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Acute Administration of Lithium Carbonate Interferes With Suckling in Neonatal Rats

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McLAUGHLIN, C. R. AND C. P. CRAMER. *Acute administration of lithium carbonate interferes with suckling in neonatal rats.* PHARMACOL BIOCHEM BEHAV 32(2) 453–458, 1989.—These studies provide an animal model for the lithium-induced decrease in suckling reported in the clinical literature that allows for more precise determination of causal mechanisms. Nine-day-old rat pups were administered lithium carbonate via either intraperitoneal (IP) injections or intragastric (IG) gavage in doses approximating that which human infants might receive via breast milk. The pups were tested for their ability to locate and attach to the nipples of an anesthetized dam. Lithium significantly increased the pups' latency to attach to a nipple. Further tests of milk extraction using oxytocin-induced milk-letdowns indicate that lithium also interferes with milk withdrawal. Tests of motor and sensory deficits using an open-field and an olfactory choice test indicated that lithium did not similarly impair these behavioral facets of suckling. Alternative mechanisms for lithium-produced suppression of suckling are discussed.

Lithium Suckling Neonate Rat

ACCOUNTS in the clinical literature suggest that lithium inhibits the "neonatal suck response" (1), although most of the available literature on perinatal exposure to lithium is retrospective, anecdotal or outdated (4). In fact, reports of a decreased suck response are apparently based on two case studies, in which several other confounding variables were present (15,16). First, both mothers presented with long premorbid histories of manic-depressive symptoms that worsened as their pregnancies progressed, necessitating psychiatric intervention and drug therapy despite pregnancy. Second, in each case several other neuroleptics and antidepressants were administered in addition to lithium carbonate. Finally, in both cases the mothers displayed signs of acute lithium toxicity following delivery, severe enough to result in stupor and coma (15,16). Toxicity may well have spread to the fetus, since lithium has been shown to freely pass the placental barrier in rats (8). In addition, evidence in the human literature suggests that approximately one-half to one-third of the maternal lithium level is present in breast milk (14).

Reports in the animal literature are also problematic. In one study, mice given lithium chloride in their drinking water refused to drink and died (10). When the concentration of lithium chloride was decreased to a level at which the animals were able to survive and mate, the serum lithium levels obtained in the dams and pups were significantly lower than those reported in the human literature. Although the pups of these lithium-treated mice were underweight, suggesting a decrement in suckling, several confounding effects of the lithium on the dams, such as maternal dehydration, malnutrition, changes in maternal behavior or teratogenic effects, were not considered. In pilot studies in our own lab, female rats given lithium carbonate-tainted water also refused to drink and lost a significant amount of weight, making them unsuitable for breeding. Therefore, to bypass some of these uncontrolled maternal variables and to systematize the earlier reports of a decreased suck response, in the current studies we administered direct, acute lithium carbonate to neonatal rats. Nipple attachment is assessed in Experiment 1, and milk extraction is investigated in Experiment 2. In addition, we explored several behavioral changes that have been shown to interfere with suckling, particularly motor impairment (Experiment 3) and sensory deficits (Experiment 4).

EXPERIMENT ONE

Suckling has been shown in rats to consist of two separate and distinct behavioral components: nipple attachment and milk extraction (2,7). To attach, the pup must find the mother, locate a nipple and draw the teat firmly into its mouth. In Experiment 1A, lithium was administered via injection. Although oral administration of lithium would have more closely approximated ingestion via breast milk, we selected intraperitoneal injections for pilot studies as well as the first experiment because it provided a more rapid and

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direct method of administration. Furthermore, IP administration eliminated the possibility of accidental suffocation inherent in IG infusions with young animals. Lithium was administered via gavage in Experiment 1B, however, to more closely approximate ingestion from breast milk and to eliminate any discomfort created by the IP injections.

METHOD

Subjects

Primiparous and multiparous female Long-Evans-derived hooded rats and their pups were used in all experiments. The females were mated in our colony with Long-Evans males (Blue Spruce, Altmont, NY). Approximately one week prior to parturition and throughout lactation, they were housed individually in plastic tub cages (24×43×28 cm). Dams were checked daily for the birth of pups, with the day of birth considered Day 0. The vivarium was maintained on a 14:10 light-dark cycle at approximately 26°C with Prolab 3000 chow and water available ad lib. All pups were 9 days of age at the time of testing.

