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Right Hemisphere Involvement in Imprinting Memory Revealed by Glutamate Treatment

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JOHNSTON, A. N. B. AND L. J. ROGERS. *Right hemisphere involvement in imprinting memory revealed by glutamate treatment.* PHARMACOL BIOCHEM BEHAV **60**(4) 863–871, 1998.—The lateralized use of the forebrain hemispheres during recall of imprinting memory was investigated using unilateral intrahemispheric injections of glutamate. Administration of glutamate to the right hemisphere 1, 3, or 6 h after exposure to the imprinting stimulus disrupted recall 8 h after the end of training, whereas the same treatment of the left hemisphere had no effect. Imprinted chicks treated with glutamate injected into the right hemisphere did not approach the imprinting stimulus in preference to an alternative, unfamiliar stimulus during a simultaneous choice test, whereas imprinted chicks treated with glutamate injected into the left hemisphere showed a preference for the imprinting stimulus. Thus, the left and right hemispheres are involved differentially in the recall of imprinting memory. Fear behavior or activity levels were not altered by glutamate treatment of either the right or left hemisphere, indicating that the effects of glutamate were specific to recall of imprinting memory. However, the amnestic effect of treatment of the right hemisphere with glutamate was transient: it was no longer evident by 48 h after the end of training. Also, glutamate had no effect when the chicks were treated 9 h after the end of training. These results suggest that regions in right hemisphere of the chick brain are involved in early (0–8 h after training) recall of imprinting memory. © 1998 Elsevier Science Inc.

Chick Glutamate Asymmetry Learning Memory Lateralization

IMPRINTING is one of a number of early learning tasks performed by chicks that involves differential use of the forebrain hemispheres [summarized in (30,39)]. The lateralized involvement of the left and right forebrain in imprinting has been shown by lesioning the intermediate medial hyperstriatum ventrale (IMHV) region in the left and then the right hemisphere or in the right and then the left hemisphere (12,30). The effect of the sequential lesioning differs according to whether it is the in the left–right or the right–left sequence. When the left IMHV region is lesioned first and the right IMHV is second, chicks show a preference for the imprinting stimulus. When the right IMHV region is lesioned first and the left IMHV is second, chicks do not show a preference for the imprinting stimulus. This indicates that the left IMHV is both a shorter and longer term store of imprinting memory, whereas the right IMHV is a temporary store only (12). There is a longer term store, presumably in the right hemisphere, to which memory is transferred from the right IMHV, an area referred to as S′ [summarized in (22,30)] located in a site as yet unknown.

Lateralized involvement of the IMHV regions during consolidation and recall of imprinting memory has been shown also by using a number of biochemical and morphological measures. For example, imprinting has been shown to cause elevation of NMDA-type receptor binding in the IMHV and the archistriatum/lobus parolfactorius in the left hemisphere, but not in the same regions in the right hemisphere (25,26, 31,32).

Nicol et al. (35) recorded from neurons in the left or right IMHV of chicks during imprinting and showed that there are electrophysiological changes associated with imprinting. They demonstrated stimulus-specific responses by neurons in the right IMHV as well as the left IMHV. A higher proportion of neurons in the right IMHV of imprinted chicks responded to the representation of the imprinting stimulus than in naive chicks. Although there are electrophysiological changes in the

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left IMHV region of imprinted chicks (8,9), these are less specific; recording of electrical activity in the right IMHV at test depends more on representation of a stimulus of the same color and shape as the imprinting stimulus, whereas activity recorded in the left IMHV was much more general, responding to a number of objects similar and dissimilar to the imprinting object (35). The proportion of recording sites from the IMHV of imprinted chicks that respond to an alternative (or nonimprinting) stimulus was greater in the left IMHV than in the right IMHV. Moreover, the specificity of responsiveness of changes in the right IMHV appeared to be related to auditory, as well as visual, aspects of the imprinting stimulus (35). Thus, electrophysiological evidence suggests that both left and right IMHV regions may involved in the long-term storage of imprinting memory but differentially, confounding earlier suggestions of the specialized role of S′.

The potential involvement of other regions in addition to the IMHV, in the left and right hemispheres can be examined by injecting of low doses of L-glutamate into the hemispheres. This pharmacological approach is beneficial when the exact anatomical site of the region/s involved in imprinting recall have not been fully described because the glutamate diffuses through a large area of the forebrain, targeting specific receptor sites in those regions. Hence, the IMHV regions and the unidentified site of longer term memory storage in the right hemisphere, region S′ may be targeted simultaneously, even though the location of S′ is not known.

Low concentrations of glutamate, of the order of 10 nmol in 1 μl of saline injected bilaterally into the IMHV regions 4 days following imprinting training have been shown to disrupt imprinting memory for at least 3 h after administration (42, 43). By injecting glutamate into the left or the right hemisphere at various times after imprinting, lateralization of memory consolidation and/or recall might be revealed, and time courses associated with changes in glutamate receptor involvement during imprinting might be obtained. Administration of low doses of glutamate into one or the other hemisphere has been used previously as a treatment to reveal lateralized hemispheric functions, for attack and copulatory behavior in chicks (39), recall of passive avoidance learning (34,36), and for performance on a task requiring search for grain against a background of pebbles (14).

