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Stimulus parameters and temporal evolution of the olfactory evoked potential in rats

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Abstract. Evoked potentials were recorded from olfactory bulb, piriform cortex and scalp in urethane anesthetized rats in response to brief odorant stimuli (amyl acetate, phenylethyl alcohol, eugenol) presented through a nasal cannula by means of a constant flow olfactometer. The effects of stimulus duration, nasal cannula position, flow rate, concentration and interstimulus interval were examined. The highest amplitude potentials were evoked by 10% amyl acetate at 20 ms duration, 1000 ml/min flow rate and a 60-s interstimulus interval with the stimulus delivered at the nares. Odorant evoked potentials from deep within the olfactory bulb consisted of a triphasic wave with major components at 60 ms (P60), 90 ms (N90) and 140 ms (P140) with the latter two reversing polarity close to the surface of the bulb. Potentials recorded from layer I of piriform cortex were of similar amplitude, but opposite in polarity to the deep olfactory bulb potentials. Recordings from the skin over the nose elicited waveforms of similar morphology to the deep olfactory bulb potentials, but one-quarter the amplitude and of opposite polarity. The evoked potentials changed with repetitive stimulation. The N90 component was not present initially and only appeared after several stimuli. The appearance of the N90 component depended on the integrity of the olfactory peduncle. Thus, olfactory evoked potentials to odorant stimuli reflect dynamic aspects of the encoding of olfactory information dependent on connections between olfactory bulb and piriform cortex.

Introduction

Olfactory bulb potentials to odorant stimuli in mammals consist of 'induced' sinusoidal waves of 15–65 Hz (Adrian, 1950) and a slow, sustained, positive potential related to inspiration upon which the sinusoidal waves are superimposed (Ottoson, 1954). The slow potential begins 50 ms after the onset of the electro-olfactogram and precedes the induced waves by 20–30 ms (Moulton and Tucker, 1964). The slow potential is thought to be presynaptic, generated at the glomerulus (Ottoson, 1959), whereas the induced waves probably represent granule cell synaptic potentials in response to repetitive activation by mitral and tufted cells (Freeman, 1972). More recently, Inokuchi *et al.* (1986) have shown that a negative waveform recorded from the surface of the brain in rats, 110 ms after odorant stimulation, depended on the integrity of prepiriform cortex, olfactory tubercle and the anterior olfactory nucleus. We will provide details of olfactory evoked potentials in rats by recording from various scalp and intracranial sites testing the role of stimulus parameters, brain lesions and the temporal evolution of these potentials with repetitive odorant stimulation.

Materials and methods

Animals

Eleven male and 10 female Sprague–Dawley rats, weighing 225–770 g and ranging from 240 to 400 days of age were used in this study. Four were anesthetized with urethane (2.5 g/kg, i.p.) and acepromazine (10 mg/kg, i.p.), and 16 were anesthetized with urethane alone. One animal was anesthetized with nembutal (2 mg/kg, i.p.) and

ketamine (60 mg/kg, i.m.) for purposes of chronic recordings. Rectal temperature was monitored and maintained at 37°C during testing. Animals were killed by nembutal overdose at the end of the recording session.

Olfactometer

Olfactory stimuli were generated by a constant flow olfactometer (Figure 1) that delivered brief puffs of odorous air interrupting an otherwise continuous flow of humidified air. Input into the olfactometer consisted of charcoal-filtered medical air at 10 p.s.i., the volume flow rate being controlled by a flowmeter. At the outlet of the flowmeter, a T-connector diverted air flow into one of two 250-ml Pyrex gas washing bottles. One of these bottles contained 100 ml distilled water through which clean air was bubbled for humidification. The other gas-washing bottle was empty and the air simply flowed through to the odorant container. Odorant solutions were placed on a cotton ball inserted into a 7.5-ml glass test tube. The shafts of two 18 gauge sterile needles were inserted into the silastic stopper and served as the inlet and outlet ports of the odorant container. A 4-cm length of tubing connected the outlet of the odorant container to the normally closed port of a three-way solenoid valve (Lee Co., #LFAA1200118H). A longer, 1-m length of tubing connected the outlet of the gas washing bottle to the normally

Fig. 1. Scheme of the constant flow olfactometer and recording apparatus used to record the olfactory evoked potentials. At the top of the figure, the relative change in air velocity measured by a hot-wire probe 2 cm beyond the tip of the nasal cannula is shown for a 40-ms puff of air (indicated by the bar on the time axis). OB = olfactory bulb, PC = piriform cortex.

open port of the solenoid. A 10-cm length of tubing (0.76 mm i.d., 1.22 mm o.d.) functioned as nasal cannula, directing the air flow from the common port of the solenoid into one nostril. The side of stimulation was randomly selected. The tubing was replaced after each animal and odorant solutions were replaced monthly.

