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# MODULATION OF SPONTANEOUS HIPPOCAMPAL SYNAPTIC EVENTS WITH 5-HYDROXYINDOLE, 4OH GTS-21, AND rAAV-MEDIATED α7 NICOTINIC RECEPTOR GENE TRANSFER

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## Abstract

One approach to treatment of negative cognitive effects associated with Alzheimer's disease and schizophrenia may involve activation of neuronal α7 nicotinic acetylcholine receptors (nAChRs). We used the α7-selective partial agonist 3-(4-hydroxy, 2-methoxy-benzylidene)anabaseine (4OH-GTS-21), the α7 modulator 5-hydroxyindole (5-HI), and recombinant adeno-associated virus (rAAV)–mediated α7 gene transfer in order to test the hypothesis whether combining these strategies would significantly increase indirect measures of α7 nAChR function, including measures of spontaneous synaptic events in CA1 pyramidal cells. 5-HI (1mM), and 5-HI (1 mM) + 4OH-GTS-21 (5 μM) increased the frequency of APV- and NBQX- sensitive currents, while 5-HI + 4OH-GTS-21 increased the frequency and amplitude of bicuculline-sensitive currents. Effects on EPSCs were blocked with tetrodotoxin (TTX) (1 μM), but not by methyllycaconitine (MLA) (50 nM). Neither TTX nor MLA reduced the potentiation of IPSC frequencies. However, TTX blocked, and in some cases MLA reduced, the potentiation of IPSC amplitudes. These data suggest that effects of 5-HI + 4OH-GTS-21 on EPSC frequency were associated with action potential-dependent transmitter release produced by 5HI, and that potentiation of IPSC amplitudes resulted at least in part, from activation of α7 nAChRs. Finally, rAAV–mediated α7 gene transfer did not alter the magnitude of effects produced by 5-HI or 5-HI + 4OH-GTS-21. Thus, although we previously showed direct measures of α7 nAChR function were enhanced by α7 gene transfer, indirect measures of α7 nAChRs function were not significantly enhanced by combining α7 gene transfer with either agonist activation or positive allosteric modulation of α7 nAChRs.

### Keywords

alpha7; gene transfer; nicotinic; rAAV; hippocampus; 5 hydroxy indole; 5-HI; GTS-21; DMXB

## 1. INTRODUCTION

Nicotinic acetylcholine receptors (nAChRs) containing the α7 subunit are expressed at high levels in the hippocampus. Unlike the high affinity nicotine-binding receptors of the brain, they can be activated by both acetylcholine and its precursor choline, but they display rapid desensitization to high concentrations of agonist, and are selectively blocked by

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methyllycaconitine (MLA) and α-bungarotoxin. Responses mediated by α7 receptors can be readily detected by agonist applications to the cell bodies of hippocampal interneurons, and indirect measurements have suggested that they also function in the dendrites of hippocampal pyramidal cells (Ji and Dani, 2000). Disruption of α7 nAChR function has been implicated in Alzheimer's disease (AD) and schizophrenia (Court et al., 1999; Freedman et al., 1997; Guan et al., 2000), leading to their recognition as potentially important therapeutic targets for the treatment of these conditions. Because of their high permeability to calcium (Seguela et al., 1993), it is likely that α7 nAChRs modulate the release of many neurotransmitters at a variety of synapses. Activation of α7 nAChRs can modulate the release of GABA (Alkondon et al., 1997; Wanaverbecq et al., 2007), glutamate (Gray et al., 1996; McGehee et al., 1995), dopamine (Schilstrom et al., 1998), and noradrenaline (Li et al., 1998). Also, it has been shown that α7 nAChRs regulate the excitability of CA1 pyramidal cells (Dani, 2000) and dentate granule cells (Frazier et al., 2003).

Several strategies are available for increasing α7 nAChR function including agonist activation, allosteric modulation, and α7 nAChR gene delivery. Agonist activation of α7 nAChRs has been demonstrated with the compound 3-(2,4 dimethoxybenzylidene)anabaseine (GTS-21 or DMXB), a selective partial agonist for rat α7 nAChRs. GTS-21 improves memory-related behaviors in aged rats (Arendash et al., 1995), aged rabbits (Woodruff-Pak et al., 1994), nonhuman primates (Briggs et al., 1997), and nucleus-basalis lesioned rats (Meyer et al., 1998a). Although GTS-21 has a lower efficacy for human α7 nAChRs than for rat α7 nAChR, its primary human metabolite, 3-(4-hydroxy, 2-methoxy-benzylidene)anabaseine (4OH-GTS-21) exhibits greater efficacy than GTS-21 for both rat and human α7 nAChRs, and compared to GTS-21, produces a better equilibrium between activation and agonist-dependent inhibition and/or desensitization (Meyer et al., 1998b). Consequently, 4OH-GTS-21 may cause more receptor activation over extended intervals than other agonists which may induce greater inhibition. Also, like GTS-21, 4OH-GTS-21 produces positive cognitive and cytoprotective effects in rats (Ren et al., 2007a) and 4OH-GTS-21 has been shown to be able to increase spontaneous firing rates in α7-expressing neurons (Uteshev et al., 2003).

Agonist activation of α7 receptors may be complicated in the treatment of AD by several factors. For example, there are reductions in hippocampal cholinergic innervation (Mesulam, 2004; Mufson et al., 2003), and α7 receptor function is reduced in animal models of cholinergic hypofunction (Thinschmidt et al., 2005). Also, there is an increase in astrocytic neurotransmitter receptor expression in AD (Teaktong et al., 2003). Thus, it may be practical to pursue alternative treatments such as gene therapy for AD. Accordingly, we recently reported (Ren et al., 2007b) a technique to elevate α7 nAChRs in a neuron-selective manner using recombinant adeno-associated virus (rAAV)-mediated delivery of α7 nAChR transgene directly into the hippocampus. We found this resulted in an increase in [$^3$H]MLA binding in wild-type and α7-receptor knockout (KO) mice, functional α7 receptors in α7 KO mice, substantial increases in the magnitude of ACh-evoked currents in stratum radiatum interneurons, and improved acquisition performance in the Morris water task (Ren et al., 2007b).

Known positive modulators of α7 nAChR include ivermectin (Krause et al., 1998), galantamine (Santos et al., 2002), PNU-120596 (Hurst et al., 2005), TQS (4,5,9b-tetrahydro-3-H-cyclopenta [c] quinoline-8-sulfonic acid amide) (Gronlien et al., 2007), and 5-hydroxyindole (5-HI) (Gurley et al., 2000; Zwart et al., 2002). However, 5-HI also produces a number of other physiological effects. For example, in the hippocampus 5-HI increases glutamate release, the amplitude of population spikes, and the amplitude of evoked excitatory and inhibitory postsynaptic potentials (Mannaioni et al., 2003). Also, 5-HI increases the frequency and amplitude of spontaneous GABAergic inhibitory postsynaptic currents (IPSCs) (Mannaioni et al., 2003; Mok and Kew, 2006). Thus, 5-HI is an effective positive allosteric modulator of α7 nAChRs that facilitates the output of both excitatory and inhibitory neurotransmission, although it is unclear from previous studies if all of the effects reported for 5-HI arise strictly from the positive modulation of α7 nAChRs.