Tests examining less specific effects of unilateral treatment with glutamate, such as increases in fear responses or alterations in the lateralized observation of the imprinting stimulus, must also be included in such a study to ensure that the unilateral glutamate administration does not alter imprinting preferences nonspecifically. This seemed particularly important as Phillips and Youngren (37) have demonstrated that treatment of 5- to 6-day-old chicks with kainic acid into the right, but not the left, archistriatum reduces fear levels, indicated by significantly decreased distress peeping and visual scanning.

METHOD

Animals and Housing

Fertile white Leghorn × New Hampshire eggs from 24 separate batches were used. For the first 16 days the eggs were incubated in an automatic turning, forced draught incubator (Multiquip, Australia) maintained at 37–38°C and 70% relative humidity. On day 17 of incubation the eggs were transferred to a completely dark hatching incubator located in a completely darkened room. The hatching incubator was monitored closely throughout the last 24–36 h of incubation. A numbered leg tag was placed on each chick shortly after hatching (performed in complete darkness). The chicks remained in the dark incubator for 36 ± 1 h, a usual delay between hatching and imprinting (3,25,26).

Imprinting

Chicks were moved directly from the darkened incubator into the imprinting apparatus. During the training period each chick was placed in a running wheel [based on the design of Bateson and Wainwright (5)], fitted with an incremental optical encoder, which counted the rotation of the wheel in both clockwise and anticlockwise directions.

The chicks were trained by exposure to either a stuffed hen (of a feral strain from North West Island, Australia) or a box (12 cm wide × 9 cm long × 23 cm high), positioned between 48 and 54 cm from the chick. The larger faces of the box were red and the smaller faces of the box were covered in black nonreflective material. Red was selected to enhance the strength of imprinting preference, as it is a preferred color for imprinting (46). These stimuli were mounted on platforms that revolved at 30 rpm for 30-s intervals interspersed by 5-s pauses. The imprinting stimulus used was randomized across chicks. The running wheels and the imprinting stimulus were contained in a light- and sound-attenuated box (120 cm wide × 120 cm long × 100 cm high) lined with absorbent black corrugated rubber.

Each chick was exposed to the imprinting stimulus for 140 min at an age of 36 ± 1 h posthatching. Immediately after training was completed each chick was returned to the dark incubator, where it remained undisturbed apart from when it was treated with glutamate or saline (vide intra).

Chicks were tested for imprinting preferences at either exactly 8, 24, or 48 h after the end of exposure to the imprinting stimulus. Testing involved a 5-min period of simultaneous exposure to both of the stimuli, situated on either side of and at 50 cm from the centrally placed running wheel. The movement of each chick towards or away from the imprinting stimulus (clockwise or anticlockwise rotation of the wheel) was recorded. Chicks were tested individually and once only. The testing apparatus was contained within a sound- and light-reduced cabinet (65 cm high × 70 cm wide × 120 cm long).

Activity levels varied during training and during testing. Chicks were not excluded on the basis of low activity levels during training as activity during training is only weakly correlated with activity during testing, if at all (4). However, a minimum activity criterion during testing was applied following the method of Bateson (3), chicks being excluded if they moved less than one-quarter of a revolution of the wheel during the 5-min test period. The experiment was continued until each treatment group contained 13 chicks that had reached the test criterion.

The % preference score was used to assess relative imprinting levels of each chick (3). Percent preference scores were calculated by dividing the revolutions of the wheel towards the imprinting stimulus by the total number of revolutions during the testing period. This ratio is independent of individual differences in activity and enables a direct comparison of the level of imprinting preference of all of the chicks (3).

Initially, chicks were exposed to the imprinting stimulus and then treated with either glutamate or saline 1, 3, or 6 h after the end of training (i.e., six groups each containing 13 chicks that satisfied training and testing criteria). They were tested 8 h after the end of training. A separate group of chicks

was then exposed to the imprinting stimulus, treated with glutamate or saline 6 h after the end of exposure, and tested 8, 24, or 48 h after the end of training. Each of these experiments also included groups of noninjected chicks that were exposed to the imprinting stimulus and then remained undisturbed until testing either 8, 24, or 48 h after the end of exposure. Another four groups of chicks were treated with glutamate or saline in the left or right hemisphere 9 h after the end of training and tested for preference for the imprinting stimulus at either 24 or 48 h after the end of training.

Intracerebral Injections

Five microlitres of 0.9% sterile pyrogen-free saline or 5 μl of 100 mM glutamate was injected into the hyperstriatum ventrale in the left or right forebrain (23). The needle of a 10-μl glass Hamilton syringe was covered with a plastic sleeve starting 3 mm above the tip of the needle, and this limited the depth of the injection to 3 mm below the surface of the cranium. The injection was placed approximately 1 mm posterior to the midway line of the rostro–caudal axis of the forebrain (from a ventral view) and between 0.5 and 1.0 mm lateral to the midline. Each chick received one injection only. The treatments were randomized across batches of chicks. The injection procedure took approximately 1 min per chick, and it was performed under dim lighting conditions (70–80 lx).