Odorant stimuli consisted of saturated vapors of pure solutions, including amyl acetate (Fisher Scientific), phenylethyl alcohol (Aldrich Chemical Co. Inc.) and eugenol (International Flavors and Fragrances, Inc.). Physiological saline was used as a control. Different stimulus concentrations of one odorant (amyl acetate) were obtained by preparing serial dilutions in diethyl phthalate (International Flavors and Fragrances, Inc.) in log steps of 100, 10, 1, 0.1 and 0.01%.

The olfactometer did not control for variables such as differences in volatility and rate of depletion of different odorants, nor for minor fluctuations in volume flow rate accompanying activation of the solenoid. The change in volume flow rate during activation of the solenoid was measured by a hot-wire sensor (Mohamed and LaRue, 1990) placed 2 cm beyond the tip of the nasal cannula with the clean air turned off. Using this method, stimulus onset occurred ~ 10 ms after solenoid activation with a rise time to maximum flow rate of < 5 ms (inset, Figure 1). We were unable to measure the change in odorant concentration over a brief 2–40 ms stimulus period and have assumed that the odorant rise time is similar to the volume flow rate curve. Based on the vapor concentration at room temperature, we estimated that a 1% solution of amyl acetate at a flow rate of 200 ml/min and 40 ms stimulus duration yields an odorant stimulus of ~ 70 p.p.m. with a total volume of 0.13 ml.

Recording sites

The head was placed in a stereotaxic instrument and a craniotomy was performed. The dura was removed to expose the entire dorsal surface of the olfactory bulb and cerebral hemisphere.

Olfactory bulb. Concentric, bipolar tungsten needle electrodes (separation = 0.5 mm) were placed 1.8 mm below the surface of the olfactory bulb in the granule cell layer under direct visualization using approximate surface coordinates: 8.0–9.0 mm anterior to Bregma, 1.2 mm lateral to midline. In one animal, odorant evoked potentials were recorded from the bulb surface and at 0.3 mm steps along the dorsal-ventral axis as the electrode was lowered into place. In another animal, four needle electrodes were placed in ipsilateral olfactory bulb, 1.8 mm below the surface, along the rostral-caudal axis at points 7, 8, 9 and 10 mm anterior to Bregma. In the one chronic animal, the olfactory bulb electrode was fixed in place by cementing the shaft to the skull with Kadon. Histological verification of electrode placement within the cellular layers of the bulb was not performed.

Scalp. Stainless steel needle electrodes were placed subcutaneously in the skin over the nose (~ 10 mm from the tip) and in the scalp over the parietal regions. All active electrodes were referenced to a subcutaneous needle electrode over the inion and a sacral electrode served as ground.

Piriform cortex. In three animals, concentric, bipolar tungsten electrodes (separation = 0.5 mm) were placed in piriform cortex using surface coordinates: 2.4 mm anterior

to Bregma, 2.8 mm lateral to midline, 6–8 mm below the dura at 14° from vertical. In the piriform cortex, evoked potentials were recorded from both poles of the electrode referenced to inion, in response to electrical stimulation of ipsilateral olfactory bulb (600 μ A current pulses, 200 μ s duration, 1 Hz rate). Localization in layer I of piriform cortex was verified by the occurrence of polarity reversal in the evoked potential recorded from the distal pole of the electrode.

Recordings

The electroencephalogram was amplified up to 20 000 times and filtered using a bandpass of 0.1 to 100 Hz (3 dB step attenuation). Evoked potentials were digitized at rates between 500 and 2000 Hz providing timebases of 256–1024 ms, including a prestimulus baseline of 10–20% of the timebase.

