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The Dopamine Innervation of the Visceral Cortex Mediates the Aversive Effects of Opiates

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ZITO, K. A., A. BECHARA, C. GREENWOOD AND D. VAN DER KOOY. *The dopamine innervation of the visceral cortex mediates the aversive effects of opiates.* PHARMACOL BIOCHEM BEHAV 30(3) 693–699, 1988.—We have previously reported that opiates acting on peripheral receptors produce aversive effects whereas opiates acting on central brain receptors produce rewarding effects [1]. The neurotransmitter dopamine (DA) has previously been implicated in both opiate reinforcing (positive) and aversive (negative) effects. We, therefore, chose to investigate the effects of disruption of DA systems on these two motivational properties of the opiate, morphine. Moreover, we sought to determine the brain site where dopamine might act as a mediator of these motivational effects. One group of rats received 6-hydroxydopamine (6-OHDA) lesions of the visceral (agranular insular) cortex to destroy dopaminergic innervation to this area. A separate group of animals were pretreated with intraperitoneal (IP) injections of the DA receptor blocker, alpha-flupenthixol (0.8 mg/kg), followed in both groups by 15 mg/kg (IP) morphine. Both 6-OHDA-lesioned and alpha-flupenthixol-pretreated subjects failed to develop the normal aversion to saccharin seen in control groups following conditioned taste aversion training with morphine. In a place conditioning paradigm, the aversive effects produced by low IP injections of morphine (acting on peripheral receptors) were blocked by 6-OHDA lesions of the visceral cortex. However, DA depletion of the visceral cortex did not disrupt the ability of animals to acquire a morphine place preference. Taken together, these results indicate that DA innervation of the visceral cortex mediates the aversive, but not the rewarding, properties of opiates.

Conditioned taste aversion Place conditioning Visceral cortex Morphine Dopamine

IT is now well established that behaviors that have been acquired or maintained by psychoactive stimulant drugs such as cocaine, amphetamine and apomorphine, can be greatly altered through manipulation of dopaminergic (DA) systems within the brain. This finding is based upon convergent evidence from a number of laboratories employing a variety of behavioral paradigms including self-administration [27, 37, 39, 40, 56–58], self-stimulation [19,32], locomotor activity [41] and place preference [48]. In addition to their rewarding properties, psychoactive agents have also been reported to possess aversive properties as measured in the conditioned taste aversion paradigm [5, 11, 13, 14, 30]. Some evidence exists for a DA involvement in the aversive properties of amphetamine [20].

Opiates have also been reported to possess both rewarding and aversive motivational effects. For example, when they are paired with visual, olfactory and textural environmental stimuli in rats, such drugs produce rewarding effects [33,51]. However, at similar doses over the same routes of administration, when paired with taste stimuli, opiates produce aversive effects [12]. A role for DA in the reinforcing effects of opiates has not been definitively established. There is evidence both to support and refute a critical role for dopamine in the positive reinforcing effects of opiates. While some investigators have suggested DA involvement in the mediation of opiate reward [6–8, 49], other researchers have suggested that DA appears not to be critical for the rewarding effects of opiates [16, 29, 54].

We have previously demonstrated that opiate activation of central brain receptors is responsible for mediating the rewarding effects of morphine, whereas morphine's aversive effects are mediated by opiates acting on peripheral gut receptors [1,3]. This anatomical dissociation is advantageous in that it permits us to individually manipulate both the positive reinforcing and negative aversive effects of opiates. Using this approach, we sought to determine which of these motivational effects of the opiate, morphine, would be affected by disruption of DA systems in the brain.

