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Sniffing in Infant Rats During Sleep and Wakefulness

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Sniffing, a behavior that enhances detection and localization of odorants, is typically assumed to require behavioral arousal. In an effort to determine whether sniffing and arousal are dissociable, dimethyl disulfide (DMDS) was presented to 8-day-old rats while respiration and behavioral state were monitored. Pups sniffed in response to the highest concentrations of DMDS, exhibiting a lower olfactory threshold when awake. Surprisingly, sniffing occurred even while pups remained asleep. Sniffing was mediated by the olfactory system, as evidenced by the abolition of sniffing when the lateral olfactory tracts were cut and the retention of rapid arousal in response to a trigeminal stimulant, acetic acid. Finally, sleeping pups presented with acetic acid awakened without sniffing. Thus, although olfactory threshold increases during sleep, sleeping does not preclude sniffing.

Sleep in mammals can be defined by using electrographic criteria—including changes in the electromyogram (EMG) and electroencephalogram (EEG)—and behavioral criteria—including a relaxed posture and increased sensory thresholds (Campbell & Tobler, 1984; Rechtschaffen & Kales, 1968). For example, active sleep (AS; or REM sleep) is characterized in part by the co-occurrence of skeletal muscle atonia, myoclonic twitching, and activation of the cortical EEG. In the infants of many mammalian species, especially altricial infants such as rats, some components of AS emerge only gradually during development (Blumberg & Lucas, 1996); for example, state-dependent changes in EEG are not detected until after 10 days of age (postnatal [P]10; Corner & Mirmiran, 1990; Frank & Heller, 1997; Gramsbergen, 1976). Therefore, at younger ages, AS is identified by using a restricted set of criteria, such as nuchal atonia and myoclonic twitching (Karlsson & Blumberg, 2002). Here, we ask whether an increased sensory threshold to an olfactory stimulus is a defining characteristic of AS in infant rats.

Sleep-related increases in sensory threshold have been studied in many different species, typically requiring subjects to exhibit arousal-related responses. For example, auditory and olfactory stimuli were presented to human adults during sleep and evidence of detection (e.g., pushing a button, changes in EEG) was assessed (Badia, Wesensten, Lammers, Culpepper, & Harsh, 1990; Bonnet, Johnson, & Webb, 1978; Bonnet & Moore, 1982; Williams, Morlock, & Morlock, 1966). Similarly, sleep-dependent changes in sensory thresholds have been measured with auditory stimuli in rats (Baust, Berlucchi, & Moruzzi, 1964), with vibratory stimuli in flies and zebra fish (Shaw, Cirelli, Greenspan, & Tononi, 2000; Zhdanova, Wang, Leclair, & Danilova, 2001), and with electrical stimuli in desert iguanas (Huntley, 1987). Under these conditions, sensory thresholds have been found to increase during sleep. Much less is known, however, as to whether stimuli that do not induce arousal are detected and processed by the sleeping brain. Relevant evidence does exist in human adults in which stimuli, especially meaningful stimuli, presented to sleeping subjects become incorporated into dreams (Berger, 1963; Dement & Wolpert, 1958). Other studies have shown that human subjects can respond behaviorally to the presentation of tones during sleep without showing EEG signs of arousal (Williams et al., 1966). Finally, human newborns are able, during sleep, to detect an odor that had previously been paired with a reinforcing stimulus (Sullivan et al., 1991).

Although there is evidence of increased sensory thresholds during sleep in human infants (Hutt, Hutt, Lenard, von Bernuth, & Muntjewerff, 1968; Murray & Campbell, 1970), we are unaware of similar work in the infants of other species. As part of a larger project aimed at characterizing sleep and its neural substrates in infant rats (Karlsson & Blumberg, 2002; Karlsson, Kreider, & Blumberg, 2004; Kreider & Blumberg, 2000), we wondered whether increased sensory thresholds accompany AS early in ontogeny. Because the auditory and visual systems of rats are immature until after P12, we turned to the olfactory system, whose vital role in such behavioral processes as nipple attachment is well known (Teicher & Blass, 1976). Furthermore, we chose to present pups with a salient olfactory stimulus, dimethyl disulfide (DMDS), a component of rat saliva and vaginal secretions that helps to guide nipple attachment (Pedersen & Blass, 1981).

