Role of the Serotonin 5-HT_{2A} Receptor in Learning

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This study reviews the role of the serotonin 5-HT_{2A} receptor in learning as measured by the acquisition of the rabbit's classically conditioning nictitating membrane response, a component of the eyeblink response. Agonists at the 5-HT_{2A} receptor including LSD (d-lysergic acid diethylamide) enhanced associative learning at doses that produce cognitive effects in humans. Some antagonists such as BOL (d-bromolysergic acid diethylamide), LY53,857, and ketanserin acted as neutral antagonists in that they had no effect on learning, whereas others (MDL11,939, ritanserin, and mianserin) acted as inverse agonists in that they retarded learning through an action at the 5-HT_{2A} receptor. These results were placed in the context of what is known concerning the anatomical distribution and electrophysiological effects of 5-HT_{2A} receptor activation in frontal cortex and hippocampus, as well as the role of cortical 5-HT_{2A} receptors in schizophrenia. It was concluded that the 5-HT_{2A} receptor demonstrates constitutive activity, and that variations in this activity can produce profound alterations in cognitive states.

The past few decades have brought an increasing awareness of serotonin's role in behavior. The development of drugs acting on the serotonergic system of brain that allow for the treatment of depression, anxiety, appetite regulation, and post-traumatic stress disorders has focused a great deal of attention on the role of serotonin in processes involving emotional states. Commensurate with our increasing understanding of the role of serotonin in behavioral processes has been the identification of at least seven serotonin (5-hydroxytryptamine; 5-HT) receptor sub-types. More recently, investigators have focused on the role of serotonin in cognitive functions, including learning and memory (Harvey 1996; Barnes and Sharp 1999; Meneses 1999, 2002; Williams et al. 2002) and in the deficits in attention and associative processes seen in schizophrenia (Meltzer 1999). Serotonin receptor subtypes that have been demonstrated to occur in brain regions capable of playing a role in learning and memory include the 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_6, and 5-HT_7 class of receptors (Barnes and Sharp 1999; Meneses 2002). Table 1 summarizes studies that have examined the effects of serotonin agonists on learning. It can be seen that agonists at the 5-HT_{1A} receptor subtype had either no effect ($n = 2$) or impaired learning ($n = 3$), whereas both 5-$HT_{2A/2C}$ and 5-HT_4 receptor agonists primarily improved learning. The majority of serotonin antagonists are reported to have no effect on learning (Table 2). Thus, all of the 5-HT_{1A} ($n = 4$) and 5-HT_3 ($n = 4$) antagonists that have been examined were found to have no effect on learning. However, two of the six 5-$HT_{2A/2C}$ antagonists impaired learning, as did one of the two 5-HT_4 antagonists.

Although the data cited above indicate that activation of serotonin receptors or their blockade can produce alterations in learning, these data do not provide the consistent outcomes that would allow for definite conclusions concerning the precise role of the various receptor subtypes in learning. However, this is not surprising, as different behavioral paradigms invoke different forms of learning that are mediated by differently distributed neuronal networks. In this regard, it is important to note that, even within a particular paradigm, alterations in the precise manner by which stimuli are presented can produce systematic differences in the brain regions undergoing learning-dependent changes. For example, it is well known that contextual fear conditioning requires hippocampal mediation, whereas signaled fear conditioning does not (Phillips and LeDoux 1994). Consequently, activation of serotonin receptors in different brain regions would be expected to have different effects, depending on the behavioral paradigm used, as well as on the precise role of the different classes of serotonin receptors in mediating activity within particular neuronal networks. On the basis of these considerations, our laboratory chose to use a single behavioral paradigm (classical eyeblink conditioning) and to restrict our studies to an examination of the role of the serotonin 5-HT_{2A} receptor in learning. This chapter details the results of studies providing evidence that the serotonin 5-HT_{2A} receptor demonstrates intrinsic activity that determines the rate of associative learning.

Serotonin 5-HT_{2A} receptors in frontal cortex and hippocampus modulate local circuitry. In contrast to the limited experimental data on other serotonin receptor subtypes, a great deal of systematic data has been collected to indicate an important role for 5-HT_{2A} receptors in modulating neuronal circuitry in medial prefrontal cortex and hippocampus. Both of these brain areas are known to be involved importantly in associative learning across a number of species and learning paradigms, including the classically conditioned eyeblink response in rabbits (Buchanan and Powell 1982; Port et al. 1985; Solomon et al. 1986; Kronforst-Collins and Disterhoft 1998; Weible et al. 2000) and humans (Clark and Squire 1998). The 5-HT_{2A} receptors are located in both the medial prefrontal cortex and hippocampus of the rat (Pazos et al. 1985), rabbit (Aloyo and Harvey 2000), primate (Jakab and Goldman-Rakic 1998), and human (Hoyer et al. 1986; López-Giménez, et al. 1998; Barnes and Sharp 1999). The 5-HT_{2A} receptors found in the rabbit frontal cortex are pharmacologically similar to human receptors (Aloyo and Harvey 2000). Electrophysiological studies in the rat (Sheldon and Aghajanian 1991; Marek and Aghajanian 1994) and primate (Williams et al. 2002) indicate that 5-HT_{2A} receptors can modulate cortical neuronal excitability, and thus, may be expected to play an important role in modulating learning. The 5-HT_{2A} receptors have been shown to be located on both the dendrites of cortical pyramidal cells as well as on interneurons (Jakab and Goldman-Rakic 1998), and mediate excitation in both neuronal types (Tanaka and North 1993; Aghajanian and Marek 1997). Thus, activation of 5-HT_{2A} receptors in cortex can produce both a direct excitation and a feed-forward inhibition of cortical pyramidal cells. In addition,