Another group of chicks used in these experiments, the "noninjected" group, did not receive an injection. The chicks in this group, randomized across batches, received training identical to that of the other chicks but they remained in the incubator relatively undisturbed for 8 h from the end of training to testing.

The chicks were sacrificed after testing and the site of injection was checked by observation of the dorsal surface of the brain and by gross dissection of the brain. Brain dissection enabled the needle tract to be traced and the terminal site of the needle to be assessed. Chicks were excluded from the analysis on the basis of injection site if the site of injection deviated from the region delineated as IMHV (23) by greater than 1 mm in any direction. Only 26 out of a total of 469 chicks used were excluded on this basis. The chicks were killed at the end of each experiment and the sex of each was determined by inspection of gonads.

Tonic Immobility Tests

Immobility tests were used as a measure of arousal or fear; modified from those of Gallup (17–19). Fifty chicks were incubated and hatched as described above and then randomly allocated to one of the five experimental groups (injected into either the left or right forebrain hemisphere with either saline or glutamate or not injected). The treatment was given 44 h after hatching, to match the age of the third group of chicks that had been imprinted. The chicks remained undisturbed for a further 2 h and then were moved from the homecage into an adjacent room where they were tested individually.

Testing involved placing each chick on its side on a bench surface that had been padded with several layers of paper towelling. The chick was immobilized by the experimenter's hands, one placed over the chick's head and the other over its body, for a total of 15 s. The hands were then removed slowly. The number of times this induction had to be performed for the chick to remain in a prone position for at least 10 s was recorded (28). Once tonic immobility had been induced in each chick, latency to vocalize, latency to move the head and latency to stand were recorded (11,27,29). Following tonic immobility testing chicks were returned to their home cages and supplied with food and water ad lib. They remained in the home cage for 2 to 2.5 h before to being tested with novel objects.

Response to Novel Objects

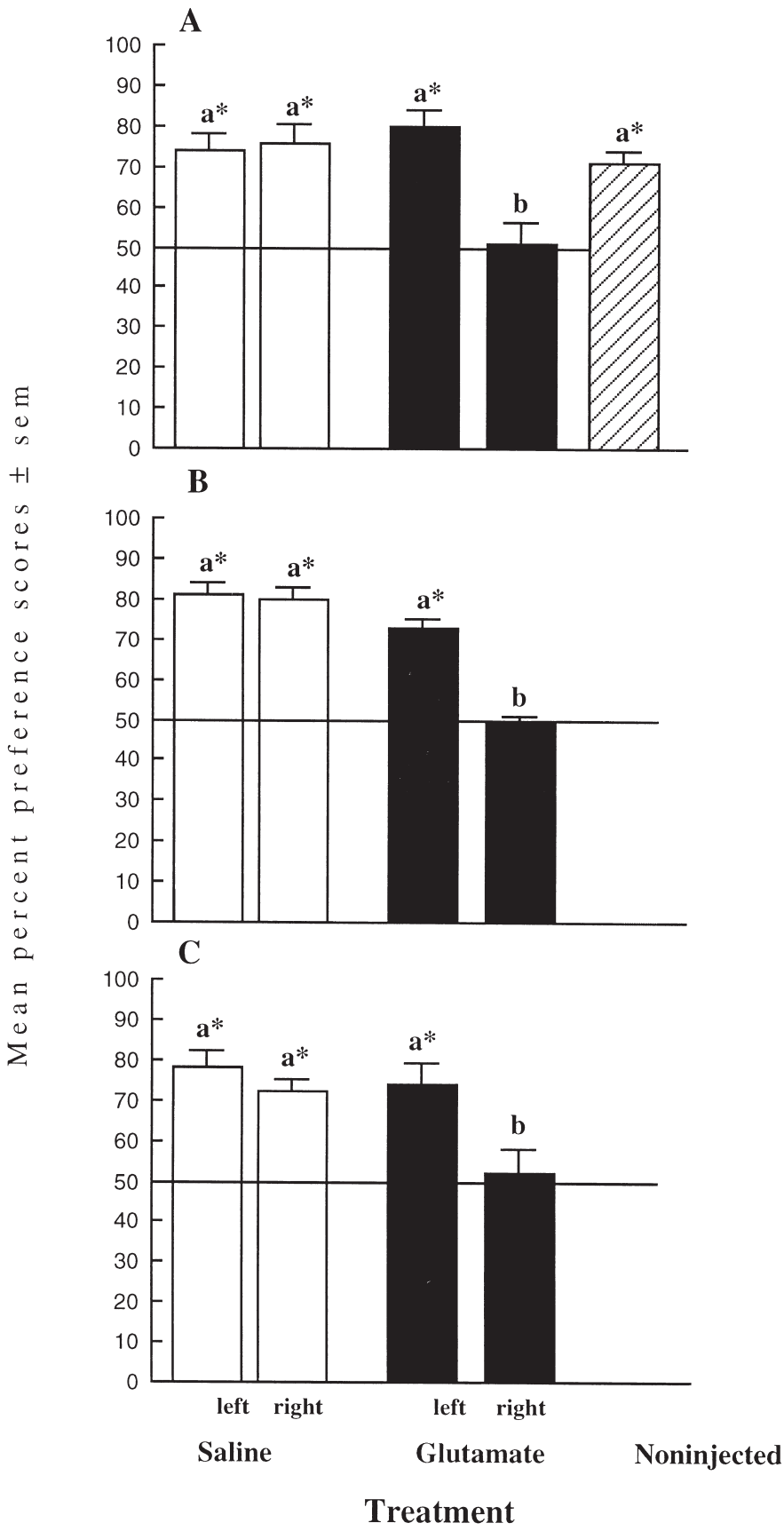
Each chick was placed in a modified home cage, situated so that it faced another home cage containing a novel object. The chick was separated from the other cage by a single sheet of clear perspex. The chick was placed in the cage facing away from the novel stimulus. The novel objects used were the stuffed hen used for imprinting (note that these chicks had not previously been exposed to this stimulus) and a live white rat. Chicks were exposed to the stuffed hen for 2 min, returned to their home cage for 15 min, and then returned to the testing situation and exposed to the rat for 2 min. The behavior of the chick during each 2-min period was recorded using a video camera. The behaviors scored were latency to move, latency to vocalize, the total number of steps taken during each 2-min exposure, and the number and duration of "observations" (fixed viewing) of the novel stimuli with the right eye and the left eye. Observation with a particular eye was recorded when the angle subtended by the chick's beak and the imaginary line between the chick's head and the novel stimulus was greater than 20°, thereby eliminating the binocular field of vision (16,44). The total time spent observing each stimulus was also recorded.