In the present report we combined 4OH-GTS-21 treatment, expected to produce direct activation of α7 nAChRs, with allosteric modulation of α7 nAChRs using 5-HI, and rAAV–mediated α7 nAChR gene transfer in order to test the hypothesis that combining these strategies would produce increases in several potential sequella of increased α7 nAChR function, specifically the frequency and amplitude of spontaneous synaptic events in hippocampal CA1 pyramidal cells. We also tested the hypothesis that increasing the function and number of α7 nAChRs through α7 gene transfer would increase the magnitude of effects produced by bath application of 5-HI, 4OH-GTS-21, and 5-HI + 4OH-GTS-21. Additionally, we compared the frequency and amplitude of spontaneous synaptic events in stratum radiatum interneurons from normal animals and animals that received α7 gene transfer.

## 2. RESULTS

### Pharmacological identification of spontaneous synaptic currents in CA1 pyramidal cells

In order to determine the pharmacological identity of spontaneous synaptic currents in CA1 pyramidal cells under our experimental conditions, we measured the effects of bath application of μM NBQX & 40 μM 2-amino-5-phosphonopentanoic acid (APV) on inward currents recorded at −60 mV (putative excitatory postsynaptic currents (EPSCs)) using a $K^+$ based internal solution (Figure 1A). The effects of 30 μM bicuculline methiodide on outward currents were recorded at 0 mV (putative inhibitory postsynaptic currents (IPSCs)) using a Cs-based internal solution (Figure 1B). The spontaneous inward currents recorded under basal conditions were completely eliminated following bath application of NBQX and APV, confirming that these currents were EPSCs mediated by AMPA and or NMDA receptors. Likewise, the putative spontaneous IPSCs recorded under basal conditions were completely eliminated following bath application of bicuculline methiodide, indicating these currents were mediated by GABA-A receptors.

### Drug effects on spontaneous EPSCs in CA1 pyramidal cells from normal animals

5-HI (1 mM) and 5-HI (1 mM) + 4OH-GTS-21 (5 μM) produced a significant increase in the frequency of EPSCs (Figure 2A, Figure 3A, Table 1). In contrast, when applied alone, 4OH-GTS-21 (5 μM) had no significant effects on the frequency or amplitude of spontaneous EPSCs (Figure 2B, Table 1). The increased frequency produced by 5-HI + 4OH-GTS-21 was reduced by application of tetrodotoxin (TTX) (1 μM) (Figure 2A). There was no effect of 5-HI + 4OH–GTS-21 on the amplitude of EPSCs (Figure 2B, Table 1), but EPSC amplitudes were significantly ($p < 0.01$) reduced when application of 5-HI + 4OH-GTS-21 was followed by TTX (Figure 2B, Table 1).

### Drug effects on spontaneous EPSCs in CA1 pyramidal cells from animals that received α7 gene transfer

As seen in cells from normal animals, 5-HI (1 mM) and 5-HI (1 mM) + 4OH–GTS-21 (5 μM) both increased the frequency of EPSCs (Figure 4A, Table 1) following α7 gene transfer. The effects produced by 5-HI were not reversed with bath application of methyllycaconitine (MLA) 50 nM, as shown in Figure 4A and Table 1. When applied alone, 4OH-GTS-21 produced no significant effect on the frequency of EPSCs (Figure 4A, Table 1), and there was no significant effect of 5-HI, 4OH-GTS-21, 5-HI + 4OH-GTS-21, or 5-HI + MLA on the amplitudes of spontaneous EPSCs (Figure 4B, Table 1).

### Drug effects on spontaneous IPSCs in CA1 pyramidal cells from normal animals

When applied alone, 4OH–GTS-21(5 μM) produced no significant change in the frequency or amplitude of IPSCs (Figure 5A,B, Table 2). In contrast, 5-HI (1 mM) + 4OH–GTS-21 (5 μM) increased the frequency and amplitude of IPSCs (Figure 5A-B, Table 2). TTX (1 μM) and MLA blocked the increase in amplitude (Figure 5B) but did not affect the increase in frequency (Figure 5A). Although in the presence of 5-HI + 4OH–GTS-21, the mean amplitude of IPSCs was 45.2 ± 7.9 pA, it should be noted that in some cases very large outward currents were recorded. These currents measured in excess of 1 nA but nevertheless were blocked with picrotoxin and bicuculline methiodide (data not shown). The increase in the frequency of IPSCs was not blocked by either TTX or MLA (Figure 5A).

### Drug effects on spontaneous IPSCs in CA1 pyramidal cells from animals that received α7 gene transfer

5-HI + 4OH–GTS-21 produced a significant increase in the amplitude and frequency of IPSCs (Figure 6A,B, Figure 3B, Table 2). These effects were not reversed with bath application of MLA (50 nM) (Figure 6A,B). In contrast, when applied alone, 4OH–GTS-21(5 μM) produced no change in the frequency or amplitude of IPSCs (Figure 6A,B, Table 2).

### Comparison of drug effects on spontaneous synaptic events in CA1 pyramidal cells from normal animals and animals that received α7 gene transfer

The magnitude of increases in frequency and amplitude produced by bath application of 5-HI and 5-HI + 4OH-GTS-21 reported above were not significantly different in cells from animals that received α7 gene transfer compared to cells from normal animals. Also, we found no significant difference in the baseline frequency or amplitude of EPSCs or IPSCs following α7 gene transfer, and no effects of MLA when applied alone (data not shown).

### Comparison of the frequency and amplitude of spontaneous synaptic events in stratum radiatum interneurons from normal animals and animals that received α7 gene transfer

There was no significant difference in the frequency or amplitude of EPSCs or IPSCs in stratum radiatum interneurons following α7 gene transfer (data not shown). In stratum radiatum interneurons with α7 gene transfer (n = 8) the frequency of EPSCs was 1.8 ± 0.9 Hz, and in control cells (n = 9) it was 1.3 ± 0.4 Hz . The amplitude of spontaneous EPSCs in GFP+ stratum radiatum interneurons following α7 gene transfer was 15.0 ± 1.4 pA, and in control cells it was 13.2 ± 1.0 pA. The frequency of IPSCs in stratum radiatum interneurons with α7 gene transfer (n = 9) was 12.4 ± 1.1 Hz, and in control cells (n = 9) it was 13.4 ± 2.2 Hz. The amplitude of IPSCs was 24.7 ± 2.5 pA in control cells and 21.0 ± 2.7 pA in stratum radiatum interneurons with α7 gene transfer.

