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The Effects of Melatonin on Isolation Distress in Chickens

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NELSON, E., J. PANKSEPP AND S. IKEMOTO. *The effects of melatonin on isolation distress in chickens.* PHARMACOL BIOCHEM BEHAV 49(2) 327-333, 1994.—Melatonin (0.1-1.0 mg/kg) reduced isolation-induced distress vocalizations (DVs) in young domestic chickens in a dose-dependent manner. This effect was unaffected by the administration of *d*-amphetamine (1.0 mg/kg) suggesting that melatonin's effects were not merely due to fatigue. The melatonin reduction in DVs was not naloxone reversible, indicating an action independent of the endogenous opioid system. However, chronic pretreatment with naltrexone facilitated the melatonin effect, suggesting a complex relationship between melatonin and the endogenous opioids in regulating distress vocalizations. Chickens exhibited a marked reduction in DVs when isolation chambers were darkened, suggesting endogenous, as well as exogenous, melatonin mediation of isolation distress; however; pinealectomy only partially reversed the darkness effect. Pinealectomized animals, like control animals, exhibited a reduction in DVs following melatonin treatment; however, the melatonin effect was shorter lasting. The implications that these results may have for socialization and emotional distress are discussed.

Melatonin Circadian Anxiety Distress vocalizations Opioids Isolation distress

IN the young of mammalian and avian species enforced social isolation can provoke vigorous emotional protest that is characterized by prolonged bouts of vocalization (28). These distress vocalizations (DVs) serve to alert parents to direct attention to finding, feeding, and comforting the lost offspring. This emotional response of the infant appears to be based on an intrinsic operating system of the brain (25) which may have important implications for certain psychiatric disorders including, depression, autism, and perhaps child abuse (28). A variety of neurochemical systems have now been found to participate in the elaboration of this emotional response, yielding potential new insights into understanding the basic psychobiological mechanisms that mediate social motivation.

Previous findings from this lab and others have suggested that endogenous opioids play a role in social reward and social attachment (27,30). Opioid agonists decrease isolation-induced distress in chickens and increase juvenile play behavior in rats, while opioid antagonists increase isolation-induced distress and decrease play. Further evidence for involvement of endogenous opioids in social behaviors comes from studies that have found changes in brain opioid activity following social interaction in both rats and primates (15,26). These results indicate that endogenous opioids may be a fundamental neurochemical component of socialization.

However, social interactions are both varied and complex, and opioid release is likely to be only one component in a complex set of neurochemistries that control social reward and separation distress (25). For example, a recent study (23) found that opioid blockade did not block social reward in juvenile rats. Besides opioids, it has been suggested that oxytocin, serotonin, and an endogenous benzodiazepine-like molecule may also be involved in social processes (5,6,11,12, 24,25,31). In addition, it has been suggested that melatonin may be directly involved in the etiology of autism (7,29), a disease that is marked by asociality. Melatonin may, therefore, contribute to the neurochemical control of social emotions.

Interestingly, a relationship has been established between melatonin and many neurotransmitters that have previously been found to modulate social behavior. Lakin et al. (17) reported a naloxone reversible circadian fluctuation in murine analgesia, suggesting a link between endogenous melatonin release and endogenous opioid activity. Likewise, Kumar et al. reported circadian fluctuations in Met-enkephalin levels that were reversed by pinealectomy (16). Additionally, several studies have found that melatonin retards the aging process in mice, and it has been suggested that this effect may be mediated through the endogenous opioids (19,32).

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Like benzodiazepines, melatonin has been found to inhibit CRF release (13), and, therefore, result in lowered plasma levels of beta-endorphin (18). As CRF administration has been found to increase rates of isolation-induced distress vocalization (25), melatonin may inhibit DVs by reducing brain CRF release. Furthermore, melatonin has been found to inhibit diazepam binding in the central nervous system, suggesting an action at the benzodiazepine-GABA receptor complex (20). Recently, it was reported that melatonin-induced analgesia was not only naloxone reversible, but also reversible with the benzodiazepine antagonist, RO15-1788 (9), suggesting a complex relationship between melatonin, opioids, and GABA. Finally, Anton-Tay et al. (3) have reported that melatonin administration increased central serotonin levels, and serotonin agonists have been found to inhibit DVs (25).

