietal cortex are able to knock out short-term memory while leaving long-term memory intact for the same task in the same animal (Izquierdo *et al.*, 1998a–c; 1999a).

The same drugs that had been studied for their effect on long-term memory in the CA1 region were given 10 min prior to a retrieval test of short-term

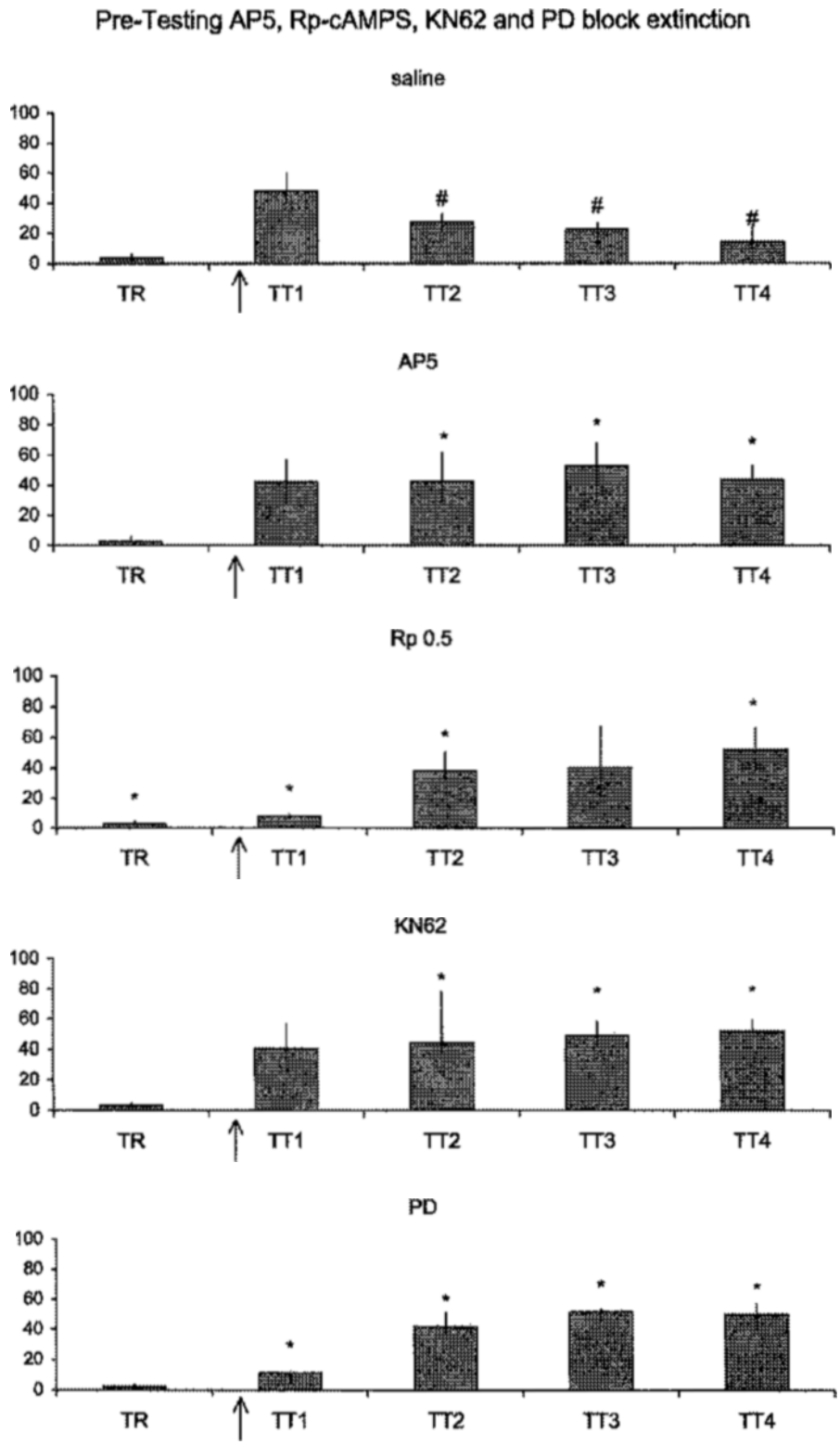

FIGURE 6 Training (Tr) and Test session (TT) retrieval performance expressed as median (interquartile step-down latency, in sec), in the one-trial avoidance task in rats. TT sessions were carried out at 24 h intervals over successive days (TT1, TT2, TT3, TT4). Animals received, 15 min prior to TT1, a bilateral infusion into the CA1 region of the dorsal hippocampus of saline, AP5 (5 μg/side), Rp-cAMPs (Rp, 0.5 μg/side), KN-62 (KN, 1.0 μg/side) nmoles), or PD098059 (PD, 50 μM). In the saline-treated animals, retrieval decreased along the successive sessions, which indicates extinction. In this and successive figures, # indicates significant differences in Mann–Whitney *U* tests ($p < 0.03$) in performance when compared with TT1. Rp and PD, but not AP5 or KN-62 attenuated retrieval in TT1, but the four drugs blocked extinction in the successive test sessions. In this and following figures, asterisks indicate significant differences in Mann–Whitney *U* tests ($p < 0.02$) relative to the corresponding session of the saline group.

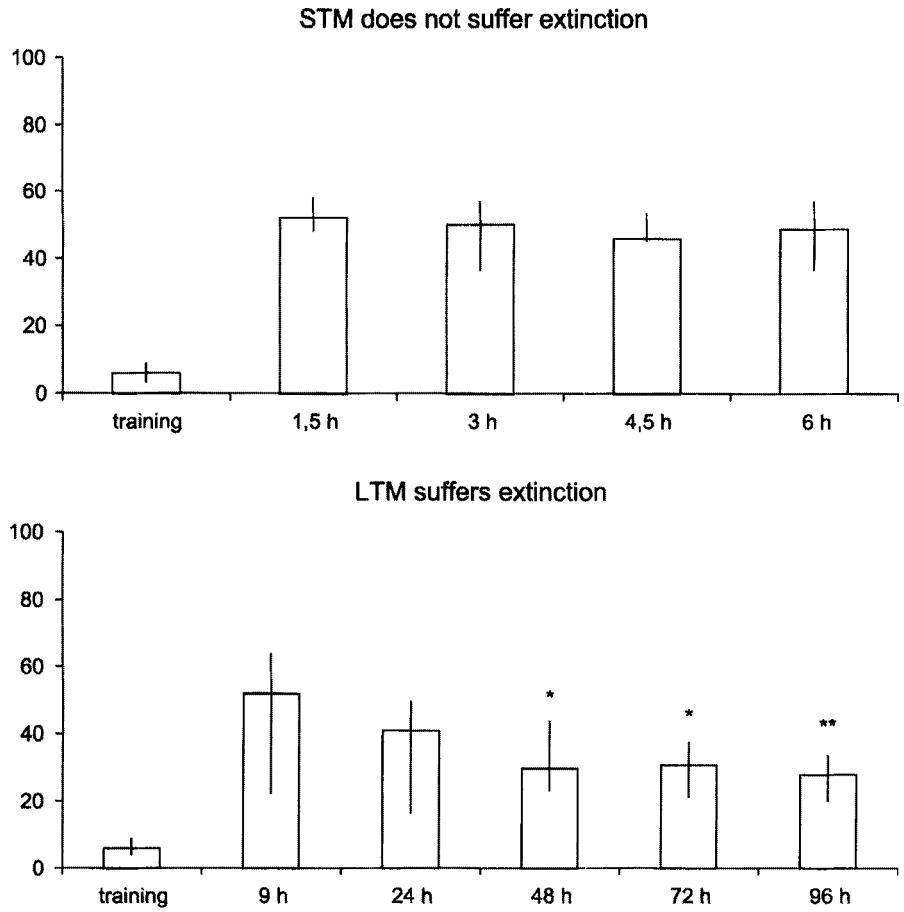

FIGURE 7 Rats were trained in one-trial step-down inhibitory avoidance using a 0.5 mA footshock and tested for retrieval 1.5, 3.0, 4.5 and 6.0 h after training (A: short-term memory, STM), or 9, 24, 48, 72 and 96 h after training (B: long-term memory, LTM). No extinction was visible upon repeated testing in the first 6 h; clear-cut extinction was seen in the long-term memory tests. Asterisk indicates significant differences from the first test session at $p < 0.02$ in Duncan test.

memory, measured 3 h after training. It was found that the retrieval of short-term memory, unlike that of the long-term type, is insensitive to most of the treatments. It is blocked only by the AMPA receptor antagonist, CNQX, and by the mGluR antagonist, MCPG (Izquierdo *et al.*, 2000). Therefore, the metabolic requirements of the retrieval of short-term memory are scarce, indeed, and it seems only to depend on the integrity of regular synaptic AMPAergic transmission in CA1.