Experiments on stimulus parameters

Amyl acetate was used to study the effect of several stimulus parameters on the odorant evoked potential recorded from olfactory bulb.

Fig. 2. Olfactory evoked potentials recorded at four electrode sites (olfactory bulb, nose and scalp over the parietal areas) in one rat to four different odorants presented at 40 ms duration, 200 ml/min flow rate and 2-s interstimulus interval. The recording sites are referenced to inion. Note the different amplitude scales for each electrode site. The components have been labeled by their polarity and approximate latency in the olfactory bulb recordings with a symbol to allow their identification at the other electrode sites ($\dagger$ = P60, $\circ$ = N90, $\ddagger$ = P140). In this figure and in all subsequent figures, positivity at the recording site is plotted upwards; the time of the stimulus is represented by the bar on the time axes; and the number of trials per average is represented by n , shown directly above the time axes.

Table 1. Latency and amplitude of olfactory bulb potentials as a function of stimulus parameter

	P60		N90		P140	
	Latency (ms)	Amplitude (μ V)	Latency (ms)	Amplitude (μ V)	Latency (ms)	Amplitude (μ V)
Odorant <i>n</i> = 1						
Amyl acetate	63	202	93	-1	136	140
Phenylethyl alcohol	100	130	121	78	150	140
Eugenol	105	117	126	66	156	120
Cannula (mm) <i>n</i> = 1						
-8	99	62	123	26	156	99
-3	70	50	98	106	155	81
0	60	150	91	240	143	354
3	60	115	87	92	138	287
8	62	30	92	85	144	138
Duration (ms) <i>n</i> = 1						
2	73	95	105	20	138	149
5	61	162	94	25	127	173
10	60	184	91	58	130	141
20	61	199	91	41	130	142
40	61	200	94	10	133	140
Flow rate (ml/min) <i>n</i> = 2						
200	116	4	150	-6	176	1
400	104	9	146	25	191	6
600	94	26	134	66	172	7
800	85	34	119	54	149	15
1000	82	57	121	64	154	3
1200	87	59	123	77	161	7
Concentration (%) <i>n</i> = 3						
0.01	147	5	180	-2	233	8
0.1	132	4	165	-3	215	8
1	104	6	134	-1	180	17
10	85	48	110	-6	150	49
100	112	21	143	-2	180	32
Interval (s) <i>n</i> = 9						
2	66	8	96	-30	144	15
30	60	7	90	-25	138	24
60	62	26	83	6	129	61
Mean (SD)	63 ($\pm$ 11)	13 ($\pm$ 14)	90 ($\pm$ 11)	-18 ($\pm$ 32)	137 ($\pm$ 16)	33 ($\pm$ 27)

Nasal cannula position. Nasal cannula position was examined at six different nasal cannula positions inside and outside the nostril: two inside the nasopharynx (within the dorsal nasal meatus) at +8 mm and +3 mm insertion, one at the opening of the nostril (0 mm), two outside the nostril at -3 mm and -8 mm away from the nostril opening, and one at -8 mm directed over the animal's head. Flow rate was maintained at 200 ml/min, stimulus duration at 20 ms and interstimulus interval at 2 s.

Duration. Stimulus durations of 40, 20, 5 and 2 ms were tested in one animal. Flow rate was maintained at 500 ml/min, interstimulus interval at 2 s and nasal cannula insertion at +3 mm.

Flow rate. Flow rates of 200, 400, 600, 800, 1000 and 1200 ml/min were tested in two animals while maintaining stimulus duration at 20 ms, interstimulus interval at 2 s and cannula position at +3 mm.

