The Role of the Central Nucleus of the Amygdala in Feeding and Monitoring Internal State Cues in the Rat

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Running Head: Feeding and Internal Cues

Abstract

These experiments examined the role of the central nucleus of the amygdala (CN) in rats' ability to use internal and external cues as signals for shock. Half of the animals received Pavlovian discrimination training prior to ibotenate lesions of the CN while the other half were trained after surgery. Rats were placed on an alternating 24hr, 0hr food deprivation schedule and were shocked under one of the two levels of deprivation in combination with different auditory stimuli. During extinction, rats were tested with deprivation cues alone, then with auditory cues alone. Percentage of freezing under each condition served as the index of discrimination.

Rats trained prior to surgery were able to learn to discriminate between shocked and non-shocked conditions. In postoperative extinction, they did not show discrimination between levels of deprivation, but did discriminate between auditory stimuli. Rats trained after surgery were unable to learn either discrimination. In a separate experiment, the amount of food and water consumed did not change, but CN rats were more active than controls.

These results suggest that the CN plays an important role in acquisition, but not retention of fear conditioning. Furthermore, it appears that there are different mechanisms that underlie the utilization of internal and external cues.

Anatomically, the amygdala consists of several nuclei that have only recently been studied separately from one another. The earliest recognized subdivisions in the amygdala were the corticomedial and basolateral nuclei (McDonald, 1992). Later work by Krettek and Price (cited in McDonald, 1992) showed further cytoarchitectural divisions that are now considered to be the main nuclei of the amygdala.

Most of the early lesion studies of the amygdala consisted of complete bilateral ablations of the area. Results from these studies showed the amygdala to be involved in emotion and fear, as well as several aspects of memory (McDonald, 1992). These findings provided a foundation for amygdaloid function, but due to the different afferent and efferent connections of the nuclei it soon became necessary to focus on them separately. The latest research on the amygdala further supports the idea that the nuclei are each involved differently in many complex brain functions.

One of the most widely studied nuclei is the central nucleus (CN). Located in the caudal third of the amygdala, it continues nearly to the caudal tip of the amygdaloid complex (Amaral et al., 1992). The CN projects to many regions of the brain including the midline nuclei of the thalamus, lateral hypothalamus, and the brain stem. This last projection is most interesting because the CN is the only nucleus of the amygdala that sends fibers to the brain stem. These fibers descend into and through the midbrain, pons, and medulla and innervate other structures such as the periaqueductal gray, parabrachial nucleus, dorsal vagal nuclei, and the reticular

formation. In addition, fibers from these structures ascend to CN (Amaral et al., 1992). Within the amygdaloid complex itself, the CN seems to be the focus of projections from other nuclei. Sensory information that enters the amygdala through the lateral nucleus is sent to the basal nucleus which then projects it to the CN (Amaral et al., 1992).

The blockade of fear conditioning by CN lesions is well documented. Heart rate conditioning (Kapp et al., 1979), fear-potentiated startle using both visual and auditory stimuli (Davis, 1992), conditioned emotional response (CER), conditioned freezing, and conditioned blood pressure response (Spevack, Campbell & Drake, 1975) have all been shown to be attenuated by these lesions. It should be noted that each of these studies examines only the conditioning and not the retention of information. Studies of retention of fear conditioning have only recently emerged. Thus, Roozendaal and associates (1993) reported that rats trained prior to surgery in either passive or active shock avoidance tasks were not impaired in extinction testing. However, rats trained following surgery were unable to acquire the avoidance behavior. This suggests that the CN is important in acquisition, but not retention of fear conditioned responses.

H.M. serves as the classic example of memory deficiencies following damage to medial temporal structures in the brain. However, as pointed out by Hebben and associates (1985), much less attention has been given to H.M.'s other deficits. They reported that following bilateral resection of the uncus, amygdala, anterior

hippocampus, and parahippocampal gyrus, H.M. was extremely impaired in identifying his metabolic state. Unlike other amnesics and controls in the study, H.M. rated his state of hunger or thirst the same both before and after a meal. In the study, there was one amnesic control who, like H.M., suffered from complete bilateral damage to the amygdala. He also exhibited difficulty rating his internal state. From this finding, Hebben et al. (1985) reported that "deficient monitoring of internal states by these two patients may be due to the involvement of the amygdala in both cases."

Animal studies of amygdala lesions have, to this point, provided conflicting evidence as to the changes in feeding behavior following surgery. Goddard (1964) reported that some researchers found no differences in food intake, some found an increase, and some found a decrease. In some cases, both a decrease and an increase occurred with the decrease always coming first. From his own research, Goddard (1964) reports a longer lasting hyperphagia following a severe, but transient increase in food consumption. The hyperphagia was characterized by constant nibbling and did not seem to involve high food motivation. Grossman and Grossman (1963) attempted to localize the region of the amygdala that is involved in the regulation of food intake. Studying the ventral aspect of the amygdala, they found that lesions always increased food intake while electrical stimulation produced the opposite effect.

In their study of eating behavior following discrete lesions of the amygdala, Schoenfeld and Hamilton (1981) reported that CN lesions did not result in a decrease in food intake. They also

found that basal and lateral nuclei damage impairs the formation of taste aversion in rats. Later research (Simbayi et al., 1986) implicated the basolateral area of the amygdala in the control of food and fluid intake after localized lesions of this area produced deficits in conditioned taste aversion. An earlier study by Box and Mogenson (1975) reports a discrete location in the area of the CN that produced temporary aphagia and adipsia. This finding directly contradicts that of Schoenfeld and Hamilton (1981). It is obvious from these reports that the effects of both amygdalectomies and more discrete lesions on food intake are as yet unclear. The only agreement seems to be that the basolateral area of the amygdala is somehow responsible for conditioned taste aversion.

