In vivo electrophysiological recordings in amygdala subnuclei reveal selective and distinct responses to a behaviorally identified predator odor

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Govic A, Paolini AG. In vivo electrophysiological recordings in amygdala subnuclei reveal selective and distinct responses to a behaviorally identified predator odor. J Neurophysiol 113: 1423–1436, 2015. First published December 4, 2014; doi:10.1152/jn.00373.2014.—Chemosensory cues signaling predators reliably stimulate innate defensive responses in rodents. Despite the well-documented role of the amygdala in predator odor-induced fear, evidence for the relative contribution of the specific nuclei that comprise this structurally heterogeneous structure is conflicting. In an effort to clarify this we examined neural activity, via electrophysiological recordings, in amygdala subnuclei to controlled and repeated presentations of a predator odor: cat urine. Defensive behaviors, characterized by avoidance, decreased exploration, and increased risk assessment, were observed in adult male hooded Wistar rats (n = 11) exposed to a cloth impregnated with cat urine. Electrophysiological recordings of the amygdala (777 multiunit clusters) were subsequently obtained in freely breathing anesthetized rats exposed to cat urine, distilled water, and eugenol via an air-dilution olfactometer. Recorded units selectively responded to cat urine, and frequencies of responses were distributed differently across amygdala nuclei; medial amygdala (MeA) demonstrated the greatest frequency of responses to cat urine (51.7%), followed by the basolateral and basomedial nuclei (18.8%) and finally the central amygdala (3.0%). Temporally, information transduction occurred primarily from the cortical amygdala and MeA (ventral divisions) to other amygdala nuclei. Interestingly, MeA subnuclei exhibited distinct firing patterns to predator urine, potentially revealing aspects of the underlying neurocircuitry of predator odor processing and defensiveness. These findings highlight the critical involvement of the MeA in processing olfactory cues signaling predator threat and converge with previous studies to indicate that amygdala regulation of predator odor-induced fear is restricted to a particular set of subnuclei that primarily include the MeA, particularly the ventral divisions.

PREDATOR ODORS are commonly utilized as stimuli in unconditioned tests of fear, as they reliably elicit fear and defensive responses in rodents (Blanchard et al. 1998; Dielenberg and McGregor 2001). Robust fear-related responses have been demonstrated with several odors including cat fur (Blanchard et al. 1990), 2,5-dihydro-2,4,5-trimethylthiazoline (a synthetic component of fox feces; Takahashi et al. 2005), fox urine (Funk and Amir 2000), and, more recently, lion and coyote urine (Ferrero et al. 2011). Studies examining the neural pathway underpinning predator odor-induced fear behavior have flagged the amygdala as playing a central role. The amygdala is a collection of several heterogeneous nuclei that are thought to play unique functional roles in the regulation of fear behavior (Pitkänen et al. 2000; Suh et al. 2003). While the involvement of the amygdala in detecting and responding to conditioned fearful stimuli or events has been well documented (Davis 1992), study of the contribution of each nucleus of the amygdala in the processing of predator odor has produced rather diverse and often conflicting results.

Activation studies measuring the protein product of the immediate-early gene c-fos both support and refute a role for the basolateral complex of the amygdala (BLA) in predator odor processing. Increased activation (Funk and Amir 2000) and unaltered activity (Dielenberg et al. 2001) have been reported in rats exposed to predator odor, while BLA lesions/inactivation have been shown to impair fear behavior to cat odor (Takahashi et al. 2007; Vazdarjanova et al. 2001). The central amygdala (CeA) has largely been thought of as non-critical for the expression of predator odor-induced fear behavior (Hunt et al. 2009; Li et al. 2004; McGregor et al. 2004). However, fox urine exposure alters monoamine activity in the CeA (Hayley et al. 2001), and ferret odor has been documented to result in the activation of a phenotypically distinct neuronal population in the CeA (Butler et al. 2011). Evidence for the role of the medial amygdala (MeA) in predator odor processing is more consistent, with lesion (Blanchard et al. 2005; Li et al. 2004; Takahashi et al. 2007) and neural activation (Dielenberg et al. 2001; Hunt et al. 2009; McGregor et al. 2004) studies indicating a pivotal role in the generation and regulation of predator odor-induced fear behavior, particularly within the posteroverentral part of the MeA. The relative importance or involvement of the remaining MeA subnuclei in predator odor processing is largely unknown and unclear.

To the best of our knowledge, a high-density multiunit recording of global amygdala activity to controlled presentations of predator odor has yet to be conducted. Given the conflicting findings in the literature and the high temporal and spatial resolution provided by electrophysiological techniques, the present study was undertaken to further clarify the role of specific amygdalar subnuclei in the processing of predator odor. Consequently, neural activity within the local amygdala network, to controlled and repeated presentations of cat urine, was examined in the freely breathing anesthetized rat. A multiunit approach was adopted to enable a global assessment of amygdalar subnucleus activity toward a predator odor. As the MeA is thought to be the first site of olfactory and vomeronasal information convergence (Samuelsen and Meredith 2009) and pivotal in regulating predator-odor induced fear, it was predicted that the MeA would demonstrate greater

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neural activity in response to a predator odor of cat urine than other amygdalar nuclei.

MATERIALS AND METHODS

Subjects. Subjects were adult male hooded Wistar rats (n = 11; Flinders University, Adelaide, SA, Australia) weighing 300–325 g at the time of behavioral testing (n = 11) and 350–375 g at the time of electrophysiological recordings (n = 6). Animals were housed in a temperature-controlled colony room under a 12:12-h reverse light cycle (lights off at 1100). Animals were housed individually with ad libitum access to food and water. Behavioral testing took place between 1300 and 1500 h, while electrophysiological recordings began at approximately 1200. Animal experimentation and care were performed in accordance with the guidelines set forth by the National Health and Medical Research Council and were approved by the institutional Animal Ethics Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Predator odor behavioral testing. In a procedure similar to Blanchard et al. (2001), rats were habituated to a clear Plexiglas box (100 × 30 × 50 cm, L × W × H) containing a cloth wrapped around a wooden block (9 × 9 × 2 cm, L × W × H) positioned in one end of the box. Habituation to the box and odor block occurred under red light conditions over three consecutive days for 10 min, of which the last session was recorded via a closed-circuit TV camera mounted on the ceiling directly above the box. During this session double-distilled water (ddH2O) was placed on the odor block, while predator odor (cat urine) was placed on the odor block on the fourth day. In a subset of animals, an additional ddH2O exposure session, followed 24 h later by an exposure session with eugenol (1:100 in mineral oil; Sigma-Aldrich, Castle Hill, NSW, Australia), was conducted to gauge behavior toward a neutral odor. Behavior was video tracked on all days with Ethovision video tracking software (Noldus, SDR Clinical Tech, Middle Cove, NSW, Australia) that was situated and operated in an adjacent room. For off-line analysis, the video tracking software allowed the test apparatus to be divided into three equal zones: far (farthest away from the cat urine block), middle (middle transient zone), and near (nearest zone that contained the urine odor block). The division of zones enabled the systematic examination and measurement of the total distance traveled, velocity, transits between the zones, the time spent in each zone, and the frequency and total duration of direct contact with the odor block. Direct contact with the odor was recorded each time the rat made an exploratory investigation of the urine block with its snout. In addition, the frequency of vigilant rearing, grooming bouts, and risk assessment behaviors was assessed by an observer blind to the treatment conditions. Vigilant rearing was defined as the rat standing on its hind legs without assistance from the walls. Risk assessment included flat-back approach and flat-back attention behaviors. Flat-back approaches were recorded when the animal approached the odor stimulus with a flattened back and hind legs extended back. Flat-back attention was defined as a stationary posture where the animal, being oriented toward the odor block, had its back flattened and/or neck stretched.

