

Pharmacological Studies of the Molecular Basis of Memory Extinction

Monica R.M. Vianna*, Martín P. Cammarota, Adriana S. Coitinho, Jorge H. Medina, and Ivan Izquierdo

Centro de Memória, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

Abstract: Retrieval procedures, if carried out without reinforcement, initiate memory extinction. The extinction of one-trial avoidance learning requires glutamate NMDA receptors, calcium-calmodulin dependent protein kinase II, cAMP-dependent protein kinase and mitogen-activated protein kinases, and, importantly, protein synthesis and gene expression in the hippocampus. The extinction of fear-potentiated startle requires NMDA receptors and mitogen-activated protein kinases in the basolateral amygdala. The extinction of conditioned taste aversion requires protein synthesis in the insular cortex. Thus, extinction is an active process that involves a variety of molecular events and—at least for the one task in which it was studied—both gene expression and protein synthesis. Insofar as in each of the tasks mentioned, the treatments were studied in a different brain region, therefore, it is still not known whether extinction in general uses those brain areas in parallel, or whether the extinction of each task is metabolically different. A role of endogenous cannabinoids in extinction has been postulated; some evidence indicate that they act on the amygdala, but indirect findings suggest that they may also exert their action in the hippocampus. When carried out using methods that enhance perception that the reinforcement is absent, extinction can be quite profound, and the animals require “de novo” gene expression and protein synthesis in the hippocampus in order to reinstall the original learning. This might be of value in the design of “exposure” therapies for the treatment of phobias and of post-traumatic stress disorders.

Key Words: Memory extinction, mechanisms of extinction, involvement of brain structures in extinction, clinical applications of extinction

INTRODUCTION

Repeated retrieval without reinforcement leads to the extinction of memories. Extinction was first described by Pavlov for classical conditioning [1], in which an initially neutral stimulus (conditioned stimulus, CS) signals the imminence of an unconditioned stimulus (US), which always elicits a response because it is “biologically significant”: eg. food, a footshock, etc. In Pavlovian conditioning, the CS-US contiguity ends up generating a response to the CS that is similar or related to that produced by the US: salivation, leg withdrawal, etc. The new response to the CS is called conditioned response (CR). Repetition of the CS alone, without the US (i.e., without “reinforcement”), leads to a diminution of retrieval: the intensity or the occurrence of the CR becomes gradually extinguished [2]. This is generally believed to consist of a new learning in which the CS-no US association predominates over the previously acquired CS-US association, and the reduction of retrieval of the previous memory results from a change in the hierarchy of possible responses to the CS. Preference for the omission or attenuation of the CR prevails over performance of the CR [2, 3].

Extinction is important clinically. Under various names (extinction, habituation, exposure, etc.) it has been used successfully for decades in the treatment of fobias, and has more recently been applied to the treatment of the post-traumatic stress disorder [4-6]. The therapeutic objective is

to dissociate a given experience from its potential or imagined dire consequences: the sight of a spider or a snake from their bite; or the witnessing of or suffering from torture, pain, prison, etc. from a repetition of such experiences [5]. Clearly, the constellation of cues for the terrorizing consequences of the experiences must be re-associated with non-harmful consequences.

Extinction is not synonymous with forgetting: forgetting consists in the actual loss of memories, be it by the lack of use of the synapses involved [cf. 7], or by the physical loss of neurons or synapses, due to the passage of time or because of some disease. Extinction is viewed as a form of learning by which the expression of some memories is inhibited because the significance of cues is altered so that a new connection of the CS with other responses may predominate [2].

In 1928, Konorski, based on the earlier findings of Thorndike [8], described another form of conditioning, which is now generally called instrumental [9]. In this type of learning, the CR determines whether the US will be delivered or not, and is therefore used as an instrument by the subject. For example, salivation to the CS can bring about the delivery of food (instrumental alimentary conditioning), or withdrawal of the leg is used by the animal to avoid a footshock (avoidance conditioning). The extinction of instrumental conditioning is usually much slower than that of the classical conditioning, particularly in avoidance situations, because the US will be absent every time that the animal makes a correct CR, and so the subjects will need many trials before they can actually perceive that the CS-no US has become a new association [9]. It should be noted here

*Address correspondence to this author at the Centro de Memória, Departamento de Bioquímica, ICBS, UFRGS, Ramiro Barcellos, 2600-anexo, (90035-003) Porto Alegre, RS, Brazil; E-mail: mrmvianna@yahoo.com

that the CR is almost invariably “useful” to the animal, regardless of whether the training paradigm is classical or instrumental. In Pavlov’s original model, in which the CS-US pairing is present on every acquisition trial, salivation is appropriate to the ingestion of food and leg withdrawal serves the purpose of diminishing the surface of the leg that receives a footshock [1]. That is precisely what made the discovery of instrumental conditioning possible: if the CR can be made purposeful, why not use it as a means to bring about the US (in alimentary tasks) or its omission (in avoidance tasks) [8, 9]?

The task most used for the study of the effect of drugs on learning and memory is a one-trial procedure called “passive” or “inhibitory” avoidance [10, 11], but it is in reality a classically conditioned task [3]. This procedure has been used in many species, from mollusks to humans; most studies have been conducted in rodents and chicks [11, 12]. The training paradigm consists of one trial of Pavlovian learning in which the animals are placed in a safe compartment or platform (CS), and as soon as they step out of it they receive a footshock (US). On test trials, the animals are placed again on the safe place (CS) and their latency to step out of it is measured. This latency is higher than that in the single training trial, and this increase is taken as indicative of a CR. By performing this CR, of course, the animals delay or prevent the occurrence of the footshock; but since learning was by contiguity and the animals had no chance to put to test the avoidance value of the CR at the time of training, this is to be viewed as a Pavlovian task. As custom has it, however, it is usually called an “avoidance” task, because the longer the test latency, the more the animals will delay the footshock. But the footshock is usually omitted in the test session of this task. Thus, actually, the test session usually consists of the initiation of extinction (a CS-no US session) [13, 14], and in this task as well as in others of its kind the animals are indeed prevented by experimental design to find out whether the CR can effectively be used as an instrument.

Since one-trial avoidance is by definition an anxiogenic task, its extinction can be viewed as a good model for the studies of human extinction of anxiogenic learning, such as that of phobias or post-traumatic stress disorders.

BRAIN AREAS INVOLVED IN THE INITIATION OF EXTINCTION

As mentioned, the formation of many memories, perhaps most, crucially involves the hippocampus [15] and its connections [16]. This includes one-trial inhibitory avoidance and similar tasks [11, 17]. Thus, the trace of the original CS-US association of these tasks must presumably lie in synapses of the hippocampus and its connections, most of which are cortical.