Because melatonin appears to be linked to many neurochemical systems that may be involved in social processes, and because isolation-induced DVs appear to undergo a circadian fluctuation (30), the present study was undertaken to systematically assess the effect of melatonin on social isolation distress in young chickens.

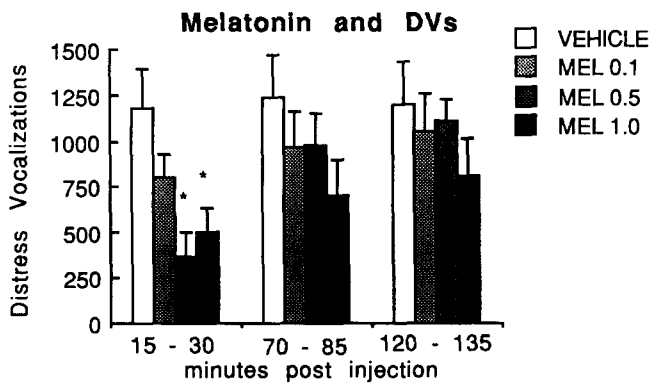

FIG. 1. Distress vocalizations for 5-day-old chicks isolated for 15-min periods 15, 70, and 120 min after receiving one of three doses of melatonin. Asterisks indicate a significant ($p < 0.05$) difference from control for specified time period. Standard error bars are indicated.

GENERAL METHOD

Subjects

Domestic chicks (*Gallus gallus*) of the cornish red rock broiler strain, ranging in age from 2 to 12 days, were used for all experiments. All chicks were housed socially in large (60 × 90 cm) housing pens. Food and water were available ad lib. The animal room was maintained on a 12 L : 12 D cycle throughout the testing period. All testing was conducted during the first half of the light cycle.

Apparatus

Isolation-induced distress vocalizations (DVs) were recorded with an automated apparatus. The apparatus consisted of 16 separate sound-attenuated lighted chambers into which individual chicks were placed for various periods of time. Each chamber contained a microphone that was designed to detect distress vocalizations and transmit the vocalizations to a computer where the data was tabulated and stored.

Drugs

Melatonin, *d*-amphetamine, naloxone hydrochloride, and morphine sulfate were used. Melatonin (and melatonin vehicle) contained 1% ethanol to facilitate dissolution. All other drugs were mixed in distilled water. All injections were given intraperitoneally (IP) in a volume of 1 ml/kg.

EXPERIMENT 1A

Thirty-two 5-day-old chicks were randomly assigned to four experimental groups. Each group received one dose of melatonin (0.0, 0.1, 0.5, and 1.0 mg/kg) prior to social separation. Chicks were weighed and injected then returned to a social condition for 15 min. All chicks were then subjected to three 15-min separation sessions that occurred 15, 70, and 120 min after the injections. After each DV collection period, chicks were returned to the social housing pens.

Results and Discussion

The number of distress vocalizations emitted by each chick (Fig. 1) was subjected to a two-factor mixed design ANOVA with melatonin dose as the between factor and time block as the within factor. A significant group by time interaction was found, $F(6, 56) = 3.89$, $p < 0.01$, as well as a significant main effect for time block,;$F(2, 56) = 17.73$, $p < 0.001$. The interaction reflected a significant group effect at the first time block, $F(3, 29) = 5.41$, $p < 0.01$. The mean DVs obtained at each melatonin dose was compared to the DVs obtained for the vehicle group for each time period using orthogonal polynomial analysis. This analysis revealed a significant reduction in DVs for each of the two high melatonin groups (0.5 and 1.0 mg/kg melatonin) and the control group at this first time block ($p < 0.01$ for both doses). No statistically reliable group difference was found at either the second or third time block for the higher doses, or at any time block for the lowest dose.