Surgical procedure. All experiments were performed in vivo on freely breathing animals 2–3 wk after behavioral testing. Long-lasting anesthesia was induced with intraperitoneal injections of urethane-ddH2O (20% wt/vol; Sigma-Aldrich). The head of the animal was fixed in place on a stereotaxic frame (David Kopf Instruments, Tujunga, CA) fitted with nanopuncture ear bars for surgery and during recording sessions. To gain access to the amygdala, craniotomies were performed over the dorsal surface of the structure. Once the dura was removed with a small incision, a 128-channel three-dimensional multunit electrode array (NeuroNexus Technologies, Ann Arbor, MI) was positioned and implanted into the amygdala. The three-dimensional electrode comprised a total of sixteen 12-mm-long shanks, arranged in a 4 × 4 matrix, with each shank containing eight electrode sites. Intershank spacing and within-shank spacing were 200 μm. Coordinates derived from the rat brain atlas (Paxinos and Watson 2007) were used to target the right and/or left amygdalar complex. These were −1.72 mm posterior/anterior to bregma, 3.2 mm lateral to the midline, and −9.0 mm ventral to the brain surface. To allow for the identification of penetration tracts, electrodes were dipped with a solution of the fluorescent dye 1,1′-dioctadecyl-3,3′,3′,–tetramethylindocarbocyanine perchlorate (DiI, 2.5 mg ml/absolute ethanol; Invitrogen) prior to each electrode penetration. A low-impedance silver reference electrode was placed under the skin and served as a reference for action potential recording. Surgery and electrophysiological recordings were conducted in a sound-attenuated chamber (Savant Technologies) fitted with an exhaust fan. Body temperature was monitored continuously with a rectal thermometer and maintained at 35–37°C by a feedback-controlled heating pad (World Precision Instruments, Sarasota, FL). Respiratory activity was also recorded by measuring chest extensions with a piezoelectric strap (MP100 Pulse Transducer; AD Instruments). The output was amplified, filtered, and fed through a window discriminator that provided pluses synchronous with the transition from exhalation to inhalation. The 128 channels were continuously sampled at 24 kHz, band-pass filtered (300–5,000 Hz), and stored on a hard drive. Binary data files containing electrode signals and experimental events were processed with TDT ActiveX software. Custom MATLAB (MathWorks) programs were used to extract spike waveforms.

Odor presentation. Odors were delivered directly to the nose through a computer-controlled eight-channel air-dilution olfactometer (35.0 × 38.1 × 29.2 cm, L × H × W; Med Associates, St. Albans, VT). Briefly, a continuous stream of filtered and humidified air was directed to the animal’s nose (1.0 l/min) during recording sessions via the polyethylene delivery tube connected to the olfactometer. The odor delivery tube was secured onto the mouthpiece of the stereotaxic frame, positioned ~3 mm in front of the animal’s nose. Odor delivery was regulated by mixing a second airstream airflow (1.5 l/min) to the glass bottles containing the odor via computer-controlled solenoid valves. The two airstreams combined for a final flow rate of 2.5 l/min. Odor delivery was calculated to occur ~2 s after activation of the solenoid valves when factoring in flow rate, internal volume of the olfactometer, internal diameter of the delivery tube, and the distance between the olfactometer and the nose (tube length). To time lock normal breathing patterns with odor stimulation, odor delivery was initiated just before inspiration at expiration ending. A total of seven different odors and a control (ddH2O) condition were presented to the rats. Examined in this report are the data obtained from two odors: undiluted cat urine and eugenol. As cat urine is largely composed of water (~95%; Cottam et al. 2002), eugenol was diluted to 1:100 in near-odorless mineral oil in an effort to equilibrate odorant intensities. Each of the eight odors was presented successively twice for 2 s, with clean air presented in between (intertrial interval range 60–80 s). The presentation of all eight odors was alternated in a pseudorandom order. Clean air presentations (range 60–80 s) occurred between each odor delivery. A total of five cycles were presented to the animal within a recording session. Thus each animal was exposed to any single odor 10 times in total. This protocol was adopted to minimize any potential effects of habituation, as higher-order sensory processing structures have been reported to habituate faster than first- or second-order structures (Wilson 1998). Furthermore, preliminary studies indicated that response adaptation occurred with more than two successive presentations of any single odor. A grand average of all 10 trials was calculated for each recording unit for each of the odor conditions (ddH2O, eugenol, and cat urine).

Collection and storage of cat urine. Cat urine was obtained from a number of female laboratory cats fed a carnivorous diet. Cats were placed into clean metabolic stainless steel cages overnight, and freshly voided urine was collected, pooled, divided into aliquots, and frozen at ~80°C. Samples were defrosted and used once per testing session.
**Histology.** At the conclusion of electrophysiological recordings, animals were given an overdose of pentobarbital sodium and perfused transcardially with 120 ml of 0.1 M phosphate-buffered saline (PBS; pH 7.4) followed by 120 ml of 4% paraformaldehyde (PFA). Brains were removed from the skull and stored overnight in 4% PFA at 4°C. Prior to sectioning, brains were transferred to a cryoprotectant solution of 30% sucrose-PBS. Full-brain serial horizontal sections (50 μm) were cut with a cryostat (Leica CM 1850; Leica Microsystems, Wetzlar, Germany) and collected on Super Frost slides. Slides were stained with NeuroTrace fluorescent Nissl stain 500/525 (catalog no. N21480; Molecular Probes, Eugene, OR). Sections were photographed with fluorescent microscopy (Nikon Eclipse 90i microscope and DS-SMC digital camera; Nikon, Kanagawa, Japan). Positions of the electrodes within the amygdalar complex were confirmed and photographed. Probe placement for each experiment was determined by mapping histological images onto standardized sections of the rat brain (Paxinos and Watson 2007).