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Table 1. Effect of Agonists at Serotonin Receptor (5-HT) Subtypes on Learning

Drug	Receptor	Task	Species	Effect	Reference
Lisuride	5-HT_{1A}	EBR	Rabbit	No Effect	Welsh et al. (1998a)
8-OH-DPAT	5-HT_{1A}	EBR	Rabbit	No Effect	Welsh et al. (1998a)
8-OH-DPAT	5-HT_{1A}	CAR	Rat	Impaired	Meneses and Hong (1994)
Buspirone	5-HT_{1A}	CAR	Rat	Impaired	Alhaider et al. (1993)
Tandospirone	5-HT_{1A}	VM	Human	Impaired	Yasuno et al. (2003)
TFMPP	5-$HT_{2A/2C}$	CAR	Rat	Enhanced	Alhaider et al. (1993)
Quipazine	5-$HT_{2A/2C}$	CAR	Rat	Enhanced	Alhaider et al. (1993)
RS67333	5-HT_4	SPL	Rat	No Effect	Fontana et al. (1997)
RS67333	5-HT_4	OD	Rat	Enhanced	Marchetti et al. (2000)
RS17017	5-HT_4	MTS	Macaque	Enhanced	Terry et al. (1998)
SL65.0155	5-HT_4	ORT	Rat	Enhanced	Moser et al. (2002)

(ACC) Aversive classical conditioning; (ASL) autoshaping learning; (CAR) conditioned avoidance response; (EBR) associative conditioning of the eyeblink response; (MTS) matching to sample; (OD) olfactory discrimination; (ORT) object recognition task; (PWL) perceptual word list; (SPL) spatial learning; (VD) visual discrimination; (VM) verbal memory; (VSL) visuo-spatial learning; (8-OH-DPAT) 8-hydroxy-2-[di-n-propylamino]tetralin; (TFMPP) 1-[m-trifluoromethylphenyl]piperazine.

the location of 5-HT_{2A} receptors in cortex and hippocampus on cholinergic (Quirion et al. 1985) and glutamatergic (Aghajanian and Marek 2000; Lambe et al. 2000; Hasuo et al. 2002) axon terminals can serve to regulate the release of these transmitters. On the basis of studies that have used intrahippocampal injections of drugs that alter glutamatergic and cholinergic transmission during trace conditioning of the rabbit's eyeblink response (Thompson et al. 1992; Weiss et al. 2000), the increased release of acetylcholine and glutamate in hippocampus would also be expected to enhance learning. Thus, activation of 5-HT_{2A} receptor would be expected to increase learning through post-synaptic actions on cortical pyramidal cells (Williams et al. 2002) as well as through heteroceptors located on presynaptic terminals of cortical cholinergic and glutamatergic neurons.

Classical Conditioning of the Rabbit's Nictitating Membrane Response: A Model of Associative Learning

Classical conditioning of the rabbit's nictitating membrane response (a component of the eyeblink) was chosen for our studies because it has been acknowledged to provide a reliable measure of associative learning and to exhibit all of the associative processes observed in humans (Gormezano et al. 1983). Consequently, it has been used widely to model a variety of clinical states characterized by deficits in associative processes, including schizophrenia (Sears et al. 2000), Alzheimer's dementia (Woodruff-Pak 2001), autism, and obsessive-compulsive disorder (Steinmetz et al. 2001). Eyeblink conditioning has also provided an excellent model for examining the neuronal circuitry underlying associative learning (e.g., Thompson 1986; Harvey and Welsh 1996; Steinmetz 2000), the receptor systems that play a major role in the acquisition and retention of associative learning (e.g., Harvey 1987, 1996; Schindler and Harvey 1990; Romano and Harvey 1992), and the molecular cascade that is responsible for the alterations in synaptic efficacy that are required for the permanent storage of learned associations (Geinisman et al. 2001; Zhen et al. 2001). The wide use of this behavioral paradigm is also due to the fact that alterations in the acquisition of conditioned responses (CRs) can be further analyzed in terms of alterations in nonassociative processes such as sensitization and pseudoconditioning, as well as alterations in performance factors.