If short-term memory is expected to fulfill its role as a stand-by for long-term memory, it should not suffer extinction. It does not, as shown in Fig. 7. These findings further illustrate the differences between the two memory types summarized elsewhere (Izquierdo *et al.*, 1999a).

Retrieval of Remote Memory

We remember not only things learned a few hours or days before, but also, and importantly, episodes and semantic knowledge acquired decades ago. These evoke different states of mind, different moods and different emotions through the years. Emotional events are usually recalled more intensely by children or old people; and, at the same time, children and old people are more prone to reacting more emotionally when recalling a given memory. For example, they are more prone to cry when recalling a funeral than 20- or 40-year olds. So it can be said that there is a developmental difference in the quality and perhaps in the quantity of retrieval; at least concerning emotionally-laden memories.

There are several treatments well-known to enhance retrieval when given prior to a test session: exposure to a novel environment (Izquierdo and McGaugh, 1985), and the systemic administration of the so-called stress hormones, epinephrine, ACTH, vasopressin and β-endorphin (Izquierdo, 1989; Izquierdo *et al.*, 1997). Here we report that, in addition, two well-known antidepressant agents with different biochemical properties, bupropion and sertraline, also enhance retrieval measured one day after training (see below). These two substances were administered *per os* 6 and 3 h prior to testing respectively, in order to mimick the time for maximum effect and the treatment conditions used in human patients.

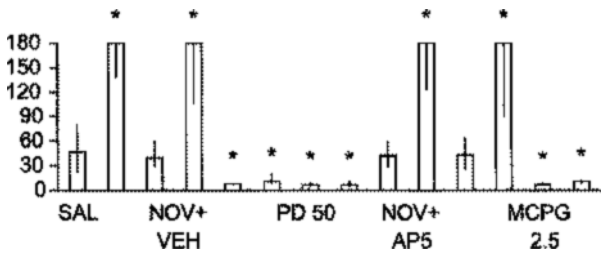

FIGURE 8 Exposure to a novel environment (5 min in a 50 × 50 × 50 cm open-field) 1 h prior to retention testing enhances retrieval. Here animals were tested 31 days after training in the one-trial task using a 0.8 mA footshock. Novelty presented 1 h before testing enhanced memory unless the animals received, 10 min prior to testing, a bilateral infusion into CA1 of Rp-cAMPs (Rp, 0.5 μg/side), PD098059 (PD, 50 μM) or MCPG (2.5 μg/side), but not of AP5 (5.0 μg/side) or CNQX (1.25 μg/side). Thus, the enhancement of retrieval caused by novelty depends on hippocampal mGluRs, PKA and MAPK.

Exposure to a novel environment (a 50 × 50 × 50 cm open field during 5 min) 1 h prior to testing has been known for some time to enhance retrieval (Izquierdo and McGaugh, 1985). The effect is now known to require mGluRs, PKA and MAPK (Izquierdo *et al.*, 2001) (Fig. 8).

Two separate groups of male Wistar rats were used. One was trained in the one-trial avoidance task at the age of three months using a single 0.5 mA footshock and tested 24 h later. The other group was also trained in this at the age of three months, but using two 0.8 mA footshocks given in close sequence. These animals were then tested at the age of 6, 9, 12, 15 and 18 months. Thus, in these animals, memory aged together with the subjects. Animals were randomly assigned to groups on each test. Results are shown in Figs. 9–15.

As shown by Gold *et al.*, (1981), retrieval performance of this task declined with time ($H = 24.7$, $p < 0.02$ in a Kruskal–Wallis test) (Figs. 9–15). Retention test performance levels were indistinguishable from those of the original training session in the last test ($p < 0.1$ in a Mann–Whitney U test, two-tailed), carried out when the memory was 19 months old and the animals were 22 months old. Since in the present experiment, the animals were rotated among treatment groups and tested more than once, at different intervals and with different treatments, it is not possible to know how much of the loss was due to simple forgetting (Gold *et al.*, 1981), and how much was due to extinction caused by the repeated testing (e.g. Vianna *et al.*, 2001). The rotation of animals across groups at each training-test interval was carried out in such a way that no animal was exposed to the same treatment twice in their life span.

Exposure to novelty 1 h prior to testing enhanced retrieval 1 day, or 3, 6, 9, 12, 15 or 19 months after training to a similar degree, regardless of the level of performance in control (untreated) animals (Fig. 9).

The effects of the stress hormones varied with the training-test interval. ACTH markedly enhanced retrieval of the avoidance task one day after training at a dose of 0.2 μg/kg. At the 3-month training-test interval, however, ACTH was effective only at a dose 5 times higher. At the 6-month interval, neither 1.0 nor 5.0 μg/kg of ACTH had any detectable effect on retrieval. At the 9-month interval, ACTH depressed retrieval at the dose of 5.0 μg/kg. At the 12-month training-test interval, ACTH enhanced retrieval, at the doses of 1.0 or 5.0 μg/kg (Fig. 10) When the memory was 15 or 19 months old, ACTH was able to enhance retention test performance again at the lowest dose, 0.2 μg/kg, as it did when memory was 1 day old (Fig. 10).

Adrenaline enhanced retrieval at a dose of 5.0 μg/kg at the 1-day training-test interval but only at a dose of 25.0 μg/kg at the 3- or 6-month intervals (Fig. 11). When the memory was 9 months old, the pre-test administration of 25.0 μg/kg of adrenaline actually depressed retrieval. However, at the 12-, 15- or 19-month training-test intervals, the drug was again effective at the lowest dose, 5.0 μg/kg (Fig. 11).

Vasopressin enhanced retrieval 1 day after training at a dose of 10 μg/kg. At the 3- or 6-month training-test intervals it was effective only at a dose 5 times higher. It had no effect, even at the higher dose, at the 9-month interval. Vasopressin enhanced retrieval at the two dose levels at the 12-month interval and at the lower dose level (10 μg/kg) at the 15- or 19-month training-test intervals (Fig. 12).

β-Endorphin enhanced retrieval at the dose of 1.0 μg/kg when the memory was acquired on the preceding day, but only at the dose of 5.0 μg/kg when the memory was 3 months old. At the 6 month training-test interval, β-endorphin had no effect on retrieval at the doses of 5.0 or even 25.0 μg/kg. When the memory was 9 months old, the drug had a depressant effect on retrieval at the dose of 25.0 μg/kg. At the 12-month interval the enhancing effect of β-endorphin reappeared, at doses of either 5.0 or 25.0 μg/kg (Fig. 13). At the 15- or 19-month training-test intervals, β-endorphin again enhanced retrieval at the lowest dose (1.0 μg/kg), as it did when the memory was just 1-day old (Fig. 13).

Bupropion (20 or 60 mg/kg, p.o.) was given p.o. 6 h prior to testing, and sertraline (3.3 or 10 mg/kg, p.o.) was given 3 h prior to testing. These times are those at which the compounds are supposed to attain maximum effect in clinical practice. Figure 14 illustrates the findings obtained with bupropion and Fig. 15 those obtained with sertraline. Both drugs, at the two dose levels studied, significantly enhanced retrieval performance at all training-test intervals, regardless of the basal performance level at different ages. At the 1 day, 3 month and 9 month training-test

