Thalamic olfaction: characterizing odor processing in the mediodorsal thalamus of the rat

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Courtiol E, Wilson DA. Thalamic olfaction: characterizing odor processing in the mediodorsal thalamus of the rat. J Neurophysiol 111: 1274–1285, 2014. First published December 18, 2013; doi:10.1152/jn.00741.2013.—Thalamus is a key crossroad structure involved in various functions relative to visual, auditory, gustatory, and somatosensory senses. Because of the specific organization of the olfactory pathway (i.e., no direct thalamic relay between sensory neurons and primary cortex), relatively little attention has been directed toward the thalamus in olfaction. However, an olfactory thalamus exists: the mediodorsal nucleus of the thalamus (MDT) receives input from various olfactory structures including the piriform cortex. How the MDT contributes to olfactory perception remains unanswered. The present study is a first step to gain insight into the function of the MDT in olfactory processing. Spontaneous and odor-evoked activities were recorded in both the MDT (single unit and local field potential) and the piriform cortex (local field potential) of urethane-anesthetized rats. We demonstrate that: 1) odorant presentation induces a conjoint, coherent emergence of beta-frequency-band oscillations in both the MDT and the piriform cortex; 2) 51% of MDT single units were odor-responsive with narrow-tuning characteristics across an odorant set, which included biological, monomolecular, and mixture stimuli. In fact, a majority of MDT units responded to only one odor within the set; 3) the MDT and the piriform cortex showed tightly related activities with, for example, nearly 20% of MDT firing in phase with piriform cortical beta-frequency oscillations; and 4) MDT-piriform cortex coherence was state-dependent with enhanced coupling during slow-wave activity. These data are discussed in the context of the hypothesized role of MDT in olfactory perception and attention.

Olfaction; mediodorsal thalamus; dorsomedial thalamus; single unit; LFP; slow-wave sleep; piriform cortex

Olfaction is a unique sensory modality in terms of its anatomic organization. In fact, for all senses except olfaction, the information from the sensory neurons necessarily passes through a thalamic nucleus before reaching the primary sensory cortex. In olfaction, sensory neurons project directly to the olfactory bulb, which, in turn, projects to the olfactory cortex, including the piriform cortex (PCX; Haberly and Price 1977), the cortical nucleus of the amygdala, the olfactory tubercle, the lateral entorhinal cortex, and the anterior olfactory nucleus (Haberly and Price 1978; Price and Powell 1971). Although there is no thalamic relay between the olfactory sensory neurons and the olfactory cortex, an olfactory thalamic nucleus exists. The mediodorsal thalamic nucleus (MDT) receives direct input from the PCX (Cornwall and Phillipson 1988; Heimer 1968; Kuroda and Price 1991; Powell et al. 1963; Price 1985; Price and Slotnick 1983) and, in turn, projects to the orbitofrontal cortex (OFC; Krettek and Price 1977), forming a transthalamic PCX-MDT-OFC pathway. The OFC also has direct reciprocal connections with the PCX (Illig 2005; Schoenbaum and Eichenbaum 1995). The MDT additionally receives direct input from other olfactory structures, such as the olfactory tubercle, the cortical nucleus of the amygdala, and the entorhinal cortex (Bay and Cavdar 2013; Krettek and Price 1974; Price 1985; Price and Slotnick 1983; Ray and Price 1992).

In other sensory systems, the thalamus is involved in many functions ranging from basic sensory information processing (McCormick and Bal 1994; Saalmann and Kastner 2009) to more complex functions, such as sensory gating and attention modulation (Coulh 1998; Newman 1995), control of sleep states (Steriade 1992), and memory processing (Jankowski et al. 2013). Despite the unusual anatomic arrangement of the olfactory pathway, does the MDT play similar roles in olfaction? Some evidence does suggest different roles for the MDT in olfaction (Tham et al. 2009). MDT single units have been shown to respond to both lateral olfactory tract and odorant stimulation (Benjamin and Jackson 1974; Inamura et al. 1984; Jackson and Benjamin 1974; Motokizawa 1974; Price 1985; Price and Slotnick 1983; Takagi 1986; Yarita et al. 1980). In addition, evidence from lesion studies has supported a role for the MDT in odor learning and memory by demonstrating, for example, that MDT lesions do not lead to anosmia but impair odor reversal learning (Eichenbaum et al. 1980; Koger and Mair 1994; Sapolsky and Eichenbaum 1980; Slotnick and Kaneko 1981; Slotnick and Risser 1990; Staubli et al. 1987). Finally, several groups have proposed a role for the MDT in olfactory attention (Plailly et al. 2008; Tham et al. 2009, 2011a,b; Veldhuizen and Small 2011). In humans, using functional magnetic resonance imaging (fMRI), Plailly et al. (2008) showed specific changes in functional connectivity in the PCX-MDT-OFC transthalamic pathway during olfactory attention processing.

To examine more closely the contributions of the MDT to olfactory perception, the present study begins a more detailed analysis of how the MDT contributes to olfaction in the rodent and how its activity is shaped by its primary olfactory afferent, the PCX. As a first step, we characterized MDT single-unit and local field potential (LFP) spontaneous and odor-evoked activity and examined the relationship between MDT activity and PCX activity in urethane-anesthetized rats.

