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Facial Wiping in the Rat Fetus: Variation of Chemosensory Stimulus Parameters

ABSTRACT: *Fetal rats reliably express a facial wiping response to novel chemosensory stimuli. Previous research has examined facial wiping as an early form of motor coordination and as a behavioral indicator of sensory responsiveness. The present study examined how variation in stimulus parameters of lemon odor infusion (concentration, volume, and infusion time) affected the wiping response of E20 rat fetuses. Infusions of higher concentration or greater volume generally elicited wiping responses of greater duration and more strokes. Most facial wipes involved strokes by single forelimbs; however, bilaterally synchronous wiping was expressed only in bouts of at least seven wipes, and was facilitated by stimuli of moderate intensity. These findings suggest that the total number of wiping strokes or bout duration are well suited as measures of overall sensory responsiveness in the fetus and that chemosensory stimulus parameters exert a permissive influence on interlimb coordination during a bout of facial wiping. © 2004 Wiley Periodicals, Inc.* Dev Psychobiol 44: 219–229, 2004.

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Facial wiping is a simple action pattern that can be observed in fetal and neonatal rats and other perinatal rodents (Robinson & Smotherman, 1992; Smotherman & Robinson, 1987). Wiping consists of coordinated movement of the forepaws as they alternately are placed in contact with the lateral surface of the head, generally behind the ears, and then pulled downward toward the nose, with contact sliding over the eyes and the sides of the face (Robinson & Smotherman, 1991). Spontaneous facial wiping occurs occasionally in fetal rats observed within or externalized from the amniotic sac on the last 2 days of gestation (E20 & E21; conception = E0), but can be reliably elicited by experimental presentation of novel chemosensory fluids or punctate cutaneous stimuli applied to the perioral region. Although both chemical and tactile stimuli are effective in eliciting wiping responses, the organization of the wiping response is affected by the method of stimulation. Intraoral infusion of chemosensory fluids with strong olfactory components, such as lemon extract, consistently elicits a coordinated, bilateral wiping response involving a series of limb strokes by the left and right forelimbs (Robinson & Smotherman, 1991). In contrast, application of a stiff bristle (von Frey filament) to the lateral vibrissal pad evokes a single unilateral stroke by the forepaw on the stimulated side of the face (Smotherman & Robinson, 1992a).

Facial wiping has proven to be interesting and useful as a subject of research in developmental psychobiology for various reasons. Facial wiping exhibits a striking ontogenetic pattern, appearing during the last few days of gestation but disappearing several days after birth in a manner analogous to the neonatal stepping response of human infants (Thelen, Fisher, & Ridley-Johnson, 1984). Experimental manipulations of pup posture and testing conditions have revealed that this ontogenetic pattern is the result of interference by other patterns of behavior that compete for expression (e.g., righting) and the influence of environmental context (e.g., contact with a substrate and gravitational loading of the limbs) (Golani & Fentress, 1985; Robinson & Smotherman, 1992;

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Smotherman & Robinson, 1989). Facial wiping also provides a striking example of coordinated motor behavior that develops before birth, but is clearly related to functional patterns of postnatal behavior such as grooming and aversion responses (Berridge, 1994; Johanson & Shapiro, 1986; Richmond & Sachs, 1980). Detailed video analysis of wiping sequences has demonstrated that interlimb coordination is not apparent in the initial limb strokes of a bout, but emerges gradually as a succession of limb strokes is performed (Robinson & Smotherman, 1991). These and other studies of fetal facial wiping have contributed to a body of evidence demonstrating that early motor behavior is not just reflexive nor divorced from sensory experience (Robinson & Kleven, in press; Robinson & Smotherman, 1994; Smotherman & Robinson, 1989).

Because facial wiping can be consistently elicited by tactile or chemosensory stimuli, wiping responses also have been used as an indicator of fetal sensory responsiveness, and of changes in sensory responsiveness following exposure to other stimuli or pharmacological agents (Robinson, Arnold, Spear, & Smotherman, 1993; Smotherman & Robinson, 1992b). For instance, fetal or neonatal rats that are experimentally exposed to milk (Smotherman & Robinson, 1992a) or amniotic fluid (Korthank & Robinson, 1998) experience a brief period of elevated activity in the endogenous opioid system. The effects of opioid activity can be conveniently and reliably measured by recording a reduced tendency for fetal subjects to express wiping responses to a perioral tactile stimulus, or a reduced number of wiping strokes in response to intraoral lemon infusion. Because pretreatment with opioid antagonists, such as naloxone, reinstate high levels of facial wiping, changes in the probability or frequency of wiping responses have been concluded to be opioid mediated and most likely the result of changes in sensory thresholds.

Although punctate cutaneous stimulation applied to the face of the rat fetus can yield a discrete measure of the presence or absence of fetal response, variation in the number of wiping strokes elicited by chemosensory stimulation may provide a more sensitive, continuous measure of fetal responsiveness. Because a bout of facial wiping can vary not only in the number of limb strokes but also in the degree of coordination between the limbs, chemosensory-induced facial wiping offers the potential to study motor as well as sensory aspects of the wiping response. In adult rats, motor components of taste-elicited behavior have been quantified in taste-reactivity tests and are known to depend on characteristics of the taste solutions. Whereas differences in the number and sequencing of mouth and tongue movements are common during taste reactions to oral infusions of sucrose, NaCl, or HCl solutions, adult rats show organized body responses such as head shaking, face washing, and forelimb flails to infusions of bitter tastants such as quinine (Grill & Norgren, 1978a). Thorough understanding of how stimulus characteristics affect the response of the animal has facilitated investigations of the control mechanisms of such highly stereotyped action sequences (Berridge & Fentress, 1985) and have contributed to our understanding of neurological impairment (Grill & Norgren, 1978b). Though motor components of the facial wiping response in the rat fetus have been described in considerable detail (Robinson & Smotherman, 1991), possible relationships between stimulus properties and response characteristics have not been examined.