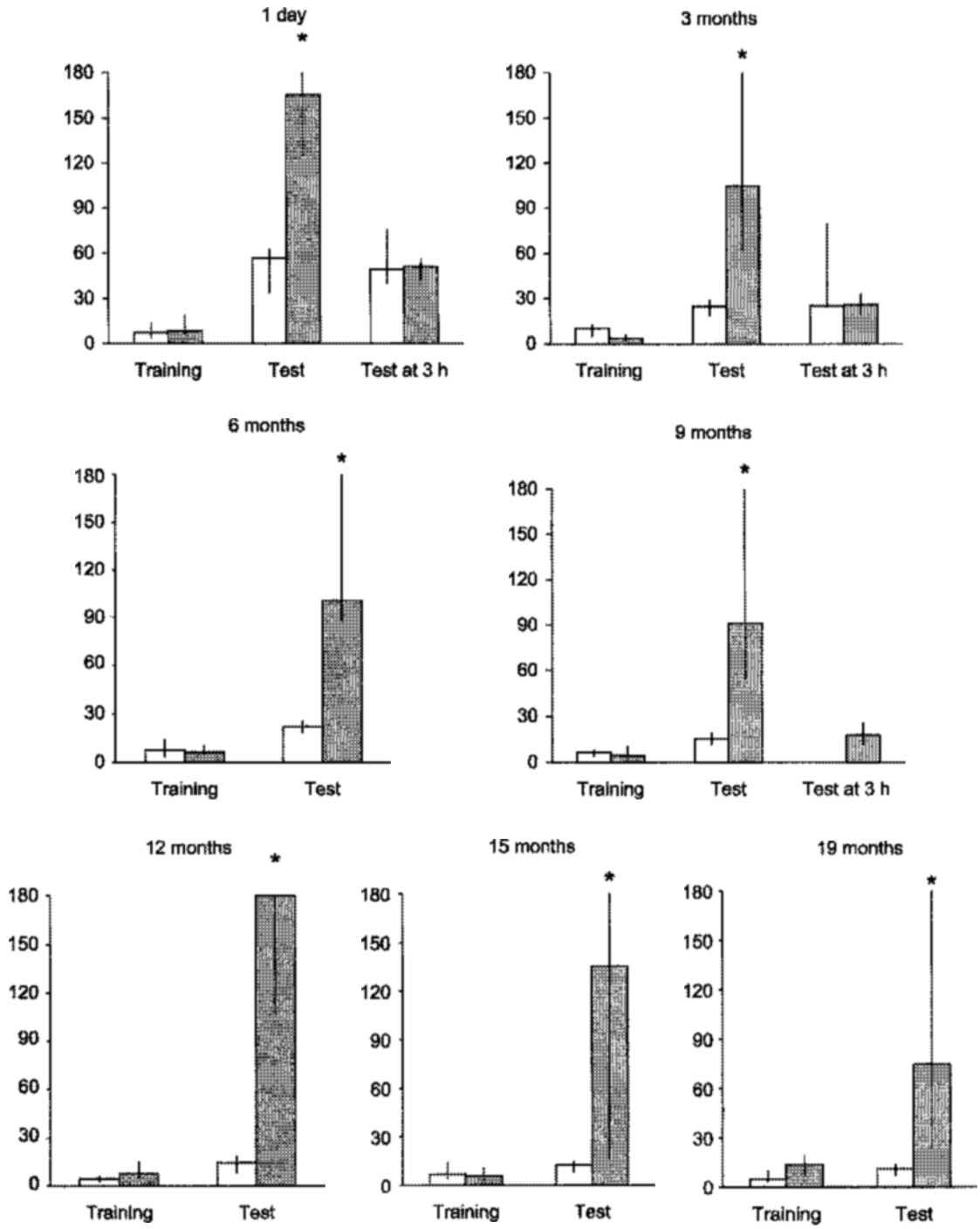

FIGURE 9 Effect of exposure to novelty 1 h prior to testing on retrieval of one-trial step-down avoidance in rats. Asterisks indicate significant differences from control groups. The animals were trained at the age of 3 months, and tested either the next day, or 3, 6, 9, 12, 15 or 19 months later. White columns correspond to animals just taken out of their home cages and tested (controls). Dotted columns correspond to animals exposed to a novel environment 1 h before the retention tests. Animals tested 1 day, 3 months or 9 months were submitted to a 2nd retention test, 3 h after the first. $N = 10$ per group. In control animals, retrieval declined with age (or with the length of the training-test interval). This decline was significant in a one-way Kruskal–Wallis ANOVA ($H = 25.2$, $p < 0.02$). At the 19-month interval, control test session values were not different from those of the training session ($p > 0.1$ in a Mann–Whitney U test). Exposure to the novel environment prior to retention testing enhanced retrieval performance at all intervals and ages ($p < 0.02$ in Mann–Whitney U tests) when compared with control test performance or with performance in a second test 3 h after the first).

interval there was a significant difference between the two dose levels of bupropion ($p < 0.05$). At the 3 month interval, there was a dose-response difference in the sertraline-treated animals ($p < 0.05$). At all other intervals, the difference in the effect of the two doses of each compound were not significantly different. The enhancement of retrieval caused by the two drugs was actually

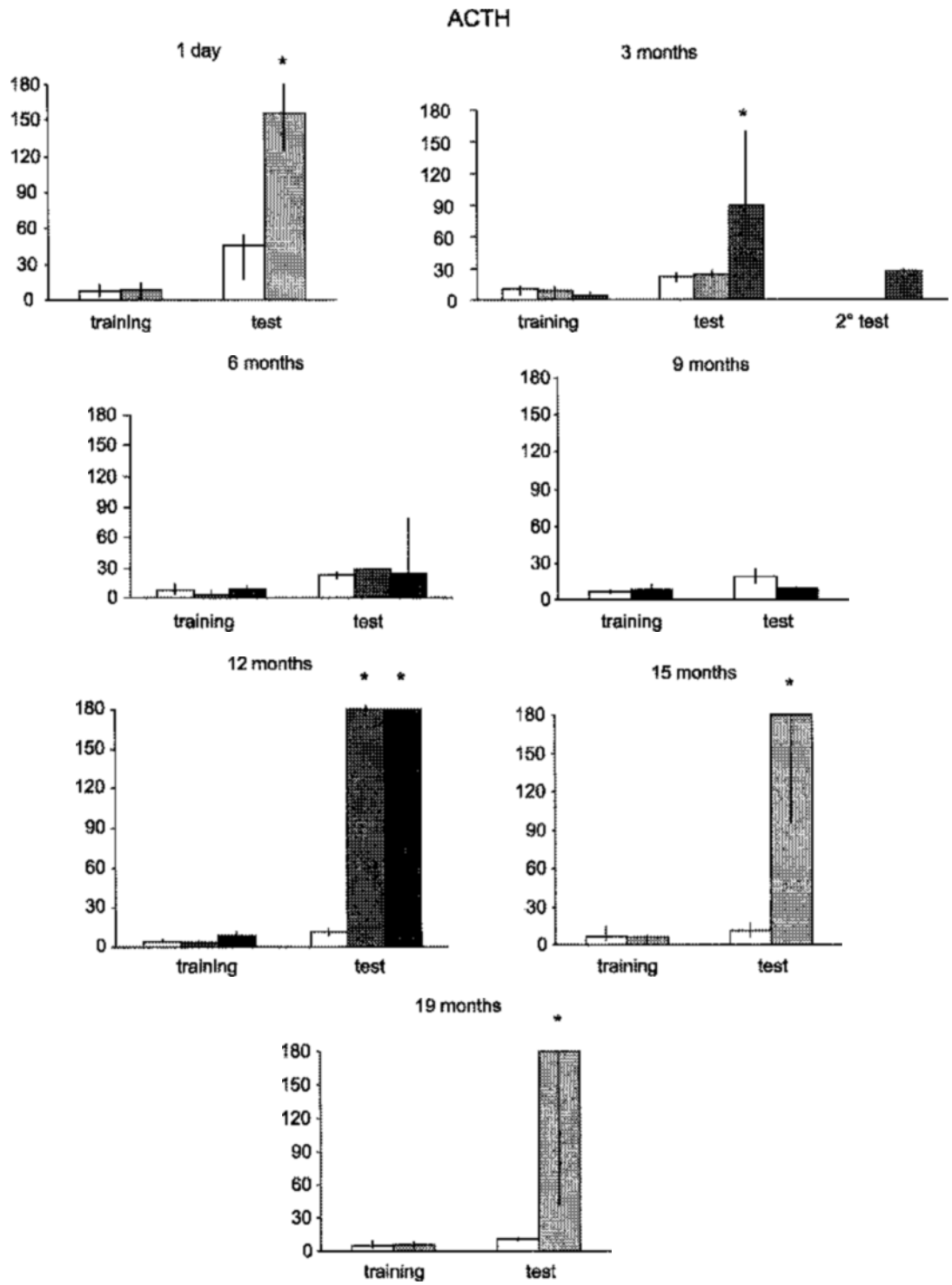

FIGURE 10 Effect of $ACTH_{1-24}$ on retrieval of one-trial inhibitory avoidance in rats, measured at different times from training: 1 day, 3, 6, 9, 12, 15 and 19 months. Asterisks indicate significant differences from saline-treated controls at a $p < 0.02$ level in Mann–Whitney U tests, two-tailed. In this and following figures, $N = 8$ or 10 per group. Training session performance of the animals selected for each particular interval are shown. Data from saline controls are shown in the white columns; data obtained with 0.2 μg/kg of ACTH are shown in the light dotted columns, data obtained using 1.0 μg/kg of ACTH are shown in the gray dotted columns, and data obtained using the higher dose of ACTH (5.0 μg/kg) are shown in the darkest columns. The effect of $ACTH_{1-24}$ was different across training-test intervals ($H = 23.4$ in a Kruskal–Wallis ANOVA, $p < 0.02$, $N = 63$). $ACTH_{1-24}$ given i.p. 10 min before retention testing enhanced retrieval of step-down inhibitory avoidance at the dose of 0.2 μg/kg, i.p. when the animals were tested one day after training. ACTH also enhanced retrieval in animals tested 3 months after training, but only at a dose 5 times higher (1.0 μg/kg). This effect was no longer seen in a second retention test carried out 3 h after the first. ACTH had no effect on retrieval when administered to animals trained 6 months before, even at a dose of 5.0 μg/kg. At the 9 month training-test interval, when the rats were 12 months old, ACTH (5.0 μg/kg) hindered retrieval. The drug became effective again in enhancing retrieval at the lower dose levels when the animals were tested 12, 15 or 19 months after training. Note that the level of retention test performance declined with age in the control (saline-treated) animals, and that at 22 months of age (19 months after training) the retention scores became indistinguishable from those of the training session.

