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The Influence of Droperidol, Diazepam, and Physostigmine on Ketamine-induced Behavior and Brain Regional Glucose Utilization in Rat

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Diazepam and droperidol are used clinically with ketamine anesthesia to reduce emergence hallucinations, vivid unpleasant dreams, and hyperexcitability. Also, there are reports that the recovery time from ketamine anesthesia is shortened after administration of physostigmine. The authors investigated the influence of diazepam, droperidol, and physostigmine pretreatment on ketamine anesthesia by measuring the brain regional activity and behavioral responses in rat. The 2-deoxyglucose brain local metabolic mapping method was used to determine regional brain functional activity. The recovery of tail flick response and righting reflex from ketamine anesthesia were prolonged by diazepam and by droperidol pretreatment, but the duration of agitation was shortened; physostigmine caused no significant change in any of these responses. Ketamine alone caused a statistically significant ($P < 0.05$) increase in the rate of glucose utilization along the hippocampal molecular layer (control $87 \mu\text{mol} \cdot 100 \text{g}^{-1} \cdot \text{min}^{-1}$; ketamine $166 \mu\text{mol} \cdot 100 \text{g}^{-1} \cdot \text{min}^{-1}$) and a decrease in medial geniculate (25%), inferior colliculus (37%), and lateral habenula (18%). Diazepam, droperidol, and physostigmine pretreatment did not significantly alter any ketamine-induced glucose use changes, except for a decreased activity in hippocampal molecular layer with diazepam pretreatment (20%) and an increased activity in the lateral habenula with droperidol pretreatment (94%, $P < 0.05$). These findings corroborate the "epileptogenic" character of ketamine anesthesia and implicate the hippocampus as a major

focus. The reduced activity in the hippocampus induced by diazepam pretreatment and the increased activity in the lateral habenula induced by droperidol pretreatment may be factors in the clinical reduction of ketamine hyperexcitability and hallucination by these drugs. (Key words: Anesthetics, intravenous: droperidol; ketamine. Antagonists, miscellaneous: physostigmine. Brain: glucose consumption, regional. Hypnotics: benzodiazepines, diazepam.)

IT IS KNOWN that anesthesia with ketamine frequently is accompanied by emergence hallucinations, hyperexcitability, and psychomotor activity. There is evidence that ketamine produces an activation of EEG patterns and seizure discharge in the hippocampus of the cat.^{1,2} Ketamine-induced electrical seizure activity has been found in the limbic and thalamic areas in humans.³ We⁴ reported that ketamine caused an increase of metabolic activity (glucose utilization) in the hippocampus and a reduction in the medial geniculate and inferior colliculus. Similar changes were found with hippocampal seizures induced by direct injection of penicillin in rats. Thus, ketamine-induced emergence hallucinations, hyperexcitability, and psychomotor activity may be related to the excitation of the hippocampus and other subcortical nuclei. There are conflicting reports that the emergence phenomena which are induced by ketamine are reduced by administration of droperidol⁵⁻⁸ and diazepam.^{9,10} Others have reported that the recovery time from ketamine anesthesia is shortened¹¹ or is not shortened¹² by physostigmine pretreatment clinically.

The quantitative 2-deoxyglucose local glucose utilization method was used to map regions of altered brain function. This method provides a means of measuring the metabolic activity in individual brain regions and in many instances reflects the level of functional activity

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in these regions.¹³ We employed this procedure to determine the influence of droperidol, diazepam, and physostigmine pretreatment on the brain regional changes produced by ketamine, and attempted to relate them to the modification of ketamine anesthesia by these drugs.