MATERIALS AND METHODS

Subjects

A total of 34 male Long-Evans rats (>200 g) obtained from Charles River Laboratories were used in the present study. All animals...
were group-housed ranging in groups of 3–4 animals in polypropylene cages. Food and water were available ad libitum. Experimental procedures were developed in accordance with, and reviewed and approved by, the Institutional Animal Care and Use Committee at Nathan Kline Institute for Psychiatric Research and National Institutes of Health guidelines for the proper treatment of animals.

Experimental Design

Animal preparation. Rats were anesthetized with urethane (1.25 g/kg ip with additional supplements as needed) and placed in a stereotaxic apparatus. The animals were placed on a heating pad to maintain constant body temperature.

Electrophysiological recordings. Single-unit recording procedures for the MDT were performed similar to previous reports (Wilson 1998; Xu and Wilson 2012). Single units were recorded using a tungsten microelectrode (1–5 MΩ), and signals were acquired (sampling rate: 10 kHz) and analyzed with Spike2 (CED). MDT units were identified by histological confirmation (Fig. 1) with coordinates ranging from lateral olfactory tract stimulations were used to determine the electrode position in the PCX. Respiration was monitored throughout the recording session with a piezoelectric device placed under the animal’s chest.

Odorant stimulation. Odors were delivered with a flow-dilution olfactometer that was positioned 2 cm from the animal’s nose. Odor vapor was added with a computer-controlled solenoid at a rate of 0.1 l/min to a constant flow of nitrogen gas (N₂) at 1 l/min. Odors were administered for 2 s per trial with at least a 30-s interstimulus interval.

We used 3 categories of odorants: mixtures and monomolecular and biological odorants. The 3 mixtures were 10c, 10c-1, and 10cR1. Components of the mixture have been detailed elsewhere (Barnes et al. 2008; Chapuis and Wilson 2012; Lovitz et al. 2012). Mixture 10c comprised 10 components: isoamyl acetate, nonane, ethyl valerate, 5-methyl-2-hexanone, isopropylbenzene, 1-pentanol, 1,7-octadiene, 2-heptanone, heptanal, and 4-methyl-3-penten-2-one. Isoamyl acetate was added to the mixture to produce 10c-1, and it was replaced with limonene in 10cR1. Mixtures were created by adding odorant components to mineral oil in amounts that provided concentrations of 100 parts per million (ppm) for all components except 1,7-octadiene, which, due to a calculation error, was at 442 ppm. Two monomolecular odorants were also used: ethyl valerate and isoamyl acetate. Ethyl valerate was used with an identical concentration as in the mixture. Isoamyl acetate was used at 2 concentrations: 1 with an identical concentration as in the mixture (ISO1) and 1 with a ppm 65-fold higher than ISO1 (ISO2). Finally, 2 biological odorants were used: fresh dirty Litter and rat Feces. These components were used pure and obtained from the animal’s own cage.

Data Analysis

Data analysis was performed using Spike2 and Excel. LFPs. For all odorant stimulations combined, power spectra [fast Fourier transform (FFT) size, 0.2048 s; Hanning window] were calculated for the 0- to 3-s period after stimulus onset and compared with the power of the 3-s prestimulus baseline. FFTs were also calculated in three respiratory cycles before odor onset and in six cycles after odor onset for two representative odors (10cR1 and dirty Litter).

To quantify the phase-locking of MDT units relative to beta cycle, raw LFPs (MDT and PCX) were band-pass filtered between 15 and 35 Hz. Beta cycles (negative troughs) were then detected using a threshold of −2 SD of the filtered signal in both PCX and MDT.

To measure the coherence between MDT and PCX LFP activities, we used a coherence script within Spike2. Values of coherence were either determined on the entire duration of the file (including both spontaneous and odor-evoked activity) or calculated for two representative odors (10cR1 and dirty Litter) on the 3-s period before the stimulus onset and the 0- to 3-s period after the stimulus onset. A ratio of the 0- to 3-s period after stimulus onset on the 3-s prestimulus period was then determined for statistical comparisons.

To quantify the troughs of slow waves (SW; MDT and visual neocortex) or sharp waves (SPWs; PCX), raw LFPs (MDT, PCX, and visual neocortex) were low-pass filtered to <20 Hz. We used a custom peak-trough-level Spike2 script to detect SW or SPW troughs using a threshold of −1 SD of the filtered signal. This SW/SPW event detection also allowed us to identify transitions between slow-wave and fast-wave activities (SWA and FWA, respectively).

Single units. Single-unit identification and analyses were all performed using Spike2 using both template-matching and principal component analyses. Individual units were identified by having at least a 2-ms refractory period in interval histograms. In a subset of our units (22/106), we observed high-frequency burst activity as described...
in nucleus reticularis thalamic, perigeniculate, and visual neocortical neurons (Gray and McCormick 1996; Kim and McCormick 1998; Mukhametov et al. 1970). For those units, we tolerated a reduced refractory period (those units having at least a 1-ms refractory period). This subset of units showed the same type of response as the other units and was thus not discarded from the analysis.