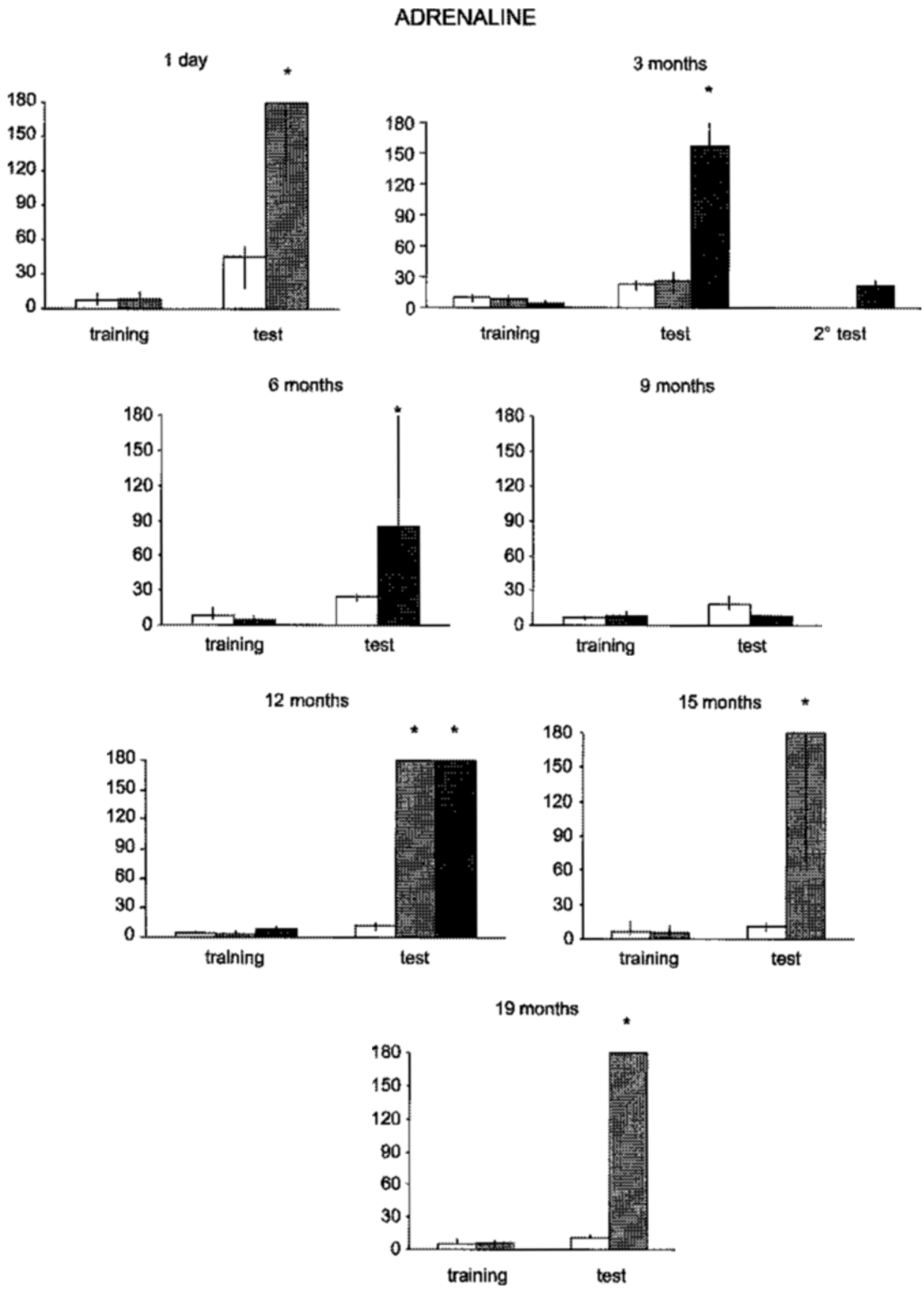

FIGURE 11 Effect of adrenaline on retrieval of one-trial inhibitory avoidance in rats, measured 1 day or 3, 6, 9, 12, 15 or 19 months after training. The white columns show data from saline-treated controls; the gray dotted columns show data obtained using 5.0 μg/kg of adrenaline, and the darker dotted columns show data obtained using 25.0 μg/kg of the drug. The effect of adrenaline was different at different training-test intervals ($H = 29.0, p < 0.02, N = 66$). Adrenaline enhanced retrieval 1 day after training at the dose of 5.0 μg/kg, ip. Adrenaline also enhanced retrieval when the tests were 3 or 6 months after training, but only at a dose of 25.0 μg/kg. In the animals with the 3 month training-test interval, the enhancing effect of adrenaline was no longer seen 3 h later. At the 9 month training-test interval, adrenaline (25 μg/kg) actually depressed retention test performance. The hormone again enhanced retrieval, even at the lowest dose, when the memory was 12, 15 or 19 months old. In this and the following figures, the control groups are the same of Fig. 1.

