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## A specific olfactory cortico-thalamic pathway contributing to sampling performance during odor reversal learning

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### Abstract

A growing body of evidence shows that olfactory information is processed within a thalamic nucleus in both rodents and humans. The mediodorsal thalamic nucleus (MDT) receives projections from olfactory cortical areas including the piriform cortex (PCX) and is interconnected with the orbitofrontal cortex (OFC). Using electrophysiology in freely moving rats, we recently demonstrated the representation of olfactory information in the MDT and the dynamics of functional connectivity between the PCX, MDT and OFC. Notably, PCX-MDT coupling is specifically increased during odor sampling of an odor discrimination task. However, whether this increase of coupling is functionally relevant is unknown. To decipher the importance of PCX-MDT coupling during the sampling period, we used optogenetics to specifically inactivate the PCX inputs to MDT during an odor discrimination task and its reversal in rats. We demonstrate that inactivating the PCX inputs to MDT does not affect the performance accuracy of an odor discrimination task and its reversal, however it does impact the rats' sampling duration. Indeed, rats in which PCX inputs to MDT were inactivated during the sampling period display longer sampling duration during the odor reversal learning compared to controls - an effect not observed when inactivating OFC inputs to MDT. We demonstrate a causal link between the PCX inputs to MDT and the odor sampling performance, highlighting the importance of this specific cortico-thalamic pathway in olfaction.

### Keywords

Mediodorsal thalamus; piriform cortex; orbitofrontal cortex; olfaction; sampling

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**Informed consent:** This article does not contain any studies with human participants performed by any of the authors.

## Introduction

Sensory perception and discrimination depend upon the capacity to accurately sample, integrate and associate the stimuli with their outcome in order to give an appropriate response to the environment. In this process, not only first order thalamic relays are essential but accumulating evidence also highlights the importance of higher order thalamic relays (Sherman and Guillery 2006). Those thalamic relays receive information from primary sensory cortical areas as opposed to first order relays which mainly receive information from the periphery (Mitchell et al. 2014). Higher order thalamic relays such as the pulvinar or the posterior medial group are known to modulate and coordinate activity in primary sensory and higher order cortical areas (Saalman et al. 2012; Mease et al. 2016).

In the olfactory system, there is no first order thalamic relay, however the primary olfactory areas including the piriform cortex (PCX) project to the mediodorsal thalamus [MDT; (Powell et al. 1963; Price and Slotnick 1983; Courtiol and Wilson 2015)] which is interconnected with the orbitofrontal cortex [OFC; (Krettek and Price 1977)]. Interestingly, some PCX neurons form giant synapses onto MDT neurons, with properties similar to the driver synapses observed in other higher order thalamic pathways (Pelzer et al. 2017). Relative to olfaction, lesions of the MDT do not affect olfactory detection, olfactory delayed nonmatching-to-sample task or simple odor discrimination, however they do impair odor preferences, difficult odor discrimination and odor reversal learning (Slotnick and Kaneko 1981; Eichenbaum et al. 1980; Sapolsky and Eichenbaum 1980; Tham et al. 2009; Zhang et al. 1998). Some of those deficits can be overcome with overtraining (Staubli et al. 1987). In addition, electrophysiological studies have demonstrated that the MDT can encode odor information (Courtiol and Wilson 2014, 2016) as well as the reward value associated to the odor (Kawagoe et al. 2007).

Importantly, we have shown that the functional coupling (spike-local field potential phase locking) within the PCX-MDT-OFC pathway changes during a two-alternative odor discrimination task (Courtiol and Wilson 2016). Notably, we observed a specific increase of functional coupling between the PCX and the MDT during the odor sampling period compared to both baseline and pre-sampling period. However, it is still unclear whether or how this increase in connectivity contributes to olfactory processing. To decipher the contribution of PCX input to MDT during odor discrimination, here we optogenetically inactivated the PCX input to MDT during the sampling period of an odor discrimination task and its reversal. As a comparison, in separate animals we inactivated the OFC input to MDT during sampling, given that our previous work demonstrated that OFC-MDT coupling is less robust than PCX-MDT coupling during sampling when compared to pre-sampling. In addition to assessing the accuracy of performance during these manipulations, we also analyzed the sampling duration. Sampling duration is a sensitive metric used to assess odor discrimination, and it has been shown that animals increase their sampling duration during difficult discriminations to attempt to maintain constant performance accuracy (Slotnick 2007; Rinberg et al. 2006; Abraham et al. 2004; Lovitz et al. 2012).

## Materials and methods

### Animals

33 Male Long–Evans rats (Envigo; 250–350 g at the beginning of the experiments) were used. Rats were single-housed in polypropylene cages on a 12 h light/dark schedule with food *ad libitum*. Once the behavioral phase started, they were placed under water restriction with access to water provided during the behavioral session and for 30 min after each session. Rats were weighed daily to ensure that their body weight was not inferior to 80% of their body weight at the beginning of the experiment. Animal care protocols and experiments were approved by the Nathan S. Kline Institute Institutional Animal Care and Use Committee and were in accordance with National Institutes of Health guidelines. All efforts were made to minimize pain and discomfort to the animals.

### Virus surgery

Naïve animals were anesthetized and kept unconscious with an isoflurane anesthesia system (E-Z Systems). A surgical level of anesthesia was maintained and monitored throughout the experiment by measuring corneal and paw pinch reflexes. In addition, local anesthetic (1% xylocaine, s.c.) was administered locally around the wound site. Temperature was maintained at 37°C throughout the surgery using a heating pad. Animals were placed on a stereotaxic device and the skull was exposed.

Bilateral holes for virus infection were made either above the PCX in two sites (we chose to inject in two sites in the PCX because it is a structure that extends broadly ventrally to the brain; 1<sup>st</sup> PCX site: AP: +3.2, ML: ±3; DV: 6.2 relative to the surface of the brain and 2<sup>nd</sup> site: AP: +1, ML: ±4.4–4.6, DV: 6.8, Fig. 2A1) or in the OFC in 1 site (AP: +3.8, ML: ±2.4–2.8; DV: 4.2–4.5, Fig. 2B1).