In rats and other mammals, sniffing is most closely identified with rapid, shallow breathing (i.e., polypnea), a mechanism that enhances transport of airborne chemicals across the olfactory epithelium (Alberts & May, 1980; Macrides & Chorover, 1972; Welker, 1962). The full expression of sniffing in adults, but not infants, includes nose retraction, protraction of the vibrissae, and head movement (Welker, 1962). Thus, sniffing is conventionally viewed as an active, investigatory behavior and is therefore typically associated with wakefulness. We asked here whether sniffing can be expressed in sleeping P8 rats and whether this particular motor response can be used to distinguish behavioral states at an

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age when state-dependent EEG activity is absent. We report that rats exhibit a higher sensory threshold to DMDS during AS but can display robust polypnea even while asleep.

Method

All experiments were performed under National Institutes of Health guidelines for the care of animals in research and were approved by the Institutional Animal Care and Use Committee of the University of Iowa.

Subjects

A total of fifty 8-day-old (25 males and 25 females) rats from 30 litters were used. Body weight ranged from 17.2 to 24.8 g. All pups were born to Harlan Sprague–Dawley rats housed in the animal colony at the University of Iowa. The pups were raised in litters that were culled to 8 pups within 3 days of birth (day of birth = Day 0). Litters and mothers were raised in standard laboratory cages (48 cm long × 20 cm wide × 26 cm high), in which food and water were available ad libitum. All rats were maintained on a 12-hr light–dark schedule with lights on at 7 a.m.

Test Environment

Pups were tested inside an electrically shielded, double-walled glass chamber (height = 17.0 cm; i.d. = 12.5 cm) with a Plexiglas lid. Air temperature inside the chamber was maintained at 35 °C by means of a temperature-controlled water circulator. Access holes in the side and lid of the chamber allowed for the passage of air through the chamber (300 ml/min), as well as the passage of plethysmograph wires and EMG electrodes. A round platform constructed of polyethylene mesh was fitted inside the chamber. The mesh allowed for the movement of air from the bottom of the chamber (where it entered) to the top of the chamber.

Olfactory Stimuli

In Experiments 1–3, stimuli were prepared by dissolving DMDS in 95% ethyl alcohol (ETOH) and then dissolving that solution (consisting of some amount of DMDS and 0.1 ml ETOH) in 10 ml of 0.9% (wt/vol) saline. The concentrations of DMDS in saline included 1 part DMDS to 100 parts saline, 1:1,000, 1:10,000, and 1:100,000. A control stimulus consisted of 10 ml saline and 0.1 ml ETOH. In Experiment 4, varying concentrations of glacial acetic acid (GAA) were prepared by diluting concentrated GAA in isotonic saline. The concentrations were 1:10, 1:100 and 1:1,000, as well as undiluted (i.e., concentrated) GAA.

Procedure

Experiment 1. Twelve male and female P8 rats, 1 from each of 12 litters, were used. On the day of testing, a pup with a visible milk band was removed from the litter, weighed, and placed in an anesthesia induction chamber. Under isoflurane anesthesia, three plethysmograph electrodes were attached to the surface of the skin with collodion; two electrodes were secured on either side of the thorax, and the third electrode was secured in the lumbar region. The pup was lightly secured in a supine position in a harness and placed inside the test chamber to recover for 90 min before testing.

Respiration was monitored with an impedance plethysmograph (UFI, Morro Bay, CA). EMG electrodes were connected to differential amplifiers (A-M Systems, Carlsborg, WA), and their signals were amplified (×10,000), with filter settings of 1–3000 Hz and 300–5000 Hz, respectively. Respiratory and EMG data were digitized at 1 kHz by means of a data acquisition system (BioPac Systems, Santa Barbara, CA) and simultaneously visualized by the experimenter during the test. A microcamera was placed above the chamber lid for monitoring and recording of behavior. Respiratory, EMG, and video data were recorded to digital videotape with a data recorder (DV8; WinTron Technologies, Rebersburg, PA).

