Pharmacology Biochemistry & Behavior, Vol 24 pp 549–554, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Glutamate-Induced Asymmetry in the Sexual and Aggressive Behavior of Young Chickens

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Received 26 October 1984

BULLOCK, S P AND L J ROGERS *Glutamate-induced asymmetry in the sexual and aggressive behavior of young chickens* PHARMACOL BIOCHEM BEHAV **24**(3) 549–554, 1986 —A unilateral injection of monosodium glutamate (MSG) into the left hemisphere of the forebrain, at doses of either 50 nmol or 500 nmol per hemisphere on day 2 post-hatch, resulted in a marked and long lasting elevation of attack and copulatory behavior in cockerels This was not observed when MSG was injected into the right hemisphere or both hemispheres, and further demonstrates functional brain asymmetry in lower vertebrates A similar asymmetry was observed after administering the higher dose of MSG to females on day 2 A significant change in copulatory performance was observed when the higher dose was injected into the left hemisphere on day 11, compared to the controls, without affecting attack behavior, whereas, the lower dose failed to induce any changes This may be due to the development of efficient brain uptake mechanisms for glutamate The possibility that the behavioral changes were induced indirectly via increased secretion of plasma androgen, which then stimulates the appropriate brain centres, was found not to be the case

Brain asymmetry Glutamate Attack Copulation Androgens Effects on males and females Chickens

MARKED behavioral changes have been found after intracranial administration of low doses of glutamate, the putative neurotransmitter, into the forebrain of the male chicken during the first week of post-hatch life Unilateral administration of glutamate into either the right or left hemisphere has revealed functional asymmetry in the forebrain hemispheres Monosodium glutamate (MSG) injected into the left hemisphere results in a long-lasting elevation in aggressive and sexual behavior, as measured by the standard hand-thrust tests for attack and copulation This elevation is not observed after MSG treatment of either the right hemisphere or both hemispheres [8,16]

Testosterone implant studies have implicated the hypothalamus in the neuroendocrine regulation of aggressive and copulatory behavior (and possibly gonadotropin release) [4,5] In mammalian species, it is known that higher centres modulate hypothalamic functions, both behavioral and hormonal [10] Administration of glutamate into the chicken forebrain suggests that higher centres may have the capacity to modulate neural activity associated with the control of attack and copulation Rogers [15] has suggested that the left hemisphere, or pathways associated with it, normally inhibit attack and copulation in the untreated, young cockerel and that glutamate removes this inhibition

Furthermore, there is evidence of a role for glutamate in the neuronal control of gonadotropin release Olney [13] has found that systemic administration of glutamate in low doses to adult male rats causes a significant elevation of the levels of serum luteinizing hormone (LH) and testosterone within fifteen minutes after treatment More importantly, Ondo *et al* [14], using a central route of administration, have shown that glutamate injected into the third ventricle of anaesthetised male rats induces a significant elevation in plasma LH without a change in the level of follicle stimulating hormone

The elevation in attack and copulation which follows glutamate treatment of the left hemisphere may result from a direct excitatory effect of glutamate on forebrain pathways linked to the hypothalamic centres for attack and copulation, or indirectly by elevating LH and androgen levels, which then stimulate the hypothalamic centres for attack and copulation to induce the behavioral changes Thus, one aim of this study was to determine whether a correlation exists between plasma androgen levels and the elevation in attack and copulation observed in the treatment group receiving MSG into the left hemisphere

In female chicks, the effect of androgen on male-type copulatory behavior has been reported to be well below the levels of copulation scored in similarly-treated male chickens [2] Female chickens were treated with MSG to determine whether sexual differentiation of the chicken influences the underlying mechanism involved in the behavioral changes

Learning of visual tasks is permanently retarded by intracranial injection of MSG during the first week of post-hatch

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life, but not if MSG is administered after this time [18] Hambley and Rogers [7] have proposed that the termination of this sensitive period may be due to the development of efficient brain uptake mechanisms The possibility that the developing brain becomes less sensitive to the effect of glutamate on attack and copulation was also investigated

METHOD

Housing Conditions

Australorp-leghorn cross cockerels and pullets were obtained from a local poultry farm on day 1 post-hatch (the day of hatching) Chicks were initially housed in groups of five or six to ensure that they learned to feed and drink They were visually isolated from each other from day 5 onwards