## 3. DISCUSSION

In the present report, 5-HI and 5-HI + 4OH-GTS-21 significantly increased the frequency of spontaneous APV- and NBQX- sensitive EPSCs. Also, 5-HI + 4OH-GTS-21 significantly increased the frequency and amplitude of spontaneous bicuculline-sensitive IPSCs, although 4OH-GTS-21 produced no significant effect when applied alone. The 5HI + 4OH-GTS-21 induced increase in EPSC frequency was blocked by TTX, but 5-HI induced effects were not blocked by application of MLA. In contrast, the 5-HI + 4OH-GTS-21 increase in IPSC frequency was not blocked or reduced by application of TTX or MLA, but 5-HI + 4OH-GTS-21 mediated increases in IPSC amplitudes were blocked by TTX and reduced by MLA in normal animals. In cells from α7 gene transfer animals, however, MLA was not effective in reducing increases in IPSC amplitudes. Finally, α7 gene transfer did not alter the magnitude of effects produced by 5-HI or 5-HI + 4OH-GTS-21, and did not affect the baseline frequency or amplitude of synaptic events in stratum radiatum interneurons. Thus, indirect measures of α7 nAChR function were not significantly enhanced by combining α7 gene transfer with either agonist activation or positive allosteric modulation of α7 nAChRs.

When applied alone, or combined with 4OH-GTS-21, 5-HI increased the frequency of EPSCs. Because there was no frequency or amplitude increase produced with the selective α7 agonist 4OH-GTS-21, and no significant difference between the magnitude of increases produced by 5-HI when applied alone and with 4OH-GTS-21 (5-HI + 4OH-GTS-21), it appears the effects of 5-HI + 4OH-GTS-21 resulted from the actions of 5-HI alone. Since the 5HI + 4OH-GTS-21 mediated increase in EPSC frequency was blocked with TTX but unchanged by MLA, our data suggest these effects were not mediated by direct activation of α7 nAChRs, but rather by increases in action potential-dependent transmitter release. This is consistent with studies that have shown 5-HI mediated increases in glutamate release, the amplitude of population spikes, and the amplitude of evoked excitatory postsynaptic potentials in the hippocampus (Mannaioni et al., 2003). However, to our knowledge, this is the first report showing that 5-HI potentiates the frequency of spontaneous EPSCs in CA1 pyramidal cells.

Our results are also consistent with studies showing that 5-HI increases the frequency and amplitude of spontaneous GABAergic inhibitory postsynaptic currents (IPSCs) (Mannaioni et al., 2003; Mok and Kew, 2006). Because TTX did not reduce the 5-HI + 4OH-GTS-21 mediated frequency increase in IPSCs (Figure 5A), our results suggest that 5-HI + 4OH-GTS-21 may have increased IPSC frequencies through mechanisms not related to action potential-dependent transmitter release, perhaps ones related to cell excitability, even though we measured no significant changes in holding currents measured at the cell body. Because TTX did not reduce the 5-HI + 4OH-GTS-21 mediated frequency increase in IPSCs (Figure 5A), increased IPSC frequencies in response to 5-HI + 4OH-GTS-21 are not related to action potential-dependent transmitter release, and the absence of significant changes in holding currents measured at the cell body (data not shown) make it unlikely that cell excitability was altered. Further, because TTX blocked, and MLA reduced the 5HI + 4OH-GTS-21 mediated increase in IPSC amplitudes (Figure 5B), this effect likely involves both activation of α7 nAChRs and increases in action potential-dependent GABA release. Curiously however, MLA did not significantly reduce the 5HI + 4OH-GTS-21 potentiation of IPCS amplitudes in cells from animals that received α7 gene transfer. Any number of functional changes or potential changes involving synaptic architecture resulting from gene transfer could have produced this difference.

With the exception of a reduction in the potentiation of IPSC amplitudes mentioned above, none of the effects of 5-HI or 5-HI + 4OH-GTS-21 were reversed or completely blocked by MLA. In this regard, our findings are consistent with Mannaioni et al. 2003, who showed that 5-HI potentiation of IPSCs in CA1 pyramidal cells was not sensitive to preincubation with MLA. In contrast, Mok and Kew 2006 showed that 5-HI potentiation of IPSCs in stratum radiatum interneurons was occluded by pretreatment with MLA, TTX, and nicotine (hypothesized to desensitize receptors). Taken together, our results and those of Mannaioni et al. 2003 and Mok and Kew 2006 demonstrate 5-HI facilitates both excitatory and inhibitory neurotransmission. Further, they show that while the 5-HI mediated potentiation of IPSCs in stratum radiatum interneurons can be mediated by activation of α7 nAChRs, IPSC potentiation in CA1 pyramidal cells is probably mediated by both an increase in action potential-dependent transmitter release and activation of α7 nAChRs.

Because the activation of α7 nAChRs has been shown to enhance transmitter release at various synapses (Gray et al., 1996; MacDermott et al., 1999; McGehee et al., 1995; Seguela et al., 1993), we tested the hypothesis that α7 gene transfer would change the frequency and/or amplitude of spontaneous IPSCs and EPSCs. Further, we tested the hypothesis that the magnitude of effects produced by 5-HI and 4OH-GTS-21 would be greater following α7 nAChR gene transfer. Although we found no change in the baseline frequency and amplitude of EPSCs and IPSCs in CA1 pyramidal cells and stratum radiatum interneurons following α7 nAChR gene transfer, the coupling of α7 nAChRs at various synapses is well established. For example, it has been shown that nAChRs occur presynaptically and postsynaptically at GABAergic synapses (Fabian-Fine et al., 2001) and more recently that α7 nAChR activation inhibits evoked GABAa receptor-mediated currents and increases the frequency of spontaneous miniature IPSCs in hippocampal interneurons (Wanaverbecq et al., 2007). Accordingly, it seems possible that changes in evoked IPSCs or miniature IPSCs could have occurred following α7 gene transfer but were not resolved in the current study. Further, because studies implementing ultrastructural resolution have shown that α7 nAChRs tend to occur away from sites of ACh release, ACh may provide a more diffuse type of hippocampal input (Descarries et al., 2004), making certain effects produced by α7 gene transfer undetectable within the present methodologies.