The changes in feeding behavior that may accompany lesions of the amygdala could be due, in part, to an inability to use internal state cues. The procedure reported by Davidson, Flynn, and Jarrard (1992) provides a method of testing whether animals are able to utilize these interoceptive cues. In their experiment, rats learned to use food deprivation intensity cues as signals for shock in three reinforced training trials. In addition, rats can learn about these cues in the presence of relevant auditory cues. The rats were placed on a diet that alternated daily between 24hr and 0hr deprivation. During training, they were placed in small conditioning boxes equipped with a grid floor that could be electrified. Rats were divided into two groups. One group was shocked in the conditioning boxes when they were 24hr food deprived and not shocked during the 0hr condition. The second group received

the reverse contingency. Both groups received three training sessions under each deprivation condition. After training was completed the rats were given one 4-minute extinction session per day for eight days. The deprivation levels were alternated as they were during training. No shocks were administered during these sessions. A videotape of the extinction sessions was scored and the percentage of freezing became the index of conditioning.

The findings from this experiment showed that the percentage of freezing during 24hr deprivation was greater for animals that had been shocked under this condition than those that did not receive a shock. The same was true for the 0hr extinction sessions. In other words, the percentage of freezing was greater in extinction sessions for rats that had been shocked under the same condition. In addition, rats were slower to extinguish the behavior under their shocked condition than they were the non-shocked condition. It is apparent from this study that "food deprivation intensity cues can acquire discriminative control over responding" (Davidson et al., 1992).

Using a similar procedure, Davidson and Jarrard (1993) tested rats with lesions of the hippocampus. In addition to the food deprivation cues, rats were trained with two auditory stimuli (i.e. tone and clicker). Half of the rats were shocked during presentation of the tone while the other half received the reverse contingency. During extinction trials when no shocks were delivered, the two stimuli, deprivation and auditory, were tested separately. Hippocampals did not exhibit greater freezing to the

shocked deprivation level thus indicating that they were unable to discriminate between their internal state. However, they had no trouble distinguishing the auditory cues and did not differ from controls in their percentage of freezing to the shocked stimulus.

In the present research, the role of the CN in the use of internal and external cues as signals for shock is examined. The procedure is similar to that of Davidson and Jarrard (1993) described above. A separate experiment considers the effects of CN lesions on eating, drinking, and activity in the rat.

Experiment 1 Method

Subjects and Apparatus

The subjects were 16 naive male Sprague-Dawley derived rats. All rats were individually housed for the duration of the experiment.

The monitoring system consisted of 18 stainless steel cages that were separated from each other by wooden insulated barriers. The boxes were controlled by a personal computer that recorded the number of contacts with the food hopper and water bottle. An ultrasonic measuring device connected to the computer was used to monitor the activity of each rat. The computer sampled each of these variables every five seconds for 23hr per day. Amount of food and water consumed each day was measured.

Procedure

Rats were placed in the system for 13 days prior to surgery in order to establish a baseline measurement for each variable. Eight rats received CN lesions while the remaining eight were

anesthetized. Following surgery, rats were returned to the system for another 18 days.

Surgery

Subjects were anesthetized by intraperitoneal (ip) injections of equithesin. CN lesions were made with bilateral injections of .08 microliters of ibotenic acid (Jarrard, 1989) at -2.0 AP, +-3.8, -7.2 DV and -3.0, +-4.1, -7.2. Four rats were sham operated. The pipette was placed at each coordinate, but no neurotoxin was administered. The other four received ip injections of equithesin.

Histology

At the end of each experiment, rats were given ip injections of chloral hydrate and then perfused with physiological saline followed by 10% formalin. The brain was removed and embedded in egg yolk before sectioning. 40 micron sections were mounted and stained with cresyl violet.

Histology was obtained from rats in Experiment 2a. Lesions appeared to be quite small and included the CN more than any other structure. Damage outside of the CN was minimal and seemed to only involve the basal and lateral nuclei of the amygdala. Bilateral damage appeared in all rats except one. This rat did show lesions in both hemispheres, but one side was much less damaged than the other.

Results

There was no difference between the groups in amount of food eaten, but the interaction of group X blocks of days showed a significant effect $F(9,117) = 5.89$, $p < .0001$. The increase by CN

rats seemed to be a rebound from the significant drop in intake the first two days following surgery. However, by the end of Block 5, this difference had disappeared.

Water intake was similar to that of food consumption except that CN rats consistently drank less than controls. Again, groups did not differ, but the group X blocks interaction was significant $F(9,117) = 4.88$, $p < .0001$. Differences between groups only occurred at Block 2.

The number of contacts with the food hopper was not significantly different between groups, $F(1,13) = 1.64$, $p = .2221$. Contacts did differ between day and night hours, $F(1,13) = 106.22$, $p < .0001$, as expected. The interaction of groups X blocks of days was significant, $F(8,104) = 4.41$, $p = .0001$, indicating that across days, there were differences between groups in the number of contacts. The triple interaction of groups X blocks X day/night was also significant, $F(8,104) = 3.57$, $p = .0011$. Both interactions seem to be due to the extremely low number of contacts by CN rats during the night immediately following surgery. Overall, CN rats appeared to have more contacts with the food hopper than did controls, but this difference lasted only to the fifth block of days.