**Data and statistical analysis.** All data are expressed as means ± SE. For all behavioral variables, paired-samples t-tests were utilized to examine differences between the no-odor and cat urine conditions only and between the far, middle, and near zones within the no-odor and cat urine odor conditions. To analyze predator odor-evoked changes in amygdala activity, the spike count of each unit’s responses to each odor (ddH2O, eugenol, and cat urine) was computed in 0.2-s bins for the first 30 s following the activation of the solenoid valves (0 s). The number of units that responded to an odor was identified by a peak/trough response (at any 0.2-ms bin) occurring in the first 10 s following odor delivery that exceeded the 95% confidence interval of the mean spiking rate over the entire 30-s window. A significant decrease in spiking activity was not observed as a primary response in any cases. χ²-Tests were utilized to examine response frequency across the major nuclei of the amygdala, followed by an examination of the standardized residuals. All data from the units responding to cat urine and the analogous responses to ddH2O and eugenol were normalized by deducting the average spike count of the last 10 s (20–30 s) from the spike count at each time bin (0.2 s). Mean ± SE spike count and the moving averages based on a 1-s sample window were computed for each nucleus for responses to cat urine, eugenol, and ddH2O. Changes in spike activity over time were examined for the three odor conditions (cat urine, ddH2O, and eugenol) separately in each nucleus with repeated-measures (RM) ANOVAs examining the average activity over 1 s, every 2 s from stimulus onset until 8 s (0, 2, 4, 6, 8 s). Planned contrasts were adopted comparing each time point against the first time point (baseline: 0 s) to determine when differences in spiking count occurred for each odor in each subnucleus. Additionally, paired t-tests were utilized to examine differences between ddH2O (or eugenol) and cat urine at the same time points mentioned above, with the significance level set at P < 0.01 to control the familywise error rate.

The analysis of the temporal characteristics of the responses, peak and response latency were examined. Peak latency was defined as the time at which the maximum spike count occurred, and response latency was defined as the start time at which the spike frequency increased above zero, beginning anytime from trial onset. To minimize false positives, spike elevations were required to occur for at least 0.6 s of a 1.0-s time window. A one-way ANOVA, followed by Bonferroni adjusted post hoc tests, was adopted to examine the temporal features of responses to cat urine in amygdala nuclei. RM ANOVA was further adopted to examine spike activity for data that were normalized on the basis of response onset. The average activity over 1 s from the time before a response was detected until 5 s after response onset (−1, 0, 1, 2, 3, 4, 5). Post hoc analysis involved planned contrasts of all time points with the first time point (−1 s) for each subnucleus. For all RM ANOVAs conducted, the assumption of sphericity was examined with Mauchly’s test. To enable the assessment of sphericity in subnucleus containing low sample sizes, specifically the BLa and BLv, the number of measures analyzed was limited to the first four time points. All violations of sphericity were corrected for with Greenhouse-Geisser correction. Significance level was set at P < 0.05 for all analysis unless otherwise stated.

**RESULTS**

Cat urine elicits defensive behavior in naive rats. The behavioral results are displayed in Fig. 1. We found that animals explored both sides of the box equally when ddH2O or eugenol was placed on the odor block, while cat urine exposure resulted in a dramatic preference for the side that did not contain the odor block (Fig. 1A). Given that only a small subset of animals were exposed to eugenol, statistical comparisons were only made between the ddH2O and cat urine exposure sessions. Only cat urine exposure resulted in a significant
decrease in the time \([t_{(10)} = 4.93, P = 0.001\), paired \(t\)-test; Fig. 1B\] and the frequency \([f_{(10)} = 2.56, P = 0.028; \text{Fig. 1C}\) of entries into the zone that contained the odor compared with the zone furthest away from odor block. When comparing between odor conditions, cat urine exposure increased the time spent in the area furthest away from the odor block \([t_{(10)} = 2.52, P = 0.030]\), decreased the time spent in the zone nearest the odor \([t_{(10)} = 2.361, P = 0.040; \text{Fig. 1B}\], but did not alter the time spent in direct contact with cat urine \((14.07 \pm 3.5\%\) compared with the ddH2O presentation \((12.56 \pm 5.6\%\). Similarly, the frequencies of entries into the middle and the near odor zones were decreased when rats were exposed to cat urine compared with the ddH2O condition \([\text{middle, } t_{(10)} = 2.50, P = 0.031; \text{near, } t_{(10)} = 2.71, P = 0.022; \text{Fig. 1C}\], despite the lack of difference in the frequency of odor investigation between the two odor conditions \([\text{ddH2O, 26.8} \pm 5.6; \text{cat urine, 13.5} \pm 2.6; \text{time} t_{(10)} = 1.87, P = 0.092]\. In addition to the avoidance demonstrated by rats toward cat urine, rats traveled significantly less \([t_{(10)} = 2.72, P = 0.022; \text{Fig. 1D}\) and moved significantly slower when exposed to cat urine \((4.7 \pm 0.35 \text{ cm/s})\) compared with no odor \([5.8 \pm 0.39 \text{ cm/s}; t_{(10)} = 2.75, P = 0.021]\). Furthermore, the two risk assessment behaviors, flat-back attention and flat-back approach, occurred more frequently when cat urine was placed on the odor block than when ddH2O was present [\text{Fig. 1E; } t_{(10)} = 2.70, P = 0.022]. Vigilant rearing remained unchanged between odor conditions; however, self-grooming increased significantly with exposure to cat urine compared with ddH2O \([t_{(10)} = 2.47, P = 0.033; \text{Fig. 1F}\).

**Cat urine elicits responses in amygdala subnuclei selectively.** Electrophysiological recordings were conducted on the left and right amygdala in a total of six rats. Consequently, 12 recording sessions were conducted with a total of 1,281 recording sites established. Histological analysis (Fig. 2) revealed that the majority of electrode recording sites reached various amygdalar nuclei (60.9%). Recordings were obtained from all divisions of the MeA \([\text{anterior ventral (MeAV), posterior ventral (MePV), posterior dorsal (MePD)}\], a number of cortical amygdaloid (CoA) nuclei \([\text{anterior (ACo), posteriolateral (PLCo), posteriomedial (MePD)}\], a number of cortical amygdaloid (CoA) nuclei \([\text{anterior (ACo), posteriolateral (PLCo), posteriomedial (PMCo)}\], basolateral (BL) nuclei \([\text{anterior (BLa), posterior (BLp), ventral (BLv)}\], basomedial (BM) nuclei \([\text{anterior (BMA), posterior (BMP)}\], and CeA nuclei \([\text{medial (CeM), capsular (CeC)}\], the amygdaloid hippocampal area (AHi), the intra-amygdaloid division of the bed nucleus of the stria terminalis (BSTIA), the anterior amygdaloid area (AAA), the amygdalopiriform transition area (APir), and intercalated nuclei of the amygdala (ICN). The total numbers of recordings obtained from the AAA, APir, and ICN were few (<2 each) and were therefore not included in any analysis. Consequently, amygdalar responses to cat urine were examined in the remaining nuclei and comprised a total of 777 multiunit clusters.