Procedure

At 9 days of age, pups were removed from their dams, weighed and marked with permanent ink. In Experiment 1A they were then administered a 2% body weight IP injection of either lithium carbonate (20, 40, 60, 80 mg/10 ml, which yielded doses of 40, 80, 120, 160 mg/kg body weight) or isotonic saline one hr prior to a nipple attachment test with an anesthetized dam. In Experiment 1B, a second group of pups was administered 4% body weight of either lithium carbonate (20, 40, 60, 80 mg/10 ml, IG which yielded doses of 80, 160, 240, 320 mg/kg) or saline by gavage, using PE-50 tubing, one hr prior to testing. Higher doses were used in Experiment 1B to compensate for the slower absorption time in the stomach while keeping maternal separation constant. The pups remained separated from their dams in plastic tubs (15 cm diameter) placed on a heating pad (37°C) until testing. Pups of approximately the same age were left with the dam until she was anesthetized (1 cc/kg sodium pentobarbital, Abbott Laboratories, IP) 15 min prior to testing. The dam was placed on her side in a slightly tilted plastic tub (17×27.5 cm), which provided the pups full access to her ventrum and all of her nipples (6). Five pups, one from each dose, were simultaneously placed in the tub with their snouts pointed toward the dam. The dependent measure was latency to attach to a nipple in a 5-min test. Pups that failed to attach within 5 min were scored as 5 min. Sixty pups from 6 litters provided an N of 12/cell in Experiment 1A and forty pups from 4 litters provided an N of 8/cell in the Experiment 1B. In this and all subsequent experiments, the person scoring behavior was blind to the drug condition.

RESULTS AND DISCUSSION

As can be seen in Fig. 1A, lithium carbonate injected IP produced a highly significant increase in latency to attach, $F(4,55)=9.06$, $p<0.01$. This increase was dose-dependent [linear trend analysis, $F(4,55)=35.73$, $p<0.01$]. Lithium particularly interfered with attachment in the 160 mg/kg group, in which no pups attached during the test. These results indicate that the acute IP administration of lithium carbonate significantly interferes with attachment in neonatal rats. Lithium also significantly increased attachment latency when given IG (Fig. 1B), $F(4,35)=5.44$, $p<0.01$. Linear trend analysis revealed a dose-dependent reduction in attachment,

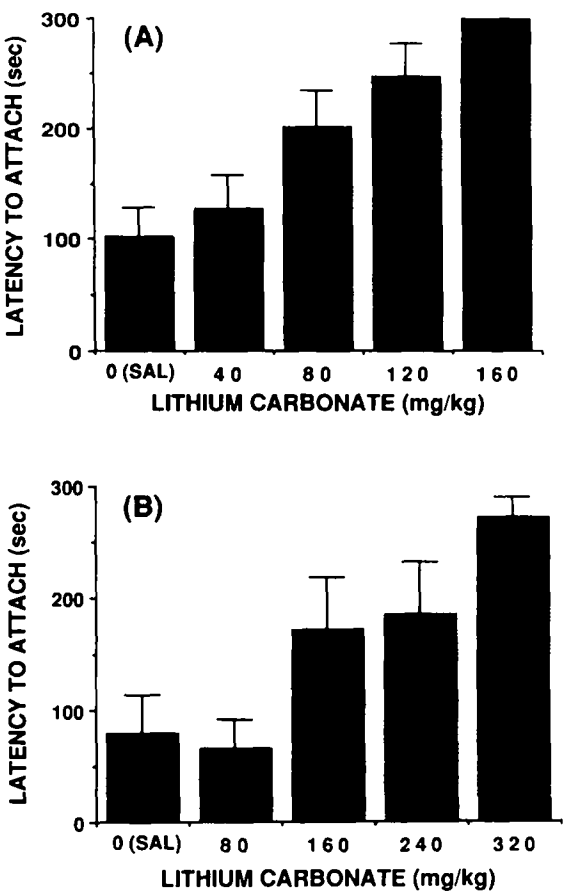

FIG. 1. Mean (±SEM) latency to attach to the nipples of an anesthetized dam following IP injection (A) or IG administration (B) of lithium or saline.