Classical eyeblink conditioning can be carried out by use of either delay or trace-conditioning procedures. In delay conditioning, the conditioned stimulus (CS) is presented for a given period of time and then its offset occurs simultaneously with onset of the unconditioned stimulus (US). The time between CS onset and US onset is referred to as the CS–US interval. One can use either short-delay procedures (e.g., a 200-msec CS–US interval) or long-delay intervals (e.g., 800–1500-msec CS–US intervals). In trace conditioning, the CS is presented for a fixed period of time (e.g., 100 msec). Offset of the CS is followed by a trace period (e.g., 500 msec), during which time no stimuli are presented. The US is then presented at the end of the trace period. Of special relevance for the studies described here, medial prefrontal cortex and hippocampus areas having a high density of 5-HT_{2A} receptors are critically involved in acquisition of the rabbit's eyeblink response during trace-conditioning procedures (Solomon et al. 1986; Gibbs and Powell 1991; McEchron and Disterhoft 1997; Kronforst-Collins and Disterhoft 1998; Romano 1999; Beylin et al. 2001) or during delay eyeblink conditioning using long CS–US intervals (Beylin et al. 2001). These brain regions are also important for trace eyeblink conditioning in the human (Clark and Squire 1998; LaBar and Disterhoft 1998). For these reasons, we chose to use this behavioral paradigm to explore the role of serotonin 5-HT_{2A} receptors in associative learning, and the results of these studies are summarized below.

Serotonin 5-HT_{2A} Receptor Agonists Enhance Associative Learning

Agonists at the 5-HT_{2A} receptor such as d-lysergic acid diethylamide (LSD), 2,5-dimethoxy-4-methylamphetamine (DOM), methylenedioxyamphetamine (MDA), and methylenedioxymethamphetamine (MDMA) enhance acquisition of the rabbit's

Table 2. Effect of Serotonin Receptor Antagonists on Learning

Drug	Receptor	Task	Species	Effect	References
WAY100135	5-HT_{1A}	SPL	Rat	No Effect	Carli et al. (1995)
WAY100635	5-HT_{1A}	VSL	Marmoset	No Effect	Harder et al. (1996)
WAY100635	5-HT_{1A}	SPL	Rat	No Effect	Carli et al. (1997a)
Spiperone	5-$HT_{1A/2A}$	CAR	Rat	No Effect	Alhaider et al. (1993)
Pindolol	5-$HT_{1A/1B}$	CAR	Rat	No Effect	Alhaider et al. (1993)
Ketanserin	5-HT_{2A}	CAR	Rat	No Effect	Alhaider et al. (1993)
Mianserin	5-$HT_{2A/2C}$	CAR	Rat	No Effect	Alhaider et al. (1993)
Cinanserin	5-$HT_{2A/2C}$	VD	Rat	No Effect	Kobayashi et al. (1995)
Metergoline	5-$HT_{2A/2C}$	PWL	Human	No Effect	Vitiello et al. (1997)
Cyproheptadine	5-$HT_{2A/2C}$	CAR	Rat	Impaired	Ma and Yu (1993)
Cyproheptadine	5-$HT_{2A/2C}$	CAR	Rat	Impaired	Titov et al. (1983)
Ritanserin	5-$HT_{2A/2C}$	ACC	Human	Impaired	Hensman et al. (1991)
MDL72,222	5-HT_3	CAR	Rat	No Effect	Alhaider et al. (1993)
WAY100289	5-HT_3	SPL	Rat	No Effect	Hodges et al. (1995)
Ondansetron	5-HT_3	ORT	Marmoset	No Effect	Barnes et al. (1990)
Ondansetron	5-HT_3	SPL	Rat	No Effect	Carli et al. (1997b)
DAU6215	5-HT_3	SPL	Rat	No Effect	Pitsikas et al. (1994)
SDZ205557	5-HT_4	ORT	Rat	No Effect	Moser et al. (2002)
RS67532	5-HT_4	OD	Rat	Impaired	Marchetti et al. (2000)

See Table 1 for abbreviations.

Table 3. Effects of 5-HT$_2$ Agonists and Antagonists on Associative Learning in the Rabbit as Measured by the Classically Conditioned Nictitating Membrane Response, a Component of the Eyeblink

Serotonin receptor agonists that enhanced associative learning

Drug	5-HT receptor	Effective doses (μmole/kg)	Reference
LSD	2A/2C	0.001–0.100, i.v.	Gimpl et al. (1979)
LSD	2A/2C	0.080–0.197, i.v.	Siegel and Freedman (1988)
DOM	2A/2C	0.3–3.0, i.v.	Harvey et al. (1982)
MDA	2A/2C	1.0–10, s.c.	Romano et al. (1991)
MDMA	2A/2C	4.1–16.5, s.c.	Romano and Harvey (1994)

Serotonin receptor antagonists with no effect on learning

Drug	5-HT receptor	Dose range (μmole/kg)	Reference
BOL	2A/2C	0.003–0.30, i.v.	Harvey et al. (1982)
BOL	2A/2C	0.06–5.8, s.c.	Romano et al. (2000)
LY53,857	2A/2C	0.067–6.7, s.c.	Welsh et al. (1998a)
Ketanserin	2A	1.0–10, s.c.	Harvey et al. (1999)

