MINIREVIEW

Neural Topography and Chronology of Memory Consolidation: A Review of Functional Inactivation Findings

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Findings on the role of subcortical and cortical structures in mnemonic processes, obtained by means of the reversible functional inactivation technique, are reviewed. The main advantage of this method (subcortical or cortical administration of local anesthetics or tetrodotoxin) is that it provides information not only on "where" but also "when" and for "how long" these processes take place, thus adding to the topographical dimension the chronological one. The review covers several types of memory (e.g., passive avoidance and spatial memory) studies examining the neural substrates of memory consolidation on the basis of the functional inactivation of the nucleus of the solitary tract, parabrachial nuclei, substantia nigra, hippocampus (dorsal and ventral), nucleus basalis magnocellularis, amygdala, medial septal area, striatum, olfactory bulb, and neocortex. The data are discussed in relation to earlier research and with respect to the anatomical and functional connectivity of the examined centers. © 1999 Academic Press

Learning is one of the “higher nervous functions.” It consists of acquiring information about the internal and external environment that produces transient or permanent behavioral modifications. Memory is the storage of retained information, and its existence is shown by the persistence of overt behavioral modifications, which can be observed and recorded. It is generally accepted that the mnemonic process is made up of several sequential phases. First, information is acquired (a memory trace, engram, is formed), and successively the engram is consolidated, stored, and retrieved as and when needed. Experimental evidence suggests that we must postulate a consolidation phase because, for some time after acquisition, the acquired information is vulnerable and may be altered or lost. This is illustrated by the findings on retrograde amnesia, which show that engram consolidation is a time-dependent process (McGaugh, 1966). Although the mechanisms underlying retrograde amnesia are not fully understood, it is nevertheless well established that the effects of amnesic interventions are inversely
related to the duration of the delay between acquisition (training session in experimental psychology protocols) and the administration of amnesia eliciting interventions. On another level, much work has been devoted to the attempts to draw a brain map correlating behavioral modifications with destruction of discrete brain structures. The first results (going back to the 19th century) were obtained as functional deficits after the destruction, accidental or pathological, of portions of the encephalon (for more information, see Brazier, 1959, and Blakemore, 1977).

From these beginnings originated the practice of controlled (i.e., experimental) neural lesions in subhuman experimental subjects. The permanent lesion technique has become increasingly accurate and specific (stereotaxic method, Horsley & Clarke 1908; kainate and ibotenate lesions sparing axons, Coyle, Bird, Evans, Gulley, Nadler, Nicklas, & Olney, 1981; selective damage to mediator-specific neurons, Jonsson, Malmfors, & Sachs, 1975). Its unavoidable drawback, however, is that the effects are irreversible.

To be more precise, there are at least two kinds of drawbacks. One is the possibility that, within limits, a reorganization of the undamaged neural circuits may take place. This is obviously a time-dependent process, which may eventually compensate for the imposed loss. In this case the observable effects of a lesion will not be permanent, differing according to the time of observation. On the other hand, even in the case of a constant functional deficit, what can be observed must be interpreted cautiously because the behavior under study may be the final result of several, perhaps sequentially arranged processes. The observable behavioral deficit may result from the loss of only one of these processes, with the others being undisturbed. This complication assumes particular relevance in memory studies, in which a lesion disrupting memory formation, consolidation, storage, or retrieval is always manifested by a failure of retrieval. A lesion blocking only the retrieval circuit may leave the other stages intact but this cannot possibly be proved by irreversible lesions. On the other hand, the reversible inactivation technique has been shown to be quite adequate for investigating memory processes and mechanisms. By means of this technique, a fully reversible functional blockade may be imposed on a given cerebral site for a known duration, after which there will be no permanent residual functional impairment. This technique has been employed to provide data for a functional mnemonic topography, based on the temporary involvement of several neural sites during the subsequent phases of memory processing. In fact, it is possible to disrupt repetitively a chosen mnemonic phase without interfering with preceding or subsequent phases (Bures & Buresova, 1990). In other words, this approach makes it possible to acquire experimental information not only on the “where” of mnemonic processing, but also on the “when” and “how long.” In particular, during consolidation, functional inactivations performed at different delays after acquisition may disclose the critical duration of the intact function at a given site. Evidently knowledge of the when and how long makes a considerable contribution to the understanding of memory and learning, which are essentially time-dependent functions.

