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ORIGINAL RESEARCH

Specific neural representation for a conceptual set of behavior: pair bonding

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Abstract: Our study examines whether ZENK expression in the nucleus taeniae (avian amygdala) serves as a predictor for pair bonding in ring doves. We were able to predict pair bonding in two experiments at 90%–100% using a quadratic discriminant analysis on principal component scores conducted on ZENK cell counts in the nucleus taeniae. This method appears to be more sensitive in predicting pair bonding than behavioral preference tests, which are subject to environmental factors such as housing conditions and testing parameters.

Keywords: pair bonding, taeniae (avian amygdala), ZENK expression, ring doves

Introduction

Pair bonding is of importance in understanding relationship dynamics between males and females within a given social species. Aside from aiding male parental care,[1] a pair bond functions in insuring paternity[1] and in aiding provisioning (Schwagmeyer and Mock D).[4] Separation from a mate pair has been shown to lead to weight increase and a diminishing of social interaction and exploratory behavior in hamsters.[5]

Monogamous pair bonded species have been shown to stray. The monogamous bank swallow, which forms lifetime pair bonds, has been seen spending time alternating between mate guarding and trying to seek out extra pair courting opportunities.[7] The right balance of these two alternating behaviors is said to be associated with producing as many offspring as possible while insuring that its mate's offspring are also his own.[8] Straying has been known to increase reproductive success in males[9,10] and females.[10–14] Paternity tests in many avian species that were once thought to be monogamous now show that this is not the case.[9,15,16] Eggs from the nests of monogamous birds had a high percentage of differing paternal DNA.[15,16]

Lifelong pair bonds are seen in animals that seek out their mate amongst a group of conspecifics despite an extended amount of separation. In a study by Morris and Erickson (1971),[17] doves that mated and reared a squab together exhibited this mate seeking behavior in an outdoor arena, amongst a large group of other doves, despite being separated from each other for over a year. In the laboratory, triad tests are used to mimic this effect by using amount of time spent on sides of a preference chamber to look at a subject's preference to spend time with their mate versus a stimulus. They serve as a proxy measurement of pair bonding in which animals that spend more time with their mate are considered to be pair bonded. We reason that a neural marker, if such a marker exists, may be a more reliable indicator of pair bonding and would establish that there is a neural representation for pair bonding in the brain and may solidify the

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importance of pair bonding as a deep rooted, evolutionary driven behavior. Currently, no study has established that there is a neural representation for pair bonding in the brain. Previous work done in zebra finch has implicated the nucleus taeniae (avian amygdala) in having a role in preening and clumping, two behaviors that are associated with courtship in finch.[18] Studies in hamsters, rats, and quail have shown that areas of the amygdala/nucleus taeniae are responsible for the mediation of sexual satiety, appetitive behavior, and consummatory behavior.[19,20]

Tract tracing work in the nucleus taeniae has shown that it has afferent connections toward several hypothalamic regions and reward system areas, including the nucleus accumbens.[21] These connections, as well as the studies mentioned above, suggest that the nucleus taeniae may be a likely candidate in encoding stimulus properties leading to pair bonding, might serve as a neural marker for such behavior, and could function in mediating pair bonding behavior in the brain.

Studies in rats, birds, and humans have shown a similarity in subcortical circuitry involved in unconscious emotional signals and suggest that subcortical structures, such as the amygdala, involved in processing emotional signals evolved early.[22] By understanding behaviors, such as pair bonding, controlled by these subcortical reward driven regions, we might begin to understand pair bonding behavior across multiple species.

The present study asks if ZENK expression in the nucleus taeniae is a reliable indicator of pair bonding, therefore having the ability to serve as a neural marker for pair bonding. A quadratic discriminant analysis, which takes into account regional specificity within the nucleus taeniae, is used to classify doves as either pair bonded or not pair bonded.

In experiment 1, we looked at whether ZENK counts in the taeniae were able to classify both male and female doves as pair bonded or not. In experiment 2, we looked if this classification was persistent when using a larger, all female group. In both experiments, doves are tested using a preference test to determine the reliability of traditional stimulus preference tests in classifying pair bonded doves.

