A Pharmacological Analysis of an Associative Learning Task: 5-HT_1 to 5-HT_7 Receptor Subtypes Function on a Pavlovian/Instrumental Autoshaped Memory

Alfredo Meneses

Depto. De Farmacobiología, CINVESTAV-IPN, México City 14330, México

Recent studies using both invertebrates and mammals have revealed that endogenous serotonin (5-hydroxytryptamine [5-HT]) modulates plasticity processes, including learning and memory. However, little is currently known about the mechanisms, loci, or time window of the actions of 5-HT. The aim of this review is to discuss some recent results on the effects of systemic administration of selective agonists and antagonists of 5-HT on associative learning in a Pavlovian/instrumental autoshaping (P/I-A) task in rats. The results indicate that pharmacological manipulation of 5-HT_{1-7} receptors or 5-HT reuptake sites might modulate memory consolidation, which is consistent with the emerging notion that 5-HT plays a key role in memory formation.

Notwithstanding phylogenetic distances between invertebrate and mammal species, convergent studies have provided significant insights into the role of serotonin (5-hydroxytryptamine [5-HT]) in mnemonic processes. Indeed, in several invertebrates, including *Aplysia* and *Hermissenda*, 5-HT plays a critical regulatory role in mediating short- and long-term associative learning (Byrne and Kandel 1996; Angers et al. 1998; Crow et al. 2001; Barbas et al. 2002; Cohen et al. 2003), which are both associated with molecular and morphological modifications. Barbas et al. (2002) and Marinesco and Carew (2002) have observed at least six different 5-HT receptor subtypes present in *Aplysia* that activate different postsynaptic responses. Notably, these studies have demonstrated that endogenous 5-HT participates in information processing, modulating short- and long-memory (possibly) via different 5-HT receptors and associated signal transduction machinery, which could ultimately lead to morphological modifications.

Future work is needed to clarify the contribution of chemical and structural changes to human memory (Arshavsky 2003). Because of, at least in part, the large phylogenetic distances, it has been difficult to compare the pharmacological and transduction profiles of the diverse 5-HT receptors so far identified in invertebrates (see Li et al. 1995; Angers et al. 1998; Crow et al. 2001; Barbas et al. 2002; Cohen et al. 2003) with those in mammals. Considering a growing scientific and public interest in mnemonic functions and dysfunctions in humans, it will be of great value when future studies attempt to integrate the results from invertebrates and vertebrates that have been used to investigate the mnemonic actions of 5-HT. Because 5-HT receptors may determine the occurrence, magnitude, and specificity of the sign of plasticity (Kirkwood 2000) in invertebrates and mammals, it might be heuristic to look for parallels among species. Recent reviews of mnemonic actions of 5-HT in mammals (Meneses 1999; Buhot et al. 2000; Dringenberg 2000) have shown that the 5-HT system plays a complex role in mnemonic processes in many different behavioral models. However, there are contradictory findings, making direct and detailed comparisons difficult. Nevertheless, it is clear that 5-HT exerts its effects on learning and memory via multiple 5-HT receptors and may produce variable results depending on 5-HT receptor subtype, site of administration (systemic or central), the drug, timing of drug administration, and behavioral tests used. Interestingly, drug effects can be examined on the same learning task across a broad range of intracerebral sites (Izquierdo and McGaugh 2000), using different learning tasks while focusing on the same site and drugs, and/or holding behavioral task and infusion site while varying the drug dosage (or with different drugs) and using the same or different species (Table 1). Finally, investigators have administered the drugs before (prelearning) or after (postlearning) the learning task and/or preretention (Fig. 1; McGaugh and Izquierdo 2000).

The aim of this review is to present and discuss a series of recent results on associative learning by using a Pavlovian/instrumental autoshaping (P/I-A) task in rats and systemic administration of selective 5-HT receptor agonists and antagonists. It is important to mention that, in contrast to systemic drug administration, the technique of intracerebral infusion is a powerful tool to determine the drug action site on the brain. Nevertheless, it has potential pitfalls, including drug diffusion (Menard and Treit 1999). Notably, central administration only indicates the involvement of a given area, whereas systemic injection provides information on a wide range of areas that might be involved and parallels traditional clinical drug administration. Finally, a combination of central and systemic drug administration could provide significant insights into memory neuronal circuitry.

Autoshaping or Sign-Tracking Learning Task

Autoshaping has been studied in detail by using behavioral and environmental approaches (Locurto et al. 1981), demonstrating that it is very sensitive to motivational, perceptual, and environmental changes (e.g., intermittent unsignaled, unconditioned stimulus presentations). In an autoshaping or sign-tracking setting (Fig. 2), a hungry animal is placed in a conditioning chamber to find food pellets (unconditioned stimulus [US]) in the food-magazine and is then given a Pavlovian sequential pairing (stimulus–stimulus [S-S]) of a lighted key or a retractable-illuminated lever (conditioned stimulus [CS]) and food (US; Fig. 2A–D,F). After a number of such presentations, the animal approaches the CS and presents instrumental responses (conditioned response [CR]), such as peck, nose-poke, and contact- or lever-press (Fig. 2C). Then, CR or autoshaped responses result

E-MAIL ameneses@msn.com; FAX 52-55-54832863.
Article and publication are at http://www.learnmem.org/cgi/doi/10.1101/lm.60503.

Table 1. Approaches Used to Study Learning and Memory

Behavioral task	Intracerebral administration sites	Drug	Timing of drug administration
Same test	Different sites	One or different doses	Prelearning
Different tests	Same site	Same or different doses	Postlearning
Same test or different tests	Same or different sites	Same drug or different drugs	Preretention

from the S-S association and are sustained by response–stimulus (R-S) association.

As in Pavlovian autoshaping, CS and US are independent of the animal's behavior. It was as though autoshaped behavior was minimally affected by instrumental responses, inasmuch as when the CR prevented delivery of the US, pigeons continued responding to CS, leading to the conclusion that autoshaped response was subserved by Pavlovian mechanisms and insensitive to instrumental contingency. Nevertheless, in rats, as in other mammals, the omission contingency is effective in reducing the CR frequency, although it rarely eliminates it (Boakes 1977), probably because the consummatory behavior and manipulative abilities of mammals are considerably more complex. Notably, the nose-poke response compared with the lever-press response becomes easier to learn for rodents, because the former is more "natural" to their behavioral patterns. To facilitate CR acquisition and reduce its variance, we adjusted the mechanical lever device to detect 10-g responses, making it more salient by using a translucid and illuminable lever (Fig. 2C). Importantly, within the continued progress of behavioral task development, a P/I-A task combines both Pavlovian and instrumental conditioning. These offer the opportunity to study hippocampus-mediated declarative memory and striatum-mediated R-S "habit formation" (Meneses 2002b; also see next section).

Furthermore, P/I-A, except for magazine training, is almost completely automatized, considerably reducing human intervention. It is sensitive to small increases or decreases in various behavioral parameters (i.e., not measuring the same event twice), including sign tracking (i.e., conditioned behavior directed toward the localized retractable and illuminated lever; CS), and goal tracking (i.e., the place where the US is delivered; Fig. 2D). The latter is quite important, as it allows the study of bidirectional expression of an enhanced or impaired memory formation. In addition, as in other associative learning tasks (Harvey 1996), autoshaped responses are rapidly observed when trials are spaced in time, and its acquisition speed is relatively constant when the CS/US intertrial interval ratio interval is constant. Therefore, the difference in CR number between the first and second autoshaping session is modest (Table 2), requiring limited training and allowing easier interpretation of results. This is a particularly important aspect, because the possibility and degree of engram manipulation are related to both the training amount and strength of posttraining treatments (see Lorenzini et al. 1999). P/I-A clearly separates training for testing sessions, and it has been useful to detect changes in memory formation elicited by drugs or aging (Meneses and Hong 1998). Importantly, a large number of serotonergic mechanisms have been tested (Tables 2–5).

Autoshaped Memory and Memory Systems: Biochemical, Physiological, and Pharmacological Evidence

P/I-A requires intact neuronal systems in the hippocampus, septum, and cortex (Oscos-Alvarado et al. 1985, 1991; Manuel-Apolinar and Meneses 2003; Meneses et al. 2003). For instance, a recent autoradiographic (using [^{3}H] 5-HT as ligand) study revealed that adult (3-month-old) trained rats expressed fewer 5-HT receptors in hippocampal CA1 and dentate gyrus than did old (9-month-old) animals. These younger rats performed better in memory tasks than did aged rats. Moreover, memory formation increased 5-HT$_4$ receptor expression in hippocampus CA2, or decreased expression in dentate gyrus in adult rats. Notably, aged rats demonstrated increased expression in hippocampal CA1 and decreased expression in CA2 and CA3. Interestingly, faster instrumental autoshaping learning was observed in knockout (KO) mice lacking 5-HT$_{1A}$ or 5-HT$_{1B}$ receptors (Pattij 2002). In addition, 8-OH-DPAT treatment facilitated autoshaped memory consolidation and increased cortical and hippocampal cAMP production (Manuel-Apolinar and Meneses 2003).

Together, these data indicate hippocampal mediation on autoshaped memory. Importantly, the lesion of some of the above-mentioned brain areas disrupt or facilitate autoshaped responses (for references, see Meneses 2002b). Moreover, physiological and pharmacological studies (Meneses and Hong 1998) using a P/I-A learning task found that 3- to 12-month-old, but not 18- and 24-month-old, spontaneously hypertensive (SHR) rats display poor learning and memory compared with that of normotensive Wistar-Kyoto (WKY) rats, indicating that hypertension and aging may have an additive detrimental effect on cognitive functions (Meneses and Hong 1998). We have also observed that nimodipine (a calcium channel blocker with well-known enhancement effects on learning) reversed memory impairment caused by aging and hypertension (Meneses and Hong 1998).