The reversible inactivation of neural sites may be obtained by different methods, physical or pharmacological: focal (Brooks, 1983; Shefchyk, Jell, & Jordand, 1984) or total cooling (Handwerker, Iggo, & Zimmerman, 1975), administrations of various active compounds including neurotransmitter agonist
(muscimol, Martin, 1991), and antagonists (glutamate receptor blockers, J erusalinsky, Ferreira, Walz, Da Silva, Bianchin, Rushel, Median, & Izquierdo, 1992), eliciting cortical spreading depression (Bures, Buresova, & Krivanek, 1974). Recently the focal injection of local anesthetics (lidocaine, tetracaine) has been employed to reversibly inactivate neural structures and/or central pathways (Albert & Madryga, 1980; Flicker & Geyer, 1982; Sandkuhler, Maisch, Gebhart, & Zimmerman, 1995). Tetrodotoxin (TTX) has been employed for the same purpose (Cahill, Coopersmith, Leon, & McGaugh, 1987; Zhuravin & Bures, 1991). It is possible to administer these compounds stereotaxically into deep subcortical neural sites. The time course and volumetric dimensions of the produced functional blockade are sufficiently well known (lidocaine: Sandkuhler, Maisch, & Zimmerman, 1987; Martin, 1991; TTX: Cahill et al., 1987, Zhuravin & Bures, 1991). Both lidocaine and TTX block the voltage-dependent sodium channels, so that they temporarily mimic the condition existing after an electrolytic lesions, but if they have a similar mode of action there is a marked difference in their efficiency. In fact, lidocaine (1 μl of 4% lidocaine) produces a short-lasting blockade (about 20 min, Sandkuhler et al., 1987; Martin, 1991), while TTX blockade (10 ng in 1 μl) is maximal 30–120 min after administration, decaying exponentially and completely disappearing within 24 h (Zhuravin & Bures, 1991). Consequently lidocaine and TTX are preferentially employed to induce short-lasting and long-lasting inactivations, respectively. Thus, independent of the specific characteristics of the employed blocking agent, for each compound there is a well-defined relationship between amount administered, volume of inactivated tissue, and inactivation duration (Zhuravin & Bures, 1991; Sandkuhler et al., 1987). According to Bures, “Although the mechanisms of the TTX induced disruption remains obscure, it is conceivable that the permanent engram is supported during several days after the association by a process requiring continued impulse activity in some cerebral area. It is likely that the plastic change is maintained by a metabolic or morphogenetic process requiring continuous supply of trophic factors released at the storage site by impulse activity. Prolonged silence of the network may decrease the concentration of the putative trophic factors below the level indispensable for fixation of the plastic modification . . . . It can be speculated that the prolonged impulse blockade in a network the connectivity of which has been recently changed by learning will interfere with the most recent modifications, the fixation of which may require continued input of neurotransmitters (e.g., catecholamines) as well synaptic drive maintaining the membrane potential of neuron at a level activating the intracellular plastic event.” (Bures, Buresova, & Ivanova, 1991)

Following this brief survey of the advantages of reversible inactivations in the study of learning and memory, we shall review recent experimental results obtained by this technique, particularly in research into the neural mechanisms of consolidation. The sites examined are arranged in topographical order, from medulla to neocortex.

NUCLEUS OF THE SOLITARY TRACT

Memory storage can be influenced by the peripheral administration of adrenergic hormones and drugs (Martinez, Jensen, Messing, Vasquez, Soumireu-Mourat, Geddes, Liang, & McGaugh, 1980). Since these compounds do not easily pass the blood–brain barrier (Weil-Malherbe, Axelrod, & Tomchick, 1959), the effect appears to be mediated by the stimulation of peripheral receptors (Introini-Collison, Saghofi, Novack, & McGaugh, 1992). The nucleus
of the solitary tract directly receives the sensorial input from visceral afferents (Sumal, Blessing, J oh, Reis, & Pickel, 1983), the stimulation of which may affect mnemonic functioning, by some not-yet-well-understood mechanism (McGaugh & Gold, 1989). According to some authors the nucleus of the solitary tract plays an essential role in memory modulation and its functional inactivation may diminish, therefore, the memory-enhancing effects of the above-mentioned peripherally administered compounds. In fact, although in Sprague-Dawley rats systemic posttraining administration of epinephrine facilitates training in a Y-maze retention paradigm, this effect is absent after lidocaine (2% in 0.5 μl) inactivation of the solitary tract nucleus (Williams & McGaugh, 1993). Furthermore, these results confirm previous results obtained using a one-trial inhibitory avoidance paradigm (Sprague-Dawley rats; footshock intensity: 0.35 mA, 0.55 s). The administration of the same lidocaine dosage in the solitary tract nucleus was followed by retention impairment. Moreover, it has been shown that lidocaine effects are time-dependent. There were amnesic effects when 0.5 ml of lidocaine (2%) was injected immediately after training, but not if it was administered 2 h later (Williams & McGaugh, 1992). These findings support the hypothesis that the neural sites receiving vagal input (like the solitary tract nucleus) mediate the memory-modulating effects of the stimulation of peripheral receptors, which appear to be especially important during the posttraining interval. Apparently both hypothalamic and amygdalar monitoring and central feedback control of the regulation of autonomic and behavioral responses are based on neural information mediated by the nucleus of the solitary tract. The regulatory influence of these structures on mnemonic storage may be altered by the absence of visceral input.