Methods

Experiment 1

The subjects were a group of ten ring doves bred and housed in an animal housing facility in the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited animal care facility at Newark, Rutgers University. The doves were equally divided into two groups: a bonded group (n = 5; 3 females and 2 males) and a nonbonded group (n = 5, 5 females). The bonded group was allowed to mate with a female (if male) and one male (if female) and rear at least one squab, as described.[23] The nonbonded group and bonded group were housed separately a week prior to the initiation of the experiment. During the experiment, the nonbonded group was housed with stimulus males that were rotated out of their cage on Monday, Wednesday, and Friday of every week to prevent the possibility of them bonding.

Stimulus preference test

Doves in both groups were put through a y-shaped preference test developed by our laboratory (Figure 1). The male dove was allowed to roam the chamber for 15 minutes preceding data collection to get acclimated with the chamber.

Afterwards, two females (if subject was male) or two males (if subject was female) were put into plexiglass contained chambers opposite each other. The males in the pair bonded group were exposed to their mate and a novel stimulus female (if male) or a novel stimulus male (if female) while the birds in the non-pair bonded group were exposed to two novel stimulus males. The subject was put behind a plexiglass compartment that allowed it to view both females (if male) or both males (if female) but not to interact with them or roam through the chamber. It was held there for an additional 15 minutes.

The plexiglass containing the subject was then lifted and the subject was allowed to roam the chamber freely for an hour. During this time, we recorded the amount of time spent on each side of the preference chamber.

The percentage of time spent with their mate was calculated by dividing total time spent with mate by total time spent with stimulus birds and mate. Birds were determined to have a preference for their mate if they spent more than 60% of time with their mate.

Immediate early gene ZENK staining

Subjects in both groups were perfused using a 4% paraformaldehyde solution. Brains were extracted and fixed in 4% paraformaldehyde solution overnight. Tissue was stored in a 30% sucrose solution for approximately 2 days postfixation.

Tissue was cut into 30 μm coronal sections, washed, and stained in accordance to procedures described by Svec et al.[18] Briefly, tissue was washed and incubated in a 1 μg/mL dilution of primary antibody anti-Egr-1 (Catalog number sc–189, Santa Cruz Biotechnology Inc., Dallas, TX, USA) overnight. The tissue was then rinsed and incubated in a 1:500 dilution of secondary biotin-sp-conjugated donkey

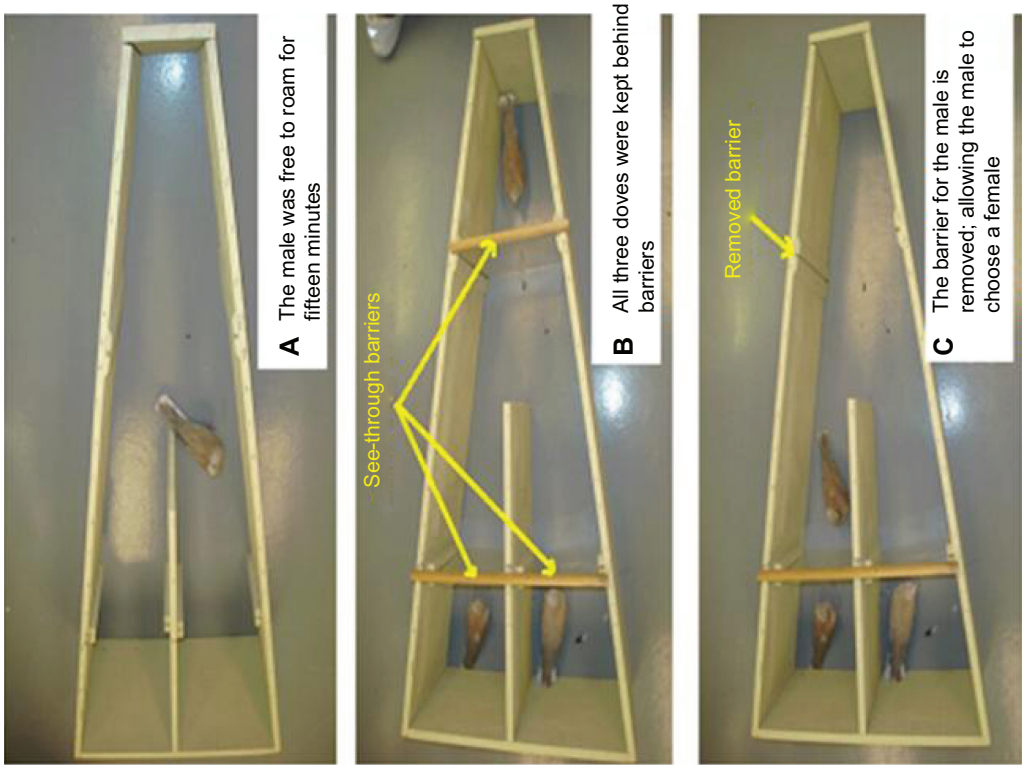

Figure 1 Behavioral preference test.