In test sessions conducted without reinforcement, as mentioned above, the original memory trace is reactivated, which causes retrieval as soon as the CS is presented [18-21]. However, if the CS is not accompanied by the US, the animals detect the new CS-no US association at the same time [3, 22, 23]. In a one-trial step-down avoidance task, this

takes place as soon as the animals step down from the safe platform (CS) and receive no footshock when they place their paws on the grid (no US) [24].

The initiation of extinction, in which a CS-no US connection supersedes or superimposes upon the original CS-US connection should somehow involve the synapses used for the original learning. By definition, extinction consists of the inhibition of a previous association and its gradual substitution by another, new association (see above). Associative learning is believed to result from synaptic changes [1, 5, 52, 58, 62]. Not surprisingly, much recent research on the mechanisms of memory extinction has focused on the hippocampus [3, 24-28], which is held responsible for the consolidation of many forms of learning, particularly those of a declarative and episodic type [11, 15]. In particular, it was recently demonstrated that the context-specificity of extinction (i.e., animals extinguish better in the apparatus in which they had originally been trained) is inhibited by the administration of the GABA_A agonist, muscimol, into the dorsal hippocampus [25].

There are tasks, however, in which other brain structures are critical for memory consolidation. In some of these tasks, the role of these other structures on extinction has been studied. The acquisition of fear-potentiated startle relies quite heavily on the basolateral nuclear complex of the amygdala [29]. Studies on the extinction of fear-potentiated startle have accordingly been carried out on the amygdala [30-33]. The consolidation of conditioned taste aversion relies on the insular cortex [34] and on the perirhinal cortex [35]. Studies on the extinction of conditioned taste aversion have been carried out only on the insular cortex [36]. The medial prefrontal cortex and the retrosplenial cortex have recently been implicated both in the acquisition and extinction of trace eye blink conditioning, a form of learning that depends on the hippocampus [37, 38]. The rostral portion of the retrosplenial cortex is critical for acquisition and its caudal part is critical for extinction of this task [38].

However, most of these studies have centered on only one structure (the hippocampus for inhibitory avoidance, the basolateral amygdala for conditioned startle, the insula for conditioned taste aversion, etc.). It is therefore possible that, as happens with consolidation [11, 16] and retrieval [18, 19, 22, 23, 28, 39], extinction could involve other structures in addition to those that have specifically been studied for each behavior. For example, the perirhinal cortex has recently been shown to participate in conditioned taste aversion [35] along with the insular cortex. There are indeed important anatomical and physiological connections between the hippocampus, the amygdala, the ento- and perirhinal cortex and other cortical areas including the insula and the frontal cortex [40, 41]. It is in principle hard to believe that the related forms of fear conditioning acquired in similarly brief session should require different arrays of brain structures for either consolidation or extinction [16, 42]. Although there may be regional specialization in the analysis of different components of each task [29, 38, 42, 43], in all probability several brain regions need to be activated at the same time in all or most of them, including the hippocampus, basolateral amygdala and the entorhinal and parietal cortex [44, 45].

ARE NMDA RECEPTORS NECESSARY FOR EXTINCTION?

While extinction is clearly a consequence of retrieval without reinforcement, the mechanisms involved in extinction and retrieval show several important differences. The retrieval of fear potentiated startle is blocked by intra-amygdala infusion of the glutamate AMPA receptor antagonist, CNQX [46] or by mGluR II receptor agonists [32] but not by that of the glutamate NMDA receptor antagonist, AP5 [47]. Similarly, retrieval of one-trial step-down inhibitory avoidance learning is hindered by the intrahippocampal infusion of CNQX but not of AP5 [48]. However, the extinction of these tasks is strongly affected by the intra-amygdala [30] and the intrahippocampal infusion of AP5 [24], respectively, when the drugs are administered on the first retrieval session. Further, in fear-potentiated startle, extinction is facilitated by the intra-amygdala infusion of D-cycloserine, a partial agonist at the glycine recognition site of the NMDA receptor complex, which would be expected to increase the function of that receptor [33].

Thus, while it is obvious that extinction is triggered or gated by the first retrieval session without reinforcement [3, 23], it is also clear that the biochemical substrates of retrieval are not identical to those of extinction, beginning at the glutamate receptor level. The former do not, and the latter do, include NMDA receptor activation in the hippocampus.

Further, NMDA receptors have repeatedly been assigned a role in associative learning [see for references 11, 16]. Extinction involves a new association (CS-no US) that superimposes upon and supersedes the original association (CS-US) [2].

However, the extinction of conditioned taste aversion does not require NMDA receptors in the insular cortex: the localized pre-test infusion of AP5 into this structure does not affect extinction [36]. Whether this means that the extinction of this task involves a different mechanism from that of the fear conditioning tasks, is not clear. Other structures, such as the perirhinal cortex [35], hippocampus and amygdala have not been studied in relation to the extinction of conditioned taste aversion. It is entirely possible that the "closure" of the CS-no US circuit for this task may take place somewhere other than the insular cortex, and that the latter plays an important, but subsidiary role.

An important experiment by Quirk and his associates has explored the relation of NMDA receptors to extinction under a different light [49]. They found that rats can extinguish both conditioned fear and a conditioned emotional response normally for 90 min under the presence of a systemically administered NMDA receptor antagonist, but they do not recall this extinction 24 h later. These findings suggest that early extinction takes place independently of NMDA receptors, but the extinction manifested at longer times does require NMDA receptors; and also suggest that the consolidation of extinction may involve off-line relearning, perhaps in prefrontal-amygdala circuits, such as those described by others [41]. The concept of simultaneous extinction processes taking place at different areas of the brain, using different biochemical mechanisms [49] may be important to inter-

pret many of the apparently discrepant data on the pharmacology of extinction, such as those mentioned above concerning conditioned taste aversion. It also points to the possibility of two separate forms of extinction, a short-term and a long-term form, that to an extent rely on different biochemical substrates. This is of course reminiscent to the recently described separation of short- and long-term memory for fear-motivated conditioning [50]. Extinction would appear to be just another form of learning with parallel short- and long-lasting memory processing [51]. The distinction between short- and long-term extinction should be further explored. So far, most studies have centered on long-term extinction, including all those that are commented in this article except [49].

THE ONSET OF EXTINCTION REQUIRES GENE EXPRESSION AND PROTEIN SYNTHESIS

It is believed that the various biochemical changes that follow after the acquisition of new learnings and culminate by two peaks of gene expression and protein synthesis [52-54] end up generating, first, cell adhesion changes [55, 56] and ultimately morphological changes at synapses [57, 58].

Does extinction also require protein synthesis? The first report showing that it does was by Flood and his collaborators in 1977, using the protein synthesis inhibitor, anisomycin and a pole jump active avoidance task [59]. They correlated the duration of the brain protein synthesis inhibition with the deleterious effect of the drug on consolidation and extinction of this task. Two recent pharmacological experiments extended this finding to specific brain regions. The infusion of anisomycin into the insular cortex at the time of the first test session blocks extinction of conditioned taste aversion [36]. Similarly, anisomycin bilaterally infused into the CA1 region either before or after the first of a series of test sessions blocks extinction of inhibitory avoidance [24].