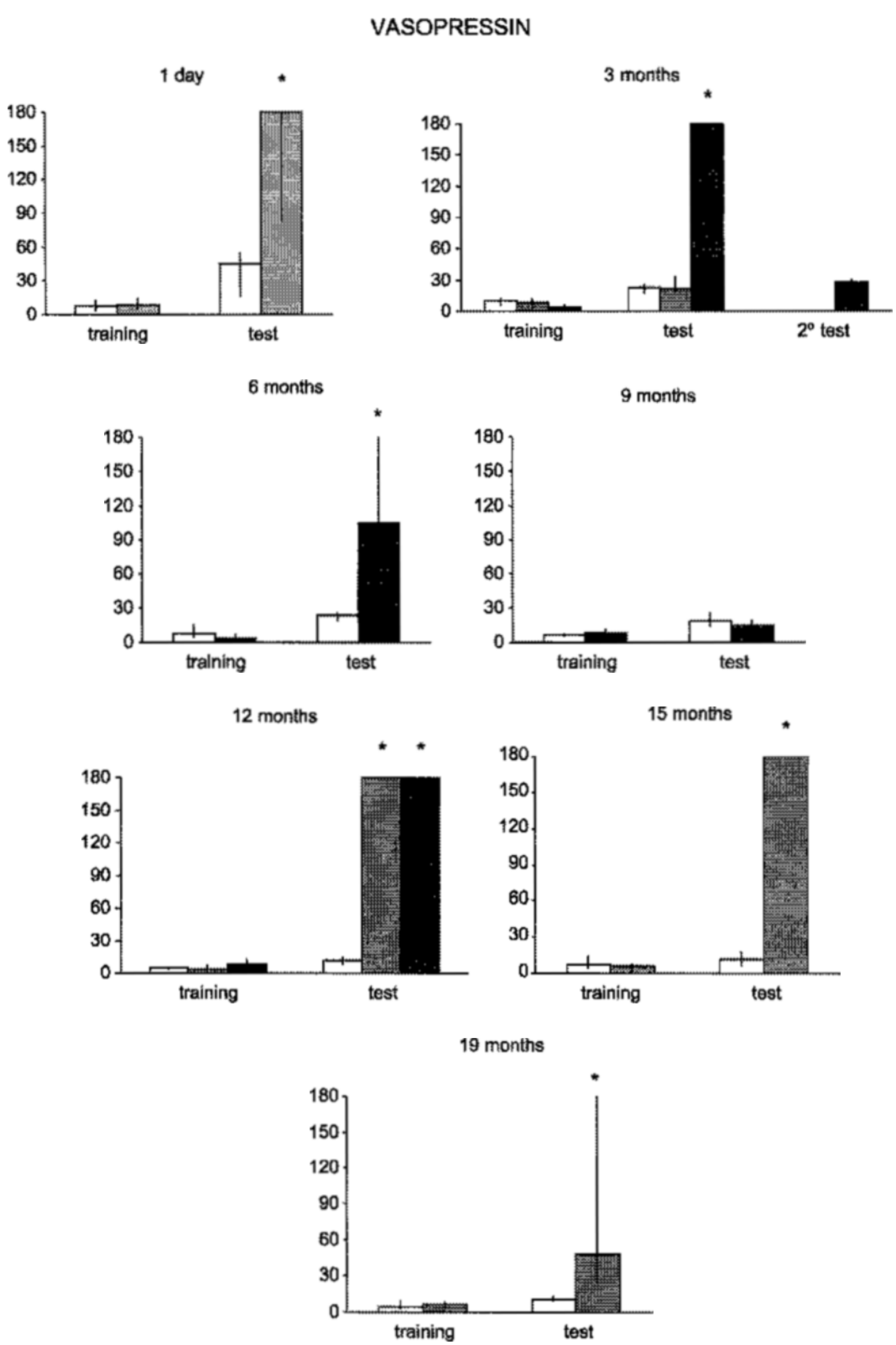

FIGURE 12 Effect of vasopressin on retrieval of one-trial inhibitory avoidance in rats, measured 1 day or 3, 6, 9, 12, 15 or 19 months after training. The white columns show data from saline controls, the light gray columns show data obtained using 10 μg/kg of vasopressin, and the darker columns show data obtained using 50.0 μg/kg of the drug. The effect of vasopressin on retrieval was different at different training-test intervals ($H = 21.9$, $p < 0.02$, $N = 64$). Vasopressin enhanced retrieval measured 1 day after training when given 10 min prior to testing at a dose of 10.0 μg/kg, ip. Vasopressin also enhanced retrieval when animals were tested 3 or 6 months after training, but only with a dose of 50.0 μg/kg. In the animals with the 3 month training-test interval, the enhancing effect of vasopressin was no longer seen in a second retention test carried out 3 h after the first. However, 3 months later, the effect of vasopressin, even at the higher dose, was no longer seen. The hormone enhanced retrieval again when memory was tested 12, 15 or 19 months after training.

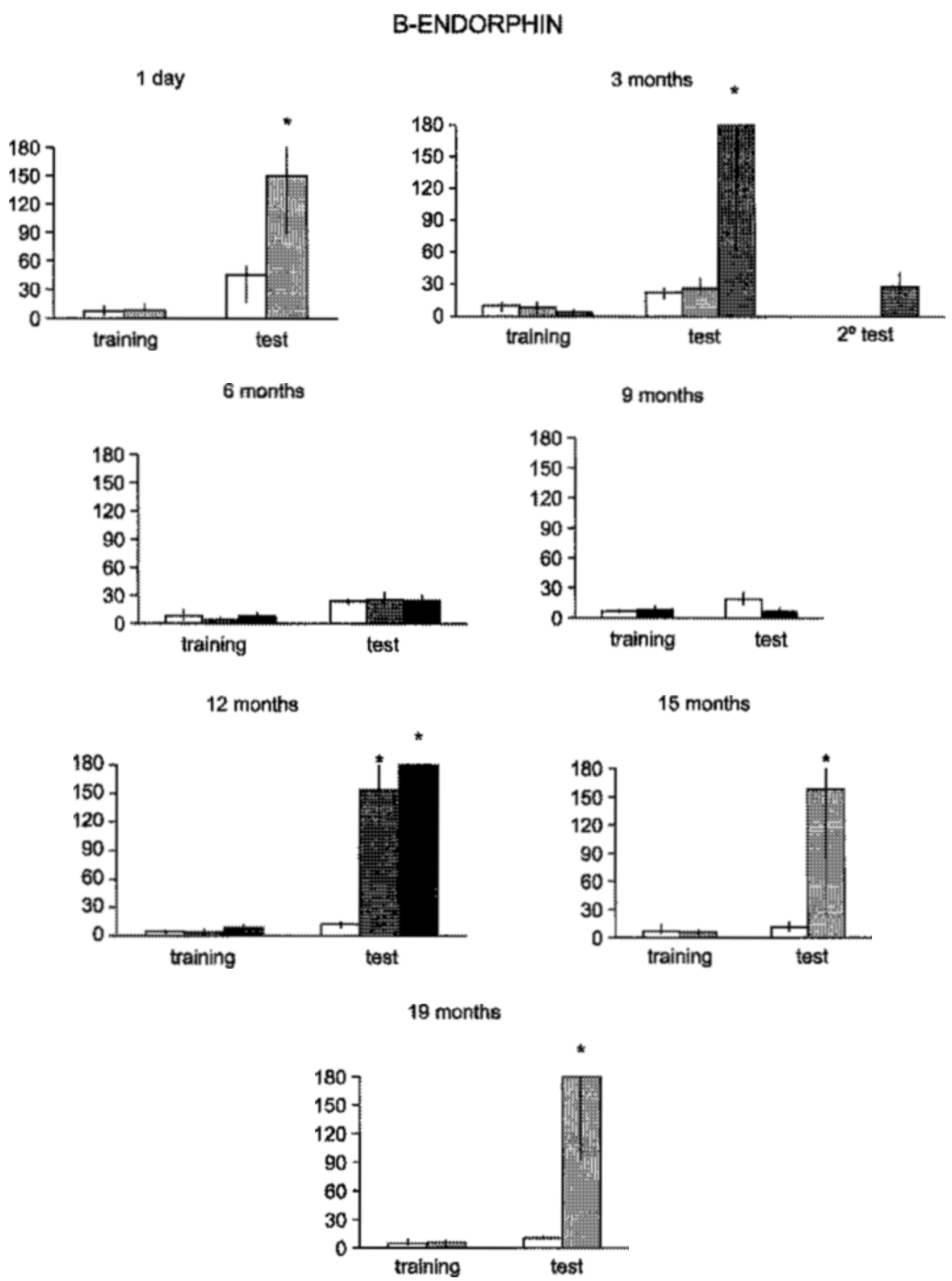

FIGURE 13 Effect of β-endorphin on retrieval of one-trial inhibitory avoidance in rats, measured 1 day or 3, 6, 9, 12, 15 or 19 months after training. Data from saline controls are shown in the white columns; data obtained with 1.0 μg/kg of β-Endorphin are shown in the light dotted columns, data obtained using 5.0 μg/kg of β-endorphin are shown in the gray dotted columns, and data obtained using the higher dose of the drug (25.0 μg/kg) are shown in the darkest columns. The effect of β-endorphin was different at different training-test intervals ($H = 31.6, p < 0.02, N = 62$). β-Endorphin enhanced retention test performance of the one-trial task at a dose of 1.0 μg/kg given ip. 10 min before testing, when the animals were tested 1 day after training. When the memory was 3 month old, a dose of 5.0 μg/kg was needed to obtain retrieval enhancement. This effect was no longer seen in a second test session carried out 3 h later. When the memory was 6 or 9 months old, not even 25.0 μg/kg of the drug were able to alter retention test performance. Three months later, however, β-endorphin enhanced retrieval again at the doses of 5.0 and 25.0 μg/kg. When the memory was 15 or 19 months old, and the rats were 18 and 22 months old, respectively, β-endorphin again facilitated retrieval at the lowest dose, 1.0 μg/kg.

more intense in the animals tested at the longer training-test intervals, i.e. in the older animals: in several of these groups all animals reached the 180 s ceiling.

The effects of all the treatments were limited to the time of retrieval. Retention test performance returned to normal 3 h after the effect of novelty or the stress hormones was observed, or 24 h after that of