anti-rabbit immunoglobulin (Ig)G antibody (catalog number 712-065-153, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) for 1 hour. Postincubation, tissues were processed using the Vectastain ABC Elite Kit (catalogue number PK–6100, Vector Laboratories, Inc., Burlingame, CA, USA) followed by visualization using diaminobenzidine with 0.0075% hydrogen peroxide. Tissue was then mounted, dehydrated, and coverslipped. Control sections without primary antibody also underwent this process.

Statistical analysis and ZENK quantification

For each bird, three sections were counted and averaged for both medial and lateral portions of each of the following regions corresponding to the pigeon brain atlas:[24] 7.50–7.25 region (more anterior), 7.25–7.00 region, and 6.75–6.50 region (more posterior). A *t*-test was done to determine if there were differences in cell counts for these regions.

Additionally, a principal component analysis was conducted on the ZENK cell counts to reduce dimensions. A linear and quadratic discriminant analysis was done on the principal component analysis to determine if pair bonded/non-pair bonded birds could be classified using principal component scores based on ZENK cell counts.

Experiment 2

The subjects were a group of sixteen female ring doves bred and housed in an animal housing facility in the AAALAC accredited animal care facility at Newark, Rutgers University. The doves were equally divided into two groups: a bonded group (n = 10) and a nonbonded group (n = 10). As in experiment 1, the bonded group consisted of females that were allowed to go through the bonding process and were housed separately a week prior to the initiation of the experiment. The nonbonded group was housed with stimulus males that were rotated out of their cage on Monday, Wednesday, and Friday of every week to prevent the possibility of them bonding.

Male subject group

An additional group of five pair bonded males were allowed to undergo the same procedure as their female counterparts. A *t*-test was done on ZENK cell counts to determine if there was a difference between male and female subjects.

Stimulus preference test

The same protocol was used as in experiment 1.

Immediate early gene ZENK staining

Similar to experiment 1, subjects in both groups were perfused using a 4% paraformaldehyde solution. Brains were

extracted and fixed in 4% paraformaldehyde solution overnight. Tissue was stored in a 30% sucrose solution for approximately 2 days postfixation. The same protocol, described above, was used for ZENK staining.

Statistical analysis and ZENK quantification

As in experiment 1, three sections were counted and averaged for both medial and lateral portions of each of the following regions: 7.50–7.25 region (more anterior), 7.25–7.00 region, and 6.75–6.50 region (more posterior) for each bird. A *t*-test was done to determine if there were differences in cell counts for these regions. A quadratic discriminant analysis was done on principal component scores of ZENK counts, as described in experiment 1.

Results

In both experiments, significant differences in cells positively labeled for ZENK were found between pair bonded birds and non-pair bonded birds in the anterior portions of regions 7.50–7.25 (experiment 1: $t = 2.49$, $P<0.05$; experiment 2: $t = 3.2$, $P<0.05$) and 7.25–7.00 (experiment 1: $t = 2.70$, $P < 0.05$; experiment 2: $t = 2.95$, $P<0.05$). Sex differences were not seen in pair bonded samples in either group.

Interestingly, ZENK expression in the pair bonded group was not correlated with time spent with the mate (experiment 1: $r[3] = 0.127$; $P>0.05$; experiment 2: $r[8] = 0.13$; $P>0.05$). Although most birds had a preference for their mate as determined by the y-shaped preference test, some pair bonded birds did not. In addition, for the pair bonded birds that did have preferences for their mates, the percentage of time spent with their mate and amount of time spent with their mate varied greatly (Table 1).

The principal component analysis conducted on ZENK cell counts showed that three components (experiment 1) and four components (experiment 2) accounted for 95% of the variance in our data. Principal component scores were assigned to the data.

In experiment 1, birds were classified using both a linear and quadratic discriminant analysis that was based on principal component scores assigned to the data. The linear discriminant analysis showed a misclassification error rate of 30% while the quadratic discriminant analysis was able to classify whether birds were pair bonded or not with 100% accuracy (Figure 2).

