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The Neurochemical Control of Crying

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PANKSEPP, J., R. MEEKER AND N. J. BEAN. *The neurochemical control of crying.* PHARMAC. BIOCHEM. BEHAV. 12(3)437–443, 1980.—The capacity of 18 drugs, including those which modify brain opioid, serotonin, norephinephrine, dopamine and acetylcholine activity, to modulate separation-induced distress vocalizations (DV) in young chicks were studied. Intraperitoneal morphine (1.7–5 mg/kg) injections were very effective in reducing DV's and naloxone (1.7–5.0 mg/kg) was found to increase DV's. Smaller bidirectional effects were also observed after pharmacological manipulation of cholinergic and serotonergic systems. Blockade of these systems with atropine (5–15 mg/kg) and methysergide (0.56 mg/kg) increased DV's while a facilitation of activity in these systems with pilocarpine (15 mg/kg) and quipazine (15 mg/kg) reduced DV's. Small reductions of DV's could also be achieved with neuroleptics such as haloperidol and chlorpromazine, and with apomorphine, suggesting that reduction of brain dopamine activity reduces DV's, while clonidine (0.56–1.7 mg/kg) was very powerful in reducing DV's, perhaps through autoreceptor reduction of brain NE activity. Chlordiazepoxide (5–15 mg/kg) was capable of reducing DV's, while imipramine (15–45 mg/kg), and pentobarbital (5–15 mg/kg) were essentially without effect. Opiate effects could be obtained as readily following intraventricular as following peripheral drug administration, while cholinergic and serotonergic agents were most effective by the peripheral route. In general, it was concluded that the opiate system had the most powerful specific effect on distress vocalization of all systems studied.

Distress vocalizations Chicks Opiates Acetylcholine Norepinephrine Serotonin Chlordiazepoxide

LITTLE work has been directed toward understanding the neurochemical controls of spontaneously occurring emotional behaviors. This probably reflects the difficulty in reliably obtaining emotional responses in laboratory animals without resorting to shocking maneuvers. However, one emotional behavior which can easily be elicited in animals and which has great species generality, is the distress vocalization (crying) that young of most avian and mammalian species exhibit in response to brief periods of social isolation. This panic response has not been extensively analyzed pharmacologically, although Scott [21] has provided evidence that in puppies many major psychoactive agents, including chlorpromazine, reserpine, meprobamate, diazepam, alcohol, sodium pentobarbital and amphetamine, do not reduce separation induced distress vocalizations (DV's) except at toxic or sedating doses. Scott found that the only drug which appeared to have a specific effect on DV's in puppies was the tricyclic antidepressant imipramine, and that was only effective in beagles but not in telomians or beagle×telomian hybrids. This separation-distress ameliorating effect of imipramine has been recently confirmed in rhesus monkeys [24], and it is noteworthy that imipramine is a member of the only psychopharmaceutical class known to reduce spontaneously occurring panic attacks in humans [10,11].

Our interest in analyzing the pharmacological control of DV's arose from an interest in understanding the neurochemical nature of social affect and social bond formation: Our initial work was based on the premise that isolation induced distress vocalization is an indicator of loneliness (which itself may be a diminutive emotional form of panic), and it was assumed that the identification of psychoactive drugs which could reduce DV's would suggest which neurochemical systems are activated by social comfort. Clearly, social stimuli themselves are very effective in inhibiting distress vocalization. For instance, young shicks do not emit many DV's in the proximity of imprinted objects [18] or in the presence of the mother hens [3]. Not only has this simple observation led many investigators to conclude that distress vocalization in a comfortable, non-threatening environment is primarily a response to isolation from social companions, but it also leads to the possibility that drugs which reduce DV's might be acting upon neural systems via which social stimuli normally quell separation distress.

Our initial drug trials were premised on the theoretical notion that neurochemical similarities may exist between social bond-formation and narcotic addiction [13]. Indeed, we have found that opiate receptor agonists (whether plant alkaloids or brain peptides) are very potent in reducing DV's in a variety of species [7, 13, 14] and that opiate blockade with naloxone can increase DV's [7,25]. Although these effects were observed at doses which produced no apparent sedation (as low as 0.125 mg/kg), it is essential to evaluate the roles of other brain neurochemical systems more fully before ascribing unique functional importance to brain opioid systems in controlling social affect.