Chick starter crumbs (Barastoc) and water were available ad lib Overhead bulbs provided constant light and warmth (25°C)

Drug Administration

Drug administration usually occurred on day 2, except for one experiment in which the chicks were injected on day 11

The intracranial injections were performed freehandedly with the animal conscious The injections were placed midway between the rostral-caudal aspects of each hemisphere and at least one millimetre on either side of the midline Sterile 25-gauge syringes were fitted with plastic stops to prevent penetration into the head of greater than three millimetres [9]

Monosodium glutamate (British Drug Houses) was dissolved in sterile pyrogen-free water and injected intracranially in a volume of 5 μl per hemisphere The effect was studied at two dose levels (50 and 500 nmol per hemisphere) within the dose range known to cause elevated attack and copulation in this strain of chickens [16] There were between 6 and 9 animals in each treatment group Control animals received 5 μl of 0 9% sterile saline per hemisphere, as an osmotic control for the effects of the sodium salt of glutamate

Testosterone oenanthate (12 5 mg, Schering A G , Berlin) was administered intramuscularly in 0 1 ml of an oil vehicle to a group of animals on day 2 (n=6), the control animals were untreated (n=3) This experiment was conducted simply to check the methods used

Behavioral Testing

The behavioral tests for attack and copulation are standard tests developed by Andrew [1] and modified by others [20,21] The test involves a rank-ordering on a scale of 0 to 10 of behavioral responses to a thrusting hand

Attack responses range from an avert gaze (no score) through to a maximum score of 10 for active sparring, pecking and leaping at the hand Copulatory responses also range from an avert gaze (no score) through to a full crouch on the hand with pelvic thrusting, circling and treading (an accumulated score of 10) The chicks were tested first for attack and then copulation Each animal was tested three times consecutively for each behavior and then a daily mean score was determined for both attack and copulation

The experimenter tested each animal without prior knowledge of scores on previous days and without checking the labelling of each treatment group for that experiment Inter-rater reliability was found to be 91% agreement for attack scoring and 94% for copulation scoring between testers

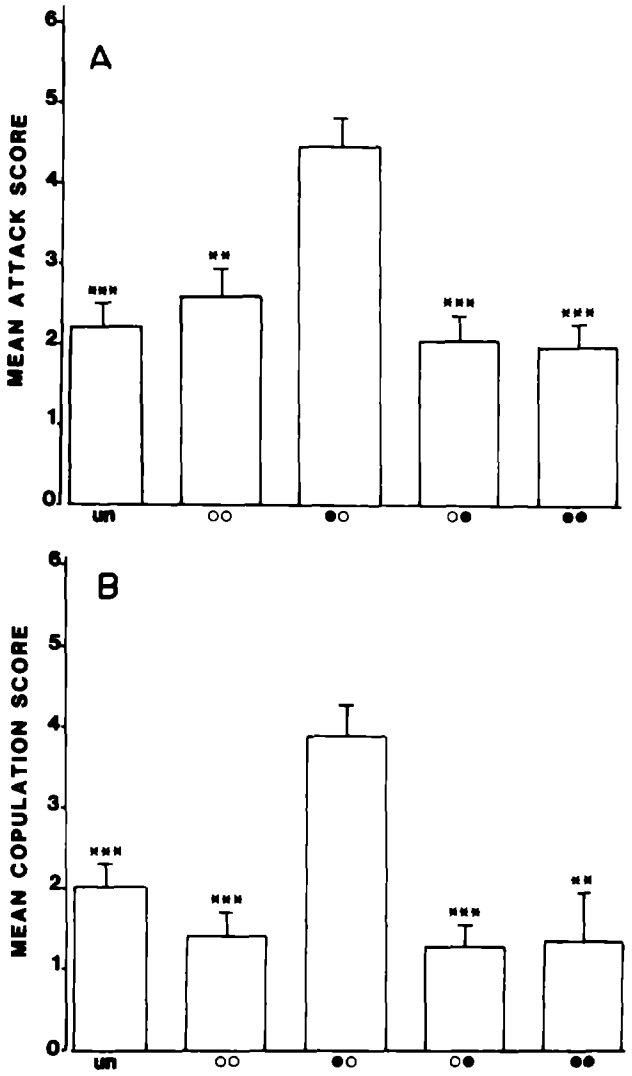

FIG 1 The observed behavioral responses for attack (A) and copulation (B) after injection of 500 nmol per hemisphere of glutamate to cockerels on day 2 post-hatch The data is represented as a mean (±standard error) for each treatment group The pair of circles below each histogram represents the chick hemispheres, the closed circle for glutamate injection and the open one for saline injection $^{**}p<0\ 01$, $^{***}p<0\ 001$ Statistical comparison of group treated in left hemisphere with each other group