$F(4,35)=19.42$, $p<0.01$. The results from Experiment 1B provide additional support for the hypothesis, since lithium given in a manner that closely approximates ingestion via breast milk also interferes with attachment. These results also suggest that the interference in attachment observed in the first experiment was not a direct result of the possible discomfort resulting from IP lithium injections. Furthermore, none of the pups were noticeably different in either their approach or response to the nipple, which included head sweeping and circling and tugging at the teat. Because the IP route of administration yielded slightly more reliable results and eliminated the risk of accidental suffocation inherent in IG infusions with young animals, IP injections were employed for the remaining experiments.

EXPERIMENT 2

Although lithium significantly interfered with attachment in Experiment 1, milk extraction may not be affected by exposure to lithium. Extraction involves the use of negative pressure to withdraw milk from the teat and may be subserved by different sensory, motor and motivational controls (2,7). Therefore, in Experiment 2 we assessed this second component of suckling independent of attachment by measuring the amount of milk each pup ingested after it had attached to a teat.

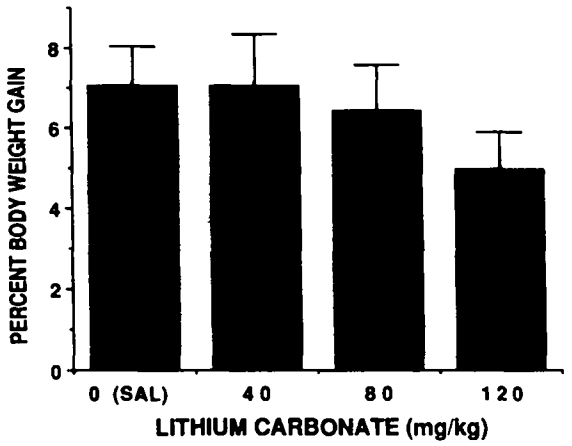

FIG. 2. Mean (±SEM) percent body weight gain following lithium administration. Pups were deprived of food and maternal contact for 6 hours prior to testing with a milk-replete, oxytocin-induced anesthetized dam. A total of ten milk-letdowns were induced at 4-min intervals.

METHOD

Procedure

Nine-day-old pups were deprived of food and their dam for a total of 6 hr. During this time, the pups were housed in heated tubs as described in Experiment 1. One hr prior to testing they were given a 2% body weight IP injection of either lithium carbonate (20, 40, or 60 mg/10 ml, IP which yielded doses of 40, 80, 120 mg/kg) or saline. The high dose used in previous experiments was eliminated, as those pups did not readily attach. Each dam was separated from her pups for 6 hours to allow milk supplies to build, anesthetized and implanted with a cannula in the ventral tail vein following the procedure as previously described (5). One pup from each dose condition was weighed and then allowed to attach to a nipple. One min after all pups attached, ten milk-letdowns were induced at 4-min intervals by intravenous oxytocin administration (0.0015 μ units in 0.1 ml isotonic saline). These milk delivery parameters approximate a typical nursing bout and provide a naturalistic situation for assessing pup behavior independent of maternal interference. After the final milk-letdown, pups were removed from the dam and again weighed to determine intake. In this experiment, the percent body weight gained was the dependent measure. Thirty-two pups from 8 litters provided an N of 8/cell. The data were analyzed within-litter using repeated measures ANOVA to account for potential differences in maternal milk supplies.

RESULTS AND DISCUSSION

As can be seen in Fig. 2, the percent body weight consumed was significantly reduced by lithium administration, suggesting that lithium also interferes with milk extraction [$F(3,21)=3.54, p<0.05$; planned comparison contrast between saline and 120 mg/kg, $F(1,7)=5.83, p<0.05$]. These results indicate that lithium significantly interferes with both elements of suckling, attachment and milk extraction, in a dose-dependent fashion. Milk extraction rate, unlike attachment latency, is sensitive to motivational (or at least activational) state in pups of this age (5,7). The results of Experiment 2 therefore suggest the possibility that lithium fundamentally affects pups' willingness to suckle. However, both dependent measures could be disrupted by sensorimotor deficits. Thus, in Experiments 3 and 4 we assessed gross motor and sensory impairment, which could contribute to suckling interference.

EXPERIMENT 3

Lithium could be interfering with suckling either by sedation or by some other form of gross motor impairment. Therefore, in Experiment 3 we attempted to assess whether the lithium-induced suppression in nipple attachment was correlated with gross motor impairment as measured in an open field modified to incorporate the smaller size and limited ambulatory abilities of young animals.