Olfactory stimuli were presented on cotton-tipped applicators dipped in the stimuli solutions. The applicators were lowered into the chamber through a hole in the lid and placed approximately 1–2 cm from the snout of the subject for 10 s. Stimuli were presented to waking or sleeping pups. Pups that were exhibiting coordinated motor activities (e.g., kicking, stretching, yawning) were considered awake; stimuli were always presented immediately after the cessation of these activities. When nuchal EMG was monitored, pups exhibited high muscle tone during these periods of wakefulness, consistent with previous findings (Karlsson & Blumberg, 2002). Pups that were exhibiting myoclonic twitching of the distal limbs and tail were considered to be in active sleep; stimuli were always presented during periods of twitching, which occur only against a background of nuchal muscle atonia (Karlsson & Blumberg, 2002). Behavioral states were monitored for 5 s before stimuli were presented.

Stimuli were presented in an order such that no concentration was presented twice in a row and presentations were alternated between behavioral states. Beyond those two stipulations, presentations were randomized without replacement. Stimuli were presented a minimum of 120 s apart.

Experiment 2. Twenty-four male and female P8 rats from 8 litters were used. Three littermates were tested each day, and each pup was assigned to a different experimental group. The procedure was identical to that described for Experiment 1, with the following changes. First, in addition to respiration, nuchal EMG was measured with two bipolar 50-μm stainless steel hook electrodes (California Fine Wire, Grover Beach, CA) inserted bilaterally into the muscle with a ground wire attached to the back skin. Second, while recovering from surgery and after 1 hr in the incubator, each littermate was intubated with commercial half-and-half (50% cream/50% milk; 3% of body weight in ml). The first subject was tested 1 hr after intubation, the second subject 5 hr after intubation, and the third subject 10 hr after intubation. All subjects were placed in the recording chamber 45 min prior to testing to allow for acclimation.

Experiment 3. Eight male and female P8 rats from 4 litters were used. Two littermates were tested each day, and each pup was assigned to a different experimental group (i.e., olfactory input isolation and sham). Pups were anesthetized with cold anesthesia (Phifer & Terry, 1986), the skull was exposed, and two holes were drilled above the olfactory bulbs with a 22-gauge needle. A blunted 25-gauge needle was inserted into each hole, and the lateral olfactory tracts were cut. Sham controls were treated identically to experimental pups, except the blunted needle was not swept across the lateral olfactory tracts. The scalp was closed, and plethysmograph and EMG electrodes were attached. Subjects recovered at 35 °C for 3 hr and were then intubated with commercial half-and-half (3% of body weight in ml). Fifteen minutes after intubation, pups were placed in the testing chamber and acclimated for 45 min before testing began. Testing consisted of counterbalanced presentations of 1:100 DMDS while pups were asleep or awake. After the last DMDS or vehicle presentation, pups were exposed to concentrated GAA to ensure that the trigeminal system was not adversely affected by olfactory input isolation. After this test, pups were overdosed with sodium pentobarbital and perfused with saline and formalin. The completeness of the nerve cuts was confirmed in all 4 pups.

Experiment 4. Six male and female P8 rats, 1 from each of 6 litters, were used. Pups were prepared for testing as described in Experiment 1, except nuchal EMG electrodes were also implanted. Testing was identical to that described in Experiment 1 except that GAA replaced DMDS as the stimulus.

Data Analysis

Data were played back from digital videotape, and the experimenter monitored the video to mark periods of onset and offset of stimulus

presentation. Then, on the basis of the digitized records of respiration and, when available, nuchal EMG, each stimulus exposure period was divided into three sections: 30 s before stimulus exposure, 10 s during exposure, and 30 s after exposure. Ten consecutive breaths were chosen from each section, and data analysis software (Acknowledge; BioPac Systems, Santa Barbara, CA) was used to calculate the average number of breaths per minute (BPM). In sleeping pups, behavioral arousal in response to odor presentation was defined as the expression of any of the characteristics of wakefulness as defined above. Analysis of variance (ANOVA) was used to test for differences in respiratory rate. Post hoc tests were paired *t* tests. Alpha was set at .05.

Results

Experiment 1: Sniffing and Arousal to DMDS During Sleep and Wakefulness

Figure 1 presents an example of polypnea in a sleeping P8 rat. Upon presentation of DMDS, the pup quickly transitioned from regular breathing to polypnea, which continued throughout the presentation period. The pup aroused (i.e., began exhibiting awake-related behavior) from AS only after the odor was withdrawn. The timing of arousal in this particular instance may have been unrelated to the presentation of the odor, however, because sleep periods in week-old rats are often shorter than 20 s (Karlsson et al., 2004).

