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Opioid Blockade and Social Comfort in Chicks[1]

JAAK PANKSEPP, N. JAY BEAN[2], PAUL BISHOP, TOM VILBERG[3] AND TONY L. SAHLEY

Department of Psychology, Bowling Green State University, Bowling Green, OH 43403

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PANKSEPP, J., N. J. BEAN, P. BISHOP, T. VILBERG AND T. L. SAHLEY. *Opioid blockade and social comfort in chicks*. PHARMAC. BIOCHEM. BEHAV. 13(5) 673–683, 1980.—When young animals are separated from their normal social environment in groups they distress vocalize (DV) less than when isolated alone. Opioid blockade with naloxone (1 mg/kg peripherally, and 1 μg centrally) increased crying more in group tested chicks than individually tested ones. The serotonin receptor blocking agent methysergide and the cholinergic blocking agent atropine sulfate produced similar effects. The testing of birds in mirrored environments also produced a reduction of DV's, and all three receptor blocking agents augmented DV's more in mirrored environments than in mirrorless ones. However, in studies evaluating changes in this comfort response by facilitation of opiate, serotonin (5 HT), and acetylcholine (ACh) activity (with morphine, quipazine and pilocarpine, respectively), only morphine was found to magnify the comforting effect of mirrors. The effects of naloxone on contact comfort, and the acquisition and expression of imprinting were further evaluated, and opioid blockade reduced all these measures of social comfort. It is concluded, that endogenous opioid activity (and to a lesser extent, 5 HT and ACh) contribute to the comfort which animals derive from their social environment.

Social comfort Imprinting Opioids Naloxone Morphine Methysergide Quipazine Atropine Pilocarpine

KINDRED animals can obtain emotional comfort from each other. Based on the fact that opiates [1,6] and opioids [10] are very effective in alleviating social separation-induced distress, and more effective than most other psychoactive drugs [7], it has been proposed that brain opioid systems help mediate the comfort which animals appear to derive from social stimuli. Accordingly brain opioids may help create social bonds which keep animals together. If so, it would be predicted that opiate antagonists would interfere with the comforting effect of social stimuli as measured by indices of separation distress such as crying. The present series of experiments tested this prediction from a variety of perspectives.

GENERAL METHODS

Subjects and Housing

Newly hatched male White Leghorn chicks were obtained within 1–6 hrs of hatching from Napoleon Hatchery Inc., Napoleon, OH. Birds were housed communally (100–200 animals in a cage with 90×90 cm floor area) and had free access to food and water from the time of arrival in the lab until testing. A total of 2052 birds were tested in these experiments. Except for Experiments 9–12 each bird was only tested once. Ambient lighting conditions varied among experiments. In Experiments 1, 3, 4, and 9 animals were housed in continuous light. In Experiments 6, 11, and 12 animals were housed in continuous darkness. In Experiment 10 half the animals were in continuous light and the other half in continuous darkness. In Experiments 2, 5, 7 and 8 animals were kept on a 12:12 hr light-dark cycle and testing was conducted during the first 4 hrs of the dark and first 4 hrs of the light cycles, respectively. In the continuous dark condition, a red-light illuminated work table (whereby the light did not directly shine into the housing chamber) was used for animal identification and injections. Room temperature was maintained at 25±1°C.

Testing Procedures

Vocalization tests in all of these experiments were conducted in 36×32×33 cm sound insulated chambers with white acoustic tile walls and ceilings, and aluminum floors. Air circulation in the chambers as well as background masking noise was provided by electric fans. The chambers were lighted with Chicago miniature bulbs (Model 313CM). Distress vocalization (DV) were automatically collected by Radio Shack sound activated relay circuitry (Catalog No. 28-131), and recorded via electromechanical counters.

In the studies with peripheral injections, animals were removed from the flock, and injected intraperitoneally with the various drug solutions in volumes of 3 cc/kg. All drugs were mixed in a distilled water carrier. Injections were given 20 minutes prior to being individually placed into the testing chambers for the separation tests. During the interval between injections and testing, animals were housed in groups

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[2]Present Address: Department of Psychology, Vassar College, Poughkeepsie, NY 12601.
[3]Present Address: Department of Psychology, University of Scranton, Scranton, PA 18510.

of 6–8 in 17×28×13 cm high white translucent-plastic holding cages.

Experiments with central injections used intraventricular drug administration whereby 3 μl of test solution was injected into unanesthetized chicks directly through the skull immediately anterior to the cerebellum using a 10 μl 70 Hamilton syringe with a stop placed 5 mm from the tip. Previous dye injection studies indicated that these injections perfuse the fourth ventricle and up to the third ventricle in most animals.

Notes on the Distress Vocalization Measure

Although isolation induced distress vocalization is a robust phenomenon in that it occurs in essentially all the chicks we test using present procedures, the measure is also quite variable within and between experiments. For unknown reasons, the control levels of vocalization may vary substantially from experiment to experiment, and herein we consider only relative differences among groups within any one experiment to be meaningful. We presently do know that the measure is very sensitive to circadian effects, and we suspect that it varies as a function of the testing season and perhaps the specific flock of animals obtained from the hatchery. There are also effects of age and various environmental stimuli such as temperature and noise which can clearly affect the measure. Finally, it has been our informal observation that chicks which have recently eaten (i.e., their crops are full) tend to exhibit lower control baselines than hungry chicks. All of these variables deserve further inquiry.

In experiments where several chicks were studied simultaneously, the total data for the group is provided. Though not typically presented, it should be noted how those numbers would change when divided by the number of animals tested. ANOVAs and *t*-tests were used for statistical comparisons.

EXPERIMENT 1

Since the aim of subsequent experiments was to test whether naloxone could block the comfort young chicks derive from each other, our initial task was to determine how the distress vocalizations of chicks are inhibited by the presence of other chicks. Since automated vocalization recording procedures were to be used throughout these experiments, the possibility arose that several chicks together would vocalize in unison, yielding a single count on the automated equipment. Therefore, validity and reliability check of the recording procedures were also undertaken.

METHOD

Experiment 1a

The DVs of 112 two- to four-day old chicks were recorded following solitary isolation (n=16), paired isolation (n=16 pairs), or social separation in groups of four (n=16 groups). Cumulative vocalizations were automatically recorded for five minute isolation periods immediately following removal of animals from the large holding pen.