The visceral cortex is part of a neural circuit that relays aversive information about opiates from peripheral opiate

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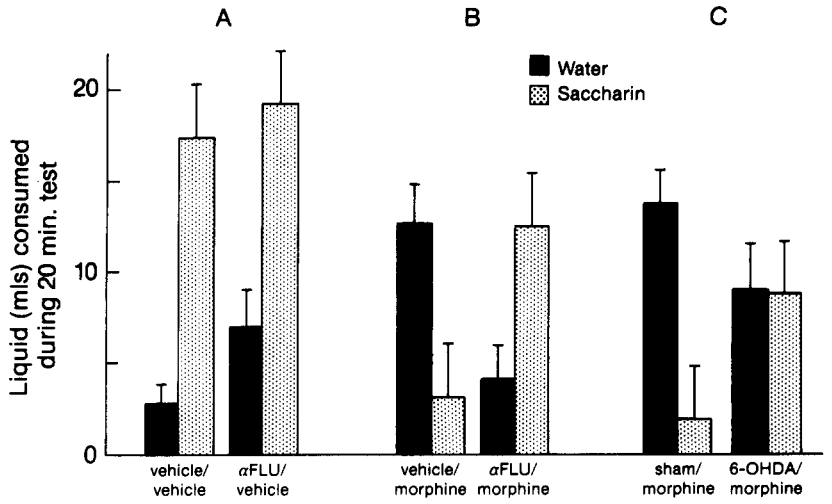

FIG. 1. The effects of alpha-flupenthixol pretreatment (panel B) or 6-OHDA lesions of the visceral cortex (panel C) on 15 mg/kg morphine-induced conditioned saccharin aversions. Panel A shows the effects of alpha-flupenthixol or vehicle pretreatment on the normal saccharin preference. Results are expressed as the mean (±SEM) amount of liquid consumed (saccharin and water) for each of the six groups during a two-bottle choice taste test.

receptors located in the gut. Lesions of the visceral cortex with the neurotoxin ibotenic acid, which selectively destroys cell bodies in this area, block conditioned taste aversions but not conditioned place preferences induced by morphine [28]. Anatomically, the visceral cortex has been defined as that area of agranular insular cortex that projects directly to autonomic areas in the medulla, and that maintains afferent and efferent connections with both the mediodorsal thalamus and the thalamic taste area in the ventromedial thalamic nucleus [24,52]. The visceral cortex also receives a dopaminergic input from the ventral tegmental area and substantia nigra [15, 17, 47] and has recently been shown to be the site of highest dopamine utilization in the brain [22]. Central dopamine pathways have been implicated as mediators of the motivational effects of many psychoactive agents [18,55]. We, therefore, thought it possible that the dopaminergic projection to the visceral cortex might also play a role in the mediation of the aversive effects of opiates. If so, then lesioning the dopaminergic innervation to the visceral cortex, or pretreating systemically with the dopamine receptor blocker alpha-flupenthixol should disrupt the aversive effects of opiates.

Here we report that both treatment with the DA receptor blocker alpha-flupenthixol, or 6-OHDA lesions of the visceral cortex, disrupt the acquisition of a morphine-induced conditioned taste aversion to saccharin. In addition, this 6-OHDA lesion also prevents the acquisition of a place aversion to low intraperitoneal doses of morphine (which act primarily on peripheral receptors) yet fails to disrupt the acquisition of a place preference seen following injections of higher doses of morphine (which act on brain receptors). Taken together, these results suggest that the aversive but not the rewarding properties of opiates are mediated by a dopaminergic pathway that innervates the visceral cortex.

METHOD

Subjects

Sixty-nine male Wistar rats (Charles River) served as subjects. Immediately upon receipt from the supplier, animals were handled and housed individually in hanging wire cages in a room maintained at 22°C and lit from 0900 to 2100 hours. Subjects were given ad lib food throughout the experiment. Rats used in the conditioned taste aversion experiment had their fluid intake restricted during taste aversion training and testing.