Materials and Methods

Male Sprague-Dawley rats, weighing 200–250 g, were used throughout the study. Cannulas were placed in the femoral vein under halothane anesthesia and the rats were allowed to fully awaken and recover for more than two hours before the experiments were started. 2-Deoxyglucose, ketamine, droperidol, and diazepam were given intravenously via this cannula. Physostigmine was injected intraperitoneally because the doses used are sometimes lethal when given intravenously. In a behavior study, seven rats received 30 mg/kg ketamine as a control. In order to observe the effects of droperidol, diazepam, and physostigmine on the duration of ketamine anesthesia, five rats received 0.2 mg/kg droperidol, six received 0.45 mg/kg diazepam, and five received 0.4 mg/kg physostigmine 5 min before ketamine injection. Time to recovery of tail flick response, time to recovery of righting reflex, time to end of ataxia, and time to end of agitation (excitation) were measured. The tail flick response was carried out in the manner described by Jansen *et al.*¹⁴ by immersing the rat tail in water at 55° C and using a cutoff time of 15 s. Agitation was defined as exaggerated responses by the animal to auditory (hand claps) and sensory (light pinching of the skin) stimuli and continuous, non-purposeful walking in wide circles in the cage. In the regional brain glucose utilization study in 35 rats, six were controls, five were treated with ketamine, four with droperidol, four with physostigmine, four with a combination of ketamine and droperidol (pretreatment), four with ketamine and diazepam (pretreatment), and four with ketamine and physostigmine (pretreatment). The method for regional brain glucose utilization was based on that of Sokoloff *et al.*¹⁵ The (¹⁴C)-2-deoxyglucose (2-DG) brain regional functional mapping method of Sokoloff *et al.*¹⁵ was done as follows: the animals were anesthetized with halothane and once under halothane anesthesia, lidocaine was infiltrated around the incision sites. The femoral vein was cannulated with a 3-cm section of intramedic PE50 polyethylene tubing inserted into a 3-cm piece of Dow Corning® silastic tubing (0.34-inch, ID) attached to a 24-cm section of S-54-HL formulation tygon tubing (0.34-inch, ID). The femoral artery was cannulated using a 5-cm section of intramedic PE10 polyethylene tubing connected to a 15-cm section of S-54-HL formulation tygon tubing (0.02-inch, ID).

The arterial cannula reached the aorta and could be cleared by two or three drops of blood. The venous cannula was carried subdermally in front of the hind leg to the midline back and forward to exit between the ears with a 7-inch stainless steel needle. The arterial cannula was passed subdermally behind the leg to exit just rostral to the base of the tail. Each cannula was anchored to the skin using a sleeve of PE205 intramedic polyethylene tubing. About 4 cm of each cannula was left protruding and plugged. The rats were allowed to recover from halothane for a minimum of two hours before experiments were begun.

Just prior to the initiation of the 2-DG procedure, 150 units of heparin were injected intravenously into each free roaming animal and control blood samples taken. The rats were placed in a plastic rodent restraining cage which allowed access to the venous cannula through a hole near the front of the cage. The arterial cannula was accessible at the open end of the cage. A pulse of 2-DG (100 μCi/kg) was given in the venous cannula and immediately flushed with saline. During and immediately following the pulse, six timed serial arterial blood samples (0.05–0.075 ml) were collected in heparinized hematocrit tubes. The rat was released from the cage after the one-minute blood sample and allowed to roam freely for the remaining 44 min. A total of 24 blood samples were taken for plasma glucose determination and scintillation counting for ¹⁴C. At the end of 45 min, the rat was decapitated and the brain quickly removed, frozen in freon 12 stored at –70° C, and bagged in plastic air-tight bags for storage at –70° C.

Five microliters of each plasma sample were pipetted into 4 ml scintillation cocktail (Research Products International Corp. #3a70*) and counted in a Hewlett-Packard® Tri-Carb Scintillation Counter. Ten microliters of plasma were used to determine plasma glucose levels with a Yellow Springs Instrument model 23A glucose analyzer (Yellow Springs, Ohio).

The brains were sectioned at 20 μm at –20° C, and immediately dried on a 55–60° C slide warmer. These sections, along with (¹⁴C)-methylmethacrylate standards were exposed to Kodak® Min-R x-ray film for 21 days. Developed films were analyzed with a densitometer (Model TBX, Tobias Assoc. Inc., Ivyland, Pennsylvania) equipped with a 0.3-mm aperture. Glucose levels and ¹⁴C concentrations were used to calculate the rate of brain regional glucose uptake according to the equation developed by Sokoloff *et al.*¹⁵ Ketamine was given 5 min before injection of 2-deoxy-(¹⁴C)-glucose (100 μCi/kg), and in the pretreatment experiments, droperidol, diazepam, or physostigmine were given 5 min before ketamine.