Responses to cat urine were detected in all amygdala subnuclei examined; however, the robustness of the response in terms of frequency was highly dependent on the location of the electrodes (Table 1). An extensive 51.7% of all total responses to cat urine occurred in MeA subnuclei. The BL- and BM-related nuclei accounted for a less substantial 18.8% of all responses to cat urine. Ten percent of responding units were located in the AHi, 6% of the responses occurred in the BSTIA, while the CoA and CeA subnuclei responded the least to cat urine and only accounted for 4.5% and 3.0% of all responses, respectively. The frequency of responses to cat urine was found to be distributed significantly differently across the major nuclei of the amygdala \([\chi^2, n = 777] = 53.78, P < 0.001, \chi^2].\) Examination of the standardized residuals confirmed that the MeA \((R = 4.7), \text{CeA} (R = -2.7), \text{and AHi} (R = -3.8)\) were the major contributors to this difference, with the frequency of responses in the MeA being higher and those in the CeA and AHi lower than the expected frequency.

In contrast to cat urine, responses to eugenol were not detected in every amygdalar subnucleus, with no responses detected in the ACo, PMCo, BLp, BMa, and CeM by the criteria adopted here. Additionally, of the structures found to demonstrate responses to eugenol, the frequency of responses was substantially lower than that of those detected toward cat urine (Table 1) and only totaled 2.7% of all recorded units. Furthermore, only 2 of the 21 eugenol-responding units (both located in the BLa) were found to also exhibit responses to cat urine, while the remaining failed to demonstrate a response toward cat urine.

**Amygdala subnuclei selectively respond to cat urine.** The average spike count and moving average spike count of MeA units during cat urine, ddH2O, and eugenol exposure are presented in Fig. 3 for each individual subnucleus. Cat urine dramatically altered the firing pattern in all MeA subnuclei \([\text{RM ANOVA; MeAV, } F(4,24) = 9.21, P < 0.001; \text{MeAD, } F(4,64) = 13.44, P < 0.001; \text{MePV, } F(3,0.597) = 9.87, P < 0.001; \text{and MePD } F(2,9.781) = 4.36, P = 0.002].\) In contrast, presentation of ddH2O and eugenol each resulted in spike activity that was relatively stable during the odor presentation period and the 18-s poststimulus period in all MeA nuclei \([\text{RM ANOVA, } P > 0.05; \text{Fig. 3}].\) The increase observed in spiking frequency during cat urine presentation was a direct consequence of odor delivery, as the increased activity occurred soon after activation of the olfactory vesicles at \(-3.6 to 4.4 s\) and after \(-1.5 s\) from the calculated latency for the odor to reach the rats nose. Planned contrasts conducted with time 0 (0–0.8 s) revealed no significant differences in spike activity at 2 s \((2.0–2.8 s), 6 s \((6.0–6.8 s), \text{ and } 8 s \((8.0–8.8 s)\) after onset of cat odor delivery in any MeA nuclei. A significant increase in activity, however, was evident in all subnuclei at 4 s \((\text{MeAV, } P = 0.003; \text{MeAD, } P < 0.001; \text{MePV, } P = 0.007; \text{MePD, } P < 0.001)\). Furthermore, the increased spike count demonstrated at 4 s was significantly elevated compared with activity demonstrated to ddH2O (all nuclei \(P < 0.001\); paired \(t\)-test). While no differences between cat urine and ddH2O were statistically evident at the remaining time points examined \((0, 2, 6, 8 s)\), a marked suppression of activity is evident in the MeAV and MePV after cat urine exposure compared with ddH2O extending from \(-6 s\) until \(8 s\) in the MeAV (Fig. 3A) and 10 s in the MePV (Fig. 3C). Paired comparisons with eugenol revealed a similar result, with no differences found in spike activity between cat urine and eugenol at \(0, 2, 6, \text{ and } 8 s,\) while a statistically significant increase in spike count was found at \(4 s\) when cat urine was presented compared with eugenol presentation for all MeA subnuclei \((\text{MeAD, MePV, MePD } P \leq 0.001, \text{MeAV } P = 0.008; \text{ paired } t\)-test). Further examination of the responses to cat urine in BL and BM amygdalar nuclei indicated an altered firing pattern in the BLa \([F(4,12) = 12.44, P = 0.001; \text{Fig. 4A}].\) Blv \([F(4,12) = 8.94, P = 0.001; \text{Fig. 4B}].\) BMa \([F(4,20) = 4.22, P = 0.012;\) doi:10.1152/jn.00373.2014 • www.jn.org

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while the spike activity of these structures was not changed with delivery of ddH\textsubscript{2}O or eugenol. The altered firing pattern in all BL/BM subnuclei to cat urine consisted of a significant increase in spike activity at 4 s only (\(P = 0.005; \text{RM ANOVA}\)) compared with 0 s, and comparisons with the remaining time points examined failed to show difference (0, 2, 6, 8 s). Interestingly, the increase in firing demonstrated at 4 s during cat urine presentation was significantly elevated compared with that of ddH\textsubscript{2}O for the BL\textsubscript{A} (\(P = 0.010; \text{paired } t\text{-test}; \text{Fig. 4A}\)) and BM\textsubscript{P} (\(P = 0.005; \text{Fig. 4D}\)), whereas only a trend in that direction was evident in the BL\textsubscript{V} (\(P = 0.063; \text{Fig. 4B}\)) and BM\textsubscript{A} (\(P = 0.058; \text{Fig. 4C}\)). Compared with eugenol, the increased firing demonstrated at 4 s when cat urine was presented was significantly different in the BM\textsubscript{P} only (\(P = 0.005\)) and approached significance for the BL\textsubscript{A} (\(P = 0.027\)), BL\textsubscript{V} (\(P = 0.042\)), and BM\textsubscript{A} (\(P = 0.011\)).