Surgery and Neurochemical Analysis

All surgery was performed under pentobarbital anesthesia (60 mg/kg IP). Subjects were placed in a stereotaxic apparatus, a scalp incision was made and two small holes were drilled in the skull to allow passage of a 1 μl Hamilton syringe. Bilateral microinjections of either 8 μg/μl 6-OHDA hydrobromide dissolved in 0.1 mg/ml ascorbic acid (dose expressed as free base), or its vehicle, were directed into the visceral cortex. The injection coordinates as taken from the atlas of Paxinos and Watson [35] were AP +0.2 mm anterior to bregma, ML ±4.8 mm and DV +5.5 mm below the dura, with the mouth bar set at −3.3 mm below the interaural line (see Fig. 1 in [28] for representative injection sites employing these coordinates). Subjects were allowed to recover from surgery for two weeks before behavioral testing. During the recovery period animals were handled periodically. Following all behavioral testing rats were sacrificed by decapitation and the visceral cortex was dissected rapidly on ice. Tissue samples were immediately frozen and stored at −70°C for later analysis. Dopamine levels were determined by high performance liquid chromatography (HPLC) according to the method of Mefford [31].

Conditioned Taste Aversion Procedure

Six groups of animals (n=8/group) were initially trained to consume water from calibrated glass drinking tubes on a limited access regime of 20 minutes each day, for a total of five training days. On the first experimental day, groups 1 and 2 received injections of alpha-flupenthixol (0.8 mg/kg IP) 2.5 hours prior to each of the conditioning sessions. Groups 3 and 4 were pretreated with the alpha-flupenthixol vehicle, whereas groups 5 and 6 (6-OHDA and sham lesion groups, respectively) received no pretreatment.

On the first conditioning trial, all groups were presented with 0.1% saccharin instead of water for the regularly scheduled 20 minute session. Immediately following the saccharin presentation, groups 1, 3, 5 and 6 were injected with 15 mg/kg (IP) morphine sulphate dissolved in physiological saline. The rats in groups 2 and 4 received vehicle injections in place of morphine. On the second experimental day, all animals were provided with water in place of saccharin for 20 minutes followed by saline injections. Treatment on subsequent experimental days continued to alternate between each of these two procedures for a total of six days (3 drug pairings). The side of the cage that the saccharin tube was placed was counterbalanced within each group but remained constant throughout the experiment for each rat. On day seven, a two-bottle choice test was administered to each animal by simultaneously presenting both saccharin and water for 20 minutes and measuring the amount of both liquids consumed for each rat.

Place Conditioning Procedure

The effects of 6-OHDA or sham visceral cortex lesions on the establishment of morphine place conditioning were evaluated with both a high dose (15 mg/kg IP) and low dose (0.05 mg/kg IP) of morphine. High doses of morphine produce place preferences for the drug-paired side, whereas low intraperitoneal doses of morphine produce place aversions [1]. The 6-OHDA- and sham-lesioned groups trained with 15 mg/kg morphine (n=8/group) were the same rats that received taste aversion conditioning two weeks earlier. The 6-OHDA- (n=11) and sham- (n=10) lesioned groups trained with 0.5 mg/kg morphine were new subjects with lesions performed identical to the rats trained with the higher dose of morphine.

Place conditioning procedures were similar to those employed by Mucha *et al.* [33]. Briefly, conditioning for each rat took place in one of two boxes which differed in color, texture and smell. One had black walls and a black Plexiglas floor which was wiped with a 2% glacial acetic acid solution prior to placing each rat inside. The second box had white walls and a wood chip floor which gave off a slight smell of wood. Each rat received injections of a drug on one day and vehicle on the next for a total of six days. When injected with morphine a rat was placed immediately in one of the boxes and on alternate days, when injected with vehicle, was placed in the other box. Each pairing lasted 30 minutes. The order of drug and vehicle presentation as well as the choice of environment rats received drug injections in, was counterbalanced for rats within each group. On the seventh (test) day each rat was placed into a larger rectangular test box which consisted of environments similar to the conditioning boxes at each end separated by a smaller grey area (neutral zone). The time each rat spent on each of the two ends was recorded over a ten-minute period.