Significant differences between control and experi-

TABLE 1. The Effects of Droperidol, Diazepam, and Physostigmine on the Duration of Ketamine Anesthesia

	Recovery of Tail Flick Response (min)	Recovery of Righting Reflex (min)	End of Ataxia (min)	End of Agitation (min)
Ketamine (n = 7)	10.0 ± 1.7	7.4 ± 1.2	35.3 ± 1.9	46.3 ± 2.9
Ketamine + droperidol (n = 5)	16.3 ± 2.4	12.5 ± 1.4†	31.0 ± 0.6	29.5 ± 1.7†
Ketamine + diazepam (n = 6)	18.0 ± 2.4*	11.7 ± 0.9*	40.2 ± 3.4	35.3 ± 3.2*
Ketamine + physostigmine (n = 5)	12.3 ± 1.7	7.8 ± 0.6	34.4 ± 1.8	38.8 ± 3.9

All values are means ± SE.

* Significant difference from the ketamine alone at the $P < 0.05$ level.

† Significant difference at the $P < 0.01$ level.

mental groups of animals were determined by first using ANOVA for multiple comparisons followed by the Dunnett *t* test where appropriate.

Results

The effects of droperidol, diazepam, and physostigmine pretreatment on the duration of ketamine anesthesia are shown in table 1. The time to recovery of tail flick response was prolonged with diazepam. Recovery of righting reflex was prolonged, and the time to end of agitation was shortened by both droperidol and diazepam. Physostigmine caused no changes in these responses. When droperidol, diazepam, or physostigmine were injected alone (no ketamine) there were no significant effects on tail flick or righting reflex, and they did not produce ataxia or agitation. Quantitative measure of glucose utilization in control, ketamine-, droperidol-, diazepam-, and physostigmine-treated rats was determined for 11 selected areas of brain (table 2). Ketamine caused an increase in rate of glucose utilization along the hippocampal fissure, and a decrease in the medial geniculate nucleus (fig. 1), lateral habenula (fig. 2), and inferior colliculus. Droperidol stimulated glucose utilization in the lateral habenula (fig. 2). Diazepam caused a decrease in the regional activity in frontal cortex, septum, hypothalamus, and medial geniculate nu-

cleus (table 2). Physostigmine caused a depression of four regions; frontal cortex, septum, hypothalamus, inferior colliculus, and a trend to increased glucose use by the superficial layer of the superior colliculus (table 2 and fig. 3), as we reported earlier.¹⁶ Regional glucose utilization in rats anesthetized with ketamine and pretreated with droperidol, diazepam, and physostigmine is shown in Table 3. These pretreatments did not significantly alter the ketamine-induced glucose utilization changes except for an increase in rate in the lateral habenula with droperidol (table 3 and fig. 2).

Discussion

The 2-DG procedure and the quantitation of brain regional glucose use developed by Sokoloff and his colleagues has become a potent new tool for identifying those brain regions whose activity changes under different functional states. Brain regions are highly heterogeneous, and the contribution of the various cellular processes can not be resolved; thus, the rate of glucose only represents a regional average. However, the procedure can provide useful information about events in the central nervous system during anesthesia. Furthermore, numerous studies have established a relationship between functional activity and energy metabolism in the central nervous system. In the work described here,

TABLE 2. Regional Glucose Utilization ($\mu\text{mol} \cdot 100 \text{g}^{-1} \cdot \text{min}^{-1}$)

	Control (n = 6)	Ketamine (n = 5)	Droperidol (n = 4)	Diazepam (n = 4)	Physostigmine (n = 4)
Frontal cortex	82 ± 3	80 ± 7	86 ± 8	65 ± 1*	64 ± 1*
Caudate	97 ± 6	109 ± 7	108 ± 11	77 ± 3	77 ± 1
Septum	69 ± 4	68 ± 6	75 ± 10	47 ± 1†	46 ± 1†
Hypothalamus	55 ± 2	53 ± 6	64 ± 7	37 ± 1†	39 ± 1†
Vent. thalamus	79 ± 4	73 ± 2	97 ± 11	68 ± 1	75 ± 2
Par. cortex	86 ± 1	86 ± 9	85 ± 8	77 ± 1	69 ± 1
Hipp. (molecular layer)	87 ± 7	166 ± 10†	106 ± 9	73 ± 3	71 ± 2
Med. geniculate	107 ± 4	80 ± 7*	132 ± 12	82 ± 4*	87 ± 2
Sup. colliculus	82 ± 7	79 ± 7	85 ± 8	72 ± 2	97 ± 6
Inf. colliculus	137 ± 9	86 ± 7†	155 ± 10	116 ± 2	103 ± 2*
Lateral habenula	88 ± 3	72 ± 4*	188 ± 9†	80 ± 1	81 ± 2

All values are means ± SE.