Experiment 1b

Four single chicks, and four groups consisting of four birds each were run as in Experiment 1a. In addition to automated recordings, birds were observed through one-way viewers and DVs were counted manually.

RESULTS AND DISCUSSION

Experiment 1a

The average DVs of individually isolated birds was 407 (±28). The number of recorded DVs from paired birds was 365 (±39), and of the quadrupled birds 327 (±44). Although the group of four birds emitted 80% as many vocalizations as individually isolated birds, the difference was not statistically reliable. Of course, if each of these group means is divided by the number of animals tested, the results indicate that paired birds vocalized on the average, 45% as often, and quadrupled birds 20% as often as individually isolated birds (p's<0.001).

Clearly the frequency of vocalizations was not determined exclusively by the simple presence or absence of conspecifics. Indeed, in the present situation, groups of birds behave quantitatively as a single animal, in that approximately the same number of vocalizations were recorded during a session regardless of the number of animals. This suggests that the birds were deriving some comfort from each other. Certainly the cumulative frequency of the response was not doubled or quadrupled in the case where twice and four times as many chicks were tested together. Alternatively, this may indicate that the DVs were inhibited by the responses of other birds, or an inability of the counters to distinguish simultaneously occurring DVs.

Experiment 1b

This experiment evaluated the reliability of the automatic recording equipment. In individual birds, machine counts totaled 340 (±72) vocalizations, while the human counts totaled 366 (±76). Groups of four chicks averaged 333 (±64) when counted by the sound activated relay circuitry and 352 (±48) when manually detected. The product moment correlation between the two counting methods was r=0.95.

Although the sound activated counters tended slightly to underestimate the frequency of vocalization, the problem was not of major importance, since the magnitude of the counting error did not depend on the number of subjects being tested. Observation of these and other animals suggested that chicks in a group suppress their vocalization in response to the calling of others. Were this not the case, one would expect four times as many birds to vocalize four times as frequently. They do not. It was visually apparent that only one bird vocalized at a time. The other birds oriented to the vocalizing bird, but did not answer his calling. Although, this inhibition could be mediated by a variety of factors, in the following experiments this reduction was taken as a measure of the capacity of social stimuli to alleviate separation distress.

EXPERIMENT 2

Since the aim of the following experiments was to determine whether opioid blockade would reduce social comfort, it was deemed necessary to further verify that the vocalizations produced by the brief periods of social isolation used herein were truly due to the absence of comforting social stimuli as opposed to being simply a transient distress response to being placed in a novel environment. To evaluate such an alternative cause for the etiology of DVs, we compared the vocalizations emitted by individually isolated chicks during two successive 10 min periods of social isolation to those emitted by chicks which were tested socially for

the first 10 minutes followed by removal of social companions for a second 10 min. If DVs are primarily induced by novelty then it would be expected that individually isolated birds should emit more DVs in the first 10 min of isolation than during subsequent periods. Furthermore, if it is simply the novelty of the test chamber that is inducing DVs, it would be expected that a chick which had been initially exposed to the test chamber with social companions should not exhibit a vigorous increase in DVs when the social companions were removed. To the contrary, if the animal was responding to cues of social isolation, the removal of conspecifics should elicit a prompt increase in vocalizations.

METHOD

Eighty 3 day old chicks were used. Sixteen animals were placed in social isolation by themselves and DVs were recorded for two successive ten min periods. Between the two periods, the test chamber was opened and the experimenter briefly inserted his hands as if to pick up imaginary conspecifics. The remaining animals were initially placed into the test chambers in groups of four. Following the first ten minutes of recording, three chicks were removed, and the amount of vocalization emitted by the remaining bird was recorded for 10 min.

RESULTS AND DISCUSSION

The average (±SEM) DVs of individually isolated chicks was 796 (±48) during the first ten minutes and 765 (±72) during the second ten minutes (N.S.). The group of four birds vocalized a total of 403 (±95) times during the first ten minutes, and the bird that remained in the test chamber after three chicks were removed vocalized 763 (±88) times during the second 10 min. The level of vocalization in the grouped birds was reliably lower than any of the other conditions, t's (30)>3.38, p's<0.01, while the levels of vocalization in the individually isolated birds did not differ under any condition. These results indicate that the DVs elicited in our situation were due more to the effects of social isolation than to simple novelty, even though the participation of the later process as contributory cannot be ruled out completely.

EXPERIMENT 3

The major aim of this experiment was to determine whether opioid blockade would reduce the mutual inhibition of DVs that grouped chicks induce in each other. In previous work, opioid blockade was found to reliably increase DVs [7]. However, blockade of serotonergic and muscarinic cholinergic systems produced similar effects, and activation of all three systems was found to reduce DVs [7]. Accordingly, social comfort could be mediated by activation of any of these systems, and the following experiment compared the effects of receptor blockade of these three systems on the DVs of pair-tested chicks.

METHOD

A total of 282 birds between 1 and 3 days of age were used. Twenty min prior to testing, animals were injected IP with either 1 mg/kg naloxone hydrochloride, 1 mg/kg atropine sulfate, 0.5 mg/kg of methysergide maleate, or the distilled water carrier. Following injection, birds were housed in a 17×28×12 cm high translucent plastic chamber in groups of 8. Due to the enforced proximity in this situation, DVs were rare during the pretest interval.

TABLE 1

AVERAGE (±SEM) DV'S/10 MIN IN ANIMALS PRETREATED WITH NALOXONE, ATROPINE OR METHYSERGIDE

Treatment (n's)	Single bird	Paired birds	% change
Control (24,26)	895 (±76)	893 (±67)	0%
Naloxone (24,24)	952 (±47)	1378 (±69)	+45%†
Atropine (22,20)	907 (±44)	1097 (±86)	+12%*
Methysergide (24,24)	983 (±52)	1353 (±66)	+38%†

*$p<0.05$.
†$p<0.001$.

The data, summarized in Table 1, indicate that following control injections, paired birds vocalized essentially the same amount as individual birds. Further, all drugs reliably increased vocalizations in the paired birds (both within and between treatments), t's(44–48)>1.88, $p<0.05$, one-tailed, with naloxone exhibiting numerically the largest effect (+54%), followed closely by methysergide (+52%), and a smaller 23% effect with atropine. Although the vocalization frequencies of individually tested birds were also slightly increased by these agents (2–10%), the effects were not reliable in this experiment.