RESULTS

Neurochemical Analysis

One subject from the sham-lesioned group died after behavioral testing and was, therefore, not included in the neurochemical analysis. The results from the HPLC assay verified that 6-OHDA infusions had, indeed, produced substantial depletions of dopamine. These assays were performed on the rats tested with the low dose of morphine (0.5 mg/kg) in the place conditioning paradigm. Compared to sham-lesioned controls, 6-OHDA treatment resulted in a significant (88%) depletion of dopamine in the visceral cortex, $t(18)=4.2$, $p<0.001$. The average DA visceral cortex content for lesioned animals was 88 ± 48 ng/g wet tissue as compared to 741 ± 163 ng/g for the sham-lesioned controls.

Conditioned Taste Aversion

Figure 1 presents the results of alpha-flupenthixol pretreatment or 6-OHDA lesions of the visceral cortex on morphine-induced conditioned taste aversions. As can be seen in panel A, pretreatment of the unlesioned control groups with vehicle or alpha-flupenthixol resulted in comparable consumption of both water and saccharin. A t-test comparing the mean percentage of total fluid intake in the form of saccharin for the two groups failed to reveal any significant differences, $t(14)=0.25$, $p=0.80$. This demonstrates that alpha-flupenthixol pretreatment alone failed to exert a significant effect on the normal preference for saccharin observed in vehicle-injected control animals.

However, pretreatment with alpha-flupenthixol was effective in preventing the normal taste aversion conditioned in rats that had saccharin intake paired with morphine injections (panel B). A significant difference in the mean percentage of total fluid intake in the form of saccharin for the two groups was observed, $t(14)=2.86$, $p<0.05$. Rats that were preinjected with alpha-flupenthixol prior to the morphine conditioning sessions consumed on average 12.5 ± 3.0 ml saccharin (64% of their total fluid intake) as compared to vehicle-pretreated controls that consumed a mean amount of 3.1 ± 1.9 ml of saccharin (20% of their total fluid intake). The ability of alpha-flupenthixol to prevent the aversive conditioning effects of morphine was not simply due to an overall increase in total fluid consumption. Vehicle- and alpha-flupenthixol-pretreated subjects did not differ significantly in the total amount of fluids consumed, $t(14)=0.37$, $p=0.72$. Thus, pharmacological blockade of DA transmission with the neuroleptic alpha-flupenthixol selectively suppressed the ability of morphine to produce a conditioned taste aversion.

A similar effect was observed in rats that received microinfusions of 6-OHDA directly into the visceral cortex. Morphine induced clear conditioned taste aversions in sham-lesioned rats, but not in rats with 6-OHDA lesions of the visceral cortex. A t-test comparing the mean percentage of total fluid intake in the form of saccharin for the two groups proved significant, $t(14)=2.7$, $p<0.05$. Sham-lesioned animals that had morphine injections paired with saccharin presentation consumed on average 1.9 (±1.2) ml of saccharin and 13.8 (±1.8) ml of water during the two-bottle choice test. In contrast, lesioned animals drank approximately equal amounts of both saccharin and water when given a choice (8.8 ± 2.4 and 9.0 ± 2.5 ml, respectively). Visceral cortex 6-OHDA lesions did not significantly alter the total amount of fluids consumed during testing [$t(14)=1.03$,

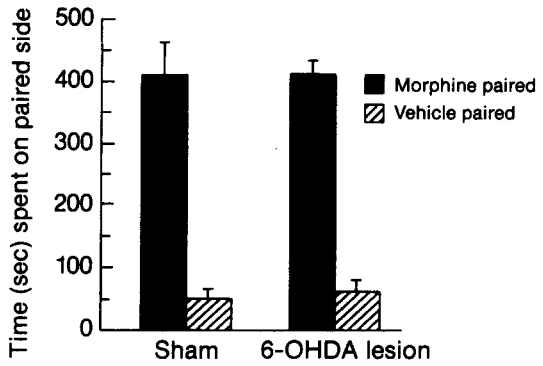

FIG. 2. The effects of 6-OHDA lesions of the visceral cortex on a 15 mg/kg morphine place preference. Results are expressed as the mean (±SEM) time (seconds) spent on both the morphine- and vehicle-paired sides recorded during a ten-minute test session.