In experiment 2, principal component scores based on cell counts were classified using a quadratic discriminant analysis. The quadratic discriminant analysis

Table 1 Time subjects spent with pair bonded mate in each experiment

Dove	Percent of time spent with mate
A	
1	67%
2	59%
3	63%
4	39%
5	61%
Female	**Percent of time spent with mate**
B	
1	72%
2	38%
3	100%
4	22%
5	62%
6	89%
7	62%
8	82%
9	54%
10	100%

Notes: (**A**) Percentages of time subjects spent with pair bonded mate in experiment 1. (**B**) Percentage of time female subject spent with pair bonded mate in experiment 2.

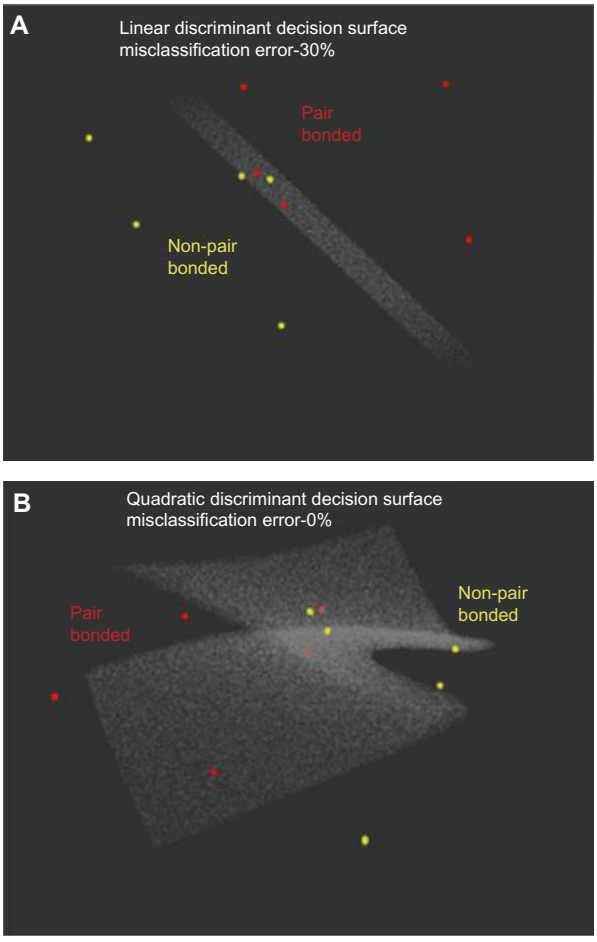

Figure 2 Results of discriminant analysis for experiment 1.
Notes: (**A**) Classification using a linear discriminant analysis for experiment 1. (**B**) Classification using a quadratic discriminant analysis for experiment 1.

was able to classify whether birds were pair bonded or not with 90% accuracy. This is consistent with preliminary data that classified doves as either pair bonded or not bonded with 100% accuracy.

Discussion

The results gained from both experiment 1 and 2 suggest that ZENK expression patterns can be used as a neural marker for pair bonding, indicating that this region encodes stimulus properties of the perceptual and motor manifestations associated with pair bonding and illustrating the region's power of predictability. ZENK counts in the anterior medial taeniae were significantly higher in pair bonded doves than in non-pair bonded doves. Additionally, classification using a quadratic discriminate analysis on principal component scores, based on ZENK counts in all regions, ranging from more anterior to posterior, in the taeniae was able to predict pair bonding with high accuracy. ZENK, a neuronal marker that is used to measure changes in behavioral states, is considered to be highly conserved among species.[25] The high predictability of ZENK expression in the nucleus taeniae for pair bonding supports that pair bonding is encoded in the rewards system and suggests that this marker for pair bonding may be retained across multiple species.

The results of our preference tests mimic that of triad tests,[26] indicating that doves do not always prefer to spend time with their mates and corroborate the observation that straying behavior is found amongst pair bonded animals. Inherent in these preference tests are experimental factors, such as housing conditions, time spent away from mate before the commencement of the test, and other procedural factors that could impact the results of the test. Although all birds were separated for a week prior to the preference test, and should, therefore, have been separated long enough to control for the "coolidge effect," this may still be one of the confounding factors that could account for birds not choosing their mates during preference tests. Because only a small percentage of pair bonded doves did not prefer spending a greater amount of time with their mate, we determined that the "coolidge effect" has little impact on preference results. These and other unknown factors could complicate the interpretation of the preference test and make it difficult to determine whether the results of the preference test are solely determined by pair bonding.