The reciprocal connections between the solitary tract nucleus and the limbic structures are well established by electrophysiological and immunochemical findings (Rogers & Fryman, 1988). For instance, neurons in the central amygdalar nuclei become active after stimulation of the vagus nerve or of the nucleus of the solitary tract, and, conversely, neurons of the nucleus of the solitary tract are activated by stimulation of the amygdala as well as by the orthodromic stimulation of the hypothalamic paraventricular nucleus (Rogers & Fryman, 1988). In addition to the projections of the nucleus of the solitary tract to limbic structures involved in memory processes, some axons from this nucleus go to the parabrachial nuclei (Kawai, Takagi, Yana i, & Tohyama, 1988; Herbert, Moga, & Saper, 1990) which play an important role in the memorization of aversively motivated behaviors (Ivanova & Bures, 1990; Tasoni, Bucherelli, & Bures, 1992). These axons project both to the amygdala (Cechetto & Calaresu, 1983) and to the solitary tract nucleus (Papas & Ferguson, 1990). Thus, peripheral physiological influences modulating the activity of brain structures involved in memory processing follow more than one route. Not only medullar structures like the nucleus of the solitary tract, but also brainstem centers, like the parabrachial nuclei, appear to cooperate with more rostral centers, through well-developed reciprocal connections.

**PARABRACHIAL NUCLEI**

As mentioned above, the parabrachial nuclei complex projects both to the nucleus of the solitary tract and to the amygdala. By means of bilateral TTX (10 ng in 1 μl) inactivation in Long-Evans rats trained in a step-through
passive avoidance response (footshock intensity: 1 mA, 5 s) it has been shown that this complex must be functionally intact for no less than 24 h in order to avoid retrograde amnesia. Unilateral inactivations were without effect (Tassoni, Bucherelli, & Bures, 1992b). On the other hand, using the same experimental procedure a memory impairment followed the unilateral functional inactivation of parabrachial nuclei if the contralateral amygdala was contemporaneously inactivated by TTX. This result has been interpreted as indicating an ipsilateral linkage between these two sites (Tassoni et al., 1992b). This hypothesis is further supported by the finding that the effect of the ipsilateral inactivation of both sites is not greater than that following the isolated inactivation of either of them (Tassoni et al., 1992b). The TTX inactivation technique was also employed to investigate the mnemonic role of parabrachial nuclei in other aversive paradigms, such as the conditioned taste aversion. It was found that their integrity was necessary during the consolidation phase of the conditioned response. In fact, in Long–Evans rats, TTX (10 ng in 1 μl) injection at this site elicited retrograde amnesia when applied 1, 2, or 4 but not 8 days after CTA acquisition (Ivanova and Bures, 1990b).

In accord with previous results, using Wistar rats trained to learn a one-trial passive avoidance response, parabrachial nuclei studies have shown that the amnesic effect is directly related to the intensity of punishment (Bucherelli & Tassoni, 1992). When weak footshocks (0.8 mA, 3 s) were delivered, functional ablation of the parabrachial nuclei was still effective when induced up to 8 days after acquisition training. On the other hand, when rats were shocked with stronger stimuli (1.2 mA, 3 s) 2 days after acquisition the functional ablation was no longer effective. Moreover, effects of TTX (10 ng in 1 μl) blockade of parabrachial nuclei on conditioned taste aversion learning indicate that, in Long–Evans rats, amnesia was considerably attenuated when TTX was used after repeated saccharin–LiCl pairings, a result consonant with the notion that overtraining increases the strength of the memory trace, its resistance to amnesic agents, or both (Ivanova & Bures, 1990b).