Serotonin receptor antagonists that impair associative learning

Drug	5-HT receptor	Effective doses (μmole/kg)	Reference
Ritanserin	2A/2C	0.067–6.7, s.c.	Welsh et al. (1998a,b)
Mianserin	2A/2C	0.1–10.0, s.c.	Romano et al. (1991)
MDL11,939	2A	0.067–6.7, s.c.	Welsh et al. (1998a,b)
Pizotifen	2A/2C	8.4–33.8, s.c.	Ginn and Powell (1986)

(LSD) d-lysergic acid diethylamide; (DOM) 2,5-dimethoxy-4-methylamphetamine; (MDA) methylenedioxyamphetamine; (MDMA) methylenedioxymethamphetamine; (BOL) d-bromolysergic acid diethylamide.

eyeblink response (Table 3). The enhancement of associative learning produced by these agonists was most likely due to increased activity at the 5-HT$_{2A}$ receptor in frontal cortex and hippocampus, because, as noted above, those brain regions have been demonstrated to mediate eyeblink conditioning in rabbits and humans. These findings are complementary with experiments in monkeys that have indicated that activation of 5-HT$_{2A}$ receptors facilitates mnemonic processes occurring in prefrontal pyramidal cells that participate in spatial working memory (Williams et al. 2002). More importantly, the enhancement of eyeblink conditioning produced by 5-HT$_{2A}$ receptor agonists occurred in the range of doses that are known to produce alterations in associative and other cognitive processes in humans (see Table 4). Consistent with our findings, it has been reported that the 5-HT$_{2A/2C}$ receptor agonists quipazine and TFMPP (1-[m-trifluoromethylphenyl]piperazine) enhanced acquisition of the conditioned avoidance response in the rat (see Table 1; Alhaider et al. 1993). However, it is not clear whether the enhancement of learning produced by 5-HT$_{2A}$ agonists in our studies might not also be produced by agonists acting at other serotonin receptor subtypes. So far, only the 5-HT$_{1A}$ receptor agonists 8-OH-DPAT and lisuride have been examined, and they had no effect on acquisition of the eyeblink response (see Table 1; Welsh et al. 1998a). However, investigators using other measures of learning have reported that 5-HT$_{1A}$ receptor agonists impair learning. Thus, as noted in Table 1, 8-OH-DPAT (Meneses and Hong 1994) and buspirone (Alhaider et al. 1993) retarded acquisition of the conditioned avoidance response in the rat and tandospirone retarded acquisition of a verbal memory task in humans (Yasuno et al. 2003). As mentioned in the introduction, it will be important in future research to determine how activation of particular serotonin receptors produces differential effects depending on the learning task used.

As shown in Figure 1, the increases in learning produced by the prototypic agonist LSD was not dependent on the modality of the CS and US or on the use of defensive eyeblink conditioning. Thus, LSD increased learning when a tone CS and air puff US was used (Fig. 1A), when a tone CS and shock US was used (Fig. 1B), when a classically conditioned appetitive jaw movement response was employed using a tone CS and water as the US (Fig. 1C), and when tone or light CSs were paired with a shock US (Fig. 1D). However, the effects of LSD on learning were critically dependent on the CS–US interval used. It is well known that the rate of acquisition of CRs during eyeblink conditioning is a function of the CS–US interval (Gormezano et al. 1983). As shown in Figure 2A, control animals characteristically demonstrate low rates of CR acquisition at short CS–US intervals, for example, 100 msec, maximum rates of acquisition at CS–US intervals of 200–400 msec, followed by low rates of acquisition at longer CS–US intervals. LSD significantly enhanced CR acquisition at short (100 msec) and long (800 msec) CS–US intervals, but failed to do so at the optimum intervals of 200 and 400 msec (Fig. 2A; Harvey et al. 1988). This is further illustrated by acquisition curves in Figure 2, B, C, and D. At the 200-msec CS–US interval, both LSD-injected and vehicle control groups acquired CRs rapidly and at the same rate (Fig. 2C). In contrast, LSD produced a robust enhancement of CR acquisition at CS–US intervals of 100 msec (Fig. 2B) or 800 msec (Fig. 2D) that generated low rates of acquisition in vehicle controls. In other studies, enhancement of eyeblink CRs was seen during trace-conditioning procedures using CS–US intervals >1000 msec (Siegel and Freedman 1988). The factors that determine the ability of LSD to enhance learning may not be due to the CS–US interval being used as such, but rather to the rate of learning generated at different CS–US intervals. Thus, on the basis of a number of experiments, it was found that LSD failed to enhance learning during procedures that generated high rates of CR acquisition in controls, regardless of the CS–US interval used, but did enhance learning during procedures that generated lower rates of CR acquisition in controls (Fig. 3). Because rate of learning is an index of task dif-

Table 4. Comparison of the Efficacy of 5-HT$_2$ Agonists on CR Acquisition and Cognitive Effects in Humans

