longer stimulus durations was unexpected but not unprecedented. The explanation offered for this type of increase with duration of visual and auditory stimuli assumes that the longer durations ". . . lengthen reaction time rather than make it faster by providing the subject with an opportunity to take a *longer* sample of sensory information than is necessary . . ." (*18*). This explanation seems appropriate for our taste data (Fig. 1) and is supported by the increase in judged intensity with longer pulses (Table 2). Since the latency of the peripheral neural response cannot be posited as a possible reason for the increases in reaction time, the concept of greater information at the longer durations fits both the intensity judgment and the reaction time observations. However, since a high sensitivity to changes over time in the concentration of gustatory stimuli has been observed (*17, 19*), with maximum sensitivity at ≤ 1 Hz (*19*), the long reaction times to our 1000-msec pulses may be related to the separation between ON and OFF with such pulses.

The data demonstrate a substantial human capacity not only to respond to, but also to receive qualitative and quantitative information from relatively brief taste pulses. The generality of these data is limited by our use of only single, relatively high, concentrations of two substances (*10, 11*). Nonetheless, it seems that the long human taste reaction times are not caused by a requirement for stimulus durations almost as long, and the initial phasic portion of the human peripheral gustatory neural response is likely to contain appreciable, but far from complete, information on the stimulus.

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1. Lightning strokes have an average total duration of 1 to 2 msec [A. D. Watt, *J. Res. Nat. Bur. Stand.* **64D**, 425 (1960)]. For perception during brief illumination, see W. S. Hunter and M. Sigler [*J. Exp. Psychol.* **26**, 160 (1940)].
2. The sounds of a dry twig snapping or a human gasping last 100 to 150 msec.
3. Minimum human simple visual or auditory reaction times requiring a finger movement are 140 to 220 msec [A. T. Welford, Ed., *Reaction Time* (Academic Press, London, 1980), pp. 1–330].
4. Participants were six paid volunteers (ages 20 to 27) who passed a screening task used in a taste reaction time experiment for reliability of taste judgments [B. Lester and B. P. Halpern, *Physiol. Behav.* **22**, 391 (1979)].
5. Two magnitude estimates [S. S. Stevens, *Psychol. Rev.* **78**, 426 (1971)] were obtained during each intensity session trial, one 100 msec before the end of the pulse and the other 5 seconds after the end of the stimulus pulse. These estimates were signaled for by one or two 100-msec, 2600-Hz sounds produced by solid-state tranducers operated by a programmable controller, which ran each trial.
6. Conductivity, < 1.8 μS; refractive index, 1.3330.
7. Liquids reached the tongue through a 10-mm by 5-mm eliptical opening in the bottom wall of a polypropylene tube and continued in the tube past the tongue. The anterior dorsum of the tongue was pressed up against a silicon rubber bead around the opening, with the anterior tip of the tongue contacting a tongue reference point located at the rear margin of the rubber bead. Participants pressed their upper central incisors down on a Plexiglas rod. On the outside of the liquid delivery tube, a distilled water flow at 9.4 ml/sec provided a surround rinse around the tongue opening. A median concentration of 4-m*M* NaCl (below human taste threshold [M. O'Mahony, *Perception* **8**, 441 (1979)] was measured in the surround rinse liquid during the flow of 500-m*M* NaCl through the delivery tube and over the tongue.
8. Kramer test, $P > .05$ [A. Kramer, G. Kahan, D. Cooper, A. Papavasiliou, *Chem. Senses Flavor* **1**, 121 (1974)]. χ^2 (24) = 19.63, $P > .75$. When all quality category columns other than salty and bitter were combined so that all expected frequencies were > 1 and only 25 percent of the cells had expected frequencies < 5, χ^2 (6) = 5.385, $P > .75$.
9. An error on a control trial occurred when a change in taste was reported for the control (water) pulse. Control error rates for 100-, 200-, 300-, and 1000-msec pulses were 0, 2, 2, and 2 percent for NaCl, 0, 0, 6, and 0 percent for saccharin across magnitude estimate sessions; 4, 0, 6, and 2 percent across quality category sessions; 8, 10, 7, and 5 percent across all reaction time sessions.
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12. Typical mammalian chorda tympani nerve response latencies to chemical stimulation of the tongue with CsCl, HCl, KCl, NaCl, or RbCl solutions are 26 to 45 msec; with sucrose, 60 to 300 msec [L. M. Beidler, in *Handbook of Perception, vol. 6A, Tasting and Smelling*, E. C. Caterette and M. P. Friedman, Eds. (Academic Press, New York, 1978), pp. 21–49; J. R. Faull and B. P. Halpern, *Science* **178**, 73 (1972); L. A. Marowitz and B. P. Halpern, *Chem. Senses Flavor* **2**, 457 (1977); M. S. Nejad, thesis, Florida State University (1961); C. Pfaffmann, *J. Neurophysiol.* **18**, 429 (1955)].
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A Benzodiazepine Receptor Antagonist Decreases Sleep and Reverses the Hypnotic Actions of Flurazepam

