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Stimulation of 5-HT_{1A} receptors in the dorsal hippocampus and inhibition of limbic seizures induced by kainic acid in rats

Marco Gariboldi, [1]Piotr Tutka, Rosario Samanin & [2]Annamaria Vezzani

Laboratory of Neuropharmacology, Mario Negri Institute for Pharmacological Research, Via Eritrea 62, 20157 Milano, Italy

1 We studied whether the stimulation of 5-HT_{1A} receptors by 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a specific 5-HT_{1A} receptor agonist, reduced electroencephalographic (EEG) seizures induced by intrahippocampal injection of 0.04 μg in 0.5 μl of the glutamate analogue kainic acid in freely-moving rats.

2 Pretreatment with 8-OH-DPAT 15 min earlier at the same site as kainic acid injection, caused a dose-dependent decrease of kainic acid-induced seizure activity. One and 10 μg significantly reduced the total time spent in seizures by 72% on average and the total number of seizures by 58% ($P<0.01$) and 43% ($P<0.05$) respectively. The latency to onset of the first seizure was increased 2.8 times ($P<0.01$) only after 1 μg 8-OH-DPAT; 0.1 μg was ineffective on all seizure parameters.

3 Systemic administration of 25, 100 and 1000 μg kg^{-1} 8-OH-DPAT significantly reduced the total number of seizures and the total time in seizures induced by intrahippocampal kainic acid by 52% and 74% on average. The latency to onset of the first seizure was delayed 1.8 times by 100 and 1000 μg kg^{-1} ($P<0.05$).

4 The anticonvulsant action of 8-OH-DPAT given intrahippocampally or systemically was significantly blocked by 5 μg, but not 1 μg WAY 100635, a selective 5-HT_{1A} receptor antagonist, administered in the hippocampus before the agonist.

5 These results indicate that postsynaptic 5-HT_{1A} receptors in the hippocampus mediate the anticonvulsant action of 8-OH-DPAT and that their stimulation has an inhibitory role in the generation of limbic seizures.

Keywords: Anticonvulsants; EEG; hippocampus; 5-HT_{1A} receptors; 8-hydroxy-2-(di-n-propylamino) tetralin; kainic acid; limbic seizures; WAY 100635

Introduction

Experimental evidence has indicated that brain 5-hydroxytryptamine (5-HT) plays a role in various models of generalized seizures. Treatments aimed at raising the extracellular 5-HT concentration such as (i) electrical stimulation of the medial raphe nucleus (Kovacs & Zoll, 1974; Lazarova *et al.*, 1979), (ii) administration of 5-hydroxytryptophan (De La Torre *et al.*, 1970; Löscher & Czuczwar, 1985); (iii) 5-HT uptake blockers (Wada *et al.*, 1993b; Yan *et al.*, 1994) reduce the severity of generalized seizures and delay their onset after electroshock or pentylenetetrazol in rodents. Conversely, a diffuse deficit of forebrain 5-HT induced by chemical or electrolytic lesions enhanced susceptibility to generalized convulsions (Kovacs & Zoll, 1974; Lazarova *et al.*, 1979). Enhancement of 5-hydroxytryptaminergic neurotransmission has also been shown to have an anticonvulsant effect in genetically epilepsy-prone rats (Yan *et al.*, 1994) and it may at least in part mediate the antimyoclonic action of some antiepileptic agents (Whitton *et al.*, 1983; 1985).

Recent findings suggest that 5-HT may affect seizures originating in limbic areas. Thus, 5-HT uptake blockers such as fluoxetine and paroxetine reduced the duration and increased the threshold of afterdischarge in fully kindled rats or cats (Wada *et al.*, 1993a,b). These effects were mimicked by intrahippocampal infusion of the specific 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (Wada *et al.*, 1993c). Autoradiographic analysis of 5-HT receptors in fully kindled rat brain showed a selective increase in 5-HT_{1A} binding in the dentate gyrus (Clark *et al.*, 1993). These findings suggest that 5-HT_{1A} receptors may have an inhibitory role in the generation of hippocampal seizures.