Therefore, P/I-A testing of adult and middle-aged SHR rats provides a useful model in drug screening for treatment of memory disorders associated with hypertension and aging (Meneses and Hong 1999) or genetic lesions (McDonald et al. 1998). Likewise, SHR rats have proven useful for the study of mnemonic dysfunctions on spatial learning (Terry et al. 2000) and attention-deficit/hyperactivity disorder (Sagvolden 2000). Animal models of cerebrovascular diseases may help to elucidate types of lesions linked to cognitive impairment and pathophysiological mechanisms of vascular origin seen in human diseases (Terry et al. 2000; Pantoni et al. 2002).

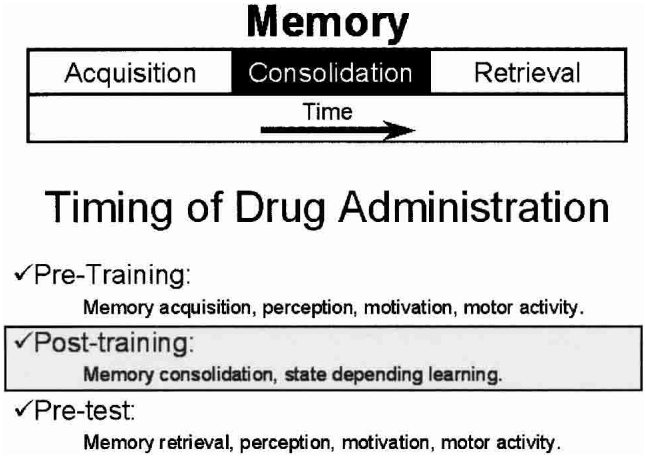

Figure 1 Cognitive processes and abilities potentially affected by time of drug administration.

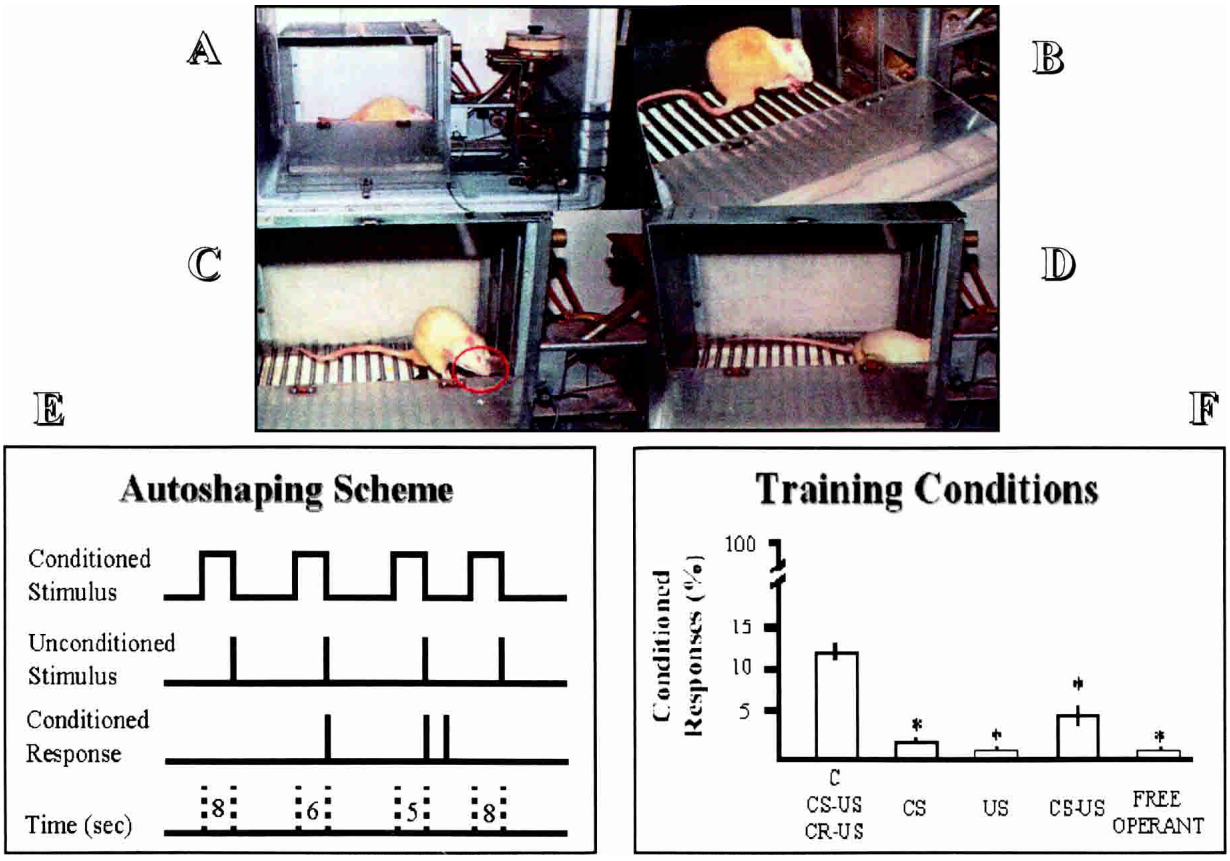

Figure 2 Autoshaping Pavlovian/instrumental. (*A*) The autoshaping chamber. (*B*) Rat at the beginning of autoshaping session. (*C*) Rat showing autoshaped lever-press response. (*D*) Rat consuming the US-food pellet. (*E*) Autoshaping Pavlovian/instrumental scheme. (*F*) Percentage of CRs during an autoshaping session after different pretraining conditions (from *left* to *right*): Pavlovian/instrumental, only CS, only US, Pavlovian autoshaping, and operant level. *Values significantly different from those of the P/I-A trained group ($P < 0.05$ by Dunnett's *t* test)

Autoshaping and Brain Memory Systems

Memory is organized into multiple brain systems. According to its duration, it is divided into short-, intermediate-, and long-term memory. Short-term memory appears to involve protein phosphorylation, whereas intermediate memory requires gene translation but not transcription. Long-term memory requires both translation and transcription (Marinesco and Carew 2002). When memory is classified according to its content, it is either declarative or nondeclarative. "Cognitive" declarative (or explicit) memory is based on conscious recall and is hippocampus-dependent, whereas nondeclarative (procedural or implicit) memory is based on stimulus-response (S-R) "habit" formation and is mediated by the caudate nucleus (for review, see Packard and Cahill 2001; Meneses 2002b).

Brain lesion studies using autoshaping and/or Pavlovian conditioning (for references, see Meneses 2002b) have shown that acquisition depends on the nucleus accumbens and specific limbic cortical afferents, including anterior cingulated cortex, basolateral and central amygdala, and ventral subiculum, but not hippocampus (nevertheless, see Good and Macphail 1994; Richmond and Colombo 2002). Indeed, even though learning of the association between a neutral discrete cue and food reward, in both Pavlovian and instrumental learning tasks, has been shown to be hippocampus-independent (see Thomas and Everitt 2001), bilateral ibotenic acid infusions into the basal forebrain disrupted autoshaped response acquisition (Steckler et al. 1993). In a similar P/I-A test of memory acquisition, an increase in the incorporation of [^{35}S]-methionine into proteins of cytoplasmic and synaptosomal fractions from caudate nucleus (autoshaping saline group) and hippocampus (autoshaping amphetamine group) relative to that of the nontrained saline control group was observed. Significant enhancement of learning was only observed in the amphetamine-treated group (Oscos et al. 1985), indicating

Table 2. Effects of Food Magazine Training Prior to Autoshaped Memory in Pre- or Posttraining–Treated Animals With 8-OH-DPAT, Scopolamine, PCPA, or PCA

	Administration			
	Pretraining		Posttraining	
	Food magazine training			
Drug (mg/kg)	Yes	No	Yes	No
Control	12 ± 1	1 ± 1	11 ± 1	0
8-OH-DPAT (0.062)	13 ± 2[a]	1 ± 1	34 ± 5[a]	0
(0.250)	16 ± 3[a]	1 ± 1	33 ± 6[a]	1 ± 1
Control	—	—	14 ± 3	—
Scopolamine (0.17)	—	—	4 ± [a]	—
PCPA (300) × 3 d	8 ± 2	—	9 ± 3	—
PCA (10) × 2 d	—	—	12 ± 3	—

Data from Meneses and Hong 1994a,b, 1995, 1999.
[a]Values significantly different from the corresponding vehicle-treated group ($P < 0.05$ by Dunnett's *t* test).