Abstract. *The benzodiazepine receptor antagonist 3-hydroxymethyl-β-carboline, which blocks several of the pharmacological actions of benzodiazepines, induces a dose-dependent increase in sleep latency in the rat. Furthermore, at a low dose that by itself does not affect sleep, 3-hydroxymethyl-β-carboline blocks sleep induction by a large dose of flurazepam. The benzodiazepine receptor may play a role in both the physiological regulation and pharmacological induction of sleep.*

Benzodiazepines are widely used in the treatment of insomnia, anxiety, seizures, and muscle disorders. In recent years a single benzodiazepine, flurazepam, has accounted for about half of all hypnotic prescriptions in the United States (*1*). The excellent correlations between the affinity of a series of benzodiazepines for specific receptor sites in the mammalian central nervous system and the potency of these compounds as anxiolytics, anticonvulsants, and muscle re-

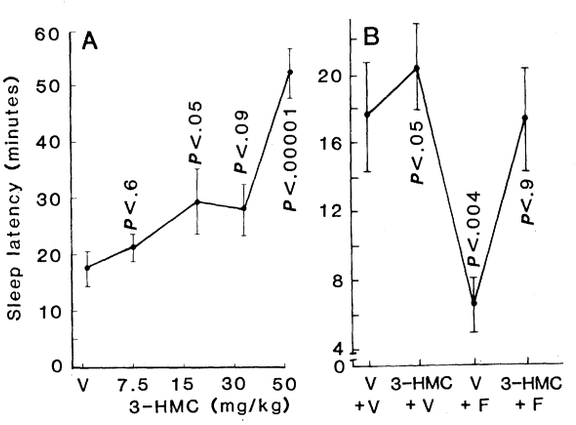

Fig. 1. (A) Effects of 3-HMC on sleep latency. The rats were administered 3-HMC at 0900 hours, and 5 minutes later EEG recordings were made for 2 hours. Overall significance by ANOVA was $P < .00001$. (B) Effects of 3-HMC on the hypnotic actions of flurazepam. The rats were administered vehicle or 3-HMC (7.5 mg/kg) 5 minutes before receiving vehicle or flurazepam (40 mg/kg). EEG recordings were performed for 2 hours after the last injection, beginning at 0905. Overall significance by ANOVA: $P < .003$. Abbreviations: *V*, vehicle; F, flurazepam.

Table 1. Effects of 3-HMC, alone and in combination with flurazepam, on sleep in rats. The animals were injected with vehicle or 3-HMC (7.5 mg/kg) 5 minutes before the administration of vehicle or flurazepam (40 mg/kg). EEG was recorded for 2 hours after the last injection. Values are minutes (means ± standard errors). N.S., not significant.