The finding from this first experiment, that melatonin decreased distress vocalizations, suggested that the melatonin-treated chicks experienced less emotional stress during social isolation than the vehicle-treated chicks. However, melatonin is reported to have soporific effects (10). The reduction in DVs may, therefore, have been the result of decreased behavioral output due to a generalized sedation rather than a specific emotional effect. Indeed, many of the chicks from the higher melatonin groups appeared moderately drowsy. Typically, chicks would be sitting down and inattentive. Because of this confounding effect, we proceeded to evaluate the potency of melatonin in the presence of *d*-amphetamine. If melatonin merely decreased DVs because of sleepiness, it was anticipated that amphetamine-induced arousal would tend to reverse the melatonin effect. Amphetamine by itself has little effect on DVs (27).

EXPERIMENT 1B

Twenty-six chicks were randomly assigned to one of three drug treatment groups: vehicle/vehicle, vehicle/melatonin, and *d*-amphetamine/melatonin. All drugs were administered successively at doses of 1 mg/kg (given IP in a volume of 1 ml/kg).

Approximately 15 min after receiving the second injection, chicks were isolated and placed in the DV recording chambers for a single 15-min test session. As an index of drowsiness, it was noted whether the chick was sitting or standing before removal from the isolation chamber.

Results and Discussion

d-Amphetamine appeared to reverse the melatonin-induced drowsiness. The melatonin/vehicle group was easily distinguishable from the other two, as most of the chicks (six out of eight) were sitting when removed from the DV boxes. Amphetamine appeared to reverse this effect. Only three of the eight amphetamine/melatonin-treated animals were sitting when removed from the DV boxes, which compares favorably with the vehicle/vehicle group in which two of the eight were sitting. Because the amphetamine/melatonin group did not appear noticeably different from the vehicle/vehicle group, it seems that amphetamine reversed the melatonin-induced drowsiness.

The number of distress vocalizations emitted during the 15-min isolation period (Fig. 2) was subjected to a one-way ANOVA, with drug treatment as a between-groups factor. This analysis revealed a significant overall difference between the groups, $F(2, 23) = 4.00, p < 0.05$. A post hoc Neuman–Keuls comparison of the three means revealed a significant difference between both melatonin groups and the control group ($p < 0.05$). No significant difference between the two melatonin groups was evident.

EXPERIMENT 2

Previous studies have reported that manipulation of the endogenous opioid systems had powerful effects on distress vocalizations (27). Several studies have also reported an interaction between melatonin and the endogenous opioid systems (14,17,19). It is, therefore, possible that the DV alleviating effects of melatonin may be mediated through the release of endogenous opioids. To evaluate that possibility, we administered melatonin to chicks in the presence and absence of the opioid antagonist naloxone.

Twenty-four 5-day-old chicks were randomly assigned to four groups. Two groups received 1.0 mg/kg melatonin followed by a vehicle or naloxone (5 mg/kg) injection while the other two groups received a vehicle injection followed by an injection of vehicle or the same dose of naloxone. Immediately following the second injection chicks were isolated and placed in the DV chambers. DVs were collected every 5 min for a 45-min period.

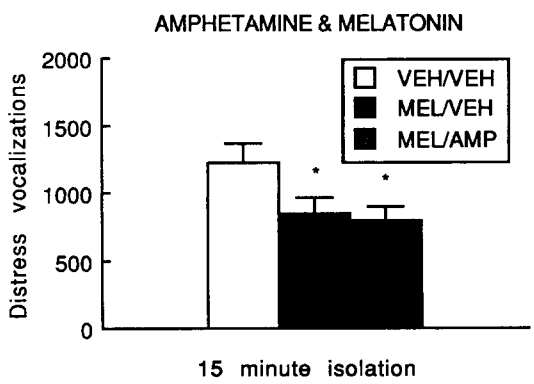

FIG. 2. Distress vocalizations for chicks collected for a 15-min period beginning approximately 15 min after receiving a two injection regimin: Vehicle/vehicle; melatonin (1.0 mg/kg)/vehicle; or melatonin (1.0 mg/kg)/amphetamine (1.0 mg/kg). Asterisks indicate significant difference from vehicle/vehicle group. Standard error bars are indicated.