Viruses AAV5-CaMKIIa-eArchT3.0-eYFP (ArchT,  $3 \times 10^{12}$  IU/mL) and AAV5-CaMKIIa-eYFP (control,  $2.5 \times 10^{12}$  IU/mL) were obtained from UNC Vector Core through an MTA with Karl Deisseroth, Stanford U. Viruses were bilaterally infused using a glass pipette at a rate of 0.1 µL/min through a Hamilton-pump in the PCX (1<sup>st</sup> PCX site: volume: 0.8 µL and 2<sup>nd</sup> site: volume: 0.5 µL) or OFC (volume: 0.5 µL). The holes in the skull were covered with a fine layer of silicone elastomer (KWIK cast; World Precision Instruments).

Three main groups of rats were thus defined: rats injected with ArchT in the PCX (PCX virus, n=9 used in the final analysis), rats injected with ArchT in the OFC (OFC virus, n=4) and controls (n=9). Controls included rats injected with control virus (either in PCX, n=2, or OFC, n=5) and rats given no virus (sham holes in the bone over the coordinates of the PCX) but implanted with optical fibers (n=2). For the tests (expert), we did not measure a significant difference in performance or sampling duration between the injected/non injected animals or between the sites of the injection, thus for statistical comparisons they are merged in a single Controls group.

For the reversal learning during which rats are connected with light every session, two of the control rats were not given light (one of them was not connected). In addition, two PCX virus rats were moved to controls because they were not connected and therefore not given

light. In fact, in order to minimize animal use, we decided to keep those two rats as controls for the reversal and not connect them since they were difficult to connect. We compared the sampling duration and performances between animals that received light or no light and between animals connected and not connected and we did not find significant differences thus for statistical comparisons they are merged in a single Controls group.

Optical fibers (Doric Lenses, MFC 200/250–0.66 ZF2.5 FLT) were then implanted bilaterally in the MDT (AP: –2.8, ML:  $\pm 1.8$  with an angle of 10 degrees to allow for placement of bilateral cannulas, DV: 5). The optical fibers were glued and cemented to the rat's skull. Two anchor bone screws were added bilaterally over the posterior visual cortex to secure the cement. Immediately after the surgery and prior to recovery, animals were treated with the analgesic buprenorphine (0.1 mg/kg, sc injection). The antibiotic enrofloxacin (5 mg/kg, sc injection) was also delivered following the surgery. For at least 3 days after the surgery, rats were complemented with the antibiotics Baytril (0.5mg/ tablet; Bacon flavor; Bio Serv, Flemington, NJ). Rats were allowed to recover for at least 8 days before starting the behavior with water restriction.

## Behavior

Odor discrimination was assessed in a two-alternative odor discrimination task (Fig. 1A). Animals received 30 min training sessions 5 days per week. The operant chamber was described previously in (Chapuis and Wilson 2011; Courtiol and Wilson 2016). In brief, it consisted of a plexiglas box with a central odor port on the central wall and two goal ports on the left and right walls (Vulintus). A trial was initiated by a nose poke into the central port which triggered the delivery of an odor terminating when the rats withdraw their nose from the central port. Rats were required to hold in the odor port for at least 0.3 s for the event to be considered a trial eligible for a reward. Water reward ( $\sim 35\mu\text{l}$ ) was delivered, depending on odor identity, upon a correct choice of the left or right goal port within 3 s. The operant chamber was controlled by Spike2 software (Cambridge Electronic Design) and all behavioral epochs were recorded through Spike2.

**Laser and stimulation:** Laser stimulation in the MDT (532nm, model: GL532T3H-200FC, Shanghai Laser & Optics Century Co. Ltd.; 10–20mW at the tip of the optical fiber) was triggered by the nose poke onset in the odor port and terminated by the nose poke withdrawal (Fig. 1A).

**Original discrimination:** All animals were first trained on a vanilla versus peppermint discrimination (pure extract; McCormick) until criterion was reached. Criterion was defined as two consecutive sessions with  $>75\%$  correct responses. Before testing the animals with the laser and in order to make sure that there was sufficient time for robust ArchT expression (at least 6 weeks), animals were habituated to perform the discrimination while connected to the fiberoptic patchcord (Doric Lenses, BFP(2) 200/220/900–0.53- FCM-2xZF2.5) and tested the day after.

**Test-expert- Original discrimination:** The animals were tested in the original vanilla-peppermint discrimination once they were expert. Optogenetic inactivation of either PCX or

OFC inputs to the MDT during the sampling period was performed during 1/3 of the session (10 minutes pseudo-randomly selected across the 30 minutes session; Fig. 1A-B). Light was presented continuously as long as the rat remained in the sampling port.

**Reversal:** The day after the test in the original discrimination, the animals started the reversal training with vanilla-peppermint and the experimental contingencies were reversed (change of the goal port side associated with the odor). During the reversal and since we had no preconceptions of the effect of the inactivation of PCX/OFC inputs to MDT, those inputs were inhibited during the sampling period on all trials at each session of the reversal (Fig. 1B).

**Test-expert- Reversal learning:** once the animals were expert in the reversal (at least two consecutive days >75% performance), they were again tested with light exposure during sampling. Optogenetic inactivation of either PCX or OFC inputs to the MDT during the sampling period was performed during 1/3 of the session in order to have a within animal design for this phase (Fig. 1B).

### Data analysis and statistics

Animals that either did not succeed to perform the original discrimination, took longer than 35 sessions to attain the criterion in the reversal learning, or were not generating trials were terminated and their data omitted from the analysis. Seven animals were removed for those reasons.