Table 3. Effects of 5-HT Receptor Antagonists on Memory Consolidation in an Autoshaping Learning Task Compare With Control Saline Treated Groups

			Effective doses	
Receptor	Treatment (mg/kg)	Effects	Impairment	Facilitatory
5-HT$_{1A}$	S-UH-301 (0.3–3.0)[a]	=		
	WAY 100135 (5.0–20.0)[a]	=		
	WAY 100635 (00.1–1.0)[a]	=		
5-HT$_{1B}$	SB-224289 (0.1–10.0)[b]	↑		5.0, 10.0
5-HT$_{1B/1D}$	GR127935 (0.1–10.0)[b]	↕	1.0	5.0, 10.0
	5-HT-moduline (50–500 μg)[c]	↑		100, 500 (i.c.v.)
5-HT$_{2A}$	MDL100907 (0.1–3.0)[d]	=		
5-HT$_{2B/2C}$	SB-200646 (2.0–40.0)[a]	=		
5-HT$_{3}$	Tropisetron (0.001–0.1)[f]	↑		0.01
	Ondansetron (0.01–1.0)[f]	↑		0.1, 1.0
5-HT$_{4}$	SDZ 205-557 (1.0–10.0)[g]	=		
	GR125487 (0.39–1.56)[g]	=		
5-HT$_{6}$	Ro 04-6790 (1.0–10.0)[h]	↑		5.0, 10.0
5-HT$_{7}$	LY2155840	=		
	DR 4004 (0.5–10.0)[i]	=		
	SB-269970 (1.0–10.0)[i]	=		

↑ indicates facilitation; ↓ indicates impairment; = indicates no effect; i.v.c. = intracerebroventricular; [a]Meneses and Hong 1999, Meneses 2002a; [b]Meneses et al. 1997, Meneses 2001b; [c]Hong et al. 1999; [d]Meneses et al. 1997; [e]Meneses 2002a; [f]Hong and Meneses 1996; [g]Meneses and Hong 1997a; [h]Meneses 2001b; [i]Meneses 2002b.

an important hippocampus, amygdala, caudate nucleus, and temporal cortex engagement, which is decreased in well-trained groups compared with the Pavlovian group. In addition, autoshaped memory requires hippocampal CA1 and dentate gyrus decrements in 5-HT receptors with increased CA2 and decreased dentate gyrus 5-HT$_4$ receptor subtype expression (Meneses et al. 2003). Although extensive Pavlovian autoshaping training (Tomie et al. 2003) failed to produce any correlation between 5-HT$_{1A}$ or 5-HT$_{2A}$ receptor expression and CR, this group demonstrated correlation between both receptors and CS–US presentations. Taken together, these data indicate that neuroanatomical, neurochemical, and behavioral effects of Pavlovian and P/I-A versions are different, although the latter could be considered more as an instance of system processing styles (i.e., S-S, S-R, and stimulus-reinforcer [S-Rf] learning; see White and McDonald 2002). Of course, further studies are required to confirm these observations.

5-HT System and Autoshaped Memory

In mammals, serotonin pathways originate in the mid- and hindbrain raphe nuclei, and ascending 5-HT fibers innervate brain areas (e.g., cortex, hippocampus, amygdala, septum; see Barnes and Sharp 1999) involved in learning and memory, which make it well placed in mediating normal and dysfunctional memory (Meneses 1999; Buhot et al. 2000). Nonetheless, the mechanisms, loci, and time window of 5-HT system involvement on mammals are unclear. What could be the role of central 5-HT in memory? Is this similar or different to that of the cholinergic system? To shed light on these crucial questions, past research has used several approaches, including pharmacological, lesion, and genetic manipulations, as well as water maze (WM) and passive avoidance (PA) learning models (Meneses 1999; Bonasera and Tecott 2000; Buhot et al. 2000). Importantly, advances in molecular biology have allowed the cloning and sequencing of at least 14 mammalian 5-HT receptors and their diverse splice variants (see Raymond et al. 2001), which have been classified into families according to their operational (pharmacological), structural (molecular), and transductional (second messenger systems) profiles (for reviews, see Hoyer et al. 1994, 2002; Barnes and Sharp 1999).

Apart from the 5-HT$_3$ receptor, this unique family member is a ligand-gated cation channel. Other receptors—5-HT$_1$, 5-HT$_2$, 5-HT$_4$, 5-HT$_6$, and 5-HT$_7$—belong to the G protein–coupled receptor superfamily. Particularly, 5-HT$_1$ receptors are functionally, but not exclusively (see Raymond et al. 2001), coupled to G$_i$ and/or G$_o$ protein; 5-HT$_2$ receptors, to G$_q$; and 5-HT$_4$, 5-HT$_6$, and 5-HT$_7$ receptors, to G$_s$. Emerging evidence indicates that 5-HT$_{1A/1B/1D//1E/1F}$, 5-HT$_{2A/2B/2C}$, 5-HT$_{3/3B}$, 5-HT$_4$, 5-HT$_{5A/5B}$, 5-HT$_6$, and 5-HT$_7$ receptors show a regional and cellular distribution within the central nervous system (Barnes and Sharp 1999) in brain areas associated to learning and memory processes (Buhot et al. 2000; Meneses 2002a), and different 5-HT markers are affected by aging and Alzheimer's disease (AD; Meneses 1999). Different 5-HT$_{1A/1B/1D//1E/1F}$ receptor activation produces hyperpolarization; 5-HT$_{2A/2B/2C}$, 5-HT$_{3/3B}$, 5-HT$_4$, and 5-HT$_7$ receptors elicit depolarization (Hoyer et al. 2002). Although there is a limited number of specific 5-HT receptor agonists and antagonists, growing evidence indicates that 5-HT serves as a link between synaptic plasticity at the receptor and postreceptor level (i.e., signal transduction pathways) during learning and memory formation.

More importantly, classification and cloning of multiple 5-HT receptors (Hoyer et al. 1994, 2002) have provided excellent opportunities to investigate 5-HT effects on learning and memory (Meneses 1999). Notably, in a P/I-A task, a large number of 5-HT–mediated mechanisms have been tested (Tables 2–5). These have taken advantage of the fact that autoshaped responses during acquisition show moderate levels in control animals, and both learning enhancement and retardation produced by 5-HT drugs are easily detectable. Indeed, Pavlovian autoshaping is better performed by rats with reduced serotonergic activity (Tomie et al. 2001), whereas P/I-A performance is unaffected by serotonergic depletion or inhibition synthesis (Table 2). Incidentally, these latter findings clearly indicate a 5-HT postsynaptic mediation of autoshaped memory.

Autoshaped Memory: Behavioral and Pharmacological Validation

During P/I-A training, the animal is placed into the experimental chamber and allowed to habituate to the environment. The animal finds and eats 50 food pellets (45 mg each) and learns where it can find food (Meneses 2002b). Immediately afterward, the program consisting of discrete trials begins. A trial consists of an illuminated retractable lever (CS) presented for 8 sec, followed by a food-pellet (US) delivery every 60 sec (Fig. 2E). When the animal presses the CS, the lever is retracted, the light is turned off, and a US is delivered immediately. This action is considered as a CR, and an increase or decrease in CR percentage in treated animals compared with control animals is considered as an enhancement or impairment of learning, respectively. The first training session consists of 10 trials lasting ~12 min, and the second session (i.e., session test) consists of 20 trials (lasting ~24 min). Usually the compounds are infused immediately after the

Table 4. Effects of 5-HT Receptor Agonists and Antagonists on Memory Consolidation in an Autoshaping Learning Task

Receptor	Effective dose (mg/kg)	Effect	Antagonized	No effect	5-HT depletion (PCA) effect
5-HT_{1A}	8-OH-DPAT (0.062)[a]	↑	WAY100635 S-UH-301	GR127935 MDL100907 SB-200646 Ro 04-6790	PCA annulled agonist effect
5-HT_{1B}	GR46611X (10.0)[b,h]	↓	5-HT-moduline SB-224289 GR127935		Eliminated
$5\text{-HT}_{1B/1D}$	TFMPP (5.0–10.0)[c]	↓	GR127935 SB-224289		Eliminated
	mCPP (5.0–10.0)[c]	↓	Ketanserin		No effect on agonist effect
5-HT_{1F}	LY344864 (5.0–10.0)[d]	↑			
5-HT_{2A}	DOI (0.01–0.1)[c,i]	↑	MDL100907	SB-200646 LY215840 (low dose)	Eliminated
$5\text{-HT}_{2B/2C}$	1-NP (0.1–5.0)[c]	↓	SB-200646		
	Mesulergine (0.2–0.4)[c]	↓	SB-200646		
5-HT_{3}	mCPBG (1.0)[e]	↓	Ondansetron Tropisetron		Eliminated
5-HT_{4}	BIMU1 (20.0)[f]	↓	GR125487D		Unaffected
	BIMU8 (5.0)[f]	↓	SDZ205-557		Unaffected
5-HT_{5}					
5-HT_{6}	Ro 04-6790 (5.0)[j]	↑	Ritanserin	WAY100635 GR127935 Ketanserin Ondansetron GR125487D	Unaffected
5-HT_{7}	8-OH-DPAT (0.0.62)[g,d]	↑	LY215840 Ketanserin Ritanserin (high dose) DR4004 SB-269970		Eliminated

↑ indicates facilitation; ↓ indicates impairment; [a]Meneses and Hong 1994a, 1999; [b]Meneses et al. 1997; [c]Meneses and Hong 1997a,c; [d]Meneses 1999; [e]Hong and Meneses 1996; [f]Meneses and Hong 1997b; [g]Meneses and Terrón 2001, Meneses 2003c; [h]Hong et al. 1999; [i]Meneses 2002a; [j]Meneses 2001b and slightly modified 8-OHDPAT effect.

first autoshaping session (in the case of long-term memory, 24 h later), thus specifically affecting memory consolidation (Fig. 1).

