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# Ibotenic Acid Lesions of the Medial Septum Retard Delay Eyeblink Conditioning in Rabbits (*Oryctolagus cuniculus*)

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S. Berry and R. Thompson (1979) reported that electrolytic lesions of the medial septum significantly retard eyeblink conditioning. However, these electrolytic lesions were nonselective and may have also damaged the subcortical inputs to the hippocampus via the fimbria–fornix. In the present study, the medial septum was selectively lesioned with ibotenic acid in rabbits (*Oryctolagus cuniculus*), whose performance in a delay eyeblink conditioning paradigm was compared with that of intact controls, sham-operated controls, and intact controls given a systemic injection of scopolamine. Rabbits with selective medial septal lesions and rabbits receiving systemic scopolamine were significantly slower to condition than were intact and sham-lesioned rabbits. This finding demonstrates that the selective removal of the medial septum retards delay eyeblink conditioning in a manner similar to the disruption seen after systemic administration of scopolamine.

One interesting feature of work from classical eyeblink conditioning is the finding that, although a hippocampal lesion does not affect delay eyeblink conditioning (Schmaltz & Theios, 1972), a disruption of hippocampal activity greatly retards delay eyeblink conditioning (Berry & Thompson, 1979; Solomon, Solomon, Vander Shaaf, & Perry, 1983). Classical eyeblink conditioning is a simple associative learning paradigm in which a neutral stimulus (the conditioned stimulus [CS], usually a tone or light) is paired with a response-evoking stimulus (the unconditioned stimulus [US], usually a corneal airpuff or periorbital eye shock). Initially, the tone elicits no behavioral response, whereas the airpuff elicits a reflexive eyeblink. By repeatedly pairing the tone and airpuff, the tone alone comes to elicit an eyeblink response (the conditioned response [CR]).

The hippocampal system is involved in a variety of classical conditioning tasks such as latent inhibition (Shohamy, Allen, & Gluck, 2000; Solomon & Moore, 1975), reversal learning after a simple two-tone discrimination (Berger & Orr, 1983; Buchanan & Powell, 1980), and complex temporal tasks such as trace conditioning (Moyer,Deyo, & Disterhoft, 1990). In trace conditioning, there is a time interval between the offset of the CS and the onset of the US. However, if the CS and US overlap and coterminate as in delay conditioning, the hippocampal system is not necessary. Schmaltz and Theios (1972) found that nonselective hippocampal lesions do not disrupt delay eyeblink conditioning. More recently, Shohamy et al. (2000) reported that ibotenic acid lesions which selectively damage the dorsal hippocampus do not disrupt simple delay eyeblink conditioning.

However, if hippocampal activity is disrupted, the acquisition of CRs in delay eyeblink conditioning is retarded. Berry and Thompson (1979) demonstrated this hippocampal disruption effect through lesions of the medial septum that disrupted hippocampal theta activity along with hippocampal activity related to CRs. Damage to the medial septum retarded classical eyeblink conditioning such that the appearance of CR responses was delayed. However, once CRs did appear, acquisition continued at a normal rate.

The medial septum is a subcomponent of the basal forebrain cholinergic system that consists of a constellation of cholinergic and GABAergic neurons. Within the rostral basal forebrain, cholinergic neurons originating in the medial septum and the diagonal band nuclei project axons that terminate within the hippocampus.

Much attention has been paid to the medial septal modulation of the hippocampus through cholinergic projections. Many eyeblink conditioning experiments involving the pharmacological administration of the cholinergic antagonist scopolamine, which blocks muscarinic receptors, have been reported (Harvey, Gormezano, & Cool-Hauser, 1983, Harvey, 1985; Moore, Goodell, & Solomon, 1976; Powell, 1979; Powell, Hernandez, & Buchanan, 1985; Salvatierra & Berry, 1989; Solomon & Gottfried, 1981; Solomon et al., 1983). Scopolamine, either administered systemically or infused directly into the medial septum, significantly retards eyeblink conditioning in rabbits (Solomon & Gottfried, 1981).

However, scopolamine administered directly to the dorsal hippocampus does not retard eyeblink conditioning (Solomon & Gottfried, 1981). Therefore, it seems that the site of action of the cholinergic disruption is within the medial septum and not the hippocampus. This would imply that the effect of lesioning the medial septum should be equivalent to blocking muscarinic receptors in the medial septum. However, the scopolamine retardation

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effect has not been directly compared with the retardation effect seen after medial septal lesions.