These data indicate that blockade of opioid, serotonergic and to a smaller degree, muscarinic cholinergic systems, can reduce the capacity of birds to inhibit the vocalization rates of others, and hence raises the possibility that activity in all these systems contributes to the comfort animals obtain from being in proximity to other animals. Although the failure to observe reliable drug effects in individually tested animals fails to replicate previous work [7], this may have been due to the high baseline levels of controls. Indeed, of all the experiments we have conducted in chicks, these have been the highest baseline vocalization values we have observed.

EXPERIMENT 4

In unpublished work, we have found that 1 μg of naloxone administered intraventricularly, can increase distress vocalizations in individually isolated chicks. To ascertain whether the effect of naloxone observed in Experiment 2 was due to a central effect, in the following experiment we evaluated the capacity of intraventricularly administered naloxone to alter DVs in individually and pair-isolated birds.

METHOD

A total of 126 one-to-two day-old birds were injected centrally with either 0, 0.2, or 1.0 μg of naloxone in 3 μl of distilled water. Animals were tested immediately following injections either individually or in pairs. There were 14 animals or pairs in each treatment conditon.

RESULTS AND DISCUSSION

The data, summarized in Table 2 indicate that the 0.2 μg dose of naloxone had no reliable effect on DV'ing, whereas the high dose increased crying by 72% and 77% respectively, in individually and pair-tested animals, respectively. There was no reliable difference between the scores of individually and pair-tested groups under any condition.

TABLE 2
AVERAGE (±SEM) DV'S/10 MIN FOLLOWING INTRAVENTRICULAR NALOXONE

Treatment	Single birds	Paired birds
Control (3 μl H_2O)	540 (±92)	477 (±62)
0.2 μg Naloxone	566 (±81)	493 (±83)
1.0 μg Naloxone	929 (±71)*	843 (±52)*

*p's<0.001 from respective controls.

Although the data indicate that naloxone can increase DVs, the failure to observe differential magnitudes of the effect in the individual and paired birds, is not consistent with the conclusion that opioid blockade selectively reduces the comfort chicks can derive from each other. Naloxone simply increased separation distress overall. Still, since a variety of factors (including ceiling effects) could have precluded the appearance of an interaction effect, the experiment was reconducted using different procedures.

EXPERIMENT 5

Since between animal and between experiment variability is high in distress-vocalization studies, the following experiment evaluated the effect of centrally administered naloxone on DVs of group tested birds using a within-animal design where drug scores were compared to individual baseline scores.

METHOD

A total of 392 birds were tested individually, in pairs, or with four animals together. An equal number of groups were tested at each of 7 equally spaced ages between 12 and 168 hrs of age. Animals were initially tested for 10 min, whereupon half the animals in each testing condition received intraventricular injections of 1 μg of naloxone, and the other half received 3 μl of the distilled water carrier. Animals were promptly returned to the test chambers and an additional 10 min of vocalization data were collected. Altogether there were 28 scores for each of the six test conditions.

RESULTS AND DISCUSSION

The mean vocalization rates, averaged across ages, before and after naloxone administration are summarized in Fig. 1. Under no condition did the control injection affect DV rates, indicating the stability of this measure within the experiment. However, all naloxone treated groups reliably increased crying. Compared to their own baseline values, individually tested birds increased vocalizations by 22%, $t(27)=3.99$, $p<0.001$, paired birds by 39%, $t(27)=4.96$, $p<0.001$, and quadrupled birds by 93%, $t(27)=5.89$, $p<0.001$. Both the relative and absolute increments of vocalizations in the quadrupled animals were larger than in the individually tested animals, t's(54)>3.85, $p<0.001$. Due to the small number of animals per age group, the relative changes as a function of age were not analyzed, but the effect was apparent at all ages, numerically the largest effects occurring at 2–5 days of age.

Not only do these results confirm the capacity of small doses of intracerebral naloxone to increase distress vocalizations, but they demonstrate that the effect can be substantially larger in group tested than individually tested animals. This suggests that opioid blockade can reduce the comfort (or vocalization inhibition) that young chicks derive from each other. It should be emphasized that this was only a partial attenuation of interanimal comfort. By no means did naloxone increase vocalizations in grouped animals to values near levels that would have been expected had these animals been individually isolated.

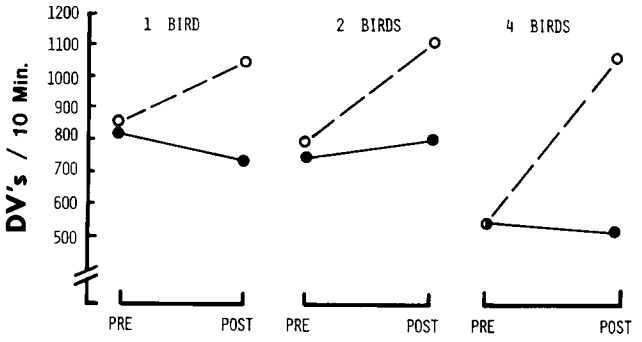

FIG. 1. Average DVs in 10 mins before and after treatment with either 1 μg of naloxone or 3 μl of the water carrier in individually isolated chicks and those tested in pairs and fours (Experiment 5). ●——● control; ○– –○ naloxone.

EXPERIMENT 6

The previous study suggested that naloxone can reduce the comfort derived from nearby conspecifics. The possibility that the observed naloxone effect merely reflects the capacity of this agent to disinhibit vocalizations among grouped birds was tested in the following series of experiments. To this end, we measured DVs in the presence of silent social stimuli. Indeed, we proceeded to eliminate the need for conspecifics in the test situation by providing social stimuli consisting of the chicks' own reflections in mirrored test environments.

METHOD

A total of 84 one-day-old chicks were tested in isolation chambers where the walls were either blank acoustic tile, or mirrored tile (totally covering all four walls). Four groups were used (n=21 per group): two groups were tested with mirrors, and two without; from each of these groups, half were tested with constant light, and the other half with lights on for a minute and off for a minute, for ten successive minutes of the test period.