Attack and copulation were tested from day 7 to 14 for all animals treated on day 2 of life In the experiments where treatment was delayed until day 11, attack and copulation were tested from day 8 or 9 to day 15 inclusively

Plasma Androgen Assays

All of the animals used in the hormonal investigation were killed by an overdose of sodium pentobarbitone (May & Baker, 0 5 ml intraperitoneal injection) on either day 14 or 15 of life Blood was withdrawn directly from the heart while it was still beating Plasma samples were refrigerated (−18°C) until assayed

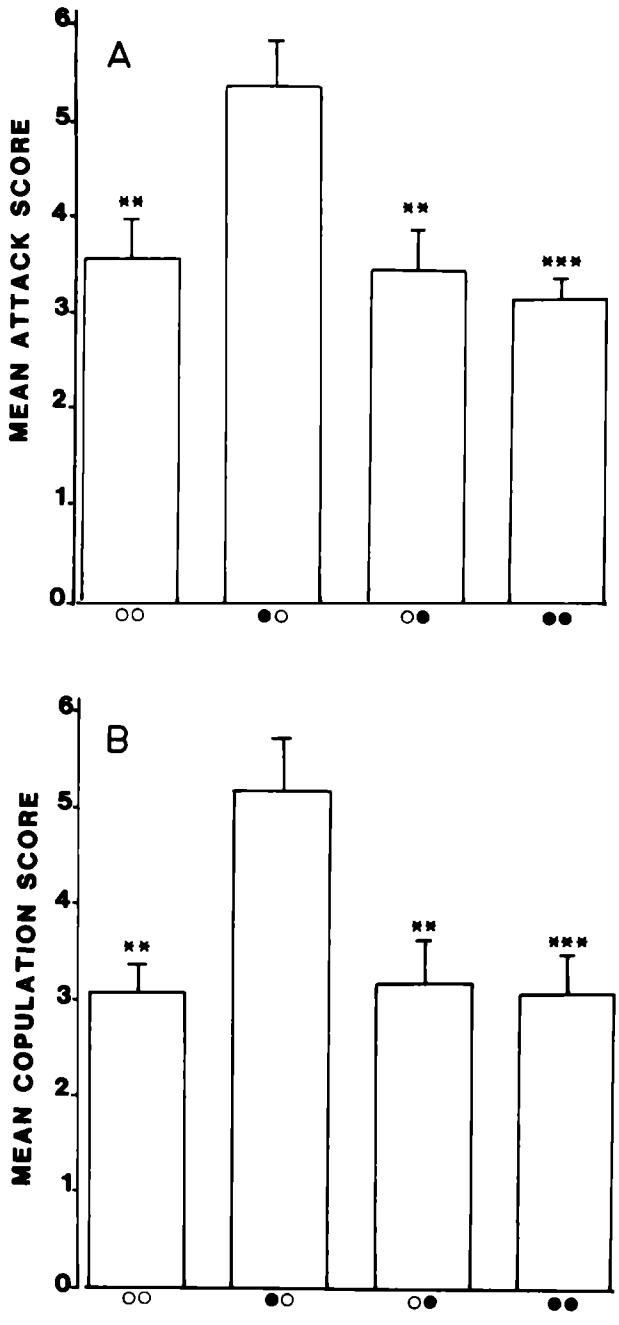

FIG 2 The observed behavioral responses for attack and copulation after injection of 50 nmol per hemisphere administered to cockerels on day 2 post-hatch Symbols as in Fig 1