Responses to the different odor stimuli were analyzed by comparing spike activity 3 s before and 3 s after stimulus onset. Spontaneous activity was calculated to be the per-second average of the prestimulus activity across all of the prestimulus activities. We also calculated odor-evoked receptive fields for individual unit. Response magnitudes within individual neurons were normalized to the response of the odor evoking the maximal response (either an excitation or a suppression that was considered as the best odor) in that unit to obtain a relative response magnitude to each odor for each unit (Xu and Wilson 2012). Finally, we also calculated the slope of the receptive fields of the unit using the slope function in Excel, based on change in response magnitude with rank order of the stimulus response (Xu and Wilson 2012).

**RESULTS**

A total of 106 MDT units were obtained from 34 male Long-Evans rats. In 18 out of these 34 animals, simultaneous LFP recordings were performed in both MDT and PCX (with 57 units recorded in the MDT). Electrode tip positions in MDT are shown in Fig. 1.

In the present study, we wanted to determine the odor response of the MDT in urethane-anesthetized rats and characterize the relationship between MDT activities and PCX LFPs.

**Odor Responses in the MDT**

The odor-evoked response of LFPs in the MDT. We observed that, in urethane-anesthetized rats, odorant stimuli induced the emergence of beta (15–35 Hz) oscillation as shown in the raw example of Fig. 2A. Confirming the raw example, we observed a significant increase of beta-band power during odor presentation (Fig. 2B). We also found a significant increase of power in other oscillatory bands ranging from 7 to 46, 51 to 53, and 65 to 70 Hz, frequencies in theta (2–12 Hz), beta (15–35 Hz), and gamma (36–80 Hz) bands [repeated-measures ANOVAs with the period as the factor and post hoc Fisher test; $n = 34$ animals]. Mean ($\pm$ SE) FFT values for each frequency band are also represented with the same color as previously (paired t-test). Although mean preodor and odor values were very close (e.g., in the gamma-frequency band), within-animal repeated-measures analyses revealed significant odor-evoked changes. C: mean ($\pm$ SE) FFT ratios between odor-evoked and prestimulus periods as a function of oscillatory frequencies ($n = 34$ animals). Although oscillatory activity was broadly elevated across frequency by odor stimulation, the most robust variations of ratio can be observed in the beta band. $*P < 0.05; **P < 0.001$.}

![Fig. 2. Odor-evoked local field potentials (LFPs) in the MDT. A, top: raw LFP trace recorded in the MDT before and during odor presentation (black horizontal line); bottom: raw LFP trace recorded in the MDT before and during odor presentation (black horizontal line); bottom: is the associated sonogram. B: mean ($\pm$ SE) fast Fourier transform (FFT) values (logarithmic scale) as a function of the period (pre-stimulus in gray and odor-evoked in black). Since FFT values decrease as a function of frequency, we split the frequencies into 3 different frequency groups, from 2 to 12 Hz (theta), 15 to 35 Hz (beta), and 36 to 80 Hz (gamma), from the left to the right, respectively (repeated-measures ANOVAs with the period as the factor and post hoc Fisher test; $n = 34$ animals). Mean ($\pm$ SE) FFT values for each frequency band are also represented with the same color as previously (paired t-test). Although mean preodor and odor values were very close (e.g., in the gamma-frequency band), within-animal repeated-measures analyses revealed significant odor-evoked changes. C: mean ($\pm$ SE) FFT ratios between odor-evoked and prestimulus periods as a function of oscillatory frequencies ($n = 34$ animals). Although oscillatory activity was broadly elevated across frequency by odor stimulation, the most robust variations of ratio can be observed in the beta band. $*P < 0.05; **P < 0.001$.](http://jn.physiology.org/)

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**Histology**

After recording, rats were overdosed with urethane and transcardially perfused with saline and 4% (wt/vol) formaldehyde. Frontal brain sections (40 μm thick) were cut using a microtome (Leica) and stained with cresyl violet to identify electrode positioning in MDT (Fig. 1).
ANOVAs, \( F(1,1089) = 5.235, \ P < 0.05 \); post hoc Fisher, \( P < 0.05 \); Fig. 2B, but it was clear from the FFT ratio (odor-evoked/prestimulus) that the major effect of odor presentation was in frequencies overlapping the beta-frequency range (Fig. 2C). We therefore focused our analysis on this frequency band.

Odor-evoked response of the units in the MDT. We observed that 54 single units out of the 106 units recorded responded (significant change in firing rate) to at least 1 odor (51%). In Fig. 3A, 3 odor-responsive units are represented. As in other regions of the olfactory system, odor-evoked responses could be either an increase of spike discharge or a decrease in response to odor. B: percentage of units responding to odors as function of MDT segments (total number of units for each segment: Medial = 29; Central = 56; Lateral = 21 units). C: depth of each unit recorded (diamonds) and group mean (± SE; circles) depending on the odor response of the unit (odor-nonresponsive units, gray; n = 52 units and odor-responsive units, black; n = 54 units). D: mean (± SE) spontaneous frequency discharge of MDT units as a function of their odor response. There was no significant difference of spontaneous activity between odor-nonresponsive (gray; n = 52 units) and odor-responsive units (black; n = 54 units; unpaired \( t \)-test).