Groups differed significantly from each other in the number of contacts with the water bottle, $F(1,13) = 17.51$, $p = .0011$, with CN rats having fewer contacts. This result is consistent with the trend for CN rats to drink less than controls. It is interesting to note that this difference occurred only during the nighttime hours,

$F(1,26) = 31.45$, $p < .0001$, and not during the day.

Figure 1 shows the mean activity for CN rats and controls across blocks of days. Groups differed from each other overall, $F(1,13) = 5.20$, $p = .0402$, with CN rats being more active.

Insert Figure 1 about here

The triple interaction of groups X blocks X day/night was also significant, $F(8,104) = 3.56$, $p = .0011$. Further analysis revealed that CN rats were more active during the day for the first four blocks of days following surgery, but returned to the normal pattern of increased activity at night for the last two blocks of days and did not differ from controls.

Experiment 2a Method

Subjects and apparatus

Sixteen naive male Sprague-Dawley derived rats were singly housed throughout the experiment.

Subjects were trained and tested in four identical conditioning chambers. Each chamber had Plexiglas sides and ceiling and aluminum end walls. The floor consisted of stainless-steel rods that could be electrified. The chambers were housed together in a sound-attenuating room and a low-light video camera was used to monitor activity in all four chambers simultaneously. All stimuli and recordings were controlled by a computer and relay equipment located in an adjacent room.

Procedure

Rats were divided into two groups of eight rats each. All subjects were then placed on an alternating 24hr and 0hr food deprivation schedule. During 24hr deprivation, food was withheld for approximately 24 hours prior to a deprivation session and was continuously available for 24 hours prior to a 0hr session. Subjects were run in four squads of four rats, each counterbalanced with respect to the original grouping. In addition, an equal number of rats from both groups were assigned to each conditioning chamber.

Training sessions lasted for 4 minutes and consisted of the presentation of an auditory stimulus, either a tone or a clicker, in conjunction with the level of deprivation. The auditory stimulus was presented for the duration of the session. Four rats were shocked under each of the possible combinations of stimuli (24hr+tone, 24hr+clicker, 0hr+tone, 0hr+clicker) following a counterbalanced order. Shock was delivered (0.5mA, 0.5 sec) at the end of the 4 minute session. Rats were removed from the conditioning chamber approximately 45s after the session ended.

All subjects received nine training sessions under each level of deprivation and auditory stimulus for a total of 18 sessions. At the end of training, half of the rats were given CN lesions identical to those described in Exp. 1.

After 10 days of recovery, subjects began extinction testing sessions. Only one stimulus was tested at a time. During testing for food deprivation level, rats were placed in the conditioning

boxes for four minutes. No shocks and no auditory stimuli were delivered. Testing continued for 10 days so that each rat received five presentations of each level of deprivation.

In order to achieve an internal state that was different from those induced by the food deprivation schedule, rats were 17 hours water deprived prior to each extinction session for auditory stimuli. For testing, subjects were returned to the conditioning boxes for four minutes. During this time one auditory stimulus was delivered. Rats received two presentations of each auditory stimulus.

The video tape recordings of all training and extinction sessions were scored. Samples were taken every two seconds so that each rat received a score every eight seconds. Freezing was determined to be the absence of all muscular movement except that which is necessary for respiration.

Results

In acquisition, there was no difference between groups in percentage of freezing, $F(1,14) = .06$, $p = .8068$. This was expected since all rats were still unoperated. The percentage of freezing across days differed $F(5, 70) = 29.43$, $p < .0001$, indicating that rats learned to discriminate between shocked and non-shocked conditions. Figure 2 shows the means for these two conditions.

Insert Figure 2 about here

Groups differed significantly in deprivation extinction,

$F(1,14) = 15.74$, $p = .0014$. Figure 3 presents the mean percentage of freezing for each group in shocked and non-shocked conditions.

Insert Figure 3 about here

It is apparent that CN rats showed no discrimination between conditions. The interaction of groups X blocks of days was significant, $F(9,126) = 3.40$, $p = .0009$. Further analysis revealed that groups differed in percentage of freezing for all but the last two presentations of the shocked stimulus (block 5). CN rats were not only unable to discriminate between the shocked and non-shocked deprivation level, but showed very little freezing overall.

In auditory extinction, CN rats froze significantly less than controls, $F(1,14) = 8.00$, $p = .0134$ (see Fig. 4). However, CN rats did differ in percentage of freezing to the shocked versus the non-shocked conditions, $F(3,42) = 11.64$, $p < .0001$. This result shows that CN rats were able to discriminate between the shocked and

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Insert Figure 4 about here

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non-shocked conditions when external, auditory stimuli were used and learning occurred before the operation.

Experiment 2b Method

Procedure

Rats from Exp. 1 served as the subjects for this experiment. The procedure is identical to that of Exp. 2a with 18 days of

acquisition and 18 days of extinction. In this experiment, CN lesions were made prior to the start of training.

Results

Rats with CN lesion froze less than controls in acquisition, $F(1,14) = 91.40$, $p < .0001$. Examining the means for each group under shocked and non-shocked conditions (see Fig. 5), it is apparent that CN rats were unable to learn the discrimination.

Insert Figure 5 about here

The interaction of groups X blocks of days was also significant, $F(5,70) = 4.55$, $p = .0012$. Further analysis revealed that the groups differed in percentage of freezing at every block. This result supports previous findings that CN lesions block acquisition of fear-conditioning.

In deprivation extinction, CN rats froze significantly less than controls, $F(1,14) = 9.50$, $p = .0081$. The interaction of groups X blocks of days, $F(13,182) = 5.08$, $p < .0001$, revealed that CN rats froze significantly less than controls during the first four blocks of the shocked stimulus and only the first block of the non-shocked. It is apparent that CN rats were not able to discriminate between the levels of deprivation as was the case in controls.

