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Operant Responding Controlled by Milk or Milk Contaminated With Alcohol as Positive Reinforcers in Infant Rats

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DOMINGUEZ, H. D., G. BOCCO, M. G. CHOTRO, N. E. SPEAR AND J. C. MOLINA. *Operant responding controlled by milk or milk contaminated with alcohol as positive reinforcers in infant rats.* PHARMACOL BIOCHEM BEHAV **44**(2) 403–409, 1993.—Infant rats during the first, second, or third week of life were tested in operant conditioning with uncontaminated milk or milk supplemented with 6.0% v/v absolute ethanol (EtOH) as the reinforcer. Relative to yoked controls, pups of each age group reinforced on a response-contingent basis exhibited a significantly higher rate of responding with either reinforcer. In terms of amount of reinforcement, milk induced a higher rate of lever pressing than did the EtOH-contaminated compound. Age-related differences in the onset of differential responding for plain milk and EtOH-contaminated milk suggested developmental changes in the effects of alcohol. In a second experiment, forced drinking of milk and EtOH-contaminated milk was compared in similar age groups. Patterns of intake resembled the patterns of operant responding controlled by the same substance in the first experiment. These experiments indicate that the presence of alcohol in milk partially inhibits the reinforcing capacity of uncontaminated milk. Nevertheless, the former compound is still effective as a positive reinforcer during the first weeks of life.

Ethanol Operant Infant learning Rat Reinforcer Developmental
Alcohol Self-administration Milk

DEVELOPING humans are liable to be exposed to alcohol through maternal consumption of the drug during pregnancy (1,11), suckling of maternal milk contaminated with ethanol (1,3) or through forced drinking of alcoholic beverages (6, 28,34). While ethanol's (EtOH) teratogenic effects have been extensively investigated during the last 16 years (1,17), relatively few studies have dealt with early ontogenetic experiences that might regulate subsequent recognition, discrimination, and acceptance of this pharmacological agent.

Altricial mammals have been observed to exhibit drastic changes in EtOH preference patterns as a function of learned and unlearned experiences with alcohol prior to weaning (10, 27). Processes related to olfactory learning involving alcohol (23,26,27,31), perception of alcohol's orosensory cues during acute intoxication (5,21,22), aversive properties of the drug (15,26), CNS sensitivity to EtOH (13,14), and patterns of metabolism (18) appear to represent early ontogenetic factors that later modulate EtOH preference.

Although human neonates can be exposed to EtOH in maternal milk, little is known about the infant's acceptance of this solution of EtOH and milk during ingestion and, especially, about the consequences of this ingestion for ongoing behavior. When the infant consumes EtOH in its milk, will its behavior in attaining it be reinforced negatively or positively, and does this depend upon level of development of brain and sensory (taste) receptors, that is, postnatal age?

The present study was designed to assess operant conditioning in infant rats with milk alone or milk contaminated with alcohol as positive reinforcers. The first experiment adapted an operant conditioning paradigm originally described by Johanson and Hall (16) for newborn rats. Reinforcement-contingent lever pressing was examined in rat pups during postnatal days 3–4, 9–10, and 15–16. A second experiment dealt with similar age groups exposed to a forced-drinking procedure, defined by intraoral infusion of milk laced with EtOH or milk alone.

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EXPERIMENT 1

First-order Pavlovian associations have been demonstrated during pre- and perinatal stages of development in the rat (30,32,33,35). Further, milk as well as sweetened solutions have been shown to act as positive reinforcers for operant learning and as an appetitive unconditioned stimulus for Pavlovian conditioning early in ontogeny (16,21). When milk is employed as a reinforcer, newborn rats are able to modify lever-pressing rate to receive such nutrient and can even acquire a discrimination on this basis (16).

In the present experiment, infant animals during the first (3–4 days old), second (9–10 days old), and third (15–16 days old) postnatal weeks of life were tested for their capacity to modify operant behavior when milk or a mixture of EtOH and milk served as reinforcers.