Given the small sample size and the similarity of responses in the ACo, PMCo, and PLCo, the results were combined as an analysis of CoA responses to cat urine, ddH\textsubscript{2}O, and eugenol (Fig. 5A). Spike activity in the CoA was altered by cat urine [\(F(4,20) = 9.77, P < 0.001; \text{RM ANOVA}\)] but not ddH\textsubscript{2}O or eugenol presentation. The change in firing to cat urine consisted of a significant increase at 4 s (\(P = 0.023\)), which was significantly elevated from the activity demonstrated in this nucleus to ddH\textsubscript{2}O (\(P = 0.006\)) and eugenol (\(P = 0.01\)). In the

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**Fig. 2.** Histological identification of recording sites. A: diagram of the 16-shank 128-channel 3-dimensional multiunit (12 mm) recording probe configured in a 4 \(\times\) 4 matrix, each probe with 8 recording sites. Intershank (horizontal) spacing was 200 \(\mu\text{m}\) and 400 \(\mu\text{m}\); within-shank (vertical) spacing was 200 \(\mu\text{m}\). Prior to electrode penetration, probes were dipped in fluorescent DiI solution to allow identification of penetration tracts after experimentation. B: serial images of Nissl-stained horizontal sections superimposed with the DiI tracks of the 3-dimensional recording array. Electrode tracks are visible as red florescence. Scale bar, 1 mm. C: schematic diagram of the horizontal plane depicting electrode recording sites as indicated by dots (showing DiI locations) constructed from histological sections and the standard stereotaxic atlas of the rat brain as a reference (Paxinos and Watson 2007). Orange dots indicate electrode sites that correspond in location to those depicted in the Nissl-stained images presented in B. D: Depiction of the anatomical range of recordings (all sessions collapsed together). Recordings were obtained from an area encompassing \(-1.80\) to \(-3.24\) mm to bregma (anterior/posterior), 2.8 to 5.4 mm lateral to the midline, 7.6 to 9.6 mm ventral to the brain surface. Orange shaded area indicates the anatomical range recorded from. Figure modified from Paxinos and Watson (2007) with permission.
AHi, increased firing over time was evident with the presentation of cat urine \(F(2.4,28.6) = 7.52, P = 0.001; \text{Fig. 5B}\), which was absent when ddH\textsubscript{2}O or eugenol was presented. Contrasts conducted with the onset of cat odor delivery revealed a significant increase at 4 s only \(P = 0.015\), which was significantly elevated compared with the activity demonstrated to both ddH\textsubscript{2}O \(P < 0.001\) and eugenol \(P = 0.007\). Responses to cat urine were scarce in the CeM; as a result, data were unable to be statistically analyzed. However, Fig. 5C depicts the activity of the few units that responded to cat urine. A clear increase in activity was evident at \(\sim 4\) s after cat urine delivery, which was absent with the delivery of ddH\textsubscript{2}O or eugenol. The BSTIA demonstrated alteration in activity similar to all amygdala nuclei examined, with a significant change in spike activity evoked by cat urine \(F(1.4,9.5) = 5.93, P = 0.029; \text{RM ANOVA; Fig. 5D}\), which was absent with ddH\textsubscript{2}O and eugenol delivery. Firing increased at 4 s after trial onset \(P < 0.001\), which was also significantly elevated compared with the spike activity demonstrated to ddH\textsubscript{2}O \(P = 0.008\) and eugenol presentation \(P = 0.016\). Temporal characteristics of response to cat urine in amygdala subnuclei. The timing of the peak response to cat urine occurred differently across the various subnuclei of the amygdala. A representative example of amygdala responses to cat urine within a single recording session indicated that the CoA was the first to demonstrate a peak response to cat urine at 1.8 s and again at 3.6 s after activation of the solenoid valves that contained cat urine. Interestingly, both the MeAV and MePV were found to peak soon after the CoA at 2.6 s, with repeated bursts of activity occurring in both these structures until 4.2 s (Fig. 6A). The MeAD peaked soon after the MeAV and MePV at 4.0 s, followed by the MePD at 4.2 s, the latter of which demonstrated increased activity occurring as early as 3.2 s. The AHi and the BSTIA were found to respond later than most MeA subnuclei, each peaking at 4.2 s after the beginning of odor delivery. In this recording session, the BM nuclei demonstrated a peak response to cat urine earlier than the AHi, BSTIA, CeM, and BL nuclei. Specifically, the Bmp peaked at 3.6 s, the Bma demonstrated a relatively low peak response at 4.0 s, while the BLa and BLv, along with the CeM, peaked latest of all amygdala nuclei at 4.4 s. CoA, all BL and BM nuclei, the CeM, BSTIA, and AHi were found to peak similarly at 4.4 s. The MePD was found to respond the latest of all nuclei examined, with peak activity first occurring at 4.6 s (Fig. 6A). A similar trend was examined when the timing of the peak response across all experimental recording sessions was examined. Within MeA nuclei, a significant difference in the timing of the peak response was found between subnuclei \(F(3,68) = 7.45, P < 0.001; \text{ANOVA}\). While it appeared that the peak response to cat urine occurred earlier for the ventral nuclei compared with the dorsal nuclei of the MeA (Fig. 6B), only the MePD was found to peak later than the MeAV and MePV (MeAV, \(P = 0.007\); MePV, \(P < 0.001\); Bonferroni post hoc test). The timing of the peak response to cat urine was also significantly different between the BL and BM nuclei \(F(3,20) = 4.69, P = 0.012; \text{ANOVA}\). The BM subnuclei appeared to demonstrate a peak response to cat urine earlier than the BL subnuclei (Fig. 6B); however, only the Bmp was found to peak earlier than the BLa \(P = 0.026\) and a trend in that direction was observed for the BLv \(P = 0.071\). Examination of the peak response across all amygdala subnuclei revealed a main effect for structure \(F(10,112) = 5.28, P < 0.001; 1\text{-way ANOVA}\). This difference was directly attributed to the MeAV, MePV, CoA, and Bmp, which demonstrated a significantly earlier peak response to cat urine compared with the MePD (MeAV, \(P = 0.036\); MePV, \(P = 0.003\); CoA, \(P = 0.001\); Bmp, \(P = 0.038\); Bonferroni post hoc) and the BSTIA (MeAV, \(P = 0.013\); MePV, \(P = 0.004\); CoA, \(P = 0.001\); Bmp, \(P = 0.016\); Fig. 6B).