In the P/I-A procedure, the training session itself results in significant increases in performance of the trained group compared with the nontrained group (Table 2; Fig. 2F). Slight modifications of the procedure may result in variable data. For instance, when animals are not trained to the food-magazine, they display a lower level of CR, whereas control animals trained with the food-magazine show significant increases on memory consolidation (Table 2; Fig. 2F), indicating modest but reliable control scores. For instance, the muscarinic antagonist scopolamine (Table 2) or N-methyl-D-aspartate (NMDA) antagonist dizocilpine (Meneses 1999) treatment impairs memory consolidation, whereas *d*-amphetamine facilitates memory consolidation (Oscos et al. 1985). These data are consistent with the notion that engram formation and manipulation are related both to the training amount and to posttraining treatment strength (Lorenzini et al. 1999; see above). In the P/I-A task, 10 trials (rather than five or 20 trials) was best at detecting the drug-induced changes on the CR (Oscos et al. 1988).

5-HT Receptor Antagonist and Agonist Effects on Autoshaped Memory

5-HT neuronal activity is tightly regulated, which results in tonic activation of target forebrain neurons providing variable activation in other brain regions (Jacobs and Azmitia 1992; Adell et al. 2002), including their coexistence in the same anatomical location (Uphouse 1997) and multiple transduction pathways. Assuming there is serotonergic tonic levels, 5-HT antagonist administration should then provide key information about the physiological role. Selective receptor antagonists for 5-HT_{1A}, 5-HT_{2A}, $5\text{-HT}_{2B/2C}$, 5-HT_{4}, or 5-HT_{7} receptors prove to have no effect on memory consolidation in the P/I-A task (Table 3). This indicates that there is not 5-HT endogenous tonic activity via these receptors. Nevertheless, 5-HT_{1B} (SB-224289, [1′-methyl-5-([2′-methyl-4′(5-methyl1-1,2,4-oxadiazol-3-yl) biphenyl-4-yl] carbonyl)-2, 3,6,7tetra-hydrospirofuro[2,3-f]indole-3,4′-piperidine]), 5-HT_{1D} (GR127935), 5-HT_{3} (tropisetron or ondansetron), or 5-HT_{6} (Ro 04-6790 [4-amino-N-(2,6-bis[methylamino]-4-pyrimidinyl)-benzenesulfonamide dihydrochloride]) receptor blockade facilitates memory consolidation (Table 3); hence, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{3}, and/or 5-HT_{6} receptors exert tonic serotonergic limits or constrain autoshaped memory consolidation. Another possibility is that there is basal activity, that is, agonist-independent activity, such as in the case of 5-HT_{2A} receptors (see Harvey 1994; Harvey et al. 2002; Meneses 2002a).

Table 5. Effects of 5-HT Drugs on the Percentage of Conditioned Responses (CR) in an Autoshaping Learning Task

Treatment (mg/kg)	CR (%)
Control	8 ± 2
Tianeptine (5.0) + phenserine (0.5)	28 ± 6[a]
Fluoxetine (5.0) + phenserine (0.5)	38 ± 10[a]

[a]Values are significantly different from control-saline; + versus only antagonist group ($P < 0.05$ by Tukey test).

Importantly, none of the 5-HT antagonists (Table 3) tested herein impaired autoshaped memory, which contrasts with the well-known cholinergic antagonism-induced amnesia (see Sarter et al. 1992) observed in many learning tasks, including autoshaping (Meneses and Hong 1997a). A parsimonious conclusion is that 5-HT and cholinergic systems may play different roles during autoshaped memory formation and behave differentially. Thus, although cholinergic activity interruption results in a poor memory, serotonergic activity—at least via 5-HT_{1B}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_3, and/or 5-HT_6—seems to restrain memory capacity and/or accessibility. Hence, autoshaped memory formation would require intact cholinergic function, whereas serotonergic function, at least via the above 5-HT receptors, would be instructive (e.g., 5-HT_{1A} receptors directly modifying the synaptic connection strengthen required for memory engram storage; Shobe 2002). This notion is consistent with 5-HT agonist data (Table 4), revealing that overstimulation of 5-$HT_{1A/7}$ (using 8-OH-DPAT), 5-HT_{1F}, and 5-HT_{2A} receptors produce better autoshaped memory consolidation, and that 5-HT_{1B}, 5-HT_{1D}, 5-$HT_{2B/2C}$, 5-HT_3, and 5-HT_4 receptors produce impaired autoshaped memory consolidation. Except for 5-HT_{1F} receptors for which we lacked a selective antagonist, selective antagonists eliminate the facilitatory (5-HT_{1A}, 5-HT_{1F}, and 5-HT_{2A} receptors) or impairment effect (5-HT_{1B}, 5-HT_{1D}, 5-$HT_{2B/2C}$, 5-HT_3, and 5-HT_4 receptors; Table 5); hence, both facilitatory and impairment effects are attributable to specific receptors. Similarly, pharmacological specificity of 5-HT_6 receptor blockade has been demonstrated, and this enhances autoshaped memory (Table 3). This effect was not altered by 5-HT_{1A}, 5-$HT_{2A/2B/2C}$, 5-HT_3, 5-HT_4, or 5-HT_7 receptor blockade (Meneses 2001b). Finally, selective and nonselective 5-HT_7 receptor antagonists, but not selective 5-$HT_{2A/2B/2C}$ antagonists, reverse the 8-OH-DPAT facilitatory effect (Meneses and Terron 2001; A. Meneses, in prep.).

Pre- and Postsynaptic 5-HT Receptor Function During Normal and Impaired Autoshaped Memory

Although 5-HT presynaptic integrity does not appear necessary for autoshaped memory (Table 2), 5-HT depletion can reliably provide us information about pre- versus postsynaptic mechanisms. For instance, some presynaptic 5-HT receptors play a significant role during autoshaped memory, as demonstrated by stimulation or blockade of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_3, and 5-HT_7, but not 5-HT_4 and 5-HT_6, receptors and resultant effects were attenuated or eliminated by p-chloroamphetamine (PCA) 5-HT depletion and/or p-chlorophenylalanine (PCPA) 5-HT inhibition synthesis (Meneses 1999, 2001a,b; 2002a,b). In addition, the 8-OH-DPAT facilitatory effect of autoshaped memory consolidation was blocked by WAY 100635 ([N-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-N-[2-pyridinyl] cyclohexanene carboxamide-6-trihydrochloride]), LY215840, ritanserin (high dose), ketanserin, DR4004 (2a-[4-(4-henyl-1,2,3,6-tetrahydropyridyl)butyl]-2a,3,4,5,tetrahydrobenzo-indol-2[1H]-one), or SB-269970, but not by GR127935, MDL100907 ([R+]-a[2,3-dimethoxyphenyl]-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol), or SB-200646 (N-[1-metyl-indolyl]N′-[3-pyridyl]urea hydrochloride), and partially by Ro 04-6790([4-amino-N-(2,6-bis[methylamino]-4-pyrimidinyl)-benzenesulfon-amidedihydrochloride]; Table 4). It seems reasonable to conclude that 5-HT_{1A} and 5-HT_7 receptors mediate the 8-OH-DPAT facilitatory effects on memory consolidation because of the following: (1) WAY 100635 is a selective 5-HT_{1A} receptor antagonist; (2) LY215840, ketanserin, and ritanserin display affinity for 5-HT_2 and 5-HT_7 receptors; (3) MDL100907 and SB-200646 are selective antagonists for 5-HT_{2A} and 5-$HT_{2B/2C}$ receptors, respectively; and (4) DR-4004 and SB-269970 are selective 5-HT_7 receptor antagonists (see Hoyer et al. 1994, 2002). Admittedly, 8-OH-DPAT displays a low intrinsic activity at 5-HT_7 receptors (Hoyer et al. 2002).

Interestingly, ondansetron, ketanserin, and Ro 04-6790, which have no affinity for the 5-HT_{1A} receptor, at doses that by themselves facilitate autoshaped memory consolidation, blocked the 8-OH-DPAT-facilitatory effect (Table 4; Meneses and Hong 1999). This is attributable to physiological antagonism indicating that some 5-HT receptors have opposite roles during memory consolidation. Importantly, when 5-HT receptors are individually examined, such findings can lead to conclusions that differ from those observed when endogenous serotonin and multiple 5-HT receptors are explored.

Notably, agonists display affinity for the subpopulation of receptors coupled to G protein, whereas antagonists can label both coupled and uncoupled receptors (Vergé and Calas 2000). Their affinity order (Ki, nM) for 5-HT is as follows: 5-HT_{1A} (3.1) > 5-HT_{1D} (4.4) > 5-HT_{5A} (6.6) = 5-HT_{5B} (6.6) > 5-HT_6 (7.0) = 5-HT_{1B} (7.0) ≥ 5-HT_4 (7.1) ≥ 5-HT_{2A} (7.2) > 5-HT_7 (8.5) > 5-HT_{2C} (11.0) ≫ 5-HT_3 (240.6; original range values were calculated from Uphouse 1997). In addition, 5-HT has varied affinity and/or potency for different receptors. Different receptors using multiple transduction pathways differ in their susceptibility to agonist-mediated desensitization/down-regulation and to changes in physiological (Uphouse 1997; Raymond et al. 2001), environmental, and behavioral states.