Performance (% correct) and sampling duration (with 10 ms resolution) were analyzed offline using Spike2. Data were analyzed at specific behavioral stages only in sessions where the animals performed at least 60 trials (including full trials, aborted trials, hit and error trials):

**- Test-expert- Original discrimination:** performance and sampling duration were compared between laser stimulation ON and OFF (within animals) and across groups (Controls, OFC virus, PCX virus). Values were statistically compared (Statview) using repeated measures ANOVA with laser stimulation as the dependent factor and group as the independent variable followed by LSD posthoc test.

**- Reversal learning stages:** Seminal work by Slotnick and Kaneko (1981) has shown that lesions of MDT impair the initial reversal learning and the performances of the MDT-lesioned group are then improved in subsequent reversals. Moreover, numerous studies demonstrated transient changes of PCX and OFC excitability and connectivity across learning (Saar et al. 1999; Cohen and Wilson 2017; Cohen et al. 2015; Martin et al. 2006). For those reasons, we chose to analyze 3 sessions during the reversal learning: the first day of the reversal, the first day the animal reached >40% and the first day the animal reached >60%. The two last sessions allowed us to examine the sampling duration and number of days to reach each stage when the performances were on average below and above chance but not at criterion yet.

Sampling durations were compared across stages (within animals) and between groups (Controls, OFC virus, PCX virus). Values were statistically compared using repeated measures ANOVA with stage as the dependent factor and group as the independent variable followed by LSD post-hoc tests. The number of days to attain each stage of the reversal was also compared between groups using a factorial ANOVA.

**- Test-expert- Reversal:** performance and sampling duration were compared between laser stimulation ON and OFF (within animals) and across groups (Controls, OFC virus, PCX virus). Values were statistically compared using repeated measures ANOVA with laser stimulation as the dependent factor and group as the independent variable followed by LSD posthoc test.

## Histology

After the completion of the behavioral experiments, animals were overdosed with urethane anesthetic. Rats were transcardially perfused with phosphate buffered saline (PBS) and with 4% paraformaldehyde in PBS. Brains were removed and stored in a 30% sucrose-4% paraformaldehyde in PBS solution before sectioning. Coronal brain sections (40  $\mu$ m thick) were collected using a microtome (Leica). Sections were placed on glass slides, mounted using DAPI-fluoromount G (Southern Biotech) and sealed with nail polish. Anatomical targeting of viral injection and infection was verified using an Epifluorescent microscopy. Indeed, expression of the enhanced yellow fluorescent protein reporter fused to the archeorhodopsin vector construct was sufficient enough to detect infected cells in PCX/OFC and their axonal fibers in MDT (Fig. 2). Track localizations of the optical fibers in the MDT were verified with the DAPI staining (Fig. 2 A2, B2). Experimental animals that displayed clear leak of viruses outside the target area were excluded from the analysis. Four animals were removed for that reason, leaving the final n's/group listed above.

## C-fos experiment

In a subset of animals (N=9 with n=6 controls and n=3 PCX-OFC ArchT injected rats), an additional experiment was performed after the completion of the behavioral tests. We used c-fos expression, a marker of neuronal activation, to confirm that the viral expression of AAV5-CaMKIIa-eArchT3.0-eYFP in the PCX or OFC resulted in neural inhibition in the MDT upon light exposure (Fig. 2C).

To do so, rats underwent a first phase of habituation. They were placed in their home cage connected to the laser with no stimulation. A tea ball with no odor was added for 30' then this tea ball was replaced by another similar one with no odor for 5'. The animals were then disconnected and stayed in their cage for 90'. This protocol was repeated for 2 days. The third day, the animal was connected in their home cage, a tea ball with no odor was added for 30' then a tea ball filled with filter paper impregnated with orange essence (McCormick) was added in the cage for 5' and the laser was turn ON simultaneously. After 5', the laser was turned OFF and the tea ball was removed. The rats remained in their home cage for 90'. The animals were then immediately overdosed with urethane anesthetic. Rats were transcardially perfused with PBS and with 4% paraformaldehyde in PBS. Brains were removed and stored in a 30% sucrose-4% paraformaldehyde in PBS solution before

sectioning 6 days later. Coronal brain sections (40  $\mu\text{m}$  thick) were collected using a microtome (Leica). Sections were placed in 12 well plates in a PBS-sodium azide solution. For the immuno-histochemistry, sections were rinsed with PBS-Triton. A primary antibody, Anti-c-Fos polyclonal rabbit 1:1000 (Synaptic Systems) in blocking solution, was added and incubated overnight. The sections were then washed with PBS-Triton and a secondary antibody Alexa 568 donkey anti-rabbit 1:1000 (Fisher Scientific) was incubated for two hours at room temperature. Sections were then washed with PBS-Triton. Finally, sections were placed on glass slides, mounted using DAPI-fluoromount G (Southern Biotech) and sealed with nail polish. Epifluorescent microscopy was used to take pictures of the sections in the MDT area. Images were then analyzed using ImageJ software. For each rat, two sections containing the MDT (anterior and posterior) were analyzed. Using translucent images from the atlas of Paxinos and Watson (2009), the MDT was delineated. An optical density threshold was then applied to assess the number of labeled cells and the total area bilaterally. Labeled cell number/total area was determined in both hemispheres and for both anterior and posterior sections and was averaged across rats. Average of controls values was made, each value of ArchT-injected rat was then divided by this controls average. One sample t-test was used to compare the values of ArchT-injected rats to one.

## Results

A total of 22 animals were analyzed in the results below. Three groups of animals were defined: PCX virus (animals injected with AAV5-CaMKIIa-eArchT3.0-eYFP in the PCX), OFC virus (animals injected with AAV5-CaMKIIa-eArchT3.0-eYFP in the OFC) and Controls (see Methods). In order to confirm the efficacy of our optogenetic inhibition manipulations following all other assays, in a subset of animals we observed that the viral expression of AAV5-CaMKIIa-eArchT3.0-eYFP in the PCX or OFC resulted in suppression of odor-evoked activity in the MDT upon light exposure with an 18% decrease in c-fos positive cells compared to Controls. This decrease is small but significant ( $t(2)=-4.837$ ,  $p=0.04$ ) and demonstrates the efficacy of our optogenetic manipulation.