Drug	Rabbit ED50[a] (μg/kg)	Human dose[b] (μg/kg)
LSD	0.8[c]	1.0[h]
DOM	52[d]	50[i]
MDMA	500[e]	250[j]
MDA	800[f]	1000[k]
BOL	No effect[d,g]	No effect[h]

See Table 3 for abbreviations.
[a]Dose of drug required to produce a half maximal enhancement of learning.
[b]Lowest dose required to obtain reliable hallucinations in humans.
[c]Gimpl et al. (1979); [d]Harvey et al. (1982); [e]Romano and Harvey (1994); [f]Romano et al. (1991); [g]Romano et al. (2000); [h]Abramson et al. (1955); Rothlin (1957); Brawley and Duffield (1972); [i]Snyder et al. (1967); [j]Cole and Sumnall (2003); [k]Marquardt et al. (1978); Shulgin (1978); Braun et al. (1980).

ficulty, it is possible that activation of the 5-HT_{2A} receptor had a proportionately greater effect as increases in task difficulty placed greater demands on attentional and associative processes.

Control experiments using the explicitly unpaired presentations of CS and US indicated that the enhancement of CR acquisition produced by LSD, DOM, MDA, and MDMA was due to an enhancement of associative learning (Harvey et al. 1982; Romano et al. 1991; Romano and Harvey 1994). For example, base-line responding in control animals was low (1%–3%), as was responding to the unpaired tone stimulus (2%–8%). LSD had no effect on these measures of nonassociative responding during either defensive eyeblink or appetitive jaw movement conditioning procedures. The amphetamine derivatives DOM, MDA, and MDMA produced only small (4%) increases in base-line responding. MDA and MDMA also did not increase responding to the CS. However, DOM did produce a small, but significant, increase in responding to the CS, the mean values being 1.6% for controls and 11% for animals injected with the highest dose of DOM (3 µmole/kg). Lower doses of DOM had no effect on responding to the CS during the unpaired procedures, although these doses (0.3 and 1.0 µmole/kg) did enhance the rate of learning. Thus, the enhancement of CR acquisition produced by the 5-HT_2 agonists listed in Table 3 represented an increase in associative learning and not to such nonassociative factors as sensitization, pseudoconditioning, or increases in base-line rates of responding.