We hypothesized that a combination of an α7 selective agonist, allosteric modulator, and α7 gene transfer would be ideal for inducing maximum activation of α7 nAChRs in the hippocampal slice. However, our data suggest that although combining these methods may have amplified certain physiological functions of α7 nAChRs, we were unable to elucidate a role for hippocampal α7 nAChRs in excitatory and inhibitory synaptic transmission with this combination of treatments. Moreover, no effects on synaptic transmission could be resolved with the α7 selective agonist 4OH-GTS-21 at the concentration tested, although this was within a concentration range shown to be efficacious with *in vitro* measures of GTS-21 mediated cytoprotection (Ren et al., 2005). Furthermore, the α7 allosteric modulator 5-HI appeared to have affected synaptic transmission primarily via non-α7- related mechanisms. Interestingly, consistent with this finding, more recent results from our laboratory show that 5-HI does not increase whole-cell currents produced by focal somatic application of 4OH-GTS-21 in stratum radiatum interneurons, although other positive modulators can increase 4OH-GTS-21 evoked responses and 5-HI can increase α7 responses to other partial agonists (Lopez et al., 2007 unpublished data). These findings suggest a complex interaction between partial agonists and positive modulators of α7 nAChRs that should be considered in future studies seeking experimental effects by combining these compounds. Finally, α7 gene transfer produced no significant effects on spontaneous synaptic events, although we previously showed direct measures of α7 function (e.g. the magnitude of ACh evoked currents) are in fact significantly affected by α7 gene transfer (Ren et al., 2007b). Thus, establishing a definitive role for the physiological function of α7 nAChRs in hippocampal synaptic transmission remains elusive but may be accomplished in future studies investigating other mechanisms or substrates.

## 4. EXPERIMENTAL PROCEDURES

### rAAV8 preparation

The recombinant adeno-associated virus serotype 8 (rAAV8) hybrid vector was used for transduction of the hippocampus because of its ability to achieve gene transfer within large tissue volumes (Klein et al., 2006). The rAAV8 vector was prepared and quantified using the methods of Zolotukhin et al. (1999), Klein et al. (2006), and Ren et al. (2007). Expression of green fluorescent protein (GFP) or rat α7 nAChRs was driven by a chicken α-actin promoter containing the human cytomegalovirus enhancer. Plasmids were propagated in SURE cells (Stratagene, LaJolla, CA, USA) and CsCl-purified. Briefly, 70% confluent human embryonic kidney 293 cells were transfected by the calcium phosphate method with AAV terminal repeat-containing GFP or rat α7 nicotinic receptor plasmid in equal molar ratios with the rAAV8 helper plasmid. After 3 days, cells and media were harvested and centrifuged at 3000×*g*. The pellets were resuspended in a solution of 50 mM Tris, pH 8.3 and 150 mM NaCl, then freeze-thawed three times. The resulting suspension was put through a discontinuous iodixanol gradient followed by a Q-sepharose column (Sigma Chemicals, St. Louis, MO, USA) to purify rAAV8. Vector doses were expressed as genomic particles (gp).

## Hippocampal injections of rAAV8-rat α7 and rAAV8-GFP

All procedures involving animals were approved by the University of Florida Institutional Animal Care and Use Committee and were in accord with the NIH *Guide for the Care and Use of Laboratory Animals*. Male Sprague Dawley rats were maintained on a 12hr day night light cycle and had food and water available ad libitum until used in experiments. At 18–22 days of age they were placed in an isoflurane containing chamber for 5 minutes prior to mounting on a stereotaxic frame. Lack of withdrawal reflex indicated readiness for making the initial incision, which followed the midline suture from just rostral to bregma, continuing back to lambda. A small hole (0.5 mm diameter) was drilled 3.6 mm caudal to bregma and 2.2 mm lateral to the midline. A 27-gauge needle attached to a 10 μl Hamilton syringe was lowered into the hippocampus and stopped at a depth 2.6–3.0 mm ventral to the skull surface. Equal volumes of rAAV8-rat α7 and rAAV8-GFP vectors [2–3 μl total volume ($10^{10}$ gp)] were delivered at 0.2 μl/min using a CMA/100 microinjection pump (CMA/Microdialysis, Solna, Sweden). The incisions were closed using surgical nylon and treated with Betadine. Animals recovered from anesthesia under a heat lamp and were monitored for signs of pain. Additional analgesic injections were administered as necessary. Animals were kept for 7–10 days in standard housing prior to use for electrophysiological experiments.

## Patch-clamping in hippocampal slices

Animals were anesthetized with halothane (Halocarbon Laboratories, River Edge, NJ) and swiftly decapitated. Transverse (300 μm) whole brain slices were prepared using a vibratome (Pelco, Redding, CA) and a high $Mg^+$/low $Ca^{2+}$ ice-cold artificial cerebral spinal fluid (ACSF) containing (in mM) 124 NaCl, 2.5 KCL, 1.2 $NaH_2PO_4$, 2.5 $MgSO_4$, 10 D-glucose, 1 $CaCl_2$, and 25.9 $NaHCO_3$ saturated with 95% $O_2$-5%$CO_2$. Prior to sectioning, a single-edge razor was used to make a longitudinal cut that separated the two hemispheres which allowed for keeping the injected and un-injected sides separate. Slices were incubated at 30°C for 30 minutes and remained at room temperature until they were transferred to a submersion chamber (Warner Instruments, Hamden, CT) for recording. Slices were perfused (2 ml/min) with normal ACSF containing (in mM) 126 NaCl, 3 KCL, 1.2 $NaH_2PO_4$, 1.5 $MgSO_4$, 11 D-glucose, 2.4 $CaCl_2$, 25.9 $NaHCO_3$, and 0.004–0.008 atropine sulfate saturated with 95% $O_2$-5%$CO_2$ at 30°C. Cells were visualized with infrared differential interference contrast (IR DIC) using a Nikon E600FN microscope that was equipped with fluorescence for viewing GFP in injected cells. In addition to confirmation of fluorescence, functional expression was confirmed in some cells using somatic application of ACh (1mM) which in all cases produced evoked-responses (data not shown). Whole cell patch-clamp recordings were made with glass pipettes (3–5 MΩ) containing an internal solution of (in mM) 125 K-gluconate, 1 KCL, 0.1 $CaCl_2$, 2 $MgCl_2$, 1 EGTA, 2 MgATP, 0.3 $Na_3$GTP, and 10 HEPES (pH 7.3 using KOH). To record inhibitory post synaptic currents (IPSCs), cells were held at 0 mV using an internal solution of (in mM): 140 $CsMeS0_3$, 8 NaCL, 1 $MgCl_2$, 0.2 EGTA, 10 HEPES, 2 Mg-ATP, 0.3 Na-GTP, and 5 QX-314 (pH 7.3 using CsOH). Prior to data collection, all cells were held at –70 mV, and -10 mV / 10 ms test pulses were used to determine series resistance (Ra), input resistance (Rm) and whole-cell capacitance (Cm). Cells with series resistances > 60 MΩ or those requiring holding currents > 250pA were not included in the final analyses

## Drug application and data analyses

Bath applications of all drugs were achieved by introducing them into the ACSF using a syringe pump (Kd Scientific, Holliston, MA) loaded with a concentrated stock solution diluted to the final concentration in the perfusion line prior to entering the recording chamber. Baseline

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recordings of spontaneous synaptic events were made for a period of 2 min. Each drug was washed in for 6 min, and the following 2 min of recording were used to measure drug effects. Drugs were applied sequentially or together as indicated in the text. Since the effects of 1mM 5-HI combined with 5 μM 4OH-GTS-21 (5-HI + 4OH-GTS-21) were not significantly different if the drugs were applied sequentially or together, in some cases data from each type of application were pooled together. 4OH-GTS-21 was synthesized and provided by Taiho Pharmaceuticals (Tokushima, Japan) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f] quinoxaline-2,3-dione (NBQX) was purchased from Tocris Cookson (Ellisville, MO). All other chemicals were obtained from Sigma (St. Louis, MO).