METHOD

Subjects

Eighty-four preweanling Wistar-derived rats born and reared at the Institute Ferreyra were tested. Pups were representative of 38 litters originally composed of 8–10 pups. At birth (postnatal day 1), pups were housed with their biologic parents in standard opaque maternity cages partially filled with wood shavings and equipped with automatic water dispenser valves. Animals had free access to rat chow (ADIABIC) and were maintained on a 14 L : 10 D cycle (light onset 0700 h). Temperature in the colony room ranged between 22 and 25°C.

Design, Apparatus, and Procedures

The present experiment was based on a 3 × 2 × 2 factorial design. The factors were age (3–4, 9–10, or 15–16 days), type of reinforcer [milk (M) or milk contaminated with EtOH (M + E)], and contingency between behavior and the reinforcer [operant behavior paired with reinforcer delivery (P) or yoked control (Y)]. The number of litters represented in each age group was as follows: 3–4 days, 14; 9–10 days, 11; and 15–16 days, 13. The number of animals randomly assigned to paired and yoked treatments for each age group and type of reinforcer was as follows: 3-4/M, 6; 3-4/M + E, 8; 9-10/M, 7; 9-10/M + E, 7; 15-16/M, 7; and 15-16/M + E, 7. The sole constraint to random selection in the distribution of pups was that from any litter no more than two animals were assigned to the different groups defined by age, reinforcer, and contingency treatment.

Twelve hours prior to conditioning, pups were deprived of maternal care, water, and solid chow. Immediately after commencement of deprivation, an oral cannula was implanted into each pup's mouth, following previously described procedures (12,21). Briefly, these devices were constructed from 5-cm sections of polyethylene (PE) tubing (Clay Adams, PE 10). A small flange (external diameter 1.2 mm) was formed in one end of the cannula by applying heat. The nonflanged end was inserted into a tungsten pin. The pin was then pulled through the medial internal surface of the right cheek. The flanged end of the cannula remained in the mouth while the remainder of this device exited from the oral cavity. Following this procedure, pups were individual weighed (±0.01 g). During the entire period of deprivation (12 h), pups were kept in standard opaque maternity cages partially filled with wood shavings and maintained at a temperature of 30–32°C through the use of heating pads.

Following deprivation, individual body weights were again recorded. Paired and yoked animals were then placed in individual opaque plastic containers. These devices varied in size in accordance with the age of animals (3–4 days, internal base diameter = 4 cm, volume capacity = 125 cc; 9–10 days, 5.5 cm, 250 cc; 15–16 days, 9.0 cm, 750 cc). A cotton-covered plastic paddle, extended into each container, was placed at different distances from the floor of the cup. For the youngest age group, this paddle was placed 1.5 cm from the floor. An upward force on the paddle was required to close a microswitch. For the 9- to 10- and 15-days groups, the distance between the paddle and floor was 1.2 cm. For these two age groups, a downward force was required to close the microswitch. Distance and directionality of the force were determined through pilot experiments. In these pilot experiments, it was observed that paddle pressing was easier to achieve through an upward probing response for youngest animals but through a downward force for older pups. Paddles were individually attached to microswitches (Omron SSL, Model 08L146, 5 A 125 V/3 A 250 V, AC) that in turn activated temporal programming and cumulative frequency recording equipment (Perosio Instruments). This instrument was connected to a peristaltic infusion pump (Manostat Cassette Pump, Standard Model). PE tubing (Clay Adams PE 50) that exited from this pump was attached to the oral cannulae of infants.

Paddle pressing performed by a paired subject activated the entire circuit and hence a given reinforcer was administered simultaneously into the oral cavities of both the paired rat and the corresponding yoked control rat. Therefore, for yoked animals there was no explicit contingency between lever pressing and reinforcer delivery. Whenever an experimental pup (P) pressed the paddle, however, the infusion pump was activated during 3 consecutive s. During this reinforcement period of 3 s, each of the paired and yoked pups received a volume of a specific reinforcer equivalent to 0.022% of its preconditioning body weight. This value was determined after analyzing pilot data indicating consistent paddle pressing for all ages during a 12-h test.