Concentration. Odorant concentration was studied in three animals. Stimuli were presented in order of increasing concentrations of 0.01, 0.1, 1.0, 10 and 100% while

Fig. 3. Olfactory bulb and nasal scalp potentials recorded along two dimensions of the olfactory bulb. (A) Potentials recorded from four electrodes placed along the rostral-caudal axis of the olfactory bulb at a depth of 1.8 mm below the surface of the bulb. The scale of '10' to '7' represents distance in millimetres from the Bregma. Note the phase reversal in the most rostral recording ('10'). (B) Potentials recorded from an electrode 8 mm anterior to Bregma at different depths in the olfactory bulb along the dorsal-ventral axis. Note the phase reversal between the surface and deep layers beginning at 0.9 mm depth. Simultaneous averages recorded from the skin over the nose are superimposed in the top trace to show the degree of variability in the scalp potentials while recording at different levels in the bulb. Potentials in both (A) and (B) were evoked by 10% amyl acetate at 20 ms duration, 1000 ml/min flow rate and 5 s interstimulus intervals. The components are indicated by the vertical interrupted lines.

maintaining flow rate at 500 ml/min, stimulus duration at 20 ms, interstimulus interval at 2 s and nasal cannula position at +3 mm.

Interstimulus interval. The effect of interstimulus interval was examined in nine rats: three at intervals of 2 s, three at intervals of 30 s, and three at intervals of 60 s. The animals had received no odorant stimulation prior to testing. Stimulus parameters remained constant at 1% concentration, 20 ms duration, 500 ml/min flow rate and +3 mm cannula position. Thirty stimuli were presented in three blocks of 10 trials each. Each block of 10 trials was followed by a 10 min rest period during which air flow through the nasal cannula was discontinued.

Data analysis

Averages of either 10–60 trials or single trials were made. Peak latencies of the evoked potential components were measured from stimulus onset to the point of maximum voltage or to a point extrapolated from the intersection of the ascending and descending limbs. Peak amplitudes of the averaged evoked potential components were defined as the difference between the maximum voltage of a component and the average voltage of the prestimulus baseline. In the interstimulus interval experiment, peak amplitudes and latencies were subjected to analysis of variance (ANOVA) for the factors of interstimulus interval (60, 30, 2 s) and stimulus block (1, 2, 3). For the analysis of single trials, component amplitudes were measured at the peak latency determined from the averaged evoked potential for each animal and subjected to ANOVA for the factors

Fig. 4. Evoked potentials recorded from piriform cortex to electrical stimulation of the olfactory bulb (left panel) showing a phase reversal between layers I and III. In the right panel, odorant evoked potentials from these two piriform sites and olfactory bulb and nose are shown. Note the phase reversal of the N90 component between layers I and III and the increase in N90 amplitude between the first ('1') and third ('3') blocks of stimuli. The odorant stimuli were presented in blocks of 10 trials using amyl acetate (20 ms duration, 200 ml/min flow rate) at 2-s interstimulus interval with a 5-min interval between stimulus blocks.

of interstimulus interval (60, 30, 2 s), stimulus block (1, 2, 3) and trial number within each block (1, 2, 3 . . . 10). Duncan's range test was used for *post hoc* analysis.

Lesion experiments

The olfactory peduncles were transected bilaterally in three rats. In two rats, evoked potentials were recorded from the ipsilateral olfactory bulb and nose, both before and after transection. In one rat, the olfactory peduncle was transected prior to odorant stimulation and evoked potentials were recorded under nembutal anesthesia from a chronically implanted olfactory bulb electrode up to 3 months after surgery. The odorant stimulus consisted of 1% amyl acetate at 20 ms duration, 500 ml/min flow rate and 60 s interstimulus interval. Complete transection of the peduncle was verified post-mortem after perfusion.

In one animal, evoked potentials were recorded from piriform cortex and nose (10% amyl acetate, 20 ms duration, 1000 ml/min flow rate, 5 s interstimulus interval) before and after aspiration of both olfactory bulbs.

Results

Recording sites

Olfactory bulb. Evoked potentials from one animal in response to amyl acetate, phenylethyl alcohol and eugenol are shown in Figure 2. Potentials were similar for each odor and consisted of a large amplitude (up to 200 μ V) triphasic wave whereas a small (40 μ V) positive wave accompanied saline stimulation. The latency of the peaks of components varied considerably between animals (up to 20 ms) and from average to average within the same animal (up to 10 ms) even when stimulus parameters were constant. We have named the three components of the olfactory bulb potential P60, N90 and P140 reflecting their polarity and approximate median latencies based on measures from the interstimulus interval experiment with amyl acetate. In the figures and tables the actual latencies of the components depicted may differ from these labels, e.g. P60 occurs at 100 ms when stimulating with phenylethyl alcohol and eugenol (Table I).