Furthermore, as 5-HT diffuses to the extracellular space, extrasynaptic 5-HT receptors activate nonsynaptic or volumetric transmission, relevant to such effects of serotonin reuptake inhibitor (SSRI) on memory. Indeed, 5-HT reuptake inhibitors (SSRI) facilitate autoshaped memory consolidation (Meneses and Hong 1995), an effect blocked by selective 5-HT_{1A} to 5-HT_7 receptor antagonists (Meneses 2002b), whereas acetylcholinesterase inhibitor administration potentiates SSRI subeffective doses (Table 5). These results are consistent with the notion that 5-HT reuptake tightly regulates synaptic plasticity rather than transmitter clearance, which is constitutively determined (Hoyer et al. 2002). Even though it has been suggested that SSRIs could primarily have effects on depression and aggressive behavior rather than on cognitive dysfunctions in AD patients, in nondemented elderly, depressed patients, and idiopathic autistic young children, treatment with SSRIs improve cognitive function (for references, see Meneses 1999). Notably, memory deficits associated with the recreational use of "ecstasy" (3,4-methylenedioxymethamphetamine [MDMA]; Morgan 1990) could be related to the loss of 5-HT reuptake sites (Meneses 1999). It is frequently neglected that SSRI-induced impairment is observed during acquisition but not during memory consolidation (Meneses and Hong 1995; Meneses 1999).

In contrast, the pre- versus posttraining administration of 5-HT_{1A}, 5-$HT_{2A/2C}$, or 5-HT_4 receptor agonists and antagonists is similar (Meneses 1999). For instance, 8-OH-DPAT administration has been reported to cause impairment or enhancement or to have no effect on learning and memory (for references, see Meneses 1999; Buhot et al. 2000). These contradictory findings could be attributable to pre- versus postsynaptic 5-HT_{1A} receptors playing opposite roles. The former has no role or facilitating performance (depending on doses, agonist versus antagonist, timing of drug administration, and behavioral task used; see Fig. 1; Meneses 1999), and hippocampal 5-HT_{1A} receptor stimulation impairs learning and memory (Buhot et al. 2000; Carli et al. 2001).

Because PCA pretreatment eliminated or attenuated the facilitatory or inhibitory effects of receptor agonists/antagonists of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_3, and 5-HT_7—but not of 5-HT_4 and 5-HT_6 (see below)—on autoshaped memory formation, intact diverse 5-HT auto- and hetero-receptors are necessary for this type

of memory. If this conclusion is correct, then it is possible that a serotonergic tone could be altered under amnesic conditions, such as AD, although evidence of 5-HT involvement is limited (see Lynes et al. 2003) and more investigation is urgently needed. Different 5-HT markers are diminished in AD patients (for references, see Meneses 1999; Porter et al. 2000; Lai et al. 2002; Versijpt et al. 2003), including raphe complex, 5-HT release and uptake/transporter, and 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_4 receptors (but see Lai et al. 2003); however, 5-HT_3 receptors remain unchanged. Furthermore, a significant increase in 5-HT_6 receptor gene 267C allele has been associated as a risk factor for AD (Tsai et al. 1999; Liu et al. 2001; but see Orlacchio et al. 2002). Likewise, although the 5-HT_{2A} receptor may play a role in amyloid precursor protein secretion (Versijpt et al. 2003), activation of human 5-HT_4 receptor (and probably of other 5-HT receptors) stimulates the nonamyloidogenic soluble form of the amyloid precursor protein (sAPPα) secretion; sAPPα displays neuroprotective activity and memory-enhancing effects and might, in turn, affect the Aβ deposition process (Robert et al. 2001), a cerebrovascular amyloid key component detected in AD brains. The above information clearly indicates that the 5-HT system plays a pathophysiological role in a deficient memory, although it is unclear if this is a result or a cause.

Nevertheless, manipulation of the 5-HT system has therapeutic potential and is strengthened by the following: Raphe nuclei are significantly affected by AD pathology (Chen et al. 2000); however, 5-HT system plasticity is the probable reason for the lack of correlation of reduced 5-HT neuron density and cognitive decline. Second, in normal humans, decreased hippocampal [^{11}C] WAY-100635 binding correlated with better memory performance, indicating that hippocampal postsynaptic 5-HT_{1A} receptors have a negative influence on explicit memory function (Yasuno et al. 2003). Third, reduced 5-HT levels and increased 5-HT_{1A} receptor density in the neocortex are markers for accelerated cognitive decline in AD; importantly, intraprefrontal and systemic 8-OH-DPAT administration selectively enhances attention and accuracy on the five-choice serial reaction time task (Winstanley et al. 2003). Finally, 5-HT_{1A} receptor/G-protein complex is functionally intact in the parietal and frontal cortex of AD patients (O'Neill et al. 1991). 5-HT receptor stimulation or blockade may have potential therapeutic benefits to treating various memory dysfunctions, as 5-HT tonic influence can be modified during dysfunctional memory induced by known amnesic treatments, such as the antimuscarinic scopolamine or NMDA antagonist dizocilpine. In fact, 5-HT_{1A} receptor agonists and antagonists normalize autoshaped memory consolidation (Meneses and Hong 1999).

Similar effects were detected after SSRIs (Meneses 2002b), 5-HT_{1B}, 5-HT_{1D} (Meneses 2001a), 5-HT_3, 5-HT_4 (Hong and Meneses 1996; Meneses and Hong 1997a,b), 5-HT_6 (Meneses 2001b), and 5-HT_7 (Meneses and Terrón 2001) receptor antagonists. SB-200646 or MDL100907 alone have no effect on memory consolidation; however, coadministration reverses memory deficits induced by cholinergic and/or glutamatergic blockade, and these findings are consistent with the notion that these antagonists have no intrinsic activity in other neuronal functions (for references, see Meneses 2002a). It could therefore be hypothesized that neutral antagonists acting at 5-HT_{2A} and 5-$HT_{2B/2C}$ (even likely at 5-HT_{1A}, 5-HT_{1B}, 5-HT_6, and 5-HT_7) receptors are promising candidates for cognitive disorder treatment, which may have additional implications in the development of antipsychotic drugs that lack cognitive side effects. Moreover, data from two other associative learning tests (the rabbit nictitating membrane reflex and the conditioned avoidance response in rat) have identified two classes of 5-$HT_{2A/2C}$ receptor antagonists, including negative antagonists that retard learning when given alone (ritanserin, MDL11939, pizotifen and cyproheptadine) and neutral antagonists (ketanserin, mianserin, and LY53857) that have no effect on learning (Harvey 1996).

The effect of negative antagonists has been explained by inverse agonism at 5-HT_2 receptors and by results of potential constitutive activity in vivo (Harvey 1996; Romano et al. 2002). Indeed, SB-200646, LY215840, and MDL100907 neutral effects may raise the possibility that the changes in memory involve inverse agonism at 5-$HT_{2B/2C}$ and/or 5-HT_{2A} receptors. Purportedly, inverse agonism can only be detected in genetically engineered systems (de Ligt et al. 2000); nevertheless, Romano and colleagues recently (2002) reported that chronic pretreatment with MDL11939 selectively up-regulated 5-HT_{2A}, but not 5-HT_{2C}, receptors in limbic cortex and hippocampus and improved the eye blink response in rabbits.

Related Findings: WM and PA

Learning and memory data reproducibility and reliability among behavioral tasks and/or laboratories are a major concern; fortunately, in WM and PA tests, several 5-HT drugs have been tested. WM and PA are two of the most widely used behavioral tools to study learning and memory (see Meneses 1999; Izquierdo and McGaugh 2000; McGaugh and Izquierdo 2000; D'hooge and De Deyn 2001). For instance, in autoshaped memory, PCA or PCPA have no effect. Similarly 5-HT depletion, PCPA, or raphe dorsalis lesions did not affect working memory (Ruotsalainen et al. 1998) or WM learning, but did aggravate scopolamine effect or nucleus basalis lesions. As observed with autoshaping and pretraining administration, 8-OH-DPAT impairs hidden-platform WM acquisition and probe trial; however, low doses of 8-OH-DPAT facilitate autoshaped responses in mice (Vanover and Barrett 1998) and rats (Table 5). Administration into the dorsal raphe of 8-OH-DPAT has no effect on WM but compensated for the deficit in spatial learning caused by impaired cholinergic or glutamatergic hippocampal transmission (Carli et al. 2001) and enhanced operant conditional discrimination (Ward et al. 1999). Operant autoshaping-task mice lacking 5-HT_{1A} or 5-HT_{1B} receptors learn faster than do wild-type mice (Pattij 2002), but 5-HT_{1A}KO mice showed deficits in hippocampal-dependent learning and memory tests (Sarnyai et al. 2000). 5-HT_{1B} KO mice showed WM enhanced-platform acquisition and probe trial performance (Buhot et al. 2000), and selective 5-HT_{1B} antagonists facilitated WM (Ahlander-Luttgen et al. 2003) and autoshaped memory (Table 3). In parallel to the finding that 5-$HT_{2B/2C}$ receptor antagonist (Table 3) had no effect on autoshaped memory, 5-HT_{2C} receptor KO mice showed normal hidden-platform and preference for the target quadrant during a probe trial. Antagonists for 5-HT_3 or agonists for 5-HT_4 receptors attenuated WM deficits in scopolamine-treated and forebrain-lesioned rats or atropine-induced deficits, respectively. Similar anti-amnesic effects were observed on autoshaped memory (Hong and Meneses 1996; Meneses and Hong 1997a). In addition, 5-HT_6 receptor antagonists SB-271046-A, SB-357134-A, or Ro 04-6790 and 5-HT_6 antisense oligonucleotide improved WM retention (Rogers and Hagan 2001; Woolley et al. 2001), whereas Ro 04-6790 enhanced autoshaped memory (Table 5). Interestingly, the 5-HT_6 receptor antagonist RO4368554 reversed the scopolamine effects on PA, object recognition, and social recognition, but not on radial arm maze or step-through PA (Szczepanski et al. 2002). On untreated rats, RO4368554 enhanced autoshaped memory but had no effect on WM performance of aged rats; hence, RO4368554 appears to enhance learning and memory, particularly in "disease models" (i.e., scopolamine-treated rats).