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Accordingly, the following experiments served two major purposes: (1) they evaluated, by the use of reputed synaptic receptor agonists and antagonists, the participation of noradrenergic, dopaminergic, cholinergic and serotonergic systems in DV'ing, and (2) by the inclusion of a large number of other psychoactive drugs, the experiments allowed us to better judge whether the large effects we have previously seen with opiates could be considered, in any sense, to be specific to brain opioid system.

The young chick was used as the model system for study, not only because we can evaluate central effects easily with direct intracranial injection of drugs [14], but also because the chick is physically quite mature at birth and thereby provides a system for studying separation distress in which there may be minimal interference from the development of related behavioral processes. For example (a) the central nervous system is morphologically differentiated [1,7], (b) levels of biogenic amines in most parts of the chick brain have reached adult values [5, 12, 15], (c) cholinesterase and alkaline phosphatase have reached the adult level of activity [17], (d) the adrenal system is functional [19], and (e) the EEG is completed [9]. In addition, the chick can walk and run as early as four hours posthatch [8,20] and activity is stable over the first three days posthatch [2].

GENERAL METHODS

Subjects

Newly hatched male White Leghorn chicks were obtained within 6 hr 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. In Experiment 1, animals were housed in continuous lighting at a chamber temperature 100° ± 2°F. In all subsequent experiments, animals were housed in a 12–12 hr light-dark cycle room maintained at 85° ± 3°F, and animals were tested during the light part of the illumination cycle. A total of 1540 birds were tested in these experiments. In Experiments 1–4 all animals were between 24 and 72 hr of age. In Experiment 5 they were five days old. Each animal was tested only once.

Procedure (Experiment 1)

Animals were tested individually in a 90×90×30 wooden box painted a flat light green, with a roof which tapered to a peak approximately 90 cm from the floor. A 200 W light bulb mounted on the ceiling of the unit maintained a constant temperature of 100° ± 2°F inside the unit. The complete enclosure of the unit (including two 27×60 cm Plexiglas windows approximately 35 cm above the floor) ensured that the test animal had a minimum of visual and auditory contact with the outside environment. DV's were detected by a microphone located inside the unit and connected to a sound-activated relay.

Prior to the present experiment, 150 one-day-old chicks were used to screen for the dosages to be used. Neurotoxicity of a drug was determined in the basis of open-field behavioral responses scored in ten categories: walking, running, jumping, startle, avoidance, eye closure, head droop, low posture, trouble standing, and body weave. Neurotoxic effects were reflected by the presence of the last five responses (which were almost never observed in saline treated subjects) as well as the absence of the first five behaviors. Drug doses were selected which produced minimal neurotoxicity. This neurotoxicity data for the drugs used in this experiment are summarized in Table 1.

TABLE 1

NEUROTOXICITY DATA FOR VARIOUS RELEASING AND BLOCKING AGENTS INJECTED INTO ONE-DAY-OLD MALE CHICKS

Drug	Dose (mg/kg)	n	Number of subjects exhibiting at least one sign of neurotoxicity	Index of neurotoxicity*
Saline	—	10	1	0.03
d-Amphetamine	5	3	1	0.30
	6 –10	5	5	0.54
Phentolamine	5	6	0	0.00
	8	5	4	0.41
	10	5	2	0.33
	15 –40	5	4	0.53
Sotalol	10	5	1	0.15
	15	7	5	0.30
	20 –25	6	3	0.35
Pilocarpine	2 – 5	6	1	0.09
	8	5	2	0.27
	10	6	3	0.65
	15 –20	7	6	0.63
Atropine	1 – 4	3	0	0.00
	5	4	1	0.19
	10 –20	8	3	0.31
Felfluramine	5	5	1	0.15
	10	6	6	0.37
	12 –20	3	2	0.44
Methysergide	0.4– 0.5	7	3	0.18
	0.6– 0.8	6	5	0.36
	1 –10	5	5	0.64
l-Amphetamine	5	6	0	0.00
	10	4	0	0.00
Haloperidol	5	4	1	0.16
	8 –10	6	5	0.40
	15	2	1	0.50

*The index of neurotoxicity represents a ratio of the number of neurotoxic symptoms to the frequency of all behaviors measured in the open field. Thus, the index is sensitive not only to the presence of neurotoxic systems, but also the absence of normal behaviors.