Treatment	*N*	Sleep latency	Non-REM sleep	REM sleep	Total sleep	Intermittent wakefulness
Vehicle and vehicle	10	17.8 ± 2.9	69.2 ± 4.0	2.2 ± 0.9	71.4 ± 3.9	30.8 ± 4.4
Vehicle and flurazepam	9	6.6 ± 1.6	79.6 ± 4.5	0.5 ± 0.2	80.1 ± 4.4	33.2 ± 3.9
3-HMC and vehicle	9	20.4 ± 2.3	61.9 ± 3.7	4.5 ± 1.3	66.4 ± 4.1	33.1 ± 3.3
3-HMC and flurazepam	9	17.3 ± 3.0	61.8 ± 4.7	0.3 ± 0.2	62.1 ± 4.8	40.6 ± 7.1
Significance*		$P < .003$	$P < .02$	$P < .004$	$P < .04$	N.S.

*Analysis of variance.

laxants suggest that the receptors mediate these actions (*2*). A similar relation between the benzodiazepine receptor and the sedative and hypnotic properties of benzodiazepines has not been as firmly established.

Since certain β-carbolines substituted at C-3 have a high affinity for benzodiazepine receptors (*3*) and can antagonize some of the pharmacological actions of benzodiazepines (*4–6*), such compounds could be useful in assessing the role of these receptors in the sleep-inducing properties of benzodiazepines such as flurazepam. We now report that in rats the benzodiazepine receptor antagonist 3-hydroxymethyl-β-carboline (3-HMC) (*4–6*), at a dose that has no intrinsic effect on sleep, reverses the sleep-inducing actions of flurazepam. Furthermore, at slightly higher doses, 3-HMC induces a state of wakefulness that is not accompanied by significant alterations in motor activity. These observations suggest that benzodiazepine receptors are involved not only in the sleep-inducing properties of benzodiazepines but also function in the physiological regulation of sleep.

Male Sprague-Dawley rats (250 to 300 g; Zivic-Miller) were maintained on a photoperiodic cycle with 12 hours of light (0800 to 2000 hours). Stainless steel screw electrodes were implanted in the dura for measurement of the electroencephalogram (EEG) and stainless steel wire electrodes were implanted in the nuchal musculature for measurement of the electromyogram (EMG). One week after surgery the rats were injected intraperitoneally with 3-HMC (7.5 to 50 mg/kg) or vehicle at 0900 hours and 5 minutes later with flurazepam (40 mg/kg) or vehicle. Two-hour recordings of EEG and EMG were performed on a Grass model 78 polygraph calibrated to 50 μV per 10 mm and with a paper speed of 10 mm/sec. One "blind" investigator then read all the records, determining the state of consciousness for each 30-second epoch (*7*). The waking state is characterized by a low-amplitude, mixed-frequency EEG in the presence of high muscle tone. Sleep without rapid eye movement (non-REM) shows high-amplitude, slower EEG, while REM sleep is recognized by low-amplitude, mixed-frequency EEG and atony in the EMG. Statistical significance was determined by a one-way analysis of variance (ANOVA) for independent groups. Differences between groups were determined by a least significant difference test when a significant drug effect was identified by ANOVA.

Flurazepam produced a characteristic reduction in the time lag from injection to sleep onset (sleep latency) and an increase in the duration of both non-REM and total sleep (Fig. 1B). The flurazepam did not elicit a significant change in REM sleep. A dose of 3-HMC (7.5 mg/kg) that has no intrinsic effect on sleep latency blocked the sleep-inducing effect of flurazepam, since latency values for animals given both drugs were not significantly different from control values (Table 1).

At slightly higher doses, 3-HMC elicited a dose-dependent increase in sleep latency ($P < .00001$) (Fig. 1A). A significant ($P < .00001$) decrease in total sleep time was due to a marked reduction in non-REM sleep ($P < .0001$). Effects on REM sleep were minor and inconsistent, although there was little REM even in the control group, presumably because the 2-hour recordings were done at a time of day when REM is minimal (*8–10*).

To ascertain whether the decreases in non-REM sleep were accompanied by significant changes in motor activity (as is observed with other stimulant drugs), rats were administered 3-HMC (50 mg/kg, intraperitoneally) and observed for 3 hours in a Motron Produkter device, which measures spontaneous locomotor activity. During the first 10 minutes there was a small decrease in motor activity followed by a modest, but transient, increase 20 to 70 minutes after injection (Fig. 2). An ANOVA did not reveal a significant drug effect, but a significant drug × time interaction ($P < .03$) was observed. However, the enhanced wakefulness observed in 3-HMC–treated rats was most evident during the first 10 minutes after injection, when this group had less motor activity than the vehicle-treated rats (*11*).