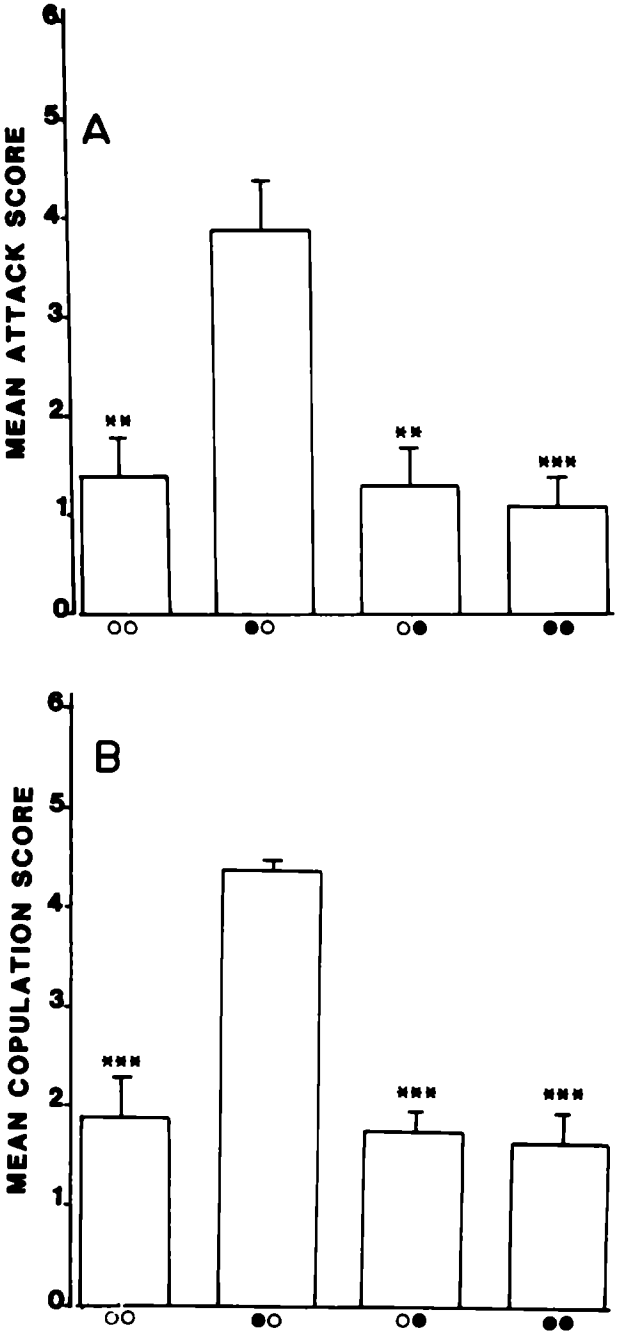

FIG 3 Attack and copulatory responses of pullets injected with 500 nmol per hemisphere on day 2 post-hatch Symbols as in Fig 1

Radioimmunoassays were used to measure plasma androgen level Details of the method have been reported previously [6]. Primary androgens detected using this method were testosterone and one of its major metabolites, 5α-dihydrotestosterone (5α-DHT) Both are capable of inducing elevated attack and copulation [20]

Two androgen antisera were made available for use in this study Both were 100% specific for testosterone, but had different cross-reactivities for 5α-DHT, one had 50% cross-reactivity and the other had 98% cross-reactivity As a consequence, interassay comparison between experiments is invalid

Body Measurements

Comb development is a sensitive bioassay for the level of circulating androgen [3] On the day of blood extraction an index of comb volume was estimated from its maximum length × maximum width × maximum height This was expressed as a ratio of comb volume to body weight