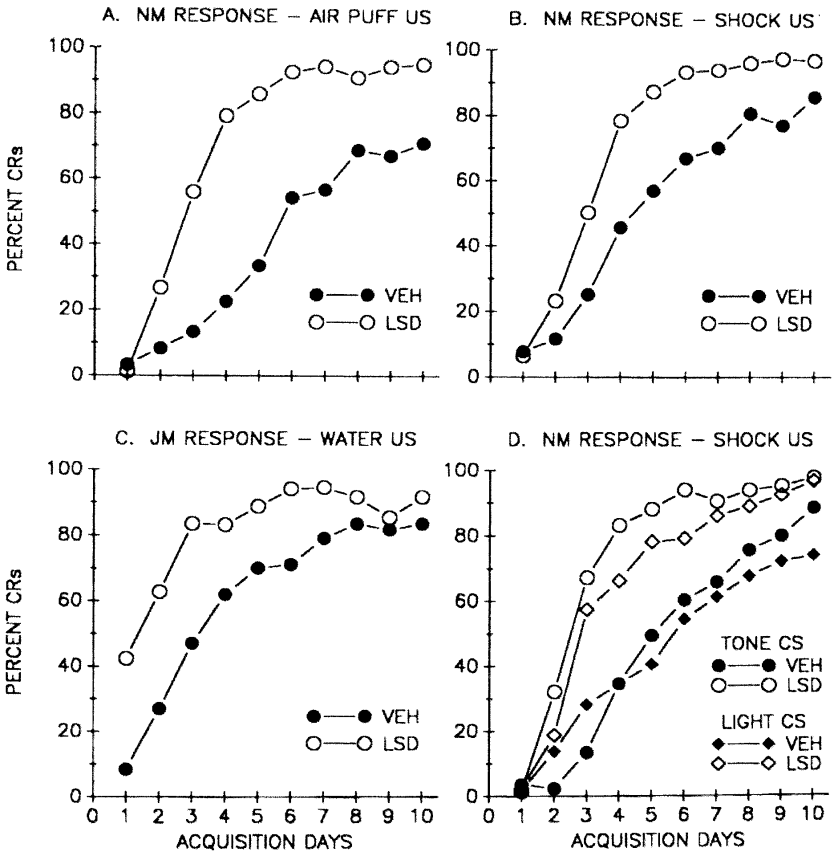

Figure 1 Enhancement of associative learning produced by the 5-HT_{2A} agonist LSD under different experimental conditions. The ordinate presents the mean percent conditioned responses (CRs) during each of 10 conditioning days derived from a minimum of 10 animals per group. (*A,B,C,D*) The dose of LSD was 13 µg/kg (0.030 µmole/kg) administered intravenously into the marginal ear vein of the rabbit. Conditioning of the NM response was based on a delay procedure using an 800-msec CS, whose offset occurred simultaneously with the onset of a 100-msec unconditioned stimulus (US). (*A*) Acquisition of the conditioned nictitating membrane (NM) response (a component of the eyeblink) during tone CS and corneal airpuff US conditioning (Schindler et al. 1985a); (*B*) acquisition of the NM response during tone CS and paraorbital shock US conditioning (Gimpl et al. 1979); (*C*) Acquisition of the appetitive jaw movement (JM) response during pairings of tone CS and water US in water-deprived rabbits. Jaw movements are produced during the ingestion of water, and during conditioning, these movements begin to be initiated to the tone CS prior to water presentation (Gormezano et al. 1980); (*D*) acquisition of the NM response to tone or light CSs paired with a shock US. The intensity of the tone CS had been adjusted so as to support the same rate of conditioning as the light CS (Schindler et al. 1985b).

Effect of Serotonin 5-HT_{2A} Antagonists on Associative Learning

As indicated in Table 3, some antagonists at the 5-HT_{2A} receptor, for example, d-bromolysergic acid diethylamide (BOL), LY53,857 and ketanserin, had no significant effect on associative learning and could, therefore, be classified as neutral antagonists. Surprisingly, some antagonists were not neutral, rather, they retarded learning in the rabbit (Table 3). As shown in Figure 4, MDL11,939, a highly selective 5-HT_{2A} antagonist, as well as the nonselective 5-$HT_{2A/2C}$ antagonists ritanserin and mianserin produced a dose-dependent reduction in CR acquisition (Welsh et al. 1998a,b; Romano et al. 2000). In a separate study, the 5-$HT_{2A/2C}$ antagonist pizotifen was also found to retard the acquisition of the rabbit's eyeblink response (Table 3; Ginn and Powell 1986). Explicitly unpaired CS–US procedures indicated that the retardation of CR acquisition produced by these drugs was due to a decrease in associative learning (Welsh et al. 1998b). In agreement with results in the rabbit, ritanserin also retarded classical aversive conditioning in humans (Table 2; Hensman et al. 1991). Another nonselective 5-$HT_{2A/2C}$ antagonist cyproheptadine impaired acquisition of the conditioned avoidance response in the rat (Table 2; Titov et al. 1983; Ma and Yu 1993). Also in agreement with our results, ketanserin was reported to have no effect on acquisition of the conditioned avoidance response in the rat; however, in contrast to our findings, these investigators also reported no effect of mianserin (see Table 2; Alhaider et al. 1993). Again, it is not yet clear how differences in outcome may be related to the learning task used or other factors.

A number of control experiments were carried out to determine the basis for the retardation of learning produced by MDL11,939, ritanserin, and mianserin in the rabbit. It was possible that these drugs were not retarding learning through an action at the 5-HT_{2A} receptor, but rather through actions at other serotonergic or nonserotonergic receptors. First, we examined whether ritanserin was acting as an antagonist at the serotonin 5-HT_{2A} receptor by examining its ability to block the effects of the 5-HT_{2A} receptor agonist LSD. Figure 5 illustrates the fact that a dose of ritanserin producing a retardation of CR acquisition was also able to block the enhancement of CRs produced by LSD. However, whereas these results could be due to the actions of ritanserin at the 5-HT_{2A} receptor (a true pharmacological antagonism), they may still have been due to a physiological antagonism mediated through actions at other receptors. Therefore, we