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SUBSTANTIA NIGRA

This structure is a constituent of two dopaminergic systems [the nigrostriatal bundle and the mesolimbic-mesocortical dopaminergic projection (Moore & Bloom, 1978)] and forms important connections with the striatum. Concerning its involvement in passive avoidance conditioning, it is believed to be subordinate to the caudate-putamen. This hierarchical arrangement was proposed on the basis of the finding that long-lasting electrical stimulation of the caudate-putamen or of the substantia nigra disrupted passive avoidance retention, while only caudate-putamen stimulation caused amnesic effects after connections with substantia nigra were cut (Fibiger & Phillips, 1976). TTX (10 ng in 1 μl, Wistar rats) inactivation did not confirm these conclusions. In fact, it was found that an intact caudate-putamen is necessary for passive avoidance consolidation (footshock intensity: 1.2 mA, 5 s) for no less than 1.5 h after acquisition (Ambrogi Lorenzini, Baldi, Bucherelli, & Tassoni, 1995). This interval is much shorter than that reported for the substantia nigra (24 h); consequently, it seems that the latter structure plays a more important role than the former structure. The functional importance of the substantia nigra is further underscored by the fact that even its unilateral TTX inactivation
disrupts passive avoidance consolidation when applied during the first 15 min after acquisition (Ambrogi Lorenzini, Baldi, Bucherelli, & Tassoni 1994b).

**HIPPOCAMPUS**

It is now generally accepted that hippocampal integrity is essential for spatial learning (O'Keefe, 1983; Redish & Touretzky, 1997). The evidence was obtained not only from lesion studies but also from functional ablation experiments, like those on working memory in a modified version of the Morris water maze task, in which hippocampal inactivation was induced by lidocaine (4%, 1 μl) (Bohbot, Othal, Liu, Nadel, & Bures, 1996).

On the other hand, the hippocampus is surely associated also with aver-sively motivated behavior. Recently, the role of the dorsal and the ventral hippocampus in consolidation of passive avoidance reaction was investigated by means by reversible TTX (10 ng in 1 μl, Wistar rats) inactivations. The two sites were studied separately because of their functional differentiation (Nadel, 1968; Moser, Moser, & Andersen, 1993). It was found that both sites are necessary for passive avoidance response (footshock intensity: 1.2 mA, 3 s) consolidation (bilateral, but not unilateral, inactivation is followed by passive avoidance response disruption). Temporal involvement of the two sites was different: dorsal hippocampus integrity was required for 90 min (Ambrogi Lorenzini, Baldi, Bucherelli, Sacchetti, & Tassoni, 1996) and the ventral for only 15 min (Ambrogi Lorenzini, Baldi, Bucherelli, Sacchetti, & Tassoni, 1997) after acquisition. The difference is thought to be due to the distinct functional characteristics of the two parts of the hippocampus. The dorsal hippocampus appears to be preferentially involved in tasks requiring the use of place strategy (Winocur & Bindra, 1976; Black, Nadel, & O'Keefe, 1977; Baker, Kesner, & Michal, 1981; Kesner & Hardy, 1983), whereas the ventral hippocampus appears to be involved in fear evaluation and elaboration. This contention is supported by the well-developed connections of the ventral hip-pocampus with amygdala (Ottersen, 1982; Moser et al., 1993).

From a general point of view, it is thought that posttraining mnemonic processing in the hippocampus is modulated by the amygdalae (Packard, Cahill, & McGaugh, 1994). Indeed, in that phase long-term potentiation is present both in the hippocampus and in the amygdala (Ikegaya, Saito, & Abe, 1995, 1996). The close functional relationship between the two structures is further supported by the finding that to avoid memory disruption the dorsal hippocampus and amygdala must be functionally intact for the same postacquisition duration (1.5 h) (Bucherelli, Tassoni, & Bures, 1992), although the amygdala projects mainly to the ventral hippocampus.

**NUCLEUS BASALIS MAGNOCELLULARIS**

This structure is a component of the cholinergic basal forebrain system (Mesulam, Mufson, Wainer, & Levey, 1983). Its role in mnemonic processing is based on both experimental and clinical evidence. Severe memory impairment in Alzheimer disease patients is accompanied by the decrease of hippocampal and cortical cholinergic acetylcholinesterase activity and by evident cell loss in the basal forebrain (Coyle, Price, & DeLong, 1983).
TTX inactivation experiments have shown that consolidation requires intact function of this structure for the longest reported duration at present: in the Wistar rat, no less than 48 h after acquisition of a passive avoidance task (footshock intensity: 1.2 mA, 3 s) in the case of bilateral TTX (10 ng in 1 µl) administration and no less than 6 h in the case of unilateral administration (Ambrogi Lorenzini, Baldi, Bucherelli, & Tassoni, 1994a). These findings are consonant with previous assessments of the importance of the nucleus basalis magnocellularis for the integration of subcortical functions (Goldman & Coté, 1991) and for regulation of neocortical activity (Lo Conte, Casamenti, Bigi, Milaneschi, & Pepeu, 1982; Riekkinen, Riekkinen, Sirvio, Miettinen, & Riekkinen, 1992). Well-developed connections with neocortex (Saper, 1984; Dunnett, Toniolo, Fine, Ryan, Bjorklund, & Iversen, 1985; Lamour, Dutar, & Jobert, 1994), and amygdala (Todd & Kesner, 1978; Emson, Paxinos, Le Gal La Salle, Ben-Ari, & Silver, 1979; Carlsen, Zaborsky, & Heimer, 1985; Dunnett et al., 1985) support the hypothesis that this structure generates both ascending and descending influences on memory processes. Its importance for memory is further underscored by the finding that passive avoidance response loss following the lesion of nucleus basalis magnocellularis was recovered after transplantation of embrional ventral forebrain grafts into neocortex (Dunnett et al., 1985; Fine, Dunnett, Bjorklund, & Iversen, 1985).