Regression analysis of the P60, N90 and P140 component amplitudes and latencies was performed to determine the effect of age, weight and elapsed time of anesthesia on the olfactory bulb potential recorded from nine animals using constant stimulus parameters. No correlation was found for these variables. The effects of gender and side of stimulation were tested by ANOVA. A significant ($P < 0.05$) effect was found for gender, with the P60 latency being 7.5 ms earlier in female than in male rats.

Evoked potentials recorded along the rostral-caudal axis of the olfactory bulb at 1.8 mm depth were similar except at the most rostral position where all of the components of the evoked potential reversed in polarity. At the rostral position, the olfactory bulb potentials resembled the nose recordings (compare recordings at '9' and '10', Figure 3A). Evoked potentials recorded along the dorsal-ventral dimension of the olfactory bulb showed potentials recorded at a depth of 1.8 mm to be of largest amplitude (Figure 3B). The peak latencies of the P60 varied up to 16 ms whereas the N90 varied 6 ms at the different recording sites. Simultaneous potentials recorded from the nose also were characterized by latency shifts suggesting that the changes within

the bulb reflect trial to trial variations rather than a systematic alteration as a function of depth of recording. The P60 component was of positive polarity throughout the depth of the olfactory bulb. In contrast, the N90 and P140 components recorded from the deep layers (1.2–1.8) became relatively isopotential at 0.6 mm depth and reversed in polarity at the surface (0). An additional short duration (25 ms), negative component was recorded at the surface just following the P60 component.

Scalp. The evoked potentials recorded from the skin overlying the nose were up to 25% of the amplitude of the deep olfactory bulb potentials and of opposite polarity (see Figures 2–4). The three components were superimposed on a slow negative potential which lasted 100–200 ms longer than the P140 waveform in the olfactory bulb. In some recordings, a positive component at 35 ms could be identified (see Figure 4, right panel, bottom traces). No reproducible potentials were seen in response to saline (Figure 2).

Evoked potentials recorded over the ipsilateral parietal scalp were both smaller than and not as reproducible as the potentials derived from nasal scalp (Figure 2). For amyl acetate, an initial negative wave occurred at 67 ms corresponding to the P60 component of the olfactory bulb potential. This was followed by a positive wave at 93 ms, corresponding to N90 of olfactory bulb. A second negative wave peaking at 114 ms was so much shorter in duration than the P140 of olfactory bulb that a second positive wave at 145 ms was thought to correspond best to the P140 component. Two additional components were unique to the ipsilateral parietal potentials: a negative wave at 290 ms latency with an amplitude of $-9 \mu\text{V}$ and a positive wave at 480 ms with an amplitude of $6 \mu\text{V}$.

The averaged potentials recorded from contralateral parietal scalp in response to amyl acetate consisted of a series of sinusoidal waves at a frequency of 17–20 Hz (Figure

Fig. 5. Olfactory bulb potentials from one rat in response to amyl acetate at different nasal cannula positions are shown in the right panel. P60 amplitude and latency are plotted in the left panel as a function of the distance of the nasal cannula from the nostril (mm); negative numbers representing distance outside the nostril and positive numbers representing distance inside the nose.

2). These waves were reliably detected during the first 350 ms of recording, but in some records were seen as long as 700 ms post-stimulation. The sinusoidal waves were preceded by a negative wave at 60 ms and a positive wave at 106 ms corresponding to the P60 and N90 components of the olfactory bulb potential, respectively. Similar sinusoidal waves were also occasionally seen in the evoked potentials recorded from the other electrodes.

Piriform cortex. Two types of evoked potentials recorded from piriform cortex are shown in Figure 4. Electrical stimulation of olfactory bulb resulted in evoked potentials similar to those reported by Stripling *et al.* (1988), consisting of an initial biphasic negative wave in layer I at 5–13 ms latency, followed by a positive wave at 29 ms. These potentials reversed in polarity in layer III. In response to the odorant amyl acetate, the potentials corresponding to the N90 and P140 of olfactory bulb consisted of a positive

Fig. 6. The effect of stimulus duration (A), flow rate (B) and concentration of amyl acetate (C) on the P60 amplitude and latency (left panels). Olfactory bulb potentials from one animal are shown in the panels on the right.

wave occurring at 108 ms and a negative wave at 160 ms which also reversed polarity between layers I and III.