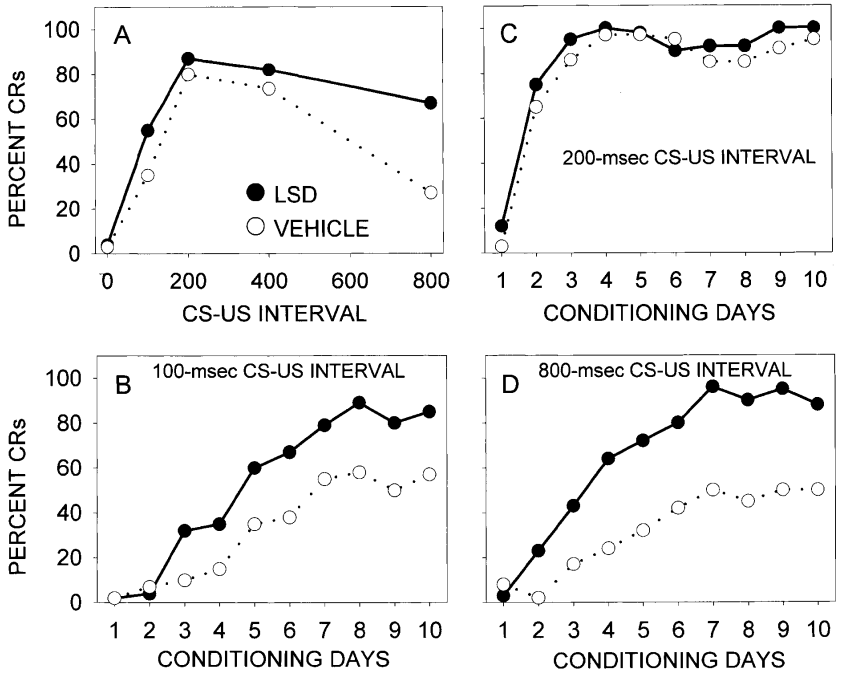

Figure 2 Enhancement of associative learning produced by the 5-HT_{2A} agonist LSD (13 µg/kg) at different CS–US intervals. Acquisition of the NM response during the pairing of a tone CS and paraorbital shock US. A trace procedure was employed using a 100-msec tone and shock US. Thus, the 0-msec CS–US interval indicates the simultaneous presentation of the stimuli and the 100 msec CS–US interval represents a delay procedure (0 trace). The 200-, 400-, and 800-msec CS–US intervals represent trace conditioning of 100, 300, and 500 msec, respectively. Data are taken from Harvey et al. (1988).

The ability of the antagonists MDL11,939, ritanserin, and mianserin to retard learning, to block the effects of agonists, and to have their own effects blocked by neutral antagonists, meets the criterion for establishing them as inverse agonists (Kenakin 1996; Strange 2002). Our identification of drugs as agonists, neutral antagonists, and inverse agonists on the basis of their effects on associative learning is supported by in vitro studies of 5-HT_{2A} and 5-HT_{2C} receptors in transfected cell lines. In such systems, LSD increases PI hydrolysis, an effect that is blocked by antagonists. Moreover, the antagonists fall into two classes. For example, as in the in vivo studies, mianserin decreased PI hydrolysis, indicating that it was an inverse agonist, whereas BOL was identified as a neutral antagonist, in that it had no effect on basal PI hydrolysis by itself, but blocked both the effects of the agonist LSD and the inverse agonist mianserin (Barker et al. 1994; Westphal and Sanders-Bush 1994; Egan et al. 1998). Similar studies have not been possible in native tissue due to the low levels of constitutive activity. However, the effects of 5-HT_{2A} agonists, neutral antagonists, and inverse agonists on associative learning not only provide the first in vivo demonstration of inverse agonism at the 5-HT_{2A} receptor, but also suggest that there is a functionally high level of constitutive (intrinsic) activity at that receptor that regulates the rate of associative learning.

examined whether the neutral 5-HT_{2A} receptor antagonist BOL could block the retardation of CR acquisition produced by mianserin. As is illustrated in Figure 6, the large retardation of learning produced by mianserin was completely reversed by the neutral antagonist BOL. Thus, the retardation of associative learning produced by mianserin was due to an action at the 5-HT_{2A} receptor.

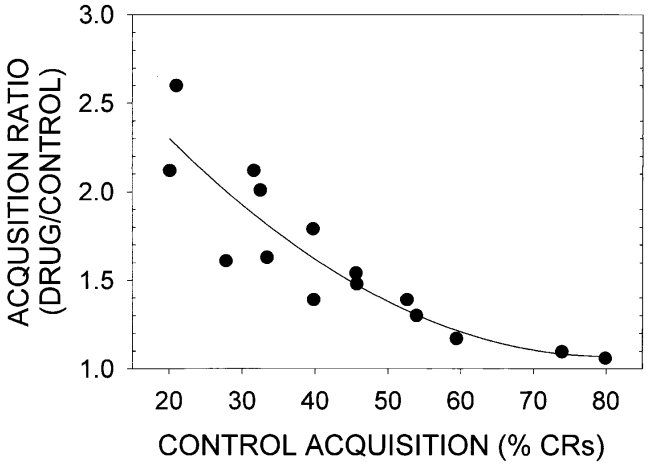

Figure 3 The relationship between the mean percentage of CRs generated by various NM conditioning procedures in control animals (abscissa) and the degree of enhancement of learning produced by LSD (13 µg/kg). The effects of LSD are expressed as an acquisition ratio, calculated as the mean percentage of CRs for animals injected with LSD divided by the mean percentage of CRs for their respective controls. Data were taken from Gimpl et al. (1979), Harvey et al. (1982, 1988), Schindler et al. (1985a,b), and Welsh et al. (1998a,b).

Implications of Inverse Agonism for Associative Learning and Psychopathology

The existence of constitutive activity at a receptor not only allows for the occurrence of both agonist and inverse agonist actions of drugs, but also raises the possibility that alterations in constitutive activity produced by genetic and/or environmental events could have profound effects on behavior. There are recent data indicating that there is a decrease in 5-HT_{2A} receptor density (Aurora and Meltzer 1991) and in 5-HT_{2A} receptor mRNA expres-