Statistical Analysis

Activity during imprinting testing was analyzed by a multifactor analysis of variance (multifactor ANOVA) using the factors treatment (glutamate or saline), time of injection (1, 3, or 6 h after training), hemisphere (left or right hemisphere) and imprinting stimulus (box or hen). A "time of test" factor was substituted for "time of injection" in the second set of experiments. A multifactor ANOVA was also performed on the raw percent preference scores, as they approximated a normal distribution (20). Tukey's tests of honestly significant difference (Tukey's HSD) were used post hoc to establish the source of any significant differences revealed by the multifactor ANOVAs (20). The χ^2 statistic was used to determine any significant variation between the relative numbers of chicks in each test group that failed to reach the test activity criterion to indicate whether treatment or delay between training and testing altered activity in testing.

Two-way ANOVAs, incorporating the factors treatment and sex, were used to examine the data related to the induction and maintenance of tonic immobility. Sex was included as a factor in the analysis of data from the tonic immobility tests as some studies indicate that male and female chicks respond differently in fearful situations (1,10,45).

Difference scores for eye use (number of observations and time spent observing with the left eye was subtracted from the equivalent measures made with the right eye) were analyzed by multifactor ANOVA. Sex was again included as a possible variable as significant sex differences have been observed in a number of these measures (15). However, as the same chicks were exposed to both of the novel objects a repeated measure was placed on the "object" factor. Where significance equal to or less than 0.05% was recorded with a multifactor ANOVA, post hoc *t*-tests were used to determine specific treatment effects.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Mean percent preference scores ± sem

RESULTS

Effects of Glutamate Treatment at Various Times After Training

There were significant main effects of both treatment (glutamate/saline/noninjected) and hemisphere (left/right) on the percent preference scores, $F(1, 121) = 34.2$, $p < 0.01$; $F(1, 121) = 34.8$, $p < 0.01$, respectively. There was also a significant interaction of these factors, $F(1, 121) = 22.8$, $p < 0.01$, but no significant effect of imprinting stimulus (box or hen) or time of injection (1, 3, or 6 h after training; see Fig. 1). Post hoc Tukey's HSD tests revealed that the significant interaction was due to lower percent imprinting scores recorded in chicks treated with glutamate in the right hemisphere for all of the times of injection ($p < 0.05$).

Percent imprinting scores control for differing activity levels. This was confirmed by the lack of correlation between activity scores during testing and percent preference scores in testing (Pearson's correlation $r = 0.11$, $p > 0.05$). Thus, the effect of treatment of the right hemisphere with glutamate was unlikely to be due to varying activity levels. Moreover, there was no significant difference in the proportion of chicks that failed to reach the test criterion of activity in the five different treatment groups ($\chi^2 = 10.4$, $p > 0.05$), indicating that the failure to reach criterion was not due to effects of the injection with either glutamate or saline. Therefore, the following data is compared to saline-injected control groups only.

The findings above were confirmed by the experiment in which chicks were injected with glutamate or saline 6 h after the end of training and tested at 8, 24, or 48 h after the end of training (see Fig. 2). There was a significant three-way interaction of treatment, hemisphere injected, and also time of testing, $F(2, 72) = 3.85$, $p = 0.04$. Post hoc tests showed that, while chicks treated with glutamate in the right hemisphere had significantly lower percent preference scores than all other groups when they were tested 8 or 24 h after training ($p < 0.05$), at 48 h after training there was no significant effect of treatment of the right hemisphere with glutamate ($p > 0.05$). Thus, by 48 h after testing, chicks that had the right hemisphere treated with glutamate showed percent preference scores significantly higher than 50%, $t(9) = 8.45$, $p < 0.01$ unlike those tested at 8 h, $t(9) = 0.35$, $p = 0.74$, or 24 h, $t(9) = -0.06$, $p = 0.96$, after training. Mean percent preference scores from all of the groups, of around 75% indicate significant preference for the imprinting stimulus, akin to those described in previous studies (3,22,26). Again, these effects were not due to disproportionate levels of exclusion of chicks due to failure to reach criterion activity levels during testing ($\chi^2 = 8.83$, $p > 0.05$.