Results and Discussion

Data were subjected to a three-factor mixed design ANOVA with melatonin and naloxone treatment as between-subject factors and trial as a within-subject factor. A significant melatonin by trial interaction effect was found, $F(7, 161) = 3.416, p < 0.05$, which appeared to be the result of a time-related decrease in DVs in the vehicle-treated chicks, which was not found in the melatonin-treated chicks (see Fig. 3). A significant main effect was also found for melatonin, $F(1, 23) = 4.32, p < 0.05$, which was the result of lower DV levels in both melatonin groups (right panel) than in both vehicle groups (left panel). No overall effects were found for naloxone, and there was no trend for naloxone to reverse the effects of melatonin.

The failure of a relatively high dose of naloxone to exert any effect on the melatonin-induced DV attenuation is evidence for an action of melatonin, which is independent of the endogenous opioids. Although melatonin does interact with opioids on some processes (14,17,19), this interaction is apparently not an essential component of the distress reducing effect of melatonin in the domestic chicken.

EXPERIMENT 3

It is possible that acute exposure to elevated levels of melatonin, as would occur following a single injection, would produce different effects in a drug-naive animal than in an animal that has been exposed to chronically elevated levels of melatonin or endogenous opioids, as might occur in a syndrome-like childhood autism. Therefore, in this experiment we analyzed the effects of melatonin in chicks following chronic exposure to melatonin, morphine, and naltrexone.

Thirty-two 2-day-old chicks were randomly assigned to four groups. Between 2 and 6 days of age chicks received three daily injections (separated by 8 h) of vehicle, melatonin, morphine, or naltrexone. All drugs were administered in a dose of 1.0 mg/kg. Chicks were kept socially housed throughout this period, with no DV testing.

At 7 and 8 days of age birds were administered 1.0 mg/kg melatonin and vehicle injections in a counterbalanced order (i.e., half the birds received melatonin first, and half the birds received vehicle first) and tested for DVs. DVs were collected for ten 10-min sessions.

Results and Discussion

The number of DVs emitted by each bird (Fig. 4) was subjected to a three-factor mixed-design ANOVA with treatment history as the between factor and receipt of melatonin or vehicle, and trial as within-subject factors.

Both melatonin, $F(1, 28) = 107.4$, and trial, $F(9, 27) = 6.77$, resulted in a significant overall effect at the $p < 0.01$ level. No significant overall effect was found for treatment history, $F(3, 28) = 1.79, p > 0.05$. A significant interaction was found for treatment history by melatonin $F(3, 28) = 19.94, p < 0.01$, and for melatonin by trial, $F(9, 27) = 5.72, p < 0.01$.

Consistent with the results from previous experiments, melatonin resulted in a decreased rate of distress vocalizations. No evidence for tolerance to chronic melatonin treatment appeared, as melatonin was equally effective in reducing DVs in melatonin-treated birds as in vehicle-treated birds. The melatonin effect was marginally potentiated in the morphine-treated birds and markedly potentiated in the naltrexone-treated birds. This was apparently due both to elevated DV