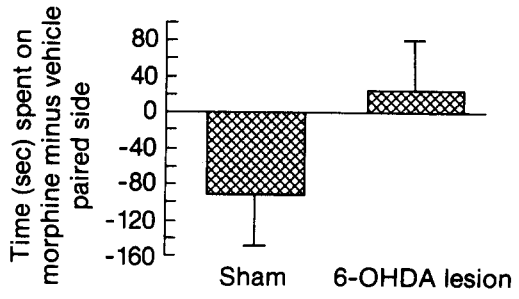

FIG. 3. The effects of 6-OHDA lesions of the visceral cortex on a 0.05 mg/kg morphine place aversion. Results are expressed as the mean (±SEM) time (seconds) spent on both the morphine- and vehicle-paired sides recorded during a ten-minute test session.

$p=0.32$, comparing 6-OHDA- and sham-lesioned groups], therefore ruling out the possibility that the 6-OHDA treatment nonspecifically compromised the sensorimotor aspects of fluid consumption. Although 6-OHDA lesions attenuated the aversion produced by morphine, animals with such lesions failed to exhibit a normal preference for saccharin as seen in the control and alpha-flupenthixol-pretreated groups. Thus, alpha-flupenthixol produced a more complete blockade of morphine-induced conditioned taste aversions than did 6-OHDA lesions of the visceral cortex.

Place Conditioning

The results from the high dose morphine place conditioning experiment are presented in Fig. 2. A t-test comparing the mean amount of time spent on the morphine minus the vehicle paired side for the two groups failed to reveal a significant difference, $t(14)=0.11$, $p=0.92$. All animals, both sham- and 6-OHDA-lesioned, showed strong place preferences for the environment in which they had received morphine injections. These results were in contrast to those obtained with a much lower dose of morphine. Such injections restrict morphine to an action on peripheral receptors and produce place aversions instead of the typical preferences seen with higher doses of the same agent. On the test day, sham control animals spent significantly less time on the drug-paired side when place conditioning sessions were paired with injections of a low dose (0.5 mg/kg) of morphine, as compared to lesioned animals, $t(19)=2.3, p<0.05$. The finding that 6-OHDA-lesioned animals spent more time on the low dose morphine-paired side as compared to sham controls indicates that such lesions were capable of attenuating the low dose place aversions (Fig. 3).

DISCUSSION

The present results indicate that either pharmacological blockade of dopaminergic systems or 6-OHDA lesions to the visceral cortex severely attenuate the aversive but not the rewarding properties of morphine. Pretreatment with alpha-flupenthixol blocked the normal taste aversion to morphine-paired solutions. Lesions to the visceral cortex with the neurotoxin 6-OHDA attenuated morphine-induced taste aversions. These lesions, which resulted in an 88% depletion of visceral cortical dopamine levels, also prevented the aversive effects of low doses of morphine in the place aversion paradigm, yet had no effect on higher dose morphine place preferences. These results are in agreement with those of Sklar and Amit [45], who report blockade of morphine, but not lithium chloride, taste aversions with the catecholamine synthesis inhibitor alpha-methyl-para-tyrosine. These findings are also consistent with our previous observation that alpha-flupenthixol pretreatment has no effect on morphine place preferences [29].

Morphine produces its positive reinforcing effects by acting on anatomically specific subpopulations of opiate receptors in the brain [36, 50, 53]. Morphine's aversive effects are produced by action on peripheral opiate receptors which relay the aversive information into and through the brain by neural pathways that allow for association of the aversive effects with conditioned taste and place stimuli [1,3]. We have identified a dopaminergic projection in the brain that is crucial for the passage or processing of opiate aversive information, yet seems independent of the brain pathways mediating the positive reinforcing effects of opiates.