The effect of DMDS concentration on latency to arousal was not significant, $F(4, 55) = 1.0$. To further explore the effect of odor presentation on arousal, we examined the number of pups that aroused from sleep during presentation of the highest concentration of DMDS (1:100) or vehicle. Four of the 12 pups in the 1:100 condition aroused during the 10-s odor presentation period, compared with 1 of 12 pups in the vehicle condition, $\chi^2(1, N = 24) = 2.3$, *ns.* Thus, even the highest DMDS concentration had a negligible effect on behavioral arousal.

Sniffing was never observed during presentation of vehicle; indeed, spontaneous sniffing (i.e., sniffing in the absence of explicit odor presentation) was a very rare event. In contrast, DMDS reliably elicited short-lasting polypnea in sleeping and waking pups in a concentration-dependent manner. Sleeping pups were less likely to sniff to decreasing concentrations of DMDS than were awake pups (see Figure 2). While sniffing, awake pups typically lifted their head and oriented toward the stimulus, whereas sleeping pups did not. ANOVA revealed significant main effects of behavioral state, $F(1, 109) = 4.6$, $p < .05$, and DMDS concentration, $F(4, 109) = 10.0$, $p < .01$. Of the 12 subjects tested in Experiment 1, 7 sniffed (defined as a 30% increase in respiratory rate) while asleep during the 10-s presentation of 1:100 DMDS. Of these 7, all sniffed before arousing and 3 sniffed at least 3 s before arousing.

For those pups that sniffed in response to odor presentation, the latency to sniffing was 3.5 ± 0.6 s for sleeping pups and 3.3 ± 0.5 s for awake pups, $t(30) = 0.4$, *ns.*

Experiment 2: Sniffing and Arousal Under Known Conditions of Nutritional State

We repeated Experiment 1 using the same methods, except that pups ($n = 8$ per group) were intubated with half-and-half after recovery from surgery and were then tested either 1, 5, or 10 hr postintubation; in addition, nuchal EMG recordings were added to provide additional confidence in our assessments of behavioral state. Figure 3 presents two examples of polypnea in a P8 rat while asleep and awake; determinations of behavioral state were made on the basis of behavior and nuchal muscle tone. It can be seen that this pup, upon presentation of DMDS, exhibited polypnea when either asleep or awake.

The basic findings of Experiment 1 were replicated, but only for pups that had been deprived of milk for 5 hr (Figure 4). Specifically, there was increased responsiveness to DMDS in the 5-hr-deprived awake pups. The overall ANOVA revealed significant main effects of postintubation time, $F(2, 210) = 6.8$, $p < .01$; concentration, $F(4, 210) = 19.4$, $p < .01$; behavioral state, $F(1, 210) = 21.9$, $p < .01$; and a significant Postintubation Time × Behavioral State interaction, $F(2, 210) = 5.1$, $p < .01$. Surprisingly, there were no significant effects of DMDS concentration or postintubation time on latency to arousal: concentration, $F(4, 105) = 1.2$; postintubation time, $F(2, 105) = 0.02$; interaction, $F(8, 105) = 0.4$.

Examination of behavior and nuchal EMG revealed that the two measures were redundant; changes in behavioral arousal always occurred simultaneously with increases in nuchal EMG, as shown previously (Karlsson & Blumberg, 2002). Once again, we assessed latencies to sniffing and arousal in pups exposed to the 1:100