The time interval between the end of training and the administration of glutamate determines whether it has an amnestic effect or not (see Fig. 3). When chicks were treated 9 h after the end of training, there was no effect of treatment, $F(1, 121) = 0.25$, $p = 0.62$, no effect of time of test, $F(1, 121) = 0.36$, $p = 0.55$, and no significant interaction of these factors, $F(1, 121) = 17$, $p = 0.68$. A one-group t-test comparison of the percent preference scores of the combined scores of all of the groups with a no-preference score of 50% demonstrated that chicks preferred the imprinting stimulus to the unfamiliar stimulus, $t(79) = 18.28$, $p < 0.01$. Thus, these chicks had consolidated and recalled the imprinting memory.

There was no effect of sex on any of the measures associated with the tests for imprinting preference. There was also no effect of imprinting stimulus on the preference of the chicks for the imprinting stimulus.

Tonic Immobility Test

There were no significant main effects of either treatment or sex on the measures of tonic immobility (see Table 1), and no significant interactions of these factors. Treatment did not alter the number of inductions required to induce tonic immobility, $F(4, 40) = 0.25$, $p = 0.91$, the latency to move the head, $F(4, 40) = 0.19$, $p = 0.94$), the latency to stand, $F(4, 40) = 0.35$, $p = 0.89$), or the latency to vocalize, $F(4, 40) = 0.26$, $p = 0.91$.

Response to Novel Objects

There was no significant main effect of any of the factors in the latency to step during the tests of responding to novel objects [sex, $F(1, 40) = 0.07$, $p = 0.80$; hemisphere treated, $F(4, 40) = 0.45$, $p = 0.77$; hen/rat novel object, $F(1, 40) = 0.02$, $p = 0.90$] and no significant two- or three-way interaction of these factors (for the sake of brevity only the three-way result is reported, $F(4, 40) = 1.78$, $p = 0.15$). There was a highly significant effect of which novel object was used on the number of steps taken, $F(1\ 40) = 8.49$, $p < 0.01$), with the rat inducing many more steps than the hen (mean number of steps = 15 ± 8 with rat compared to 6 ± 5 with the hen; $p < 0.05$). There was no significant main effect of treatment or sex, $F(4, 40) = 0.78$, $p = 0.55$; $F(1, 40) = 0.05$, $p = 0.94$, respectively, on the number of steps taken during the test, and no significant two- or three-way interaction of these measures [three-way interaction; $F(4, 40) = 0.72$, $p = 0.69$].

A similar pattern of findings was recorded for the latency to vocalize in response to the two novel objects. The latencies to vocalize were log10 × transformed and analyzed using a three-way ANOVA, which showed that, while the rat induced a much greater latency to vocalize (14.6 ± 8.2 s to the rat compared to 5.6 ± 2.2 s to the hen; $F(1, 40) = 6.33$, $p = 0.02$), there was no significant main effect of sex, $F(1, 40) = 2.03$, $p = 0.16$, or treatment, $F(1, 40) = 1.21$, $p = 0.32$. There were also no significant two- or three-way interaction of these factors [three-way interaction; $F(4, 40) = 0.78$, $p = 0.54$]. The significant effect of the novel object on the latency to vocalize was not reflected in the mean number of vocalizations performed during the novel object test. That is, there was no main effect of treatment, $F(4, 40) = 1.85$, $p = 0.14$, sex, $F(1, 40) = 1.60$, $p = 0.21$, or novel object used, $F(1, 40) = 0.78$, $p = 0.38$. There were also no significant two- or three-way interaction of these factors [three-way interaction; $F(4, 40) = 1.20$, $p = 0.33$]. Thus, there seemed to have been no effect of either treatment or sex on any of the behaviors associated with responses to novel objects. As only eight chicks preened and six chicks defecated during the tests, these results were not included in the analysis.