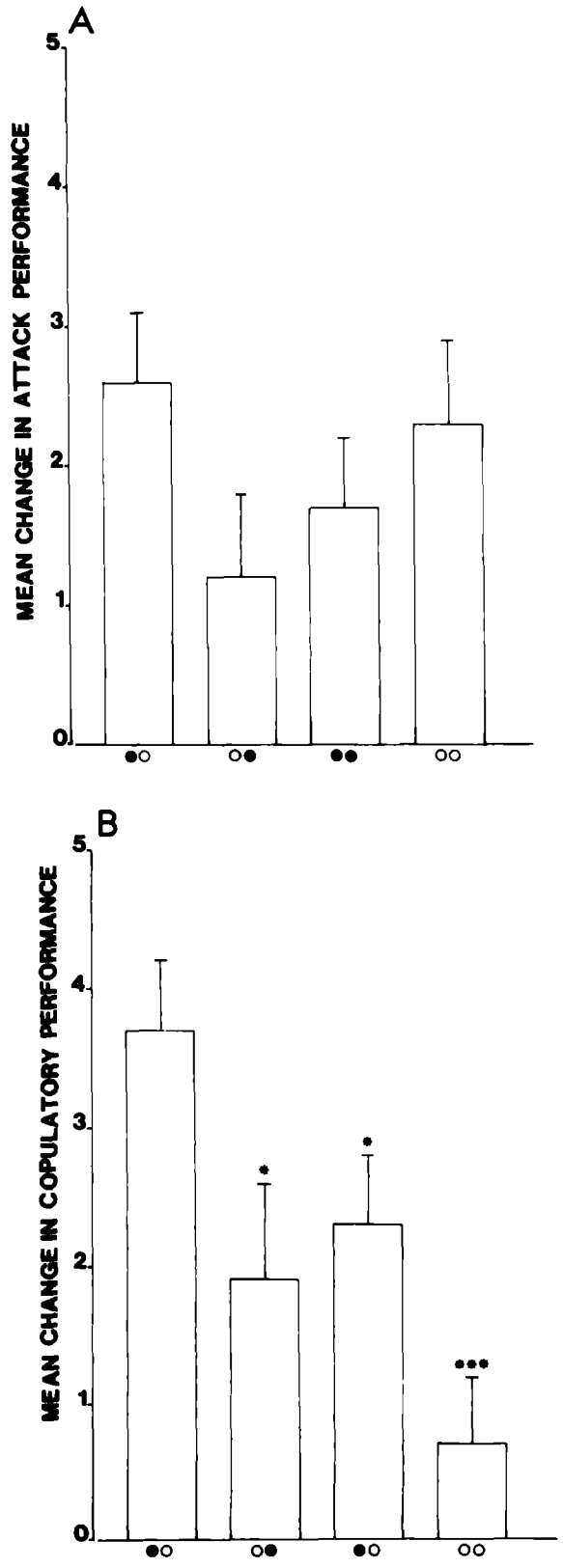

FIG 4 Change in attack and copulatory performance after cockerels are administered with 500 nmol per hemisphere on day 11 post-hatch *$p<0.05$, ***$p<0.001$

Statistical Analysis

The behavioral data were derived from an ordinal ranked scoring system and were analysed by non-parametric statistics [19] The individuals' daily mean scores were collapsed over the testing days and represented as an overall mean of the treatment group A Kruskal-Wallis test for heterogeneity was used and if significant heterogeneity was found ($p<0.05$), multiple Mann-Whitney U tests (one-tailed) were used to detect actual significance levels, since the direction of the MSG-induced effects had already been established [8,21] Diagrammatically, the data were shown as an overall treatment group mean (±standard error)

In the experiments where glutamate was administered on day 11, the data were separated into two four-day periods The daily mean scores for each individual were collapsed within each period to become two overall means The change in performance, the difference between these means for each group, was analysed by the Kruskal-Wallis test and, if significant, was followed by multiple Mann-Whitney U tests

The plasma androgen data indicated homogeneity of variance and was analysed by parametric statistics An ANOVA (one-way) was used to detect significant differences ($p<0.05$) between treatment groups, followed by Student's t-test (two-tailed) The comb development data indicated heterogeneity of variance and was transformed to logarithms The statistical analysis then followed the same procedure as for the plasma androgen assays

RESULTS

When the higher dose of glutamate (500 nmol per hemisphere) was administered intracranially to cockerels on day 2 post-hatch, there was found to be significant heterogeneity between groups for attack and copulation (Kruskal-Wallis $p<0.001$ for each behavior) The attack and copulation scores were elevated in cockerels given glutamate into the left hemisphere compared to the other treatment groups [individual comparisons with the saline, right and bilateral treatments $p<0.001$ for all comparisons except for bilateral group (copulation), $p<0.01$], but there were no significant differences between any of the other groups (Fig 1)