* Significant difference from the control at the $P < 0.05$ level.

† Significant difference at the $P < 0.01$ level.

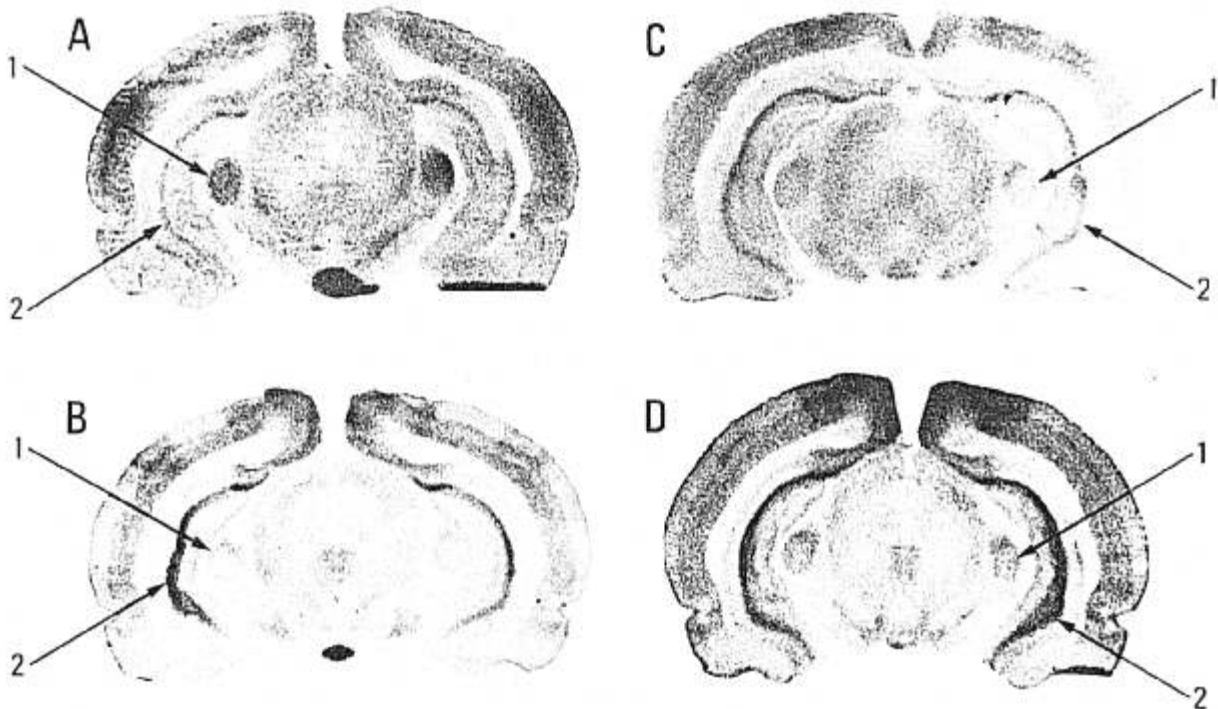


FIG. 1. Representative autoradiographs at the level of the hippocampus and medial geniculate. (A) Control. Ketamine (30 mg/kg) caused an increase in activity in the hippocampal molecular layer and a decrease in the medial geniculate nucleus (B). Diazepam pretreatment of ketamine anesthesia caused a decrease in the regional activity in the hippocampal molecular layer compared with that of ketamine alone (C). Droperidol pretreatment of ketamine anesthesia did not discernibly eliminate any ketamine-induced changes in the hippocampal molecular layer and medial geniculate nucleus (D). The numbered structures are (1): medial geniculate nucleus; and (2) hippocampal molecular layer.

11 regions were selected for quantitation after a general survey of the autoradiographs and those areas that seemed to be relevant and large enough for reliable identification were measured. Although the rate of glucose use of brain regions appears to be closely coupled to "functional activity," correlation of behavioral changes to changes in the rate of brain regional glucose use presents an important but difficult challenge. Even simple behaviors involve complex interactions of several brain systems with excitatory and inhibitory features. However, information available from the 2-DG procedure may contribute to understanding the neuroanatomical basis of some behaviors.