Signals were digitized using an Axon Digidata 1322A and sampled at 20 kHz on a Dell computer using Clampex version 8 or 9. Data analysis was done with Clampfit version 8 or 9, Excel 2000, and GraphPad/Prism version 3.02. Spontaneous synaptic events were analyzed using Clampfit event detection (threshold search in manual mode). Statistical analyses used paired and unpaired one-tailed Student's t-tests where appropriate. Data are expressed as mean ± SEM.

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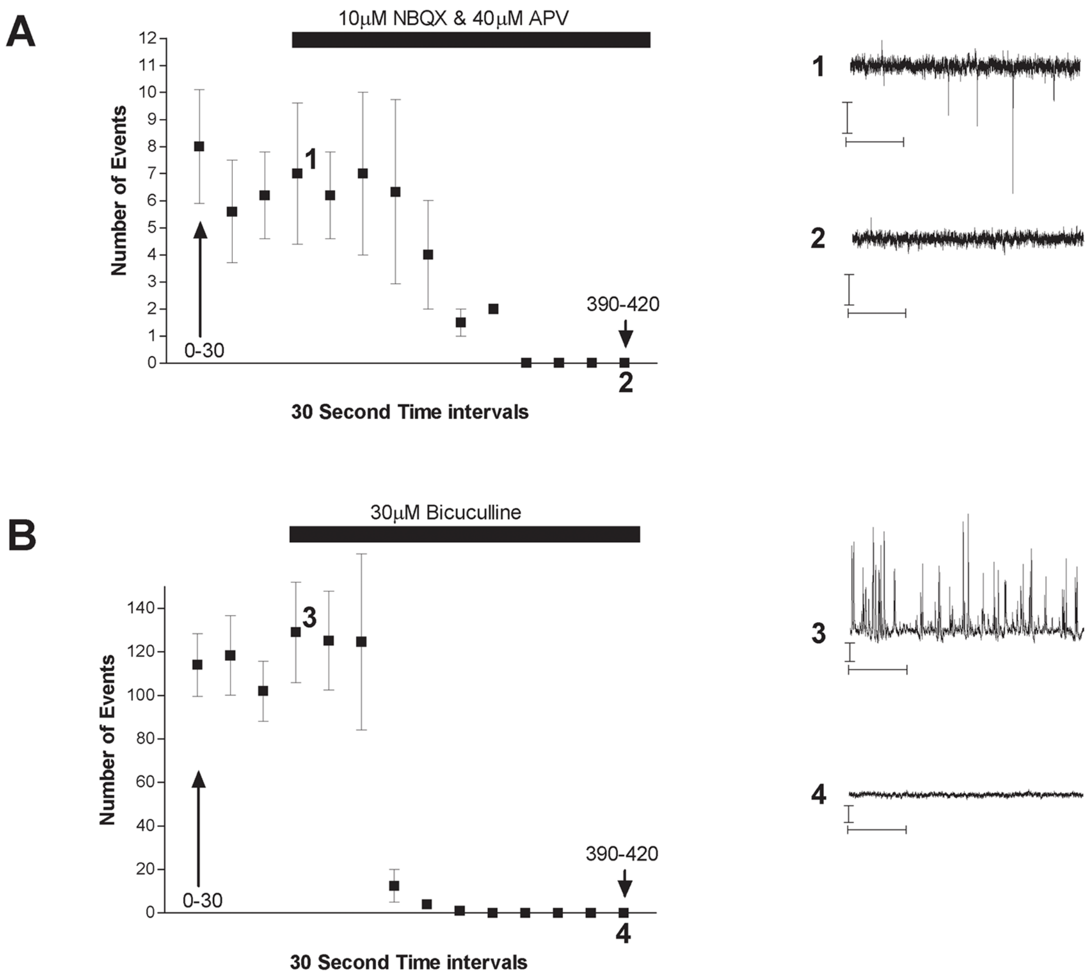


**FIGURE 1.**

Excitatory spontaneous synaptic currents in CA1 pyramidal cells at –60mV and inhibitory synaptic currents at 0mV are blocked by NBQX + APV and bicuculline respectively. The scatter plots in A and B were derived from averaging the number of events in 30s intervals (±SEM). Baseline rates were recorded for 2 min (120s) before bath application of the specified antagonist (thick black bars). A: Scatter plot (left) showing group means ±SEM (n = 5) and recordings from individual cells before (1) and following bath application of 10mM NBQX and 40mM APV (2) (note the absence of events shown in 2). B: Scatter plot (left) showing group means ±SEM (n = 5) and recordings from individual cells before (3) and following bath application of 30mM bicuculline (4) (note the absence of events shown in 4). Scale bars: (1 & 2: 10pA and 2.5 s) (3 & 4: 20pA and 2.5 s.). Any apparent differences in time course of the bath applied drugs are likely to reflect the relative potencies of the specific agents and their ability to penetrate the tissue.