However, whether this action applies to other types of experimental limbic convulsions has not been investigated. The high density of 5-HT_{1A} receptors in the hippocampus (Pompeiano *et al.*, 1992), together with the electrophysiological evidence that these receptors mediate the inhibitory effects of 5-HT there (Ropert, 1988; Baskys *et al.*, 1989; Beck & Choi, 1991; Ghadimi & Jarolimek, 1994), prompted us to study whether stimulation of 5-HT_{1A} receptors by 8-OH-DPAT reduced EEG seizure activity induced by intrahippocampal infusion of kainic acid. This acute model of seizures was chosen because of (i) its similarities with temporal lobe epilepsy in man (Ben-Ari, 1985), (ii) the pivotal role receptors of excitatory aminoacids in CNS play in various convulsive syndromes (Schwarcz & Meldrum, 1985) and (iii) the functional interactions between 5-hydroxytryptaminergic and glutamatergic neurotransmission in limbic areas (Corradetti *et al.*, 1992; Sizer *et al.*, 1992).

Methods

Male Sprague-Dawley rats (210-250 g, Charles River, Italy) were used. The animals were housed at constant temperature (23°C) and relative humidity (60%) with fixed 12 h light/dark cycles and free access to food and water. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, 18 Febbraio 1992) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, 12 December 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

Placement of cannulae and electrodes

Surgical procedures were as described elsewhere (Vezzani *et al.*, 1986). Briefly, two screw electrodes were placed bilaterally

[1]Pressent address: Department of Pharmacology, University of Lublin, Lublin, Poland.
[2]Author for correspondence.

over the parietal cortex, together with a ground lead positioned over the nasal sinus, and bipolar depth electrodes were placed bilaterally in the dorsal hippocampus under Equithesin anaesthesia (1% pentobarbitone/4% (vol/vol) chloral hydrate; 3.5 ml kg^{-1}, i.p.) An injection guide cannula (22 gauge) was positioned unilaterally on the top of the dura for intrahippocampal infusion of drugs. The coordinates from bregma for implantation of the electrode were (nose bar 2.5 mm below the interaural line): 3.5 mm posterior to bregma, 2.4 mm lateral to the midline and 2.9 mm below the dura. The electrodes were connected to a multipin socket (March Electronics, New York) and were secured to the skull, together with the guide cannula, by acrylic dental cement. The animals were allowed at least five days to recover from the surgical procedure before the start of the study.

EEG recordings and drug injections

The procedures for recording the EEG and intracerebral injection of drugs have been described previously (Vezzani *et al.*, 1986). Briefly, the animals were left to acclimatize in a Plexiglas cage and an EEG recording (4-channel EEG polygraph, model BP8, Battaglia Rangoni, Bologna, Italy) was made for at least 30 min, to assess the spontaneous EEG pattern.

Kainic acid was dissolved in 1 N NaOH, the solution was neutralized (pH 7.4) and brought to the final volume with 0.1 M phosphate-buffered saline (PBS). Kainic acid (0.04 μg in 0.5 μl) was infused slowly (60 s) in the dentate gyrus, in the region of granule cells, through an injection needle (28 gauge) which extended 2.9 mm below the guide cannula. This was the smallest dose found to induce EEG seizures in all the animals (Vezzani *et al.*, 1991).

For assessing the effect of intrahippocampally administered 8-OH-DPAT (0.1, 1, 10 μg in 1 μl), the drug was dissolved in PBS and infused 15 min before kainic acid. For assessing the effect of systemic 8-OH-DPAT (25, 100, 1000 μg kg^{-1}), the drug was injected subcutaneously (1 ml kg^{-1}) 30 min before intrahippocampal kainic acid. WAY 100635, a selective 5 HT_{1A} receptor antagonist (Fletcher *et al.*, 1994), was dissolved in PBS and infused in the hippocampus (1 or 5 μg in 1 μl) 20 min before kainic acid at the same site.

The same dose ranges had been used previously to assess the role of 5-HT_{1A} receptors in the hippocampus on acquisition and performance of a spatial task in a water maze (Carli *et al.*, 1992; Carli & Samanin, 1992; 1995). As previously shown (Arvidsson *et al.*, 1981; Hjorth *et al.*, 1982), 8-OH-DPAT given systemically to rats induced a marked flat body posture particularly evident at the doses of 100 and 1000 μg kg^{-1}. This effect was not observed when the drug was administered in the hippocampus. After kainic acid injection, the behavioural effect of 8-OH-DPAT was no longer apparent and the rats showed the typical behavioural sequelae induced by the convulsant drug (see below). The EEG recordings were made continuously for at least 180 min after kainic acid infusion and the animal's behaviour was checked visually.