The drugs and doses employed in this experiment are summarized in Table 2. All injections were delivered intraperitoneally in equal volumes of physiological saline (1 cc/kg) 30 min prior to the test session, except haloperidol which was administered one hour prior to testing. Immediately following the injection, the bird was marked and returned to the housing unit with the other birds until the time of testing. Test sessions were 10 min.

Procedure (Experiments 2–5)

Tests in these subsequent experiments were conducted in 36×32×33 cm sound insulated chambers with white acoustic tile walls, ceilings, and aluminum floors. Air circulation in

the chambers as well as background masking noise was provided by Xerox (Model 127P196) electric fans. The chambers were lighted with Chicago miniature bulbs (Model 313CM) and kept at temperature of 85° ± 3°F. Vocalization frequencies during the 10 min test periods were automatically collected by Radio Shack sound activated relay circuitry (Catalog No. 28-131), and recorded via electromechanical counters.

In the studies using peripheral injections (Experiments 2 and 4), 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 distilled water carrier. Injections were given 20 min prior to the animals being placed individually into the testing chambers for the 10 min tests. During the interval between injections and testing, animals were housed in groups of six in 17×28×13 cm high white translucent-plastic holding cages.

Experiment 3 and 5 employed 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 701 Hamilton syringe with a stop placed 5 mm from the tip. Employing a dye, we previously found that virtually all of the injections entered the fourth ventricle and the dye usually extended upward as far as the third ventricle. Usually, the anterior vermis of the cerebellum and either one or both occipital cortical areas were deeply stained. The injection procedure did not harm the animal in any obvious way, and separate control studies indicated that the procedure has a negligible effect on DV rates. Animals were removed from the flock, injected and immediately placed into the test chambers where DV's were recorded for 10 min.

In general, we found that the experimental condition used in Experiments 2–4 yielded higher and somewhat less variable DV rates than those in Experiment 1. The specific drugs and doses used in these experiments are presented with the results. Due to the high data variability, a Chi-square statistic was employed for data evaluation in Experiment 1. In all subsequent experiments, statistics refer to *t*-tests for independent groups.

RESULTS AND DISCUSSION

Experiment 1

Results. The results are summarized in Table 2. Five statistically reliable effects were observed. Both d- and l-amphetamine produced moderate (48–51%) increases in DV's. Fenfluramine, methysergide and atropine produced substantial (77–192%) increases.

Discussion. Activation of catecholaminergic systems (d-and l-amphetamine), or blockade of either serotonergic (methysergide) or cholinergic (atropine) systems can increase the emotion(s) or expressive process(es) accompanying social isolation. The specificity of these effects is not clear since neither blockade of alpha- or beta-adrenergic systems (with sotalol and phentolamine, respectively) nor the blockade of dopamine systems (with haloperidol) reliably reduced crying. However, the numerical changes were in the appropriate direction. The fact that fenfluramine, which is thought primarily to be a serotonin releasing agent [4], reliably increased vocalizations is not consistent with the data that serotonin blockade reduces DV's, unless it is assumed that the fenfluramine effect is mediated by serotonin autoreceptors [6] which functionally reduced brain serotonin activity. The fact that the indirect cholinergic agonist pilocarpine also slightly increased DV's is not consistent with the atropine results. It is possible that some of these incongruencies are due to nonspecific stress produced by some of the drugs. There was some indication for this with d-amphetamine and fenfluramine since in a small number of birds distress vocalizing was noted while still in the presence of the other birds.

TABLE 2

DISTRESS VOCALIZATION FREQUENCIES DURING 10 MIN OF SOCIAL ISOLATION (EXP. 1)

Treatment	n	Mean ± SEM	% Change from control
Saline	144	300 (±35)	
d-Amphetamine (5 mg/kg)	60	443 (±60)	+48%*
l-Amphetamine (5 mg/kg)	60	454 (±71)	+51%*
Sotalol (MJ 1999) (5 mg/kg)	36	176 (±59)	−41%
Phentolamine (5 mg/kg)	36	232 (±116)	−23%
Haloperidol (10 mg/kg)	36	231 (±51)	−23%
Fenfluramine (5 mg/kg)	60	532 (±74)	+77%*
Methysergide (0.5 mg/kg)	60	783 (±93)	+161%†
Pilocarpine (5 mg/kg)	36	378 (±90)	±26%
Atropine sulfate (10 mg/kg)	60	817 (±96)	+172%

*$p<0.05$.
†$p<0.001$.