Concerning the PA and 5-HT system (for references, see Meneses 1999; Izquierdo and McGaugh 2000), PA acquisition, con-

solidation, and retrieval may be impaired by 5-HT_{1A} or 5-HT_{1B} (Ahlander-Luttgen et al. 2003) receptor stimulation, whereas blockade of 5-HT_{1A} or 5-$HT_{2A/2B/2C}$ receptors has no effect. 5-HT_3 antagonists or 5-HT_4 agonists (systemic or central) improved PA learning and/or prevented scopolamine- or hypoxic-induced amnesia. In addition, 5-HT posttraining injections into the dorsal and ventral striatum selectively produced strong amnesia in PA (Prado-Alcala et al. 2003). Izquierdo and colleagues have demonstrated that both cyclic adenosine monophosphate (cAMP) and protein kinase C (PKC) changes are necessary for the initiation and continuity of long-term memory consolidation in the PA task, which is modulated by diverse neurotransmission systems and transduction pathways, including 5-HT_{1A} synapses in the CA1 hippocampal area, as well as the entorhinal and posterior parietal cortex (see Izquierdo and McGaugh 2000; McGaugh and Izquierdo 2000). In this regard, we found that the 8-OH-DPAT facilitatory effect on autoshaped memory was accompanied by hippocampal cAMP increases, the former blocked by WAY100635 and DR4004 (Meneses et al. 2002), indicating 5-HT_{1A} and 5-HT_7 receptor participation. Hippocampal cAMP activates cAMP-dependent protein kinase (PKA) and phosphorylation of the transcription factor CREB (cAMP-response element binding protein); both PKA and CREB have been implicated directly in long-term plasticity and memory formation (Izquierdo and McGaugh 2000). Although our observation of cAMP increase may be surprising, it should be noted that 5-HT_{1A} receptor both inhibits and activates adenylyl cyclase (Raymond et al. 2001) and, consequently, cAMP production. It should be noticed that in an in vivo microdialysis study, systemic administration of 8-OH-DPAT increased hippocampal cAMP, mediated by 5-HT_{1A} receptors (Cadogan et al. 1994).

An Initial Proposal of 5-HT Receptor Function During Memory Formation

In intact animals, there is no apparent 5-HT endogenous tone involving 5-HT_{1A}, 5-HT_{2A}, 5-$HT_{2B/2C}$, 5-HT_4, or 5-HT_7 receptors during an autoshaped memory consolidation; however, via 5-HT_{1B}, 5-HT_{1D}, 5-HT_3, or 5-HT_6 receptors, serotonergic tone limits or constrains its consolidation. Interruption of cholinergic activity results in a poor memory formation, whereas 5-HT function is instructive, being facilitatory (via 5-HT_{1A}, 5-HT_{1F}, and 5-HT_{2A} receptors) or inhibitory (via 5-HT_{1B}, 5-HT_{1D}, 5-$HT_{2B/2C}$, 5-HT_3, and 5-HT_4 receptors). This indicates that a basal activity exists that is agonist-independent during consolidation, at least in regard to 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_3, and 5-HT_6 receptors. Because selective antagonists blocked the facilitatory and amnesic effects, both effects are attributable to specific receptors.

Considering that 5-HT affinity for these receptors ranged from 4.4 to 240.0 nM (see above), it is possible that during memory formation, extracellular 5-HT concentrations may present regional-dependent variations. Thus, presynaptic 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_3, and 5-HT_7 receptors play a significant role during autoshaped memory formation and although an intact 5-HT system is not necessary for memory consolidation, these findings suggest that individual presynaptic 5-HT receptors may modulate memory formation. Under dysfunctional memory formation (e.g., cholinergic and glutamatergic blockade, or 5-HT agonism or antagonism), serotonergic function is modified. For instance, otherwise silent 5-HT_{1A} receptors (as showed by selective antagonists) in intact animals become active to agonists and antagonists and normalize autoshaped memory consolidation. Similar scenarios were detected after SSRIs, 5-HT_{1B}, 5-HT_{1D}, 5-HT_3, 5-HT_4, 5-HT_6, and 5-HT_7 antagonist treatment. Because these receptors show varying affinity for the endogenous neurotransmitter, it is possible that under memory-altered conditions in humans and impaired-memory animal models, 5-HT extracellular levels and/or 5-HT receptor number, affinity, sensitivity, and transduction pathways may be altered. Thus, multiple 5-HT receptors and endogenous 5-HT concentrations may have the potential for decoding and drive-signal pattern. Indeed, in an autoradiographic binding study, memory formation induced significant increases of [^{3}H]-5-HT radioligand receptor binding in the cortex, hippocampus, and amygdala, an effect modified by age (Meneses et al. 2002). Although these data strongly indicate a direct link between 5-HT receptors and memory formation, it must be emphasized that the above observations were obtained on autoshaped memory formation; in other learning tasks, opposite effects were reported.

Final Remarks

The present pharmacological data have provided clear-cut evidence that 5-HT_{1-7} receptor subtypes and its uptake sites participate in autoshaped memory formation. In addition, these 5-HT mechanisms are able to normalize an impaired memory consolidation provoked by cholinergic and glutamatergic antagonists, or 5-$HT_{1B/1D/2A-2C/7}$ agonist/antagonist. Apparently, 5-HT receptors operating at pre- and postsynaptic levels may modulate memory processes. Selective drugs for different 5-HT receptors will allow us to define with more precision the role of the 5-HT system in learning and memory. We are aiming to expand our pharmacological and methodological approaches to other learning and memory tasks.

ACKNOWLEDGMENTS

I thank the pharmaceutical companies and Institutes for their generous gifts, including AstraArcus, Boehringer Ingelheim (F. Borsini), Eli Lily, Gerontology Research Center, National Institutes of Health (D. Ingram and N. Greig), Glaxo, Glaxo-Wellcome, GlaxoSmithKline (D. Thomas), Institute Pasteur (G. Fillion), Meiji Seika Kaisha (T. Hiranuma), Merrel Dow, Novartis (D. Hoyer), Sandoz Pharma, Servier (M. Millan), SmithKline-Beecham (G. Price), and Wyth Research. I thank Sofia Meneses-Goytia for language review and design of tables and figures. Also, Rafael Villalobos revised language. I thank Drs. D.K. Ingram and B. Soumireu-Mourat for their warm hospitality and friendship during my postdoctoral and visiting professor stays, respectively. This research was, in part, supported by CONACYT grant 39534M. I want to thank J.L. McGaugh and Sherrie Ma for their final review of the language, which is highly appreciated. I would also like to thank the two anonymous reviewers for their helpful comments, and J.H. Byrne for his guidance and valuable assistance.

REFERENCES

Adell, A., Celada, P., Abellán, M.T., and Artigas, F. 2002. Origin and functional role of extracellular serotonin in the midbrain raphe nuclei. *Brain Res. Rev.* **39:** 154–180.

Ahlander-Luttgen, M., Madjid, N., Schoot, P.A., Sandin, J., and Ogren, S.O. 2003. Analysis of the role of the 5-HT_{1B} receptor in spatial and aversive learning in the rat. *Neuropsychopharmacology* **28:** 1642–1655.

Angers, A., Storozhuk, M.V., Duchaine, T., Castellucci, V.F., and DesGroseillers, L. 1998. Cloning and functional expression of an *Aplysia* 5-HT receptor negatively coupled to adenylate cyclase. *J. Neurosci.* **18:** 5586–5593.

Arshavsky, Y.I. 2003. Cellular and network properties in the functioning of the nervous system: From central pattern generators to cognition. *Brain Res. Rev.* **41:** 229–267.

Barbas, D., Zappulla, J.P., Angers, S., Bouvier, M., Castellucci, V.F., and DesGroseillers, L. 2002. Functional characterization of a novel serotonin receptor (5-HT_{ap2}) expressed in the CNS of *Aplysia californica*. *J. Neurochem.* **80:** 335–345.

Barnes, N.M. and Sharp, T. 1999. A review of central 5-HT receptors and their functions. *Neuropharmacology* **38:** 1083–1152.