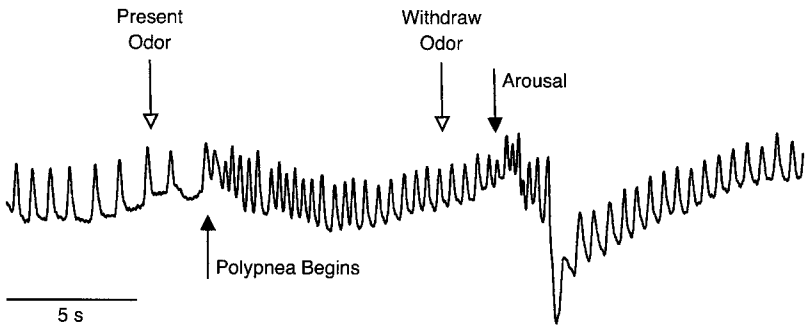

Figure 1. Example of polypnea in response to odor stimulation in a sleeping 8-day-old rat from Experiment 1. Dimethyl disulfide (1:100) was presented for 10 s as indicated. At the time of odor presentation the pup was in active sleep, as determined by the expression of myoclonic twitching. In this example, sleep continued throughout the period of odor stimulation, and behavioral arousal occurred only after the odor was withdrawn.

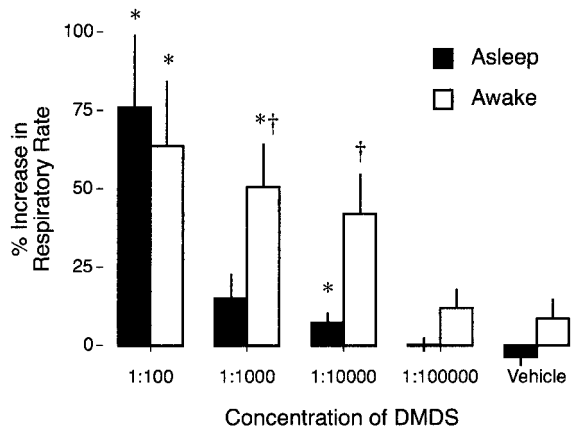

Figure 2. Mean (± *SEM*) percent changes in respiratory rate in response to varying concentrations of dimethyl disulfide (DMDS) in 8-day-old rats from Experiment 1. Pups were less sensitive to DMDS when asleep than when awake. * significant difference from vehicle; † significant difference from asleep ($p < .05$).

concentration of DMDS (combining the data for the 1-, 5-, and 10-hr-deprived pups). From a total of 24 pups, 13 sniffed while asleep within the 10-s presentation period, and all of these sniffed before arousing. Moreover, 10 of these pups sniffed at least 3 s before arousing, and 5 of these sniffed at least 8 s before arousing.

Experiment 3: Effect of Olfactory Input Isolation on Response to DMDS

To establish that the sniffing observed in Experiments 1 and 2 was mediated by stimulation of the olfactory system, we cut the lateral olfactory tracts bilaterally in 4 P8 rats; 4 littermates experienced sham surgeries. Pups were then tested as before but were presented only with the 1:100 concentration of DMDS. Olfactory input isolation abolished sniffing in response to DMDS, whereas shams were unaffected (Figure 5). Repeated measures ANOVA revealed a significant main effect of group, $F(1, 12) = 6.6$, $p < .05$; time, $F(2, 24) = 23.8$, $p < .01$; and Group × Time interaction, $F(2, 24) = 27.5$, $p < .01$. None of the pups with nerve cuts awakened at odor presentation; 2 of the sham pups did awaken, but only toward the end of the 10-s odor presentation period.

Finally, to ensure that the nerve cuts did not interfere with the functioning of the trigeminal system, we presented concentrated GAA, a trigeminal stimulant, at the end of each test. In all cases, pups with nerve cuts exhibited rapid and robust arousal responses to GAA.