As the peak response to cat urine did not always coincide with the onset of activity, the latency of the first response to cat urine was examined. A significant main effect was found within the subnuclei that comprise the MeA \(F(3,68) = 4.28, P = 0.007, \text{ANOVA}\) and the BL/BM amygdala \(F(3,20) = 9.17, P = 0.001, \text{ANOVA}\). Within the MeA, the response latency in the MePV was significantly earlier than the MePD \(P = 0.005, \text{Bonferroni post hoc}\). The MePV and the MePD each did not respond any differently from the anterior portions of the MeA (Fig. 6C). Within the baso-amygdala, activity in response to cat urine occurred earlier in the Bmp than in the

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**Table 1. Total number of units recorded in the amygdala complex and frequency and percentage of responses to cat urine odor and eugenol in each amygdala subnucleus**

<table>
<thead>
<tr>
<th>Recording Site</th>
<th>Total Units Recorded</th>
<th>Unit Responses to Cat Urine (%)</th>
<th>Unit Responses to Eugenol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdaloid hippocampal area (AHi)</td>
<td>218</td>
<td>13 (5.9%)†</td>
<td>6 (2.8%)</td>
</tr>
<tr>
<td>Anterior cortical amygdaloid area (ACo)</td>
<td>8</td>
<td>2 (25.0%)</td>
<td>0</td>
</tr>
<tr>
<td>Posteriomedial cortical amygdaloid nucleus (PMCo)</td>
<td>20</td>
<td>3 (15.0%)</td>
<td>0</td>
</tr>
<tr>
<td>Posterolateral cortical amygdaloid nucleus (PLCo)</td>
<td>8</td>
<td>1 (12.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Medial amygdaloid nucleus, anterior dorsal (MeAD)</td>
<td>63</td>
<td>16 (25.4%)*</td>
<td>1 (1.6%)</td>
</tr>
<tr>
<td>Medial amygdaloid nucleus, anterior ventral (MeAV)</td>
<td>20</td>
<td>7 (35.0%)*</td>
<td>1 (5.0%)</td>
</tr>
<tr>
<td>Medial amygdaloid nucleus, posterior dorsal (MePD)</td>
<td>100</td>
<td>28 (25.7%)*</td>
<td>2 (1.8%)</td>
</tr>
<tr>
<td>Medial amygdaloid nucleus, posterior ventral (MePV)</td>
<td>69</td>
<td>21 (30.4%)*</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>Basolateral amygdaloid area, anterior part (BLa)</td>
<td>22</td>
<td>4 (18.2%)</td>
<td>2 (9.1%)</td>
</tr>
<tr>
<td>Basolateral amygdaloid area, posterior part (BLp)</td>
<td>8</td>
<td>1 (12.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Basolateral amygdaloid area, ventral part (BLv)</td>
<td>11</td>
<td>4 (36.4%)</td>
<td>1 (9.1%)</td>
</tr>
<tr>
<td>Basomedial amygdaloid area, anterior part (BMa)</td>
<td>21</td>
<td>6 (28.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Basomedial amygdaloid area, posterior part (BMp)</td>
<td>65</td>
<td>10 (15.4%)</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Central amygdaloid nucleus, capsular part (CC)</td>
<td>36</td>
<td>1 (2.7%)†</td>
<td>2 (5.6%)</td>
</tr>
<tr>
<td>Central amygdaloid nucleus, medial division (CeM)</td>
<td>48</td>
<td>3 (6.3%)†</td>
<td>0</td>
</tr>
<tr>
<td>Bed nucleus of stria terminalis, intra-amygdaloid division (BSTIA)</td>
<td>51</td>
<td>8 (15.7%)</td>
<td>2 (3.9%)</td>
</tr>
</tbody>
</table>

*\(\chi^2\) analysis of standardized residuals \((Z > 1.96); \) †\(\chi^2\) analysis of standardized residuals \((Z < -1.96)\).
BLa (P = 0.028), BLv (P < 0.001), and BMa (P = 0.005), all three of which responded similarly in time to each other (Fig. 6C). Examination of the response latency among all amygdala subnuclei also indicated a significant main effect of subnucleus [F(10,112) = 8.00, P < 0.001, ANOVA]. Responses to cat urine in the BSTIA were found to occur significantly later than those located in the ACo, all MeA subnuclei, and the BMp (P = 0.04 to <0.001, Bonferroni post hoc). Multitrials located in the AHi responded later to cat urine compared with those in the ACo, MeAV, MeAD, MePV, and BMp (P = 0.024 to <0.001). As noted above, units in the MePV responded earlier than those located in the MePD (P = 0.036) and additionally to all BL/BM structures apart from those located in the BMp (P = 0.001 to 0.015). Furthermore, responses in the CoA were found to occur significantly earlier than the BMa (P = 0.028, Bonferroni post hoc).

Characteristics of spike activity in amygdala subnuclei to cat urine. Given that the response latency differed between and within amygdala subnuclei, all responses were matched relative to the onset of activity to examine the pattern of activity within each nuclei. As expected, a significant effect of time was observed in the spiking activity over the first 5 s in all

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Fig. 3. Mean (±SE) peristimulus histograms and the 5-point moving average spike count per stimulus repetition (0.2-s bins) during cat odor presentation trials and the analogous responses to ddH2O and eugenol in the medial nucleus of the amygdala (MeA), anterior ventral division (MeAV, n = 7; A), anterior dorsal division (MeAD, n = 16; B), posterior ventral division (MePV, n = 21; C), and posterior dorsal division (MePD, n = 28; D). Increased spike frequency was observed in all MeA subnuclei after cat urine exposure (black), while ddH2O (blue) and eugenol (green) presentation did not alter spike activity. The altered activity to cat urine was significantly elevated compared with ddH2O and eugenol exposure. Gray shading represents the time of odor delivery, 2 s. **P < 0.01, ***P < 0.001, repeated-measures (RM) ANOVA; aP < 0.001, paired t-test comparing ddH2O and cat odor; bP < 0.001, paired t-test comparing eugenol and cat odor; cP < 0.001, paired t-test comparing eugenol and cat odor.
MeA subnuclei [MeAV, $F(5,30) = 11.31, P < 0.001$; MeAD, $F(5,75) = 15.02, P < 0.001$; MePV, $F(3.0,59.7) = 20.04, P < 0.001$; MePD, $F(3.4,92.3) = 14.44, P < 0.001$; RM ANOVA; Fig. 7A]. This difference was largely attributable to the predictable increase in activity over the first second that a response was detected [MeAV, $F(1,6) = 14.97, P = 0.008$; MeAD, $F(1.15) = 74.38, P < 0.001$; MePV, $F(1.20) = 58.98, P < 0.001$; MePD, $F(1.27) = 29.38, P < 0.001$; planned contrast with −1.0 s (time before response onset); Fig. 7B]. Interestingly, activity continued over an additional second in the posterior portions of the MeA [MePV, $F(1,20) = 22.34, P < 0.001$; MePD, $F(1.27) = 29.95, P < 0.001$] while activity returned to baseline in the anterior portions of the MeA.

Additionally, while the dorsal divisions of the MeA returned to baseline after the increase, the ventral divisions demonstrated a substantial decrease in activity after activation (Fig. 7A and B). In the MeAV, suppression of spike activity occurred from 3 to 3.8 s after the onset of activity [$F(1,6) = 7.27, P = 0.036$]. In the MePV, inhibition occurred over an extended period beginning at 3 s [$F(1,20) = 4.86, P = 0.039$] and extending until 4.8 s [$F(1,20) = 11.52, P = 0.003$; Fig. 7B].