Similar effects were observed when cockerels received the lower dose (50 nmol per hemisphere) and in pullets which received the higher dose (500 nmol per hemisphere), both on day 2 The level of heterogeneity between groups was $p<0.01$, except for attack in cockerels given the lower dose, which was $p<0.05$ In individual comparisons with the left treated group for each behavior, the results for both sexes were similar ($p<0.001$ for all comparisons except the saline and right treated groups (attack), which was $p<0.01$) Refer to Figs 2 and 3 for cockerels and pullets respectively

When the higher dose of glutamate (500 nmol per hemisphere) was administered intracranially on day 11, a comparison was made between the change in performance of each group before and after administration of glutamate Significant heterogeneity was found for copulation (K-W $p<0.01$), but not for attack For copulatory behavior, the left-treated group showed a significant change in performance compared to the other treatment groups (individual comparison $p<0.001$ for the saline control and $p<0.02$ for the right and bilaterally-treated) while there were no significant differences between the other groups (Fig 4) However, there were no significant behavioral effects when cockerels received the lower dose (50 nmol per hemisphere) of glutamate

TABLE 1

EFFECTS OF MSG TREATMENT ON PHYSIOLOGICAL PARAMETERS

	UN	○○	●○	○●	●●
50 nmol/hemisphere		0 66 ± 0 33	0 50 ± 0 17	0 47 ± 0 15	0 35 ± 0 12
(male)		0 37 ± 0 10	0 28 ± 0 05	0 45 ± 0 10	0 36 ± 0 04
500 nmol/hemisphere	1 21 ± 0 20	1 04 ± 0 19	1 21 ± 0 10	1 39 ± 0 20	1 24 ± 0 13
(male)	0 40 ± 0 11	0 39 ± 0 10	0 31 ± 0 08	0 78 ± 1 46	0 65 ± 0 35
500 nmol/hemisphere		0 30 ± 0 10	0 25 ± 0 08	0 27 ± 0 09	0 39 ± 0 12
(female)		0 18 ± 0 04	0 25 ± 0 05	0 35 ± 0 12	0 30 ± 0 06

Summary of the effects of MSG treatment (for day 14 post-hatch) on plasma androgen levels (ng/ml), upper figures, with the corresponding comb development data (comb volume/body weight) immediately below Each dose of MSG was administered on day 2 post-hatch to cockerels or pullets

Data is represented as a means ± standard errors

The closed circle represents the hemisphere injected with MSG, while the open circle indicates a saline injection

UN is the untreated control group

Testosterone treatment (12 5 mg/animal) caused significant elevations in both attack and copulation (Mann-Whitney $p<0$ 01) It also enhanced comb development, $t(7)=14$ 23, $p<0$ 001, and plasma androgen level, $t(7)=4$ 81, $p<0$ 01, whereas, glutamate treatment failed to alter comb development or androgen level (Table 1)

Moreover, there was no significant correlation between plasma androgen level and overall attack (Spearman ranked test n=19, corr coef =0 300) or copulation score (corr coef =0 044) in the group which received glutamate into the left hemisphere Similarly, there was no correlation between plasma androgen level and comb development after glutamate treatment (Spearman n=49, corr coef =0 045)

DISCUSSION

The data presented confirms earlier findings that intracranial administration of MSG to the left hemisphere during the first week of post-hatch life induces long-term changes in aggressive and sexual behavior of the young cockerel [8,16] This effect was induced at two dose levels, 500 and 50 nmol per hemisphere Treatment of the right hemisphere or both hemispheres was without effect

Female chicks treated with intracranial glutamate (into the left hemisphere) exhibited increased attack and copulation as readily as their male counterparts treated on day 2 Sexual dimorphism has been found in brain regions and neuronal pathways related to hormone-dependent behavior, such as canary song [12] Although other investigations have found that female chicks treated with testosterone during the first week of post-hatch life do not show the elevated levels of attack and copulation which occur in similarly-treated male chicks ([2], C E Young, unpublished observations), in our laboratory we have found no differences in the aggressive or copulatory performance of male and female chickens treated with testosterone during the first week of post-hatch life This appears to be due to the intensity of the stimulus presentation (S P Bullock and R J Andrew, unpublished observations) In any case, from the data presented here it can be said that the MSG-induced elevation in attack and copulation can be elicited independently of the sexual differentiation of the chick brain during the first weeks of post-hatch life