Stimulus parameters

The three components of the evoked potential changed systematically in response to changes in stimulus parameters. Only the changes in the P60 component are presented below with the effects on amplitudes and latencies for all three components contained in Table I.

Nasal cannula position. Nasal cannula positions at the opening (0) and 3 mm within the nostril (+3 mm) yielded olfactory bulb potentials of the greatest amplitude (Figure 5). P60 latency increased and P60 amplitude decreased as the cannula was moved away from the nostril. No evoked potential could be recorded when the air flow was directed over the animal's head.

Duration. There was a rapid growth in the amplitude of the P60 component as stimulus duration increased reaching an asymptotic value of 20 ms duration (Figure 6A). P60 latency shortened between stimulus durations of 2 and 5 ms, but not with further increments to 40 ms.

Flow rate. The latency of P60 decreased and the amplitude increased with increasing flow rate up to 1000 ml/min (Figure 6B).

Concentration. The P60 latency decreased and P60 amplitude increased with increasing concentrations of amyl acetate, up to the 10% solution (Figure 6C).

Interstimulus interval. Interstimulus interval affected only the P140 amplitude. P140 was larger at the 60-s interstimulus interval than either the 30-s or 2-s intervals ($P = 0.009$). Analysis of the P60 and N90 components revealed no significant differences for interstimulus interval, but an inverse trend for the N90 component was

Fig. 7. The mean amplitude and standard deviation of the P60, N90 and P140 components from nine animals are plotted as a function of interstimulus interval in the left panel. Three blocks of 10 trials were presented either at 2-, 30- or 60-s interstimulus intervals. Significant effects were found for the P140 amplitude only (asterisk denotes $P = 0.009$). In the right panel, averages of the evoked potentials from the three blocks are superimposed for each group.

seen with it being smallest at the 60-s interval and increasing at shorter intervals. The superimposition of the averages from the three blocks of trials in Figure 7 shows the form of the evoked potentials varied from block to block, and in some the definition of the P60–N90 component was obscured. These changes were investigated by examining the single trials comprising the averages.

Single trials

The evoked potentials at the time of the N90 component changed on a trial-by-trial basis as the stimulus was repeated. The grand averages of the nine animals (Figure 8, middle panel) show that on the first block of trials, the N90 component was not evident till the fourth trial and then increased in amplitude through the remaining trials comprising the stimulus block. In the second block of stimulation (after a 10 min rest period), the N90 component was present on the first trial and continued to increase in amplitude with further trials. In the third block, the N90 component, evident on the first trial, increased slightly in amplitude on further trials. The mean P60–N90 peak-to-peak amplitude from the single trials of all nine animals is plotted in the left

Fig. 8. Grand averages of the olfactory bulb potentials from nine rats for each single trial (middle panel) and an example of the single trials from one of the rats (right panel) are shown. The development of the N90 component (indicated by the vertical interrupted line) varied between animals, but was usually not seen until the fourth odorant presentation in the first block of stimuli. After 10-min rest periods, second and third blocks of stimuli were presented. The N90 component was usually present on the first trial of each of the subsequent blocks and continued to increase slightly in amplitude with repeated stimulation. In the left panel, the mean P60–N90 peak-to-peak amplitudes from the single trials are plotted with the standard error for the last trial of each stimulus block (indicated by the horizontal line).

panel of Figure 8. Statistical analysis (ANOVA) of the data from the individual trials comprising the grand averages showed that the P60–N90 peak-to-peak amplitude differed significantly across trials ($P = 0.001$) with the first two trials being less than subsequent trials. Examining the results from the first stimulus block in each animal separately (an example of one of them is plotted in Figure 8, right panel) revealed the N90 component to appear on the first trial in one animal, the second trial in four animals, the third trial in one animal, the sixth trial in one animal, and the eighth trial in the remaining two animals. The N90 component, once established, appeared earlier on subsequent blocks of trials even with a 10 min intervening period without stimulation: on the first trial in seven of nine rats in block 2 and on the first trial in six of nine rats in block 3.