Nuclei comprising the BL/BM amygdala also demonstrated altered activity to cat urine over time [BLa, $F(3,9) = 30.26, P < 0.001$; BLv, $F(1,2.3) = 10.36, P = 0.036$; BMa, $F(5,25) = 30.26, P < 0.001$; BMp, $F(5,125) = 30.26, P < 0.001$; RM ANOVA; Fig. 7C].
Spike activity in the BLa \( F(1,3) = 90.89, P = 0.002 \), BLv \( F(1,3) = 12.30, P = 0.039 \), BMa \( F(1,5) = 6.87, P = 0.047 \), and BMP \( F(1,8) = 17.30, P = 0.003 \) was elevated over the first second when compared with baseline activity (Fig. 7D). Similarly, a main effect of time was found in spike activity in the CoA, AHi, and BSTIA \( \text{CoA, } F(5,30) = 5.86, P < 0.001; \text{AHi, } F(2.7,36.7) = 19.03, P < 0.001; \text{BSTIA, } F(5,35) = 17.53, P < 0.001; \) Fig. 7E]. This was due to an increase in spike activity at response onset (0 to 0.8 s) \( \text{CoA, } F(1,6) = 24.92, P = 0.002; \text{AHi, } F(1,12) = 61.21, P < 0.001; \text{BSTIA, } F(1,7) = 58.70, P < 0.001; \) Fig. 7F]. No further differences in spike activity were detected over time for these subnuclei.

**DISCUSSION**

The relative contribution of the structurally heterogeneous amygdalar nuclei in processing chemosensory cues related to defensive behavior has yet to be fully established. Here we demonstrate, in an in vivo electrophysiological examination of
multiunit amygdala activity, that amygdala neurons selectively respond to the cues present in a behaviorally validated predator odor, cat urine. Cat urine, similar to other utilized predator odors (Dielenberg and McGregor 2001; Takahashi et al. 2005), reliably elicited defensive responses in rats, characterized by avoidance, increased immobility, risk assessment, and displacement behavior to the odor. High sample variability likely accounts for disparate findings concerning the suitability of cat urine as a predator odor (Blanchard et al. 2003; Fendt 2006). However, these and recent reports (Kabitzke and Wiedenmayer 2011; Xu et al. 2012) indicate that cat urine is capable of triggering hard-wired defensive responses and, therefore, components of the antipredator neural circuit. Indeed, the behaviorally relevant odor of cat urine was capable of stimulating responses in the amygdalar complex, while an odor that lacks emotional salience, eugenol, largely failed to trigger amygdala activity. Furthermore, we found that half of all responses to predator urine originated from the MeA and occurred earlier than most of the nuclei examined. As the MeA projects heavily to areas of the hypothalamic defensive network (Canteras et al. 2004; Xie et al. 2012).

Fig. 6. A: representative example of peristimulus time histograms showing the spiking count per stimulus repetition (0.2-s bins) of channels (Ch) of the 3-dimensional probe located in various amygdala subnuclei during cat odor delivery from a single electrophysiological recording session. The CoA, MePV, and MeAV are the first to demonstrate a peak response to cat urine, while the peak response occurred latest in the BLa, BLv, and CeM. Gray shading represents the time of odor delivery (2 s); green bars denote peak response. B: scatterplot depicting the timing of the peak response to cat urine of all recorded units and the mean ± SE timing of the response peak to cat urine in the MeAV (n = 7), MePV (n = 21), MeAD (n = 16), MePD (n = 28), CoA (n = 7), BLa and BLv (n = 4 each), BMa (n = 6), BMp (n = 10), AHf (n = 13), CeM (n = 3), and BSTIA (n = 8). Gray shading represents the time of odor delivery. *Significant difference from MeAV and MePV, †significant difference from BMa, ‡significant difference from MeAV, MePV, CoA, and BMp, P < 0.05, Bonferroni post hoc test. C: mean ± SE response latency to cat urine in all amygdala nuclei; CeM not included in statistical analysis. *Significant difference from CoA, MeAV, MeAD, MePV, MePD, and BMp, †significant difference from CoA, MeAV, MeAD, MePV, and BMp, ‡significant difference from CoA and MePV, §significant difference from MePV, P < 0.05, Bonferroni post hoc test.
NEURAL ACTIVITY IN AMYGDALA TO PREDATOR ODOR

1995) and is the first site of olfactory convergence (Meredith 1998), it is in an excellent position to integrate olfactory cues signaling predators and engage excitatory or inhibitory events in downstream targets that regulate defensive responses. These findings are consonant with gene activation studies (Dielenberg et al. 2001; Meredith and Westberry 2004) and converge with lesion studies (Li et al. 2004; Masini et al. 2009) to highlight the critical involvement of the MeA in predator odor-generated fear.

Increased activity to predator urine was detected in all MeA subdivisions. The activity observed in the MePV and anterior MeA reinforces the well-established involvement of these nuclei in predator odor-induced defensive behavior (Dielenberg et al. 2001; Meredith and Westberry 2004). As these nuclei share projection pathways similar to the major components of the defensive circuit (Pardo-Bellver et al. 2012), increased activation likely reflects a functional role concerned with generating defensive responses. The modulation of these targets is likely to be glutamatergic, as predator odor activates glutamatergic populations in the MeA (Butler et al. 2011) and specifically stimulates glutamatergic MePV efferents that innervate the glutamatergic neurons of the dorsomedial ventromedial hypothalamus (VMHdm), a critical component of the defensive circuit (Choi et al. 2005). Coupled with the γ-aminobutyric acid (GABA)ergic absent MeAV (Poulin et al. 2008), the MePV would exert a particularly powerful excitatory influence on the defensive circuit when activated by predator odor.

Responses to predator urine were also detected in the MePD, implicating a potential contribution of this subnucleus in predator odor processing that is surprising given its connection with reproductive rather than defensive circuits (Canteras et al. 1995). Despite most reports failing to demonstrate MePD activation by predator odor (Choi et al. 2005; Dielenberg et al. 2001), activation has been documented after live predator (Martinez et al. 2011) and predator odor (Masini et al. 2005) exposure, commensurate with the present results. The functional relevance of this activity is unknown until the phenotypic characteristics of the activated neurons can be established. The delayed responses relative to the MePV, however, likely indicate that the functional role of MePD activity is not directed toward the generation of defensive behavior. Therefore, we speculate that this activity may reflect a potential