Does this need for protein synthesis rely on pre-existing mRNAs or does it involve newly synthesized mRNAs and therefore the expression of "new" proteins? The answer to this question is critical in order to establish whether extinction really involves new learning or consists rather merely in the inhibition of a previously consolidated response. Again, pharmacological experiments came to the rescue. The infusion of the RNA polymerase II inhibitors, DRB and -amanitin into the CA1 region of the rat prior to the first of a series of test sessions blocks extinction of the one-trial inhibitory avoidance task; infusions of these drugs (or anisomycin) 1 or 3 h after the test has no effect [24]. Therefore, extinction, like other new learnings (eg.[52, 54]) does require gene expression and the synthesis of "new" proteins. Unlike consolidation of the original task, however, the need for gene expression and protein synthesis in the case of extinction appears to be just at the time of training [24], and not in two peaks [54].

In a recent paper on contextual fear conditioning and water maze learning, the subcutaneous administration of anisomycin hindered consolidation but not extinction of the tasks [60]. Peripheral administrations of anisomycin are much less effective than infusions of the drug into the brain

in order to reduce brain protein synthesis [see 59]. Thus, it is possible that extinction is simply somewhat less sensitive to brain protein synthesis inhibition than consolidation [3, 26].

SIGNALING PATHWAYS INVOLVED IN EXTINCTION

The activation of glutamate NMDA receptors that occurs during memory consolidation or in the induction of long-term potentiation (LTP), triggers a hyperactivation of various signaling pathways, of which the best studied are those mediated by calcium/calmodulin dependent protein kinase II (CaMKII) [61], cAMP-dependent protein kinase (PKA) [53] and the mitogen-activated protein kinase (MAPK) family [53, 62].

The importance of these pathways for consolidation has been ascertained in pharmacological experiments using localized brain infusions of specific inhibitors of the enzymes. Consolidation of one-trial inhibitory avoidance requires a dual activation of PKA, firstly right after training and again 2-6 h later [16, 24, 53, 63], an early activation of CaMKII [61] and a late activation of MAPKs [62], all in the hippocampus. The PKA and MAPK changes require intact NMDA receptors at the onset of the consolidation process [62]. The consolidation of contextual and auditory fear conditioning requires the participation of PKA and MAPK in periventricular structures of the brain [64]. Fear-conditioned startle requires PKA and the MAPK cascade in the basolateral nucleus of the amygdala [31]. Conditioned taste aversion requires the integrity of the MAPK cascade in the insular cortex [34, 36].

Are any of these metabolic pathways necessary for extinction? This was studied in some detail in the hippocampus for one-trial inhibitory avoidance. When given bilaterally into CA1 either prior to, or right after, the first of a series of 4 retrieval tests, AP5 the PKA inhibitor, Rp-cAMPs, the CaMKII inhibitor, KN62, and the MAPK inhibitor, PD098059 blocked extinction of this task [26]. The effect was seen in spite of the fact that Rp-cAMPs and PD098059 hindered retrieval in the first test, and AP5, Rp-cAMPs and PD098059 hindered retrieval in the second test session. The blockade of retrieval in the 3rd and 4th test sessions by effect of AP5, Rp-cAMPs, KN62 and PD098059 was seen regardless of whether the 2nd test was carried out or omitted [26].

The extinction of fear-potentiated startle is hindered by PD098059 given into the basolateral amygdala prior to retention testing, suggesting that this molecular pathway is also essential in that area for extinction [31].

In the conditioned taste aversion task, the pre-test administration into the insular cortex of neither PD098059, nor, for that matter, scopolamine or AP5, has some effect on extinction; interestingly, the beta-blocker propranolol hinders extinction [36]. All these substances had been shown to disrupt consolidation of this task when given into that structure [34].

So again, as had been discussed above for other drugs, the findings on the pharmacology of extinction so far are

somehow scattered. More work has been done on the hippocampus using one-trial avoidance than in any other structure or task. The basolateral amygdala and fear-potentiated startle come second. However, nobody has decided to explore more than one structure in each task, which would be necessary in order to establish whether different biochemical systems acting in various brain areas control extinction. In fact, many studies on consolidation [11] and retrieval [18, 19] have shown that these two processes are controlled by many brain areas, and not just by one. The case of retrieval is of particular importance, since there is no doubt that extinction stems from retrieval: it is precisely on the first retrieval test when the animals perceive the CS-no US connection and begin to extinguish [1-3, 9, 24, 26].

ENDOGENOUS CANNABINOID SYSTEM AND EXTINCTION [71]

Endogenous cannabinoids (endocannabinoids) and their receptor CB1 are found, usually in GABAergic interneurons, in many places in the brain [65, 66], including the cerebellum, substantia nigra, globus pallidus [67], the basolateral amygdala [68] and the hippocampus [69]. Accordingly, the systemic administration of CB1 agonists produces symptoms related to the functions of these brain areas, such as catalepsy, immobility, ataxia, impairment of the performance of complex motor behaviors [67], sensitization to the psychomotor effects of amphetamine [70] and memory deficits similar to those produced by neurochemical lesions of the hippocampus [71]. Knock-out mice lacking CB1 receptors retain memory for longer times than their wild-type counterparts [72]. Endocannabinoids facilitate LTP induction in the CA1 region of the hippocampus [73]. Importantly, these substances are released in the hippocampus and cerebellum from depolarized postsynaptic neurons in a calcium-dependent manner [74] and act retrogradely on presynaptic receptors to suppress inhibition or excitation [73-75], which probably underlies their influences on LTP.

Recently, Marsicano, Lutz and their co-workers [76] have shown, using both CB1 knockout mice and the CB1 antagonist SR141716A, that endocannabinoids control extinction of auditory conditioned fear. Both in SR141716A-treated wild-type mice and in the CB1 knockouts, short- and long-term extinction are markedly depressed. The data correlate with the finding that CB1 knockout or SR141716A-treated mice present a decrease of GABA release from axon terminals; and, further, with an increase of endocannabinoid levels in the basolateral amygdala (but not in medial prefrontal cortex) seen upon re-exposure to the CS (a tone) in test sessions.

The important discovery of a possible endocannabinoid control of extinction mediated by CB1 receptors in the basolateral amygdala [76] opens an entirely new pathway for the investigation of the mechanisms of this behavioral process. In view of the findings indicating a role for endocannabinoid/CB1 mediated electrophysiological effects in the hippocampus [74, 75], of the evidence suggesting that the hippocampus is crucially involved in extinction [3, 24, 26], and of the similitude of CB1 mediated memory deficits and those of hippocampal lesions [71], it would be interesting to

reproduce the findings of Marsicano *et al.* [76] studying the hippocampus instead of the basolateral amygdala.