There is another open issue from the medial septal literature that is based on the nature of the medial septal lesions of Berry and Thompson (1979). Their lesions were electrolytic in nature and could have damaged not only the targeted medial septal neurons, but also axons passing close to the site of the lesion, as well as the source of these axons. The electrolytic lesions of Berry and Thompson may have damaged the axons that pass bidirectionally between the medial septum and the hippocampus via the fimbria–fornix and thereby disrupted hippocampal activity. Port and Patterson (1984) found that fimbrial lesions disrupted sensory preconditioning in rabbit eyeblink conditioning. Thus, there remains some question as to whether the Berry and Thompson lesion effect is due to damage to neurons in the medial septum or damage to fibers of passage in that region.

Therefore, we made a selective lesion of the medial septum through the injection of ibotenic acid (Jarrard, 1989) to determine whether the selective loss of medial septal neurons would retard eyeblink conditioning. We also compared these selective medial septal lesion results to the retardation seen after systemic scopolamine injections.

## Method

### *Subjects*

A total of thirty-four male New Zealand albino rabbits were used in this study. They were individually housed in the Association for the Assessment and Accreditation of Laboratory Animal Care-approved Rutgers Animal Facility. Rabbits had ad lib access to food and water and were maintained on a 12-hr light–dark cycle. Eight rabbits served as unoperated controls. Twenty rabbits underwent surgery in aseptic conditions, in which 11 received ibotenic acid lesions of the medial septum and 9 received sham surgeries (in which only the vehicle solution was injected). All surgical and testing procedures were compatible with the American Psychological Association's policy on animal research. Six rabbits were intact controls given a systemic injection of scopolamine before each daily conditioning session.

### *Selective Lesion Surgery*

After delivery to our housing facility, rabbits were allowed 1 week of recovery before surgery. Rabbits were weighed, and a baseline respiration rate was taken. They were then given a subcutaneous injection of Xylazine (6.0 mg/kg). Fifteen minutes later, they were given an intramuscular injection of Ketamine (60.0 mg/kg). After another 15 min, they were given a 1 cc intramuscular injection of a 2:1 Ketamine/Xylazine mixture. This last injection was repeated hourly until the end of the surgical procedure. The rabbit's head was shaved and scrubbed with a Betadine scrub, followed by isopropyl alcohol and Betadine prep solution.

The rabbit was placed in a standard stereotaxic head holder (David Kopf Instruments, Tujunga, CA). An incision was made along the midline of the skull. The skin and muscle were deflected to expose the skull and the bregma and lambda landmarks. The head was leveled so that lambda was 1.5 mm higher than bregma. Two small holes were drilled in the skull above the site of the hippocampus, exposing the dura. Two additional small holes were drilled for screws to anchor a dental acrylic headstage.

The stereotaxic coordinates for injection sites were calculated in the anterior– posterior plane from bregma, in the medial–lateral plane from the midline, and in the dorsal–ventral plane from the skull surface. Injections were made at the following coordinates: AP −3.0, ML ±0.5, DV −9.0, −11.0 (injection = 0.15 μl); AP −2.0, −1.0, ML ±0.5, DV −9.0, −11.0 (injection = 0.20 μl); and AP −1.0, ML ±1.0, DV −11.0, −13.0 (injection = 0.10 μl).

The rabbits that received selective medial septal lesions were injected with ibotenic acid (10 μg/μl; Sigma, St. Louis, MO). Rabbits in the sham lesion group were injected only with the saline vehicle solution. For each injection, the needle was lowered to the injection site, and 1 min was allowed to elapse before injection began. The injection took place over a 2-min period. After the injection, 2 min were allowed to elapse before the needle was moved to the next injection site. This procedure was repeated for all the injection sites.

After all the injections, the holes in the skull were sealed with bone wax. Two stainless steel anchoring screws were mounted in the skull, and the exposed skull was covered with dental acrylic. A bolt was attached in the acrylic for the mounting of the eyeblink detector assembly during conditioning. Rabbits were allowed 1 week for recovery from the surgery before the initiation of behavioral conditioning.

### *Materials*

The rabbits were restrained in standard restraint boxes in individual conditioning chambers. Each chamber contained a speaker, air hose assembly, and eyeblink detection system.