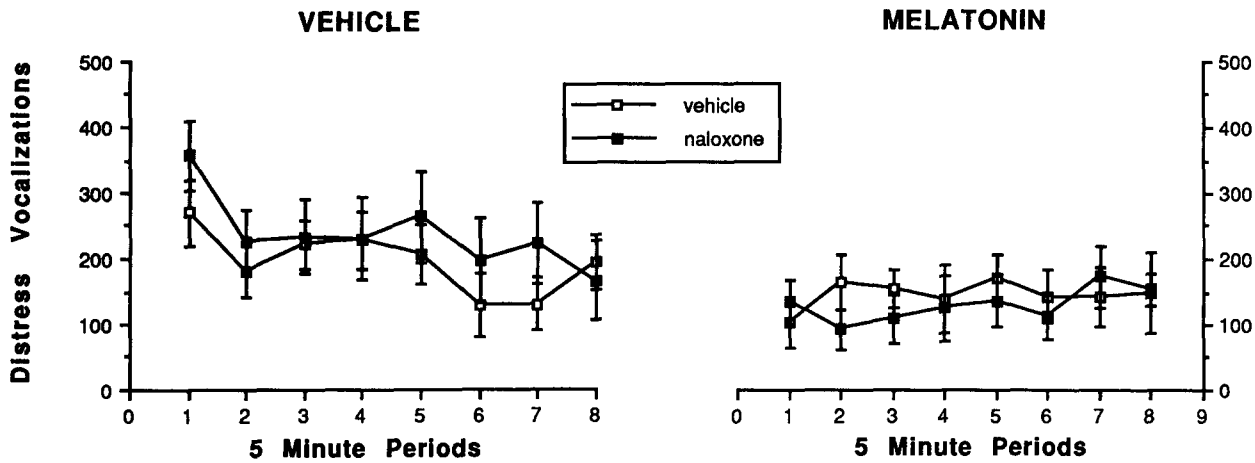

FIG. 3. Distress vocalizations emitted across eight 5-min periods for chicks receiving vehicle (open squares) or 5.0 mg/kg naloxone (closed squares) in conjunction with vehicle (left panel) or 1.0 mg/kg melatonin (right panel). Melatonin produced a significant reduction in DVs, but no significant effect for naloxone was found. Standard error bars are indicated.

rates following vehicle treatment in the birds with a naltrexone history, and to a more sustained depression of DVs following melatonin treatment in both groups.

EXPERIMENT 4

In the final experiment, we tested the effects of social isolation on a group of chicks following removal of the pineal gland, a primary source of endogenous melatonin.

Sixteen 4-day-old chicks were randomly assigned to pinealectomy or control groups. Chicks in the experimental group were anesthetized with sodium pentobarbital (40 mg/kg), the pineal glands were exposed by local craniotomy and removed by aspiration. Chicks in the control group were subjected to anesthesia and sham surgery but the pineal gland was left intact. All chicks were then allowed 5 days to recover from the surgery. At 9 days of age, distress vocalizations were collected for three 80-min sessions. For half of each session the DV boxes were lighted and for the other half the boxes were dark. The order of exposure to light and dark conditions was counterbalanced within groups. Between each testing session, chicks were returned to housing pens for an 8-min period. All data were collected during the light phase of the light-dark cycle.

When chicks were 13 days of age (one control was not tested), 0.5 mg/kg melatonin and vehicle were administered IP over a 2-day period, in a counterbalanced fashion. Immediately following injection chicks were placed in the isolation chambers. DVs were collected every 10 min for a 90-min period. One chick from the pinealectomy group died prior to the melatonin treatment, leaving 7 in the pinealectomy group and 15 overall.

Results and Discussion

Data were analyzed with a four-factor mixed-design ANOVA with group, session, lighting condition, and trials as factors.

As summarized in Fig. 5, in both groups DVs decreased across trials faster during the dark phase than the light phase, resulting in fewer DVs overall in the dark than the light conditions. This resulted in a significant interaction effect for lighting by trials, $F(3, 42), = 8.2, p < 0.001$. A significant main effect for lighting condition was also found, $F(1, 14) = 32.1$, $p < 0.001$. The light by groups interaction did not reach significance, although a strong trend was found, $F(1, 14) = 4.3$, $p < 0.06$, indicating a tendency for dark conditions to have a greater effect on the intact pineal group than on the pinealectomized group. A trend was found for a group main effect, $F(1, 14) = 4.5$, $p < 0.06$, indicating a tendency for the pinealectomized animals to have lowered DVs across both lighting condition and trials. Finally, the mean for each trial was compared at the different lighting conditions for each group using orthogonal polynomials. For the control group, a significant reduction was found during the dark condition at the second, third, and fourth trials (all $p < 0.01$). For the pinealectomized group, the dark condition resulted in a significant reduction only at the fourth trial ($p < 0.01$).