Analysis of the EEG

Seizures were measured in this study by EEG analysis. They have been found to be the most significant, reproducible and quantifiable of the epilepsy-like sequelae induced by the excitatory aminoacids when administered into the rat hippocampus, and provide a sensitive measure of the anticonvulsant activity of drugs (Vezzani *et al.*, 1986; 1991).

The EEG recording for each animal was analysed visually to detect any activity different from baseline. Seizures consisted of the simultaneous occurrence of at least two of the following alterations in all four leads of recordings: high frequency and/or multispike complexes and/or high voltage synchronized spike or wave activity. Spiking was often observed when seizures had subsided.

The quantitative parameters chosen to characterize EEG seizures were the latency to the first seizure (onset), the total number of seizures (e.g. the number of ictal episodes occurring in the three hours of recording) and the total time spent in seizures which was determined by adding together the duration of all ictal episodes during the EEG recording period.

The EEG tracings from animals receiving the various drugs and those receiving kainic acid alone were compared visually. 8-OH-DPAT given at the various doses either intrahippocampally or systemically did not modify *per se* the EEG tracing as compared to baseline recording. Shortly after administration, kainic acid induced stereotype behaviour, such as sniffing, gnawing and rearing, which lasted about 1 hour. 'Wet dog shakes' were frequently observed shortly after kainic acid injection and during EEG ictal episodes.

Histological analysis

The animals were killed by decapitation after the EEG recording and the brains were removed and frozen on dry ice. Forty μm coronal sections were cut at the level of the dorsal hippocampus with a cryostat. The sections were visually analysed to verify the correct position of the electrodes and the

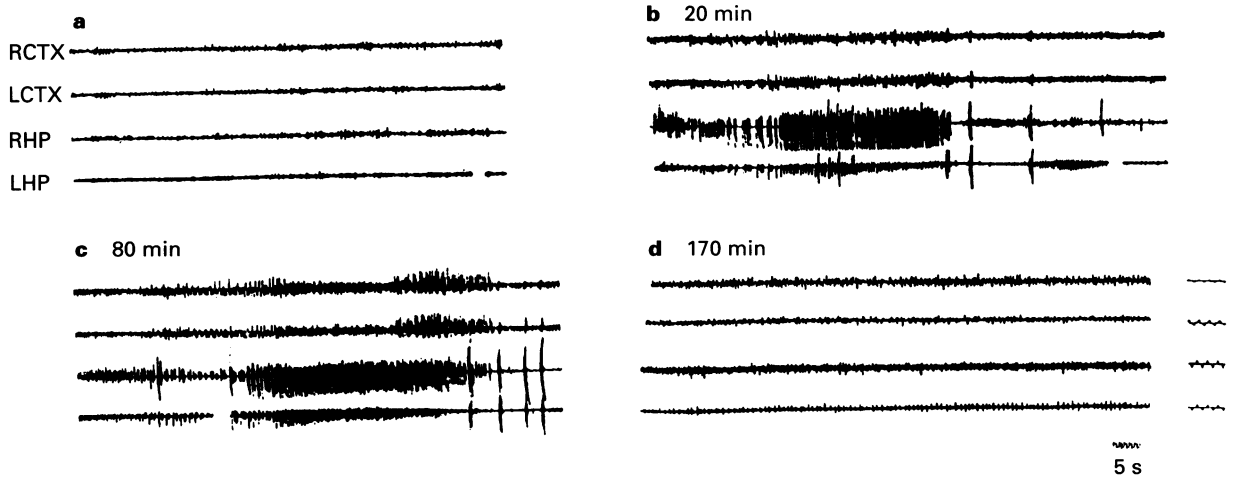

Figure 1 Representative sections of the EEG tracing from a rat injected with 0.04 μg/0.5 μl kainic acid in the left dorsal hippocampus. (a) Control period, before kainic acid injection; (b,c) typical seizures; (d) spiking activity. RCTX and LCTX represent right and left cortex; RHP and LHP represent right and left hippocampus. Time elapsed after injection of kainic acid is indicated on the recording.

track of the injection needle. Rats in which the implantation was not correct were excluded from the study.