The dopaminergic innervation of the visceral cortex (part of the agranular insular cortex) seemed a good candidate as a possible mediator of opiate aversive effects for three reasons. First, it is the primary sensory cortex for information from the visceral mucosa [24]. Second, it receives a metabolically active dopamine projection [17, 22, 47]. In fact, homovanillic acid:dopamine ratios of agranular insular cortex are higher than those reported for any other brain region to date [22]. Third, we had previously demonstrated that destruction of cell bodies in the visceral cortex with the selective neurotoxin ibotenic acid, blocks taste aversions but not place preferences produced by morphine [28]. Therefore, given that neuroleptic treatment blocks morphine taste aversions, it was not unexpected that 6-OHDA lesions of the visceral cortex attenuated the formation of opiate-induced conditioned taste aversions.

In this study we have assessed the effects of either dopamine depletion or neuroleptic pretreatment on the acquisition of associations between morphine and various conditioned stimuli. In the conditioned taste aversion experiment, both pharmacological and lesion evidence suggest that the pairing between morphine injection and stimuli

associated with the sensation of taste was not acquired. In the low dose conditioned place aversion experiment, 6-OHDA lesions of the visceral cortex disrupted the conditioned associations of morphine with visual, olfactory and tactile stimuli. The finding that such lesions block low dose morphine place aversions is important because it suggests that the observed deficit is motivational rather than due to a loss in the ability to process particular cues in the environment such as tastes or visual and tactile conditioned stimuli. Moreover, animals that had sustained significant depletions of dopamine in the visceral cortex were able to acquire a preference for an environment that had been paired with morphine injections. This suggests that disruptions of the dopaminergic innervation to the visceral cortex do not produce a general impairment in associative learning or in the acquisition of the cues of the place conditioning paradigm.

It remains possible that 6-OHDA lesions of the visceral cortex disrupt taste aversions in general. Therfore, in order to determine whether lesioned animals were still able to demonstrate an unconditioned aversion to aversive tastes and to further verify that our animals were still able to taste, two groups of previously lesioned animals (6-OHDA visceral cortex and sham-lesioned; n=7/group) were tested for their ability to demonstrate a taste aversion to low concentrations of quinine solutions. Baseline water consumption was first measured for all animals for two consecutive 24-hour periods. On the third day both groups of animals were presented with a 0.01% solution of quinine instead of water for 24 hours. During this test, not only did these animals consume comparable amounts of quinine, $t(13)=1.08$, $p=0.3$, but both groups consumed significantly less quinine than water on the preceding day [$t(12)=6.3$, $p<0.001$, $t(14)=3.6$, $p<.05$, for control and 6-OHDA groups, respectively]. This result clearly indicates that the lesioned animals were capable of taste discriminations and that the observed behavioral deficit did not involve the unconditioned motivational properties of taste stimuli themselves.

It may still be argued that the reinforcing effects of morphine are actually enhanced by decreasing DA action, thus counteracting the aversive effects of the drug in these situations. If this were the case, then we might expect 6-OHDA-lesioned animals to display a stronger preference for the environment paired with high doses of morphine as compared to sham controls. However, during testing, both groups exhibited nearly identical preferences for the environment that was previously paired with morphine injections, clearly ruling out this possibility.

Thus, it is clear that 6-OHDA (present results) and ibotenic acid lesions of the visceral cortex [28] produce a deficit that is specific to the aversive motivational properties, and not the positive reinforcing effects of opiates. It is not simply that opiate taste aversions are more easily disrupted than opiate place preferences since we have recently found that ibotenic acid brain stem lesions of the nucleus tegmenti pedunculopontis (TPP) block opiate place preferences without touching the conditioned taste aversions produced by morphine [2]. Furthermore, the effect appears to be specific to opiates as even large lesions of the lateral cortex in rat do not completely abolish the ability to acquire conditioned taste aversions to lithium chloride although in certain cases the taste aversions are partially attenuated [9, 10, 21, 23, 25, 26, 28].