Presentations of the stimuli and recording of behavioral responses were controlled by a PC. The computer housed an interface board (Keithley Metrabyte, Keithley Instruments, Taunton, MA), which triggered a set of relays that controlled the presentation of the tone CS and airpuff US.

Eyeblinks were monitored with an electronic sensor that consisted of an LED and a phototransistor (for technical details see Thompson, Moyer, Akase, & Disterhoft, 1994). The LED emitted a beam of infrared light that was reflected off the cornea, and the reflectance of this beam was converted to a DC voltage by a phototransistor. The eyeblink signal was filtered (between 0.1 Hz and 1.0 kHz) and amplified (×100) by a differential AC amplifier (A-M Systems, Everett, WA).

When the rabbit closed its eye, the reflectance of the infrared beam changed and was recorded as an eyeblink. Any movement greater than 0.5 mm during the pre-CS period caused the training trial to be discarded from analysis. A CR was scored if movement of greater than 0.5 mm was seen during the CS period. Each trial's behavioral record was displayed on the computer screen. The computer analyzed the behavioral data and delivered the data for each block of 50 trials.

### *Stimuli*

The CS was a 450-ms, 90-dB, 1000-Hz tone delivered from a speaker located 25 cm forward of the rabbit's ears. The US was a 50-ms, 3-psi corneal airpuff delivered by means of a rubber hose attached to the eyeblink detector assembly and aimed at the rabbit's cornea. All training trials consisted of a paired presentation of the tone and airpuff, such that the tone onset preceded the airpuff onset by 400 ms and the two stimuli coterminated. There was a pseudorandom intertrial interval that was no shorter than 25 s.

### *Design and Procedure*

Before acquisition, all rabbits were adapted to the conditioning chamber and restraint box for two daily sessions. On the 1st day of adaptation, the rabbit was placed in the restraint box in the conditioning chamber for 30 min. On the 2nd day of adaptation, the rabbit was placed in the restraint box in the conditioning chamber, with the eyeblink detector aimed at its cornea, for 45 min.

Scopolamine hydrobromide (Sigma) was mixed with 0.9% (wt/vol) sterile saline vehicle (2.0 mg/ml). Each day, rabbits were given subcuta-

neous injections into the scruff of the back at a dosage of 2.0 mg/kg, 10 min before the start of acquisition training.

Rabbits received a total of 500 paired presentations of the CS tone and US airpuff over five consecutive daily training sessions. The training criterion was defined as eight CRs out of nine consecutive trials.

After completion of conditioning, the rabbits were overdosed with an intravenous injection of sodium pentobarbital via the marginal ear vein and perfused via the ascending aorta with 1 L of 0.9% saline solution followed by 1 L of a 10% (vol/vol) Formalin solution. The brain was then removed and preserved in a 30% (wt/vol) sucrose–10% Formalin solution for 1 week. Serial coronal 80-micron sections through the medial septum were taken, mounted on slides, and stained with cresyl violet. The lesions were analyzed for size and location by viewing under a microscope.

### *Data Analyses*

The behavioral data were analyzed for the percentage of CRs, grouped in 50-trial blocks, with a repeated measures analysis of variance. To test for the expected medial septal lesion and scopolamine effects, we made planned pairwise comparisons between each of the experimental conditions (medial septal lesion and scopolamine) and the control conditions (intact and sham lesioned). We also made planned pairwise comparisons between the two experimental conditions (medial septal lesion and scopolamine) and between the two control conditions (intact and sham lesioned) to compare the equivalence of our experimentally induced disruptions of septohippocampal processing.

The size and location of the medial septal lesions were assessed by viewing the slices under a microscope and drawing the area of damage for each lesion onto a template of a corresponding sham-lesioned section. The area of the lesion was then calculated as a percentage of the area of the sham-lesioned section.

## Results

### *Histology*

Of the 20 rabbits that underwent surgery, 11 received ibotenic acid injections to the medial septum, and 9 had sham lesions that did not damage the medial septum. Overall, sham-lesioned controls had no damage to the medial septum, whereas the selective septal lesions destroyed on average 67% (range = 45%–90%) of the intact medial septum.