Odor response-tuning of MDT units. A: example of 3 odor-responsive units. The top half shows waveform activity of odor-responsive units with lines representing the spikes sorted and the average waveform shape on the top. The bottom half shows raster plots (lines) of responsiveness to each presentation of an odor (3 presentations) and peristimulus time histograms (PSTH). Raster plots and PSTHs show reliability of responses. Data for rasters and PSTHs were obtained by extraction of single-unit activity from the raw recordings. Odorant presentation time is represented in the gray area lasting for 2 s. Units can show either an increase of spike discharge or a decrease in response to odor. B: percentage of units responding to odors as function of MDT segments (total number of units for each segment: Medial = 29; Central = 56; Lateral = 21 units). C: depth of each unit recorded (diamonds) and group mean (± SE; circles) depending on the odor response of the unit (odor-nonresponsive units, gray; n = 52 units and odor-responsive units, black; n = 54 units). D: mean (± SE) spontaneous frequency discharge of MDT units as a function of their odor response. There was no significant difference of spontaneous activity between odor-nonresponsive (gray; n = 52 units) and odor-responsive units (black; n = 54 units; unpaired \( t \)-test).

Fig. 4. Odor response-tuning of MDT units. A: percentage of odor-responsive units as a function of the number of odor to which they responded [global \( \chi^2(4) = 4.582, \ P < 0.001 \); the category of response to 1 odor was significantly different from all of the others categories: 1 vs. 2 odors, \( \chi^2(1) = 22.09, \ P < 0.001 \); 1 vs. 3 odors, \( \chi^2(1) = 28.66, \ P < 0.001 \); 1 vs. 4 odors, \( \chi^2(1) = 39.51, \ P < 0.001 \); and 1 vs. 5 odors, \( \chi^2(1) = 49.63, \ P < 0.001 \); n = 54 odor-responsive units]. B: percentage of single units tested with each odor that showed a significant response (change in firing rate) to that odor. Individual cells were tested with at least 5 of the full set of 8 odors (see MATERIALS AND METHODS), and \( \chi^2 \)-test was used to make comparisons between each odor. Percentage of responses to odor a (10c) was significantly different from odor b (\( P < 0.01 \)) and odor c (\( P < 0.001 \)). Odor c (Feces) was significantly different from odor d (\( P < 0.05 \)) and odor a (\( P < 0.001 \)). From the left to the right, the raw number of units responsive out of the units tested for each odor is: 9/54; 9/54; 21/54; 14/54; 14/54; 10/38; 3/38; and 7/23. C: percentage of units tested responsive to each odor category (\( \chi^2 \)). * \( P < 0.05 \); ** \( P < 0.01 \); *** \( P < 0.001 \). D: single-unit odor-receptive fields. Response magnitude of each unit to each odor (n = 54 odor-responsive units) is normalized to the best odor within each unit. Therefore, a measure of 1 is the response to the best odor with subsequent responses being expressed as a percentage of that response strength. E: receptive field slopes (calculated as described in MATERIALS AND METHODS) of each odor-responsive unit. Larger slopes (further from 0) represent more narrow tuning. AU, arbitrary units.
range from strong to more moderate excitatory responses to suppressive responses (from left to right, Fig. 3A). The MDT can be anatomically separated into 3 segments: medial, central, and lateral (Price 1985); we thus compared the percentage of odor-responsive units between those 3 MDT segments (Fig. 3B). This percentage was not significantly different between the medial, central, and lateral segments [odor-responsive units out of the units recorded in the medial, central, and lateral segments: 17/29, 28/56, and 9/21, respectively; \( \chi^2(2) = 1.254, P = \text{not significant (N.S.)} \)]. We also tested whether the position of the electrode relative to the anteroposterior axis (relative to −3.0 bregma) and its depth could be related to the odor response of the unit. We were unable to detect an effect of the anteroposterior axis [odor-responsive units out of the units recorded in the anterior and posterior portions: 31/55 and 23/51, respectively; \( \chi^2(1) = 1.343, P = \text{N.S.} \)] or the depth [unpaired \( t \)-test, \( t(104) = 1.616, P = 0.1092 \); Fig. 3C]. Finally, there was no detectable difference in spontaneous activity rates between odor-responsive and odor-nonresponsive units [unpaired \( t \)-test, \( t(104) = 1.295, P = 0.19 \); Fig. 3D].

We also analyzed the relationship between MDT single-unit activity and respiration. We observed that 53% (56/106 units) of MDT single units present a significant relationship with the respiratory cycle (Rayleigh test). Out of these units with a significant phase-locking to respiration, we observed that 54% (30/56 units) were not responsive to odors and

![Fig. 5. Conjoint increase of beta oscillations during odor presentation in both piriform cortex (PCX) and MDT. A, top: raw LFP trace recorded in the PCX (gray) before and during odor presentation (gray box); bottom is the raw LFP trace simultaneously recorded in the MDT (black). B: mean (± SE) of the FFT ratios between odor-evoked and prestimulus periods as a function of oscillatory frequencies and of the structure recorded (MDT, black; PCX, gray; repeated-measures ANOVA and post hoc Fisher test, \( P < 0.05 \); \( n = 18 \) animals). C: mean (± SE) FFT ratios [value of each FFT in 1 respiratory cycle (c) divided by the mean of the FFT values during the 3 respiratory cycles of the prestimulus period] in the MDT (black; left) and in the PCX (gray; right; repeated-measures ANOVAs, 1-sample post hoc \( t \)-test; \( n = 18 \) animals). D: mean (± SE) coherence between MDT and PCX (dark gray diamonds; \( n = 18 \) animals) or between MDT and visual neocortex (light gray circles; \( n = 14 \) animals) measured over the entire duration of the recording. The coherence between MDT and PCX is significantly higher than the coherence between MDT and visual neocortex (repeated-measures ANOVAs, post hoc Fisher test, \( P < 0.05 \)). E: mean (± SE) ratio of coherence (odor-evoked/prestimulus period) between PCX and MDT. Whatever the frequency, this ratio is superior to 1, meaning that odor presentation increases the coherence between PCX and MDT. *\( P < 0.05 \); **\( P < 0.01 \), ***\( P < 0.001 \).](http://jn.physiology.org/doi/10.1152/jn.00741.2013)
46% (26/56 units) were responsive to our odor set. Thus having a significant relationship relative to the respiratory cycle did not predict odor responsiveness.