It would also be interesting to discount the various reported effects of the endocannabinoid/CB1 receptor system on motor control [67, 70] from the effects observed concerning memory consolidation [71] and particularly from those on extinction [76]. All other drugs studied for their effect on extinction when given into hippocampus or amygdala (AP5, Rp-cAMPs, PD098059, KN62, anisomycin, DRB, -amanitin) have convincingly been shown not to affect locomotor and orienting activity measured in an open-field and locomotor and pro- or anti-conflict behavior measured in an elevated plus maze [see above and 18, 24, 26] when administered into the same structures in which they have an effect on extinction.

IS THERE A ROLE FOR GABAergic TRANSMISSION IN EXTINCTION?

Since extinction involves the inhibition of a previously learned CR, it seems only logical to think that GABAergic receptors might play a role in extinction. The possibility of such a role is strengthened by the findings on endocannabinoids mentioned above [76], since the drugs affect GABAergic transmission and are present mostly in GABAergic neurons [66, 68, 76].

Obviously, GABA_A receptors must be involved in extinction. The question is how, where and when? The facts that different drugs have been used, that they were given at different times, and that all of them were given systemically, prevent a conclusion. The blocker of GABA_A receptor chloride channels, picrotoxin, enhances on-going extinction of one-trial inhibitory avoidance when given on the third of 3 retention tests [77]. However, in context-dependent fear conditioning to a tone [78], the beta-carboline FG7142 (a partial antagonist at the benzodiazepine site of GABA_A receptors) interferes with the onset of extinction and also reverses extinction once initiated, i.e. it causes reinstatement of the extinguished response [79]. The inhibitory effect of the benzodiazepine, midazolam, on fear conditioning itself is reversed when rats are tested in a different context; which has been viewed as an effect parallel to that of extinction [80, 81].

Certainly GABA_A receptor agonists and antagonists, both direct and indirect, should be studied for their effect on extinction when given on the first of a series of extinction tests [i.e., 24, 26, 31] into specific brain structures that play a role in extinction, like the hippocampus and basolateral amygdala.

“FULL” EXTINCTION

Given that extinction is not synonymous with forgetting (see above), how complete can extinction be? Can it be carried to the point in which the original CR can only be reinstalled through a process requiring again gene expression and protein synthesis, as if it were again a new learning?

Extinction is viewed as a consequence of the development of a response to a CS-no US association that supercedes an existing CS-US connection [2, 82], obviously a way in which extinction could be enhanced (and the original CR could fully be inhibited) is by strengthening the “no US” component in successive test sessions. This was done in a recent experiment conducted in our laboratory [27]. Rats were regularly trained in a conventional one-trial step-down avoidance task using a 0.5 footshock. On subsequent test sessions, spaced 24 apart, the animals were placed on the platform (CS) and then allowed to explore the apparatus freely for 30 sec once they stepped down. Extinction reached an asymptotic level in which step-down latency was indistinguishable from training session latencies in the 4th test. Further, if the extinction procedure was interrupted on the 5th session, there was no spontaneous recovery over a period of 8 days [27]. Spontaneous recovery is often used as an indication that CRs are not erased by extinction, but they are merely attenuated [2, 82]. (Extinction may however persist beyond recovery [82]).

Further, and importantly, retraining in the avoidance task 1 day after the last (5th) extinction session using the “enhanced no-US” mode caused a re-installment of the original CR; but this required gene expression and protein synthesis: it was prevented by DRB, -amanitin and anisomycin [27]. In addition, in animals extinguished using this enhanced mode, expression of the memory trace was not enhanced by the classic retrieval enhancers, systemic ACTH₁₋₂₄ or adrenaline [21, 22], and intrahippocampal Sp-cAMPs [18], a stimulant of PKA [27]. The retrieval enhancers are known to work only when there are enough remnants of the original CR so that this can be reactivated, even if this occurs months later [22].

Therefore, yes, by strengthening the “no-US” component of the extinction paradigm, extinction can be made quite complete, to a point at which there is no spontaneous recovery of the original CR, reinstallation of the original CR requires gene expression and protein synthesis, and its retrieval cannot be enhanced by any well-known retrieval enhancers [27].

THERAPEUTIC POTENTIAL OF MEMORY EXTINCTION

The observation that by strengthening its “no-US” component extinction can considerably be strengthened, offers a new perspective for the treatment of post-traumatic stress disorder and other ailments for which extinction has been reported to be beneficial, like phobias. Extinction treatment procedures, whatever the name that they are disguised with (i.e., habituation, exposure, flooding, etc.) consist in the exposure to visual or other material from a traumatic experience (or, in the case of phobias, a pseudo-experience) omitting the reinforcement [4-6]. Simple exposure and simple omission can sometimes be undesirable, since they may merely revive the horror of the original experience. The “no-US” side should be enhanced, either by increasing its duration or by psychotherapeutic procedures. Exposure to scenes of August 8, 1945 to somebody present that day in Nagasaki is likely to reevoke fear and desperation unless “no-US”

factors related to the therapeutic exposure are stressed and somehow enhanced. Some use benzodiazepines to reduce anxiety at the time of exposure [5, 6]; which, based on the extinction-like effect of benzodiazepines commented above [80], might help. However, it is far better to devise psychotherapeutic manners of rationally reducing the US value of what is shown or discussed. If not, there is a danger that the tranquilization induced by the benzodiazepines might establish a form of state-dependency, and that in the absence of the drug the patients may relive the horror inherent to the CS. Animal experiments suggest that a hypervaluation of the “no-US” component of the extinction procedure may be a useful way to enhance extinction [27].

IS ENHANCED RETRIEVAL THE SAME THING AS REDUCED EXTINCTION?

In much of the early literature on the effect of ACTH, adrenaline, vasopressin and other substances on memory processes, the drugs were administered prior to a retention test and they enhanced retrieval, an effect which was described by many as “reduced extinction” (eg., 13, 14); see [21, 22].

While it is true that reduced extinction is expressed by an enhancement of retrieval [eg. 3, 24, 26] and enhanced extinction is expressed as a reduction of retrieval [77, 81, 82], extinction itself can only be detected by its gradual progression over repeated testing [1, 9]. Retrieval may suffer ups or downs along an extinction curve [3, 26], which may result from performance rather than from real learning changes. Very often, retrieval can temporarily be inhibited by treatments that actually also reduce extinction, like intrahippocampal Rp-cAMPs, AP5, PD098059 [26] or anisomycin [24].

Therefore, it is preferable to restrict the use of the terms “enhanced (or depressed) retrieval” to studies that measure retrieval alone, without measuring extinction [eg., 13, 14], and to reserve the terms “enhanced (or depressed) extinction” to studies in which extinction is measured specifically over several test sessions.

AND WHAT ABOUT LONG-TERM DEPRESSION?