Animals required  $15\pm 5$  sessions of training (including the different stages) before reaching criterion performance in the original discrimination (vanilla-peppermint).

Animals were first tested on the original discrimination once they were expert (Fig. 3A). Optical inactivation of the PCX or OFC inputs to MDT was performed during the sampling period for 1/3 of the session (Fig. 1). There was no significant effect of the optical inactivation on the performance (optical inactivation:  $F(1,19)=2.656$ ,  $p=0.12$ ). There was a main effect of group with the OFC group displaying slightly decreased performance compared to the two other groups (group:  $F(2,19)=3.554$ ,  $p=0.049$ ; post hoc test: controls vs. PCX virus  $p=0.48$ , controls vs OFC virus  $p=0.016$ , OFC virus vs PCX virus  $p=0.0519$ ) but this effect was not related to the optical inactivation as there was no interaction between the group and the optical inactivation (optical inactivation \* group:  $F(2,19)=1.584$ ,  $p=0.23$ ). In addition, all groups had performance accuracy superior to 80%.

Following the test in the original discrimination, the rats started the reversal training on their next session. During the learning of the reversal, the OFC PCX inputs to the MDT were



inactivated during the sampling period and across all the trials of the session. We measured the number of sessions necessary to attain each stage of the reversal from the beginning of the reversal: 1<sup>st</sup> day the rats attain a performance >40%, >60% and criterion. We found no significant difference between groups in the numbers of days to reach each stage (to attain >40%,  $F(2,19)=2.662$ ,  $p=0.096$ ; to attain >60%,  $F(2,19)=1.825$ ,  $p=0.188$ , to attain criterion,  $F(2,19)=0.296$ ,  $p=0.75$ ; Fig. 3B), demonstrating that all animals acquired the reversal task at the same rate.

Finally, once the animals were expert in the reversal, they were tested as described for the original initial discrimination. Again, performance accuracy was not affected by inactivation of either MDT input pathway during the sampling period (main optical inactivation effect:  $F(1,18)=0.386$ ,  $p=0.54$ , group\*optical effect:  $F(2,18)=1.293$ ,  $p=0.3$ ; Fig. 3C). Contrary to the test in the original discrimination, there was no difference between the 3 groups (main group effect:  $F(2,18)=0.725$ ,  $p=0.5$ )

These results demonstrate that performance accuracy (percent correct) of the rats when they are expert in the original discrimination and in the reversal are not affected by the optical inactivation of PCX or OFC inputs to the MDT during sampling. The number of days to reach the criterion of reversal is also not affected by this inactivation.

Various studies (Lovitz et al. 2012; Slotnick 2007; Rinberg et al. 2006; Abraham et al. 2004) suggested that odor sampling duration may be a more sensitive metric to assess odor discrimination difficulty than simple choice accuracy. We thus measured sampling duration during our different tests and learning stages.

During the test in the original discrimination (expert), sampling durations were not affected by the inactivation, during the sampling period, of either the PCX or OFC inputs to the MDT (main optical inactivation effect:  $F(1,19)=0.27$ ,  $p=0.61$ ; Fig. 4A). There was no difference between groups or interactions between groups and optical stimulation (main group effect  $F(2,19)=0.716$ ,  $p=0.5$ ; group\*optical effect:  $F(2,19)=0.549$ ,  $p=0.59$ ). However, while there was no effect on the inactivation of the OFC or PCX inputs to the MDT on the performance accuracy during the reversal learning, we did observe a significant difference of sampling duration between the three groups ( $F(2,19)=3.785$ ,  $p=0.04$ , LSD post hoc test: controls vs. PCX virus  $p=0.01$ , controls vs OFC virus  $p=0.5$ , OFC virus vs PCX virus  $p=0.16$ ; Fig. 4B). Animals with silenced PCX input to MDT during odor sampling spent significantly more time sampling than any other group. This effect was consistent across stages with no interaction between group and stage (group \* stage:  $F(4,38)=0.899$ ,  $p=0.47$  and no main effect of stage:  $F(2,38)=0.797$ ,  $p=0.46$ ). Importantly, there was an average increase of sampling durations (across the three stages) of 130 ms between PCX virus and Controls (Fig. 4B). Finally, we demonstrate that this effect vanishes when the rats are expert in the reversal with no significant effect of PCX or OFC inputs to MDT inactivation during the test phase of the reversal, after attaining criterion performance (main optical inactivation effect:  $F(1,18)=0.038$ ,  $p=0.85$ ; main effect of group:  $F(2,18)=1.605$ ,  $p=0.23$ ; optical inactivation\*group  $F(2,18)=1.104$ ,  $p=0.35$ ; Fig. 4C).

## Discussion

It has long been known that MDT receives input from the olfactory cortex (Powell et al. 1963). Subsequent lesion studies highlighted a role of the MDT in olfactory behavior [for review see (Tham et al. 2009) and (Courtiol and Wilson 2015)]. Notably, MDT lesions cause simple odor discrimination while impairing odor reversal learning (Slotnick and Kaneko 1981). More recently, we have shown that MDT units can encode various aspects of an odor discrimination task and importantly that the functional connectivity between PCX, MDT and OFC is dynamic with an increase of PCX-MDT coupling during the sampling period (Courtiol and Wilson 2016). However, the importance of this change in coupling for odor-guided behavior was unknown.