RESULTS AND DISCUSSION

The average (±SEM) vocalization rate of birds tested in the continuously lit "blank" chambers was 781 (±37) while in the mirrored chambers it was 591 (±57), $t(40)=2.81$, $p<0.01$. The total frequency of DVs in the two remaining conditions (with intermittent light) were not reliably different ("blank" condition: 805 (±46), and mirrored condition (747 (±42)), but a minute-by-minute breakdown of the data (Fig. 2) clearly indicated the efficacy of the mirrors in reducing DVs. During the dark periods the animals cried more vigorously. That such a trend was not apparent in animals tested in the "blank" boxes indicated that it was not simply the presence or absence of light that was controlling the inci-

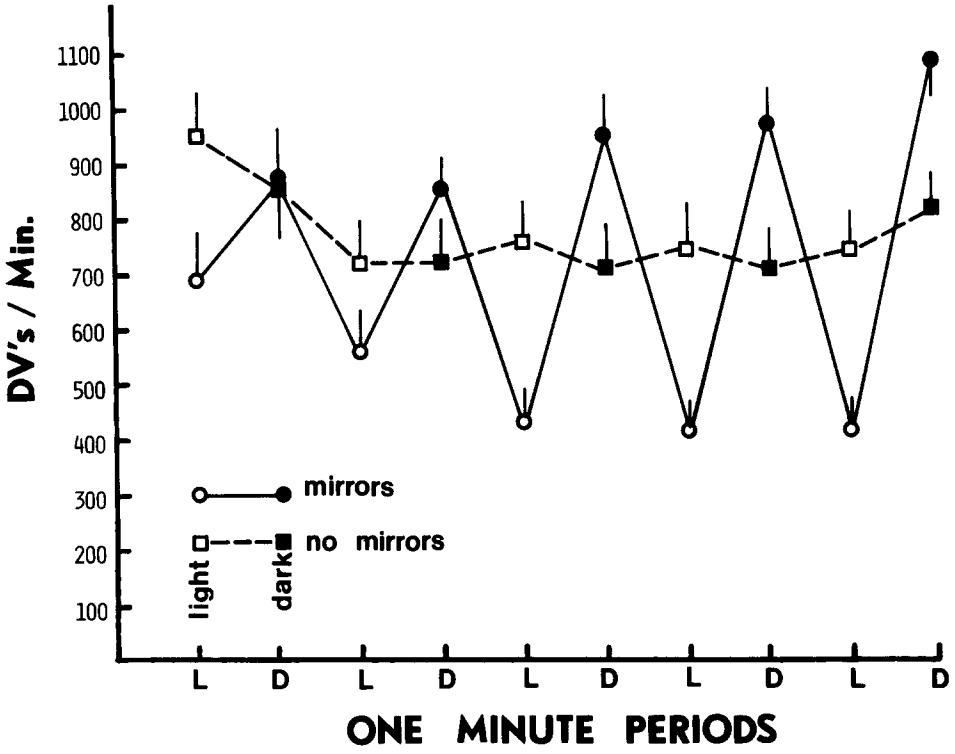

FIG. 2. DVs during successive minutes in chicks tested with intermittent light and dark periods in mirrored and non-reflective test chambers (Experiment 6).

dence of crying. Rather, the mirrors and presumably the social images reflected therefrom, reduced DVs. Although we cannot be certain that the crucial factor in attentuating DVs was solely the reflected image, the fact that this inhibition was due to *some* kind of social comfort response is suggested by the contrast effect that was apparent during intermittent light. Similar effects can be obtained with other incentives [8,9], and the tendency of the animals to cry more in darkness only when they had just experienced mirrored images, suggests that the stimuli which were present in the mirrored environment were positive social incentives to the birds.

EXPERIMENT 7

Since a mirrored environment appears to comfort young chicks, the following experiment reevaluated the capacity of naloxone, and the other agents used in Experiment 2, to reduce comfort. To further evaluate the specificity of any effects, we also evaluated agonists for these systems, namely morphine, quipazine and pilocarpine, which in previous work have been found to reduce DVs in individually isolated chicks [7].

METHOD

A total of 284 1–2 day old chicks were tested either in mirrored or non-mirrored test chambers 20 min following the intraperitoneal injection of the following substances: naloxone (1 mg/kg), methysergide (0.5 mg/kg), atropine (5 mg/kg), a mixture of the preceding three drugs at the same doses, and morphine (1 mg/kg), quipazine (10 mg/kg), and pilocarpine (10 mg/kg). The number of animals run under each condition are summarized in the data table.

RESULTS AND DISCUSSION

The mirrors reduced DVs in control animals by 29% (Table 3). The reductions in naloxone and atropine treated animals, though smaller (18% and 13%, respectively) were still statistically reliable. The smaller 8% reduction in methysergide treated animals was not. The three drugs combined yielded essentially identical results between mirror and no-mirror conditions. Comparing the drug data to control data, it can be noted that these drugs all increased vocalizations reliably under the mirror condition (t's>3.30, p's<0.01), whereas under the no mirror condition only atropine reliably increased DVs in the present experiment, $t(46)=2.80$, $p<0.001$.

Of the agonists, morphine was the only drug which reduced DVs under both testing conditions, t's(34)>5.49, p's<0.001. Quipazine reliably reduced DVs only under the no-mirror condition, $t(34)=2.56$, $p<0.01$, and the reduction of DVs following pilocarpine was not reliable under either condition in this experiment. The only instance where the mirror effect was still statistically apparent was the 60% reduction observed in morphine treated animals.