Groups differed significantly in auditory extinction, $F(1,14) = 16.48$, $p = .0012$, with CN rats freezing less compared to controls. CN rats exhibited almost no freezing to either stimulus. These results suggest that CN rats were not able to discriminate

between auditory cues in extinction due to their inability learn the original discrimination.

Discussion

The results from Experiment 1 show that selective lesions of the CN have very little effect on feeding behavior in the rat. While CN rats exhibited increased activity, there was no difference in the amount of food and water consumed compared to controls. Since all rats in the present experiment, including controls, showed a decrease in food and water intake immediately following anesthesia it is apparent that the surgical procedure itself, regardless of the lesion, has some effect on feeding. The increase in food consumption following this drop seemed to be an attempt to return body weight to near preoperative level. The results of the present experiment agree with Schoenfeld and Hamilton (1981) and suggest that other areas of the amygdala may be more important in control of feeding behavior. Selective lesions of other areas of the amygdala will be necessary in order to establish nuclei are involved in feeding behavior.

Pascoe, Supple, and Kapp (1991) present the CN as the location where CS and US information meet, are associated and the accompanying CR is formed. They use the example of conditioned bradycardia, but it seems likely that the CN would act in a similar manner for any type of Pavlovian conditioning. If this is the case, then lesions of the CN would block the formation of CS-US associations and the corresponding CR would not be exhibited. The results from Experiment 2b lend support for this hypothesis.

Rats trained prior to surgery (intact CN) were able to learn the fear-conditioning discrimination and later exhibit retention of this learning in postoperative testing. However, those trained following CN lesions were unable to acquire the CR at all. These results suggest that rats trained before surgery made the CS-US association, exhibited the CR (freezing), and then stored this information elsewhere in the brain. Rats trained postoperatively were not able to make the CS-US association and thus never exhibited the CR.

This explanation aptly describes the fear-conditioning aspect of the experiment, but there is also the matter of the internal and external cues. Animals trained before surgery did not show differential levels of freezing between shocked and non-shocked levels of deprivation during extinction testing. In fact, they showed almost no freezing whatsoever. This finding suggests that rats were not afraid of the context (room, apparatus, etc.), regardless of deprivation level or auditory stimulus. If these rats were not able to monitor their deprivation level, then they would show little freezing to the shocked condition. In addition, the CN rats would be expected to differ from controls in the number of contacts with food in Experiment 1 because they would not be able to determine their hunger level until they sampled the food (Davidson & Jarrard, 1993). Since there was no difference in feeding behavior in Exp. 1, it seems more likely that the deprivation cues act a modulators of the effectiveness of the CS-US association (see Davidson, 1993) and that amygdala damage disrupts

this association (Pascoe, Supple & Kapp, 1991) leaving the deprivation cues with nothing to modulate.

The same rats were able to discriminate between auditory cues, however, their percentage of freezing to the shocked stimulus was significantly less than controls. If CN rats were not afraid of the context, as stated above, then they would not be expected to show any freezing. The fact that they did freeze in the presence of the same contextual cues when the previously shocked auditory stimulus was presented indicates that the association between the auditory cue and the shock was retained. Obviously, external, auditory cues act differently than internal, deprivation cues. In addition, the auditory stimulus must be processed differently than the contextual cues since CN rats did freeze to the former.

The present experiments indicate that while the CN is crucial in the formation of CS-US associations in fear-conditioning paradigms, it is not necessary for retention of this information. Further, it seems that, in controls, food deprivation cues modulate these associations thus resulting in increased freezing to a previously shocked level of deprivation in controls. External auditory cues must not have the same modulatory influence since lesioned rats were able to show discriminative responding to them. The mechanisms that underlie the utilization of internal and external cues are, as yet, not fully understood, but there is no question that the CN provides a critical step in this process.

REFERENCES

Amaral, D.G., Price, J.L., Pitkanen, A., & Carmichael, S.T. (1992). Anatomical organization of the primate amygdaloid complex. In J.P. Aggleton (Ed.), The Amygdala (pp. 1-66). New York: Wiley-Liss, Inc.

Box, B.M. & Mogenson, G.J. (1975). Alterations in ingestive behaviors after bilateral lesion of the amygdala in the rat. Physiology and Behavior, **15**, 678-688.

Davidson, T.L. (1993). The nature and function of interoceptive signals to feed: toward integration of physiological and learning perspectives. Psychological Review, in press.

Davidson, T.L., Flynn, F.W., & Jarrard, L.E. (1992). Potency of food deprivation intensity cues as discriminative stimuli. Journal of Experimental Psychology: Animal Behavior Processes, **18**, 174-181.

Davidson, T.L & Jarrard, L.E. (1993). A role for hippocampus in the utilization of hunger signals. Behavioral and Neural Biology, in press.

Davis, M (1992). The role of the amygdala in conditioned fear. In J.P. Aggleton (Ed.), The Amygdala (pp. 255-306). New York: Wiley-Liss, Inc.

Goddard, G.V. (1964). Functions of the amygdala. Psychological Bulletin, **62**, 89-109.

Grossman, S.P. & Grossman, L. (1963). Food and water intake following lesions or electrical stimulation of the amygdala. American Journal of Physiology, **205**, 761-765.

Hebben, N., Corkin, S., Eichenbaum, H., & Shedlack, K. (1985). Diminished ability to interpret and report internal states after bilateral medial temporal resection: Case H.M. Behavioral Neuroscience, **99**, 1031-1039.