AMYGDALA

There is an important body of evidence showing that the amygdalar complex plays a role in emotionally loaded memory (Davis, 1992). It has been shown that posttraining manipulations of the amygdala affect retention of a number of aversively motivated tasks. Recently it was reported that lidocaine (2%, 0.5 µl) infusion into the amygdala immediately after acquisition of an inhibitory avoidance task or of a shift in reward magnitude impairs subsequent retention. In the latter paradigm rats trained to run in a straight alley for a large food reward transiently exhibit longer latencies after reward magnitude has been reduced (the so called “Crespi effect,” or “negative behavioral contrast,” Crespi, 1942; Flaherty, 1982). It has been shown that Sprague–Dawley rats receiving, immediately after reduction of reward magnitude, bilateral infusion of lidocaine (2% solution, 0.5 µl/side) into the amygdala exhibited significantly shorter latencies on the second day of reduced reward training than did rats given phosphate buffer infusions. The results suggest that lidocaine blockade of the amygdala impairs the memory of reward magnitude reduction (Salinas, Packard, & McGaugh, 1993).

According to some reports, mnemonic involvement of amygdala is lateralized (Coleman-Mesches & McGaugh, 1995a, 1995b). Unilateral infusion of lidocaine (2%, 1 µl) attenuated the reduced reward–memory effect in Sprague–Dawley rats, but the effect was more marked following inactivation of the right amygdala than after inactivation of the left amygdala (Coleman-Mesches, Salinas, & McGaugh, 1996).

It was proposed that reduction in reward magnitude elicits aversive emotional responses similar to those induced by punishment (Amsel, 1958, 1962). Amygdalar involvement in aversively motivated learning is indicated by the necessity of this structure for the full expression of an inhibitory performance. In fact, postacquisition TTX (10 ng in 1 µl) inactivation of both amygdalae is

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followed by severe impairment of passive avoidance retention in Long-Evans rats (footshock intensity: 1 mA, 5 s) (Bucherelli et al., 1992), while unilateral inactivations are without effect (Tassoni, Bucherelli, & Bures, 1992). The amnesic effect was significant for acquisition–TTX administration delays up to 90 min. At longer delays (6 h) no amnesia was observed. These data confirm the time window of amygdalar involvement reported by authors using selective pharmacological blocking agents (Izquierdo, Medina, Blanchin, Walz, Zanatta, Da Silva, Bueno E Silva, Ruschel, & Paczko, 1993; Jerusalinsky et al., 1992). Furthermore, it has been shown by passive avoidance studies that there is a subnuclear functional specificity within the amygdala: amnesia induced by postraining amygdalar inactivation appears to be mediated by the selective blockade of the basolateral complex. In fact, in Sprague-Dawley rats injection of 0.25 μl of lidocaine (4% dissolved in phosphate-buffered saline) into the basolateral complex disrupts passive avoidance consolidation (footshock intensity: 0.45 mA, 1 s), while injection of the same compound into the central nucleus has no effect (Parent & McGaugh, 1994): the amnesic effects of lidocaine were present for acquisition–administration delays of up to 6 h and were absent after a 24-h delay. Moreover, immediate posttraining infusion of the same dosage of lidocaine impaired the retention performance of rats trained with stronger footshocks (0.7 mA) but not if the lidocaine was injected 6 h after training.

From these results it may be concluded that the amygdalar basolateral complex is active immediately after acquisition and is subsequently indispensable for the consolidation of inhibitory learning for durations inversely related to the intensity of punishment (more severe stimulation accelerating the consolidation process) (Gold, Hankins, Edwards, Chester, & McGaugh, 1974). Its functional integrity appears to be necessary for at least 6 h. These conclusions are consistent with other results showing that amygdalar central and basolateral nuclei mnemonic involvement varies according to the type of learning (Kesner, Walser, & Winzenried, 1989; Ambrogi Lorenzini, Bucherelli, Giachetti, Mugnai, & Tassoni, 1991; Hatfield, Graham, & Gallagher, 1992).