Two alternative reinforcers were employed. For approximately 50% of the subjects, milk (San Regim, SanCor, 1.5% fat content, with supplement of vitamins A and D) served as the positive reinforcer. For the remaining infants, this nutrient was contaminated with 190 proof ethanol (6% v/v purchased from Porta Co.). These reinforcers were kept in glass beakers covered with Parafilm paper.

Each conditioning session was performed during 12 consecutive h (0800–2000 h). As in the original study performed by Johanson and Hall (16), there was no attempt to shape paddle responding in any of the pups. Lever pressing was recorded every 2 h. At similar intervals of time, the oral cannulae as well as the tubing exiting from the infusion pump were examined to ensure adequate flow of the reinforcers. Temperature in the plastic containers was kept throughout the entire session at 28–30°C. These values were achieved through the use of a 75-W electric bulb placed above the containers and heating pads placed below. After termination of the conditioning session, body weights were again recorded.

RESULTS AND DISCUSSION

Relative body weight loss during the schedule of deprivation prior to conditioning sessions was found not to vary as a function of age. Percent body weight loss for the different age groups was as follows: 3–4 days, 3.94 ± 0.46; 9–10 days, 4.02 ± 0.35; and 15–16 days, 4.06 ± 0.38 (mean ± SE).

During conditioning sessions, paired pups were observed to be more active than corresponding yoked controls. Initially and independently of age and reinforcer, the number of lever presses for yoked and paired animals was similar. Four to 6 h after commencement of the session, rate of responding began to differ between contingency treatments. Direct observation of animals indicated that at this point yoked pups began to exhibit marked decrements in overall body movements. On the contrary, and until the end of the session, paired pups exhibited bursts of motor activity that correlated with increased paddle pressing.

Responding for the different groups is shown in Fig. 1 in terms of cumulative responses. As can be observed, the number of responses achieved by the end of the session was always higher for paired pups than for corresponding yoked controls. Also, during the last hours of the sessions paired animals reinforced with milk tended to exhibit higher response rates than when reinforced with milk plus EtOH. These observations were confirmed by a 3 × 2 × 2 × 6 mixed analysis of variance (ANOVA) and subsequent posthoc comparisons (Fisher's tests with a probability of type I error set at 0.05). The ANOVA took into account the following between-group factors: age (3–4, 9–10, and 15–16 days), reinforcer (M or M + E), and contingency [paired (P) or yoked (Y)]. The within-group factor was time of measurement (2, 4, 6, 8, 10, and 12 h). As could be expected due to the cumulative nature of the recording, the session interval factor was highly significant, $F(5, 180) = 157.27$, $p < 0.001$. Age and contingency were also found to significantly affect cumulative responses, $F(2, 36) = 6.05$, $p < 0.005$, and $F(1, 36) = 34.47$, $p < 0.001$, respectively. The interactions between age and interval as well as between contingency and interval also exerted significant effects, $F(10, 180) = 7.39$, $p < 0.001$, and $F(5, 180) = 30.20$, $p < 0.001$, respectively. A borderline significant interaction was observed between reinforcer and interval, $F(5, 180) = 2.03$, $0.10 > p > 0.05$.

Posthoc comparisons indicated that at all ages after 6 h of reinforcement paired subjects given milk as the reinforcer exhibited significantly higher responses than their yoked controls. This difference also achieved significance at the 4-h interval for 9- to 10- and 15- to 16-day old infants. Animals reinforced with milk contaminated with EtOH were also found to exhibit significantly higher responses when compared to their yoked controls. Among pups 3–4 and 15–16 days of age given this substance as the reinforcer, those for whom it was response contingent responded more than yoked controls at the 6-, 8-, 10-, and 12-h intervals. Rats 9–10 days old exhibited similar differences but significance was achieved only at the 10- and 12-h recordings. This last group of animals, especially when considering paired treatments, was found to perform significantly fewer lever responses than did the younger and older groups. This age-related differences was observed with both reinforcers. In general, 15- to 16-day-old pups emitted higher response rates than those 3–4 and 9–10 day olds. It is difficult to interpret the absolute differences in response rates, however, in view of the difference in the response requirements for the youngest and older animals.