Jarrard, L.E. (1989). On the use ibotenic acid to lesion selectively different components of the hippocampal formation. Journal of Neuroscience Methods, **29**, 251-259.

McDonald, A.J. (1992). Cell types and intrinsic connection of the amygdala. In J.P. Aggleton (Ed.), The Amygdala (pp. 67-96). New York: Wiley-Liss, Inc.

Pascoe, J.P., Supple, W.F. & Kapp, B.S. (1991). In J.L. Martinez, Jr. & R.P. Kesner (Eds.), Learning and Memory: A Biological View (pp. 359-407). San Diego: Academic Press, Inc.

Roozendaal, B., Koolhaas, J.M. & Bohus, B. (1993). The central amygdala is involved in conditioning but not in retention of active and passive shock avoidance in male rats. Behavioral and Neural Biology, **59**, 143-149.

Schoenfeld, T.A. & Hamilton, L.W. (1981). Disruption of appetite but not hunger or satiety following small lesions in the amygdala in rats. Journal of Comparative and Physiological Psychology, **95**, 565-587.

Simbayi, L.C., Boakes, R.A. & Burton, M.J. (1986). Effects of basolateral amygdala lesions on taste aversion produced by lactose and lithium chloride. Behavioral Neuroscience, **100**, 455-465.

Spevack, A.A., Campbell, C.T. & Drake, L.T. (1975). Effect of amygdalectomy on habituation and CER in rats. Physiology and Behavior, **15**, 199-207.

Wynne, L.C. & Solomon, R.L. (1955). Traumatic avoidance learning: Acquisition and extinction in dogs deprived of normal peripheral autonomic function. Genetic Psychology Monographs, **52**, 241-284.

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Figure Captions

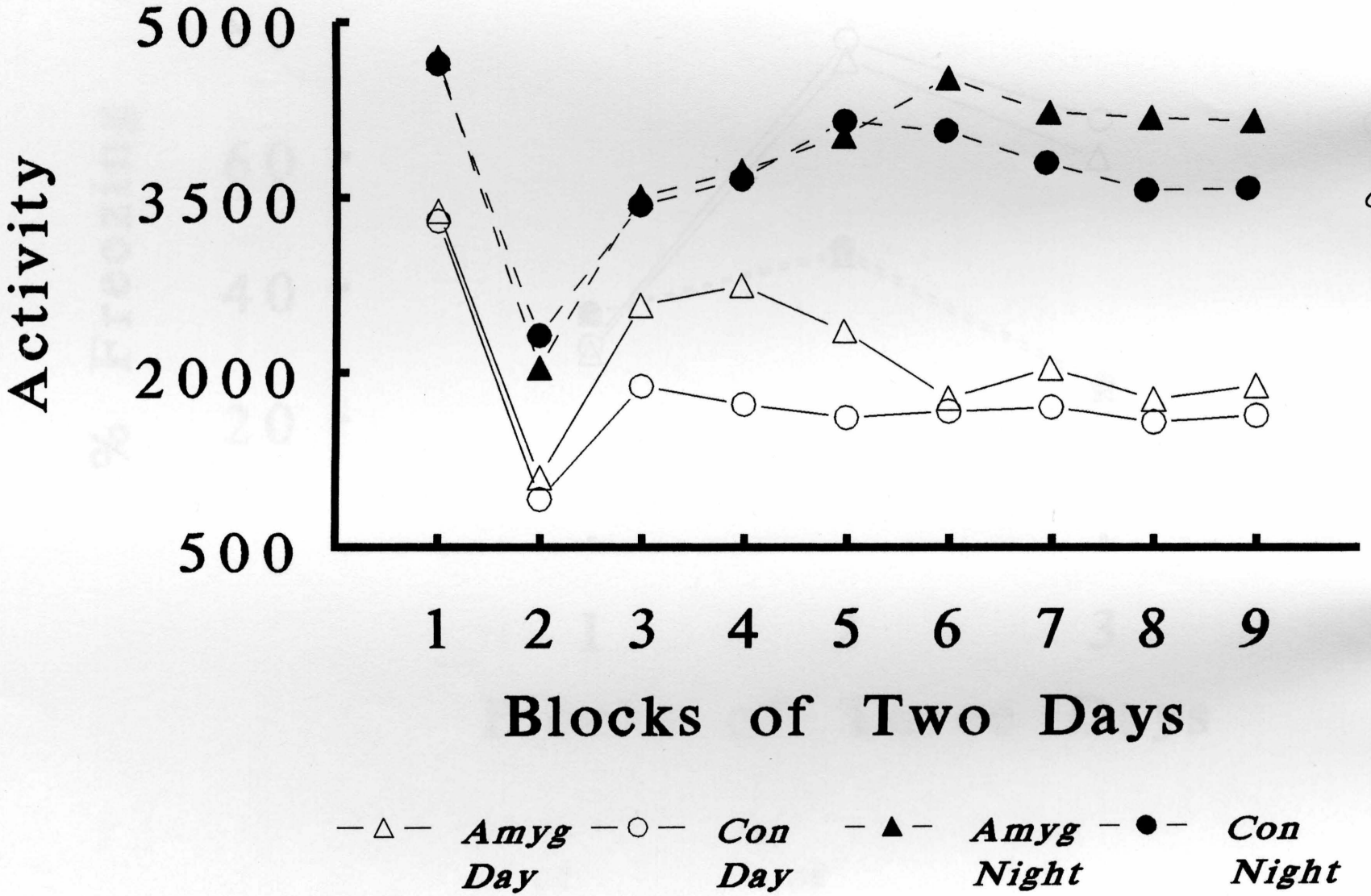
Figure 1. Mean activity for all rats during the day and night. Block 1 represents the baseline established prior to surgery and all other blocks are postoperative measurements.