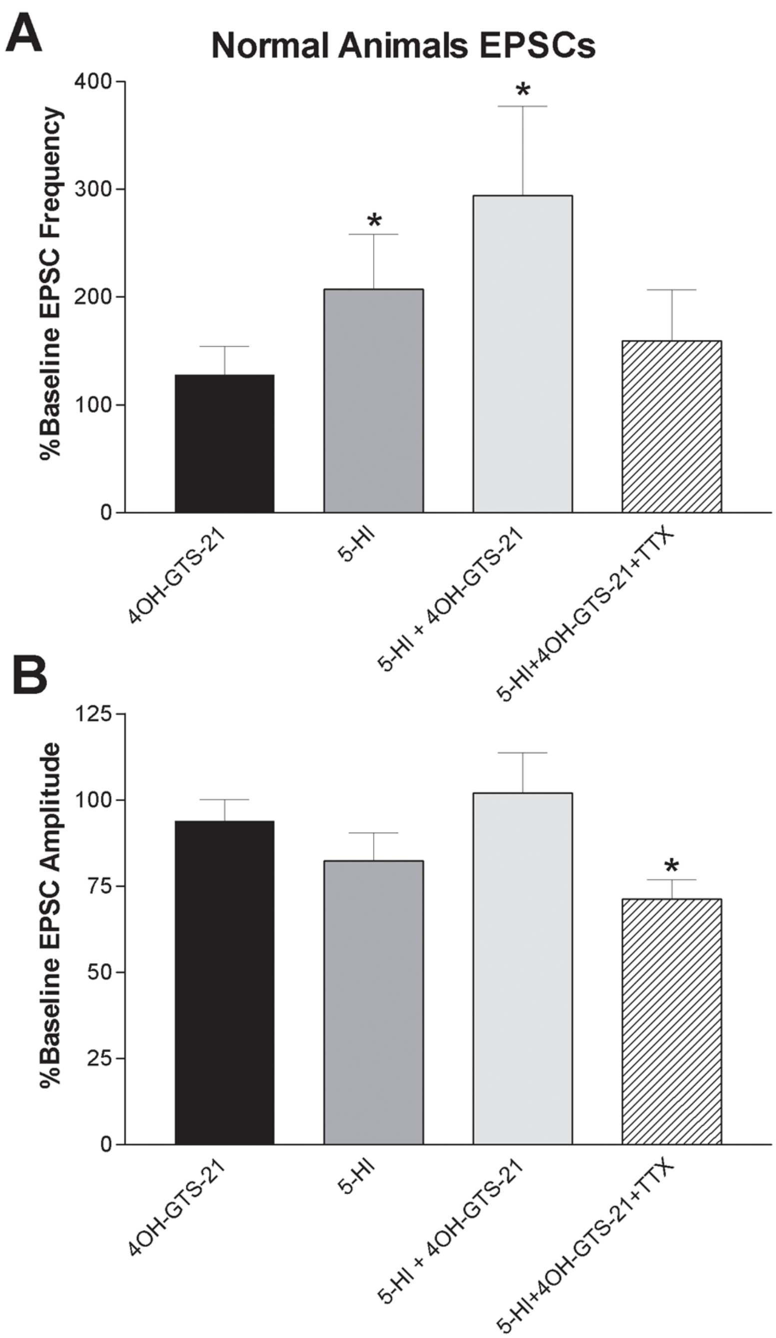


**FIGURE 2.**

Group data showing the effects of 4OH-GTS-21 (black bar), 5-HI (dark gray bar), 5-HI + 4OH-GTS-21 (light gray bar), and 5-HI + 4OH-GTS-21 + TTX (hatched bar) on the frequency (**A**) and amplitude (**B**) of spontaneous EPSCs in hippocampal CA1 pyramidal cells from normal animals. * indicates statistically significant changes from baseline amplitude and/or frequency where $p < 0.05$.

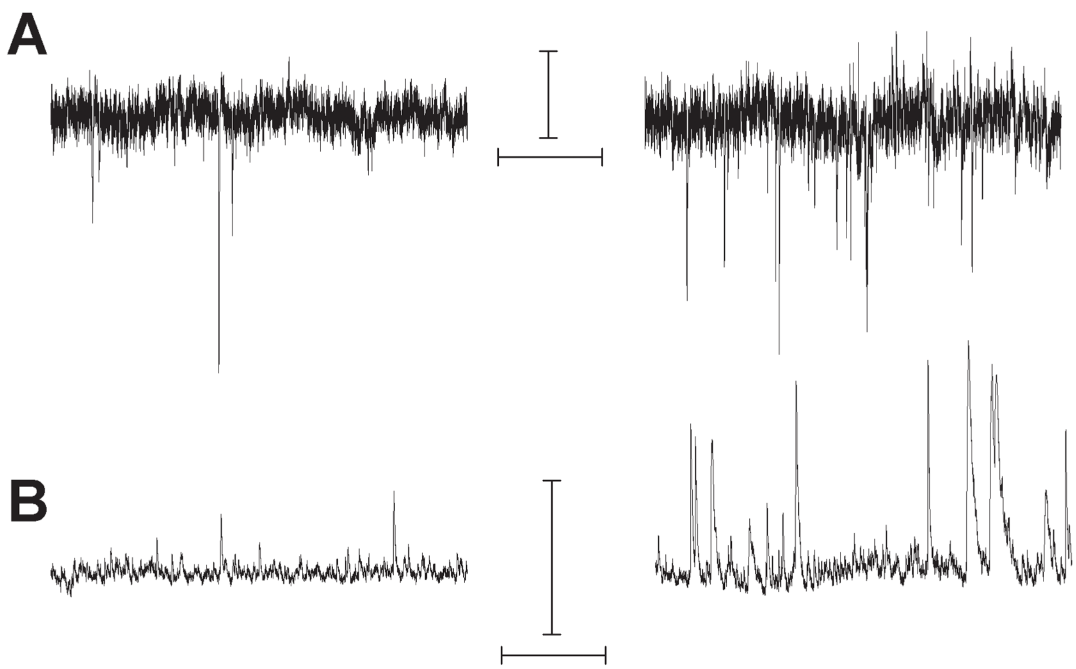


**FIGURE 3.**
Examples of the modulation of spontaneous synaptic events by 5-HI + 4OH-GTS-21, which produced an increase in the frequency of spontaneous EPSCs in hippocampal pyramidal cells (**A**) and an increase the frequency and amplitude of spontaneous IPSCs in hippocampal pyramidal cells (**B**). Baseline synaptic events are shown on the left, and events in the presence of 5-HI + 4OH-GTS-21 are shown on the right. In A the record was taken from a normal animal, and in B from an animal that received alpha 7 gene transfer. Scale bars: A: (10 pA and 2.5 sec) B: (100 pA and 2.5 sec).