After the 500 nmol dose of glutamate is administered to cockerels on day 11, there is a significant change in copulatory performance in the left-treated group compared to the controls The direction of the asymmetry is the same as for glutamate treatment on day 2 Yet, there was no effect of glutamate on attack In contrast to its ability to induce marked elevations of attack and copulation in the left-treated group when injected on day 2, the 50 nmol dose of intracranial glutamate produced no significant change in performance of attack or copulation in any of the treatment groups when injected after the first week of post-hatch life

These data suggest that there may be a sensitive period (of some sort) for the glutamate-induced elevation of attack and copulation, as has been found previously for the ability of glutamate to cause retarded visual learning Visual learning is retarded only when the injection occurs during the first week of post-hatch life (day 2) After this time, doses greater than 1000 nmol per hemisphere failed to retard learning unless administered with a neuronal uptake blocker such as ouabain [18] Whereas the 50 and 500 nmol doses induced elevated attack and copulation when injected on day 2, only the higher dose induced a behavioral change on day 11 This is consistent with the Rogers and Hambley hypothesis [7], that brain uptake systems are relatively inefficient during the first week so that any excess of glutamate is removed more slowly, therefore exerting a more prolonged extracellular influence on neurones

When the higher dose was administered after the first week of post-hatch life, only copulatory behavior was affected It is possible that the development of more efficient uptake systems has reduced the effective dosage of glutamate so that treatment on day 11 affects only copulation and not attack Andrew [2] has suggested that either the threshold dose for copulation is lower than for attack, since doses as low as 1 mg testosterone oenanthate facilitate copulation while having no effect on attack, or that these behaviors were affected via different routes The latter pro-

posal is supported by testosterone implant studies which show that only copulatory behavior is induced after implants are placed in the preoptic area of the capon hypothalamus, while aggressive behavior is induced after implants are placed within a narrow vertical region extending from the paleostriatum to the lateral diencephalon [4]

After unilateral injection of MSG into the left hemisphere no correlation between plasma androgen levels and the observed behavioral changes was found. It is possible that since we are measuring androgen level (testosterone and a significant proportion of 5α-DHT), we are overshadowing any correlation with either of these androgens alone Such an assay may be too crude to detect subtle differences in androgen levels of the order of picogram per ml, sufficient to induce the behavioral changes However, within the limits of our experiment, it seems unlikely that the elevation of attack and copulation is induced via stimulation of hypothalamic regions by increased circulating androgen

Administration of glutamate has been shown to interact with specific perceptual input to lead to retarded learning Not receiving this input after treatment protects the animal from learning deficits By a similar subtle interaction of glutamate with the developing brain and the fact that brain uptake systems are not sufficiently well developed in the first week of post-hatch life, glutamate may lead to changes in neural connectivity which directly affect forebrain pathways feeding on to one or more lower centres which initiate attack and copulation

Exogenously administered testosterone causes elevated attack and copulation and may be acting on this same system although in a manner and site of action different from that of glutamate Testosterone has been used to reveal asymmetrical control for attack and copulation Recently we have shown [17] that when testosterone-treated chickens are tested monocularly for copulation, the behavior can only be elicited when the subject is using its left eye, but not when it is using its right eye Due to the nature of optic pathways in the chick (where information entering one eye is largely processed by the contralateral hemisphere) it was concluded that the right hemisphere activates copulatory performance and the left hemisphere suppresses it In other words, testosterone-treatment may release attack and copulation by causing a switch of the dominance from the left hemisphere (in controls) to the right hemisphere

Similarly, Howard *et al* [8] postulated that the way the underlying mechanism by which treatment of the left hemisphere with glutamate has its effect is by causing a long lasting imbalance between the hemispheres In the untreated control animal, the left hemisphere may suppress these behaviors and glutamate treatment of this hemisphere removes the suppression

However, a recent study has shown lateralisation of behavioral responsiveness to hormones in lower brain centres (i e , the hypothalamus) of the female rat [11] This suggests that it may be premature for us to localise the asymmetrical nature of the testosterone effect to some area of the forebrain, where glutamate is thought to have its action, without further study, since it may be acting on lower brain centres such as the hypothalamus

Glutamate has been shown to be a useful tool for research into brain function at the endocrinological level and the behavioral level Given that glutamate and testosterone induce similar behavioral changes in the young chicken, glutamate may also prove to be valuable in providing some link with the action of testosterone in the brain

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