For the open field test we selected two measures of locomotor and exploratory behavior. 'Squares entered' were scored as the number of times the pup entered a square with all four paws and 'snout pokes' were scored as the number of time the pup's snout crossed a line. 'Squares entered,' the first measure, reflects general translational movement or locomotion and is similar to an open field test with adult rats. We noted during pilot experiments, however, that the pups would often remain in one square, pivoting about their hindquarters while sweeping their noses from side to side, possibly searching for olfactory cues. Therefore, 'snout pokes,' the second measure, combines both motor and sensory components and provides an index of general exploratory behavior.

Because the high dose of lithium carbonate produced the greatest impairment in the attachment tests, animals in this condition were of particular interest as motor and sensory deficits, if any, would be expected to be greatest in this group. Therefore, the high dose (160 mg/kg) was reintroduced in Experiments 3 and 4.

METHOD

Apparatus

The open field (38×38 cm) consisted of a piece of clear Plexiglas with 28.5 cm high sides placed over a piece of white paper marked into 25 squares (7.5×7.5 cm), which were slightly larger than a typical pup.

Procedure

Fifty pups were removed from their dam and administered a 2% body weight IP injection of either lithium carbonate (20, 40, 60, 80 mg/10 ml which yielded doses of 40, 80, 120, 160 mg/kg) or isotonic saline one hr prior to testing. Each pup was placed into the open field for 5 min. The number of squares entered was recorded for all pups; the number of 'snout pokes' was recorded for 40 of the pups, equally distributed across drug conditions. The apparatus was wiped with a dry cloth between each test to remove any olfactory cues that may have been left by the previous pup.

RESULTS AND DISCUSSION

Lithium did not significantly interfere with either gross locomotion or exploratory behavior as measured by 'squares entered' and 'snout pokes,' although a marginal dose-dependent trend was observed in 'snout pokes' (Table 1) ['Squares entered,' $F(4,45)=0.85, p=0.50$; linear trend analysis, $F(4,45)=3.10, p=0.08$. 'Snout pokes,' $F(4,35)=1.12, p=0.36$; linear trend analysis, $F(4,35)=4.00, p=0.05$]. These re-

TABLE 1

MEAN (±SEM) SCORES FOR OPEN FIELD BEHAVIORS OF PUPS GIVEN LITHIUM OR SALINE

		Dose (mg/kg, IP)				
		Saline	40	80	120	160
Squares Entered	Mean	3.7	3.5	3.3	2.4	1.7
	(SEM)	0.8	0.6	1.2	0.9	1.0
Snout Pokes	Mean	58.1	60.5	48.9	42.2	38.9
	(SEM)	10.9	4.6	9.1	10.4	8.4

sults indicate that the doses employed to interfere with nipple attachment and milk extraction in Experiments 1 and 2 are not sufficient to significantly disrupt either gross motor or exploratory behaviors as measured in an open field.

EXPERIMENT 4

The marginal dose-dependent trend observed in 'snout pokes' in Experiment 3 suggests that some element of that measure may be sensitive to lithium. As we stated earlier, the 'snout pokes' measure appears to combine both sensory and motor elements. One possible hypothesis would be that lithium is impairing sensory function, specifically olfaction, in the pup. In Experiment 4A we used a choice test to assess whether lithium had impaired the pup's ability to locate the dam independent of contact with her (13). Because olfaction is critical for nipple attachment in rats (3), Experiment 4B was conducted to distinguish between olfactory and thermal characteristics of the maternal stimulus that may have been present in Experiment 4A and specifically to assess the impact of lithium on olfactory discrimination.

METHOD

Apparatus

A clear Plexiglas box (15×37.5×7 cm) divided into two 15×15 cm square areas with a central 7.5×15 cm neutral area was used for testing. A wire mesh lid with 5 cm high walls covered the entire box. The pup was able to move freely in the lid over the two compartments and the neutral area. The lid was permeable to both thermal and olfactory cues emanating from the box below. In Experiment 4A an anesthetized, lactating dam was placed in one side of the box and clean pine shavings were placed in the other side. In Experiment 4B soiled bedding from the home nest was used in place of the dam; clean bedding was again used on the other side.