To characterize further the odor response of MDT units, we determined the number of odors capable of inducing a significant change in each MDT unit. As shown in Fig. 4A, a majority of MDT units (63%; 34/54 odor-responsive units) responded to only 1 odor out of our odor set. We next calculated the percentage of odor-responsive units relative to each of the odor stimuli used (Fig. 4B). First, we observed that MDT units as a whole were able to respond to all odorants used in this experiment; thus MDT units do not respond to only 1 specific odor. Second, the percentage of odor responses was significantly higher to 10c and lower to Feces [10c vs. ethyl valerate, $\chi^2(1) = 6.65, P < 0.01$; 10c vs. 10c-1, $\chi^2(1) = 6.65, P < 0.01$; 10c vs. Feces, $\chi^2(1) = 11.11, P < 0.001$; Feces vs. ISO1, $\chi^2(1) = 4.818, P < 0.05$; Feces vs. 10cR1, $\chi^2(1) = 4.818, P < 0.05$; Feces vs. Litter, $\chi^2(1) = 4.548, P < 0.05$; Feces vs. ISO2, $\chi^2(1) = 5.31, P < 0.05$]. Third, we pooled odorants relative to their categories, monomolecular, mixture, and biological, and found no significant effect of the odor category on the percentage of odor responses [$\chi^2(2) = 2.954, P = N.S.;$ Fig. 4C]. Finally, we looked at the effects of odorant concentration. Both pure odorants (ethyl valerate and ISO1) and components of odorant mixtures were diluted in mineral oil to a concentration of 100 ppm based on vapor pressure. Thus mixtures were at a higher total concentration than pure monomolecular odorants. We did not detect a relationship between the percentage of odor-responsive single units and the intensity of the odorant (data not shown). In further support of this concentration insensitivity, as shown in Fig. 4B, there was no significant difference between ISO1 and ISO2 [$\chi^2(1) = 0.165, P = N.S.$].

As a further examination of the specificity with which MDT units respond to odors, we determined the odorant receptive field for each single unit, as described in Xu and Wilson (2012). We first determined the most effective stimulus (evoking the greatest change in firing rate compared with baseline). We then used the magnitude of the response to the best odor to normalize response magnitudes to the other stimuli tested to derive an odor-receptive field (Fig. 4D). As demonstrated previously, it is possible to observe both excitatory and inhibitory activities (Fig. 4D). By measuring the slope of the receptive fields for each unit (Fig. 4E), we were able to quantify the selectivity of each unit to our odor set. Single units varied in receptive field slope, although the variation was uniform and did not appear to distinguish subgroups of broad or narrowly tuned cells but rather demonstrated a consistent high selectivity to our odor set.

Relationship Between PCX and MDT Activities

The PCX is the main olfactory input to the MDT (Heimer 1968; Powell et al. 1963; Price 1985; Price and Slotnick 1983). We thus wanted to characterize the relationship between MDT activities and LFPs in PCX.

The odor-evoked response of LFPs in both MDT and PCX. As seen in the MDT, we observed that odorant presentation evoked large beta oscillations in the PCX (Fig. 5A). As in the MDT, the increase of beta power was significant compared with prestimulus activity. The increase of power was also significant in other frequencies: from 4 to 58 Hz, at 65 Hz, and from 70 to 78 Hz [repeated-measures ANOVAs, $F(1,561) = 10.935, P = 0.001$, post hoc Fisher test], but again the ratio of frequency power between prestimulus and odorant periods clearly shows an increase in the beta-frequency range (Fig. 5B). The increase of beta-oscillation power was significantly greater in the PCX compared with that simultaneously recorded in the MDT [repeated-measures ANOVAs, $F(1,561) = 345.752, P < 0.0001$, post hoc Fisher test; Fig. 5B]. We next explored the temporal relationship between beta oscillations recorded simultaneously in MDT and PCX (Fig. 5C). We calculated the FFT values in three respiratory cycles before odor onset and in six respiratory cycles after odor onset. FFTs were calculated individually for each respiratory cycle from the transition between the exhalation and the inhalation to the next exhalation-inhalation transition. We observed a significant effect of the respiratory cycle for both the MDT (Fig. 5C, left) and the PCX [Fig. 5C, right; repeated-measures ANOVAs, MDT: $F(8,136) = 5.178, P < 0.0001$; PCX: $F(8,136) = 4.371, P < 0.0001$]. The post hoc one-sample t-test revealed a significant relationship relative to the phase of beta oscillation in the MDT (black; total units recorded: 106 and 29). By measuring the slope of the MDT unit, beta cycles detected on the MDT LFP signal filtered between 15 and 35 Hz, and an example of the MDT single-unit spike distribution relative to the phase of beta oscillation in the MDT. Using this distribution, Rayleigh tests were used to determine the significance of individual unit activity phase relationship to simultaneously recorded LFP beta oscillation. R: percentage of MDT units with a significant phase-locking to LFP beta oscillation recorded either in the MDT (black; total units recorded: 106 and 29 units significantly locked to beta in MDT) or in the PCX (gray; total units recorded: 57 and 9 units significantly locked to beta in PCX; $\chi^2$). C: percentage of odor-responsive units and odor-nonsensitive units of the units locked to either beta in the MDT (black) or in the PCX (gray; $\chi^2$).
significant increase of beta-band oscillation power during the first cycle following odor onset in the PCX (Fig. 5C, right) but not until the second respiratory cycle following odor onset in the MDT (Fig. 5C, left). The significant increase of beta-oscillation power thus appeared first in the PCX and then followed in the MDT.