It must be stressed, however, that the amygdala does not participate in the consolidation of all forms of aversive conditioning. For instance, the amygdala does not play a role in the consolidation of the conditioned taste aversion long-term engrams. In this aversive paradigm amygdalar TTX (1 μl of 10 ng diluted in physiological saline) inactivation was followed by amnesic effects only when induced within 1.5 h after application of the toxic US (0.15 M LiCl, 2% body weight), i.e., when there were visceral symptoms of poisoning, and was ineffective when applied at the 6-h postacquisition delay. Thus, the amygdala appears to be involved in the association of gustatory short-term memory with the visceral symptoms of poisoning, but there is no evidence indicating that it participates in the consolidation of the long-term engram of conditioned taste aversion (Roldan & Bures, 1994).

**MEDIAL SEPTAL AREA**

The medial septal area, consisting of the medial septum and the vertical limb of the diagonal band, is the main subcortical source of cholinergic projections to the hippocampus via the fimbria–fornix complex (McKinney, Coyle, & Hedreen, 1983). Irreversible lesion studies have shown that this neural com-
plex is an important component of the central circuits implementing passive avoidance conditioning (Wishart & Mogenson, 1970; Thomas, 1972). Functional inactivation of the medial septal area has yielded contrasting results in two studies using the same inactivating agent (TTX). In one, TTX (5 ng in 1 μl) administered to Long-Evans rats 5 or 90 min after PAR acquisition elicited significant amnesia, but there was no amnesic effect when TTX was applied 360 min after acquisition (Rashidy-Pour, Motaghed-Larijani, & Bures, 1996). In another study, in Wistar rats postacquisition TTX (5 ng in 0.5 μl) administration did not disrupt consolidation of passive avoidance response, but interfered with acquisition and retrieval (Ambrogi Lorenzini, Baldi, Bucherelli, & Tassoni, 1996). The contrasting results may be due to the different paradigms employed. In the first study the rats were subjected to a single, short punishment (1 mA, 1.5 s), while in the second they received two sets of unavoidable footshocks (1.2 mA, 3 s). In agreement with other results it is conceivable that the memory trace in the second study was stronger than in the first study and was, therefore, less easily disrupted.

Close connections between the medial septal area and the hippocampus are well known (Bland & Bland, 1986). Irreversible lesions either of the medial septal area or of septo-hippocampal connections were generally followed by learning deficits similar to those observed after hippocampal damage (Gray & McNaughton, 1983). On the other hand, the absence of amnesic effects caused by postacquisition inactivation were taken as evidence of the functional independence of these two structures, at least during consolidation of passive avoidance engrams (Ambrogi Lorenzini et al., 1996). In rats, the effects of 0.5 μl of tetracaine (2%) inactivation of the medial septal area on radial maze performance were used to assess contribution of this structure to distinct aspects of spatial information processing. It was concluded that “septal activation is required for acquisition of spatial information, but septal inactivation failed to affect storage or maintenance of spatial information” (Mizumori, Perez, Alvarado, Barnes, & McNaughton, 1990).

Data on the role of the medial septum in spatial learning were provided by a study describing in Long-Evans rats the effects of TTX (10 ng in 1 μl) or lidocaine (4%, 1 μl) inactivations of the medial septum on reference and working memories in the Morris water maze. Posttraining administration of TTX or lidocaine failed to affect consolidation of reference and working memories, while preacquisition or preretrieval administration caused amnesic effects (Rashidy-Pour, Motamedi, & Motaghed-Larijani, 1996). It may be concluded that functional integrity of the medial septal area is necessary for acquisition and retrieval of spatial reference memory, but its involvement in postacquisition is unlikely (Rashidy-Pour et al., 1996).

**STRIATUM**

In addition to the hippocampus and amygdala, the dorsal striatum may also play a role in mnemonic processing. It has been suggested that although these three structures act in parallel, each of them may mediate acquisition of a distinct type of information. Three systems have been proposed: the first, including the hippocampus, acquires information on relationships among stimuli and events. The second, including the amygdala, mediates rapid acquisition of behaviors based on biologically signifi-
cant events possessing affective connotations. The third, including the
striatum, mediates formation of reinforced stimulus–response associations
(Izquierdo et al., 1993; McDonald & White, 1993).

All three sites can influence memory by cholinergic–dopaminergic interac-
tion (Gasbarri, Introini-Collison, Packard, Pacitti, & McGaugh, 1993). Some
findings indicate that posttraining modifications of dopaminergic activity in-
fluence retention: intracerebroventricular administration of dopamine im-
proves retention of an inhibitory avoidance response (Haycock, Van Burskirk,
Rayn, & McGaugh, 1977). These structures could constitute, alone or with
other structures, a mnemonic circuit parallel to the limbic system.