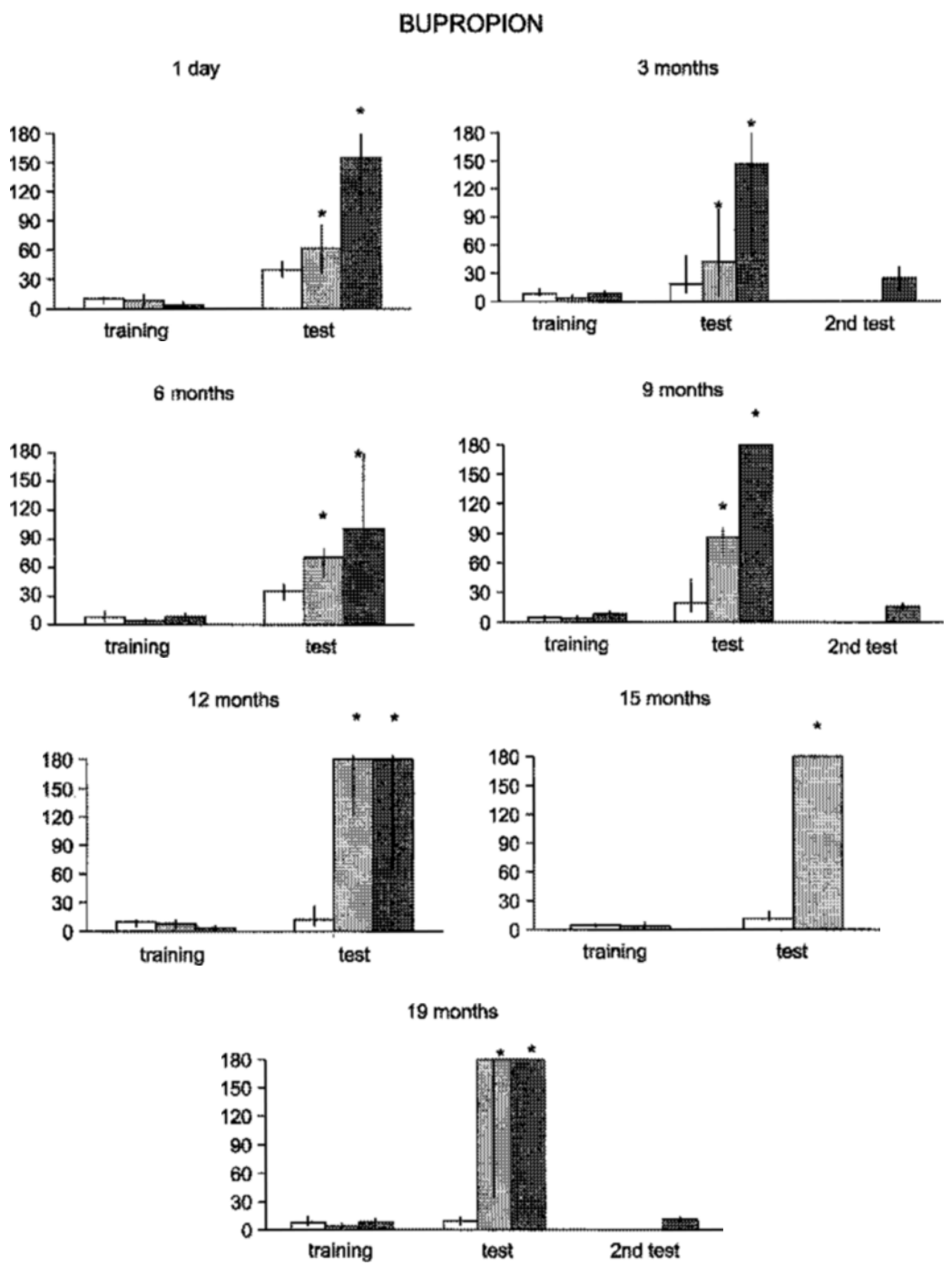

FIGURE 14 Same as preceding figures, except that here the animals received, 6 h prior to the test session, saline (white columns) or bupropion (20 mg/kg, gray columns, or 60 mg/kg, dark columns), p.o. In the 3, 9 and 19 month groups a second test session was carried out 24 h after the first. Note that retrieval declined with the training-test interval (or with age) in the control animals. Bupropion enhanced retention test performance at the two doses and at all training-test intervals. The differences between the groups tested under each dose level were significant at $p < 0.05$ level in the 1 day, 3 months and 9 months groups. Performance on the 2nd test 24 h after the first was indistinguishable from that of control groups, and significantly different from those of the first test at the 3, 9 or 19 month training-test intervals. The enhancing effect of bupropion on retrieval was more marked at the higher dose of each compound in the tests performed 15 or 19 months after training ($H = 17.3$, $p < 0.05$, $N = 70$).

bupropion or sertraline was measured. Therefore, the effects did not carry over to the next period of testing.

Thus, if tested every three months, memory of the avoidance task declines markedly, but does not disappear. Its retrieval, however, can be enhanced by novelty, ACTH, β-endorphin, vasopressin, epinephrine, bupropion and sertraline, the stress hormones, or by antidepressants. Bupropion and sertraline were increasingly effective with age. Novelty was similarly effective across ages. The stress hormones were very effective when the memory was new and the subjects were young, and then lost effectiveness

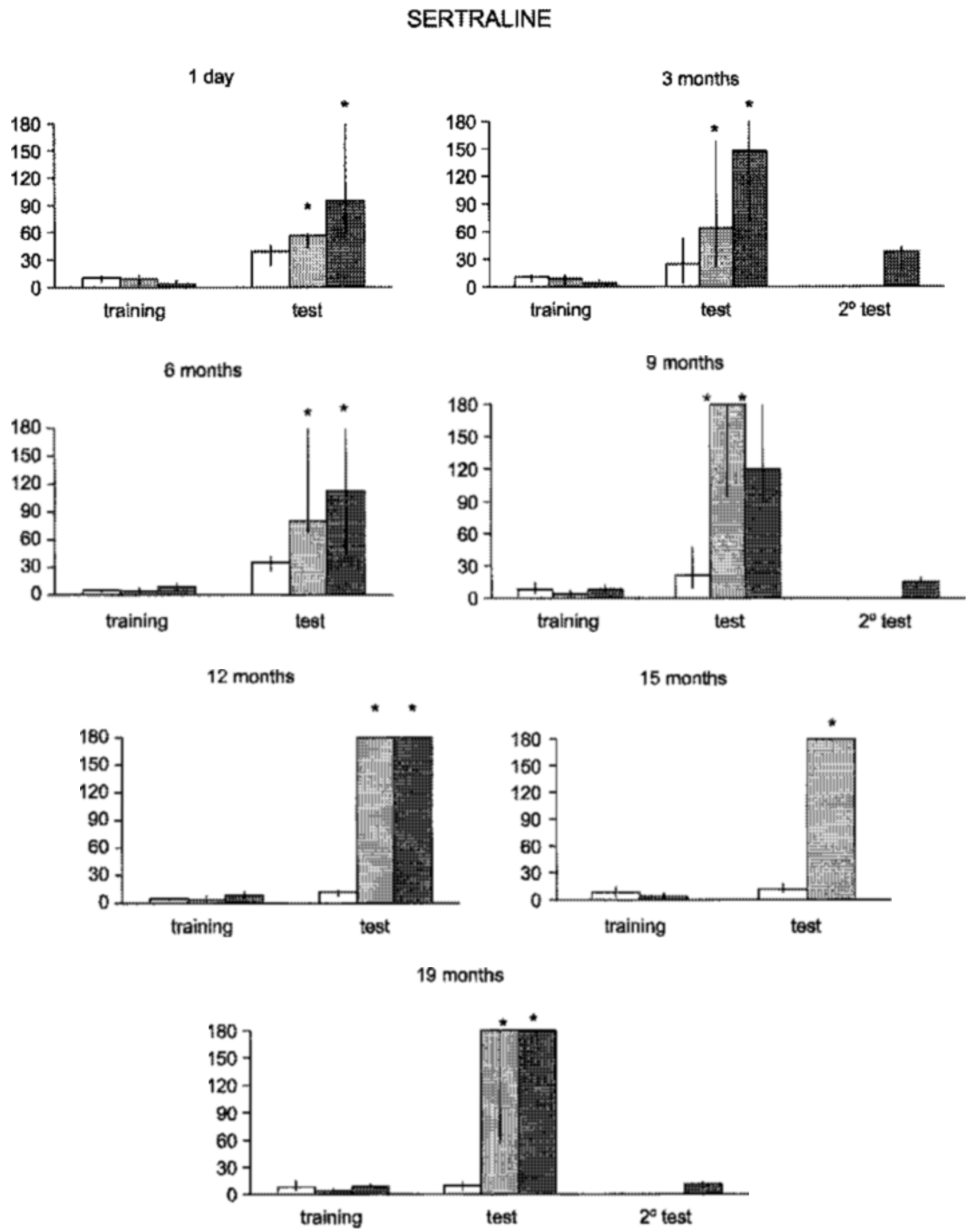

FIGURE 15 Training and test session step-down latencies of animals trained in step-down inhibitory avoidance 1 day, or 3, 6, 9, 12, 15 or 19 months before. The animals received, 3 h prior to the test session, saline (white columns) or sertraline (3.3 mg/kg, gray columns, or 10 mg/kg, dark columns), p.o. In the 3, 9 and 19 month groups a second test session was carried out 24 h after the first. As in Fig. 1, retrieval declined with the training-test interval (or with age) in the control animals. Sertraline enhanced retention test performance at the two doses and at all training-test intervals. The differences between the groups tested under each dose level were significant at $p < 0.05$ level in the 3 month groups. Performance on the second test 24 h after the first was indistinguishable from that of control groups, and significantly different from those of the first test at the 3, 9 or 10 month training-test intervals.

through time; but they recovered their anti-amnestic effect at an age which, in the rat, may be considered to be advanced.