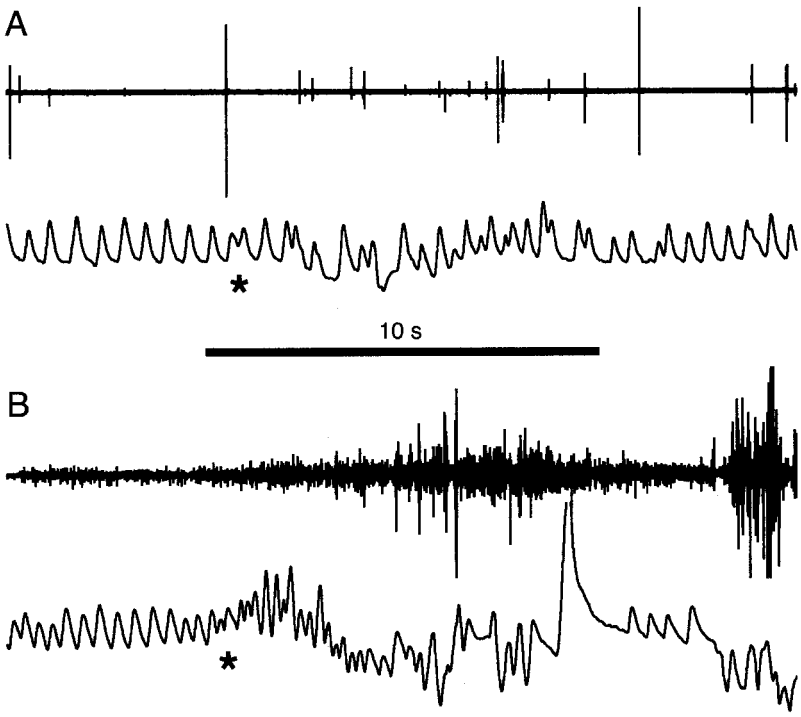

Figure 3. Nuchal electromyograph (EMG; upper traces) and respiration (lower traces) during two dimethyl disulfide (DMDS) presentations in an 8-day-old rat from Experiment 2. DMDS (1:100) was presented for 10 s, as indicated by the thick horizontal bar. Onset of polypnea is indicated by asterisks. A: This pup was in active sleep before, during, and after odor presentation, as indicated by nuchal atonia. The pup also exhibited myoclonic twitching at the time of odor presentation (twitches in the EMG record can be seen). B: This pup was awake before, during, and after odor presentation, as indicated by high muscle tone. The pup also exhibited awake behavior before odor presentation. EMG amplitudes are arbitrary but identical in the two records.

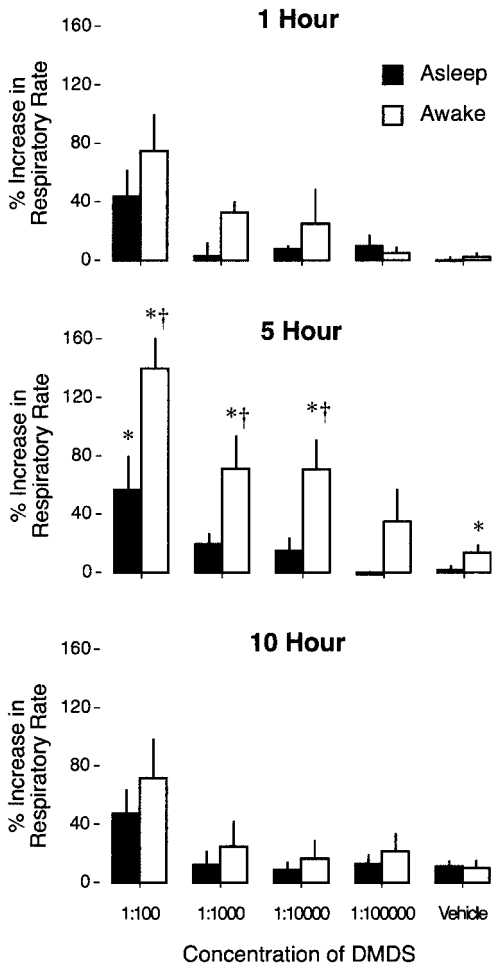

Figure 4. Mean (± *SEM*) percent changes in respiratory rate in response to varying concentrations of dimethyl disulfide (DMDS) in 8-day-old rats from Experiment 2. Pups were tested 1, 5, and 10 hr postintubation. Pups were less sensitive to DMDS when asleep than when awake, especially 5 hr postintubation. * significant difference from vehicle; † significant difference from asleep ($p < .05$).

Experiment 4: Effect of a Trigeminal Stimulant on Sniffing and Arousal

In order to assess whether a chemosensory stimulus can evoke arousal without first evoking a sniffing response, we presented varying concentrations of GAA to P8 rats ($n = 6$). The experimental design was the same as that used in Experiment 1. Tests using various concentrations of GAA evoked rapid arousal from sleep in a concentration-dependent manner. Specifically, concentrated GAA evoked arousal in every sleeping pup and did so within 1.8 ± 0.9 s of stimulus presentation, the 1:10 dilution of GAA evoked arousal in 2 pups, and vehicle presentation produced arousal in only 1 pup. Sniffing was never observed in response to concentrated GAA and was evoked on only a few occasions in response to presentation of diluted GAA (1:10, 2 awake pups and 2 sleeping pups; 1:100, 1 awake pup; 1:1000, 2 awake pups).