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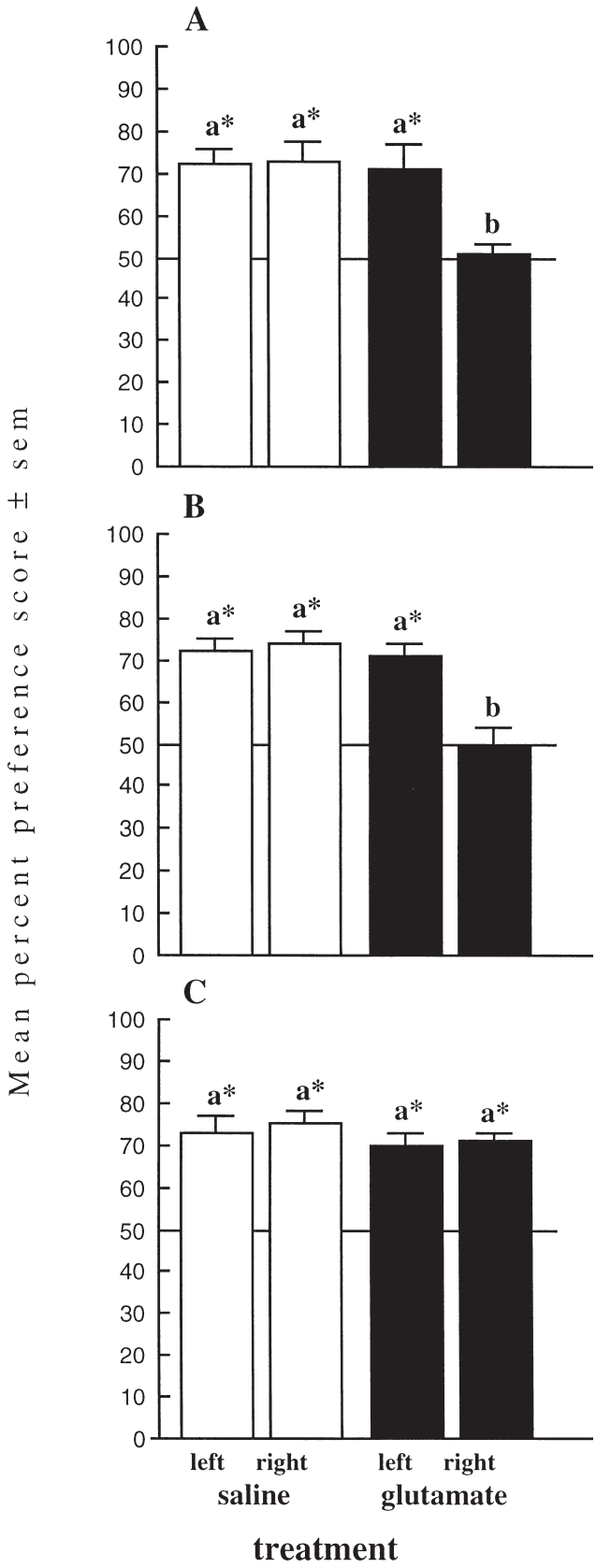
FIG. 1. The mean ± SEM percent preference scores of chicks tested 8 h after training. The chicks were injected at either 1 h (A), 3 h (B), or 6 h (C) after the end of training. Only the groups treated with glutamate in the right hemisphere differed significantly from any of the other groups. *indicates mean percent preference scores that were significantly greater than 50%. Bars superscripted with the same character are not significantly different from each other, bars superscripted with differing characters are significantly different from each other. Significance was accepted when $p < 0.05$, $n = 13$ chicks per group



Monocular Viewing of the Novel Object

There was no significant main effect of treatment, $F(4, 80) = 0.25$, $p = 0.91$, sex, $F(1, 80) = 0.26$, $p = 0.61$, or object, $F(1, 80) = 0.32$, $p = 0.57$, on the amount of time chicks spent viewing the novel object with either eye. There was also no significant two- or three-way interaction of these factors [three-way; $F(4, 80) = 1.66$, $p = 0.17$].

Treatment did not effect the total amount of monocular viewing, $F(4, 80) = 0.10$, $p = 0.95$, and there was no significant main effect of sex, $F(1, 80) = 0.35$, $p = 0.55$, or object, $F(1, 80) = 2.21$, $p = 0.14$. There was a significant interaction of sex and object, $F(1, 80) = 5.53$, $p = 0.02$, although there was no other significant two- or three-way interaction [three-way interaction; $F(4, 80) = 0.26$, $p = 0.90$]. Post hoc analysis of the differential eye use data indicated that female chicks exhibited significantly more monocular viewing (watching an object with one eye only) than males, $t(52) = 3.03$, $p < 0.01$, for females; $t(44) = -0.65$, $p = 0.52$, for males). Female chicks viewed the rat more frequently with the left eye (mean difference in looks = -2 ± 1.2) and the hen more frequently with the right eye (mean difference = 1.3 ± 1), whereas male chicks used both eyes to the same extent (rat = 1 ± 0.8; hen = 1 ± 1.1). There were no other significant interactions.

DISCUSSION

Unilateral administration of glutamate into the left or right hemisphere of the forebrain has different effects on the preference of chicks for the imprinting stimulus. This result may be interpreted as differential effects on recall of imprinting memory. Recall was prevented by administration of glutamate into the vicinity of the IMHV region of the right hemisphere 1, 3, or 6 h after training, whereas the same treatment of the left forebrain hemisphere had no effect on recall. This was a somewhat unexpected finding, given that previous studies of imprinting have demonstrated a role of the glutamatergic mechanisms in the left, but not the right, forebrain hemisphere (25,26,31,32). However, the right hemisphere may use glutamatergic mechanisms in different regions and according to a different time course than those in the left hemisphere. It should be noted also that chicks injected with glutamate into the right hemisphere do not access the memory present in the left IMHV, even though they are tested binocularly and thus have visual input to both hemispheres.