Lesions

In two animals, the P60–N90 component which had appeared during repetitive stimulation disappeared after transection of the olfactory peduncle for the remainder of the experiment (Figure 9A). In the animal in whom the olfactory peduncle was transected chronically prior to odorant stimulation, the ipsilateral olfactory bulb potentials showed only a sustained positive component (P140) without a P60 or N90 waveform ever appearing over the three months of testing. Aspiration of the olfactory bulbs was accompanied by the loss of olfactory evoked potentials from piriform cortex and nose (Figure 9B).

Discussion

This study shows that the morphology of evoked potentials recorded from olfactory bulb changes with stimulus repetition. The N90 component is not present on the first stimulus trial in all but one of the nine animals in whom single trials were recorded, and becomes of robust amplitude only by about the seventh trial. Obliteration of the P60–N90 component of the olfactory bulb potential or its failure to develop after transection of the olfactory peduncle indicates that connections between olfactory bulb

Fig. 9. Evoked potentials from olfactory bulb and nose (A) and piriform cortex and nose (B) in response to amyl acetate before and after specific lesions. In (A), note the disappearance of the P60–N90 component following transection of the olfactory bulb peduncle.

and piriform cortex are necessary for the appearance of this component. The relevance of this interconnection for olfactory processes has also been suggested by the studies of Chaput (1983) and Potter and Chorover (1976) who showed changes in odorant evoked mitral cell activity in olfactory bulb following transection of the lateral olfactory tract. Stripling *et al.* (1988) have shown an increase in the amplitude of a positive component of the piriform cortex potential elicited by rapid repetitive electrical stimulation of the olfactory bulb lasting up to 30 days. This positive component occurs at the approximate latency of inhibitory post-synaptic potentials in pyramidal cells to electrical stimulation of the lateral olfactory tract (Haberly and Bower, 1984). The component of the piriform cortex potentials at ~ 90 ms in response to odorant stimulation recorded in the present study shares a number of features with the positive component identified by Stripling *et al.* (1988) evoked by electrical stimulation. The orientation and phase of the components in the two studies were similar and both increased with repetitive stimulation. Computer simulation of the olfactory bulb–piriform cortex circuit (Ambros-Ingerson *et al.*, 1990) has suggested that the development of long-term potentiation in cortical neurons clustered by shared stimulus characteristics and shared mitral cell populations perform a hierarchical categorization of olfactory stimuli. ‘Learning’ in this model occurs over several iterations of the sampling cycle. Grajski and Freeman (1989) have found transient changes in the spatial pattern of the electroencephalogram recorded from the surface of the olfactory bulb during the first four to six non-reinforced presentations of a novel odor followed by the development of a stable spatial electroencephalographic pattern with continued odorant stimulation. The observation in the present study that the N90 component developed only after the first few stimuli is a clear demonstration of such dynamic processing in olfactory circuits using natural odorant stimuli.

The polarity reversal of the primary olfactory evoked potential components between deep and superficial layers of olfactory bulb in the present study are similar to findings in other mammals employing electrical stimulation of olfactory mucosa (Yamamoto, 1961) and primary olfactory nerve (Martinez and Freeman, 1984). Olfactory bulb potentials share a negative–positive–negative waveform at the surface and in the superficial layers of the bulb down to the external plexiform layer and a positive–negative–positive waveform below the mitral cell body layer. Martinez and Freeman (1984) have determined that the first component reflects both depolarization of granule cells and periglomerular cell activity. Since waveform morphology and laminar distribution are similar to those elicited by electrical stimulation of primary olfactory bulb afferents, the granule and periglomerular cells may also be involved in the generation of odorant evoked potentials. Further conclusions regarding the generators of the olfactory bulb potentials are limited because of the lack of histological verification of electrode placement relative to the cellular layers of bulb.