Discussion

The present results demonstrate the feasibility of using sniffing in infant rats as an indicator of olfactory system modulation by behavioral state. Experiment 1 demonstrated that P8 rats are more sensitive to varying concentrations of DMDS when awake than when asleep. Experiment 2, in which the nutritional state of the pups was controlled, again demonstrated state-dependent olfactory sensitivity, but only for pups that had been intubated 5 hr previously. These results are consistent with those of Experiment 1, in which pups were not intubated after surgery and were therefore deprived of milk for at least 2 hr. The weak responses to DMDS by 1-hr-deprived pups can perhaps be explained as resulting from satiation (Gervais, Araneda, & Pujol, 1984; Gervais & Pager, 1979), whereas the weak responses of the 10-hr-deprived pups may have resulted from lethargy caused by prolonged deprivation. Finally, as shown in Figure 4, the olfactory sensitivity of sleeping pups was relatively constant across deprivation time, and it was primarily the sensitivity of awake pups that was modified by deprivation.

These results also provide an additional defining characteristic of AS in infants, namely, increased olfactory threshold in relation to the awake state. We distinguished between the two behavioral states—awake and AS—by using nuchal EMG and/or behavior. Because muscle tone remains elevated for many seconds after a bout of coordinated motor activity, and because myoclonic twitching occurs only against a background of nuchal atonia (Karlsson & Blumberg, 2002), behavior alone can reliably distinguish between the two behavioral states. Adding nuchal EMG as a measure of state merely added confidence in our behavioral observations.

We used DMDS because it is a salient, familiar olfactory stimulus for infant rats (Pedersen & Blass, 1981). Because the vast majority of chemosensory stimulants activate both the olfactory and trigeminal systems (Hummel & Livermore, 2002), it was important that we establish the mediating role of the olfactory system in the sniffing responses observed in Experiments 1 and 2. We did this in Experiment 3 by showing that transections of the lateral olfactory tracts abolished sniffing to DMDS in both awake and sleeping rats and that this olfactory input isolation did not prevent arousal by the trigeminal stimulant, acetic acid. Next, we showed in Experiment 4 that concentrated acetic acid produced rapid arousal responses in sleeping infants without evoking prior sniffing; inhibition of sniffing and activation of arousal are expected responses if the role of nasal trigeminal reflexes is to limit damage produced by chemosensory irritants (Hummel & Livermore, 2002). All together, these results demonstrate that sniffing and arousal by nasal chemosensory stimulation are dissociable.

Even at high concentrations, DMDS did not evoke reliable arousal responses in sleeping infants, a finding that may have been due to the biological significance, familiarity, or pleasant associations of DMDS for the infant. It is possible, for example, that odors produced by natural predators or neutral odors that have become associated with unpleasant stimuli might be better able than DMDS to evoke arousal via the olfactory system in infants (Cattarelli & Chanel, 1979; Van Twyver & Garrett, 1972). On the other hand, it is interesting that sleeping human newborns exhibited conditioned arousal responses (i.e., head-turning) to an odor that was previously paired with a positively reinforcing stimulus (Sullivan et al., 1991). Regardless, even though DMDS did not