Although the results were not unambiguous, they lend themselves to the following conclusions: (1) chicks are still able to obtain perceptual comfort from their reflections when treated with naloxone at 1 mg/kg and atropine at 5 mg/kg, even though the comfort appears to be slightly reduced. (2) Following serotonin blockade, this perceptual comfort is largely abolished, and when all three systems are blocked, the comfort is completely blocked. (3) Stimulation of acetyl-

TABLE 3
AVERAGE (±SEM) DV'S/10 MIN IN EXPERIMENT 7

Treatment (n's)	No mirrors	Mirrors	% Change
Control			
H_2O (20, 20)	760 (±55)	542 (±52)	−29%*
Antagonists			
Naloxone (20, 20)	881 (±59)	727 (±43)	−18%†
Methysergide (16, 16)	835 (±51)	765 (±53)	− 8%
Atropine (20, 28)	920 (±28)	801 (±49)	−13%†
N +M + +A (16, 16)	818 (±58)	810 (±69)	− 1%
Agonists			
Morphine (16, 16)	309 (±60)	125 (±28)	−60%*
Quipazine (16, 16)	522 (±77)	369 (±73)	−29%
Pilocarpine (16, 16)	622 (±61)	522 (±75)	−16%

*$p<0.01$.
†$p<0.05$.

choline systems also appears to attenuate the comfort chicks derive from their reflections, while under a low dose of morphine, this perceptual comfort is magnified. With a serotonin receptor agonist, the relative efficacy of mirrors to reduce DVs was the same as in control animals, but due to the variability of the data, the effect was not reliable. Although these data suggest that all three systems tested participate in controlling the degree of apparent comfort chicks can derive from their own image, the bidirectional effects obtained with opiate agonists and antagonists does suggest that the opiate system may have relatively specific control over this process, while the contribution of the other systems may be more non-specific.

EXPERIMENT 8

Although several of the previous experiments indicate that naloxone is especially effective in increasing DVs when chicks are in the presence of social stimuli, the present failure to consistently replicate the previously reported finding of increased DVs in individually isolated chicks [7] is troublesome. For instance, in our previous work, 1.7 and 5.0 mg/kg of naloxone increased DVs in individually tested birds by 45% and 40% respectively, while in Experiments 3 and 7 of the present series, 1 mg/kg produced only modest increases of 6% and 16%, neither of which was statistically reliable. Accordingly, the following experiment was conducted to re-evaluate the effects of naloxone in individually isolated birds as a function of being tested in mirrored and nonmirrored environments.

METHOD

The DVs of 102 2-day old chicks were measured during a 10 minute isolation period 20 min following IP injection of 0.0, 2.5 or 5.0 mg/kg of naloxone. Half the animals were tested in mirrored and half in the nonmirrored environments. Seventeen animals were tested under each of the six conditions.

RESULTS AND DISCUSSION

The average (±SEM) DVs of birds tested in the plain boxes were 669 (±70), 831 (±62) and 758 (±46) for the 0.0, 2.5 and 5.0 mg/kg doses of naloxone, respectively. Chicks tested in the mirrored boxes DVed 291 (±72), 458 (±89) and 439 (±67) times respectively. In relative terms, naloxone increased DVs by 63% and 45% in mirror-tested birds, and by 24% and 13% in the plain boxes. However, the within group variance was again very high and none of these trends was statistically reliable. However, when all data from control animals is compared to naloxone treated ones, the overall 29% increase in DVs was reliable, $t(100)=2.21$, $p<0.025$.

Overall, these results suggest that naloxone can increase DVs in individually isolated birds, but the effect can be fragile. In the present experiment, clear differential effects of naloxone were not observed as a function of being tested in the presence of social cues. We do not believe that this negates the findings of the previous experiments, but merely indicates that the effects which were observed are not obligatory in character. The general conclusion which can be reached is that opioid blockade can increase DVs, that the probability of observing this effect is higher if animals are tested in a social environment, and that subtle undefined factors exist which can block the clear expression of the effect. Although additional work is needed to clarify the processes operating in this situation, perhaps this state of affairs reflects the difficulties which are to be encountered in trying to bring subtle emotional processes under experimental control. Perhaps all studies of spontaneously occurring emotional behaviors have to tolerate substantial data variability.

EXPERIMENT 9

To seek convergent evidence for the idea that opioid systems can participate in the mediation of social comfort, in the following experiment, we determined whether opioid blockade could attenuate contact comfort induced by direct somatosensory stimulation. The simplest measure of contact-comfort we have been able to devise is the tendency of young chickens to rapidly close their eyes when held gently in the cupped hands of a human (Fig. 3). In such a "simulated nest" all young chicks rapidly close their eyes and exhibit behavioral somnolence which has outward similarities to an "opiate nod." In the following experiment we sought to determine whether the speed of stable eye closure in this situation was affected by naloxone and morphine.

METHOD

Experiment 9a

Using blind procedures, the capacity of naloxone to reduce the latency for 18 two-day old chicks to keep both eyes closed for 30 continuous seconds was measured using the approach depicted in Fig. 3. Half of the birds were tested in counterbalanced fashion with 1 mg/kg naloxone and saline, and half with 5 mg/kg naloxone and saline. Drugs were given IP 30 min before testing.

Experiment 9b

An additional 11 animals were tested in a counterbalanced fashion on two successive days with saline and 1 mg/kg of morphine administered IP 30 min before testing.

RESULTS

In this first experiment, the lower naloxone dose increased the latency to sleep by 100% from 30 to 76 sec, while

FIG. 3. The typical eye closure of infant chicks held gently in cupped hands (Experiment 9).

the higher dose increased the latency by more than 200% from 39 to 124 sec ($p<0.01$). In the second experiment, the morphine hastened eye closure from an average of 47 sec to 9 sec, $t(11)=2.60$, $p<0.05$.

These results suggest that naloxone can reduce while morphine can increase comfort arising from gentle somatosensory contact. This pattern of results is similar to that observed with the mirrors in Experiment 7.

EXPERIMENT 10

Presumably, social bonding operates through brain systems which mediate social affect. Accordingly, it would be predicted that social learning would be modulated by opiate drugs. In previous work we have failed to observe any major effect of either naloxone or morphine on spatial learning for a social reward (rat pups running home), even though extinction of this behavior was markedly impeded by morphine and facilitated by naloxone [5]. These findings suggest that learned expressions of social motivation are strengthened by high, and weakened by reduced opioid activity. In an imprinting context it is predicted that naloxone should attenuate the acquisition and expression of imprinting. Since the aim of the following experiments was to measure the effects of opiates on imprinting, we proceeded to develop an experimental situation whereby imprinting could be evaluated using a distress vocalization measure. It has been demonstrated that birds DV less in the presence of objects to which they are imprinted [3,4] and in the following experiments we attempted to utilize the birds own reflection as the