We next examined the coherence between the PCX and the MDT. As a control, in 14 out of the 34 animals, MDT LFP and a nonolfactory cortical area, visual neocortex, were recorded. The coherence across all of the recording sessions was measured. As shown in Fig. 5D, the coherence between the PCX and the MDT was significantly greater than between the visual neocortex and the MDT, and this was true for frequencies ranging from 7 to 31 and 39 to 78 Hz, frequencies in both the beta- and gamma-band ranges [repeated-measures ANOVAs, F(1, 999) = 338.589, P < 0.0001, post hoc Fisher test]. Given this specificity, we then focused on the coherence between MDT and PCX and tested whether odorant stimulation could modify MDT-PCX coherence. The ratio (odor/prestimulus period) of coherence between PCX and MDT is presented in Fig. 5E. We observed a significant difference of coherence between the prestimulus and the odor periods. In fact, we observed a main ANOVA effect of odor [repeated-measures ANOVAs, F(2, 178) = 4.381, P < 0.05]: odorant presentation globally increases the coherence between the PCX and the MDT with a specific effect at 17, 39, 48, and 65 Hz revealed by the post hoc Fisher tests.

Spikes-LFP relationship. To characterize further the relationship between PCX and MDT, we examined the relationship between the MDT units activity and beta oscillation either recorded in the MDT or in PCX (Fig. 6A). First, we observed that 27% of MDT units were significantly locked to beta oscillations recorded simultaneously in the MDT (Rayleigh test, 29/106 units). In contrast, only 16% of MDT single units had activity phase-locked to simultaneously recorded PCX beta oscillations (Fig. 6B; 9/57 units), although this difference was only a trend and did not reach statistical significance [χ²(1) = 2.775, N.S.]. Finally, we examined whether the units phase-locked to beta oscillation were or were not responsive to odors. As shown in Fig. 6C, there is no significant difference in the percentage of odor-responsive (52%, 15/29 units) and odor-nonresponsive units (48%, 14/29 units) that were locked to MDT beta oscillation [χ²(1) = 0.068, P = N.S.]. For the relationship between MDT units and PCX beta oscillations, the picture was slightly different. We observed that the strong majority of MDT single units phase-locked to PCX beta oscillation were odor-responsive (67%, 6/9 units; Fig. 6C), although with the small number of units, this effect was not significant [χ²(1) = 2, P = N.S.].

Relationship between SPWs in PCX and SWA in MDT. By definition, the PCX presents SPWs during thalamocortical SWA in unanesthetized animals and under urethane anesthesia (Manabe et al. 2011; Murakami et al. 2005; Narikiyo et al. 2014; Wilson 2010). Here, we also observed SWA in MDT (Fig. 7A) and wanted to know whether a relationship between MDT-SW and PCX-SPWs could exist as has been reported in other PCX targets (Manabe et al. 2011; Narikiyo et al. 2014). We detected each trough of PCX-SPWs and SW in MDT (Fig. 7A). PCX-SPWs events were then plotted relative to MDT-SW events in a peri-event time histogram. We observed that both events were tightly locked with a majority of PCX-SPWs occurring just before or simultaneously to SW troughs in the MDT (Fig. 7B). We next compared the MDT-SW events to the visual neocortex-SW events as control. As expected, we also observed a strong relationship between the occurrence of the MDT-SW and visual neocortex-SW events. However, it should be noted that the PCX-SPWs were significantly more narrowly aligned with MDT-SW than the visual neocortex-SW [repeated-measures ANOVAs, F(1, 1740) = 6.465, P < 0.05, post hoc Fisher test; Fig. 7B].