The chemosensory stimulus most commonly used in previous studies of facial wiping is a fluid mixture of lemon extract in isotonic saline (Korthank & Robinson, 1998; Smotherman & Robinson, 1987). Fetal rats also show facial wiping responses to intraoral infusions of citral or cyclohexanone in liquid or gas phase (Smotherman & Robinson, 1990a), but do not respond to isotonic saline, sucrose, lactose, milk, or amniotic fluid, suggesting that the fetal rat is responsive to novel olfactory characteristics of the solution (Smotherman & Robinson, 1990b, 1992b). However, it is not known how chemosensory stimulus qualities may be related to the number or organization of forepaw strokes expressed during a wiping response.

Considering the utility of the chemosensory-evoked facial wiping response in the study of sensory capacities and behavioral organization in the fetal rat, a better understanding of how chemosensory stimulus parameters influence this response is critical to studies that propose to modify fetal sensory thresholds or measure motor coordination. The aim of the present study was to evaluate the effects of particular chemosensory stimulus parameters on the form of the wiping response and the expression of motor coordination by the E20 rat fetus. Specifically, we investigated how variation in the concentration, volume, or rate or duration of infusion affected the number and form of facial wiping strokes and the likelihood of fetal rats to express unilateral or bilaterally coordinated wiping responses. Parametric manipulation of the eliciting chemosensory stimulus and behavioral analysis of the facial wiping response may reveal psychophysical dependencies of this response on stimulus properties, thus contributing to our understanding of sensorimotor integration and the expression of motor coordination in the mammalian fetus.

METHODS

Subjects

Subjects were fetuses of pregnant Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) that were time-mated in our laboratory. A total of 363 rat fetuses from 40 pregnancies were included as subjects. During the 4-day breeding period, an adult male and 3 adult female rats were housed in standard breeding cages (38 × 48 × 20 cm) maintained in the animal care facilities at the University of Iowa. Vaginal smears were taken daily to identify the day of conception. The first day that sperm were detected in a smear was designated as E0 (Birth occurs on E22.) After removal of the male, pregnant females remained housed in groups of 3 on a 12:12 hr light:dark photoperiod (lights on at 0700 hr). Food and water were available ad libitum. Care and use of experimental animals were in accordance with guidelines established by the NIH (National Institutes of Health, 1986) and were approved by the Institutional Animal Care and Use Committee at the University of Iowa.

Prenatal Preparation

Individual rat fetuses were tested on Day 20 of gestation. To allow for direct observation and manipulation of fetal subjects, the pregnant female rat was surgically prepared by a procedure standard in our laboratory for externalization of the uterus and fetuses (Smotherman & Robinson, 1991a). The female received a 100-μl injection of 100% ethanol into the spinal cord between the first and second lumbar vertebrae while under brief ether anesthesia. This procedure (chemomyelotomy) eliminates neural activity at the low thoracic level of the spinal cord and prevents sensation in the lower portion of the body. The pregnant female then was placed in a plastic holding apparatus and immersed to chest depth in a buffered isotonic saline solution maintained at body temperature (37.5°C). The uterus of the female was externalized through a midabdominal incision. The female was monitored throughout the experiment to ensure that the spinal blockade was complete.

A small incision was made in the uterus, and individual rat fetuses that were selected as subjects were delivered into the bath. Removal of fetal membranes (amnion and chorion) permitted direct observation of the fetus. Umbilical circulation and placental attachment to the uterus remained intact. Fetuses were monitored throughout the test session, and only fetuses that appeared well-oxygenated and pink in color served as subjects. Testing did not begin until a 20-min period had elapsed to allow the female and fetuses to acclimate to the bath environment.

Presentation of the Chemosensory Stimulus

Subject fetuses were fitted with an intraoral cannula made of PE-10 polyethylene tubing. One end of the cannula had a flanged tip that rested on the midline of the tongue in a midanterior position (Kehoe & Blass, 1985). The other end of the cannula was attached to an automated infusion pump that delivered the chemosensory fluid into the mouth of the fetus. Parameters of fluid infusion that were manipulated included concentration of fluid (1:2 or 1:1 dilution of Schilling brand pure lemon odor extract in isotonic saline, or 1:0 undiluted extract), volume of fluid (10, 20, or 50 μl), and infusion time (1, 2, 5, or 10-s pulse). The 1:2 lemon dilution previously has been shown to elicit facial wiping in fetal rats (Smotherman & Robinson, 1989).

Experimental Design

In the initial experimental design, 288 fetal subjects were assigned to 1 of 36 groups ($n = 8$) that resulted from the factorial combination of manipulated stimulus parameters. Multiple subjects were provided by each pregnancy. However, to avoid conflation of group effects and litter effects, no more than 1 subject per litter was assigned to a particular experimental group (Holson & Pearce, 1992), with the order of testing counterbalanced across pregnancies. Parameters of the lemon infusion that were varied included concentration (1:0, 1:1, or 1:2 dilution), volume (10, 20, or 50 μl), and infusion time (1, 2, 5, or 10-s pulse).

As a follow-up to this initial factorial design, 75 additional fetuses from 10 pregnancies were observed to provide data on variability in facial wiping responses. These fetal subjects were exposed to a reduced range of stimulus parameters resulting in lemon infusions of moderate intensity. The volume of lemon mixture and infusion time were held constant at 10 μl and 5 s, respectively. Three groups ($n = 25$) received infusion of a different concentration of lemon: 1:0, 1:1, or 1:2 dilution.

Behavioral Observations

Test sessions began with infusion of lemon mixture into the mouth of the fetal subject. Behavioral scoring commenced at the time of infusion, and the occurrence of facial wiping was recorded for the next 60 s. In this study, a forelimb movement was scored as a facial wipe only if the paw made contact with some portion of the face. A distinction was made between unilateral (single-paw) and bilateral (dual-paw) facial wipes. A typical wiping response consists of multiple movement cycles of each forelimb, with each cycle comprising a stroke phase in which the paw is moved from ear to nose in contact with the side of the face and a placing phase in which the paw is moved from nose to ear without contacting the face (Robinson & Smotherman, 1991). In the present study, unilateral wipes were scored when the stroke phase of one paw did not overlap with the stroke phase of the opposite paw, and bilateral wipes were scored when both paws were simultaneously in contact with the face during some portion of the wiping cycle. By this scoring convention, unilateral wipes occurred (a) when only one limb expressed wiping during the bout, (b) when wiping by one limb occurred as the opposite limb remained motionless, or (c) when both limbs were exhibiting wipes with an alternated pattern of coordination (i.e., the relative phase lag between limbs was approximately 25–50% of cycle duration). Conversely, bilateral wipes occurred only when the pattern of coordination between the limbs was nearly synchronized (i.e., relative phase lag was approximately 0–25%).