When comparing the performance attained with the different reinforcers, posthoc comparisons indicated that at all ages, and especially during the last two interval recordings, paired subjects infused with milk emitted more cumulative responses than did paired animals reinforced with milk plus EtOH. Equally clear at all ages, the solution of milk and EtOH had a significant positive reinforcement effect: At each age, responding was greater for rats given this substance on a response-contingent basis than for yoked controls. In each age group, yoked controls were found not to significantly differ as a function of the reinforcer to which they were exposed, indicating no effect of the EtOH contamination of milk on rate of nonreinforced responding.

Cumulative responding was also analyzed for each age group separately as a function of contingency, reinforcer, and interval of time. The corresponding ANOVAs confirmed the results previously stated. Nevertheless, it is interesting to observe that subsequent posthoc comparisons revealed an apparent age-related difference in the performance of paired pups receiving milk in comparison to paired subjects reinforced with milk plus EtOH. At 3–4 days during the first 2 h, response-contingent pups reinforced with milk alone responded significantly more frequently than those reinforced with milk plus EtOH. This initial difference persisted throughout the entire conditioning session. In the 9- to 10-day-old group, this

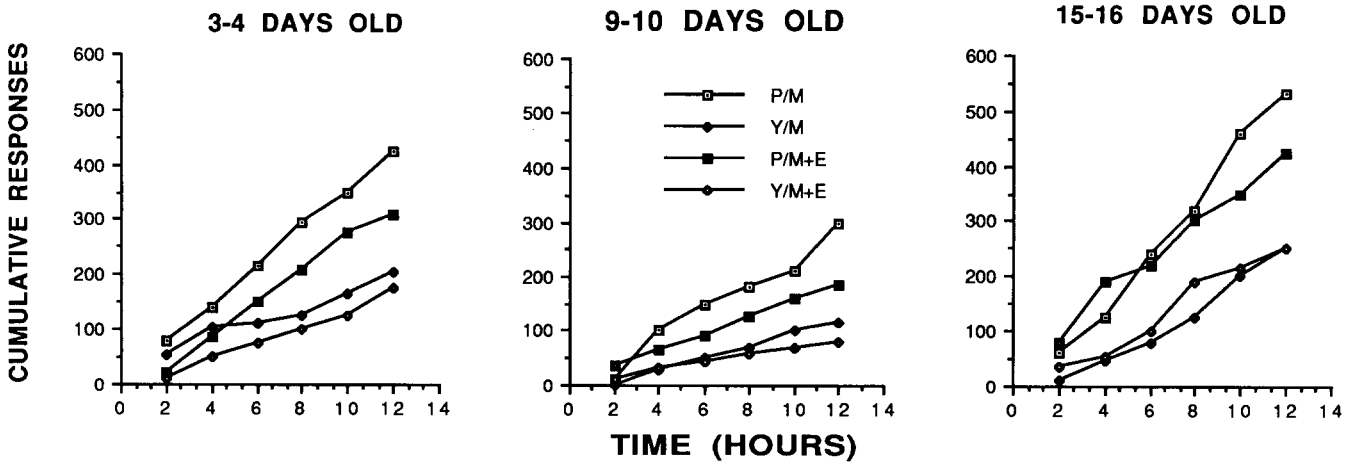

FIG. 1. Cumulative number of operant responses into the paddles as a function of age (3–4, 9–10, or 15–16 days), reinforcer [uncontaminated milk (M) or milk contaminated with EtOH (M + E)], contingency treatment [paired (P) or yoked (Y)], and recording interval (2, 4, 6, 8, 10, or 12 h).

difference did not attain significance until 6 h into the test, while in the oldest subjects (15–16 days) this significance was attained only in the last two recording intervals. The older the organism, the less the difference between the reinforcing effects of milk and milk + EtOH early in conditioning and the more experience required with the solution before a difference emerged.