It has been proposed many years ago that emotionally-laden learnings, through the release of stress hormones and brain neuromodulators, may set up "states" that, if reproduced at the time of testing, will facilitate retrieval (Zornetzer, 1978; Izquierdo, 1984; 1991). The present task acquired is known to be accompanied by the release of the stress hormones

studied here (see Izquierdo, 1989 for references). Thus, one way of looking at the present findings is that a young and an old age rats are more sensitive to a revival of the neurohumoral component(s) of the emotional state than are middle-aged rats. State dependency on endogenous substances, hormones or neurotransmitters, is of course, a peculiar way by which retrieval of a given memory can get mixed with that of others, or with new memories of the same general kind. When confronted with a dangerous or otherwise aversive situation, subjects will re-create the hormonal and neurohumoral conditions that were present in other dangerous or aversive circumstances, and this will help them to recall strategies of escape or confrontation. While this serves a clear-cut adaptive strategy (it is useful to remember how to flee when something potentially dangerous takes place), it may also confuse the memories of one episode with that of others (Izquierdo, 1984; 1989).

Since the animals in the groups tested at 6–18 months of age were rotated across groups over their life span, each of them was tested several times. Therefore, the gradual decline seen with aging in these animals could, in principle, be attributed to a very protracted extinction, rather than to a real loss of memory with age. It is true that at some point in their life all of them received one or other retrieval-enhancing treatment, but then these effects were, as mentioned above, short-lived.

FINAL COMMENT

The findings reported here show that retrieval depends on various modulatable molecular events taking place in several regions of the rat cortex during a short time. But it has profound and very long-lasting influences in subsequent behavior. The main influence is to initiate extinction, which is a new learning that not only uses the biochemical systems of retrieval, but recruits others, including NMDA receptors, CaMKII, gene transcription and protein synthesis.

But extinguished memories do not really disappear; they can be revived by appropriate enhancing treatments of various sorts given before testing. The effects of these treatments vary with age; novelty or two different antidepressant drugs are effective at all ages, the latter increasingly so with age. Stress hormones, on the other hand, are more effective in the younger and older animals. The theoretical and clinical implications of this should be studied.

In the daily life of humans, retrieval often occurs in the presence of the acquisition, retrieval or extinction of other memories. This may result in curious mixtures through which we often confuse faces, names, circumstances or events. This happens more often as time goes by: aged persons possess many more memories than younger persons with which newer ones can be mixed. When retrieval occurs in isolation, it tends to produce extinction; when it occurs concomitantly with the processing of other memories, it may lead to a form of new learning that is enriched and also filled with inexactitude.

A FEW THOUGHTS

How strange indeed are our dealings with reality. We perceive it and analyze it in a minute account through our extraordinarily fast and accurate working memory system (Goldman-Rakic, 1996). Immediately after that we process it through two separate memory systems: one metabolically more economic but of short duration, and another one, parallel, metabolically quite intricate but much longer lasting (Izquierdo *et al.*, 1999a; Izquierdo, 2001). As a result of this we change the shape and function of the pertinent synapses with remarkable precision (Geinisman, 2002). Then we recall the function of those modified circuits and retrieve the memories in surprising detail. But afterwards we let them slide into a kind of oblivion. We make of our brain a map of fragments of faded memories. And, every now and then, for reasons of mood or emotion that we often do not understand, we might bring them back; perhaps changed or mixed with other memories. For mood and emotion were also present when we acquired and when we formed each memory, but often in different proportion (Izquierdo, 1989).

No wonder we fail so often to grasp reality and have so many doubts about its meaning.

Acknowledgements

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References

Artiges, E., Salame, P., Recasens, C., Poline, J., Attar-Levy, D., De La Paillère-Martinot, M.L., Danion, J.M. and Martinot, J.L. (2000) "Working memory control in patients with schizophrenia, a study during a random number generation task", *Am. J. Psychiatry* **157**, 1517–1519.

Baddeley, A. (1997) Human Memory, Theory and Practice (Allyn and Bacon, Boston).

Barros, D.M., Izquierdo, L.A., Mello e Souza, T., Ardenghi, P.G., Pereira, P., Medina, J.H. and Izquierdo, I. (2000) "Molecular signaling pathways in the cerebral cortex are required for

retrieval of one-trial avoidance learning in rats", *Behav. Brain Res.* **114**, 183–192.

Barros, D.M., Mello e Souza, T., De David, T., Choi, H., Aguzzoli, A., Madche, C., Ardenghi, P., Medina, J.H. and Izquierdo, I. (2001) "Simultaneous modulation of retrieval by dopaminergic D_1, β-noradrenergic, serotoninergic1 A and cholinergic muscarinic receptors in cortical structures of the rat", *Behav. Brain Res.* **124**, 1–7.

Barros, D.M., Pereira, P., Medina, J.H. and Izquierdo, I. (2002) "Modulation of working memory and of long- but not short-term memory by cholinergic mechanisms in the basolateral amygdala", *Behav. Pharmacol.*, In press.

Beninger, R.J., Dringenberg, H.C., Boegman, R.J. and Jhamandas, K. (2001) "Cognitive effects of neurotoxic lesions of the nucleus basalis magnocellularis in rats: differential roles for corticopetal versus amygdalopetal projections", *Neurotox. Res.* **3**, 7–21.

Berman, D.E. and Dudai, Y. (2001) "Memory extinction, learning anew and learning the new: dissociations in the molecular machinery of learning in the cortex", *Science* **291**, 2417–2419.

Bernabeu, R., Bevilaqua, L., Ardenghi, P., Bromberg, E., Schmitz, P., Bianchin, M., Izquierdo, I. and Medina, J.H. (1997) "Involvement of hippocampal D1/D5 receptor-cAMP signaling pathways in a late memory consolidation phase of an aversively motivated task in rats", *Proc. Natl Acad. Sci. USA* **94**, 7041–7046.

Cahill, L. and McGaugh, J.L. (1998) "Mechanisms of emotional arousal and lasting declarative memory", *Trends Neurosci.* **21**, 294–299.

Cahill, L., Weinberger, N.M., Roozendaal, B. and McGaugh, J.L. (1999) "Is the amygdala a locus for 'conditioned fear'? Some questions and caveats", *Neuron* **23**, 227–228.

Cammarota, M., Bernabeu, R., Levi de Stein, M., Izquierdo, I. and Medina, J.H. (1998) "Learning-specific, time-dependent increases in hippocampal Ca^{2+}/calmodulin-dependent protein kinase II activity and AMPA GluR1 subunit immunoreactivity", *Eur. J. Neurosci.* **10**, 2669–2676.

Cammarota, M., Bevilaqua, L.R.M., Ardenghi, P., Paratcha, G., Levi de Stein, M., Izquierdo, I. and Medina, J.H. (2000) "Learning-associated activation of nuclear MAPK, CREB and Elk-1, along with Fos production, in the rat hippocampus after a one-trial avoidance learning: abolition by NMDA receptor blockade", *Mol. Brain Res.* **76**, 36–46.

Corcoran, K.A. and Maren, S. (2001) "Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction", *J. Neurosci.* **21**, 1720–1726.

Danion, J.M., Meulemans, T., Kauffmann-Muller, F. and Vermaat, H. (2001) "Intact implicit learning in schizophrenia", *Am. J. Psychiatry* **158**, 944–948.

Dudai, Y., Rosenblum, K., Meiri, N., Miskin, R. and Schul, R. (1995) "Correlates of taste and taste aversion learning in the rodent brain", In: McGaugh, J.L., Bermúdez-Rattoni, F. and Prado-Alcalá, R., eds, Plasticity in the Central Nervous System (Erlbaum, Mahwah), pp 161–170.

Eccles, J.C. (1957) The Physiology of Nerve Cells (Johns Hopkins Press, Baltimore).

Egan, M.F., Goldberg, T.E., Kolachova, B.S., Callicott, J.H., Mazzanti, C.M., Straub, R.E., Goldman, D. and Weinberger, D.R. (2001) "Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia", *Proc. Natl Acad. Sci. USA* **98**, 6917–6922.

Eldridge, L.L., Knowlton, B.J., Furmanski, C.S., Bookheimer, S.Y. and Engel, S.A. (2000) "Remembering episodes: a selective role for the hippocampus during retrieval", *Nature Neurosci.* **3**, 1149–1152.

Falls, W.A., Miserendino, M.J. and Davis, M. (1992) "Extinction of fear-potentiated startle: blockade by infusion of NMDA antagonist into the amygdala", *J. Neurosci.* **12**, 854–863.