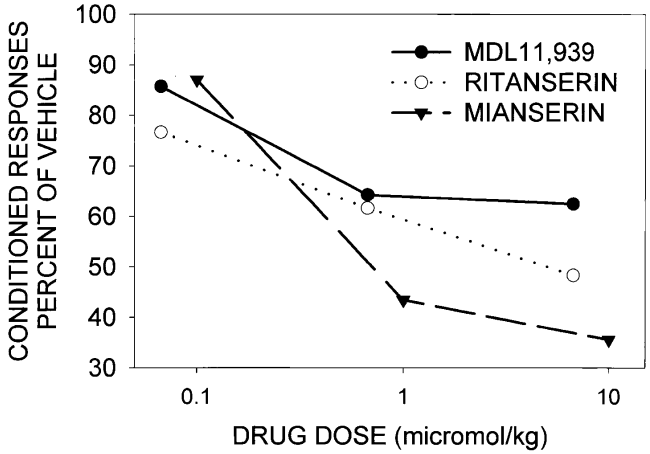

Figure 4 Retardant effect of MDL11,939, ritanserin, and mianserin on acquisition of NM response as a function of dose. Drugs were injected subcutaneously 1 h prior to each conditioning session. Conditioning used the pairing of a tone CS with an air puff US. Drug effects are expressed as the percentage of vehicle controls. Data are taken from Welsh et al. (1998b) and Romano et al. (2000).

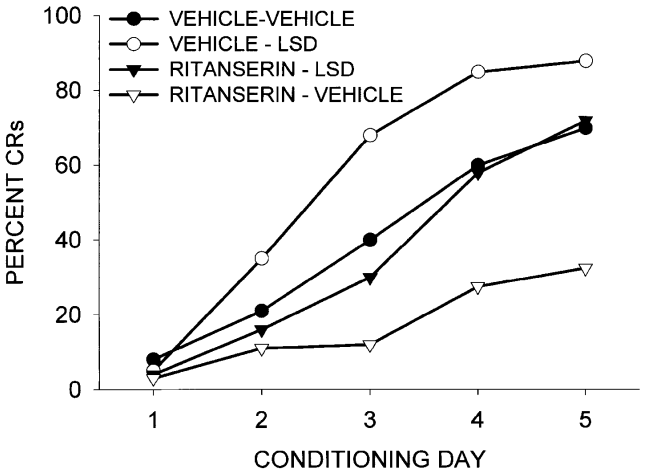

Figure 5 Antagonism by ritanserin (1 μmole/kg) of the enhancement of CR acquisition produced by LSD (0.030 μmole/kg). Ritanserin was injected subcutaneously 60 min prior and LSD intravenously 20 min prior to each acquisition session. Acquisition of the NM response was measured during the pairing of a tone CS and air puff US. Data are taken from Welsh et al. (1998a).

sion in frontal cortex of schizophrenic patients (Burnet et al. 1996; Hernandez and Sokolov 2000), and that neuroleptic treatment elevates the level of 5-HT_{2A} mRNA in schizophrenics to or above that of controls (Hernandez and Sokolov 2000). Additional studies have led to the conclusion that the antipsychotic actions of most drugs are due to their inverse agonist actions at serotonin 5-HT_{2A} receptors (Meltzer 1999; Weiner et al. 2001) due to the ability of inverse agonists to upregulate 5-HT_{2A} receptors in frontal cortex (Milligan and Bond 1997; Leurs et al. 1998; Hernandez and Sokolov 2000; Strange 2002). These proposals are supported by the finding that chronic administration of the inverse agonist MDL11,939 produces an up-regulation of 5-HT_{2A} receptors in the frontal cortex of the rabbit with no change in the density of 5-HT_{2C} receptors (Aloyo et al. 2001). Moreover, the up-regulation of the 5-HT_{2A} receptor was accompanied by increased responsiveness to the behavioral effects of 5-HT_{2A} receptor agonists (Aloyo et al. 2001). In conclusion, the data cited in this review indicate

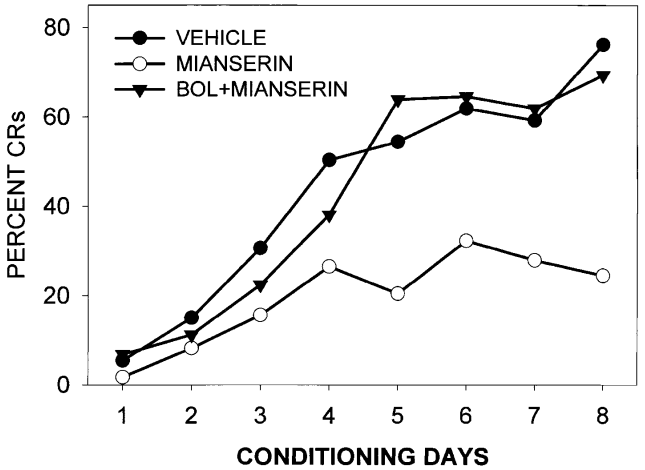

Figure 6 Antagonism by BOL (5.8 μmole/kg) of the retardant effects of mianserin (10 μmole/kg) on acquisition of the NM response. Mianserin was injected 1 h and BOL 20 min prior to each conditioning session by use of the pairing of a tone CS and air puff US. All injections were subcutaneous. Data are taken from Romano et al. (2000).

that the serotonin 5-HT_{2A} receptor is involved importantly in learning, and that alterations in this receptor can lead to abnormalities in cognitive functions in both humans and experimental animals.

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