Alternatively, it may be that the memory store in the left hemisphere, although intact, is not able to be accessed. Indeed, it is possible that functional recall of the imprinting stimulus may cycle from left to right hemisphere, particularly during the relatively early stages (<24 h) after exposure to an imprinting object, in a manner akin to that described for recall of passive avoidance learning (2,3). Andrew (2) has described a pattern of "trace reactivation" following training on the passive avoidance task, in which different elements of the memory are reactivated at different times after training in the left and right hemispheres. The memory stores held in the left and

FIG. 2. This figure presents the mean percent preference scores ± SEM of chicks injected 6 h after training and tested at 8 (A), 24 (B), or 48 (C) h after the end of training. Data are presented as in Fig. 1. Groups with mean percent preference scores that are significantly different from each other are indicated by different letters. Those that did not differ significantly from each other are annotated with the same letter. *indicates groups that differed significantly from a "no preference" score of 50%. $n = 10$ per group.

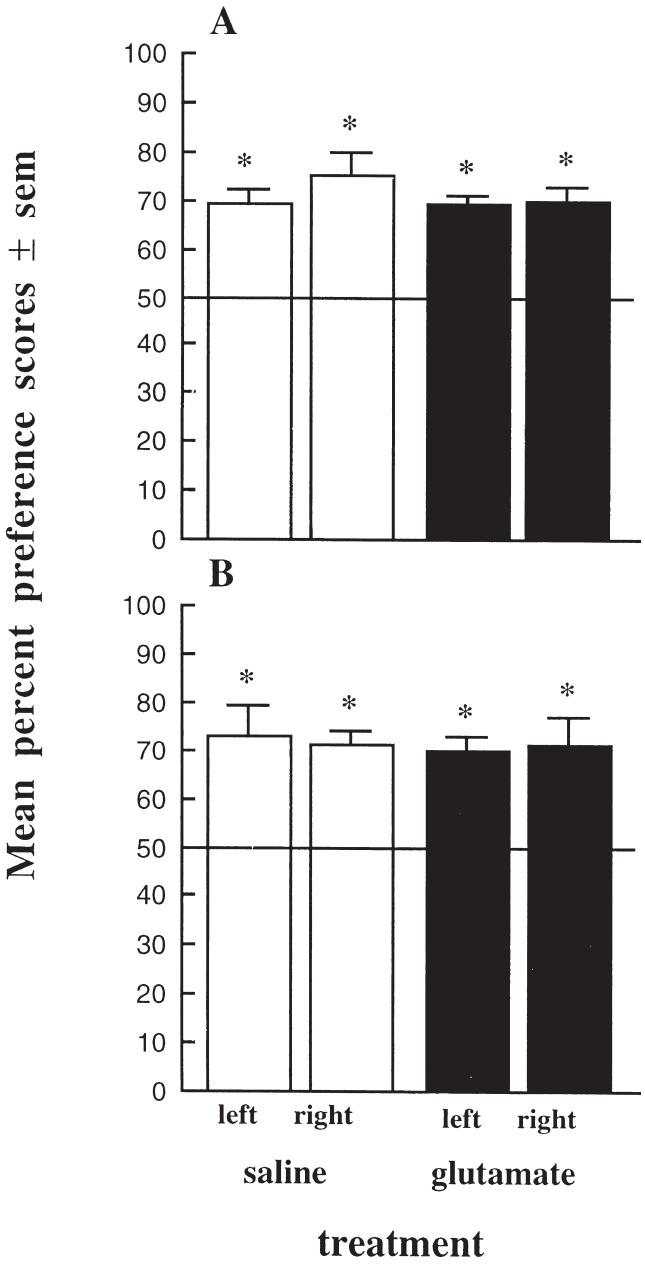


FIG. 3. The mean percent preference scores ± SEM of chicks injected 9 h after training and tested at 24 (A) or 48 (B) h after the end of training are presented. Mean percent preference scores did not differ significantly between groups. Data are presented as in Fig. 1. n = 10 chicks per group.

right hemispheres have different cycles of activity so that periods of exceptionally high or exceptionally low recall are evident when the chicks are tested so that they primarily access one hemisphere only (33). If the chicks are asked to recall from one hemisphere when the other is active, the outcome is as if no memory store is present. Moreover, if the memory stores are in flux, "moving" from one hemisphere to the other, the chicks also present behaviorally as if they do not recall the task (2). If the same pattern of memory cycling occurs following imprinting, it may be that 8 h after training is a time at which the memory store in the left hemisphere cannot be accessed and the chick is entirely reliant on the memory store in the right hemisphere. The only possible indicator against memory cycling following imprinting, compared to that following passive avoidance training (2), is that imprinting is a less precisely timed learning event, and it is much less likely that such specific time-dependent hemispheric patterns of recall would be constantly revealed.