Cowen *et al.* (*5*) reported that 3-carbomethoxy-β-carboline (β-CCE) (5 mg/kg, intravenously) produces a transient reversal of flurazepam-induced decreases in motor activity. Similarly, Oakley and Jones (*6*) noted that the doses of β-CCE (50 to 100 mg/kg, intraperitoneally) necessary to antagonize the anticonvulsant actions of diazepam are far greater than might be predicted from the affinity of this compound for benzodiazepine receptors in vitro [inhibition constant (K_i), ~ 1 n*M*]. These observations suggest that the relatively transient action of β-CCE in rats is due to rapid metabolism of

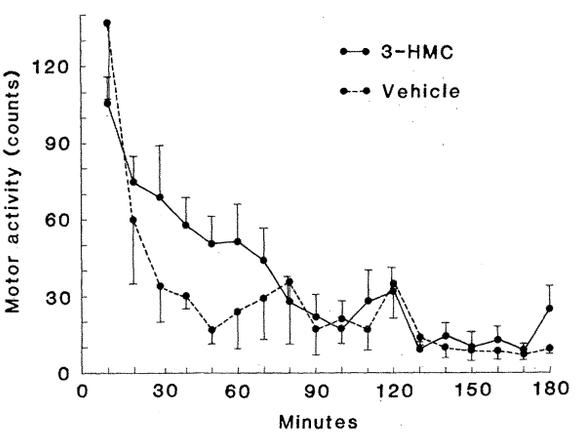

Fig. 2. Effects of 3-HMC on motor activity. The rats were administered 3-HMC (50 mg/kg), and motor activity was measured in a Motron Produkter apparatus. Analysis of variance revealed no overall drug effect, but a significant drug × time interaction ($P < .03$).

the compound to inactive β-carboline-3-carboxylic acid (K_i, ~ 25,000 nM). This has been confirmed in our laboratories, and has stimulated the development of substantially longer lived, pharmacologically active β-carboline derivatives (*12*).

We have demonstrated that 3-HMC, which antagonizes the anxiolytic and anticonvulsant actions of diazepam, also antagonizes the sleep-inducing properties of the benzodiazepine flurazepam. Thus the hypnotic actions of flurazepam may be mediated through interaction with the benzodiazepine receptor. At slightly higher doses, 3-HMC increased wakefulness by significantly increasing sleep latency and reducing non-REM (but not REM) sleep (Fig. 1A and Table 1). Thus 3-HMC is not merely a benzodiazepine antagonist but exerts a pharmacological action on sleep opposite that produced by benzodiazepines (*13*). Although other drugs (such as amphetamines and methylxanthines) can reduce sleep (*14*), they also invariably cause profound alterations in behavior and motor activity (*15*). Compounds that reduce sleep without eliciting major changes in motor activity may, therefore, be more properly termed "somnolytics." The suggestion that benzodiazepine receptors (and, by implication, the endogenous substrates that subserve these receptors) are involved in both physiological and pharmacologically induced sleep could lead to the development of β-carbolines or related compounds for treating human sleep disorders, especially those characterized by excessive somnolence.