We then examined the relationship between MDT single-unit activity and SW occurring in the MDT and SPWs occurring in the PCX. We observed that 83% of MDT single units had a significant change of activity relative to the occurrence of the SW in the MDT (78/94 units in total recorded in the MDT...
under SWA and 82%, 45/55 units, recorded under SWA with simultaneous recording in the PCX; repeated-measures ANO-VAs, $P < 0.05$). This was also true when looking at the relationship between MDT unit activities and PCX-SPWs, with 82% of units having a significant change of activity relative to PCX-SPWs. Figure 8A shows the mean normalized spikes count of MDT units relative to either SW in MDT (left) or SPWs in PCX (right). As shown in this figure, MDT units can present a biphasic discharge related to either SW in MDT or SPWs in PCX. Visual inspection of each unit individually revealed that, in fact, some units can present biphasic responses relative to the trough of either SW in the MDT or SPWs in the PCX (31%, 14/45, for the MDT and 29%, 13/45, for the PCX; Fig. 8A). However, these kinds of responses were not universally shared by all of the MDT units. In fact, as shown in Fig. 8B, we observed that some MDT single-unit discharges could precede (11%, 5/45, for the MDT and 20%, 9/45, for the PCX), be simultaneous with (20%, 9/45, for both MDT and PCX), or follow the MDT-SW or PCX-SPWs (38%, 17/45, for the MDT and 31%, 14/45, for the PCX). It has to be noted that units 2 and 5 were recorded in the same animal; the difference in phase relative to the MDT-SW or PCX-SPWs is thus not related to animal-specific state. A further example is shown in Fig. 8C, where units 6 and 7 were recorded simultaneously and showed different profiles relative to MDT-SW or PCX-SPWs, confirming that differences in phase are not related to a variation of state between animals or within animals. Finally, we also examined the relationship between MDT single-unit activity and SW occurring in the visual neocortex and observed that a majority of MDT units (78%, 28/36 units; Fig. 8D) have a significant change of activity relative to the SW in the visual neocortex, consistent with the global nature of SWA.

**Relationship Between MDT Odor-Evoked Activities and Brain State**

It has previously been demonstrated that state (FWA vs. SWA) has an effect on PCX odor-evoked activity (Murakami et al. 2005; Wilson 2010; Wilson and Yan 2010). Here, we tested whether MDT odor-evoked activity could similarly be state-dependent. MDT activities (units and LFP) and PCX LFPs were recorded under both FWA and SWA states. Figure 9A presents a raw LFP trace recorded in the MDT under both states. A total of 13 single units were recorded under both states where we were able to present all of our odorants 3 times in each state for within-cell analyses. In total, we recorded 44 units across both states, but since the number of odor presentations was different between both states, we focused our results on these 13 units that allowed testing across states within cells. We observed that MDT single-unit spontaneous activity was significantly decreased under SWA compared with FWA [paired $t$-test, $t(12) = 2.348$, $P < 0.05$; Fig. 9B]. Associated with this decrease in spontaneous activity, the frequency discharge of MDT units during the odor period was also significantly decreased under SWA compared with FWA [paired $t$-test, $t(12) = 2.423$, $P < 0.05$]. We then compared the

Fig. 8. Relationship among MDT single-unit activity, LFP SWA in the MDT, and PCX-SPWs. A. top: mean ($\pm$ SE) normalized MDT units discharge relative to SW in MDT (black; left) and SPWs in PCX (gray; right; $n = 55$ units recorded with both PCX and MDT under SWA). Bottom: example of 2 MDT units that present biphasic activities relative to SW in MDT and SPWs in PCX. B. example of 3 MDT units spike discharge relative to SW in MDT and SPWs in PCX (same colors as in A). Unit 3 presents an increase of discharge that precedes the troughs of SW in MDT and the SPWs in PCX, unit 4 presents an increase of discharge simultaneous to the troughs of SW in MDT and the SPWs in PCX, and unit 5 presents an increase of discharge following the troughs of SW in MDT and the SPWs in PCX. C. example of 2 simultaneously recorded units that present 2 different relationships with the troughs of SW in MDT. D. mean ($\pm$ SE) normalized MDT units discharge relative to SW in visual neocortex ($n = 36$ units recorded with both visual neocortex and MDT under SWA).
percentage of odor responses under both states. We observed a slight, nonsignificant decrease in the number of MDT single units showing significant odor response under SWA compared with FWA [8 odor-responsive units out of 13 units under SWA and 6 under FWA; \( \chi^2(1) = 0.62, P = \text{N.S.}; \) Fig. 9C]. These observations were also true when taking into account the full set of 44 units in an across-cells comparison. Finally, we examined the coherence between PCX and MDT under both states and observed that SWA significantly increased the coherence between these two structures compared with FWA [repeated-measures ANOVAs, \( F(1,429) = 359.254, P < 0.0001; \) post hoc Fisher test, \( P < 0.05; \) Fig. 9D, left]. As a control, we also compared the coherence between the MDT and the visual neocortex under both states (Fig. 9D, right). First, as described previously, we observed that the coherence between the MDT and the PCX was significantly higher than that between the MDT and the visual neocortex in both states [repeated-measures ANOVAs, \( F(1,495) = 178.546, P < 0.001 \) with no significant interaction between the state and the area, \( F(1,495) = 0.375, P = 0.54 \)]. Second, the global increase in coherence observed during FWA was also observed between the MDT and the visual neocortex [repeated-measures ANOVAs, \( F(1,66) = 61.010, P < 0.001; \) Fig. 9D, right] but to a lesser extent than between the MDT and the PCX with only three values being different between SWA and FWA (post hoc Fisher test, \( P < 0.05 \)).

**DISCUSSION**

In all senses except for the olfactory sense, the thalamus is known to be involved in various functions ranging from basic sensory information to attention modulation. Because of the specific organization of the olfactory pathway (i.e., no direct thalamic relay between sensory neurons and primary cortex), olfactory thalamus was often overlooked in understanding olfactory function, especially in nonhuman animal models (Shepherd 2005). In fact, it has been hypothesized that either the olfactory bulb (Kay and Sherman 2007) or PCX (Murakami et al. 2005) may serve many thalamic functions in olfaction. Here, we provide evidence that updates the implication of the thalamus in olfactory processing.