Procedure

At 9 days of age, pups were removed from their dams and administered a 2% body weight IP injection of either lithium carbonate (20, 40, 60, 80 mg/10 ml which yielded doses of 40, 80, 120, 160 mg/kg) or isotonic saline one hr prior to testing and handled in the same manner as described in Experiment 1. The pups were then placed on the cover over the central neurtral zone, and their movements were recorded for 150 sec. The amount of time each pup spent on the side with the dam (Experiment 4A) or the home bedding (Experiment 4B) was the dependent measure, and a pup was scored as having made a choice when all four paws had crossed out of the neutral area. Eighty pups from 8 litters provided an N of 8/cell in each experiment.

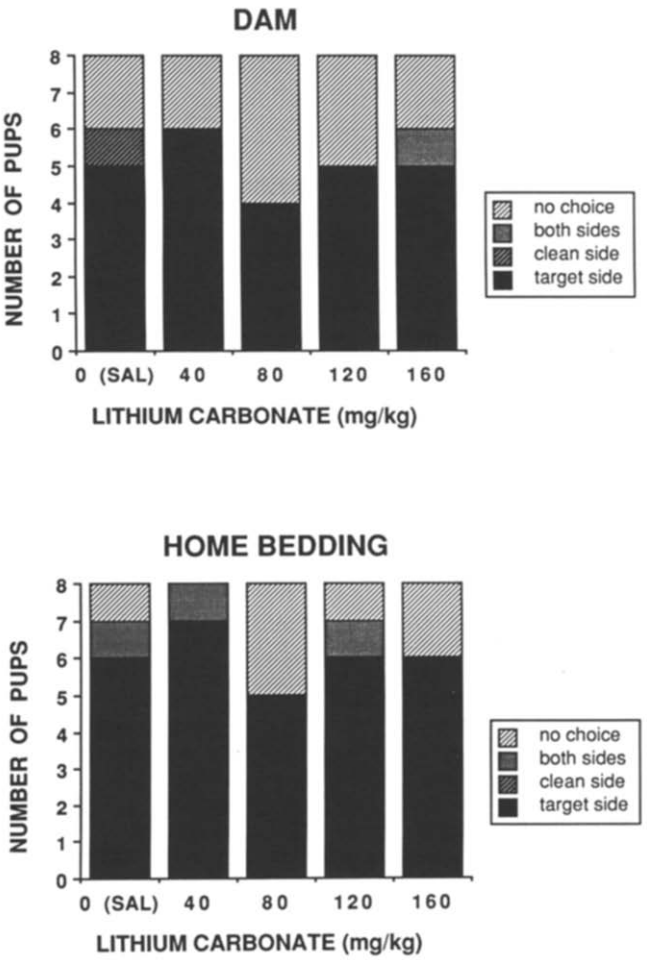

FIG. 3. Number of pups in each drug condition choosing the target side, the clean side, or both sides or making no choice. The target in the upper panel was an anesthetized dam and in the lower panel was bedding from the nest.

RESULTS AND DISCUSSION

The choices made by pups in each condition are illustrated in Fig. 3. There was no relationship between dose and ability to select the target side. As can be seen in Table 2, lithium did not significantly impair the pups' ability to find the dam [$F(4,35)=1.26$, $p=0.31$; linear trend analysis for the quadratic term, $F(4,35)=0.76$, $p=0.10$]. Furthermore, lithium did not impair the pups' ability to locate soiled bedding from the nest [$F(4,35)=2.12$, $p=0.10$; linear trend analysis for the quadratic term, $F(4,35)=3.08$, $p=0.09$]. Doses of lithium that interfered with suckling did not impair either the pups' ability to locate the dam or their olfactory function.

These tests also confirm the lack of motor debilitation demonstrated in Experiment 3, as the pups had to locomote to find either the dam or the nest material. Because making a choice involved moving at least a full body length to reach a

TABLE 2

MEAN (±SEM) TIME SPENT OVER EITHER AN ANESTHETIZED DAM (EXPERIMENT 4A) OR HOME BEDDING (EXPERIMENT 4B) BY PUPS GIVEN EITHER LITHIUM OR SALINE

		Dose (mg/kg, IP)				
		Saline	40	80	120	160
Dam (sec)	Mean	40.9	87.1	44.2	46.9	82.1
	(SEM)	21.5	19.8	21.6	18.0	18.9
Bedding (sec)	Mean	94.6	91.9	33.6	71.9	75.9
	(SEM)	21.7	20.0	21.8	18.2	19.1

TABLE 3

MEAN (±SEM) LATENCY TO MAKE A CHOICE BETWEEN EITHER AN ANESTHETIZED DAM (EXPERIMENT 4A) OR HOME BEDDING (EXPERIMENT 4B) AND CLEAN BEDDING BY PUPS GIVEN EITHER LITHIUM OR SALINE