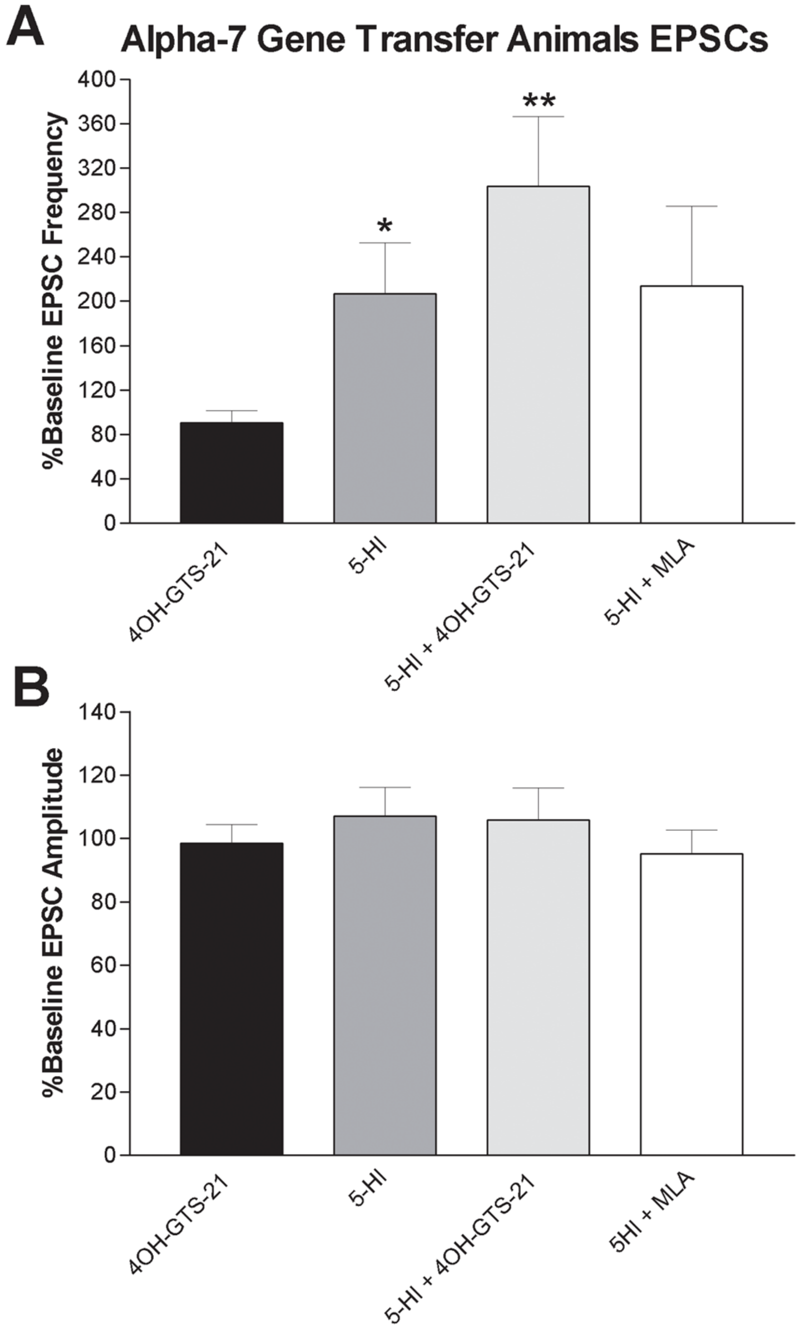


**FIGURE 4.**

Group data showing the effects of 4OH-GTS-21 (black bar), 5-HI (gray bar), 5-HI + 4OH-GTS-21 (light gray bar), and 5-HI + MLA (white bar) on the frequency (**A**) and amplitude (**B**) of spontaneous EPSCs in hippocampal CA1 pyramidal cells from animals that received alpha 7 gene transfer. .* indicates statistically significant changes from baseline amplitude and/or frequency where $p < 0.05$ and ** where $p < 0.01$.

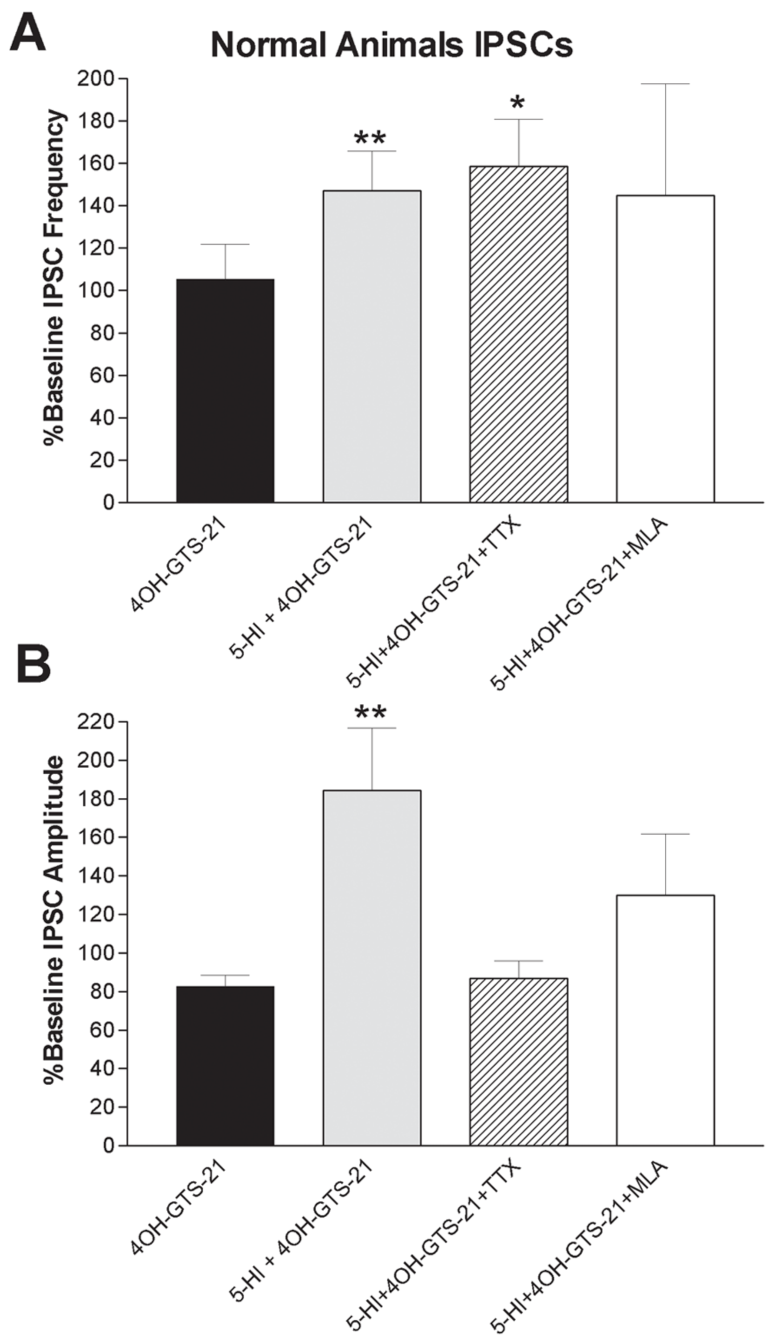


**FIGURE 5.**

Group data showing the effects of 4OH-GTS-21 (black bar), 5-HI + 4OH-GTS-21 (light gray bar), 5-HI + 4OH-GTS-21 + TTX (hatched bar), and 5-HI + 4OH-GTS-21 + MLA (white bar) on the frequency (**A**) and amplitude (**B**) of spontaneous IPSCs in hippocampal CA1 pyramidal cells from normal animals.* indicates statistically significant changes from baseline amplitude and/or frequency where $p < 0.05$ and ** where $p < 0.01$.