Each instance of unilateral or bilateral facial wiping was recorded by entering a category code into a computer with custom software (written by S.R.R.) that served as a real-time event recorder. This method of categorizing and recording fetal behavior provided information on the time of occurrence (±0.1 s) and category of the wiping movement, permitting calculation of the time interval between wipes. In previous fetal research in which behavioral sessions were recorded on videotape, repeated scoring of the same video segment resulted in interrater reliabilities of the occurrence of behavioral events in excess of .90 and intrarater reliabilities exceeding .95 (Robinson, Blumberg, Lane, & Kreber, 2000). Additional measures that were derived from scoring records included duration of the wiping bout (time between first and last wipe of bout), number of wiping events (the sum of unilateral and bilateral wipes), and percentage of total wiping strokes that were bilateral (calculated with unilateral wipes scored as one stroke and bilateral wipes as two strokes).

Data Analysis

The presence or absence of a facial wiping response during the test session was compiled for all subjects and analyzed by nonparametric chi-square test of independence. Additional chi-square analyses were carried out to compare the presence or absence of certain response characteristics (e.g., bilateral facial wipes and total wiping events). For subjects that showed one or more facial wipes, the effects of chemosensory stimulus parameters on fetal behavior were examined by comparing the number of single or unilateral wipes, the number of bilateral wipes, percent bilateral wiping strokes, and duration of wiping bout by analysis of variance (ANOVA). Post hoc comparisons between groups were conducted with the Fisher PLSD test. An alpha level of $p < .05$ was adopted to determine significance in all statistical tests.

In most analyses, behavioral measures were summed over the 60-s test session. However, additional analyses were conducted to investigate changes in interlimb coordination within a wiping bout. Robinson & Smotherman (1991) reported that the phase relationship between the forelimbs changes throughout the wiping bout, with the two limbs becoming synchronized during the middle of the wiping bout. Thus, the percentage of bilateral strokes was higher during the last two thirds of the wiping bout. To make a comparable assessment of interlimb coordination, we partitioned each wiping bout into three segments that contained equal numbers of wiping events. Segments of the wiping bout then were included as a factor in multi-way ANOVA.

RESULTS

Effects of Concentration, Volume, and Infusion Time on Facial Wiping Responses

Facial wiping was not expressed by all fetuses during the 60-s test session. Following lemon infusion, 250 of 288 fetuses (86.8%) showed a facial wiping response (Table 1). For a general summary of the overall pattern of responsiveness, a 2 (wipe vs. no wipe) $\times$ 36 (combinations of concentration, volume, and infusion time) chi-square test of independence indicated significant variation in the overall expression of facial wiping across conditions, $\chi^2(35, N = 288) = 85.01$, $p < .0001$. A 2 (wipe vs. no wipe) $\times$ 4 (1-, 2-, 5-, 10-s) chi-square test showed no significant variation in the pattern of responsiveness across the different infusion times ($p = .99$). However, two chi-square tests revealed significant differences in the occurrence of wiping responses at different concentrations and volumes, concentration: 2 (wipe vs. no wipe) $\times$ 3 (1:2, 1:1, 1:0), $\chi^2(2, N = 288) = 28.44$, $p < .0001$, and volume: $\chi^2(2, N = 288) = 22.98$, $p < .0001$.

Table 1. Number of Fetuses Expressing a Facial Wiping Response or Showing No Wiping Response (Wipe/No Wipe) to Lemon Infusions of Different Concentration and Volume

	Concentration		
Volume	1:2	1:1	1:0
10 μl	15/17	24/8	32/0
20 μl	25/7	29/3	32/0
50 μl	31/1	30/2	32/0

Note. Data are collapsed across four infusion times (1, 2, 5, or 10 s).

To further characterize differences among concentration and volume conditions, a series of simplified chi-square tests was conducted. A 2 (wipe vs. no wipe) $\times$ 3 (1:2, 1:1, 1:0 *or* 10, 20, 50 μl) chi-square test was conducted at each concentration and volume of lemon, collapsing across infusion times. Fetuses that received infusions of the 1:1 and 1:0 concentrations consistently showed a facial wiping response whereas fetuses that received infusions of the 1:2 concentration were more likely to express facial wiping at increased volumes, $\chi^2(2, N = 96) = 21.20$, $p = .0001$. Fetuses that received infusions of 10 or 20 μl of lemon did not consistently express facial wiping, but were more likely to show a facial wiping response at stronger concentrations of lemon, $\chi^2(2, N = 96) = 23.47$, $p = .0001$, and $\chi^2(2, N = 96) = 8.26$, $p = .02$, respectively. Fetuses that received infusions of 50 μl consistently expressed a wiping response. Thus, stimulus parameters affected the likelihood of the fetus to show a facial wiping response.

Characteristics of the Facial Wiping Response. The following analyses for describing characteristics of the facial wiping response and coordination within a bout of facial wiping are based on fetuses that expressed at least one facial wipe during the test ($n = 250$). Facial wiping responses were compared in a 3 $\times$ 3 $\times$ 4 (Concentration $\times$ Volume $\times$ Infusion Time) ANOVA. Comparisons for wiping-bout duration indicated a main effect of concentration, $F(2, 214) = 28.31$, $p < .0001$. Post hoc comparisons revealed that fetuses in the 1:0 concentration group expressed wiping bouts of significantly longer duration than fetuses in the 1:2 and 1:1 concentration groups (ps $< .0001$; Figure 1).