In this report, and following the study of Slotnick and Kaneko (1981), we demonstrate that the inactivation of PCX or OFC inputs to MDT during the sampling period of an odor discrimination task and its reversal does not affect the performance accuracy or the number of days to attain the criterion (or the different stages of the reversal). However, the inactivation of PCX inputs, but not OFC inputs, to MDT induces longer sampling duration compared to controls during reversal learning. We thus demonstrate that the contribution of PCX input to MDT is critical to maintain optimal sampling performance during the learning of the new odor-side contingencies. These results corroborate and deepen the initial observation by Slotnick and Kaneko (1981) that the MDT and its PCX inputs are important for the learning of new odor-rule contingencies. Importantly, they also strengthen the hypotheses driven by our spike-local field potential phase locking data that the specific engagement between PCX and MDT during the sampling period is actually relevant for odor-guided behavior.

### Relationship between sampling duration and performance: speed-accuracy tradeoff?

Speed-accuracy tradeoff is a general phenomenon observed across sensory modalities and shared by various species (Heitz 2014). In olfaction, the speed-accuracy tradeoff seems to depend on the task and its parameters (Khan and Sobel 2004; Kepecs et al. 2007; Slotnick 2007; Frederick et al. 2017). For example, mice forced to sample the odorants longer increase their performance accuracy (Rinberg et al. 2006). Abraham et al. (2004) also showed that when very similar odorant mixtures are used, mice increase their discrimination sampling time while maintaining a high level of response accuracy. At the opposite, keeping short discrimination times can lead to a drop in performance in difficult odor discrimination (Uchida and Mainen 2003). Sampling durations can also vary during the acquisition of the two alternative odor discrimination task and are longer in 'good' vs. 'poor learners' (Lefevre et al. 2016; Lovitz et al. 2012). Sampling duration, response accuracy and difficulty of the tasks are thus intermingled.

We show that when the PCX inputs to MDT are inactivated, performance is not affected however the animals stay longer in the odor port (on average, across the three learning stages, 130ms). This outcome is consistent with a need to sample odors longer during the reversal learning to maintain optimal performance accuracy (as the same level as the other groups), and this extended sampling is no longer needed when they are expert in the task, when the task becomes easy. In behaving rats performing a two-alternative odor

discrimination task, the rats usually sniff around 6–8Hz (sniff cycles: 125–166ms) during the odor sampling period (Courtiol et al. 2014; Lefevre et al. 2016; Kepecs et al. 2007). The increase of sampling duration observed here is sufficient to allow the rat to add another sniff. This idea fits with the speed-accuracy tradeoff concept. Indeed we can hypothesize that the suppression of PCX inputs to MDT leads to a disrupted computation of the olfactory object or association between the odor and its output in the MDT making it more difficult for the animal to either discriminate the odorants or associate them with certainty to the goal port location. The additional time provided by another snapshot of the stimuli can either be used to integrate more olfactory information over time or/and to improve the decision making process (Frederick et al. 2017; Slotnick 2007).

### **PCX/OFC inputs to MDT inactivation: effect on performances**

We did not observe light-induced differences in performance accuracy across our different tests and reversal learning. There may be several explanations. First, lesions of the MDT do affect performances in the reversal learning however in the present study, we only inactivated the fibers from the PCX or the OFC to the MDT. The inactivation of those MDT inputs might be compensated by the other olfactory inputs converging to the MDT [e.g., olfactory tubercle, amygdala, etc. (Krettek and Price 1974; Price and Slotnick 1983; Bay and Cavdar 2013)]. In addition, it has been shown that lesions of the PCX itself do not affect simple odor discrimination but impair complex olfactory discrimination and delayed nonmatching to sample task (Staubli et al. 1987; Zhang et al. 1998). Second, Staubli et al. (1987) have shown that the effects of MDT lesions on odor discrimination can decrease with extensive training and Eichenbaum et al. (1980) showed that lesions of the MDT only affect difficult odor discrimination. We might have observed an effect on performances if a more difficult odor discrimination was used. In those difficult discriminations which might increase the attentional load, the MDT might be used to compare olfactory cortical (PCX) to neocortical activity (OFC) when the odors are difficult to disambiguate (Plailly et al. 2008; Zelano et al. 2011). Additional work will be required to explore these alternatives. Finally, it has to be noted that the MDT inputs were only inactivated during the reversal and when the animals were expert in the original discrimination, thus we cannot rule out a contribution of PCX/OFC inputs during the acquisition of initial odor discrimination or new odor pair discrimination, notably in the transfer of the rules.

Regarding our results with the OFC to MDT inactivation, it is known that animals with lesions of the OFC acquire initial odor discrimination normally while they are impaired in odor reversal learning (Schoenbaum et al. 2002). The OFC thus seems as one of the critical structures needed for odor reversal learning. In this study, we specifically inactivated the OFC input to the MDT during the sampling period and we did not observe a significant effect during the tests and reversal. This corroborates our electrophysiological results showing a significant increase of coupling between OFC and MDT during the period following odor sampling and not during the sampling period when compared to the pre-sampling period (Courtiol and Wilson 2016). Our results can be interpreted in several ways. First, the OFC to MDT coupling might not be critical for the acquisition of the odor information (sampling) but to maintain the olfactory information acquired and make a decision (post-sampling). In this line of view, Schoenbaum et al. (2003) have shown that

OFC lesions affect response latency between the end of odor sampling and the reward port response. Second, the MDT to OFC and not OFC to MDT connections might be important for the reversal. As the opposite of the *'non reciprocal'* PCX to MDT connection, the OFC and MDT are interconnected and the flow of information can be critical. For example, with the prefrontal cortex-MDT connections, recent selective opto/chemogenetic manipulations have demonstrated the complexity of their interactions. Indeed, the MDT can amplify and sustain activity in the prefrontal cortex underlying rule representation, working memory and attentional control [(Schmitt et al. 2017; Miller et al. 2017); for review see Parnaudeau et al. (2018)]. Moreover, blocking MDT to prefrontal cortex connectivity has been shown to induce deficits in maintenance of working memory and in adapting to change in goal value and action/outcome contingencies. In contrast, blocking prefrontal cortex to MDT connectivity impairs the choice phase of a delayed nonmatch-to-sample task and the ability to adapt to the updated goal value but not instrumental contingency (Bolkan et al. 2017; Alcaraz et al. 2018). Future work will be required to further elucidate the role of MDT-OFC coupling during odor discrimination.