Glutamate treatment of either the left or the right hemisphere is unlikely to induce amnesia by transiently affecting the mechanisms specifically associated with retrieval of imprinting memory, in a manner akin to that described for recall of passive avoidance learning [(41); see also (34)], as it was administered at least 1 h before the chicks were reexposed to the imprinting object for testing. Summers et al. (41) have shown that transient memory losses can be produced by administering glutamate after presenting a reminder of the visual element of the taste aversion task; indicating a separate pharmacological mechanism for consolidation and recall of the memory trace, but only when the glutamate was administered immediately after reexposure, not when chicks were injected with glutamate 5–10 min before the presentation of the reminder. Thus, effects of the protocol used in the current imprinting study in which chicks were injected at least 1 h before representation of the imprinting stimuli (during preference testing) are unlikely to be due to acute disruption of specialized glutamatergic recall mechanisms. Memory consolidation in passive avoidance learning in day-old chicks has, however, been shown to involve differential use of regions in the left and right forebrain (40). For example, an injection of glutamate into the right lateral neostriatum 5 min before training causes a progressive amnesia, evident soon after training (36).

The amnestic effect following glutamate treatment of the right hemisphere was transient. When glutamate was administered to the right hemisphere 6 h after the end of training, the chicks demonstrated a preference for the imprinting stimulus when tested at 48 h after training (but not at 8 or 24 h after training). Therefore, glutamate treatment of the right hemisphere produces a temporary inability to recall, rather than a blockage of the consolidation of imprinting memory. A longer term memory may be able to be accessed by 48 h after glutamate treatment of the right hemisphere.

Injection of glutamate into the left hemisphere did not affect imprinting preferences at any of the testing times. The lack of effect of glutamate when it is administered to the left forebrain hemisphere suggests that the biochemical mechanisms that have been related to consolidation of memory [e.g., the increase in glutamate and particularly MK-801 binding (25,26,31,32)] are not altered by treatment with glutamate. Although this study did not address the dynamics of the biochemical processes associated with long-term consolidation of imprinting memory, it is tempting to suggest that the memory consolidation processes that occur in the left hemisphere, particularly the left IMHV (6,35), may be set in train very rapidly following or even during training. Nevertheless, it is surprising that the glutamate injections do not disturb the biochemical cascade initiated in the left hemisphere by the exposure to the imprinting stimulus. The roles of the left and right hemisphere in memory consolidation and recall must be very different and/or follow different time courses to those described for passive avoidance learning (2,40).

TABLE 1
INDUCTION AND MAINTENANCE OF TONIC IMMOBILITY

Treatment	Mean Number of Inductions ± SEM	Mean Latency to Move Head ± SEM (s)	Mean Latency to Stand ± SEM (s)	Mean Latency to Vocalize ± SEM (s)
Glutamate left hemisphere	6.0 ± 0.8	112 ± 27	150 ± 26	185 ± 32
Glutamate right hemisphere	6.1 ± 1.9	110 ± 43	148 ± 45	162 ± 31
Saline left hemisphere	5.9 ± 1.2	91 ± 22	135 ± 24	169 ± 21
Saline right hemisphere	5.4 ± 2.1	122 ± 25	146 ± 24	158 ± 27
Noninjected	4.9 ± 1.4	109 ± 26	122 ± 28	148 ± 24

The first column presents the mean number of handling procedures needed to induce tonic immobility and the other columns present various latency scores once tonic immobility had beed induced. $n = 10$ chicks per group. All figures indicate the mean ± SEM.

It is unlikely that the amnestic effect of administering glutamate into the right hemisphere is due to nonspecific effects on behavior, because unilateral treatment with glutamate into either hemisphere did not alter fear behavior or responses to novel objects. Glutamate treatment also did not alter activity during testing, indicating that glutamate treatment is unlikely to have altered the response of the chicks to the novelty of the testing chamber used in the imprinting tests (7) or to have altered arousal levels in any other way (38). Furthermore, given that unilateral glutamate treatment of either hemisphere did not alter the chicks' responses to novel objects or the duration and number of periods of viewing ("looks") at novel stimuli, it is unlikely that the effects of glutamate on recall are due to a change in distractibility or impaired visual ability.

When chicks were treated with glutamate in either the left or the right hemisphere 9 h after training and tested 24 or 48 h after training, they demonstrated preference scores greater than 50%. Therefore, imprinting memory may not be vulnerable to glutamate treatment by 9 h after training. The absence of vulnerability to glutamate 9 h after training supports, in part, the hypothesis of Horn et al. (21,22,24), who suggested that imprinting memory is stored in the left and right hemispheres of an intact chick by a two stage process. The first stage involves a rapidly formed memory store located in the left IMHV region, whereas the second stage involves a longer process that may involve the right IMHV and results in a memory store in the unidentified S′ region approximately 6 h after training (13,21,22,24). It would follow that the recall mechanisms affected by glutamate must access memory before it enters the S′ store in the right hemisphere. Longer term memory held in S′ appears to be unaffected by glutamate treatment. However, an alternative explanation for these results may be that as the duration between training and the administration of glutamate is increased, the effect of glutamate administration decreases (possibly to a point where it is not effective at all) in a manner akin to that described by Summers et al. (41). They showed that, as described earlier, the duration of glutamate-induced amnesia for passive avoidance learning decreased as the interval between training and the presentation of a visual reminder stimulus (similar to that used during training) increased.

More research is required to describe the mechanisms associated with the differing roles of the left and right forebrain hemispheres in the consolidation and/or recall of imprinting memory. However, the data from this study demonstrates that the right hemisphere is involved and, in fact, that its function is crucial for either early recall or consolidation at some stages of imprinting memory formation.

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