Comparisons of the number of unilateral wipes among experimental groups indicated main effects of concentration, $F(2, 214) = 3.98$, $p = .02$, and volume, $F(2,$

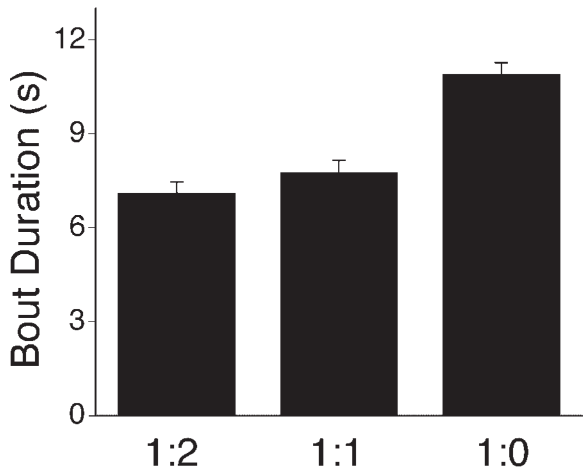

FIGURE 1 The effect of lemon concentration (1:2 = 1:2 dilution in saline, 1:1 = 1:1 dilution, 1:0 = pure lemon extract) on the duration of facial wiping bouts. Bars show mean duration; vertical lines depict *SEM*.

214) = 7.76, $p = .0006$. The 1:0 concentration of lemon elicited significantly more unilateral wipes than the 1:2 concentration, and the 50-μl volume infusion elicited significantly more unilateral wipes than the 10- or 20-μl volume infusions ($p < .05$). There also was a significant Concentration × Volume interaction, $F(4, 214) = 4.11$, $p = .003$ (Fig. 2, top). Post hoc comparisons revealed a similar pattern of response to different volumes at the 1:2 and 1:1 concentrations of lemon. Fetuses that received 10-μl infusions exhibited significantly fewer unilateral wipes than fetuses that received infusions of greater volumes at both the 1:2, $F(2, 68) = 6.02$, $p = .004$, and 1:1 concentrations, $F(2, 80) = 5.13$, $p = .008$. For fetuses in the 1:0 concentration group, different volume infusions did not produce significant differences in the number of unilateral wipes.

Comparisons of the number of bilateral wipes indicated main effects for concentration, $F(2, 214) = 12.91$, $p < .0001$, and for volume, $F(2, 214) = 4.34$, $p = .01$. The 1:0 concentration evoked significantly more bilateral wipes than the 1:2 or 1:1 concentrations, and the 20-μl volume elicited significantly more bilateral wipes than the 10- or 50-μl volume infusions ($p < .05$). Additionally, there was a significant interaction between the two factors, $F(4, 214) = 3.54$, $p = .008$ (Fig. 2, bottom). Post hoc comparisons revealed that at the 1:2 concentration, fetuses that received 20- or 50-μl volume infusions performed significantly more bilateral wipes than fetuses that received 10-μl infusions, $F(2, 68) = 3.88$, $p = .03$. At the 1:0 concentration, fetuses that received 50-μl volume infusions performed significantly fewer bilateral wipes than fetuses that received lesser volume infusions, $F(2, 93) = 4.70$, $p = .01$. There were no

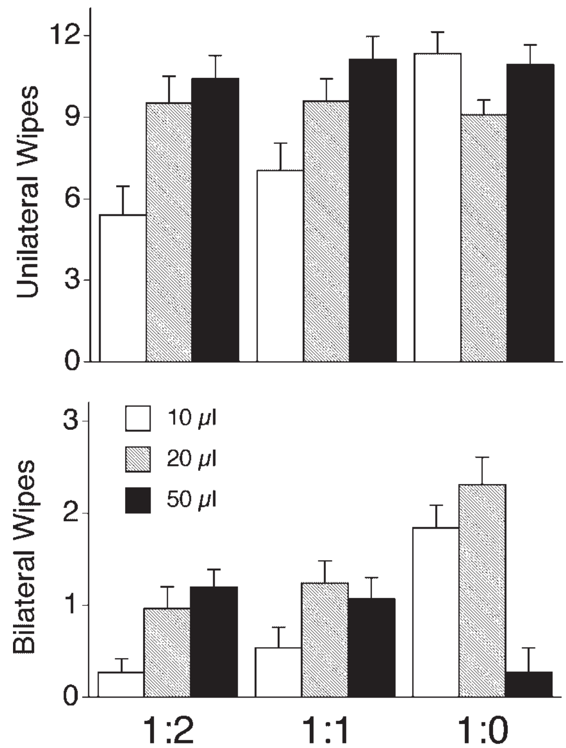

FIGURE 2 Effects of lemon concentration (1:2, 1:1, or 1:0 dilution) and infusion volume (10, 20, or 50 μl) on the number of unilateral facial wipes (top panel) or bilateral wipes (bottom). Bars show mean frequency of each category of wiping; vertical lines depict *SEM*.

differential volume effects at the 1:1 concentration of lemon.

The initial analysis comparing bilateral wipes also indicated a significant Concentration × Infusion Time interaction, $F(6, 214) = 2.32$, $p = .03$. There was a simple main effect of concentration, $F(2, 238) = 10.74$, $p < .0001$, with the highest concentration of lemon mixture (1:0) evoking significantly more bilateral wipes than the two diluted mixtures. However, there was no simple main effect for infusion time. Post hoc comparisons of means revealed that the 10-s infusion time elicited significantly more bilateral wipes when paired with the 1:0 concentration of lemon mixture, $F(2, 59) = 12.22$, $ps < .0001$. All other post hoc comparisons were not significant.

Coordination within a Bout of Facial Wiping. To determine if chemosensory stimulus parameters play a role in the expression of interlimb coordination during the facial wiping response of the rat fetus, we compared the percentage of bilateral strokes across bout segments and stimulus properties in a $3 \times 3 \times 3 \times 4$ (Segment × Concentration × Volume × Infusion Time) ANOVA. The analysis indicated a main effect for bout segment, F(2,

622) = 12.02, $p < .0001$. Fetuses expressed significantly higher rates of bilateral strokes during the second and third segments of the bout than during the first segment (Fig. 3).