Boakes, R.A. 1977. Performance on learning to associate a stimulus with positive reinforcement. In *Operant-Pavlovian interactions* (eds. H. Davis and H.M.B. Hurwitz), pp. 67–101. Lawrence Erlbaum

Associates, Hillsdale, NJ.
Bonasera, S.J. and Tecott, L.H. 2000. Mouse models of serotonin receptor function: Toward a genetic dissection of serotonin systems. *Pharmacol. Ther.* **88:** 133–142.
Buhot, M.C., Martin, S., and Segu, L. 2000. Role of serotonin in memory impairment. *Ann Med.* **32:** 210–221.
Byrne, J.H. and Kandel, E.R. 1996. Presynaptic facilitation revisited: State and time dependence. *J. Neurosci.* **16:** 425–435.
Cadogan, A.K., Kendall, D.A., and Marsden, C.A. 1994. Serotonin 5-HT_{1A} receptor activation increases cyclic AMP formation in the rat hippocampus in vivo. *J. Neurochem.* **62:** 1816–1821.
Carli, M., Balducci, C., and Samanin, R. 2001. Stimulation of 5-HT_{1A} receptors in the dorsal raphe ameliorates the impairment of spatial learning caused by intrahippocampal 7-chloro-kynurenic acid in naive and pretrained rats. *Psychopharmacology* **158:** 39–47.
Chen, C.P.L., Eastwood, S.L., Hope, T., McDonald, B., Francis, P.T., and Esiri, M.M. 2000. Immunocytochemical study of the dorsal and median raphe nuclei in patients with Alzheimer's disease prospectively assessed for behavioral changes. *Neuropathol. Appl. Neurobiol.* **26:** 347–355.
Cohen, J.E., Onyike, C.U., McElroy, V.L., Lin, A.H., and Abrams, T.W. 2003. Pharmacological characterization of an adenylyl cyclase serotonin receptor in *Aplysia*: Comparison with mammalian serotonin receptors. *J. Neurophysiol.* **89:** 1440–1455.
Crow, T., Xue-Bian, J.J., Siddiqi, V., and Neary, J.T. 2001. Serotonin activation of the ERK pathway in *Hermissenda*: Contribution of calcium-dependent protein kinase C. *J. Neurochem.* **78:** 358–364.
D'Hooge, R. and De Deyn, P.P. 2001. Applications of the Morris water maze in the study of learning and memory. *Brain Res. Brain Res. Rev.* **36:** 60–90.
de Ligt, R.A., Kourounakis, A.P., and IJzerman, A.P. 2000. Inverse agonism at G protein-coupled receptors: (patho)physiological relevance and implications for drug discovery. *Br. J. Pharmacol.* **130:** 1–12.
Dringenberg, H.C. 2000. Alzheimer's disease: More than a "cholinergic disorder": Evidence that cholinergic-monoaminergic interactions contribute to EEG slowing and dementia. *Behav. Brain Res.* **115:** 235–249.
Good, M. and Macphail, E.M. 1994. Hippocampal lesions in pigeons (*Columba livia*) disrupt reinforced preexposure but not overshadowing or blocking. *Q. J. Exp. Psychol. B* **47:** 263–291.
Harvey, J.A. 1996. Serotonergic regulation of associative learning. *Behav. Brain Res.* **73:** 47–50.
Hong, E. and Meneses, A. 1996. Systemic injection of *p*-chloroamphetamine eliminates the effect of the 5-HT_3 compounds on learning. *Pharmacol. Biochem. Behav.* **53:** 765–769.
Hong, E., Orozco, G., Meneses, A., and Fillion, F. 1999. Effect of 5-HT-moduline, an endogenous peptide, in associative learning. *Proc. West Pharmacol. Soc.* **42:** 37–38.
Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., and Humphrey, P.P.A. 1994. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.* **46:** 157–203.
Hoyer, D., Hannon, J.P., and Martin, G.R. 2002. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.* **71:** 533–554.
Izquierdo, I. and McGaugh, J.L. 2000. Behavioural pharmacology and its contribution to the molecular basis of memory consolidation. *Behav. Pharmacol.* **11:** 517–534.
Jacobs, B.L. and Azmitia, E.C. 1992. Structure and function of the brain serotonin system. *Physiol. Rev.* **72:** 165–229.
Kirkwood, A. 2000. Serotonergic control of developmental plasticity. *Proc. Natl. Acad. Sci.* **97:** 1951–1953.
Lai, M.K., Tsang, S.W., Francis, P.T., Esiri, M.M., Hope, T., Lai, O.F., Spence, I., and Chen, C.P. 2003. [^{3}H]GR113808 binding to serotonin 5-HT_4 receptors in the postmorten neocortex of Alzheimer disease: A clinicopathological study. *J. Neural Transm.* **110:** 779–788.
Lai, M.K.P., Tsang, S.W.Y., Francis, P.T., Keene, J., Hope, T., Esiri, M., Spence, I., and Chen, C.P. 2002. Postmortem serotonergic correlates of cognitive decline in Alzheimer's disease. *Neuroreport* **13:** 1175–1178.
Li, X.C., Giot, J.F., Kuhl, D., Hen, R., and Kandel, E.R. 1995. Cloning and characterization of two related serotonergic receptors from the brain and the reproductive system of *Aplysia* that activate phospholipase C. *J. Neurosci.* **15:** 7585–7591.
Liu, H.C., Hong, C.J., Liu, C.Y., Lin, K.N., Tsai, S.J., Liu, T.Y., Chi, C.W., and Wang, P.N. 2001. Association analysis of the 5-HT_6 receptor polymorphism C267T with depressions in patients with Alzheimer's disease. *Psychiatry Clin. Neurosci.* **55:** 427–429.
Locurto, C.M., Terrace, H.S., and Gibbon, J., eds. 1981. *Autoshaping and conditioning theory*. Academic Press, New York.
Lorenzini, C.G.A., Baldi, E., Bucherelli, C., Sacchetti, B., and Tassoni, G. 1999. Neural topography and chronology of memory consolidation: A review of functional inactivation findings. *Neurobiol. Learn. Mem.* **71:** 1–18.
Lynes, S.A., Zarow, C., and Chui, H.C. 2003. Neuron loss in key cholinergic and aminergic nuclei in Alzheimer disease: A meta-analysis. *Neurobiol. Aging* **24:** 1–23.
Manuel-Apolinar, L. and Meneses, A. 2003. 8-OH-DPAT facilitated memory consolidation and increased hippocampal and cortical cAMP production. *Behav. Brain Res.* (in press).
Marinesco, S. and Carew, T.J. 2002. Serotonin release evoked by tail nerve stimulation in the CNS of *Aplysia*: Characterization and relationship to heterosynaptic plasticity. *J. Neurosci.* **22:** 2299–2312.
McDonald, M.P., Wong, R., Goldstein, G., Wientraub, B., and Crawley, J.N. 1998. Hyperactivity and learning deficits in transgenic mice bearing a human mutant thyroid hormone β1 receptor gene. *Learn. Mem.* **5:** 289–301.
McGaugh, J.L. and Izquierdo, I. 2000. The contribution of pharmacology to research on the mechanisms of memory formation. *Trends Pharmacol. Sci.* **21:** 208–210.
Menard, J. and Treit, D. 1999. Effects of centrally administered anxiolytic compounds in animal models of anxiety. *Neurosci. Biobehav. Rev.* **23:** 591–613.
Meneses, A. 1999. 5-HT system and cognition. *Neurosci. Biobehav. Rev.* **8:** 1111–1125.
———. 2001a. Could the 5-HT_{1B} receptor inverse agonism affect learning consolidation. *Neurosci. Biobehav. Rev.* **25:** 193–201.
———. 2001b. Effects of the 5-HT_6 receptor antagonist Ro 04-6790 on learning consolidation. *Behav. Brain Res.* **118:** 107–110.
———. 2002a. Involvement of 5-$HT_{2A/2B/2C}$ receptors on memory formation: Simple agonism, antagonism, or inverse agonism? *Cell. Mol. Neurobiol.* **22:** 675–688.
———. 2002b. Tianeptine: 5-HT uptake sites and 5-HT_{1-7} receptors modulate memory formation in an autoshaping Pavlovian/instrumental task. *Neurosci. Biobehav. Rev.* **26:** 309–319.
Meneses, A. and Hong, E. 1994a. Mechanisms of action of 8-OH-DPAT on learning and memory. *Pharmacol. Biochem. Behav.* **49:** 1083–1086.
———. 1994b. Modification of 8-OH-DPAT effects on learning by manipulation of the assay conditions. *Behav. Neural. Biol.* **61:** 29–35.
———. 1995. Effects of fluoxetine on learning and memory involve multiple 5-HT systems. *Pharmacol. Biochem. Behav.* **52:** 341–346.
———. 1997a. Effects of 5-HT_4 receptor agonists and antagonists in learning. *Pharmacol. Biochem. Behav.* **56:** 347–351.
———. 1997b. A pharmacological analysis of serotonergic receptors: Effects of their activation or blockade in learning. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **21:** 273–296.
———. 1997c. Role of 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors in learning. *Behav. Brain Res.* **87:** 105–110.
———. 1998. Spontaneous hypertensive rat: A potential model to identify drugs for treatment of learning disorders. *Hypertension* **31:** 968–972.
———. 1999. 5-HT_{1A} receptors modulate the consolidation of learning in normal and cognitively impaired rats. *Neurobiol. Learn. Mem.* **71:** 207–218.
Meneses, A. and Terron, J.A. 2001. Role of 5-HT_{1A} and 5-HT_7 receptors in the facilitatory response induced by 8-OH-DPAT on learning consolidation. *Behav. Brain Res.* **121:** 21–28.
Meneses, A., Terron, J.A., and Hong, E. 1997. Effects of 5-HT receptor antagonists GR127935T (5-$HT_{1B/1D}$) and MDL 100,907 (5-HT_{2A}) in the consolidation of learning. *Behav. Brain Res.* **89:** 217–223.
Meneses, A., Manuel, L., Castillo, E., Gonzalez, R., Castillo. C., and Hong, E. 2002. Is 5-HT_{1A}, 5-HT_4 & 5-HT_7 receptors role related to cAMP & cGMP levels during memory consolidation? *Soc. Neurosci.* Abstract 586.12.
Meneses, A., Manuel, L., Rocha, L., Castillo, E., and Castillo, C. 2003. 5-HT receptors expression in rat brain during memory formation. *Soc. Neurosci.* Abstract 460.12.
Morgan, M.J. 1990. Memory deficits associated with recreational use of "ecstasy" (MDMA). *Psychopharmacology (Berl)* **141:** 30–36.
O'Neill, C., Cowburn, R.F., Wiehager, B., Alafuzoff, I., Winblad, B., and Fowler, C.J. 1991. Preservation of 5-hydroxytryptamine1A receptor–G protein interactions in the cerebral cortex of patients with Alzheimer's disease. *Neurosci. Lett.* **133:** 15–19.
Orlacchio, A., Kawarai, T., Paciotti, E., Stefani, A., Orlacchio, A., Sorbi, S., St. George-Hyslop, P.H., and Bernardi, G. 2002. Association study of the 5-hydroxytryptamine(6) receptor gene in Alzheimer"s disease. *Neurosci. Lett.* **125:** 13–16.
Oscos, A., Martinez, J.L., and McGaugh, J.L. 1988. Effects of post-training *d*-amphetamine on acquisition of an appetitive autoshaped lever press response in rats. *Psychopharmacology (Berl)* **95:** 132–134.
Oscos-Alvarado, A., Camacho, J.L., Meneses, A., and Aleman, V. 1985.