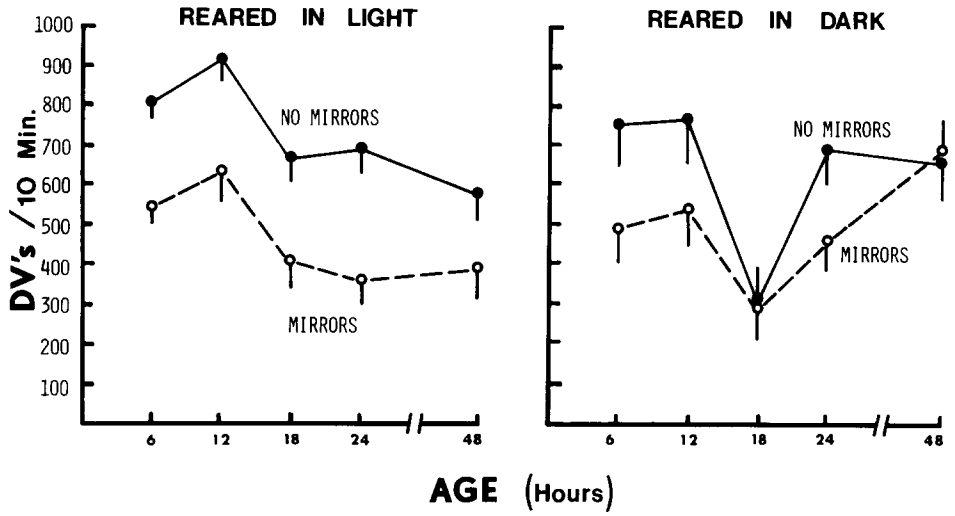

FIG. 4. Average DVs in 10 min of light and dark reared chicks tested in mirrored and non-reflective test chambers (Experiment 10a).

imprinting stimulus. Indeed, it is plausible that the reduced vocalizations observed in Experiments 6 and 7 in response to mirrored environments may have reflected an effective response to the presence of an already imprinted object (e.g., other birds). Therefore, prior to evaluating the effects of naloxone on imprinting, we proceeded to analyze the ontogenetic effects of mirrors on vocalization rates in birds.

METHOD

Experiment 10a

A total of 180 birds between the ages of 6 and 48 hrs were tested for isolation induced DVs in mirrored and non-mirrored chambers. Half the birds were communally housed in darkness prior to the test, while half the birds were communally housed in continuous light prior to testing. Four groups of nine birds each were tested under each of these conditions at 6, 12, 18, 24, and 48 hrs of age. The separation test was 10 minutes in duration.

Experiment 10b

Due to the results obtained from the dark reared group, four additional age groups (16, 38, 62, and 86 hrs) were studied. Two groups of birds (n=9 in each) were tested repeatedly at each of these time periods—one group in mirrored the other in non-mirrored boxes. At the 38, 62, and 86 hr measurement periods, additional independent groups of birds (n's=9–12 per group) were also tested under each condition.

RESULTS AND DISCUSSION

Data for Experiment 10a are summarized in Fig. 4. The overall effects of mirrors, $F(1,160)=33.2$, $p<0.001$, age, $F(4,160)=3.29$, $p<0.001$, and the lighting condition by age interaction, $F(4,160)=4.21$, $p<0.005$, were reliable. Indeed, in light reared animals mirrors reduced DVs at every age except 48 hrs, t's$(16)>2.76$, p's<0.01, whereas in dark reared animals the mirrors reliably reduced DVs only at 6 and 24 hrs of age, t's$(16)>1.84$, p's<0.05. This lighting condition by mirrors interaction approached significance, $F(1,160)=3.51$, $p=0.063$, suggesting that mirrors may be differentially effective in controlling the DVs in light and dark reared animals.

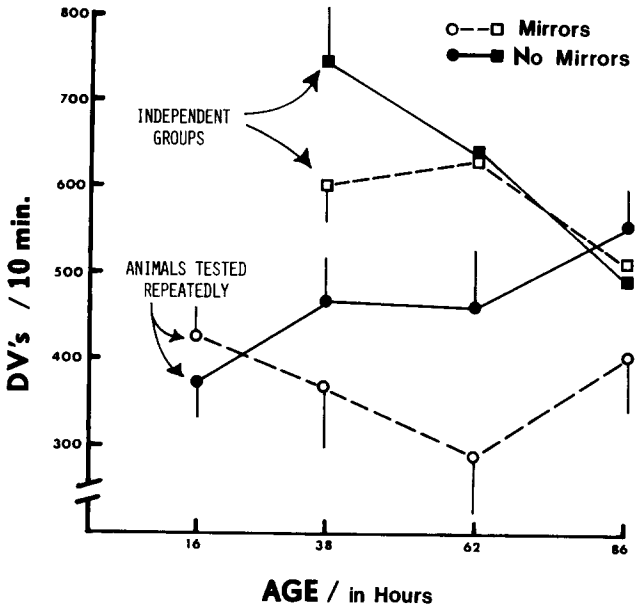

FIG. 5. Average DVs in 10 min of dark reared chicks tested either repeatedly or a single time at the indicated ages (Experiment 10b).

It is possible that the disappearance of the mirror effect at 18 hrs of age in dark reared animals was due to circadian variables, since decrements in DVs were also observed in the light reared animals. However, since the 48 and 24 hr tests were conducted at the same time of day, it would seem that the abolition of the mirror effect after 2 days of dark rearing may have been a permanent developmental effect. Experiment 10b further evaluated this possibility.

The data for Experiment 10b (Fig. 5), where all animals were housed in darkness, indicates that mirrors were ineffective at 16 hrs of age (confirming the 18 hr data of the

previous experiment). However, the mirror effect in these animals became apparent during repeated testing. Tests run on independent groups, revealed that mirrors were effective in reducing DVs at 38 hrs of age (perhaps corresponding to the presence of such an effect at 24 hr in the previous experiment), but they had no effect at subsequent time points.

Taken together, these experiments suggest that the capacity of mirrors to reduce DVs is ultimately due to an imprinting effect. Initially, both dark and light reared animals exhibit an unconditional capacity to be quieted by their own reflections, but this effect disappears in dark reared animals by two days of age. Accordingly, the data agree reasonably well with the assertion that there is a sensitive period in altricial birds for imprinting, that corresponds to the first few days of life [2].