The overall analysis also indicated main effects of concentration, $F(2, 622) = 10.23$, $p < .0001$, and volume, $F(2, 622) = 5.58$, $p = .004$. Fetuses that were infused with 1:0 or 20 µl of the lemon mixture expressed significantly higher percentages of bilateral strokes than fetuses that received infusions at other concentrations or volumes. The interaction between concentration and volume also was significant, $F(4, 622) = 3.25$, $p = .01$ (Fig. 4). Post hoc comparisons revealed that for fetuses in the 1:0 concentration group, infusions of the greatest volume (50 µl) resulted in a significantly lower percentage of bilateral strokes than for fetuses that received infusions of lesser volumes, $F(2, 285) = 6.82$, $p = .001$. Volume effects on the percentage of bilateral strokes at the 1:2 and 1:1 concentrations of lemon were not statistically significant.

Additionally, the analysis indicated a significant interaction between the concentration of the lemon solution and infusion time for percentage of bilateral strokes, $F(6, 622) = 2.78$, $p = .01$. Post hoc analyses indicated that the 1:0 concentration elicited a higher percentage of bilateral strokes than the two diluted mixtures when delivered over a 10-s infusion time, $F(2, 180) = 9.53$, $p = .0001$. There was no significant main effect for infusion time.

In summary, chemosensory stimulus parameters did affect the characteristics of the facial wiping response of

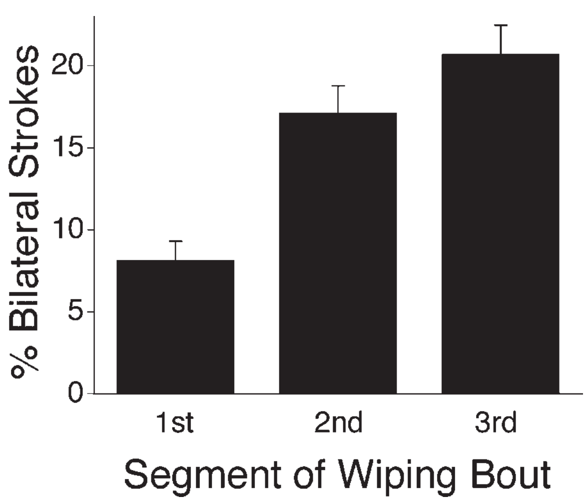

FIGURE 3 Changes in the relative occurrence of bilateral wipes within a bout of facial wiping. Bilateral strokes are expressed as a percentage of the total wiping strokes within three consecutive segments of the wiping bout. In each bout, segments (1st, 2nd, and 3rd) were delineated to contain an equal number of wiping events. Bars show mean percentage of wiping strokes that was bilateral in each segment; lines depict *SEM*.

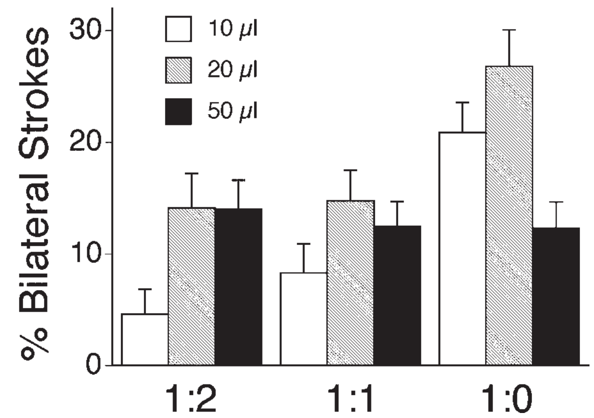

FIGURE 4 Effects of lemon concentration and infusion volume on the relative occurrence of bilateral wiping, expressed as a percentage of total wiping strokes. Bars show mean percentage of strokes that was bilateral during the bout; vertical lines show *SEM*.

the rat fetus. The stimulus properties of concentration and volume of lemon mixture affected the number of unilateral and bilateral wipes and the percentage of bilateral strokes. Findings suggest that the concentration and volume of a complex chemosensory fluid, such as lemon, contribute to overall stimulus intensity. In general, the stronger the intensity of an aversive chemosensory stimulus infused into the mouth of a rat fetus, the more likely it was that the expression of facial wiping strokes was promoted. However, this trend was not evident for bilateral wiping, which was depressed at the strongest stimulus intensity. Additionally, the likelihood of a rat fetus to coordinate the two forelimbs and express bilateral wiping strokes was shown to increase as the wiping bout progressed (see Fig. 3). This implies that the expression of bilateral wiping may be a function of the continuation of the wiping bout.

Moderate Stimulus Intensity and the Emergence of Bilateral Forelimb Coordination

To more adequately address whether increased bilateral forelimb coordination (i.e., the occurrence of bilateral wiping) is simply a function of the volume and the concentration of lemon or a continuation of the wiping bout, we examined the relationship between stimulus intensity and bilateral wiping in additional fetal subjects (see Methods). Infusion time and volume of infusion were held constant (at 5 s and 10 µl, respectively), and only the concentration of lemon fluid was varied. Importantly, stimulus parameters were selected that assured the incidence of bilateral facial wiping, to provide a sufficiently large sample to characterize the spatiotemporal emergence of bilateral wiping in real time. Although stimulus intensity was varied, all stimulus intensities were considered to be relatively moderate and not so intense as to interfere with bilateral wiping (e.g., 1:0 concentration at 50 µl, Fig. 4). This, along with a considerable increase in the number of fetuses tested, permitted a measure of variability in response to different stimulus intensities and provided better temporal resolution for examining response characteristics.

Not all fetuses showed facial wiping. An overall 2 (wipe vs. no wipe) × 3 (1:2, 1:1, 1:0) chi-square analysis indicated significant variation in the tendency for fetuses to show facial wiping, $\chi^2(2, N = 75) = 18.14$, $p = .0001$. Eleven of 25 (44%) fetuses in the 1:2 concentration group showed facial wiping, 20 of 25 (80%) fetuses in the 1:1 concentration group showed facial wiping, and 24 of 25 (96%) fetuses in the 1:0 concentration showed facial wiping. The following analyses are based only on fetuses that displayed a facial wiping response ($n = 55$).