Figure 2. Percentage of freezing for all rats during acquisition of Exp. 2a. Both groups are shown although there were no lesioned animals. Open markers represent freezing during shocked condition and filled markers show freezing during the non-shocked (N S) condition.

Figure 3. Percentage of freezing for both groups (lesioned: Amyg, control: Con) during deprivation extinction testing in Exp. 2a. Open markers show freezing under shocked condition and filled markers represent freezing to the non-shocked condition.

Figure 4. Percentage of freezing for both CN lesioned rats and controls in auditory extinction testing in Exp. 2a. Open markers show freezing under shocked condition and filled markers represent freezing to the non-shocked condition.

Figure 5. Percentage of freezing for both lesioned (Amyg) and control (Con) animals in acquisition for Exp. 2b. Freezing to the shocked condition is shown by open markers and freezing to the non-shocked condition is represented by filled markers.


Activity
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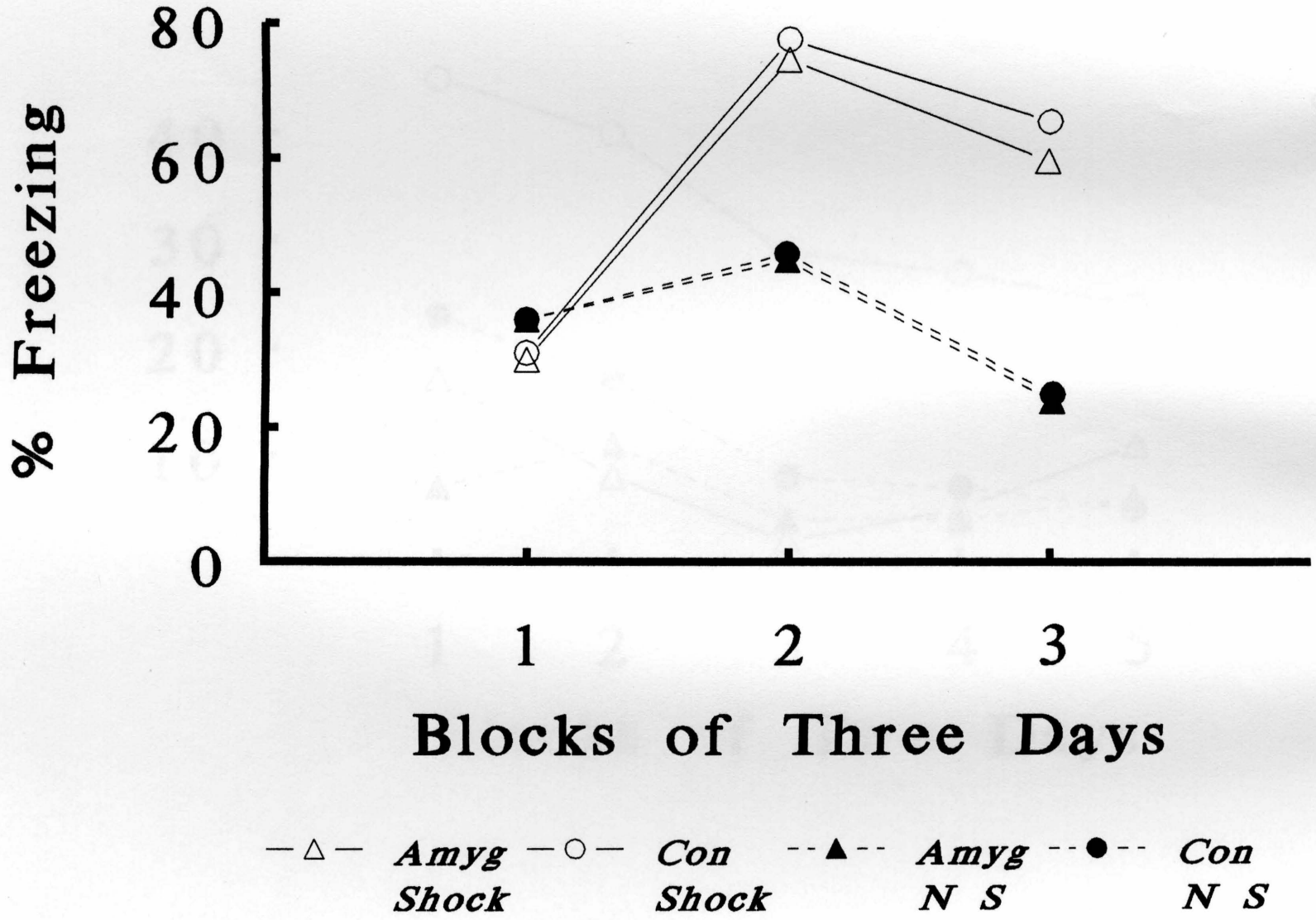
Blocks of Two Days
Amyg Day
Con Day
Amyg Night
Con Night

Figure 1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—○— Con Shock
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--●-- Con N S