EXPERIMENT 11

Although the previous experiments with mirrors suggest that the capacity of mirrors to reduce DVs is partially due to imprinting, in the following experiment we directly tested this proposition by employing the paradigmatically correct train-test procedure typically used in studies of imprinting.

METHOD

After being transported to the laboratory, 36 newly-hatched birds were housed communally in darkness. At 24 hr of age, half the animals were isolated for 10 min in the continuously lit plain test boxes, and half in the mirror test boxes. Animals were returned to their dark homes and tested at 4 days of age. Half of each training group was tested in mirrored, and half in non-mirrored chambers. On Day 5, all animals were retested on their respective conditions, 20 min after receiving an intraperitoneal (IP) injection of 2.5 mg/kg naloxone hydrochloride.

RESULTS

During training, animals in the plain boxes vocalized 668 (±61) times in 10 min and those in mirrored boxes vocalized 277 (±40) times, $t(34)=5.34$, $p<0.001$. Data for the two imprinting tests are summarized in Fig. 6. Both the main effects of mirrors, $F(1,28)=14.49$, $p<0.001$, and of the training conditions, $F(1,28)=4.92$, $p<0.05$, were reliable, while the interaction was not. Although this does not provide conclusive statistical support for an imprinting effect, the fact that the largest suppression of vocalization was observed in animals trained with mirrors and tested with mirrors does suggest that some imprinting occurred with the mirrors. Thus, animals trained in plain and tested in mirrored boxes, showed a reduction in DVs compared to the control group, but the effect did not quite reach statistical significance, $t(16)=1.94$, $p<0.10$. However, the vocalization level of this group was reliably higher than of the mirror-mirror group, $t(16)=2.50$, $p<0.05$.

When animals were retested with naloxone, the mirrors no longer produced a quieting effect. This suggests that naloxone can reduce the quieting effects of an imprinted stimulus, and may suggest that the naloxone effects we observed in the previous studies may have been at least partially due to naloxone reducing the calming effects of imprinting stimuli.

EXPERIMENT 12

In the last experiment of this series, we evaluated the capacity of naloxone and morphine to modify the acquisition of imprinting, using the above procedures. From previous theoretical considerations, it was predicted that naloxone should attenuate imprinting [6].

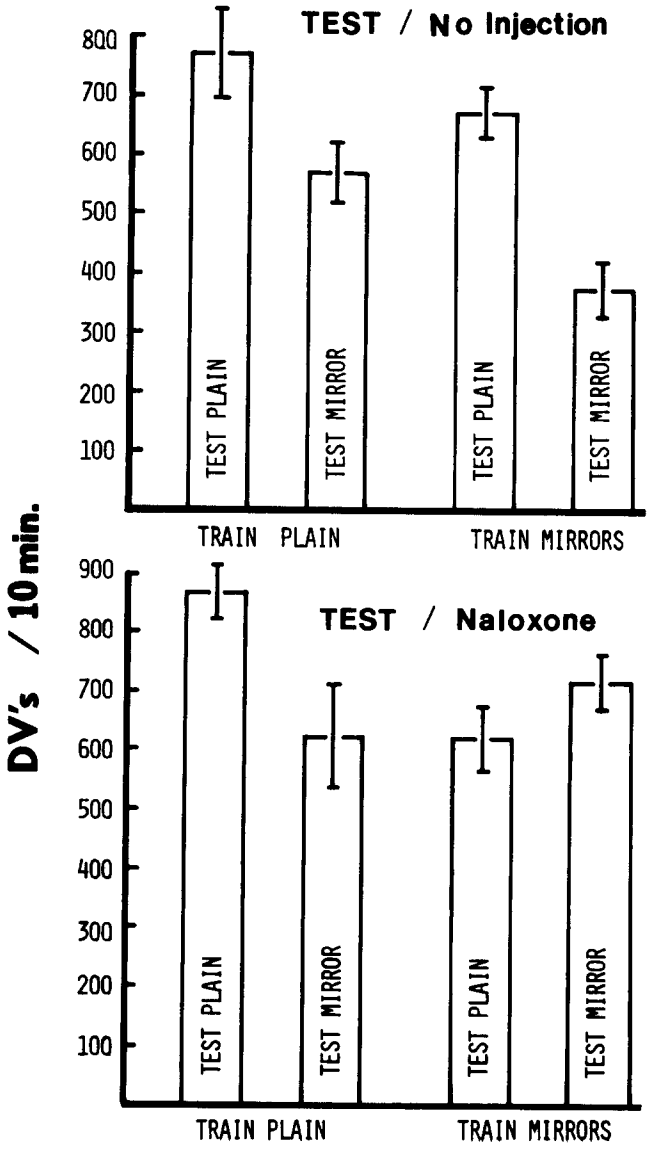

FIG. 6. Average DVs in 10 min of dark reared chicks exposed to mirrored or non-reflective test chambers at 1 day of age and retested under each of the conditions under naloxone or no-injection test conditions at 4–5 days of age (Experiment 10).

METHOD

A total of 108 newly hatched birds were group-housed in darkness upon arrival at the laboratory. At 24 hr of age, one third of the animals were injected with naloxone (2.5 mg/kg), one third with morphine (1 mg/kg) and one third with the distilled water carrier 20 min before being individually placed into mirrored chambers for a 20 min conditioning session.

Following this session, and immediately prior to being returned to their normal housing situation, each animal was injected with 2.5 mg/kg of naloxone. This maneuver was

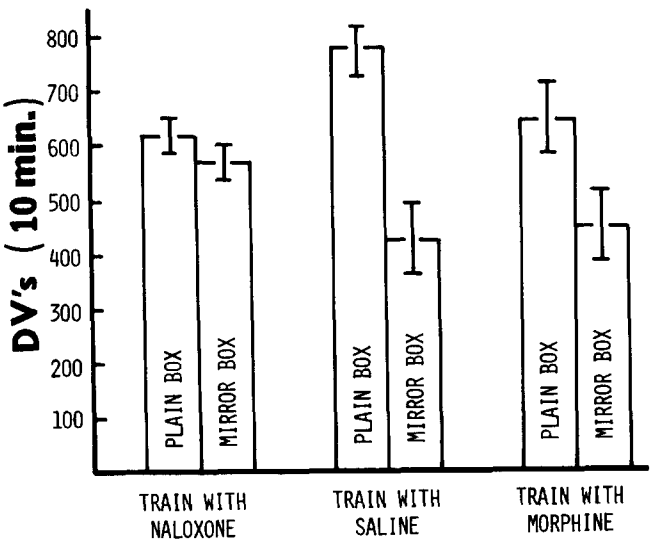

FIG. 7. Average DVs in 10 min of dark-reared chicks exposed to mirrored test chambers at 1 day of age following treatment with naloxone (2.5 mg/kg), saline, or morphine (1 mg/kg) and retested at 3 days of age either in mirrored or non-reflective test chambers (Experiment 11).

undertaken to insure a relatively equivalent experience with opioid blockade for each animal prior to being retested at a later time. On day three, animals in each of the training conditions were divided in half, with one group being retested in a mirrored environment and the other in a plain environment. The test period was 10 min in duration.