A one-way ANOVA indicated a significant effect of concentration on wiping-bout duration, $F(2, 52) = 12.45$, $p < .0001$. The longest bout durations occurred in the 1:0 concentration condition (Fig. 5, top). Additionally, a series of one-way ANOVAs indicated a significant effect

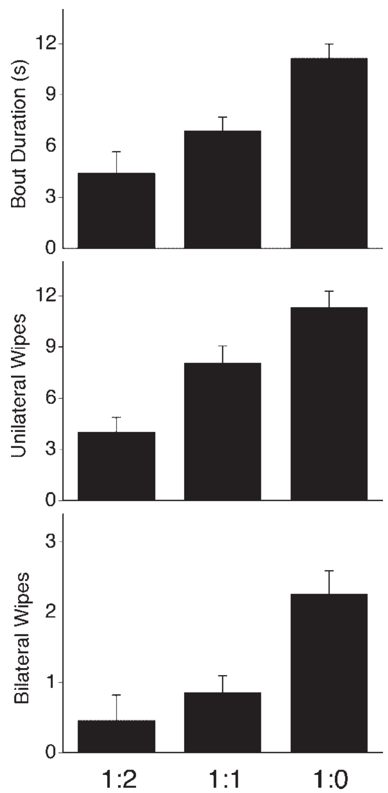

FIGURE 5 Effects of lemon concentration on the duration of wiping bouts (top panel), the number of unilateral facial wipes (middle), and the number of bilateral wipes (bottom) at moderate stimulus intensities (10 µl volume delivered in 5-s infusion time). Bars show means; vertical lines depict *SEM*.

of concentration on the number of unilateral wipes, $F(2, 52) = 10.64$, $p < .0001$, and bilateral wipes, $F(2, 52) = 8.48$, $p = .0006$. Fetuses at each of the lemon concentrations performed significantly more unilateral wipes (Fig. 5, middle) than fetuses at a lower concentration; however, fetuses that received infusions of the highest concentration of lemon (1:0) performed significantly more bilateral wipes than fetuses that received infusions of the two diluted lemon concentrations, 1:2 or 1:1 (Fig. 5, bottom). Therefore, although concentration (and thus stimulus intensity) appears to influence the duration of the wiping bout and number of unilateral wipes in a somewhat linear or progressive fashion, it alone cannot explain the differences in bilateral wipes among groups. The number of bilateral wipes expressed during a wiping bout did not increase at each increased concentration of lemon or stimulus intensity.

Earlier it was suggested that bilateral wipes do not typically occur at the outset of the wiping bout, but become more likely as the bout proceeds. Therefore, if the probability of bilateral wiping is dependent on the length of the wiping bout (defined as the number of wiping events after infusion), we should expect to see more bilateral wipes as the length of the wiping bout increases. To examine this, we plotted the conditional probability of a bilateral wipe occurring on Wiping Event *N*, in bouts that consisted of at least *N* wipes (Fig. 6). Such a plot can be used to gauge the likelihood of a bilateral wipe occurring

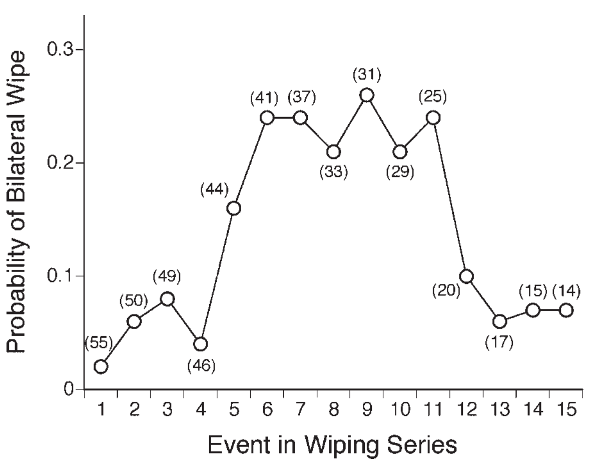

FIGURE 6 Conditional probability of bilateral facial wiping during a wiping bout. Points depict the conditional probability of a bilateral wipe occurring on wiping Event *N*, in bouts that consist of at least *N* wipes. Thus, the sixth point indicates that among the 41 bouts that comprised six or more wiping events, bilateral wiping occurred as the sixth event in 10 bouts (24.4%). The number in parentheses above each point shows the number of bouts containing at least *N* wipes. Note that the probability of bilateral wiping shows a sharp increase between Events 4 to 6 and a subsequent decrease between Events 11 to 12.

at each wiping event in the bout.[1] The incidence of a bilateral wipe versus a unilateral wipe, and thus the probability of a bilateral wipe, on a particular wiping event in the bout was not uniform across the wiping bout, as indicated by an overall chi-square test of independence, $\chi^2(15, N = 548) = 33.29$, $p = .004$. The probability of a bilateral wipe during the first four wiping events was low (All four probabilities were below .10.) and then began to increase at the fifth event in the bout. The probability of a bilateral wipe occurring on the 6th through 11th event was greater than .20 at each event. After the 11th wiping event, the probability of bilateral wiping decreased again. Thus, it appears that bilateral wiping is most likely to be expressed starting at about the fifth or sixth event of the wiping bout, after the expression of several unilateral wipes. This suggests that the performance of bilateral wiping is dependent on the number of wipes that preceded it. It also indicates that for bouts that are longer than 11 events, bilateral wiping is not maintained until the end of the wiping bout, but is followed by the performance of unilateral wipes.