To assess the role of the striatum in mnemonic processing, TTX (10 ng in 1
μl) was employed to inactivate the caudate-putamen of Wistar rats either
totally, or, on the basis of its peculiar morphology, to inactivate separately each
of its three parts (anterior, intermediate, posterior). It was shown that only the
intermediate part was necessary for formation of passive avoidance response
memories (footshock intensity: 1.2 mA, 3s) (Ambrogi Lorenzini et al., 1995).
The other two subdivisions (anterior and posterior) were not involved in
passive avoidance response memorization (Ambrogi Lorenzini et al., 1995;
Bermudez-Rattoni, Introini-Collison, & McGaugh, 1991 (3 ng in 0.5 μl citrate/phosphate buffer; footshock intensity: 0.53 mA, 0.7 s, Sprague-Dawley
rats). Since PAR retention was also disrupted by the inactivation of the
nucleus accumbens and globus pallidus, it was proposed that all main parts of
the corpus striatum contribute to PAR consolidation (Ambrogi Lorenzini et al.,
1995). Experimental evidence obtained in the above studies again indicates a
negative correlation between the amnesic effect of reversible inactivation and
the intensity of stimulation. Retention deficits after the lidocaine inactivation
of striatum (2%, 1 μl, Wistar rats) were observed only after very low footshock
intensities (footshock intensities: 0.2, 0.3 mA). One interpretation is that
stronger footshocks (0.4 mA) induce activation of several parallel elements of
the consolidation circuit, which cannot be disrupted as in the case of circum-

**OLFACTORY BULB**

Does the olfactory bulb play a role in the consolidation of newly acquired
olfactory information? This question has been addressed recently by Mouly,
Kindermann, Gervais, and Holley (1993). Instead of adequate chemical olfac-
tory stimuli, multisite electrical stimuli (50 Hz) were employed, administered
by means of electrodes implanted close to the output neurons of the olfactory
bulb (large mitral cells). The apparent information was perceived as olfactory
(Mouly, Vigouroux, & Holley, 1985; Mouly & Holley, 1986). Lidocaine (2%, 2 μl)
was injected in Wistar rats into the olfactory bulb at different delays after each
training session, and the effects on acquisition and retention of a conditioned
response (olfactory learning) were observed. Posttraining inactivation of olfac-
tory neurons moderately impaired the acquisition of the associative task in a
daily training schedule and severely impaired its retention after 5 days. There
was mnemonic disruption when lidocaine was administered immediately after
each training session, but not after a 2-h delay. It was concluded that the
immediate lidocaine administration caused a retention impairment, with the
impairment exhibiting a temporal gradient: it begins after 24 h (slight alteration of final acquisition scores) and becomes more severe after 5 days.

NEOCORTEX

The mnemonic role of the neocortex in the consolidation phase was studied using both TTX [Bermudez-Rattoni, Introini-Collison, & McGaugh, 1991 (3 ng in 0.5 μl of citrate/phosphate buffer: footshock intensity: 0.35 mA, 0.7 s, Sprague-Dawley rats); Tassoni, Bucherelli, & Bures, 1992c (10 ng in 1 μl of saline solution, footshock intensity: 1 mA, 1 s, Long-Evans rats)] and lidocaine (2%, 1 μl, Wistar rats; footshock intensity: 0.4 mA) inactivations (Perez-Ruiz et al., 1989). The inactivating compounds were administered during the postacquisition period in several areas (insular, frontal, parietal, perirhinal). TTX inactivation of insular cortex significantly impaired conditioned performance of a one-trial inhibitory avoidance task (Bermudez-Rattoni et al., 1991). Frontal or parietal TTX inactivations did not significantly disrupt retention (Bermudez-Rattoni et al., 1991; Tassoni et al., 1992c). Administration of lidocaine (2%, 1 μl) in the parietal cortex had no amnesic effect (Perez-Ruiz et al., 1989). Similar results were obtained when a spatial learning task was employed (Bermudez-Rattoni, Introini-Collison, & McGaugh, 1991). Thus it may be concluded that insular cortex plays a role in aversive and spatial learning, consistent with the assessment of the insular cortex as a multimodal brain area concerned with the perception of temporal patterns of several classes of sensory stimuli (Colavita, Szeligo, & Zimmer, 1974; Colavita & Weisberg, 1979).