The amygdala, the mammalian counterpart to the nucleus taeniae, has been tied to social and survival behaviors such as fear[27,28] and processing of emotional memories.[29,30] The nucleus taeniae has also been linked to fear in birds;[31] however, the specific region of the taeniae involved has not been specified. This, together with work that analyzed courtship-like behavior in this region,[18] indicates that separate areas of the nucleus taeniae are involved in mediating pair bonding associated behavior. Interestingly, a lesion study in ring doves has found that taeniae lesioned females will nest coo (a courtship behavior that mediates the reproductive–endocrine system) at a higher rate than nonlesioned doves, bypassing the natural fear response females normally have toward unfamiliar male doves. This suggests that the nucleus taeniae can exact an influence on the rate of courtship behavior by controlling fear factors. Svec et al[18] have shown that other courtship-like behaviors, such as preening, are associated with ZENK expression in the taeniae. The taeniae, therefore, is an integrative hub of various factors contributing to the establishment of pair bonding. Whether the nucleus taeniae functions to aid in a behavioral manifestation of the discrimination of mates from nonmates and inhibits females from performing this behavior before they become bonded has not been explored, however, this may be the case and is currently under study.

The taeniae is ideally connected for behaviors associated with reproductive strategies via various distinct regions of the archistriatum, the nucleus accumbens, and other areas responsible for visual and olfactory input (Cheng et al 1999).[21] In follow-up lesion studies, discrete regions of the taeniae as well as regions associated with reproductive and courtship behavior will be lesioned to evaluate the connectivity of the taeniae that support the maintenance and formation of pair bonding through its afferent inputs.

Our study has served to highlight the importance of regional versus global analysis when it comes to cell quantification. Previous studies that assessed sexual behavior, fear behavior, courtship behavior, pair bonding-associated behavior, and other forms of social behavior in general[18,21,31,32] have measured the taeniae globally, ie, they analyzed the taeniae as a whole rather than measuring regions of interest in the taeniae that might be implicated in a particular behavior. In this experiment, we used a classifier analysis procedure on ZENK expression cell counts in all regions, and did so by discriminating between different regions of the nucleus taeniae. Statistical differences in cell counts, however, were only seen in the most anterior region.

In summary, we have demonstrated an exceptional predictive power of ZENK expression in the nucleus taeniae for pair bonding that appears to override any confounding factors of testing procedures common in behavioral measurement.

Disclosure

The authors report no conflict of interest in this work.