Experiment 2

Rationale. Essentially, the major aim of this experiment was to replicate, clarify and extend the major findings of the previous experiment using procedures which substantially elevated the vocalizations in control animals. In addition, to further evaluate the role of serotonergic activity, a direct receptor agonist, quipazine was employed, and to further evaluate noradrenergic participation, the direct alpha-adrenoreceptor agonist clonidine [1] was studied. To provide direct comparative data for our opiate hypothesis [13], morphine and naloxone conditions were also included. Various doses of each agent were used, and the number of animals tested under each condition are indicated with the summary of results in Table 3.

Results and discussion. Basically, many of the results are consistent with our previous findings. Morphine reduced and naloxone increased DV's as we have previously observed. Methysergide increased DV's but only at the low does. Although fenfluramine still increased DV's, the fact that quipazine yielded the expected reduction is consistent with the possibility that serotonin has a specific inhibitory role in the control of distress vocalizations.

The effects of d- and l-amphetamine were not consistent with the results obtained in Experiment 1. Both agents slightly reduced DV's except at the highest d-amphetamine dose which clearly produced toxic effects in the three animals tested. Perhaps the different results obtained in this experiment and Experiment 2 were due to testing temperature. Amphetamine, at high doses, produces hyperthermia, and the higher test temperature of Experiment 1 may have added to the drug induced distress to yield high DV's. Clonidine reduced DV'ing, but the high dose also produced severe ataxia and wing droop, while the low dose animals

TABLE 3

DISTRESS VOCALIZATION FREQUENCIES (± SEM) DURING 10 MIN OF SOCIAL ISOLATION (EXPERIMENT 2)

Treatment	n's	Drug doses (mg/kg) 0.56	1.7	5.0	15.0
H_2O (3 μl/g)	12		545 (±71)		
d-Amphetamine	8,10,3		484 (±93)	386 (±87)	876 (±467)
Clonidine	8,11	275 (±116)*	245 (±105)*		
l-Amphetamine	11,10			328 (±86)	470 (±84)
Fenfluramine	10,8			730 (±91)	830 (±94)*
Methysergide	8,10	914 (±95)†	677 (±53)		
Quipazine	10,10			450 (±357)	272 (±84)*
Atropine	9,11			785 (±59)*	740 (±85)*
Pilocarpine	11,11			638 (±85)	280 (±78)*
Morphine	8,5		255 (±80)†	1 (±0.2)‡	
Naloxone	8,8		793 (±99)*	765 (±62)*	

*$p<0.05$.
†$p<0.01$.
‡$p<0.001$.

appeared somewhat sedated but normal. We suspect that these effects of clondine were due to profound autoreceptor inhibition of brain NE activity.

Experiment 3

In the following experiment we evaluated the effects of drugs used in Experiments 1 and 2 to modify DV's when administered directly into the brain. We also tested one additional dopamine agonist, apomorphine, as well as a local anesthetic (procaine) and a general anesthetic (sodium pentobarbital).

Results. The findings are summarized in Table 4. Intraventricular morphine at both 5 and 15 μg doses reduced DV's to 26% and 6% of control levels. Naloxone did not have a reliable effect, perhaps because the control DV rates were very high in this experiment. Neither fenfluramine nor methysergide reliably changed DV rates, but the high dose of quipazine reduced DV's to about half of control levels. Similar to trends in Experiment 2, l-amphetamine tended to reduce crying by 40% following the 15 μg dose. Neither the beta-adrenoreceptor antagonist, propranolol, or the alpha-receptor antagonist, phentolamine, affected DV rate. Clonidine was again extremely powerful in reducing DV's and the effect was accompanied by somnolence at the higher dose, whereas at the lower dose (0.56 μg) animals, though still drowsy, could be easily aroused. The high dose of d-amphetamine increased DV's by 25%, whereas apomorphine reduced DV's by 33 and 52%, respectively, at the low and high doses. Haloperidol at the high dose reduced DV's by 31%. Atropine reliably increased DV's by 16% at the 15 μg dose and pilocarpine reduced DV's by 42% at the higher dose. Neither dose of procaine reliably changed DV's although there was a trend for increased crying at both doses. Although, pentobarbital increased DV's by 22% at the 15 μg dose, the trend was not statistically reliable.