The results from this experiment suggest that removal of the pineal gland does not completely eliminate the reduction in distress vocalizations produced by darkness. However, the DV rate in both lighting conditions was substantialy lower in the pinealectomized chicks than in controls, and the pinealectomized animals did have an attenuated darkness effect relative to controls, suggesting that pineal melatonin may contribute to the control of distress vocalizations.

The melatonin data were analyzed with a three-factor mixed-design ANOVA with group, drug, and trial as the factors. Significant group by drug, $F(1, 13) = 5.9$, $p < 0.05$, and drug by trials, $F(8, 104) = 2.5$, $p < .05$, interactions were found. As summarized in Fig. 6, the group by drug interaction can largely be explained by group differences in response to melatonin during the last 40 min of testing. The drug by trials effect can be explained by a tendency for melatonin to be less effective in both groups with the passage of time. This is particularly true for the last 40 to 50 min of testing. A significant main effect was found for drug, $F(1, 13) = 10.8$, $p < 0.01$. This difference is a result of melatonin reducing DVs in both groups.

The results from this experiment suggest that exogenous melatonin has a similar effect on pinealectomized and control chicks. The only notable difference found between these two groups was the postmelatonin effect. Although the control

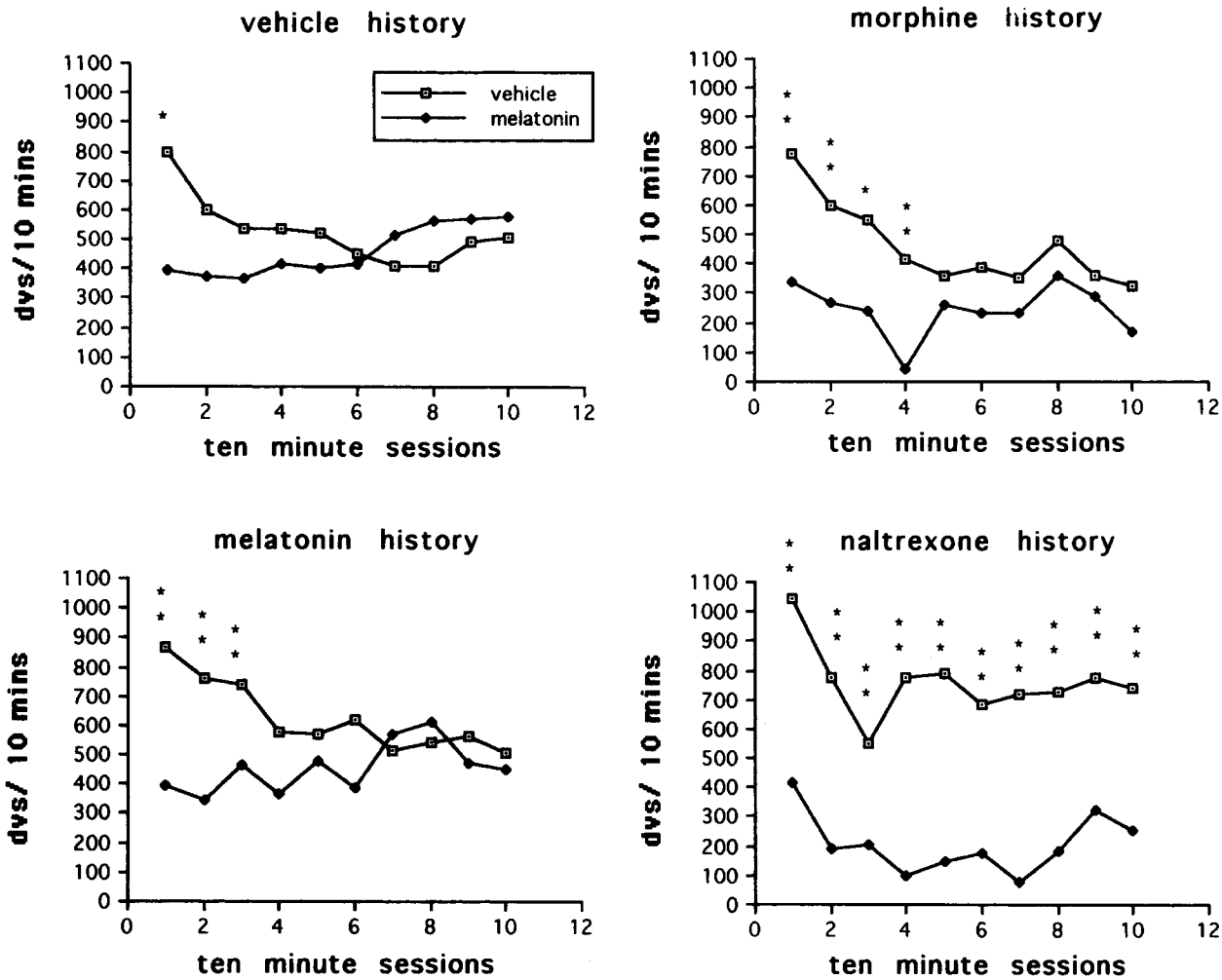