Next, we examined whether there were differences in the incidence of bilateral wipes over the course of the wiping bout for bouts of different lengths. For this analysis, each wiping bout was sorted into one of three categories (of approximately equal *N*s) by bout length: short, medium, or long. Short bouts consisted of fewer than 7 wipes ($n = 18$), medium length bouts consisted of 7 to 11 wipes ($n = 17$), and long bouts consisted of more than 11 wipes ($n = 20$). Bilateral wiping was compared across the different bout lengths using separate chi-square tests of independence at two intervals of the wiping bout. The first interval comprised Events 1 to 6 of the wiping bout; the second interval comprised events 7 to 11 of the bout. (These intervals were not chosen arbitrarily, but corresponded to the three bout-length categories.)

Bilateral wiping was not equally likely across the three bout lengths within the first interval as indicated by a 2 (bilateral vs. unilateral wipe) × 3 (short, medium, long bout) chi-square test, $\chi^2(2, N = 285) = 8.50$, $p < .05$. Of the first six wiping events of the bout, none of 63 (0%) wipes were bilateral for short bouts, whereas 12 of 102 (11.8%) were bilateral for medium length bouts and 15 of 120 (12.5%) wipes were bilateral for long bouts. Thus, all bilateral wipes that occurred during the first few wipes of the wiping bout occurred in bouts longer than seven wipes. Within the second interval (Events 7–11), short bouts were excluded from the analysis because, by definition, they terminated before the seventh event. A 2 (bilateral vs. unilateral wipe) × 2 (medium vs. long bout) chi-square test indicated that the incidence of bilateral wiping was not significantly different between bout lengths within the second interval, $\chi^2(1, N = 155) = .237$, $p > .60$. For Wiping Events 7 to 11, 14 of 55 (25.5%) were bilateral in medium-length bouts, and 22 of 100 (22%) were bilateral in long bouts. Therefore, during this interval of the wiping bout, bouts of different lengths did not differ in their incidence of bilateral wipes. For bouts that were longer than 11 events (long bouts), an additional 13 bilateral wipes (of 107 total wipes; 12% bilateral) were performed after the 11th wiping event in the bout. Thus, by classifying bouts by length, it becomes evident that the distribution of bilateral wipes during a wiping bout is different for bouts of different lengths. No bilateral wipes occurred during bouts of short length, and bilateral wipes occurred more often in the middle of the bout for medium and long bouts. Nonetheless, overall the majority of bilateral wipes happened between the 7th and 11th wiping events of the bout. An unpaired *t* test comparing the number of bilateral wipes between medium and long bouts revealed that the two bouts did not differ in their frequency of bilateral wipes ($p > .05$). Thus, fetuses that performed at least seven wipes during a bout of facial wiping performed an approximately equal number of bilateral wipes during the bout, regardless of bout length.

Examination of the percentage of fetuses that exhibited a wiping bout of at least seven events suggests that stimulus parameters exert their effects on bilateral facial wiping indirectly, mainly through the prolongation of the wiping bout. Only 12% (3) of the fetuses in the 1:2 concentration group expressed more than seven total wiping events while the percentage of fetuses in the 1:1 and 1:0 concentration groups that expressed more than seven total wiping events reached 52% (13) and 84% (21), respectively, $\chi^2(2, N = 75) = 26.03$, $p = .0001$.

Korthank & Robinson (1998) suggested that bouts that consist of few wipes are partially accounted for by an increase in the latency to the initial wipe of the bout. A one-way ANOVA comparing latency to first wipe (in seconds) indicated a significant effect of concentration, $F(2, 52) = 27.90$, $p < .0001$, with each stronger concentration of lemon producing a significantly shorter latency to the first wipe than any of the weaker concentrations. A Pearson product–moment correlation assessing the relationship between the latency to the first wipe and bout duration was .69 ($r^2 = .48$), which was significant, $F(1, 53) = 48.46$, $p < .0001$. Therefore, a longer latency to the first facial wipe of the bout is associated with shorter bout durations.

To summarize, the expression of bilateral facial wiping occurring in a single test session is dependent on the number of wipes performed by the fetus. When fewer than seven total wiping events were expressed by fetuses, there appeared to be little opportunity for coordination of the

[1]Note that Figure 6 has been truncated at 15 wiping events. Because a small number of bouts were longer than 15 wipes, such bouts provided an unreliable estimate of probability. In the analysis, all events in bouts longer than 15 wipes were collapsed.

forelimbs to be established, and therefore for bilateral facial wiping to occur. Interlimb coordination thus became more likely as the wiping bout progressed and usually emerged subsequent to the expression of several unilateral wipes (Robinson & Smotherman, 1991). Although chemosensory stimulus intensity did not directly determine the incidence of bilateral wiping, it did influence the duration of the wiping bout, the latency to the first wiping stroke, and the number of unilateral wipes. Therefore, stimulus intensity contributed to the likelihood that bilateral wipes would be expressed.

DISCUSSION

The present study confirms that the facial wiping response of the E20 rat is influenced by characteristics of the eliciting chemosensory stimulus. Previous studies of fetal responsiveness have demonstrated that the fetal rat can discriminate among qualitatively different chemosensory stimuli such as milk, lemon extract, dimethyl disulfide, and isotonic saline (Smotherman & Robinson, 1992b), and that they can detect and respond to stimuli in olfactory, gustatory, and trigeminal modalities (Smotherman & Robinson, 1990a,b, 1991b). This report extends these findings by showing that fetuses also can make finer discriminations among quantitative differences in their exposure to the same chemosensory stimulus. Intraoral infusions of lemon extract that varied in concentration, volume, rate of delivery, and infusion time differed in their effectiveness to evoke facial wiping responses. Therefore, the ability to detect differences in chemosensory stimulus quality develops before birth in the rat.

Parametric variation of the concentration, volume, and infusion time produced differences in characteristics of the wiping response. The interaction of volume and concentration of lemon influenced the number of unilateral wipes, bilateral wipes, and percentage of bilateral wipes during the facial wiping bout. The concentration of lemon also affected the likelihood that the fetus would show bilateral wiping, though indirectly, by affecting the latency to the first wipe of the bout and the duration of the wiping bout. Parametric variation of infusion time, however, did not produce systematic differences in the wiping response across groups. Although there was an interaction between concentration and infusion time, it appears to have been driven by the concentration of the stimulus. While volume and infusion time determined the rate of fluid infusion into the mouth of the fetus, the range of infusion times selected in these experiments may not have been large enough to show evidence of infusion-rate effects on the facial wiping response.