Discussion. In general, these results are in good agreement with the findings of Experiment 2. The major difference was in the failure of fenfluramine and methysergide to affect DV's by the central route. This may indicate that the brain perfusion from ventricular drug delivery was not as extensive as following peripheral injections, or that the effects following peripheral injections are due to a non-brain site of action. In any case, the ability of most agents administered intraventricularly to produce effects similar in direction to

TABLE 4

DISTRESS VOCALIZATION FREQUENCIES (± SEM) DURING 10 MIN OF SOCIAL ISOLATION (EXPERIMENT 3)

Treatment	n	Dose 5μg	15 μg
H_2O	16	784 (±40)	
Morphine	12,12	181 (±62)‡	32 (±11)‡
Naloxone	8,8	845 (±78)	822 (±96)
d-Amphetamine	6,8	871 (±93)	985 (±84)*
Apomorphine	6,8	525 (±97)†	379 (±76)‡
Haloperidol (10 & 30 μg)	6,8	685 (±82)	545 (±78)†
l-Amphetamine	6,8	896 (±26)	480 (±67)†
Clonidine (0.56 & 1.67 μg)	6,6	349 (±82)†	105 (±41)‡
Propranalol	6,8	803 (±96)	893 (±65)
Phentolamine	6,8	648 (±118)	828 (±68)
Fenfluramine	6,8	624 (±80)	783 (±96)
Quipazine	6,8	579 (±157)	404 (±39)‡
Methysergide	6,6	791 (±110)	793 (±77)
Atropine sulfate	6,8	660 (±65)	909 (±69)
Pilocarpine	6,8	705 (±91)	454 (±41)†
Procaine	6,8	872 (±81)	840 (±87)
Sodium pentobarbital	6,8	796 (±104)	960 (±163)

*$p<0.05$.
†$p<0.01$.
‡$p<0.001$.

TABLE 5

DISTRESS VOCALIZATION FREQUENCIES (± SEM) DURING 10 MIN OF SOCIAL ISOLATION (EXPERIMENT 4)

		Peripheral injection			Central injection	
Treatment	n	5 mg/kg	15 mg/kg	45 mg/kg		
Saline	12,12	821 (±67)			764 (±74)	3 μl
Morphine	12,12	115 (±57)†			78 (±29)	5 μg
Chlordiazepoxide	12,12,12	316 (±84)*	133 (±52)†		382 (±85)	15 μg
Chlorpromazine	12,12	839 (±68)	896 (±93)			
Imipramine	12,12		849 (±106)	462 (±128)*		
Pentobarbital	12,12	959 (±76)	431 (±69)*			

*$p<0.01$.
†$p<0.001$.

those following peripheral injections suggest that most of the previous drug effects we observed were due to direct CNS effect. Further, considering the extent of brain perfusion by these injections, it seems likely that the critical sites of action are around the periventricular strata of the brainstem.

Experiment 4

Rationale. In the following experiment, we evaluated the effects of several major psychoactive compounds which are effective in alleviating various major classes of psychiatric disorders. Since haloperidol was found to be somewhat effective in reducing DV's (Experiment 2), in the present experiment we studied the effects of a different antipsychotic, chlorpromazine. As a representative antianxiety agent, we evaluated the effects of chlordiazepoxide, and the antidepressant we chose to study was imipramine, since in studies of other species this agent had been found to be partially effective in reducing separation distress [21,24]. Finally, pentobarbital was analyzed as a representative sedative, and a morphine group was included for comparative purposes. Drugs which proved efficacious when administered peripherally were subsequently tested centrally.

Results. The findings are summarized in Table 5. Chlorpromazine was ineffective at both doses. Chlordiazepoxide (CDP) reduced DV's, though not by as much as morphine ($p<0.05$ at the 5 mg/kg dose). The animals at the 15 mg/kg dose of CDP appeared severely sedated. Although at the lower dose they remained alert and mobile, they still appeared to be more quite than the animals treated with morphine at 5 mg/kg. Imipramine was completely ineffective at the lower dose, and the moderate reduction at the high dose may have been a nonspecific effect since four of the 12 animals tested were left uncoordinated. These four birds accounted for most of the DV reduction—their average DV's being 85 (±32) vs 650 (±151) for the remaining eight birds ($t(10)=2.76$, $p<0.05$). Pentobarbital increased DV's slightly at the lower dose, and reduced them by 50% at the higher dose. Centrally, CDP at 15 μg did reduce DV's by 50%, but the effect was reliably smaller than the corresponding 15 mg/kg peripheral dose ($t(22)=2.40$, $p<0.05$). Morphine at 5 μg was substantially more effective than 15 μg of CDP in reducing DV's.