Ketamine is known as a "epileptogenic anesthetic"^{17,18} which induces both excitatory and depressant actions in the brain. Winters *et al.*¹ demonstrated an activation of EEG patterns in cats after ketamine injection, which showed a continuous 1-5 Hz hypersynchrony with spikes during catalepsia, and seizure discharge in the dorsal hippocampus. Kayama and Iwama² also found EEG patterns of seizure activity in the neocortex and hippocampus after ketamine injection in cats, which indicated that ketamine stimulated the neocortex, hippocampus, and other subcortical nuclei. Ferren-Allado *et al.*³ demon-

strated that patients receiving ketamine developed electrical seizure activity in the limbic and thalamic areas with uncorrelated behavioral manifestation. In this study, ketamine caused a striking increase in the rate of glucose utilization in the hippocampus, as reported earlier.⁴ These results taken together support the concept that ketamine is an "epileptogenic anesthetic" and implicate the hippocampus as a major focus of hyperactivity.

The depression of glucose use caused by physostigmine in several regions indicates that cholinergic systems of central nervous system tend to reduce metabolic activity. There are conflicting reports that ketamine anesthesia is shortened with physostigmine pretreatment in humans¹¹ and rats,¹⁹ or prolonged in humans¹² and rats.²⁰ In the experiments reported here, physostigmine tended to reduce the time of "agitation," but this did not reach statistical significance and it had no effect on the recovery of the tail flick, righting reflex, or ataxia (table 1). Also, physostigmine pretreatment did not prevent any of the ketamine-induced glucose use changes.

The general suppressing action of diazepam on regional brain glucose use may be related to its enhance-

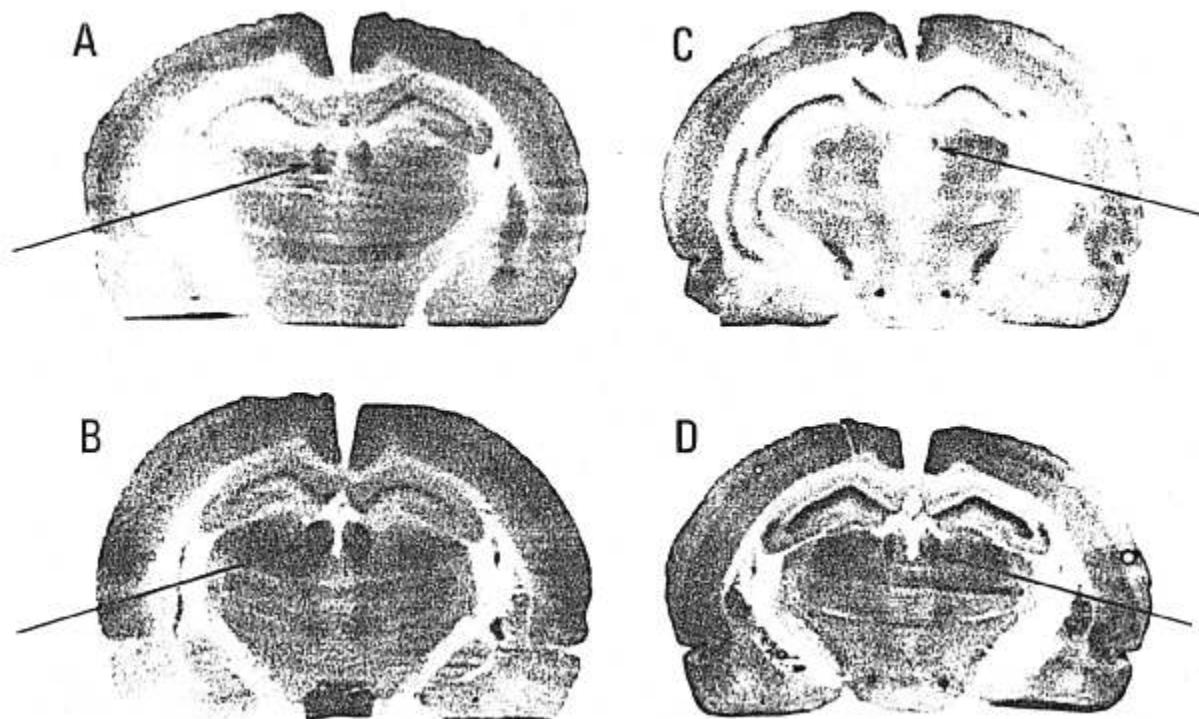


FIG. 2. Representative autoradiographs at the level of the habenula. Lateral habenula is indicated by arrow. (A) Control. The relative optical density in the lateral habenula was strikingly increased by droperidol (0.2 mg/kg) (B). Ketamine (30 mg/kg) caused a decrease in activity of the lateral habenula (C). Droperidol had little influence on the ketamine actions except for preventing the depression of the lateral habenula (D).

ment of ketamine anesthesia and reduction of excitation. Although not quite reaching statistical significance, diazepam, as well as physostigmine, tended to reduce ketamine stimulation of the hippocampus, which may have a bearing on the clinical reduction of hyperexcitability.