No attempt was made to assess the effect of 8-OH-DPAT on kainic acid-induced neurotoxicity.

Statistical analysis

Significant differences between experimental groups and their respective controls were determined by factorial analysis of variance (ANOVA 2x2), followed by Tukey's test for unconfounded means or by Duncan's test for multiple comparisons.

Drug sources

Kainic acid was purchased from Sigma Chemical Co. (St. Louis, MO); 8-OH-DPAT was purchased from RBI (Natick, MA, U.S.A.); WAY 100635 (N-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyzidinyl) cyclohexane carboxamide) was kindly provided by Dr R. McArthur (Pharmacia-Upjohn, Nerviano, Italy); chloral hydrate was purchased from Bracco (Milano, Italy) and sodium pentobarbitone was obtained from Abbott (Saint-Remy-Sur Avre, France).

Results

Figure 1 shows traces from a typical EEG pattern induced by unilateral intrahippocampal injection of 0.04 μg (0.2 nmol) kainic acid in the dentate gyrus of freely-moving rats. This dose selectively damaged CA3-CA4 neurones in 75% of the animals, as previously observed (French *et al.*, 1982) (not shown).

8-OH-DPAT 1 and 10 μg significantly reduced the total time spent in seizures by 72% on average ($P<0.01$). (Table 1). The number of seizure episodes was decreased by respectively 58% ($P<0.01$) and 45% ($P<0.05$) by the two doses. The onset to the first seizure episode was significantly increased, by 2.8 times ($P<0.01$), only after 1 μg 8-OH-DPAT. None of the seizure parameters were significantly modified by 0.1 μg 8-OH-DPAT.

To check whether the protective effect of 8-OH-DPAT on kainic acid-induced seizures was mediated by stimulation of 5-HT_{1A} receptors, the rats were pretreated in the hippocampus with WAY 100635, a selective 5-HT_{1A} receptor antagonist, 5 min before 8-OH-DPAT infusion. Table 2 shows that 5 μg WAY 100635 did not affect kainic acid-induced seizure activity *per se* but significantly antagonized the anticonvulsant effect of 8-OH-DPAT on all seizure parameters ($F=6.5-6.7$, $P<0.01$ and $F=4.0$, $P<0.05$). WAY 100635 1 μg did not modify the anticonvulsant effect of 8-OH-DPAT (not shown).

Table 3 shows the dose-response effect of systemically administered 8-OH-DPAT on kainic acid-induced seizures. At doses between 25 and 1000 μg kg^{-1}, the drug significantly reduced the total number of seizures and the total time spent in seizures by, respectively, 52% and 74% on average ($P<0.01$). The onset to the first seizure was significantly delayed by 100 and 1000 μg 8-OH-DPAT ($P<0.05$). The dose of 100 μg kg^{-1} was chosen for the subsequent experiments.

To assess whether the anticonvulsant effect was mediated by 5-HT_{1A} receptors in the hippocampus, we treated the rats with 5 μg WAY 100635 in the dentate gyrus 20 min before giving kainic acid. The effect of systemic 100 μg 8-OH-DPAT on the number and duration of seizures was significantly antagonized by WAY 100635 ($F=7.1$, $P<0.01$; $F=7.4$, $P<0.05$) (Table 4).

Discussion

The present study showed that intrahippocampal or systemic administration of 8-OH-DPAT, a specific 5-HT_{1A} receptor agonist, to rats results in protective effects against seizure activity induced in the hippocampus by kainic acid. In accordance with our findings, 5-HT and 8-OH-DPAT were found to counteract the epileptiform activity induced by bicuculline and kainic acid in hippocampal slices (Salgado & Alkadhi, 1995).

Kainic acid provokes seizures by direct activation of glutamate receptors of the kainate subtype (McDonald & Johnston, 1990) situated on pyramidal and granule neurones in the hippocampus. Its convulsant activity, however, also depends on release of glutamate as indicated by biochemical (Ferkany & Coyle, 1983) and pharmacological studies (Zhang *et al.*, 1990; MacGregor & Stone, 1992).