## Conclusions

The MDT is defined by its strong reciprocal connections with the prefrontal cortex (Groenewegen 1988; Alcaraz et al. 2016) and has been shown to contribute to a large panel of cognitive functions including goal-directed behavior and working memory (Alcaraz et al. 2014; Mair et al. 2015; Parnaudeau et al. 2013). This study, along with previous lesion studies, also highlights a role of the MDT in olfactory processing. We show here the distinct involvement of different MDT inputs (PCX vs. OFC) during odor reversal learning. We demonstrate that PCX to MDT and not OFC to MDT coupling during the sampling of the odorant is important for optimal performance during odor discrimination reversal learning. This study brings further attention to the contribution of a thalamic nucleus in olfaction.

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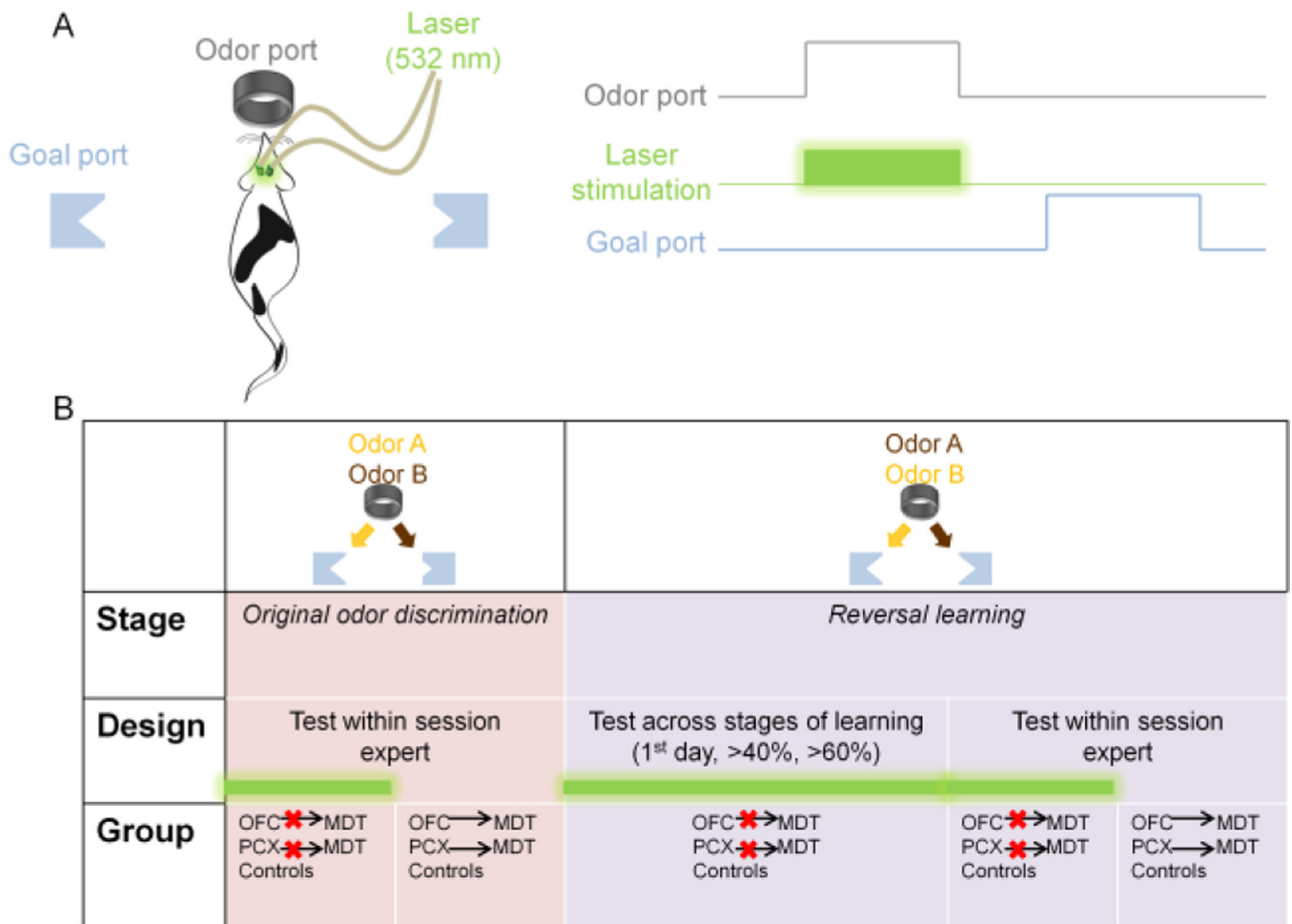
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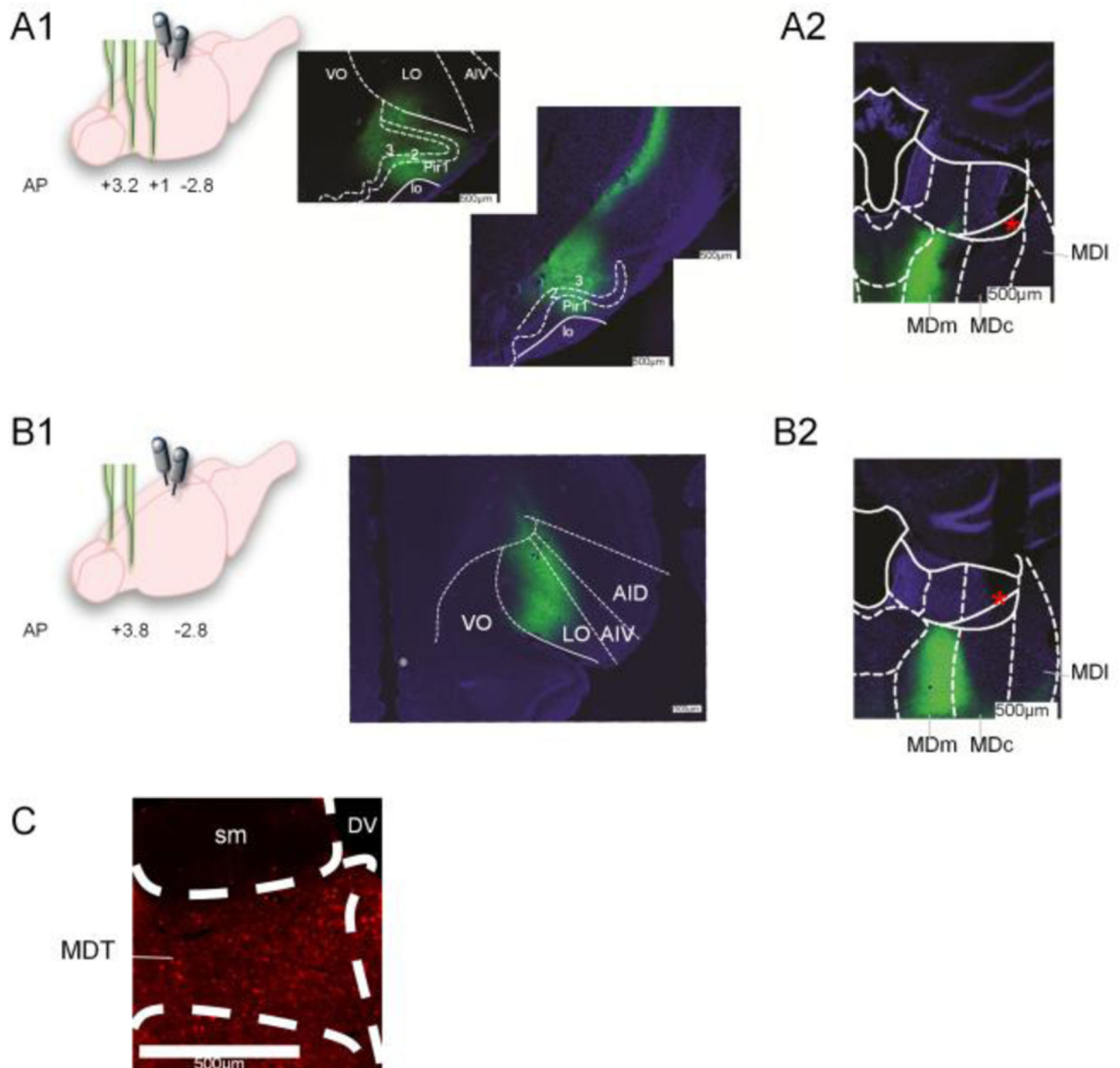
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**Figure 1. Design of the task.**