Figure 2

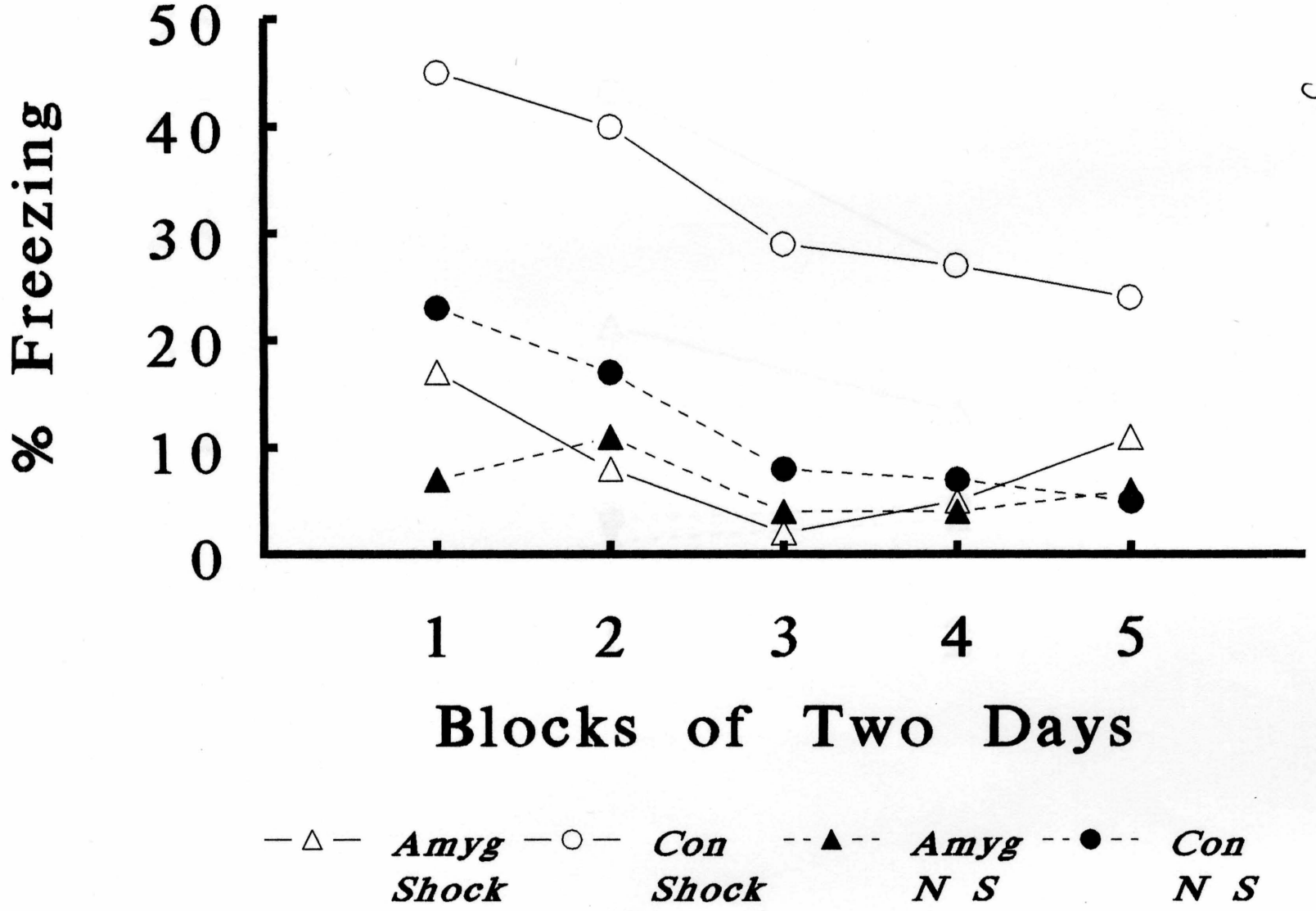
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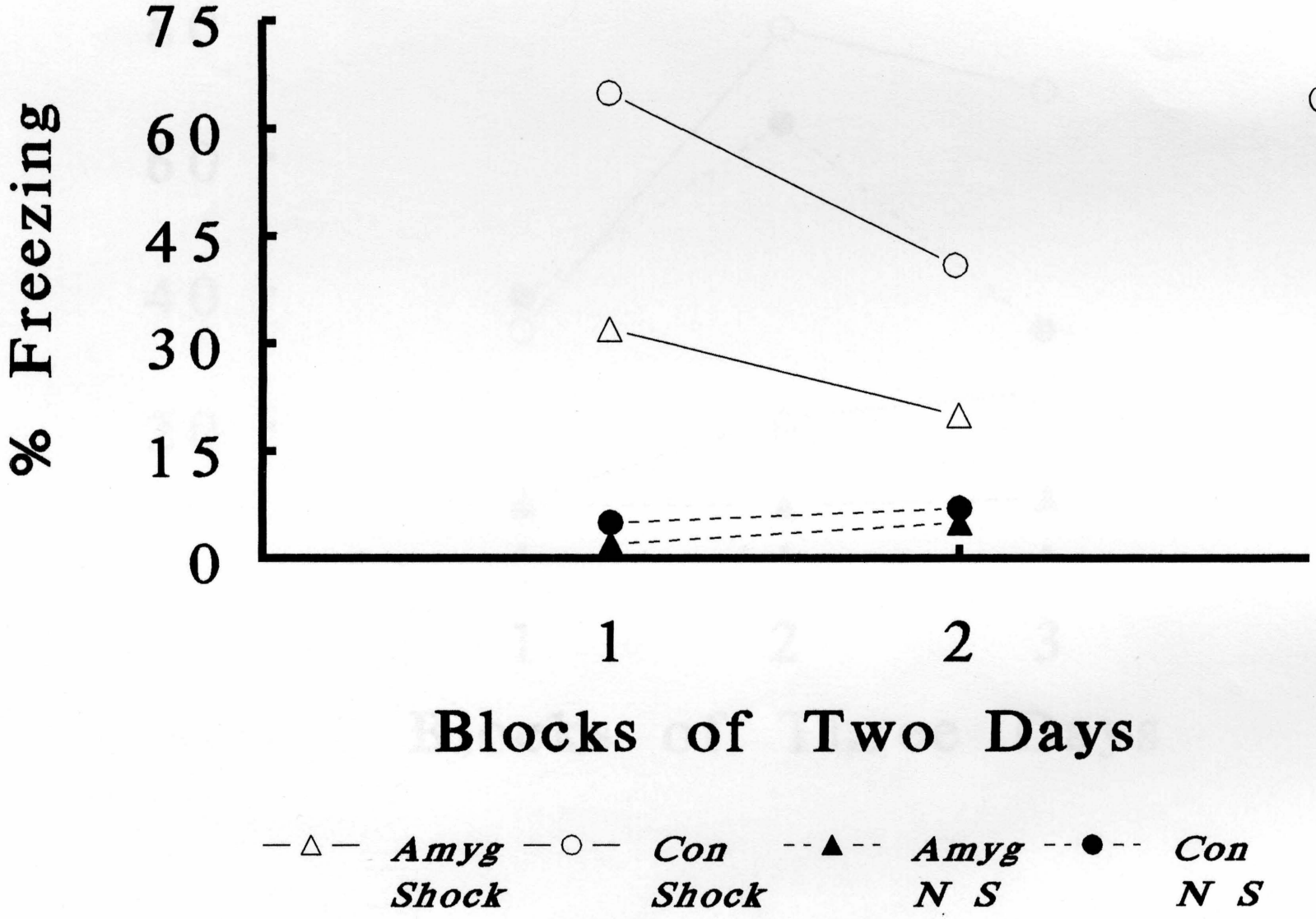
Figure 3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Blocks of Two Days
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Figure 4