Figure 1A shows the minimal extent of a medial septal lesion, and Figure 1B shows the maximal extent of a medial septal lesion. The histology templates were adapted from a stereotaxic atlas of the rabbit brain (Urban & Richard, 1972). Figure 1C shows a sham lesion that left the medial septum intact, and Figure 1D shows a

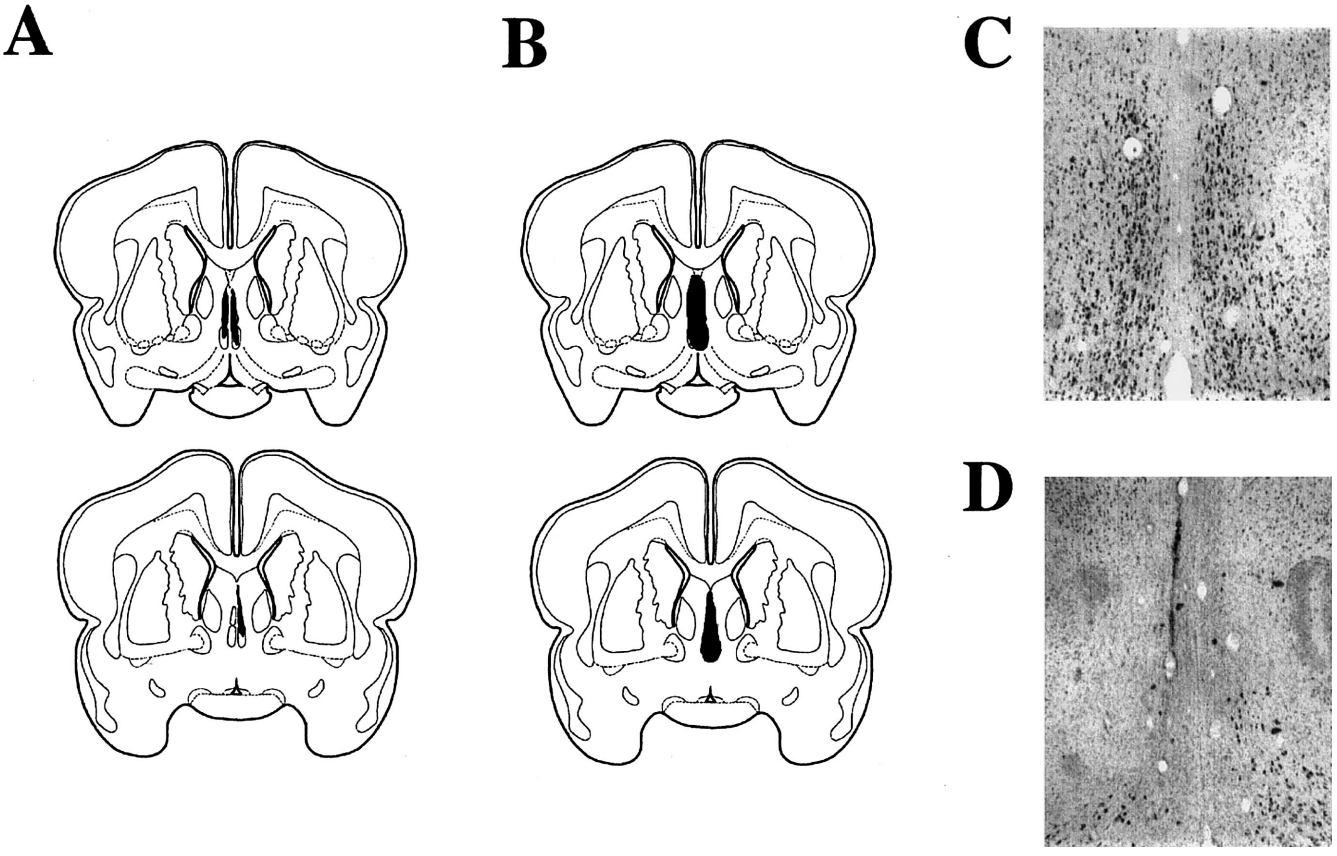


*Figure 1.* Histology of medial septal lesions. A: Location and size of the minimal medial septal lesion, represented by the black area. B: Location and size of the maximal medial septal lesion, represented by the black area. From *A Stereotaxic Atlas of the Rabbit Brain*, by I. Urban and P. A. Richard, 1972, Springfield, IL: Charles C Thomas. Copyright 1972 by Charles C Thomas. Adapted with permission. C: A photomicrograph of an intact medial septum at a magnification of ×40. D: A photomicrograph of a representative selectively lesioned medial septum at a magnification of ×40.

representative lesion that destroyed most of the medial septal neurons.