FIG. 4. Distress vocalizations collected for ten 10-min blocks following administration of 1.0 mg/kg melatonin and vehicle for chicks who had received three daily injections of vehicle, melatonin (1.0 mg/kg), morphine (1.0 mg/kg), or naltrexone (1.0 mg/kg) over a 3-day period. Asterisks indicate significant difference between vehicle and melatonin. Single asterisks indicate a $p < 0.05$, and double asterisks indicate a $p < 0.01$ level of significance.

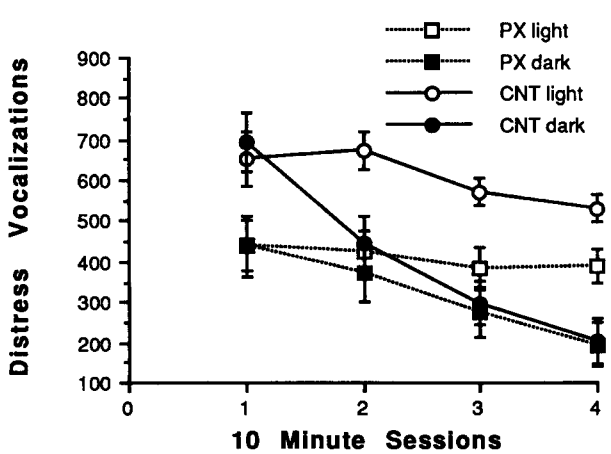

FIG. 5. Distress vocalizations averaged across three sessions for four 10-min trials in a lighted box and four 10-min trials in a dark box. Squares represent pinealectomized chicks and circles represent DVs from control chicks. Standard error bars are indicated.

animals were returning to baseline approximately 50 min after receiving the melatonin injection, the pinealectomized animals were above baseline an hour after receiving melatonin. The reason for this rebound effect is not clear.

GENERAL DISCUSSION

This series of experiments found that melatonin markedly decreased isolation-induced distress vocalizations in young chicks, and that this effect was at least partially independent of its soporific effects. This effect is similar to, but apparently independent of the effects of activation of the endogenous opioid system, as naloxone did not reverse the suppression in DVs following melatonin administration. However, the effects of melatonin may have been slightly prolonged in chickens that had previously received chronic naltrexone or morphine treatment. Although the most pronounced effect of chronic naltrexone treatment was a potentiated rate of vocalizations in the vehicle-treated animals.