Statistical tests (four-way mixed ANOVA followed by Fisher's tests, $p < 0.05$) in which absolute number of lever presses per interval of time (not cumulative) served as a dependent variable confirmed this age-dependent effect. While the two oldest groups of paired pups given milk + EtOH as their reinforcer responded more frequently during the first 2 h than those given milk (although not a statistically significant difference), 3- to 4-day-old pups exhibited an opposite and significant difference. In other words, the youngest pups responded less frequently at the beginning of the test when reinforced with milk + EtOH than when reinforced with milk alone. For the 9- to 10-day-old subjects, this difference emerged as significant at the 4-h interval, and for the 15- to 16-day old group it attained significance 6 h after commencement of the test. The statistical values attained in the corresponding ANOVA for the following factors and interactions were as follows: age, $F(2, 36) = 8.69$, $p < 0.001$; contingency, $F(1, 36) = 39.47$, $p < 0.001$; interval, $F(5, 180) = 2.37$, $p < 0.05$; and age × reinforcer × interval, $F(5, 180) = 1.84$, $p = 0.06$.

Percent body weight change (% BWC) between preconditioning and postconditioning is listed for each group in Table 1. As could be expected, body weight changes of paired and yoked animals were positively and significantly correlated [Pearson's overall correlation coefficient: $r(40) = 0.65$, $p < 0.01$]. A three-way ANOVA (age × reinforcer × contingency) was employed to further analyze these scores. Only age was found to significantly affect % BWC. As can be observed in Table 1, increases in body weight resulting from reinforcement were inversely related to age. The 15- to 16-day-old age group gained significantly less weight, proportional to their original weight, than did the youngest pups (3–4 days). Nine-day-old rats were found to exhibit intermediate weight increases relative to those registered in younger and older groups.

TABLE 1

PERCENT BODY WEIGHT CHANGE (% BWC) BETWEEN POSTCONDITIONING AND PRECONDITIONING AND ABSOLUTE ETOH (g/kg) INTAKE DURING CONDITIONING

Groups	% BWC	EtOH (g/kg)
3-4 P/M	4.78 ± 2.24	–
3-4 Y/M	4.61 ± 1.88	–
3-4 P/M + E	2.92 ± 1.56	4.72 ± 0.84
3-4 Y/M + E	4.44 ± 2.07	–
9-10 P/M	1.13 ± 0.93	–
9-10 Y/M	2.13 ± 0.98	–
9-10 P/M + E	0.85 ± 0.64	2.44 ± 0.39
9-10 Y/M + E	0.69 ± 0.63	–
15-16 P/M	1.03 ± 0.66	–
15-16 Y/M	−0.10 ± 0.90	–
15-16 P/M + E	1.03 ± 1.02	6.17 ± 1.14
15-16 Y/M + E	0.76 ± 0.70	–

Values represent means ± SE. P, paired; Y, yoked; M, milk; M + E, milk contaminated with EtOH.

Absolute EtOH intake (g/kg) was also taken into consideration for pups that self-administered milk mixed with EtOH. This index was calculated when taking into consideration the total number of paddle responses performed by each subject, its preconditioning weight, the fact that each paddle press resulted in the infusion of 0.022% of the preconditioning weight, and the relative weight contribution of EtOH in the delivered reinforcer. As can be observed in Table 1, a nonmonotonic age function was attained in this ingestion index, which seems to be related to the number of lever presses performed by each age group. The oldest animals, which emitted the highest number of lever presses, were also found to exhibit the highest values of absolute EtOH intake. The intermediate age group (9–10 days), which emitted the least responding, exhibited the lowest intake of EtOH. These observations were confirmed by a one-way ANOVA, $F(2, 19) = 4.88$, $p < 0.025$.