Although the laminar distribution of the potentials evoked by these various methods is similar, the peak latencies are different. Martinez and Freeman (1984) have obtained latencies of ~ 12 ms for the first surface-negative component evoked by electrical stimulation of the primary olfactory nerve. In the present study, using odorant stimuli, the initial component of the olfactory bulb potentials begins as early as 30 ms after stimulus onset (marked by the opening of the solenoid). Thus, considering travel time of the odorant stimulus through the nasal cannula and nasopharynx (~ 10 ms) and the conduction time along primary olfactory nerve (~ 10 ms), we estimate that perireceptor

events can occur as rapidly as 10 ms. This is more consistent with measurements of photoreceptor potential latencies of 3–15 ms (Barash *et al.*, 1988) than the 140–150 ms transduction times measured in salamander olfactory epithelium with extracellular recordings (Getchell, 1986) and in isolated olfactory receptor cells using voltage clamping (Firestein and Werblin, 1989).

The difference in the latencies of olfactory potentials for amyl acetate, phenylethyl alcohol and eugenol could be related to a number of factors. The onset latency of the olfactory bulb potential correlates inversely with the logarithm of the vapor pressures (Stull, 1947) for the three odorants tested. Since odorant thresholds were not determined, latency differences for the three odorants may also be related to disparities in stimulus intensity level. Differences in trigeminal activation between odorants might also contribute to the variation in latency. Kobal and Hummel (1988) have found that latencies of human olfactory potentials recorded from the scalp to vanillin, a 'pure' olfactory stimulant, are reduced by 50–150 ms when CO₂, a trigeminal stimulant, is mixed with the vanillin. In the present study, phenylethyl alcohol and eugenol, also 'pure' olfactory stimulants (Doty *et al.*, 1978), yield latencies that are 40 ms longer than those produced by amyl acetate, which activates both olfactory and trigeminal nerves.

Evoked potential amplitudes and latencies change systematically with changes in stimulus parameters. The increase in component amplitudes with increasing flow rate corresponds to comparable changes in amplitude of the electro-olfactogram (Moulton and Tucker, 1964), amplitude of the slow bulb potential (Gault and Leaton, 1963), amplitude and frequency of the induced waves of the bulb (Domino and Ueki, 1960) and amplitude of human chemosensory evoked potentials (Kobal and Hummel, 1988). Døving (1987) has found that the response frequency of olfactory bulb neurons to 1% amyl acetate increases with increasing flow of suction from 5 ml/min to 500 ml/min, but do not increase further at flow rates of 1000 ml/min. Døving estimates that rats inhale ~1 ml per sniff over a 50-ms period at a mean inhalational flow rate of 1200 ml/min. The results of the present study are consistent with this estimate.

The amplitude of the P140 component from olfactory bulb is significantly affected by interstimulus interval, being greater at intervals of 60 s than at 2- and 30-s intervals. These results are comparable to those obtained by Ottoson (1956), who has observed a 20% reduction in amplitude of the electro-olfactogram for repetitive stimulation at intervals of 20–60 s and a 50% reduction at intervals of <10 s. Single unit recordings from olfactory mucosa (Getchell, 1986) and from olfactory bulb (Potter and Chorover, 1976) have shown similar, slowly changing properties. However, the study by Potter and Chorover (1976) demonstrates that the recovery of mitral cell firing to odorant stimulation takes much longer (15–30 min) than the recovery of the electro-olfactogram (30–90 s). Thus, although the effect of interstimulus interval on the P140 amplitude may be due to both peripheral and central processes, the relatively rapid 'recovery' of P140 amplitude is more consistent with processes of fatigue/adaptation in olfactory receptor neurons due to desensitization in the chemoreceptive membrane than with habituation occurring centrally in the olfactory bulb or cortex.

The results of this study show that olfactory evoked potentials can be reliably recorded from the olfactory bulb, piriform cortex and scalp of anesthetized rats on response to odorant stimuli. The design of the olfactometer enables the use of brief, intermittent, controlled odorant stimuli with a rapid stimulus onset. The changes seen in the olfactory

bulb potential with increasing distance of the nasal cannula from the opening of the nostril demonstrates that the evoked potential is related to changes in the air flow and not due to auditory, visual or vibratory stimulation time-locked to solenoid activation. The long-latency, low-amplitude, monophasic olfactory bulb potential seen in response to saline may reflect contributions from either olfactory or non-olfactory chemosensory afferents. However, the loss of the triphasic waveform after olfactory bulb ablation indicates that olfactory structures are the major contributors to the scalp evoked potential.

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