Freud, S. (1952) "The neuro-psychoses of defense", The Standard Edition of the Complete Psychological Works of Sigmund Freud (Hogarth Press, London) **Vol. 3**, p 45.

Geinisman, Y. (2002) "Learning-induced alterations in synaptic ultrastructure", In: Riedel, G. and Platt, B., eds, Cellular Mechanisms of Memory Formation (Elsevier, Cambridge), In press.

Geyer, M.A., Krebs-Thomson, K., Braff, D.L. and Swedlow, N.R. (2001) "Pharmacological studoes of prepulse inhibition models f sensorimotor gating deficits in schizophrenia: a decade in review", *Psychopharmacology* **156**, 117–154.

Gold, P.E. (1986) "The use of avoidance training in studies of modulation of memory storage", *Behav. Neural Biol.* **46**, 87–98.

Gold, P.E., McGaugh, J.L., Hankins, L.L., Rose, R.P. and Vasquez, B.J. (1981) "Age dependent changes in retention in rats", *Exp. Aging Res.* **8**, 53–58.

Goldman-Rakic, P. (1996) "Regional and cellular fractionation of working memory", *Proc. Natl Acad. Sci. USA* **93**, 13473–13480.

Hall, J., Thomas, K.L. and Everitt, B.J. (2001) "Cellular imaging of zif268 expression in the hippocampus and amygdala during contextual and cued fear memory retrieval: selective activation of hippocampal CA1 neurons during the recall of contextual memories", *J. Neurosci.* **21**, 2186–2193.

Izquierdo, I. (1989) "Different forms of posttraining memory processing", *Behav. Neural Biol.* **51**, 171–202.

Izquierdo, I. and McGaugh, J.L. (1985) "Effect of a novel experience prior to training or testing on retention of an inhibitory avoidance task in mice: involvement of an opioid system", *Behav. Neural Biol.* **44**, 228–238.

Izquierdo, I. and McGaugh, J.L. (2000) "Behavioral pharmacology and its contribution to the molecular basis of memory consolidation", *Behav. Pharmacol.* **11**, 517–534.

Izquierdo, I., Da Cunha, C., Rosat, R., Jerusalinsky, D., Quillfeldt, J.A., Ferreira, M.B.C. and Medina, J.H. (1992) "Neurotransmitter receptors involved in memory processing by the amygdala, medial septum and hippocampus of rats", *Behav. Neural Biol.* **58**, 16–25.

Izquierdo, L.A., Schröder, N., Ardenghi, P., Quevedo, J., Bevilaqua, L., Netto, C.A., Izquierdo, I. and Medina, J.H. (1997) "Systemic administration of ACTH or vasopressin in rats reverses the amnestic effect of posttraining β-endorphin but not that of intrahippocampal infusion of protein kinase inhibitors", *Neurobiol. Learn. Mem.* **68**, 197–202.

Izquierdo, I., Izquierdo, L.A., Barros, D.M., Mello e Souza, T., de Souza, M.M., Quevedo, J., Rodrigues, C., Sant'Anna, M.K., Madruga, M. and Medina, J.H. (1998) "Differential involvement of cortical receptor mechanisms in working, short- and long-term memory", *Behav. Pharmacol.* **9**, 421–427.

Izquierdo, I., Medina, J.H., Vianna, M.R., Izquierdo, L.A. and Barros, D.M. (1999a) "Separate mechanisms for short- and long-term memory", *Behav. Brain Res.* **103**, 1–11.

Izquierdo, I., Schröder, N., Netto, C.A. and Medina, J.H. (1999b) "Novelty causes time-dependent retrograde amnesia for one-trial avoidance in rats through NMDA receptorand CaMKII-dependent mechanisms in the hippocampus", *Eur. J. Neurosci.* **11**, 3323–3328.

Izquierdo, L.A., Viola, H., Barros, D.M., Vianna, M.R.M., Furman, M., Levi de Stein, M., Szapiro, G., Rodrigues, C., Choi, H., Madche, C., Medina, J.H. and Izquierdo, I. (2001) "Novelty enhances retrieval: molecular mechanisms involved in rat hippocampus", *J. Neurosci.* **13**, 1464–1467.

James, W. (1890) Principles of Psychology (Holt, New York).

Kandel, E.R. and Squire, L.R. (2000) "Neuroscience: breaking down scientific barriers to the study of brain and mind", *Science* **290**, 1113–1120.

Konorski, J. (1948) Conditioned Reflexes and Neuron Organization (University Press, London).

McGaugh, J.L., Castellano, C. and Brioni, J. (1990) "Picrotoxin enhances latent extinction of conditioned fear", *Behav. Neurosci.* **104**, 264–267.

Netto, C.A. and Izquierdo, I. (1985) "On how passive is inhibitory avoidance", *Behav. Neural Biol.* **43**, 337–339.

Pavlov, I.P. (1956) Conditioned Reflexes (Dover, New York).

Pereira, P., Ardenghi, P.G., de Souza, M.M., Choi, H., Moletta, B. and Izquierdo, I. (2001) "Effects of infusions of the tyrosine kinase inhibitor radicicol into the hippocampus on short- and long-term memory of the inhibitory avoidance task", *Behav. Pharmacol.* **12**, 299–302.

de Quervain, D.J.K., Roozendaal, B. and McGaugh, J.L. (1998) "Stress and glucocorticoids impair retrieval of long-term memory", *Nature* **394**, 787–790.

Quevedo, J., Vianna, M.R.M., Roesler, R., de Paris, F., Izquierdo, I. and Rose, S.P.R. (1999) "Two time-windows of anisomycin-induced amnesia for inhibitory avoidance training in rats:

protection from amnesia by pretraining but not pre-exposure to the apparatus", *Learn. Mem.* **6**, 600–607.

Ramón y Cajal, S. (1893) "Neue Darstellung vom histologischen Bau des Zentralnorvös System", *Arch. Anat. Physiol. (Anat.)*, 319–428.

Regan, C. (2002) "Cell adhesion molecules", In: Riedel, G. and Platt, B., eds, Cellular Mechanisms of Memory Formation (Elsevier, Cambridge), In press.

Riccio, D.C. and Richardson, R. (2000) "The status of memory after experimentally induced amnesias: gone but not forgotten", *Physiol. Psychol.* **12**, 59–72.

Szapiro, G., Izquierdo, L.A., Alonso, M., Barros, D., Paratcha, G., Ardenghi, P., Pereira, P., Medina, J.H. and Izquierdo, I. (2000) "Participation of hippocampal metabotropic receptors, protein kinase A and mitogen-activated protein kinases in memory retrieval", *Neuroscience* **99**, 1–5.

Taubenfeld, S.M., Wiig, K.A., Bear, M.F. and Alberini, C.M. (1999) "A molecular correlate of memory and amnesia in the hippocampus", *Nature Neurosci.* **2**, 309–310.

Vianna, M.R.M., Barros, D.M., Silva, T., Choi, H., Madche, C., Rodrigues, C., Medina, J.H. and Izquierdo, I. (2000a) "Pharmacological demonstration of the differential involvement of protein kinase C isoforms in short- and long-term memory formation and retrieval in rats", *Psychopharmacology* **150**, 77–84.

Vianna, M.R.M., Izquierdo, L.A., Barros, D.M., Ardenghi, P., Pereira, P., Rodrigues, C., Moletta, B., Medina, J.H. and Izquierdo, I. (2000b) "Differential role of hippocampal cAMP-dependent protein kinase in short- and long-term memory", *Neurochem. Res.* **25**, 621–626.

Vianna, M.R.M., Szapiro, G., McGaugh, J.L., Medina, J.H. and Izquierdo, I. (2001) "Memory retrieval of a fear-motivated task initiates extinction requiring protein synthesis in rat hippocampus", *Proc. Natl Acad. Sci. USA* **21**, 12251–12254.

Walz, R., Roesler, R., Barros, D.M., de Souza, M.M., Rodrigues, C., Sant'Anna, M.K., Quevedo, J., Choi, H.K., Neto, W.P., DeDavid e Silva, T., Medina, J.H. and Izquierdo, I. (1999) "Effects of post-training infusions of a mitogen-activated protein kinase kinase inhibitor into the hippocampus or entorhinal cortex on short- and long-term retention of inhibitory avoidance", *Behav. Pharmacol.* **10**, 723–730.

Zornetzer, S.F. (1978) "Neurotransmitter modulation and memory: a new neuropharmacological phrenology?", In: Lipton, M.A., DiMascio, A. and Killam, K.F., eds, Psychopharmacology–A new generation of Progress (Raven Press, New York), pp 537–649.