Several conclusions may be derived from this set of results: a) It is clear that all age groups were capable of establishing associations between paddle responding and reinforcement; b) At all ages, milk was found to be highly reinforcing; c) milk plus EtOH appeared to be significantly less reinforcing than milk alone, although it is conceivable that EtOH-induced intoxication may have impaired paddle responding enough to contribute to this difference; d) of major importance to this study, however, milk plus EtOH was an effective reinforcer for instrumental learning; e) there were age-related differences in the point at which responding reinforced by milk alone diverged from responding reinforced by milk plus EtOH: The older the pup, the later it was in the conditioning session until milk contaminated with EtOH induced less responding than that observed with milk alone. This must be qualified in terms of the youngest pups (3–4 days), which emitted less responding for milk plus EtOH than for milk alone from almost the beginning regardless of contingency; also, relative to older pups, 3- to 4-day-old pups did not indicate a clear divergence of responding between paired and yoked-control groups until later in training. This age-dependent difference does not appear to correspond to age-related differences in neophobic responses to a novel flavor. Flavor neophobic responses seem to increase rather than decrease with age (20,36,37). It is difficult to interpret the initial low response rate exhibited by 3- to 4-day-old pups given milk plus EtOH or the earlier appearance of this deficit in learning with milk plus EtOH as the reinforcer in 9- to 10- relative to 15- to 16-day-old rats as due to an early reflexive neophobia not observable at older ages.

An interesting relationship can be found, however, between these age-related differences in response to milk plus EtOH and the development of the ability to absorb and metabolize EtOH. After receiving equivalent alcohol doses, 1- to 4-day-old subjects exhibit higher maximum blood alcohol concentrations than pups of 6–10 days, which in turn show higher blood levels than those of older ages (15–60 days) (18). It is conceivable that after initial exposure to the drug age differences were attained in terms of maximum blood and brain levels, as well as in the duration of exposure to EtOH. Such differences could in turn modulate alcohol self-administration.

In Experiment 1, it was observed that lever pressing also varied with age independent of the reinforcer. Nine- to 10-day-old pups responded significantly less than younger or older subjects. Although the present experimental design does not permit us to dismiss the possibility that age-related motor ca-

pacities were responsible for this, the next experiment indicated instead that age-related differences in response to the infusion of reinforcers were an important factor.

EXPERIMENT 2

The main purpose of this experiment was to compare intake of the same reinforcers by rats of the same ages as in Experiment 1 but unrestricted by the prerequisite of instrumental behavior. We applied a forced drinking paradigm developed to test the infant rat's learned and unlearned responses to gustatory stimuli (8,9,36,37). This experimental approach allowed us to a) further examine possible differences in the appetitiveness of the reinforcers and b) determine whether the age differences in operant response rate are related to age-related variation in acceptance of the infused substances.

METHOD

Subjects

One-hundred pups were sampled from 16 litters. Housing and rearing conditions for these animals replicated those described for Experiment 1.

Design, Apparatus, and Procedures

Pups were randomly assigned to one of six groups defined by age (3–4, 9–10, or 15–16 days) and nature of the substance infused [milk alone (M) or milk mixed with EtOH (M + E)]. The following numbers of infants were assigned randomly to each group, with the same constraints as in Experiment 1: 3-4/M, 14; 3-4/M + E, 13; 9-10/M, 20; 9-10/M + E, 14; 15-16/M, 19; and 15-16/M + E, 20.

As in experiment 1, all animals were orally cannulated 12 h before commencement of testing procedures. The test was performed by individually placing subjects in clear Plexiglass chambers (15 × 7 × 15 cm) fitted with stainless steel grid floors. Ambient temperature was kept at 30–32°C through the use of heating pads placed beneath the floor. The oral cannulae were attached to tubing exiting from a peristaltic infusion pump set to deliver 5.5% of the preinfusion weight of each subject during a 30-min interval. The infused solutions (milk or milk plus 6% v/v EtOH) were delivered using pulsating administration. Each pulse had a duration of 3 s (interval between pulses: 10 s). Prior to liquid infusion, bladders were voided and defecation was stimulated by stroking the anogenital region of infants with cotton swabs. Following this procedure, individual body weights were recorded, and then again 10, 20, and 30 min after starting the test. The amount of ingestion was expressed in terms of percentage body weight gain [100 × (postinfusion weight–preinfusion weight)/preinfusion weight].