CONCLUDING REMARKS

As pointed out in the Introduction, the fully reversible functional inactivation of circumscribed neural sites is a useful tool for plotting the brain map of mnemonic processes. In fact, by means of this technique it is possible to superimpose on the spatial map (topography of the memory-related centers and circuits obtained by means of permanent lesions) the temporal dimension (chronology of the duration during which a given neural structure must participate in memory processing). A further advantage of this technique derives from the possibility of employing more than one inactivating agent like lidocaine and TTX, to name only two. Both compounds block the voltage-dependent sodium channels, the first for a very short period (minutes), the second for much longer period (hours). This means that there are tools appropriate for the quantitative analysis of mnemonic phenomena with quite different time courses. Amnesic affect magnitude and/or duration are related to the administered dosage of the inactivating compound (for lidocaine sometimes a 2% concentration was ineffective, but the 4% concentration was effective). Generally speaking, this means that it may be risky to draw definitive conclusions on the function of a given neural site just from the results of a simple dosage experiment. In fact, it is well known that the possibility and degree of engram manipulation are related both to the amount of training and to the strength of posttraining treatments.

By means of this technique it has been possible not only to characterize the sequential involvement of neural sites in memory processes, but also to define
with more precision the functional relationship between neural sites, to clarify
the hierarchical relationships between anatomically connected structures, and
to determine the relative roles of the specific subcomponents of complex cen-
ters (hippocampus, amygdala, caudate-putamen), thus improving our under-
standing of mnemonic processing. It is nevertheless true that not all reported
findings are completely in accord (Rashidy-Pour et al., 1996; Ambrogi Loren-
zini et al., 1996). Some of these discrepancies can be explained by the different
experimental protocols employed and particularly by the intensities of the
aversive stimuli. It has been repeatedly confirmed by several of the reviewed
papers (Gold et al., 1975; Parent and McGaugh, 1994; Bucherelli & Tassoni,
1992) that there is a positive correlation between footshock intensity and the
speed and robustness of engram consolidation. It may be more useful to
administer lidocaine (or its related compounds) for preacquisition investiga-
tion, because the short inactivation may disrupt only the acquisition phase,
without overlap with the early consolidation period. On the same grounds,
lidocaine may be quite useful for a fine assessment of consolidation time
course, in order to reveal recruitment or drop-out phenomena, when neural
structures are recruited only some time after the beginning of consolidation.
On the other hand, a limit to lidocaine usefulness derives from the short
duration of the induced inactivation compared to the longer duration of TTX.
In some instances this short duration may be insufficient to cause retrograde

There are other studies in which the reversible inactivation technique has been
used and in which the inactivating compounds were either neurotransmitter
antagonists or more or less specific inhibitors of subcellular processes. We have
restricted the present review to studies in which either local anesthetics (lidocaine
or tetracaine) or TTX was employed, because by means of these compounds one
obtains a generalized blockade of neuronal function, due to their action on voltage-
dependent sodium channels. Therefore this kind of inactivation is quite distinct
from those caused by selective interferences related to specific neurotransmitter
action or to specific subcellular mechanisms. It must be underscored that the use
of the latter compounds has contributed to the understanding of the specific
mechanisms underlying mnemonic functions. By using such techniques informa-
tion is obtained, not so much about the function of a neural site, but rather about
the function(s) of some of its neuronal components or of some subcellular me-
chanism. Thus, these results lie outside the scope of the present review. A further
consideration may be added. Functional inactivations induced by TTX or local
anesthetics can reveal the critical spatial and chronological characteristics of a
mnemonic process. It is on the basis of this information that more specific studies
can be designed. For instance, how should one evaluate the finding that to learn
a given conditioned response (e.g., rat's passive avoidance) during consolidation
more than one subcortical site is critically active at the same time? In order to go
beyond the legitimate conclusion that each site is necessary, more complex exper-
imental designs may be employed. Combined inactivation, either uni- or bilatera-
of more than one site may give useful information on the influences that single
sites of an hypothetic circuit may exert on one another (Tassoni et al., 1992).

As stated in the Introduction, the reviewed findings were presented in
caudo-rostral topographical sequence. This kind of presentation appeared to be
the simplest one, given the ascending polarization of sensory input and of its
successive elaborations. Concerning topography, it may be observed that very
caudal structures like the nucleus of the solitary tract play an important role in mnemonic processing.

Results concerning engram consolidation have been reviewed. In fact, engram consolidation is the mnemonic phase during which the reversible inactivation technique may be, and has been, employed most advantageously on the largest number of sites, as shown by the reported findings. It must be pointed out, however, that the same technique, conditions permitting, makes it possible to examine the neural processes implementing acquisition and retrieval of memories (Ivanova & Bures, 1990b; Mizumori et al., 1990; Bielavska & Bures, 1994; Coleman-Mesches et al., 1995; Ambrogi Lorenzini et al., 1996a, 1996b, 1997; Floresco, Seamans, & Phillips, 1996; Ambrogi Lorenzini, Baldi, Bucherelli, Sacchetti, & Tassoni, in press).

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