Discussion. In general, these results affirm that morphine may have a specific effect on distress vocalization. None of the pharmacological agents we tested, including those which produced more apparent sedation than morphine (CDP and the high dose of pentobarbital), were as effective as morphine in inhibiting crying.

From this data, it can also be concluded that the CDP and morphine were probably affecting different brain areas in producing their quieting effects. While 5 mg/kg morphine and 15 mg/kg CDP given peripherally were approximately equipotent in reducing DV's, 5 μg of morphine was substantially more potent than 15 μg CDP administered intraventricularly. This suggests that the CDP sensitive system is more widespread in the brain than the morphine sensitive system.

Experiment 5

Rationale. In the previous experiments the capacity of clonidine and chlordiazepoxide to reduce DV's was clearcut. In this last experiment we determined whether these effects may be mediated via changes in brain opioid activity by determining whether their effects would be counteracted by naloxone.

Methods. One half the birds tested in this experiment were pretreated with 1 mg/kg of naloxone intraperitoneally, and the control animals were injected with equivolumetric amounts of distilled water carrier. Ten minutes later sub groups of animals were injected intraventricularly with 0.57 μg of clonidine, 15 μg of chlordiazepoxide, 15 μg of morphine sulfate, or 3 μl of the water carrier. To assure naloxone perfusion of the relevant brain areas, naloxone pretreated animals were given the above intracranial injections in a vehicle which contained 1 μg of additional naloxone. Within a minute of the intracranial injections, animals were individually placed in the test chambers and crying was recorded for 10 min periods.

Results. The data are summarized in Table 6. As expected from previous work, naloxone by itself reliably increased DV's by 38% ($t(14)=1.87$, $p<0.05$). The suppression of DV's by morphine was substantially reversed by naloxone, even though the rate of 395 DV's/10 min was still reliably lower than the 589 DV's/10 min of the control animals. The profound suppression of DV's by clonidine was not reliably reversed by naloxone. The fact that 0.57 μg of clonidine reduced DV's substantially more in these animals as opposed to those in Experiment 3 may have been due to age differences (5 vs 1 day old, respectively). The slight suppression of DV's by 15 μg of CDP seemed to be reversed

TABLE 6

DISTRESS VOCALIZATION FREQUENCIES (± SEM) DURING 10 MIN OF SOCIAL ISOLATION (EXPERIMENT 5)

Treatment (intracranial)	n	Pretreatment (intraperitoneal) Saline	Naloxone (1 mg/kg)
H_2O (3 μl)	8,8	589 (±84)	812 (±89)*
Morphine (15 μg)	8,8	63 (±32)†	395 (±45)*
Clonidine (0.57 μg)	6,6	5 (±9)†	21 (±11)†
Chlordiazepoxide (15μg)	8,8	302 (±130)*	433 (±125)

Different from Saline-H_2O.
*$p<0.05$.
†$p<0.001$.

somewhat by naloxone, but the trend was not statistically reliable, and may merely reflect the normal potentiation of DV's that was observed in controls.

Discussion. In general, it seemed that the clonidine effect was independent of brain opioid activity. Although the data suggest that CDP suppression of DV's may be partially effected through changes in opioid activity, substantial additional work on that question would be needed before any clear conclusion could be reached.