The dopaminergic projection to the visceral cortex is part of a neural circuit that is involved with the processing of opiate aversive information. It is known that the dopaminergic projection to the visceral cortex takes origin from both the ventral tegmental (A10) and substantia nigra (A8) areas of the midbrain [4, 15, 17, 44]. This projection takes two separate courses, one passes rostrally through the medial edge of the nucleus accumbens before coursing caudally through the cortex to the agranular insular region; the other passes laterally over the optic tract directly to the visceral cortex [4,44]. The rostral dopaminergic projection may be of particular importance as 6-OHDA lesions in the region of the nucleus accumbens attenuate the development of a conditioned taste aversion to morphine [46]. However, the relative importance of the rostral versus lateral dopaminergic pathways from the midbrain to the visceral cortex should be viewed with caution since specific lesions of the lateral pathway have not yet been attempted. Moreover, there is some evidence to suggest that there may be limited collateralization of midbrain dopamine cells to the mediorostral forebrain and to the agranular insular cortex [17]. This being the case, then it is possible that both pathways may be carrying identical information.

If midbrain dopamine fibers relay information pertaining to the aversiveness of opiates to the visceral cortex, then it remains to be determined how this information is transmitted to the midbrain from the nucleus of the solitary tract (NST), the primary visceral sensory area in the medulla. One candidate pathway is the projection from the NST to the parabrachial nuclei, one of the most extensive projections of the NST [34]. In fact, lesions within the parabrachial nuclei are able to attenuate the aversive effects of both morphine and lithium chloride (Gons, Martin, Bechara and van der Kooy, in preparation). An alternate route is an ascending noradrenergic pathway that makes up the majority of the direct projections from the NST to the diencephalon [43]. Indeed, lesions of this and other ascending noradrenergic pathways by means of pontine 6-OHDA injections attenuate taste aversions produced by morphine [38]. It might be argued that noradrenergic projections to the visceral cortex were the critical ones lesioned in the present study. This seems unlikely for two reasons. First, the neuroleptic pretreatments which also block opiate aversions are very specific in blocking dopamine neurotransmission. Second, although the noradrenergic perikarya present in the NST project to the midbrain and diencephalon, it is the cell bodies in the locus coeruleus that provide the major innervation of the cortex [43]. It should also be noted that numerous indirect multisynaptic pathways may contribute to the transmission of ascending information concerning the aversive properties of opiates.

Collectively, these results suggest that there are two anatomically separable systems, one which originates in the brain, and the other in the periphery, that serve to mediate the rewarding and aversive effects of opiates, respectively. Moreover, based on lesion evidence, the neural systems processing these two motivational effects appear to be parallel and completely independent. Even at a cortical level there appears to be little interaction between the aversive and rewarding effects of opiate action. This does not rule out the possibility of interactions between the rewarding and aversive effects of other psychoactive drugs. In fact, this may be the case for psychomotor stimulants. For example, Roberts and Zito [42] have developed one such model to explain stimulant self-administration behavior. According to this view, self-administration is regarded as being in a constant state of equilibrium, being controlled by not only the reward-

ing, but also the aversive effects of the drug. Systemic pharmacological treatments such as neuroleptics, which result in increases in self-administration rates, might affect both the positive and negative effects of the self-administered drug. Conversely, lesions to specific brain nuclei or pathways may disrupt either the rewarding or aversive effects of the drug independently. The final determination of the motivational basis for various manipulations will not be as straightforward for stimulants as it is for opiates, because there has not yet been as clear an anatomical separation of the rewarding and aversive substrates for stimulants as there has been for opiates.

In conclusion, the present study demonstrates that normal dopaminergic function is critical for the perception of the aversive, but not the rewarding effects of opiates. Moreover, the precise locus of this dopaminergic function is the dopaminergic innervation of the visceral cortex. This cortical DA input is involved in processing the aversive motivational properties of opiates before their learned association with any particular conditioned sensory stimulus.

ACKNOWLEDGEMENTS

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