Several years ago, Tsumoto [83] called attention to the possibility that long-term depression (LTD) could be in charge of forgetting. He used the term ‘forgetting’ perhaps improperly. Although electrophysiologists and others often confuse forgetting with extinction, it is now widely accepted that both pertain to different processes. Forgetting is the actual loss of memories, usually the result of chronic disuse [eg., 18] or neuronal loss. Extinction is instead a form of learning based on a CS-no US association, which causes the inhibition of responses previously learned to a CS-US association [see above and 2,3].

However, the postulation of Tsumoto [83] still stands, and may be rephrased. Inasmuch as memory consolidation depends on a sequence of cellular and molecular processes that is in many respects similar to those described for LTP [11, 16], can extinction involve an LTD-like sequence of

events? In principle it could. LTD occurs in many regions of the brain, requires in many cases, but not all, NMDA receptors, and involves to various extents the signaling pathways mentioned above *à propos* memory consolidation and extinction [84]. The possibility that LTD-like processes may be involved in the generation of extinction deserves investigation.

FINAL COMMENT

Extinction is a very important behavioral process. Without it, cognition as we know it would certainly be impossible: our brain would be constantly clogged with learned response choices that may be inadequate most of the time. We would attribute danger to situations whose dangerous nature has long been lost, or appetitive value to stimuli that no longer have it. In addition, as mentioned above, extinction is of therapeutic importance in the treatment of disorders in which fear-motivated behavior triggers undue and undesirable reactions, such as phobias and the post-traumatic stress disorder. Extinction can be quite complete and, for behavioral purposes, lead to an effective erasure of the previous learning.

Recent research has indicated that extinction is indeed a new learning that superimposes upon, and overrides, previously acquired learnings. Extinction relies on molecular changes in brain areas associated with the previous acquisition and consolidation of those learnings. In different behavioral models in rodents, the hippocampus, basolateral amygdala and insular cortex have been shown to be implicated in extinction; however, the experiments so far have not established whether one or other of these structures predominates for each task or all of them (and possibly others) are involved in the generation of different forms of extinction. Notwithstanding, NMDA receptors (at least in hippocampus and amygdala), signaling pathways such as those mediated by CaMKII and PKA (in the hippocampus) and MAPKs (in hippocampus and amygdala) are required for extinction. So is gene expression (in the hippocampus) and protein synthesis (at least in hippocampus and insular cortex). It will be necessary to examine in detail the need for all these biochemical processes in different brain areas in the extinction of different tasks. Some findings have suggested that there is an early form of extinction that is not dependent on NMDA receptors from which animals progress to long-lasting NMDA receptor-dependent extinction. Others suggest a key role for endocannabinoids in the specific control of extinction, acting presumably in the basolateral amygdala.

As has been previously described *in extenso* for the process of memory consolidation [see references in 11, 16], the findings that revealed the main molecular characteristics of extinction have been pharmacological. Like all specifically timed behaviors, extinction (which starts at the first notice of a CS-no US association) is not readily amenable to investigation using brain-lesioned, knockout or transgenic animals: observed effects may be attributed to influences on memory processes other than extinction.

Finally, there has been considerable speculation on the possibility that extinction must use the same neuronal cir-

cuits and/or molecular mechanisms used for consolidation of the tasks that are extinguished [eg. 85]. Indeed, as outlined above, much of the pharmacological work on extinction was carried out on the same structures that had previously been shown to be responsible for extinction of a variety of tasks: the insula for conditioned taste aversion [36], the basolateral amygdala for fear-potentiated startle [30,33], and the dorsal hippocampus for one-trial avoidance [3, 24, 26]. However, while a few of the molecular processes involved in extinction match those involved in consolidation, notably and importantly gene expression and protein synthesis [24], many do not; and, in addition, there are indications that other brain areas aside from those that had been used in consolidation may be activated at the time of extinction. No doubt, the same synapses whose function (and morphology, perhaps, [58]) have been changed by consolidation must somehow be partly inhibited or gated out by extinction [86]. But this is a far cry from saying, let alone demonstrating that extinction is built into, or sustained by changes in, those synapses. There is simply not enough data to support this speculation and build it up into a sustainable hypothesis. A much simpler case for co-existence of two different forms of memory in the same synaptic network has been posed for short- and long-term retention of the *same*, rather than a different or opposite memory [51] or LTP [87,88], with the *same* input and output, but there is no procedure available yet to test that hypothesis. Further research will no doubt throw some light into this question.

ACKNOWLEDGEMENTS

Work supported by research grants from CNPq (Brazil) to I.I. and CONICET, Argentina, to J.H.M.

NOTE ADDED IN PROOF

In a work carried out after this paper was submitted, we observed that the BLA regulates extinction of one-trial inhibitory avoidance in the rat [88]. The infusion into the BLA of DRB, a-amanitin or Rp-cAMPs prior to the first of 5 daily test sessions, at the same doses that had been studied in the hippocampus [26], inhibits extinction. Thus, both CA1 and the BLA are in charge of extinction of this task. The participation of the amygdala in extinction of inhibitory avoidance does not include a role for MAPK [88], in which it differs both from its effect on the hippocampus in extinction of this task [26], and its own role in extinction of fear-motivated startle [31].

ABBREVIATIONS

ACTH	=	Adrenocorticotrophic hormone
AMPA	=	-amino-3-hydroxy-5-methyl-4-isoxazole propionate
AP5	=	D(-)-2-amino-5-phosphono pentanoate
CaMKII	=	Calcium-calmodulin-dependent protein kinase type II

cAMP	=	Cyclic'-3'5'-adenosylmonophosphate
CB1	=	Cannabinoid type I receptor
CNQX	=	6-cyano-7-nitroquinoxaline-2,3-dione
CR	=	Conditioned response
CREB	=	Cyclic adenosylmonophosphate binding protein response element
CS	=	Conditioned stimulus
ERK	=	Extracellular signal-regulated protein kinase
GABA _A	=	Gamma-aminobutyric acid type A receptor
KN-62	=	1-(N,O,bis[4-isoquinolinesulfonyl]-N-methyl-L-tyrosyl)-4-phenylpiperazine
LTP	=	Long-term potentiation
MAPK	=	Mitogen-activated protein kinase
MCPG	=	-methyl-4-carboxyphenylglycine
mGluR	=	Metabotropic glutamate receptors
NMDA	=	N-methyl-D-aspartate
PD098059	=	2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one
PKA	=	Cyclic adenosine monophosphate-dependent protein kinase
PKC	=	Calcium-dependent protein kinase
Rp-cAMPs	=	Rp-isomer of cAMP
Sp-cAMPs	=	Sp-isomer of cAMP
US	=	Unconditioned stimulus

REFERENCES

- [1] Pavlov, I.P. (1927) *Conditioned Reflexes*. Oxford, Oxford University Press.
- [2] Rescorla, R.A. (2001) Retraining of extinguished Pavlovian stimuli. *Journal of Experimental Psychology: Animal Behavior Processes*, **27**, 115-124.
- [3] Vianna, M.R.M., Szapiro, G., McGaugh, J.L., Medina, J.H., Izquierdo, I. (2001) Retrieval of memory for fear-motivated training initiates extinction requiring protein synthesis in the rat hippocampus. *Proceedings of the National Academy of Sciences USA*, **98**, 12251-12254.
- [4] Beckett, W.S. (2002) Post-traumatic stress disorder. *New England Journal of Medicine*, **346**, 1495-1498.
- [5] van Minnen, A., Wessel, I., Dijkstra, T., Roelofs, K. (2002) Changes in PTSD patients' narratives during prolonged exposure therapy: a replication and extension. *Journal of Traumatic Stress*, **15**, 255-258.