The present results demonstrate that single units in the MDT are responsive to a wide variety of odorants, including molecular, mixture, and biological stimuli. Individual neurons, however, are very narrowly tuned, with most cells responding to a single stimulus within our test set. The temporal structure of MDT neural activity is shaped by both respiration and network oscillations, particularly in the beta-frequency band. Importantly some MDT neurons also fired in phase with beta-oscillatory activity within the primary olfactory afferent of the MDT, the PCX. Finally, the relationship between MDT neural activity and PCX activity was state-dependent, with SW sleep-related MDT activity strongly in phase with PCX SPWs. These results confirm and extend previous work on the olfactory characteristics of MDT neurons (Benjamin and Jackson 1974; Imamura et al. 1984; Jackson and Benjamin 1974; Motokizawa 1974; Price 1985; Price and Slotnick 1983; Takagi 1986; Yarita et al. 1980). They further form a basis for ongoing work in awake, performing animals to test hypotheses regarding the role of MDT in odor attention and perception based on human lesion and imaging data (Asai et al. 2008; Piaily et al. 2008; Potter and Butters 1980; Sela et al. 2009; Tham et al. 2011a,b).

Beta oscillations are a widely described phenomenon across different circuits and senses and are often associated with interarea communication (Engel et al. 2001; Tallon-Baudry et al. 2001). Functionally, it has been shown that visual attention synchronizes beta-frequency oscillations in cat lateral geniculate nucleus of the thalamus and visual primary cortex (Wrobel et al. 1994). Here, we showed that in response to odors in anesthetized animals, both the MDT and the PCX could express beta oscillations; these oscillations were coherent between the two structures, and importantly some MDT units fired in phase with PCX beta (Figs. 2, 5, and 6). Thus, in analogy with the other senses, beta oscillations could provide an efficient mechanism for olfactory information transfer between the PCX and the MDT. The PCX-MDT-OFc circuit may play an important role in attention to olfactory stimuli (Piaily et al. 2008; Tham et al. 2011a,b; Veldhuizen and Small 2011), and coherent beta oscillations across this triumvirate may promote this process. Ongoing work in awake animals will assess this. It should to be noted that although our focus here was on the large beta oscillations, we also observed a significant coherence between the PCX and the MDT in the gamma-oscillation frequency band. Gamma oscillations thus...
could also contribute to information transfer from the PCX to the MDT (Gray et al. 1989).

At the single-unit level, we observed that 51% of MDT units were responsive to odors, and the percentage of odor-responsive units was not significantly different across the 3 MDT anatomic subregions (Fig. 3), although precise localization of subdivision boundaries was not always obvious. This last result is intriguing because it has been shown that the olfactory structures project both to the medial and central segments of the MDT but not to the lateral segment (Bay and Cavdar 2013; Cornwall and Phillipson 1988; Price 1985; Price and Slotnick 1983; Ray and Price 1992). Several reasons could explain our result, but perhaps the most relevant is that, as argued by Krettek and Price (1974), the MDT subdivisions in the rat mediodorsal nucleus are not as prominent as in primates. Relative to single-unit odor response specificity, the narrow tuning of the MDT units described here (Fig. 4) is similar to the sparse odor responses in the PCX (Poo and Isaacson 2009) and to the narrow tuning observed in both the olfactory tubercle and the lateral entorhinal cortex (Payton et al. 2012; Rampin et al. 2012; Xu and Wilson 2012). This result is in contrast to the studies of Yarita et al. (1980) and Imamura et al. (1984), who found much broader odor-tuning responses of the MDT than described here; however, they used a different set of odors, species, and level of anesthesia, making direct comparison difficult [Yarita et al. (1980) in awake monkey and Imamura et al. (1984) in artificially ventilated pentobarbital-anesthetized rabbits]. Nevertheless, a common feature with those earlier reports is that MDT units can be responsive to biological, monomolecular, or mixture odors.

A growing body of evidence suggests a critical role of SWA in memory consolidation and formation (Rasch and Born 2013). The PCX presents SPWs during thalamocortical SWA (Murakami et al. 2005; Wilson 2010). We demonstrated that the MDT could also display SWA and that the MDT-SWA, at both LFP and unit levels, is tightly related to both PCX-SPWs and visual neocortex-SWA (Figs. 7 and 8). However, we showed that these relationships and notably the coherence between the MDT and the PCX is greater than that between the MDT and the visual neocortex (Fig. 9). PCX-SPWs have been shown to trigger activity in the olfactory bulb (Manabe et al. 2011; Murakami et al. 2005; Tham et al. 2011a,b) and the known links between MDT dysfunction and pathology (Baxter 2013; Hazlett et al. 2004; Mitchell and Chakraborty 2013; Parnaudeau et al. 2013), understanding mechanisms of MDT olfactory function is increasingly important. The present results suggest strong links between MDT activity and its primary olfactory afferent, the PCX, as well as important state changes in this relationship. Future work in awake animals will be necessary to clarify the contribution of MDT to olfaction.

REFERENCES


**ODOR PROCESSING IN THE MEDIODORSAL THALAMUS**