RESULTS AND DISCUSSION

The average number of DVs emitted during training for control, naloxone and morphine groups were 817 (±60), 1007 (±66) and 599 (±99), respectively, $F(2,105)=7.6$, $p<0.001$. The difference between naloxone and saline animals was reliable, $t(70)=2.20$, $p<0.05$, while the difference between saline and morphine did not quite reach significance, $t(70)=1.55$, $p<0.10$.

The test data are summarized in Fig. 7. The effect of mirrors, $F(2,102)=19.78$, $p<0.001$, and the mirrors by drug interaction, $F(2,102)=3.88$, $p<0.05$, affirm that imprinting does occur to the mirrors, and further, that training with naloxone reduces this imprinting effect. Thus, the data support the hypothesis that opioid blockade can attenuate imprinting. By comparison, a low dose of morphine has little effect on this process.

GENERAL DISCUSSION

These experiments provide converging evidence for the hypothesis that brain opioids mediate social affect and social attachments in young chicks. Naloxone was capable of reducing the capacity of young chicks to inhibit each other's separation induced distress vocalizations, and this effect was still apparent when the stimulus employed was the mirrored image of the test animal. A similar reduction of comfort was observed in a situation where touch was used as the soothing stimulus.

Although naloxone has numerically increased DVs in every experiment which we have conducted in chicks, it should be emphasized that the effect can be statistically fragile. At times the effect is lost with no apparent major modification of experimental parameters. At times this may be due to ceiling effects in control groups, but we also occassionally fail to obtain the effect when control DV levels are low. Perhaps this variability cannot be surmounted, since emotional expression may be molded as much by fleeting emotional processes in the brain of the organism as by the static environmental constraints imposed by the experimenter. In order for DVs to be sustained, animals need to be housed in a social environment, and in such communal housing situations we have little control over the momentary states that individual animals may be experiencing, but which may have marked influences on their responses to separation. Also, there are certain factors which we suspect may be relevant, but which have yet to be brought under control. For instance, chicks which have just eaten or consumed water may be differentially responsive to naloxone. Also, we suspect that seasonal variables may be operative. Our impression is that chicks tested during summer months are less responsive to naloxone than those tested in winter. Clearly, a great deal of work is needed to clarify the factors operating in this situation.

Still, we can conclude that naloxone can increase separation-induced DVs, especially when chicks are tested in a social context. However, it must be emphasized that even under the best of circumstances, the comfort derived from social stimuli is never completely inhibited by naloxone. Socially-tested chicks still vocalize substantially less following naloxone than they would if tested individually. It is unlikely that this partial inhibition of social comfort by naloxone is simply due to the failure of naloxone to occupy a sufficient number of brain opioid receptors: The capacity of naloxone to increase DVs was not increased by administering substantially larger doses of naloxone (Experiment 8). In general, with the DV measure employed herein, 1 mg/kg produces about as large an effect as can be obtained, and often, the effects are smaller at higher doses, perhaps because of accruing agonist effects of this drug. Accordingly, it is reasonable to conclude that activities of other neurochemical systems also participate in the mediation of social comfort.

The evaluation of other systems which have previously been implicated in distress vocalization, namely serotonin and acetylcholine [7], indicated that both may also participate in the comfort animals derive from each other. Indeed, when comfort was elicited with mirrors (Experiment 7), methysergide had the largest effect in reducing the comfort response and simultaneous treatment with all three blocking agents abolished the comfort response completely. Although activity in these three systems may suffice to account for the comforting effects of self-reflected social stimuli, a more thorough pharmacological evaluation of this behavioral measure will be needed to reach substantive conclusions. It is noteworthy, however, that the specificity of opiate manipulations on the comfort response appears to have been greater than following manipulation of the other systems. Activation and inhibition of opiate activity had reciprocal effects on the comfort response. To the contrary, activation of serotonin systems with quipazine did not magnify the comfort response, and activation of cholinergic systems with pilocarpine slightly attenuated the comfort response. The tendency of opioid antagonists to reduce and for opiate agonist to increase comfort suggests that activity in opioid

systems may organize information which specifically mediates social comfort.

The fact that naloxone could attenuate the acquisition and expression of imprinting, while morphine had no such effect, further affirms the possibility that brain opioid activity may participate in the formation of social bonds. Still, it should be noted that we employed a relatively little used measure of imprinting, namely reduction of DVs [3,4] and it would be important to conduct similar experiments with more traditional measures of following behavior. Although the domestic chick is not the species of choice when following is used as the measure of imprinting [2], in preliminary experiments, we have been able to train young chicks to follow humans in a naturalistic imprinting situation. Naloxone appears to precipitate internal conflict in such birds. Although naloxone treated chicks vigorously follow the experimenter (indicating they still recognize and are attracted to the imprinting stimulus), unlike normal chicks, they emit frequent DVs while near the experimenter, as if not obtaining normal comfort from being close. Indeed, naloxone treated animals often leave the immediate vicinity of the experimenter, while normal chicks remain magnetized by the experimenter's feet.

In summary, the present results provide support for the idea that brain opioids participate in social comfort and perhaps in social bond formation. Although opioids may be more influential than serotonin and acetylcholine systems in mediating these processes, the extent to which opioids exert a primary as opposed to a subsidiary function must remain uncertain in view of the present level of development in this field of inquiry. Clearly, a substantial amount of additional work is needed in this new area of research for a thorough understanding of the underlying control processes to be achieved.

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