GENERAL DISCUSSION

The present data suggest which neural systems, beside opioid based ones [7, 13, 14], are important in the control of isolation induced distress vocalizations. However, all conclusions must be tempered by several possible weaknesses in the present analysis: (1) It is known that most drugs act on more than one brain process, and in the absence of concurrent neurochemical data, the present results provide only indirect evidence for the participation of the specified neurochemical systems in the control of crying. Accordingly, our conclusion can only be as accurate as the reputed major actions of the drugs we have employed. (2) In the absence of more extensive analysis of the various control parameters used, we cannot exclude the possibility that some of the drugs found to be without effects might have reliable effects on crying in other conditions. For instance, animals tested in Experiment 1 exhibited increased DV's following d-amphetamine and fenfluramine while in Experiment 2 they did not. This may have been due to the different testing conditions employed; in this case we suspect the environmental temperature. (3) Finally, the specificity of the drug effects which were observed must presently remain in doubt. Although one aim of this work was to understand which brain systems control emotion(s) generated by social isolation, the drugs could have acted at many points within the intervening systems (sensory, perceptual, emotional, motor), and the present results do not permit the specification of the locus of action precisely. Agents which decreased DV's are, of course, most suspect on specificity grounds. However, even among drugs which increased DV's, one might question whether the drugs actually did increase the sense of social isolation or merely produced some other state of internal distress which funneled into the final common pathway of our dependent measure. With respect to such a possibility we can only note that the drugs which increased DV's did not consistently elicit vocalizations when the animals were in the presence of other animals just prior to testing. Although fenfluramine and d-amphetamine exerted such an effect in a few animals of Experiment 1, a similar trend was not observed in subsequent experiments using cooler testing conditions. In any case, considering the various interpretive problems that could arise with the present data, these experiments should be viewed only as a preliminary attempt to determine which neurochemical systems could be especially important in the control of isolation induced crying. The most compelling data for neurochemical specificity which can be obtained from the present approach is when both agonists and antagonists reliably modify the dependent measure in opposite directions.

Of the 18 non-opiate drugs which were studied in these experiments, only clonidine was as effective as morphine in reducing isolation induced distress-vocalizations. Combining this with the failure of Scott [21] to find a drug (except for imipramine) which effectively reduced crying in dogs, we can conclude, within the constraints of our inability to properly evaluate potency differences between different kinds of drugs, that the opioid system may have considerable neurochemical specificity in the control of distress vocalizations. Our failure to observe a quieting effect of imipramine in chicks contrasts to previous data in dogs [21] and monkeys [24] and may indicate an important species difference, perhaps with respect to degree of cephalization.

The present data also suggest that serotonin and acetylcholine are important in the modulatory control of DV's. Blockade of serotonin activity increased DV's, while stimulation of the system with quipazine was effective in reducing crying. Similarly, blockade of cholinergic systems increased DV's and activation of cholinergic systems with pilocarpine could reduce DV's. The fact that agonists and antagonists of both systems exerted opposing effects highlights the likelihood that these systems exert endogenous control over crying. The data further suggest that serotonergic and cholinergic controls are exerted at levels of the neuroaxis other than where opiates act. Intraventricular morphine was as effective as peripherally administered morphine, suggesting a prominent periventricular site of action. Serotonin and acetylcholine manipulations were generally less effective via the ventricular route than the peripheral routes, suggesting deeper tissue or non-central sites of action.

Catecholaminergic systems seemed to exert little specific control over DV's. In the first experiment where baseline DV's were low, both d- and l-amphetamine increased DV's, whereas in subsequent experiments where baselines were much higher, these agents had little effect, or at times reduced DV's. Still the data indicate that reduction of both noradrenergic and dopaminergic activity probably can reduce DV's. Both haloperidol and chlorpromazine reduced DV's and central apomorphine may have produced the same effect by dopamine autoreceptors inhibition of brain dopamine activity [23]. Similarly, low doses of clonidine reduced DV's, and this may also have been a similar autoreceptor mediated phenomenon [22]. Certainly clonidine was very effective in sedating animals, and considering the arousal which amphetamine produced, it is most reasonable to conclude that the clonidine was having its effect by reducing brain adrenergic activity. Considering that clonidine had such a powerful effect on DV's, further extensive analysis of this drug (especially at nonsedating doses) is indicated.

The fact that chlordiazepoxide was effective in reducing DV's in chicks is inconsistent with studies performed on dogs [21]. Whether this is a true species difference cannot be

determined at the present time, but the data do suggest that fear may contribute to the distress vocalizations elicited by the isolation procedures we employed.

Of all the manipulations performed, the capacity of morphine to reduce separation distress stood out. The morphine treated animals remained alert during these tests, and even those animals which assumed a comfortable roosting posture during testing would rapidly arise and try to escape capture when being removed from the testing chambers. The strong capacity of morphine to reduce DV's is unlikely to be explained by sedation, not only because of straightforward behavioral observations, but because agents such as pentobarbital and librium which produce clear sedation were not as efficacious in reducing DV's as morphine. Although, as with every other behavioral process that has been studied, there are multiple neurochemical influences which control crying, we remain impressed by the power with which opiates can reduce DV's, and thus take the present data to affirm the idea that brain opioid systems exert a most influential neurochemical control over emotions which arise from social isolation. However, the precise functional roles of each of the neurochemical systems studied herein remain to be specific.

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