Table 1 Dose-response effect of intrahippocampal 8-OH-DPAT on kainic acid-induced seizure activity

Treatment	*Dose* (μg)	*Onset* (min)	*No. of seizures*	*Times of seizures* (s)
Control	–	10.8±1.6	14.4±1.2	1219.0±127
8-OH-DPAT	0.1	11.0±3.0	16.3±2.3	1229.5±208
	1.0	30.9±5.8**	5.9±1.4**	305.0±88.6**
	10	14.3±1.2	8.1±1.6*	374.4±88**

Data are mean±s.e.mean ($n=6-11$). 8-OH-DPAT was infused unilaterally in the dorsal hippocampus in 1 μl phosphate-buffered saline (PBS) 15 min before 0.04 μg kainic acid in 0.5 μl PBS. Controls received 1 μl PBS 15 min before kainic acid. *$P<0.05$; **$P<0.01$ vs controls by Duncan's test.

Table 2 Antagonism by WAY 100635 of the anticonvulsant effect of intrahippocampal 8-OH-DPAT on kainic acid-induced seizure activity

Treatment	*Dose* (μg)	*Onset* (min)	*No. of seizures*	*Times of seizures* (s)
Control	–	12.8±2.3	13.8±1.6	1096.0±146
8-OH-DPAT		32.5±6.4**	5.4±1.5**	287.7±100**
WAY 100635		10.5±1.0	13.2±1.1	1185.0±155
WAY 100635+	5.0			
8-OH-DPAT	1.0	17.8±2.6[a]	11.0±3.4[b]	721.0±79[c]

Data are mean±s.e.mean ($n=5-11$). 8-OH-DPAT (1 μg) was infused in the dorsal hippocampus in 1 μl PBS 15 min before 0.04 μg kainic acid in 0.5 μl PBS. WAY 100635 (5 μg) was infused in 1 μl at the same site 20 min before kainic acid or 5 min before 8-OH-DPAT. Controls received corresponding injections of PBS before kainic acid. The data were analysed by factorial analysis of variance (ANOVA 2×2) followed by Tukey's test [a] $F(1,36)=6.7$, $P<0.01$; [b] $F(1,36)=6.5$, $P<0.01$; [c] $F(1,36)=4.0$, $P<0.05$). **$P<0.01$ vs controls.

Table 3 Dose-response effect of systemic 8-OH-DPAT on kainic acid-induced seizure activity

Treatment	*Dose* ($\mu g\ kg^{-1}$ s.c.)	*Onset* (min)	*No. of seizures*	*Time of seizures* (s)
Control	–	10.6±3.3	15.0±1.1	1248.0±98.0
8-OH-DPAT	25	10.7±1.4	8.3±1.6**	399.8±100**
	100	19.0±4.5*	6.0±1.0**	220.0±98.6**
	1000	19.2±3.7*	7.0±1.0**	343.0±66.0**

Data are mean±s.e.mean ($n=6-11$). 8-OH-DPAT was dissolved in PBS and injected subcutaneously (1 ml kg^{-1}) 30 min before 0.04 μg kainic acid in 0.5 μl PBS. Controls received PBS 30 min before kainic acid. *$P<0.05$; **$P<0.01$ vs controls by Duncan's test.

Table 4 Antagonism by intrahippocampal WAY 100635 of the anticonvulsant effect of systemic 8-OH-DPAT on kainic acid-induced seizure activity

Treatment	*Dose* (μg)	*Onset* (min)	*No. of seizures*	*Time of seizures*
Control	–	10.7±2.0	12.4±1.0	1014.0±119
8-OH-DPAT		19.3±3.6**	6.3±1.0*	335.0±83**
WAY 100635		10.7±1.2	12.1±1.0	1036.0±83
WAY 100635+	5			
8-OH-DPAT	100	12.3±2.6[a]	11.0±3.4[b]	721.0±79[c]

Data are mean±s.e.mean ($n=6-11$). 8-OH-DPAT (100 μg kg^{-1}) was dissolved in PBS and injected subcutaneously (1 ml kg^{-1}) 30 min before intrahippocampal 0.04 μg kainic acid in 0.5 μl PBS. WAY 100635 (5 μg) was infused in 1 μl in the hippocampus 20 min before kainic acid or 10 min after 8-OH-DPAT. Controls received corresponding injections of PBS before kainic acid. The data were analysed by factorial analysis of variance (ANOVA 2×2) followed by Tukey's test [a] $F(1,36)=2.9$, NS; [b] $F(1,36)=7.1$, $P<0.01$; [c] $F(1,36)=7.4$, $P<0.05$. *$P<0.05$; **$P<0.01$ vs controls.