- [6] Rothbaum B.O, Schwartz, A.C. (2002) Exposure therapy for posttraumatic stress disorder. *American Journal of Psychotherapy*, **56**, 59-75.
- [7] Eccles, J.C. (1964) *The Physiology of Synapses*. Springer, Berlin.
- [8] Thorndike, E.L. (1911) *Animal Intelligence*. New York, Macmillan.
- [9] Konorski, J. (1948) *Conditioned Reflexes and Neuron Organisation*, London, London University Press.
- [10] Gold, P.E. (1986) The use of avoidance training in studies of modulation of memory storage. *Behavioral and Neural Biology*, **46**, 87-98.
- [11] Izquierdo, I., McGaugh, J.L. (2000) Behavioural pharmacology and its contribution to the molecular basis of memory consolidation. *Behavioural Pharmacology*, **11**, 517-534.
- [12] Rose, S.P.R. (1995). Time-dependent biochemical and cellular processes in memory formation. In: McGaugh, J.L., Bermúdez Ratonni, F., Prado Alcalá, R.A., Eds., *Plasticity in the Central Nervous System: Learning and Memory*, Mahway, Erlbaum. pp. 171-184.
- [13] de Wied, D. (1966) Inhibitory effect of ACTH and related peptides on extinction of conditioned avoidance behavior in rats. *Proceedings of the Society for Experimental Biology and Medicine*, **122**, 28-32.
- [14] de Wied, D. (1970) Preservation of a conditioned avoidance response by lysine vasopressin. *Journal of Endocrinology*, **48**, xlv-xlvi
- [15] Eichenbaum, H. (1999) The hippocampus and mechanisms of declarative learning. *Behavioural Brain Research*, **103**, 123-133.
- [16] Izquierdo, I., Medina, J.H. (1997) Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiology of Learning and Memory*, **68**, 285-316.
- [17] Lorenzini, C.A., Baldi, E., Bucherelli, C., Sacchetti, B., Tassoni, G. (1996) Role of dorsal hippocampus in acquisition, consolidation and retrieval of rat's passive avoidance response: a tetrodotoxin functional inactivation study. *Brain Research*, **730**, 32-39.
- [18] Barros, D.M., Izquierdo, L.A., Mello e Souza, T., Ardenghi, P.G., Pereira, P., Medina, J.H., Izquierdo, I. (2000) Molecular signalling pathways in the cerebral cortex are required for retrieval of one-trial avoidance learning in rats. *Behavioural Brain Research*, **114**, 183-192.
- [19] Barros, D.M., Mello e Souza, T., De David, T., Choi, H., Aguzzoli, A., Madche, C., Ardenghi, P., Medina, J.H., Izquierdo, I. (2001) Simultaneous modulation of retrieval by dopaminergic D₁, -noradrenergic, serotonergic 1A and cholinergic muscarinic receptors in cortical structures of the rat. *Behavioural Brain Research*, **124**, 1-7.
- [20] Izquierdo, L.A., Barros, D.M., Medina, J.H., Izquierdo, I. (2000) Novelty enhances retrieval of one-trial avoidance learning in rats 1 or 31 days after training unless the hippocampus is inactivated by different receptor antagonists and enzyme inhibitors. *Behavioural Brain Research*, **117**, 215-220.
- [21] Izquierdo, L.A., Schröder, N., Ardenghi, P., Quevedo, J., Bevilacqua, L., Netto, C.A., Izquierdo, I., Medina, J.H. Systemic administration of ACTH or vasopressin in rats reverses the amnesic effect of posttraining β -endorphin but not that of intrahippocampal infusion of protein kinase inhibitors. *Neurobiology of Learning and Memory*, **68**, 197-202.
- [22] Izquierdo, I., Schröder, N., Netto, C.A., Medina, J.H. (1999) Novelty causes time-dependent retrograde amnesia for one-trial avoidance in rats through NMDA receptor and CaMKII-dependent mechanisms in the hippocampus. *European Journal of Neuroscience*, **11**, 3323-3328.
- [23] Izquierdo, L.A., Barros, D.M., Medina, J.H., Izquierdo, I. (2002) Stress hormones enhance retrieval of fear conditioning acquired one day or many months before. *Behavioural Pharmacology*, **13**, 203-214.
- [24] Vianna, M.R.M., Muller-Igaz, L., Coitinho, A.C., Medina, J.H., Izquierdo, I. (2002) Memory extinction requires gene expression in rat hippocampus. *Neurobiology of Learning and Memory*, in press.
- [25] Corcoran, K.A, Maren, S. (2001) Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *Journal of Neuroscience*, **21**, 1720-1726.
- [26] Szapiro, G., Vianna, M.R.M., McGaugh, J.L., Medina, J.H., Izquierdo, I. (2002) The role of NMDA glutamate receptors, PKA, MAPK and CAMKII in the hippocampus in extinction of conditioned fear. *Hippocampus*, in press.
- [27] Cammarota, M.P., Bevilacqua, L.R.M., Kerr, D., Medina, J.H., Izquierdo, I. (2002) Installment, extinction and reinstatement of a simple memory task in rats. *Journal of Neuroscience*, in press.
- [28] Izquierdo, I., Vianna, M.R.M., Izquierdo, L.A., Barros, D.M., Szapiro, G., Coitinho, A.S., Müller, L., Cammarota, M., Bevilacqua, L.R.M., Medina, J.H. (2002) Memory retrieval and its lasting consequences. *Neurotoxicity Research*, **4**, 573-593.
- [29] Davis, M. (1992) the role of the amygdala in fear-potentiated startle. *Trends in Pharmacological Sciences*, **13**, 35-41.
- [30] Falls, W.A., Miserendino, M.J., Davis, M. (1992) Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. *Journal of Neuroscience*, **12**, 854-863.
- [31] Lu, K.T., Walker, D.T., Davis, M. (2001) Mitogen-activated protein kinase cascade in the basolateral nucleus of the amygdala is involved in extinction of fear-potentiated startle. *Journal of Neuroscience*, **21**, RC162.
- [32] Walker, D.L., Davis, M. (2002) The role of amygdala glutamate receptors in fear learning, potentiated startle and extinction. *Pharmacology, Biochemistry and Behavior*, **71**, 379-392.
- [33] Walker, D.L., Ressler, K.J., Lu, K.T., Davis, M. (2002) Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine assessed with fear-potentiated startle in rats. *Journal of Neuroscience*, **22**, 2343-2351.
- [34] Dudai, Y. (2002) Molecular basis of long-term memory: a question of persistence. *Current Opinion in Neurobiology*, **12**, 211-216.