A- Rats performing a two-alternative odor discrimination task were connected to a 532nm laser. Bilateral laser stimulation occurred during the sampling period in the central odor port. B- Behavioral sequences- Animals were first tested in the original odor discrimination (pink) when they were expert. During this session and for the three groups, the laser was turned ON (inactivation of PCX/OFC inputs to MDT) during the sampling period for 1/3 of the session. The next day, the animals started the reversal. During the reversal learning, PCX/OFC inputs to MDT were bilaterally inactivated during the sampling period on all trials at each session of the reversal (violet, left). Once the animals were expert in the reversal, they were tested in a protocol similar to the test of the original discrimination (1/3 of the session with PCX/OFC inputs to MDT inactivated during odor sampling)

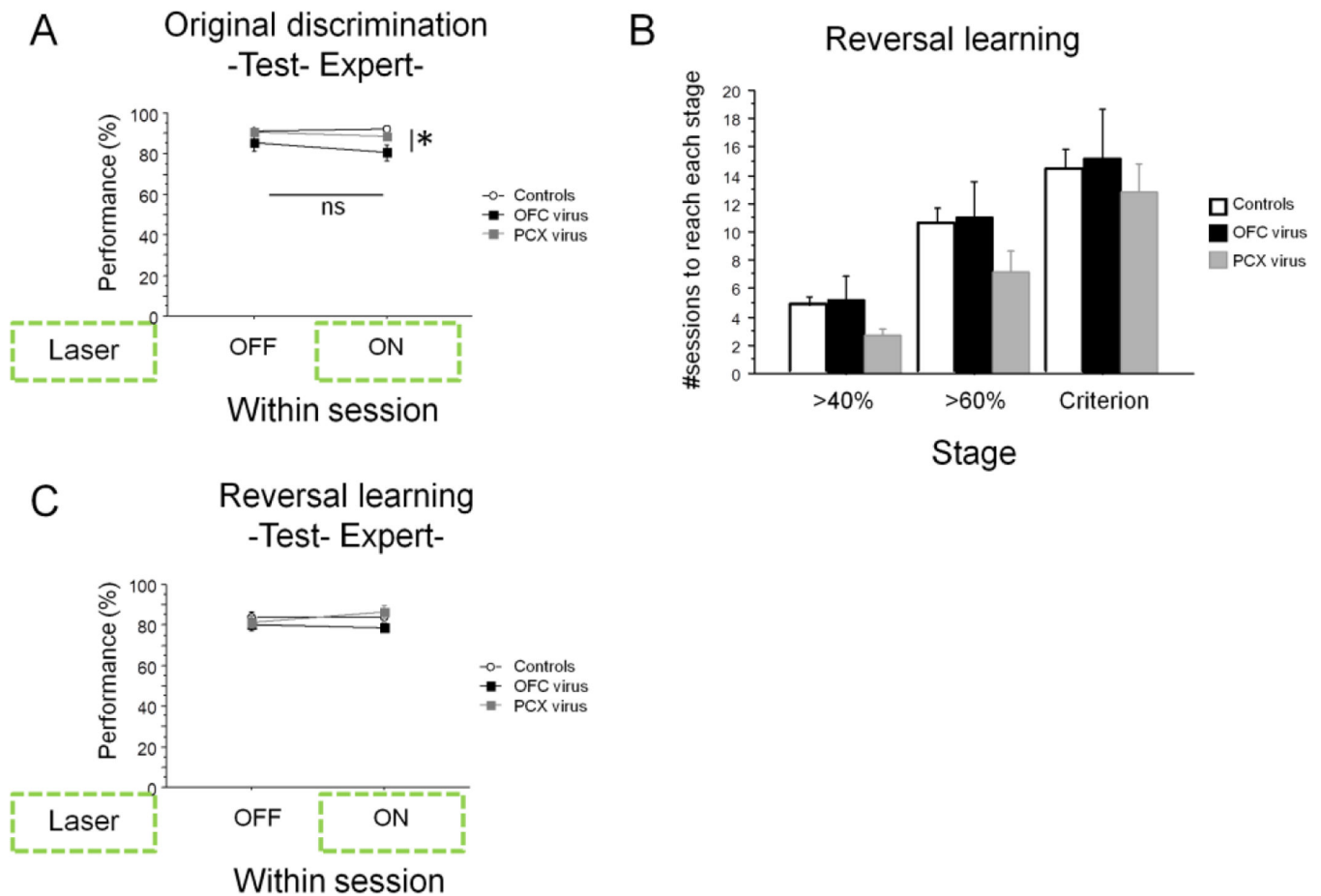




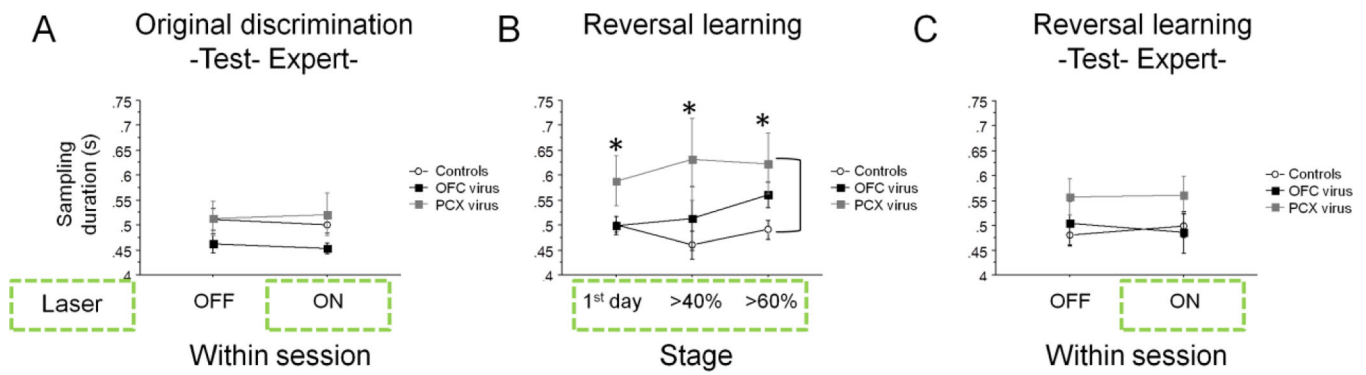
**Figure 2. Implantation design and histological confirmation.**

Design of the PCX (A1-left) or OFC (B1-left) virus injection and implantation of bilateral cannulas in the MDT. Example of the infected areas in the PCX in two sites (A1-right) and in the OFC (B1-right). A2- Labeled olfactory cortex fibers in the MDT and track of the optical fiber (\*). B2- Labeled OFC fibers in the MDT and track of the optical fiber (\*)- C- Example of labeled c-fos cells in the MDT of a control rat.