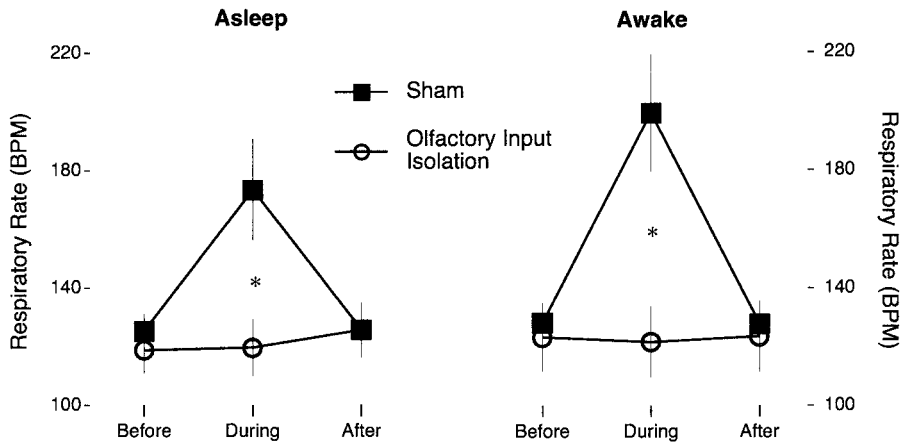

Figure 5. Effect of olfactory input isolation (by transection of the lateral olfactory tracts) on respiratory responses to dimethyl disulfide (DMDS) in 8-day-old rats from Experiment 3. Respiratory rate (breaths per minute; BPM) was measured before, during, and after presentation of DMDS (1:100). Pups were asleep or awake at the time of odor stimulation. Data are means (± *SEM*). * significant difference between sham controls and experimental pups ($p < .05$).

arouse sleeping infant rats, its acquired meaning during early development may underlie the sniffing responses to this odorant seen in Experiments 1 and 2. The relationships among sniffing, arousal, and an odor's familiarity and biological significance remain unclear (Alberts & May, 1980).

The present study demonstrates that infant rats can process an olfactory stimulus and trigger a motor response—sniffing—independent of the background tone of postural muscles. Of course, the possibility remains that presentation of DMDS to sleeping pups triggered microarousals—invisible to our measures of behavior or nuchal EMG—prior to sniffing. Such a possibility, however, is difficult to reconcile with our observations that some sleeping pups exhibited polypnea even as they continued to exhibit myoclonic twitching.

It is also possible that polypnea during sleep was merely the result of sleep-related changes in respiration or myoclonic twitching of the respiratory muscles. We think this possibility is unlikely for several reasons. First, in the present experiments, polypnea occurred only during odor presentation, even in sleeping pups (see Figures 1, 3, and 5). Second, polypnea was more likely to occur in response to higher concentrations of DMDS (see Figure 2), an unlikely relationship if the polypnea was a by-product of sleep-related respiratory changes. Third, transection of the lateral olfactory tracts in Experiment 3 abolished polypnea in sleeping pups, even though these pups continued to exhibit myoclonic twitching. Finally, a study of respiratory activity in P9 rats found that whereas respiratory irregularity increased during periods of myoclonic twitching, mean respiratory rate did not (Karlen & Blumberg, 1999). Thus, the available data indicate that arousal, as commonly defined, is not a prerequisite for sniffing.

How might behavioral state modulate the threshold for sniffing to olfactory stimuli? Odorants in the nasal mucosa are detected by sensory neurons that provide input to mitral cells located within the main olfactory bulb (MOB). Olfactory bulb activity is influenced by respiration (Kay & Laurent, 1999; Macrides & Chorover, 1972), enhanced by familiar odors (Coopersmith & Leon, 1984), and modulated by behavioral and nutritional state (Gervais & Pager, 1979). Moreover, mitral cell activity in response to odorants is influenced in a reversible fashion by an animal's prior experience with that odorant, evidence of efferent modulation of mitral cell activity by other parts of the brain (Kay & Laurent, 1999).

The MOB receives a dense projection of noradrenergic fibers from the locus coeruleus (LC; Shipley, Halloran, & de la Torre, 1985), a large part of which is already present at birth in the rat (McLean & Shipley, 1991) and plays a functional role in learning during the 1st postnatal week (Sullivan, Wilson, & Leon, 1989; Wilson & Leon, 1988). The LC appears able to modulate the sensitivity of mitral cells in the MOB (Jiang, Griff, Ennis, Zimmer, & Shipley, 1996) and has long been implicated as a modulator of arousal and sleep (Aston-Jones, Chen, Zhu, & Oshinsky, 2001; Pace-Schott & Hobson, 2002). The LC responds more vigorously to stimuli that arouse an animal from sleep than to those that do not (Aston-Jones & Bloom, 1981). Thus, the LC may mediate state-dependent changes in olfactory sensitivity through modulation of mitral cell activity or through modulation of other components of the olfactory system, such as piriform cortex (Bouret & Sara, 2002).

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