The post-trial effect of amphetamine in memory and in cerebral protein amino acid incorporation in the rat. In *Contemporary psychology: Biological processes and theoretical issues* (ed. J.L. McGaugh), pp. 123–129. Elsevier Science, Amsterdam, The Netherlands.

Oscos-Alvarado, A., Meneses, A., Aleman, V., Camacho, J.L., Meneses, A., and Ortega, A. 1991. In: *Neuroquímica del aprendizaje y la memoria: Un enfoque molecular* (ed. V.A. Colotla), pp. 13–36. La Investigación del comportamiento en México. UNAM, México City, Mexico.

Packard, M.G. and Cahill, L. 2001. Affective modulation of multiple memory systems. *Curr. Opin. Neurobiol.* **11:** 752–756.

Pantoni, L., Palumbo, V., and Sarti, C. 2002. Pathological lesions in vascular dementia Alzheimer's disease: Vascular etiology and pathology. *Ann. N.Y. Acad. Sci.* **977:** 279–291.

Pattij, T. 2002. "5-HT_{1A} receptor knockout mice and anxiety: Behavioral and physiological studies." Ph.D. thesis, Universiteit Utrecht, The Netherlands.

Porter, R.J., Lun, B.S., Walker, L.L., Gray, J.M., Ballard, C.G., and O'Brien, J.T. 2000. Cognitive deficit induced by acute tryptophan depletion in patients with Alzheimer's disease. *Am. J. Psychiatry* **157:** 638–640.

Prado-Alcala, R.A., Ruiloba, M.I., Rubio. L., Solana-Figueroa, R., Medina, C., Salado-Castillo, R., and Quirarte, G.L. 2003. Regional infusions of serotonin into the striatum and memory consolidation. *Synapse* **47:** 169–175.

Raymond, J.R., Mukhin Y.V., Gelasco, A., Turner, J., Collinsworth, G., Gettys, T.W., Grewal, J.S., and Garnovskaya, M.N. 2001. Multiplicity of mechanisms of serotonin receptor signal transduction. *Pharmacol. Ther.* **92:** 179–212.

Richmond, J. and Colombo, M. 2002. Hippocampal lesions, contextual retrieval, and autoshaping in pigeons. *Brain Res.* **928:** 60–68.

Robert, S.J., Zigaza, J.L., Fischmeister, R., Gardier, A.M., and Lezoualc, F. 2001. The human serotonin 5-HT_4 receptor regulates secretion of non-amyloidogenic precursor protein. *J. Biol. Chem.* **276:** 44881–44888.

Rogers, D.C. and Hagan, J.J. 2001. 5-HT_6 receptor antagonists enhance retention of a water maze task in the rat. *Psychopharmacology* **158:** 114–119.

Romano, A.G., Quinn, J.L., Fernando, G.S., Dave, K.D., Aloyo, V.J., and Harvey, J.A. 2002. Chronic MDL11,939 upregulates 5-HT_{2A} receptors and enhances associative learning in the rabbit. *Soc. Neurosci.* Abstract 9.1

Ruotsalainen, S., Miettinen, R., Macdonald, E., Riekkinen, M., and Sirvio, J. 1998. The role of the dorsal raphe-serotonergic system and cholinergic receptors in the modulation of working memory. *Neurosci. Biobehav. Rev.* **22:** 21–31.

Sagvolden, T. 2000. Behavioral validation of the spontaneously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (AD/HD). *Neurosci. Biobehav. Rev.* **24:** 31–39.

Sarnyai, Z., Sibille, E.L., Pavlides, C., Fenster, R.J., McEwen, B.S., and Tóth, M. 2000. Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin$_{1A}$ receptors. *Proc. Natl. Acad. Sci.* **97:** 14731–14736.

Sarter, M., Hagan, J., and Dudchenko, P. 1992. Behavioral screening for cognition enhancers: from indiscriminate to valid testing: Part I. *Psychopharmacology (Berl)* **107:** 144–159.

Shobe, J. 2002. The role of PKA, CaMKII, and PKC in avoidance conditioning: Permissive or instructive? *Neurobiol. Learn. Mem.* **77:** 291–312.

Steckler, T., Andrews, J.S., Marten, P., and Turner, J.D. 1993. Effects of NBM lesions with two neurotoxins on spatial memory and autoshaping. *Pharmacol. Biochem. Behav.* **44:** 877–889.

Szczepanski, K.V., Vivian, J.A., Dorsch, K., Blokland, A., Hedley, I., Lieben, C.K.J., Martin, J.R., Secchi, R.L., Sik, A., Sung, E., et al. 2002. Precognitive effects of the 5-HT_6 receptor antagonist in rats. *Soc. Neurosci.* Abstract 290.29.

Terry, A.V., Hernandez, C.M., Buccafusco, J.J., and Gattu, M. 2000. Deficits in spatial learning and nicotinic-acetylcholine receptors in older, spontaneously hypertensive rats. *Neuroscience* **101:** 357–368.

Thomas, K.L. and Everitt, B.J. 2001. Limbic-cortical-ventral striatal activation during retrieval of a discrete cocaine-associated stimulus: A cellular imaging study with γ protein kinase C expression. *J. Neurosci.* **21:** 2526–2535.

Tomie, A., Aguado, A.S., Pohorecky, L.A., and Benjamin, D. 2001. Individual differences in Pavlovian autoshaping lever pressing in rats predicts stress-induced corticosterone release and mesolimbic levels of monoamines. *Pharmacol. Biochem. Behav.* **65:** 509–517.

Tomie, A., Di Poce, J., Aguado, A., Janes, A., Benjamín, D., and Pohorecky, L. 2003. Effects of autoshaping procedures on ^{3}H-8-OH-DPAT-labeled 5-HT_{1a} binding and ^{125}I-LSD-labeled 5-HT_{2a} binding in the rat brain. *Brain Res.* **975:** 167–178.

Tsai, S-J., Liu, H-C., Liu, T-Y., Wang, Y-C., and Hong, C-J. 1999. Association analysis of the 5-HT_6 receptor polymorphism C267T in Alzheimer's disease. *Neurosci. Lett.* **276:** 138–139.

Uphouse, L. 1997. Multiple serotonin receptors: Too many, not enough, or just the right number. *Neurosci. Biobehav. Rev.* **21:** 679–698.

Vanover, K.E. and Barrett, J.E. 1998. An automated learning and memory model in mice: Pharmacological and behavioral evaluation of an autoshaped response. *Behav. Pharmacol.* **9:** 273–283.

Vergé, D. and Calas, A. 2000. Serotonergic neurons and serotonin receptors: Gains from cytochemical approaches. *J. Chem. Neuroanat.* **18:** 41–56.

Versijpt, J.J., Van Laere, K.J., Dumont, F., Decoo, D., Vandecapelle, M., Santens, P., Goethals, I., Audenaert, K., Slegers, G., Dierckx, R., et al. 2003. Imaging of the 5-HT_{2A} system: Age-, gender-, and Alzheimer's disease-related findings. *Neurobiol. Aging* **24:** 553–561.

Ward, B.O., Wilkinson, L.S., Robbins. T.W., and Everitt, B.J. 1999. Forebrain serotonin depletion facilitates the acquisition and performance of a conditional visual discrimination task in rats. *Behav. Brain Res.* **100:** 51–65.

White, N.M. and McDonald, R.J. 2002. Multiple parallel memory systems in the brain of the rat. *Neurobiol. Learn. Mem.* **77:** 125–184.

Winstanley, C.A., Chudasama, Y., Dalley, J.W., Theobald, D.E.H., Glennon, J.C., and Robbins, T.W. 2003. Intra-prefrontal 8-OH-DPAT and M100907 improve visuospatial and decrease impulsivity on the five-choice serial reaction time task in rats. *Psychopharmacology* **163:** 304–314.

Woolley, M.L., Bentley, J.C., Sleight, A.J., Marsden, C.A., and Fone, K.C. 2001. A role for 5-ht6 receptors in retention of spatial learning in the Morris water maze. *Neuropharmacology* **41:** 210–219.

Yasuno, F., Suhara, T., Nakayama, T., Ichimiya, T., Okudo, Y., Tacaño, A., Ando, T., Inoue, M., Maeda, J., and Suzuki, K. 2003. Inhibitory effect of hippocampal 5-HT_{1A} receptors on human explicit memory. *Am. J. Psychiatry* **160:** 334–340.

A Pharmacological Analysis of an Associative Learning Task: 5-HT$_1$ to 5-HT$_7$ Receptor Subtypes Function on a Pavlovian/Instrumental Autoshaped Memory

Alfredo Meneses

Learn. Mem. 2003, **10:**
Access the most recent version at doi:10.1101/lm.60503