The natural diurnal fluctuation of distress vocalizations (30) is probably due at least in part to lighting effects, as it was found that chicks emit fewer distress vocalizations in a darkened box than in a lighted one. However, the lighting

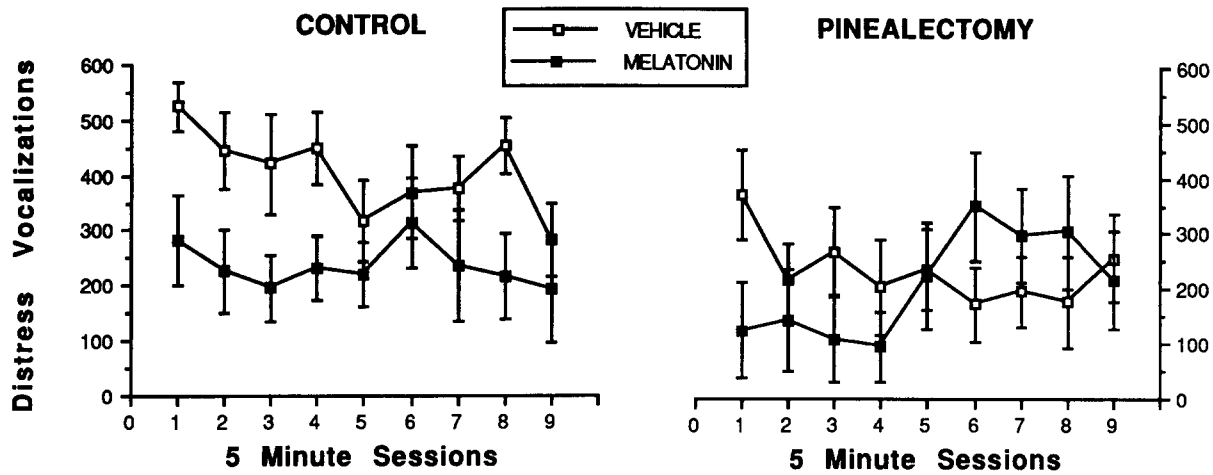

FIG. 6. Distress vocalizations for nine consecutive 5-min periods following treatment with vehicle or 1.0 mg/kg melatonin in control (left panel) and pinealectomized chicks (right panel). Standard error bars are indicated.

effect on DVs does not appear to be completely driven by the pineal gland as pinealectomy attenuated, but did not eliminate this phenomenon. The persistence of lighting effects in pinealectomized animals could be explained by conditioning, an extra pineal source of melatonin, or by some other unknown circadian or diurnal factor. The attenuation of the dark effect in pinealectomized animals, however, does suggest that pineal melatonin is contributing to the DV suppressant effect of darkness.

The general lack of a reversal of the dark effect following pinealectomy, in combination with the lack of a reduction in DVs following the low dose of melatonin in Experiment 1, suggests that melatonin's modulatory effect on DVs is a supraphysiologic effect. If this is, indeed, the case, then it is likely that endogeonous melatonin does not play a critical role in the modulation of social distress. As many previous reports have indicated that melatonin produces antianxiety effects (1,2,4, 8,20-22), which are similar to benzodiazepines, and as benzodiazepines have been found to reduce DVs in both rats and chickens (11,25), it is possible that at supraphysiologic doses, melatonin is reducing DVs through a general reduction in anxiety. However, from the present data, it cannot be argued that melatonin, at physiological levels, plays a key role in modulating separation distress circuits.

Finally, a finding worth noting in this study was the interaction between opioids and melatonin. Although melatonin's effect was not naloxone reversible, chronic naltrexone, and to a lesser extent, chronic morphine treatment did potentiate and prolong the effects of melatonin, suggesting an interaction between these systems at some level. Many previous findings have suggested a synergistic relationship between melatonin and opioids (14,17,19,32), and the results from this study provide evidence for a new and possibly indirect relationship between these neurochemical systems.

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