References

1. Mock DW, Fujioka M. Monogamy and long-term pair bonding in vertebrates. *Trends Ecol Evol*. 1990;5(2):39–43.
2. Marlow FW. A critical period for provisioning by Hadza men: Implications for pair bonding. *Evol Hum Behav*. 2003;24(3):217–229.
3. Quinlan RJ. Father absence, parental care, and female reproductive development. *Evol Hum Behav*. 2003;24(6):376–390.
4. Schwagmeyer PL, Mock D. How Consistently are Good Parents Good Parents? Repeatability of Parental Care in the House Sparrow, Passer domesticus. *Ethology*. 2003;109(4):303–313.
5. Crawley JN. Evaluation of a proposed hamster separation model of depression. *Psychiatry Res*. 1984;11(1):35–47.
6. Marazziti D, Dell'Osso B, Baroni S, et al. A relationship between oxytocin and anxiety of romantic attachment. *Clin Pract Epidemiol Ment Health*. 2006;2:28.
7. Beecher MD, Beecher IM. Sociobiology of bank swallows: reproductive strategy of the male. *Science*.1979;205(4412):1282–1285.
8. Barash D, Lipton J. *The Myth of Monogamy*. New York: Henry Holt and Company; 2001.
9. Wetton JH, Burke T, Parkin DT, Cairns E. Single-locus DNA fingerprinting reveals that male reproductive success increases with age through extra-pair paternity in the house sparrow (*Passer domesticus*). *Proc Biol Sci*. 1995;260(1375):91–98.
10. Forstmeier W, Kempenaers B, Meyer A, Leisler B. A novel song parameter correlates with extra-pair paternity and reflects male longevity. *Proc Biol Sci*. 2002;269(1499):1479–1485.
11. Dunn PO, Robertson RJ, Michaud-Freeman D, Boag PT. Extra-pair paternity in tree swallows: why do females mate with more than one male? *Behav Ecol Sociobiol*. 1994;35(4):273–281.
12. Lubjuhn T, Strohbach S, Brün J, Gerken T, Epplen JT. Extra-pair paternity in great tits (Parus major) – a long term study. *Behaviour*. 1999;136(9):1157–1172.
13. Gray EM. Female red-winged blackbirds accrue material benefits from copulating with extra-pair males. *Anim Behav*.1997;53(3):625–639.
14. Kempenaers B, Verheyen GR, Van den Broeck M, Burke T, Van Broeckhoven C, Dhondt A. Extra-pair paternity results from female preference for high-quality males in blue tit. *Nature*.1992;357:494–496.
15. Birkhead TR, Burke T, Zann R, Hunter FM, Kurpa AP. Extra-pair paternity and intraspecific brood parasitism in wild zebra finches *Taeniopygia guttata*, revealed by DNA fingerprinting. *Behav Ecol Sociobiol*. 1990;27(5):315–324.
16. Griffith SC, Owens IP, Thuman KA. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Mol Ecol*. 2002;11(11):2195–2212.
17. Morris RL, Erickson CJ. Pair bonding maintenance in the ring dove (Streptopelia risoria). *Animal Behavior*. 1971;19(2):398–406.
18. Svec LA, Licht KM, Wade J. Pair bonding in the female zebra finch: a potential for the nucleus taeniae. *Neuroscience*. 2009;160(2):275–283.
19. Absil P, Braquenier JB, Balthazart J, Ball G. Effects of lesions of nucleus taeniae on appetitive and consummatory aspects of male sexual behavior in Japanese quail. *Brain Behav Evol*. 2002;60(1):13–35.
20. Parfitt DB, Newman SW. Fos-immunoreactivity within the extended amygdala is correlated with the onset of sexual satiety. *Horm Behav*. 1998;34(1):17–29.
21. Cheng M, Chaiken M, Zuo M, Miller H. Nuclues taenia of the amygdala of birds: anatomical and functional studies in ring doves (Streptopelia risoria) and European Starlings (Sturnus vulgaris). *Brain Behav Evol*. 1999;53(5–6):243–270.
22. Tamietto M, de Gelder B. Neural bases of the non-conscious perception of emotional signals. *Nat Rev Neurosci*. 2010;11(10):697–709.
23. Morris RL, Erickson CJ. Pair bond maintenance in the ring dove (Streptopelia risoria). *Anim Behav*. 1971;19(2):398–406.
24. Karten HJ, Hodos W. *A Stereotaxic Atlas of the Brain of the Pigeon: Columba Livia*. Baltimore, MD: Johns Hopkins Press; 1967;696.
25. Long K, Salbaum JM. Evolutionary conservation of the immediate-early gene ZENK. *Mol Biol Evol*. 1998;15(3):284–292.
26. Clavijo Alex, Cheng MF. The role of hypothalamus in pair bonding. Poster presented at: Annual Biomedical Research Conference for Minority Students; Nov 2007; Austin, Texas.
27. Maren S, Fanselow MS. The amygdala and fear conditioning: has the nut been cracked? *Neuron*. 1996;16(2):237–240.
28. Campeau S, Davis M. Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *J Neurosci*. 1995;15(3):2301–2311.
29. Cahill L, Babinsky R, Markowitsch HJ, McGaugh J. The amygdala and emotional memory. *Nature*. 1995;377(6547):295–296.
30. Hamann S. Cognitive and neural mechanisms of emotional memory. *Trends Cogn Sci*. 2001;5(9):394–400.
31. Brito I, Britto LR, Ferrari EA. Induction of Zenk protein expression within the nucleus taeniae of the amygdala of pigeons following tone and shock stimulation. *Braz J Med Biol Res*. 2011;44(8):762–766.
32. Thompson RR, Goodson JL, Ruscio MG, Adkins-Regan E. Role of the archistriatal nucleus taeniae in the sexual behavior of male Japanese quail (Coturnix japonica): a comparison of function with the medial nucleus of the amygdala in mammals. *Brain Behav Evol*. 1998;51(4):215–229.