Many of these parametric effects on the facial wiping response may be summarized within the concept of stimulus intensity. Generally, more facial wipes were elicited by infusions of greater intensity. That is, fetuses showed more wiping at higher concentrations and volumes of infusion. Temporal aspects of the wiping response, such as latency to the first wipe and the duration of the wiping bout, also were directly affected by stimulus intensity; however, the likelihood of the fetus to show bilateral facial wiping did not increase in relation to stimulus intensity. Conditions permitting the expression of bilateral wiping were optimal at moderate stimulus intensities. When stimulus intensity was very low (e.g., 10-μl infusion of concentration 1:2; Fig. 4), bilateral wipes often did not occur during the wiping bout. When stimulus intensity was moderate (e.g., 20 μl of 1:0), bilateral wipes occurred more often between the 6th and 11th events of the bout. But when stimulus intensity was very high (e.g., 50 μl of 1:0), the incidence of bilateral wipes again was low. Because bilateral wiping was not clearly related to stimulus quality, it would appear to be a poor index of fetal responsiveness to stimulation; however, facial wiping in general, and the total number of wiping strokes in particular, increased monotonically with stimulus intensity, and therefore should prove useful as a general measure of fetal responsiveness in future studies.

The present study confirmed that bilateral facial wiping in the rat fetus is not merely a reaction to a stimulus of adequate intensity, but a task of interlimb coordination that becomes assembled in real time. As suggested by previous reports (e.g., Robinson & Smotherman, 1991), interlimb coordination between the forelimbs tends to emerge near the middle of the wiping bout after the performance of several unilateral wipes. This process may be analogous to the ‘‘warm-up’’ phenomenon described by Golani, Bronchti, Moualem, & Teitelbaum (1981), in which infant rats initiate exploratory behavior by first engaging in small movements of the head and successively recruit movements of greater amplitude involving the forelimbs and other parts of the body. Though bilateral wipes in principle could occur at the beginning of the wiping bout, but actually did in only a few cases, the probability of a bilateral wipe occurring before a unilateral wipe was very low. A novel finding of this study is that unilateral wipes also followed the expression of bilateral wipes in bouts that consisted of a large number of wiping events. This suggests that interlimb coordination during facial wiping was fluid and that a synchronized phase relationship between limbs was not maintained once it was achieved.

The tendency for a minimum number of wiping strokes to occur before bilateral wiping was expressed suggests the hypothesis that stimulus intensity exerts a permissive influence on the expression of bilateral facial wiping. For example, low-intensity lemon infusions resulted in longer latencies to the initial wipe of the bout and produced bouts of shorter duration, thereby generating responses that typically consisted of only a few unilateral wipes with no opportunity for the emergence of bilateral wiping. In contrast, moderate-intensity lemon infusions resulted in wiping responses with shorter latencies and bouts of longer duration, permitting bilateral wipes to be expressed after an initial series of unilateral strokes. Understanding this pattern of the emergence of bilateral facial wiping in real time is important to consider in any interpretation of fetal wiping responses to stimuli of varying quality.

The observation that bilateral facial wiping diminishes at high stimulus intensities is paradoxical. Although bout durations were long and many unilateral wipes were expressed, very intense lemon infusions usually resulted in few or no bilateral facial wipes. The absence of bilateral wiping may have been due to the recruitment of additional motor components or behavior patterns that interfered with simultaneous bilateral paw–face contact. In tracing the developmental origins of the facial wiping response, Robinson & Smotherman (1991) showed that E19 rat fetuses reliably performed facial wipes to lemon infusion when tested inside the amniotic membranes, but failed to show facial wiping when tested outside the membranes within the water bath. They suggested that the fetal membranes stabilize the head, a component of the response that appears necessary to establish paw–face contact. Likewise, stabilization of the head near the midline of the body may facilitate bilateral wiping in the E20 fetus by providing a nonmoving target for simultaneous placement of the two forepaws against the face. Thus, in the present study, fetuses that received very intense lemon infusions may have produced lateral movements of the head, similar to the head-shaking behavior of older animals during aversion sequences (Grill & Norgren, 1978a; Johanson & Shapiro, 1986), and consequently could not initiate or maintain bilateral wiping. It also is possible that these fetuses expressed wiping strokes at or near the maximum rate, which may have interfered with the temporal modulation of wiping strokes necessary to bring left and right forelimbs into phase (Robinson & Smotherman, 1991). It therefore is unclear whether highly intense lemon infusions interfere with bilateral facial wiping by evoking additional motor components or by disrupting the timing of interlimb coordination.

Just as the adult rat is responsive to variations in chemosensory stimulus properties (Grill & Norgren, 1978a), the experiments reported here demonstrate that the E20 rat fetus also is responsive to different qualities of a chemosensory stimulus. One should expect that characteristics of a response are related to characteristics of the eliciting stimulus if the response is functional. In adult rats, aversion sequences are longer and include more behavioral elements in response to aversive stimuli of greater intensity. However, for a given stimulus, the form and magnitude of response remains consistent within and between individuals. Grill & Norgren (1978a) related this consistency of response to its presumed function, specifically that "a single presentation of an aversive stimulus immediately elicits behavior(s) designed to reduce stimulus intensity" (p. 276). Whether the facial wiping response of the developing rat or the function of facial wiping is developmentally related to more mature forms of taste-aversion responses in adult animals has not been resolved (Schwartz & Grill, 1985). The organization of facial wiping responses in the rat clearly is more plastic and unstable during the prenatal period than it is during aversion or grooming sequences in adulthood (Berridge, 1990; Grill & Norgren, 1978a). Although fetal responses were more variable, components of the facial wiping response nevertheless were sensitive to parametric variations in chemosensory stimuli. Facial wiping by the rat fetus therefore may be less effective than adult aversion responses, but still have functional significance during the perinatal period.

NOTES

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