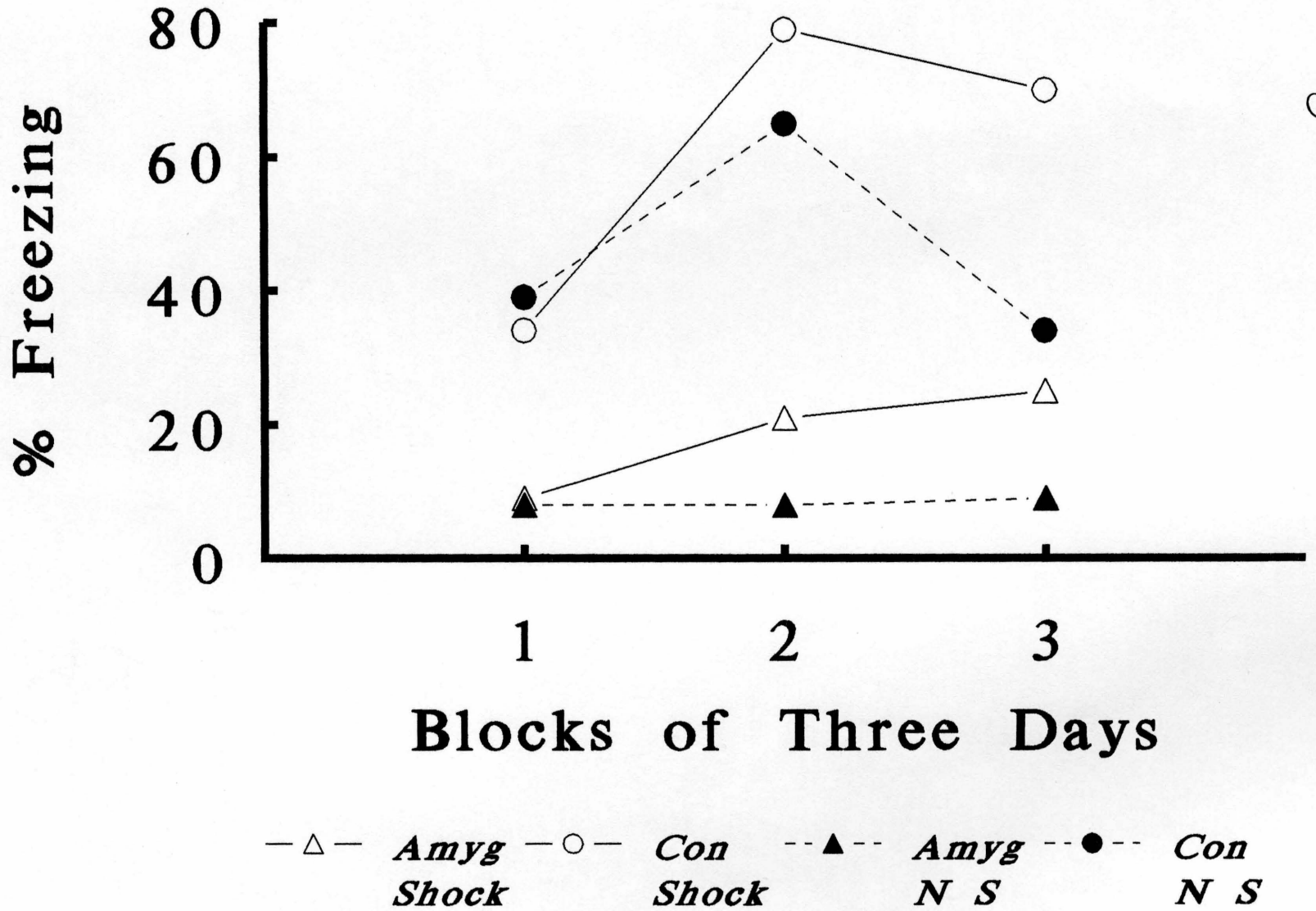
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Figure 5