### *Behavioral Results*

The 20 rabbits that had undergone septal lesion surgery were analyzed as a medial septal-lesioned group ($n = 11$) and a sham-lesioned control group ($n = 9$) and were compared with the intact control group ($n = 8$) and the scopolamine group ($n = 6$).

All rabbits gave more CRs to the tone as training progressed, $F(4, 120) = 176.82$, $p < .01$. Figure 2 shows the learning curves for the intact controls, the sham-lesioned controls, the selective medial septal-lesioned group, and the intact group that received systemic scopolamine injections. Overall, there was a significant difference in conditioned responding between the four groups, $F(3, 30) = 14.26$, $p < .01$. There was a significant Group × Session interaction, $F(12, 120) = 7.06$, $p < .01$.

*Medial septal lesions.* Pairwise comparisons revealed that the medial septal-lesioned rabbits exhibited significantly fewer CRs than either the intact controls, $F(1, 17) = 17.40$, $p < .01$, or the sham-lesioned controls, $F(1, 18) = 26.67$, $p < .01$. There was also a Group × Session interaction for the medial septal-lesioned group and the intact control group, $F(4, 68) = 6.21$, $p < .01$, and for the medial septal-lesioned group and the sham-lesioned group, $F(4, 72) = 10.78$, $p < .01$.

*Scopolamine.* Rabbits receiving scopolamine exhibited fewer conditioned responses than did either intact controls, $F(1, 12) = 18.65$, $p < .01$, or the sham-lesioned controls, $F(1, 13) = 28.73$, $p < .01$. There was also a Group × Session interaction for the scopolamine group and the intact control group, $F(4, 48) = 4.58$, $p < .01$, and for the medial septal-lesioned group and the sham-lesioned group, $F(4, 52) = 8.72$, $p < .01$.

There were no significant differences or interactions in conditioned responding between the intact control group and the sham-lesioned group or between the scopolamine group and the medial septal-lesioned group ($p > .05$).

## Discussion

Our selective ibotenic acid lesions of the medial septum replicated the Berry and Thompson (1979) electrolytic medial septal lesion retardation effect. All rabbits eventually exhibited CRs; therefore, the effect of our selective medial septal lesions was to retard, not block, eyeblink conditioning. Overall, medial septal-lesioned rabbits were significantly slower to acquire conditioned eyeblinks as compared with both unoperated controls and sham-lesioned controls. This medial septal lesion retardation effect did not significantly differ from the retardation effect observed after administration of systemic scopolamine. Therefore, the medial septal lesion effect can be obtained with a selective ibotenic acid lesion that spares fibers of passage through the fimbria–fornix from the basal forebrain to the hippocampus.

In addition, it was not necessary to completely lesion the medial septum to obtain a significant lesion effect. Quantitative analysis of medial septal lesions indicates that at least a 45% loss of cholinergic neurons is sufficient to disrupt eyeblink conditioning. This corresponds to Berry and Thompson's (1979) medial septal lesions, which damaged 35%–50% of the medial septum. Therefore, it appears that incomplete damage of the medial septum is capable of disrupting delay eyeblink conditioning.

The role of the medial septum in learning and memory has been explored in detail by both qualitative theories and computational models. Many of these have focused on modulation of the hippocampus by acetylcholine. Hasselmo (1995) interpreted the release of acetylcholine as a means whereby the dynamics of the hippocampus could vary on a continuum between two modes: the storing of new information or the recall of previously stored information.