lo= lateral olfactory tract; LO= lateral orbital cortex; VO= ventral orbital cortex; AIV/AID= agranular insular cortex, ventral and dorsal part respectively; MDI= MDT lateral part; MDc= MDT central part; MDm= MDT medial part; sm= stria medullaris of thalamus; DV= dorsal 3<sup>rd</sup> ventricle.



**Figure 3. Performance accuracy is not affected by PCX/OFC inputs to MDT inactivation –**  
 A- Effect of inactivation of PCX or OFC inputs to MDT on performance accuracy during the test in the original discrimination (Controls n= 9; OFC virus n= 4; PCX virus n= 9). B. Effect of inactivation of PCX or OFC inputs to MDT on the number of days to attain the odor reversal stages (Controls n= 11; OFC virus n= 4; PCX virus n= 7, see methods) – Factorial ANOVA for each stage. C. Effect of inactivation of PCX or OFC inputs to MDT on performance accuracy during the test of the reversal (Controls n= 9; OFC virus n= 4; PCX virus n= 8 -one of the 9 original rats in A was stopped before the final test)- \*= p<0.05- Repeated measures ANOVA



**Figure 4. Inactivation of PCX inputs, and not OFC inputs, to MDT induces longer sampling duration during odor reversal learning.**

A- Effect of inactivation of PCX or OFC inputs to MDT on sampling duration during the test in the original discrimination (Controls n= 9; OFC virus n= 4; PCX virus n= 9). B- Effect of inactivation of PCX or OFC inputs to MDT on sampling duration across reversal learning stages (Controls n= 11; OFC virus n= 4; PCX virus n= 7). C- Effect of inactivation of PCX or OFC inputs to MDT on sampling duration during test of the reversal (Controls n= 9; OFC virus n= 4; PCX virus n= 8). \*= p<0.05- Repeated measures ANOVA