		Dose (mg/kg, IP)				
		Saline	40	80	120	160
Dam (sec)	Mean	109.1	62.9	105.8	103.1	68.0
	(SEM)	21.5	19.8	21.6	18.0	21.9
Bedding (sec)	Mean	49.7	48.8	116.4	82.0	74.1
	(SEM)	20.8	11.3	17.5	16.4	18.5

side, a pup that was motorically impaired would have spent less time on any side. Therefore, an additional measure, 'latency to make a choice,' can be extrapolated from the choice test data [total time of test (150 sec) − time spent on side = latency to choice]. The 4 animals that went to both sides were excluded from the analysis because their latency to make a choice could not be extrapolated. It might be assumed, however, that the fact that these pups went to both sides does indicate a lack of motor impairment. As can be seen in Table 3, lithium did not significantly increase the pups' latency to find the dam [$F(4,34)=1.18$, $p=0.34$; linear trend analysis, $F(4,34)=0.339$, $p=0.56$]. A marginally significant effect of lithium on latency to locate the bedding from the nest was observed [$F(4,32)=2.62$, $p=0.053$; linear trend analysis, quadratic term, $F(4,32)=3.65$, $p=0.065$]. As can be noted in Table 3, however, this effect is due largely to an unpredicted increase in latency in the 80 mg/kg group (by the Student-Newman-Keuls procedure, the 80 mg/kg group is significantly different from the saline group at $p<0.05$). The high doses produced no significant effect. Therefore, by using 'latency to make a choice' as a third measure of locomotor behavior, we have again shown that lithium does not significantly interfere with gross motor behavior at doses which disrupt suckling.

GENERAL DISCUSSION

These results demonstrate that lithium carbonate, when given acutely to neonatal rats, significantly decreases both nipple attachment and milk ingestion. Furthermore, these tests indicate that this interference is not a direct result of either a gross motor impairment or anosmia. We suggest, therefore, three possible explanations for the mechanism by which lithium suppresses suckling. First, these results may be due to very subtle motor impairment, particularly in the facial movements used to grasp and suck the teat. Although unquantified observations suggest that after reaching the dam all pups responded to and approached the nipple in a similar manner (sweeping, circling, and tugging at the teat), it is possible that lithium may have disrupted the specific motor sequence necessary to finally grasp and suck the teat effectively. Similarly, suppression of facial movements could interfere with the pups' ability to withdraw milk available in the teat.

A second possibility is that the pups may have been suffering gastrointestinal distress as a result of the lithium administration. This appears to be a likely cause for the clinical observations of a decreased suck response. During pregnancy the maternal glomerular filtration rate (GFR) is greatly increased necessitating a larger lithium intake to maintain a therapeutic blood level (9). The maternal GFR returns to normal, however, shortly before parturition. This drop requires another dose adjustment to prevent toxicity. In the reports cited (15,16), the drop in maternal GFR was not anticipated, resulting in both maternal and infant intoxication. Signs of acute lithium toxicity reported in the infants included crying, cyanosis, decreased muscle tone, and an enlarged liver (15,16). Therefore, in light of the possible GI distress experienced by these infants, we suggest that the decreased suck response may reflect a combination of lithium intoxication and anorexia.

Studies of the ontogeny of amphetamine-induced anorexia in rats found marked weight loss and decreased attachment only after Day 15 (11). Amphetamine-induced anorexia was observed in pups as young as 5 days, however, when ingestion was observed independent of suckling. This suggests that the behavior of suckling might be sufficiently strong so as to mask the anorexia (11). Other mechanical and pharmacological manipulations known to impair adult ingestive behaviors have little effect on suckling in neonatal rats, again highlighting the robustness of the suckling response [reviewed in (2)]. Acute motor or motivational effects that directly affect *performance* within the suckling context, however, could interfere with nipple attachment and milk withdrawl.

Finally, one agent, the serotonergic antagonist metergoline, has been shown to inhibit suckling among infant rats in much the same manner as reported here. Previous reports suggest that the administration of metergoline to 3-to 8-day-old rat pups significantly interfered with attachment to an anesthetized dam and other suckling-related behaviors (12). The similarity of the results reported here suggests that lithium action may also be mediated by serotonin. We are currently exploring this possibility in more detail.

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