Results and Discussion

The results are illustrated in Fig. 2. Independent of the infused solution, 9- to 10-day-old pups revealed less relative increase in body weights than younger or older pups. There was also a tendency, in all age groups, for body weight increases to be slightly smaller when infused with milk plus EtOH than when infused with milk alone. A three-way ANOVA (age × substance infused × recording interval) confirmed these impressions. This analysis indicated significant main effect of age and interval, as well as a borderline effect attributable to the substance infused, $F(2, 84) = 6.86$, $p < 0.005$; $F(2, 180) = 450.34$, $p < 0.001$; and $F(1, 94) = 2.91$, $0.10 > p > 0.05$, respectively.

The forced consumption profile resulting from this test closely resembles the profile of responding in Experiment 1, in which equivalent substances served as positive reinforcers for instrumental learning. The intermediate age group (9–10 days) not only made fewer instrumental responses but also consumed less of both substances in this forced drinking procedure relative to younger or older pups. With both dependent variables (lever pressing and forced intake), higher scores were registered with milk alone than with milk plus EtOH as the ingested nutrient.

GENERAL DISCUSSION

Appetitive instrumental learning was found to occur in infant rats during the first, second, and third postnatal weeks of life. As previously reported (16), milk served as a reinforcer in maternally and nutritionally deprived rat pups. Milk contaminated with alcohol was also found to act as a positive

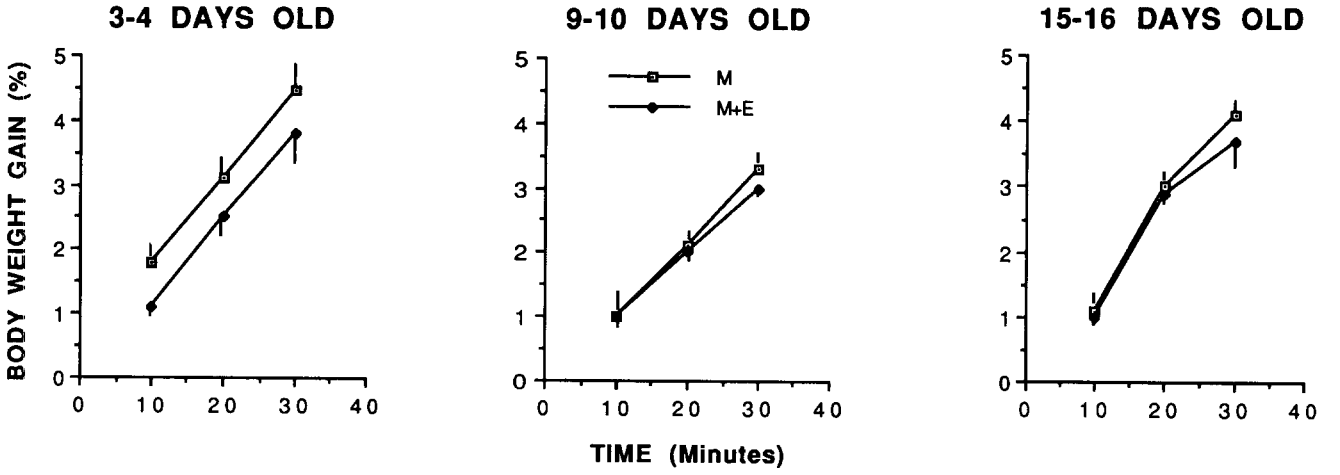

FIG. 2. Percent body weight gain in a forced intake test as a function of age (3–4, 9–10, 15–16 days), substance infused [uncontaminated milk (M) or milk contamined with EtOH (M + E)], and recording interval (10, 20, or 30 min). Vertical bars represent SEM.