The pattern of brain regional glucose utilization induced by ketamine has some similarities to that of lidocaine-induced pre seizure activity reported by Ingvar and Shapiro.²¹ Both ketamine and lidocaine induced an intense activation of the hippocampus and depression

of the inferior colliculus and medial geniculate. However, lidocaine caused an intense activation of Ammon's Horn, whereas ketamine's action was most pronounced on the molecular layer. Also, lidocaine appears to cause a greater depression of the cortex than ketamine. The increased activity in the lateral habenula caused by droperidol may be an important clue to its anti-psychotic effects, and since this persisted with ketamine, it may be involved in the modification of ketamine anesthesia. In this connection, Wechsler *et al.*²² demonstrated that glucose utilization in the lateral habenula was decreased

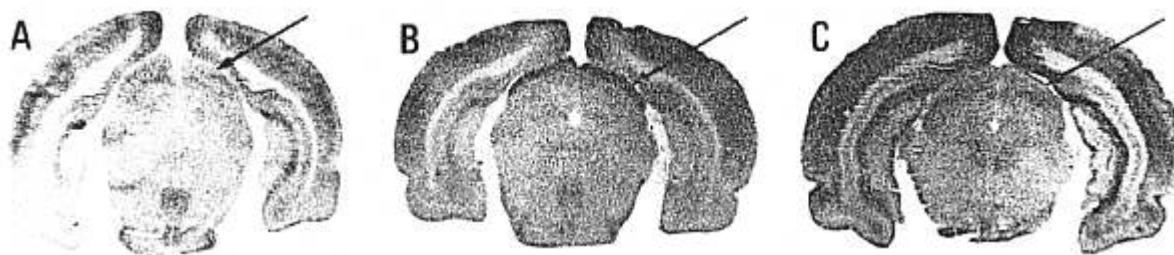


FIG. 3. Representative autoradiographs at the level of the superior colliculus. Superior colliculus is indicated by arrow. (A) Control. Physostigmine (0.4 mg/kg) caused an increase in the regional activity in the superior layer of the superior colliculus (B). Ketamine tended to neutralize the physostigmine action in the superior colliculus (C).

TABLE 3. Regional Glucose Utilization ($\mu\text{mol} \cdot 100 \text{g}^{-1} \cdot \text{min}^{-1}$)

	Ketamine (n = 5)	Ketamine + Droperidol (n = 4)	Ketamine + Diazepam (n = 4)	Ketamine + Physostigmine (n = 4)
Frontal cortex	80 ± 7	64 ± 7	80 ± 7	73 ± 6
Caudate	109 ± 7	113 ± 9	113 ± 9	100 ± 2
Septum	68 ± 6	60 ± 6	63 ± 6	59 ± 2
Hypothalamus	53 ± 6	47 ± 8	46 ± 1	42 ± 2
Vent. thalamus	73 ± 2	68 ± 8	78 ± 3	70 ± 2
Par. cortex	86 ± 9	75 ± 8	89 ± 7	75 ± 2
Hipp. (molecular layer)	166 ± 10	155 ± 20	132 ± 9	140 ± 3
Med. geniculate	80 ± 7	72 ± 7	76 ± 6	71 ± 4
Sup. colliculus	79 ± 7	63 ± 8	82 ± 6	76 ± 2
Inf. colliculus	86 ± 7	86 ± 13	102 ± 7	92 ± 2
Lateral Habenula	72 ± 4	140 ± 16*	79 ± 7	69 ± 5

All values are means ± SE.

* Significant difference at the $P < 0.01$ level.

by amphetamine. We²³ found that haloperidol, a dopamine receptor blocker, increased the rate of glucose utilization in the lateral habenula and prevented the reduction of activity in the lateral habenula caused by amphetamine. We suggest that the increased activity in the habenula with antipsychotic drugs may account for their clinical reduction in hallucinations, vivid unpleasant dreams, and hyperexcitabilities induced by ketamine.

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