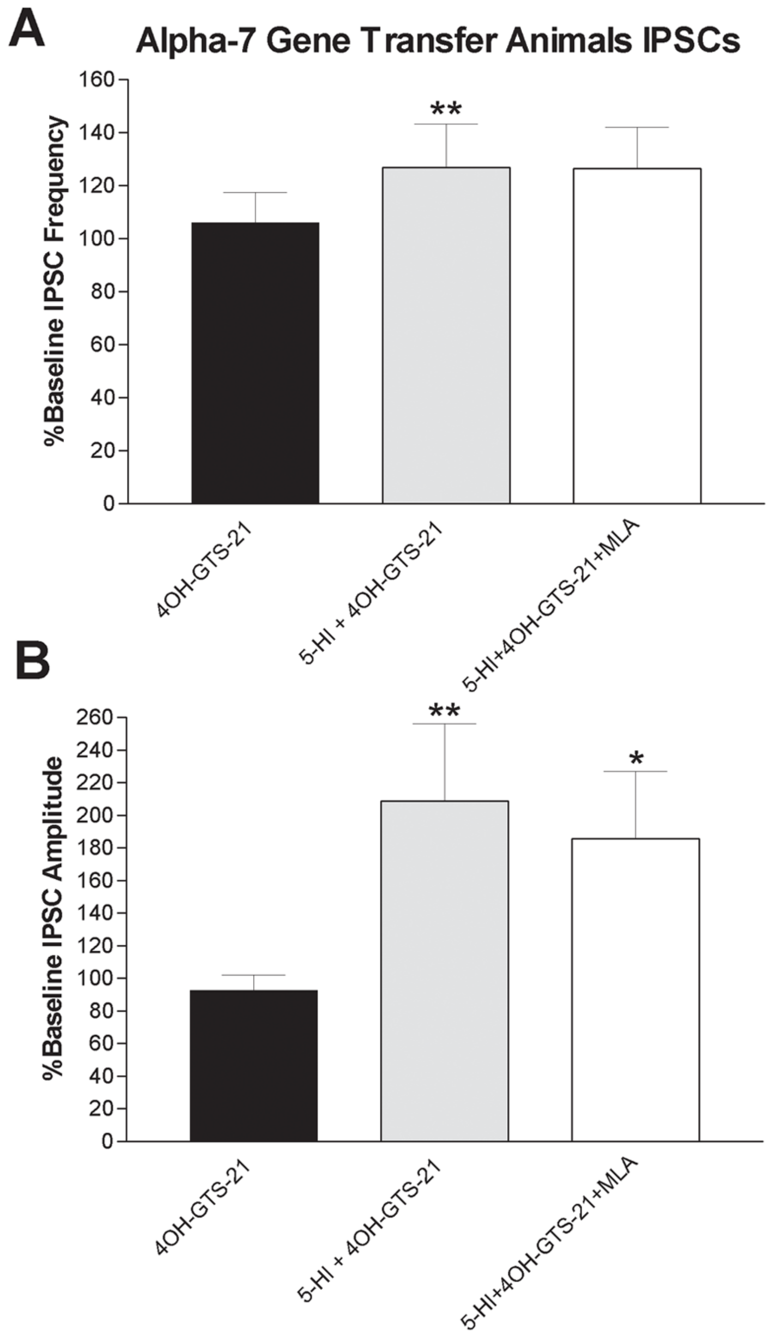


**FIGURE 6.**

Group data showing the effects of 4OH-GTS-21 (black bar), 5-HI + 4OH-GTS-21 (light gray bar), and 5-HI + 4OH-GTS-21 + MLA (hatched bar) on the frequency (**A**) and amplitude (**B**) of spontaneous IPSCs in hippocampal pyramidal cells from animals that received alpha 7 gene transfer. * indicates statistically significant changes from baseline amplitude and/or frequency where $p < 0.05$ and ** where $p < 0.01$.

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## TABLE 1

**(EPSCs)**

Raw data showing the frequencies and amplitudes of EPSCs ± SEM in CA1 pyramidal cells from normal animals and animals that received α7 gene transfer in baseline and drug treatment conditions.

| TREATMENT | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Normals** | **Baseline Hz** | **Treatment Hz** | **t-test** | **Baseline pA** | **Treatment pA** | **t-test** | **N** |
| 4OH-GTS-21 | 0.21 ± 0.04 | 0.27 ± 0.06 | - | 15.2 ± 0.7 | 14.2 ± 1.0 | - | 9 |
| 5-HI | 0.21 ± 0.03 | 0.44 ± 0.11 | * | 17.3 ± 1.7 | 14.2 ± 1.4 | - | 9 |
| 5-HI + 4OH-GTS-21 | 0.21 ± 0.04 | 0.62 ± 0.17 | * | 15.2 ± 0.7 | 15.5 ± 1.8 | - | 9 |
| 5-HI + 4OH-GTS-21 + TTX | 0.26 ± 0.07 | 0.41 ± 0.12 | - | 20.1 ± 1.8 | 14.3 ± 1.1 | * | 5 |
| **Gene Transfer** | | | | | | | |
| 4OH-GTS-21 | 0.31 ± 0.04 | 0.28 ± 0.03 | - | 16.9 ± 0.9 | 16.2 ± 1.0 | - | 22 |
| 5-HI | 0.48 ± 0.02 | 1.0 ± 0.22 | * | 17.4 ± 1.8 | 18.6 ± 1.6 | - | 8 |
| 5-HI + 4OH-GTS-21 | 0.26 ± 0.03 | 0.80 ± 0.16 | ** | 17.5 ± 1.2 | 18.6 ± 1.8 | - | 10 |
| 5-HI ± MLA | 0.39 ± 0.44 | 0.84 ± 0.28 | - | 16.6 ± 1.8 | 15.8 +1.3 | - | 4 |

* $p < 0.05$,

** $p < 0.01$.

## TABLE 2

**(IPSCs)**

Raw data showing the frequencies and amplitudes of IPSCs ± SEM in CA1 pyramidal cells from normal animals and animals that received α7 gene transfer in baseline and drug treatment conditions.

| TREATMENT | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Normals** | **Baseline Hz** | **Treatment Hz** | **t-test** | **Baseline pA** | **Treatment pA** | **t-test** | **N** |
| 4OH-GTS-21 | 1.8 ± 0.3 | 1.9 ± 0.3 | - | 33.9 ± 3.1 | 28 ± 1.9 | - | 8 |
| 5-HI + 4OH-GTS-21 | 2.2 ± 0.2 | 3.2 ± 0.4 | ** | 24.5 ± 2.5 | 45.2 ± 7.9 | ** | 13 |
| 5-HI + 4OH-GTS-21 + TTX | 2.8 ± 0.2 | 4.4 ± 0.6 | * | 23.2 ± 1.8 | 20.1 ± 2.1 | - | 5 |
| 5-HI + 4OH-GTS-21 + MLA | 1.5 ± 0.8 | 2.2 ± 0.8 | - | 38.6 ± 6.1 | 50.2 ± 12.4 | - | 3 |
| **Gene Transfer** | | | | | | | |
| 4OH-GTS-21 | 2.1 ± 0.2 | 2.2 ± 0.2 | - | 30.9 ± 2.8 | 23.6 ± 2.0 | - | 8 |
| 5-HI + 4OH-GTS-21 | 2.3 ± 0.22 | 2.9 ± 0.4 | ** | 25.4 ± 4.0 | 53.0 ± 12.1 | ** | 8 |
| 5-HI + 4OH-GTS-21 + MLA | 2.5 ± 0.4 | 3.2 ± 0.4 | - | 31.9 ± 6.7 | 59.3 +13.1 | * | 4 |

* $p < 0.05$,

** $p < 0.01$.