- [35] Tassoni, G., Lorenzini, C.A., Baldi, E., Sacchetti, B., Burcherelli. (1999) A peculiar pattern of temporal involvement of rat perirhinal cortex in memory processing. *Behavioral Neuroscience*, **114**, 875-881.
- [36] Berman, D.E., Dudai, Y. Memory extinction, learning anew and learning the new: dissociations in the molecular machinery of learning in cortex. *Science* **293**, 2417-2419.
- [37] Beylin, A.V., Gandhi, C.C., Wood, G.E., Talk, A.C., Matzel, L.D., Shors, T.J. (2001) The role of the hippocampus in trace conditioning: temporal discontinuity or task difficulty? *Neurobiology of Learning and Memory*, **76**, 447-461.
- [38] Weible, A.P., McEchron, M.D., Disterhoft, J.F. (2002) Cortical involvement in acquisition and extinction of trace eyeblink conditioning. *Behavioral Neuroscience*, **114**, 1058-1067.
- [39] Hall, J., Thomas, K.L., 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. *Journal of Neuroscience*, **21**, 2186-2193.
- [40] van Hoesen, G.W. (1985) Neural systems of the non-human primate forebrain implicated in memory. *Annals of the New York Academy of Sciences*, **444**, 97-112.
- [41] Dringenberg, H.C., Saber, A.J., Cahill, L. (2001) Enhanced frontal cortex activation in rats by convergent amygdaloid and noxious sensory signals. *NeuroReport* **12**, 2395-2398.
- [42] Izquierdo, I., da Cunha, C., Rosat, R., Jerusalinsky, D., Ferreira, M.B.C., Medina, J.H. (1992) Neurotransmitter receptors involved in memory processing by the amygdala, medial septum and hippocampus of rats. *Behavioral and Neural Biology*, **58**, 16-26.
- [43] Jerusalinsky, D., Ferreira, M.B.C., Walz, R., da Silva, R.C., Bianchin, M., Ruschel, A.C., Zanatta, M.S., Medina, J.H., Izquierdo, I. (1992) Amnesia by post-training infusion of glutamate receptor antagonists into the amygdala, hippocampus and entorhinal cortex. *Behavioral and Neural Biology*, **58**, 76-80.
- [44] Sacchetti, B., Lorenzini, C.A., Baldi, E., Tassoni, G., Burcherelli, C. (1999) Auditory thalamus, dorsal hippocampus, basolateral amygdala, and perirhinal cortex in the consolidation of conditioned freezing to context and to acoustic conditioned stimulus in the rat. *Journal of Neuroscience*, **19**, 9570-9578.
- [45] Ardenghi, P., Barros, D.M., Izquierdo, L.A., Bevilacqua, L., Schröder, N., Quevedo, J., Rodrigues, C., Madruga, M., Medina, J.H., Izquierdo, I. (1997) Late and prolonged memory modulation in entorhinal and parietal cortex by drugs acting on the cAMP/protein kinase A signalling pathway. *Behavioural Pharmacology*, **8**, 745-751.
- [46] Kim, M., Campeau, S., Falls, W.A., Davis, M. (1993) Infusion of the non-NMDA receptor antagonist CNQX into the amygdala blocks the expression of fear-potentiated startle. *Behavioral and Neural Biology*, **59**, 5-8.
- [47] Campeau, S., Miserendino, M.J., Davis, M. (1992) Intra-amygdala infusion of the N-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle. *Behavioral Neuroscience*, **106**, 5069-5074.
- [48] Izquierdo, I., Bianchin, M., Bueno e Silva, M., Zanatta, M.S., Walz, R., Ruschel, A.C., da Silva, R.C., Paczko, N., Medina, J.H. (1993) CNQX infused into rat hippocampus or amygdala disrupts the expression of memory of two different tasks. *Behavioral and Neural Biology*, **59**, 1-4.
- [49] Santini, E., Muller, R.U., Quirk, G.J. (2001) Consolidation of extinction learning involves transfer from NMDA-independent to NMDA-dependent learning. *Journal of Neuroscience*, **21**, 9009-9017.
- [50] Izquierdo, I., Barros, D.M., Mello e Souza, T., de Souza, M.M., Izquierdo, L.A., Medina, J.H. (1998) Mechanisms for memory types differ. *Nature*, **393**, 635-636.
- [51] Izquierdo, I., Ardenghi, P.G., Barros, D.M., Bevilacqua, L., Izquierdo, L.A., Medina, J.H., Mello e Souza, T., Pereira, P. (2000) Consolidation of short- and long-term memory. In: Gold, P.E., Greenough, W.T. (Eds.): *Memory Consolidation*. Washington, American psychological association, pp. 79-112.
- [52] Matthies, H. (1989) In search of the cellular mechanism of memory. *Progress in Neurobiology*, **32**, 277-349.
- [53] Bernabeu, R., Bevilacqua, L., Ardenghi, P., Bromberg, E., Schmitz, P.K., Bianchin, M., Izquierdo, I., 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. *Proceedings of the National Academy of Sciences of the U.S.A.*, **94**, 7041-7046.
- [54] Müller Igaz, L., Vianna, M.R.M., Medina, J.H., Izquierdo, I. (2002) Two time periods of hippocampal mRNA synthesis are required for memory consolidation of fear-motivated learning. *Journal of Neuroscience*, **22**, 6781-6789.
- [55] O'Malley, O'Connell, C., and Regan, C.M. (1998). Ultrastructural analysis reveals avoidance conditioning to induce a transient increase in hippocampal dentate spine density in the 6 hour posttraining period of consolidation. *Neuroscience*, **87**, 607-613.
- [56] O'Connell, C., Gallagher, H.C., O'Malley, A., Bourke, M., and Regan, C. (2000). CREB phosphorylation coincides with transient synapse formation in the rat hippocampal dentate gyrus following avoidance learning. *Neural Plasticity*, **7**, 279-289.
- [57] Ramón y Cajal. S. (1893) Neue Darstellung von histologischen Bau des Zentralnervös System, *Archives für Anatomie und Physiologie (Anatomie)*, pp. 319-428.
- [58] Geinisman, Y., Berry, R.W., Disterhoft, J.F., Power, J.M., van der Zee, E. (2001) Associative learning elicits the formation of multiple-synaptic boutons. *Journal of Neuroscience*, **21**, 5568-5573.
- [59] Flood, J.F., Jarvik, M.E., Bennett, E.L., Orme, A.E., Rosenzweig, M.R. (1977) Protein synthesis inhibition and memory for pole jump active avoidance and extinction. *Pharmacology, Biochemistry and Behavior*, **7**, 71-77.
- [60] Lattal, A.M., Abel, T. (2001) Different requirements for protein synthesis in acquisition and extinction of spatial preferences and context-evoked fear. *Journal of Neuroscience*, **21**, 5773-5780.
- [61] Cammarota, M., Bernabeu, R., Levi de Stein, M., Izquierdo, I., Medina, J.H. (1998) Learning-specific, time-dependent increases in hippocampal Ca²⁺ / calmodulin-dependent