reinforcer, although slightly less effectively than uncontaminated milk (Experiment 1). Age-related differences were observed in terms of the relative effectiveness of the two reinforcers. The younger the pup, the sooner during training a significant difference emerged between responding to milk plus EtOH and responding for milk alone. In other words, the older the pup, the more experience was required (with either the operant contingencies or the milk-EtOH solution) to differentiate the effects of the alternative reinforcers. This effect appears not to be attributable to ontogenetic differences in neophobic responses. Prior studies suggest an opposite ontogenetic course for flavor neophobic responses, that is, reflexive neophobia appears to increase rather than decrease with age (20,36,37). Also, the hypothesis concerning differences in neophobia does not appear to receive experimental support from the results of Experiment 2. During the initial exposure to the substances of the forced intake test (Experiment 2; see Fig. 2, 10-min recording interval), there is no support for an effect mediated by ontogenetic change in neophobic responses. The age-related differences in self-administration of milk plus EtOH vs. milk alone might, however, be a function of ontogenetic change in the pharmacokinetics of alcohol. Enzymatic immaturity might yield a higher accumulation and duration of alcohol in blood and brain, and hence higher intoxication, in younger animals than in older ones (18).

In the two oldest age groups (9–10 and 15–16 days), it was observed that, relative to similar behavior reinforced with uncontaminated milk, lever pressing reinforced by milk plus EtOH progressively decreased beginning about 6 h into the training session. Progressive divergence in responding might indicate either (or both) motor impairment induced by alcohol or learning the aversive consequences of EtOH as an interoceptive unconditioned stimulus. Motor impairment has been described in tilting-plane and open-field tests after preweanling rats were exposed to 1.25 and 2.0 g/kg EtOH dose, respectively (13,14,19). Such doses were attainable in Experiment 1 of the present study. At the end of the testing session, all paired pups subjected to milk supplemented with EtOH had self-administered more than 2.0 g EtOH/kg body weight. Decrements in responding for milk plus EtOH might also be attributable to an acquired association between the flavor of the contaminated solution and EtOH's postabsorptive effects. This possibility is supported by abundant evidence for acquisition of conditioned aversion to a flavor paired with EtOH (4,7,29), including tests with infant rats (21,26). In addition, perception of the conditioned flavor seems not to be strongly disrupted under a state of ethanol intoxication (25,26).

An unexpected result was the nonmonotonic variation in response rate as a function of age. Nine- to ten-day-old pups responded less frequently than younger or older subjects. This pattern was independent of the type of reinforcer employed (Experiment 1). As described in the Method section of Experiment 1, preliminary experiments led to a procedure in which upward lever pressing was reinforced in 3- to 4-day-old pups but downward responses were reinforced in older subjects. Perhaps differences in the motor capacities and posture of 9- to 10- and 15- to 16-day-old pups, or differences in their vision, were responsible for this effect (2,24). Although plausible, the results of Experiment 2 indicate that the age-related differences in response rate were related instead to acceptance of intraoral infusions. In this experiment, in which the reinforcers were infused independently of behavior, the age-related nonmonotonic function was still observed. Forced consumption in 9- to 10-day-old infants was significantly lower than that observed in younger and older pups. Moreover, the difference in control of operant responding by plain milk and EtOH-contaminated milk (Experiment 1) was manifested in the differential intake of these substances in the forced drinking test (Experiment 2). It therefore appears that infant operant behavior reflects acceptance patterns of the response-contingent reinforcers.

There seems no remaining doubt that milk containing a significant amount of alcohol is capable of reinforcing behavior in physiologically immature organisms. It is likely, moreover, that EtOH is readily detected in the milk-EtOH compound by the infant. In recent experiments, we observed that 11-day-old pups demonstrate the capacity of selectively responding to alcohol when this agent is presented in a flavor configuration (Dominguez, Bocco, Chotro, and Molina, unpublished observations). Further, infants are capable of detecting alcohol simultaneously presented with oral delivery of nonethanol-sweetened solutions (21). Under these experimental circumstances, the simultaneous association between alcohol and a sweet taste was sufficient to promote subsequent increases in alcohol intake and EtOH odor preferences. There is also evidence suggesting that associations may develop in the infant between the simultaneously presented elements of a compound [sucrose and alcohol; the presence of sucrose increases subsequent alcohol preference while EtOH's interoceptive effects decrease subsequent sucrose preference (15)]. Further research must clarify whether the configuration of milk and alcohol yields similar changes in subsequent acceptance of each separate element, a significant issue related to the more general question of possible reinforcement and behavioral consequences resulting from human neonatal exposure to EtOH when present in maternal milk.

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