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8. No evidence of EEG spiking was observed after administration of 3-HMC, suggesting that this compound does not produce seizure activity. Recently, however, other β-carbolines that bind to benzodiazepine receptors with high affinity (for example, β-CCE) were shown to elicit tonic and clonic convulsions (*9, 10*).
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11. The effects of smaller doses of 3-HMC (< 50 mg/kg) on motor activity are not known but presumably are even smaller than those observed with the higher doses. No evidence of stereotypical behavior was observed when 3-HMC (50 mg/kg) was administered daily for 3 weeks (P. Skolnick *et al.*, unpublished observations).
12. In an in vitro study, 3-HMC and β-CCE were incubated in rat plasma (37°C) at concentrations of 100 to 200 nmole/ml. Equal 500-μl aliquots were removed at intervals and treated with 25 μl of perchloric acid to precipitate the protein. After centrifugation, portions of the supernatant were withdrawn and the amount of pharmacologically active compound remaining was determined by an assay based on the displacement of [^{3}H]diazepam [P. Skolnick, S. Paul, F. Goodwin, *Arch. Gen. Psychiatry* **36**, 78 (1979)]. Greater than 80 percent of the assayable material was lost after 2.5 minutes of incubation with β-CCE. In contrast, 3-HMC appeared more stable, since approximately 70 percent of the original activity was still present after 30 minutes of incubation. Parallel experiments at 0° to 4°C confirmed the enzymatic nature of this degradation, since 70 percent of the initial activity of β-CCE was still present after a 15-minute incubation. Thus, despite the modest affinity of 3-HMC for benzodiazepine receptors (K_i, ~ 1470 nM) compared to that of β-CCE (K_i, ~ 1 nM) (*3*), the former compound appeared more suitable for sleep studies due to its slower rate of degradation. Other β-carbolines substituted at C-3, such as 3-acetyl-β-carboline, have now been synthesized. They may also prove valuable in defining the role of benzodiazepine receptors in sleep because of their high affinities (K_i, ~ 50 nM) and relative resistance to metabolic degradation.
13. The increased wakefulness observed after 3-HMC administration is blocked by the benzodiazepine receptor antagonist CGS 8216 [A. Czernik *et al.*, *Life Sci.* **30**, 363 (1982)] at a dose (5 mg/kg) that has no intrinsic action on sleep (W. B. Mendelson *et al.*, unpublished observation). Also, the convulsant action of β-CCE is antagonized by Ro 15-1788 and CGS 8216 (*9*). These findings strongly support the hypothesis that both the antagonism of the hypnotic actions of flurazepam and the increased wakefulness observed after 3-HMC injection are mediated by benzodiazepine receptors.
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16. We appreciate the technical assistance of D. Carroll.

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Nigral Transplants Reinnervating the Dopamine-Depleted Neostriatum Can Sustain Intracranial Self-Stimulation

Abstract. *Transplants of embryonic substantia nigra reinnervated the striatum and were able to sustain intracranial self-stimulation in rats with brain lesions induced by 6-hydroxydopamine. Dopaminergic drugs and alterations in current intensity produced typical changes in response rates. Animals with electrodes implanted into cortical grafts or into the denervated striatum failed to exhibit self-stimulation. These findings suggest that transplanted dopamine neurons convey specific, temporally organized information axonally to the striatum.*

A procedure for transplanting dopamine (DA) cells from the substantia nigra to ectopic cortical sites was recently described (*1*). Such grafts can reinnervate the host brain extensively and ameliorate several behavioral deficits produced by 6-hydroxydopamine (6-OHDA)–induced depletions of DA in the host, including spontaneous and drug-induced rotation, sensorimotor impairments, and akinesia (*1, 2*). However, it is not clear whether transplanted neurons simply provide a tonic release of DA into the striatum or whether the grafts release DA from their terminals on activation of the cell bodies. The phenomenon of intracranial self-stimulation (ICSS) (*3*) may provide a useful means for investigating this question, since (i) DA systems of the brain have been implicated in ICSS (*4*), (ii) there is a requirement that the animal integrate a specific input with its behavior, and (iii) brain stimulation can provide such an input to the DA cell bodies in the graft. We report here that DA-rich nigral grafts can sustain self-stimulation. This suggests that transplanted DA neurons may indeed transmit specific information to the reinnervated striatum.

The right nigrostriatal pathway in 20 young adult female rats of the Sprague-Dawley strain was lesioned with 6-OHDA, and cavities were made through the right parietal cortex and corpus callosum, exposing the dorsal surface of the caudate-putamen. Three weeks later grafts of embryonic substantia nigra (N = 14) or embryonic isotopic cortex (N = 6) were placed into the cavities (*5*). After 4 months bipolar stimulating elec-