Thus, stimulation of 5-HT_{1A} receptors in the hippocampus may reduce hippocampal excitability and cause anticonvulsant effects acting on one or both of these mechanisms.

Neuroanatomical evidence shows a dense innervation of 5-hydroxytryptaminergic fibres to the hippocampus mainly originating from the median raphe forebrain nucleus. These fibres project to the stratum radiatum of the hippocampus proper, weakly into the stratum oriens, and to the polymorphic cell layer of the dentate gyrus (Azmitia & Segal, 1978; Oleskevich & Descarries, 1990). This innervation conforms well to the 5-HT_{1A} receptor distribution in the CA1 area and the dentate gyrus as assessed by mRNA expression and receptor binding studies (Miquel *et al.*, 1991). These receptors are mainly located on dendritic projections of pyramidal and granule neurones (Pompeiano *et al.*, 1992). It therefore seems likely that the anticonvulsant action of 8-OH-DPAT is mediated by direct inhibition of neuronal excitability due to the effect of the drug on membrane ionic conductances. There is, in fact, electrophysiological evidence that 5-HT can hyperpolarize pyramidal CA1 and CA3 neurones and granule cells (Ropert, 1988; Baskys *et al.*, 1989; Beck & Choi, 1991; Ghadimi & Jarolimek, 1994) by increasing Ca^{2+}-dependent K^+ conductance.

However, effects on glutamate release may also be involved in the anticonvulsant action of 8-OH-DPAT. Thus, pyramidal and granule cell hyperpolarization may result in diminished glutamate release at the corresponding presynaptic terminals (e.g. Schaffer's collaterals and mossy fibres). This would be compatible with previous studies showing that 5-HT can inhibit the release of glutamate in the cerebellum (Maura *et al.*, 1988).

Functional interactions between 5-hydroxytryptaminergic and glutamatergic neurotransmission in limbic areas have been demonstrated previously. In particular, 5-HT_{1A} receptor activation blocks long-term potentiation induced by primed burst stimulation in the CA1 region (Corradetti *et al.*, 1992) and in the entorhinal cortex, 5-HT was able to reduce the depolarization induced by pulses of glutamate during blockade of synaptic transmission (Sizer *et al.*, 1992). These studies provide further evidence of a postsynaptic action of 5-HT mediated by 5-HT_{1A} receptors.

These receptors are heterogeneously distributed in the CNS and the density of these sites is high in the raphe nuclei where they act as presynaptic somatodendritic 5-HT receptors (Pompeiano *et al.*, 1992). Their stimulation markedly reduces extracellular 5-HT concentrations in various brain regions including the hippocampus (Invernizzi *et al.*, 1992; 1995). It is therefore interesting that 8-OH-DPAT had anticonvulsant activity on kainic acid-induced seizures also when given systemically to rats. This implies that stimulation of presynaptic 5-HT_{1A} receptors does not significantly affect seizure activity originating in the hippocampus.

These findings have important implications for new therapeutic strategies aimed at counteracting limbic seizures particularly refractory to classical anticonvulsant treatment (Elwes *et al.*, 1984; Mattson *et al.*, 1985). A recent study in patients with complex partial seizures showed that fluoxetine, a 5-HT reuptake blocker, when administered as an adjunctive drug, significantly reduced seizure frequency or even completely abolished daily seizures (Favale *et al.*, 1995).

Further investigation is therefore warranted to clarify the pathophysiology of epilepsy involving changes in central 5-hydroxytryptaminergic neurotransmission and the effects of stimulation of the different 5-HT receptor subtypes on various kinds of limbic epilepsy.

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