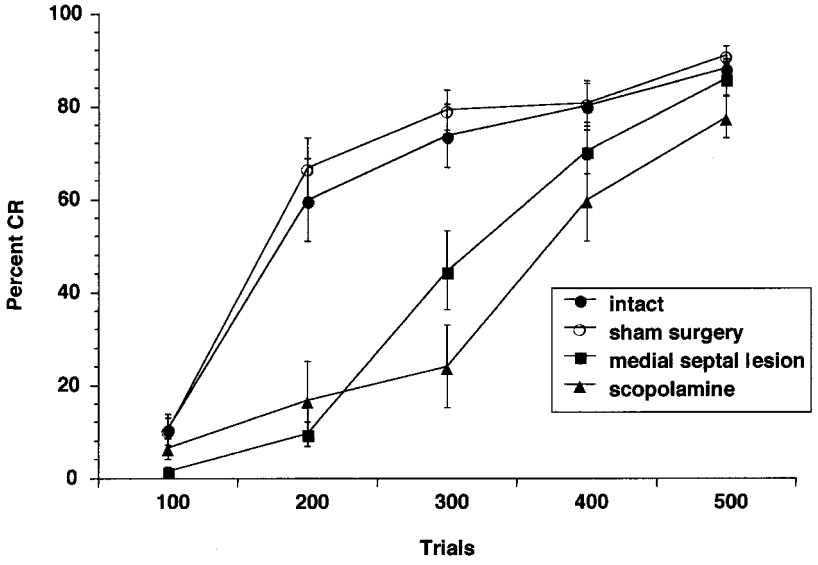


*Figure 2.* Behavioral results from the intact, sham-lesioned, systemic scopolamine, and selective medial septal-lesioned groups. All groups exhibited conditioned responses (CRs), but the selective medial septal-lesioned group and the systemic scopolamine group were slower to acquire CRs than either the intact or sham-lesioned groups.

On the basis of the Hasselmo (1995) theory, Myers et al. (1996) proposed a septohippocampal computational model that can account for the effects of septohippocampal disruption. In the Myers et al. (1996) septohippocampal model, the amount of storage in the hippocampal region network is governed by the learning rate parameter in that network. The rate at which information is transferred from hippocampal to cortical storage is governed independently by the learning rate of the lower layer of the cortical network. Therefore, altering the hippocampal learning rate is equivalent to selectively reducing hippocampal storage without affecting hippocampal recall and transfer to the cortex. Thus, this simple mechanism of adjusting hippocampal learning rate is enough to affect processing in the way hypothesized by Hasselmo (1995) to occur after septal damage.

In addition to the cholinergic septohippocampal projections, the medial septum also sends GABAergic projections to the hippocampus (Brazhnik, Vinogradova, Stafekhina, & Kitchigina, 1993; Freund & Antal, 1988), which may allow the hippocampus to switch between theta and sharp wave states (Berry & Thompson, 1979; Buzsaki & Eidelberg, 1983). Hippocampal waves operate in two distinct modes: alternating periods of theta waves (rhythmic, 4–8 Hz oscillations), and sharp waves characterized by nonrhythmic bursting activity (Fox, Wolfson, & Ranck, 1983). Theta waves occur during exploratory behavior such as walking and sniffing, whereas sharp waves occur during such behaviors as grooming and eating (Vanderwolf & Leung, 1983).

Buzsaki (1989) suggested that this alternation between theta and sharp-wave states corresponds to two phases of hippocampal-system processing. Theta represents a storage phase, in which incoming information is stored in the hippocampus. Sharp waves indicate a recall or consolidation phase, during which stored hippocampal memories are reinstated for gradual transfer to neocortex; this reinstatement is the septum-mediated recall function that Hasselmo proposes. Thus, the cholinergic and GABAergic septohippocampal inputs may interact and provide complementary functions.

The findings presented here were from selective lesions that only damaged medial septal neurons, but they were also nonspecific in that they probably damaged both cholinergic and GABAergic neurons. Recently, a more specific lesion technique was developed that allows for the selective lesioning of basal forebrain cholinergic neurons with 192 IgG saporin (Heckers et al., 1994; Wiley, Oelmann, & Lappi, 1991). Saporin is a ribosome-inactive cytotoxin that, when conjugated with the same monoclonal antibody used for p75-nerve growth factor neurons, can be used to recognize and destroy only cholinergic basal forebrain neurons. Studies in rats have shown that selective cholinergic lesions disrupt some forms of learning (Baxter, Bucci, Gorman, Wiley, & Gallagher, 1995). If this selective cholinergic lesion technique could be successfully applied to rabbit eyeblink conditioning, it would help to complete our understanding of the septal modulation of hippocampal learning in eyeblink conditioning.

At present, we have presented evidence that loss of medial septal neurons retards rabbit eyeblink conditioning in a manner similar to that observed after systemic administration of scopolamine. However, future work needs to be done to further explore and differentiate the specific effects of cholinergic and GABAergic modulation of the hippocampus in eyeblink conditioning.

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