- protein kinase II activity and AMPA GluR1 subunit immunoreactivity. *European Journal of Neuroscience*, **10**, 2669-2676.
- [62] Cammarota, M., Bevilaqua, L.R.M., Ardenghi, P.G., Paratcha, G., Levi de Stein, M., Izquierdo, I., 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. *Brain Research Molecular Brain Research*, **76**, 36-46.
- [63] Vianna, M.R.M., Izquierdo, L.A., Barros, D.M., Ardenghi, P., Pereira, P., Rodrigues, C., Moletta, B., Medina, J.H., Izquierdo, I. (2000) Differential role of hippocampal protein kinase A in short- and long-term memory. *Neurochemical Research*, **25**, 621-626.
- [64] Schafe, G.E., Nadel, N.V., Sullivan, G.M., Harris, A., LeDoux, J.E. (1999) Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. *Learn Mem.*, **6**, 97-110.
- [65] Marsicano, G., Lutz, B. (1999) Expression of the cannabinoid receptor CB1 in distinct neuron populations in the adult mouse forebrain. *European Journal of Neuroscience*, **11**, 4213-4225.
- [66] Wilson, R.I., Nicoll, R.A. (2002) Endocannabinoid signaling in the brain. *Science* **296**, 678-692.
- [67] Rodríguez de Fonseca, F., Del Arco, I., Martín-Calderón, J.L., Gorriti, M.A., Navarro, M. (1998) Role of the endogenous cannabinoid system in the regulation of motor activity. *Neurobiology of Disease*, **5**, 483-501.
- [68] Katona, I., Rancz, E.A., Acsády, L., Ledent, C., Mackie, K., Hajos, N., Freund, T.F. (2001) Distribution of CB1 cannabinoid receptors in the amygdala: their role in the control of GABAergic transmission. *Journal of Neuroscience*, **21**, 9506-9518.
- [69] Porter, A.C., Sauer, J.M., Knierman, M.D., Becker, G.W., Berna, M.J., Bao, J., Nomikos, G.G., Carter, C., Bymaster, F.P., Leese, A.B., Felder, C.C. (2002) Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *Journal of Pharmacology and Experimental Therapeutics*, **301**, 1020-1024.
- [70] Gorriti, M.A., Rodríguez de Fonseca, F., Navarro, M., Palomo, T. (1999) Chronic (-)-delta9-tetrahydrocannabinol treatment induces sensitization to the psychomotor effects of amphetamine in rats *European Journal of Pharmacology*, **365**, 133-142.
- [71] Hampson, R.E., Deadwyler, S.H. (1998) Role of cannabinoid receptors in memory storage. *Neurobiology of Disease*, **6**, 474-482.
- [72] Reibaud, M., Obinu, M.C., Ledent, C., Parmentier, M., Boehme, G.A., Imperato, A. (1999) Enhancement of memory in cannabinoid CB1 receptor knock-out mice. *European Journal of Pharmacology*, **379**, R1-2.
- [73] Carlson, G., Wang, Y., Alger, B.E. (2002) Endocannabinoids facilitate the induction of LTP in the hippocampus. *Nature Neuroscience*, **5**, 723-724.
- [74] Ohno-Shosaku, T., Tsubokawa, H., Mizushima, I., Yoneda, N., Zimmer, A., Kano M. (2002) Presynaptic cannabinoid sensitivity is a major determinant of depolarization-induced retrograde suppression at hippocampal synapses. *Journal of Neuroscience*, **22**, 3864-3872.
- [75] Wilson, R.I., Nicoll, R.A. (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature*, **410**, 566-592.
- [76] Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., Hofmann, C., Zieglgänsberger, W., DiMarzo, V., Lutz, B. (2002) The endogenous cannabinoid system controls extinction of aversive memories. *Nature* **418**, 530-534.
- [77] McGaugh, J.L., Castellano, C., Brioni, J. (1990) Picrotoxin enhances latent extinction of conditioned fear. *Behavioral Neuroscience*, **104**, 264-267.
- [78] Westbrook, R.F., Iordanova, M., McNally, G., Richardson, R., Harris, J.A. (2002) Reinstatement of fear to an extinguished conditioned stimulus: two roles of context. *Journal of Experimental Psychology: Animal Behavior Processes* **28**, 97-110.
- [79] Harris, J.A., Westbrook, R.F. (1998) Evidence that GABA transmission mediates context-specific expression of learned fear. *Psychopharmacology*, **140**, 105-115.
- [80] Harris, J.A., Westbrook, R.F. (2001) Contextual control over the expression of fear in rats conditioned to fear under a benzodiazepine. *Psychopharmacology*, **156**, 92-97.
- [81] Harris, J.A., Westbrook, R.F. (1999) The benzodiazepine midazolam does not impair Pavlovian fear conditioning but regulates when and where fear is expressed. *Journal of Experimental Psychology: Animal Behavior Processes*, **25**, 236-246.
- [82] Quirk, G.J. (2002) Memory for extinction of conditioned fear is long-lasting and persists following spontaneous recovery. *Learning and Memory*, in press.
- [83] Tsumoto, T. (1993) Long-term depression in cerebral cortex: a possible substrate of "forgetting" that should not be forgotten. *Neuroscience Research*, **16**, 263-270.
- [84] Linden, D.J., Connor, J.A. (1995) Long-term synaptic depression. *Annual Review of Neuroscience*, **18**, 319-357
- [85] Weddell, S. (2002) Forgetting those painful moments. *Neuron*, **22**, 815-817.
- [86] Schwaerzel, M., Heisenberg, M., Zars, T. (2002) Extinction antagonizes olfactory memory at the subcellular level. *Neuron*, **22**, 951-960.
- [87] Frey J.U., Morris, R.G.M. (1998) Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation. *Trends in Neuroscience*, **21**, 181-187.
- [88] Vianna, M.R.M., Coitinho, A.S., Medina, J.H., Izquierdo, I. (2003) Molecular mechanisms in the basolateral amygdala control extinction of inhibitory avoidance. *Neurobiology of Learning and History*, in press.