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Fig. 7. A, C, and E: mean ± SE 5-point moving average spike count per repetition (0.2-s bin resolution) from response onset (0) to cat urine in the MeA subnuclei (A): MeAV (n = 7), MePV (n = 21), MeAD (n = 16), MePD (n = 7); basolateral and basomedial subnuclei (C): BLa and BLv (n = 4 each), BMa (n = 6), BMe (n = 10); and CoA (n = 7), AHi (n = 13), CeM (n = 3), and BSTIA (n = 8) (E). *P < 0.05, **P < 0.01, ***P < 0.001, RM ANOVA examining 1-s averages beginning from 1 s before response detection until 5 s after response detection (−1, 0, 1, 2, 3, 4, 5 s), except for BLa and BLv, where 4 points were examined (−1, 0, 1, 2) given the low sample size. B, D, and F: mean ± SE peristimulus time histograms of spike count per repetition (0.2-s bins) when the onset of response latency has been controlled for in MeA subnuclei (B), BL/BM nuclei (D), and CoA, AHi, CeM, and BSTIA (F). As expected, all nuclei examined demonstrated a significant increase in activity over the first second after response detection. Additionally, responses in the MePV and MePD exhibited significant suppression following the increase in spike activity. *P < 0.05, **P < 0.01, ***P < 0.001, RM ANOVA and planned contrast with the time before response onset (−1.0 to 0.8).
means by which incoming chemosensory information may inhibit reproductive activity during times of predation threat. As MePD GABAergic projection neurons are not stimulated by predator odor (Choi et al. 2005), this may potentially occur via excitatory projections that synapse onto inhibitory neurons of the reproductive circuit.

Distinct firing patterns were evident among MeA subnuclei after predator odor exposure, potentially indicating the underlying neurocircuitry involved in predator odor-induced defensiveness. For instance, the pattern of excitation followed by suppression observed in the MeAV and MePV after odor detection is suggestive of feedback inhibition, as both divisions share strong reciprocal connections with defensive targets such as the VMHdm and bed nucleus of the stria terminalis (BNST) (Novaes and Shamah-Lagado 2011; Pardo-Bellver et al. 2012), any one of which could provide feedback input to the MeAV and MePV. The BNST is a viable candidate for the suppression of firing observed in the ventral MeA as it is largely GABAergic (Cullinan et al. 1993) and known to mediate autonomic, endocrine, and behavioral responses to unconditioned fear (Walker and Davis 1997). Consequently, it is plausible that increased ventral MeA activity to predator odor additionally stimulates GABAergic cells in the BNST that in turn decrease MeA output, thereby providing the necessary regulatory mechanism required to dampen the largely excitatory ventral MeA. Given that the MeAV and MeAD are thought to serve as filters and evaluators of chemosensory information (Meredith and Westberry 2004), the suppression observed in the MeAV may be relatively brief compared with the MePV to specifically enable the MeAV to be more receptive to any additional chemosensory information related to the initial threat and adjust behavioral responses accordingly.

The findings from this study raise questions concerning the respective roles of the main (MOS) and accessory (AOS) olfactory systems in predator odor processing. The odorants contained in cat urine were delivered to the animals through respiration, thereby bypassing the AOS, which requires active sniffing (Luo et al. 2003). Consequently, the robust amygdala responses evoked by cat urine suggest an important role for the MOS in processing predator odors. This is in contrast with the view that the vomeronasal system exclusively detects predator odors (McGregor et al. 2004; Meredith and Westberry 2004), although it does not exclude the possibility that the AOS contributes equally or more so in predator odor detection. Indeed, the AO bulb (Ben-Shaul et al. 2010) and MO bulb (MOB) (Kobayakawa et al. 2007) are both involved in predator odor detection, and both appear necessary for predator odor-induced defensive behavior (Masini et al. 2010). Consequently, both systems may play overlapping roles in predator odor detection. In the present study, responses to predator urine were detected in the CoA, which is expected given that odor delivery occurred via the MOS, which heavily innervates CoA nuclei, particularly the ACo (Scalia and Winans 1975). Interestingly, CoA and MeA responses to predator urine were indistinguishable temporally, suggesting that chemosensory information transfer from the MOB to the MeA may not occur exclusively through the CoA. Information transfer might occur from the MOB to the MeA directly via the sparse projections recently identified (Kang et al. 2009). However, it is highly possible that differences in the speed of information transmission between the CoA and MeA may have been too swift to detect with the analysis adopted here.

Predator urine elicited relatively fewer responses in the BL nuclei of the amygdala, and these were delayed compared with the MeA. Alongside the observed activation of a select neuronal population in the BLA by predator odor (Butler et al. 2011), these results indicate a noncritical role for this complex in fear behavior generation. BL amygdala responses to predator urine may alternatively reflect a function related to olfactory fear conditioning, as both the MeA and BL amygdala are necessary for predator odor-induced conditioned fear (Takahashi et al. 2007). Interestingly, some clusters of units located in the BLA were found to respond to both cat urine and eugenol, and may indeed reflect activity of a neural population concerned with olfactory fear learning. While the lateral amygdala (LA) was not sampled in the present study, it may also play a role in olfactory fear learning given that it is considered the major sensory interface of the amygdala to fear conditioning (LeDoux 2003) and serves as an additional amygdala target for olfactory information (Sah et al. 2003). The present results do, however, provide strong evidence toward the involvement of the BMP in predator odor-induced fear. Predator urine elicited responses in the BMP that were temporally similar to those of the MeAV and MePV. Given that the BMP shares moderately dense reciprocal connections with the MeAV and MePV (Novaes and Shamah-Lagado 2011), and projects to the same defensive targets (VMH and BNST; Petrovich et al. 1996), the temporal features of this response indicate that the BMP could serve as an additional indirect pathway for the MeA to influence the expression of defensive behavior.

A distinct lack of response in the CeA was found upon exposure to cat urine, indicating that it is unlikely to function as the principal amygdalar output station coordinating behavioral responses to predator odors as has been demonstrated for conditioned fear. Indeed, fear behavior remains unaltered by CeA lesions (Li et al. 2004) and neuronal activation markers unchanged by predator odors (Dielenberg et al. 2001; Masini et al. 2005), commensurate with the present findings. Despite the scarcity of responses within the CeA, the few responses identified in the present study do suggest at least detection of cat urine, which occurs substantially later than other amygdala nuclei. This may reflect activity of a specific glutamatergic neuronal population documented to be stimulated by predator odor exposure (Butler et al. 2011). While the CeA may not appear to be critically involved in the generation of defensive behavior, the BNST has been implicated in coordinating behavioral responses to predator odor via its connections to various defensive areas (Xu et al. 2012). Interestingly, the intra-amygdaloid part of the BNST, the BSTIA, demonstrated robust activity to predator urine, indicating a role for this subnucleus in predator odor processing that has thus far been undocumented. The BSTIA may serve as an additional node in the antipredator defensive circuit, as it receives dense innervation from the MeA (Pardo-Bellver et al. 2012) and demonstrated delayed firing to predator urine compared with the MeA. Consequently, the BSTIA is in a position to respond to highly processed information from the MeA and provide supplemental input into other antipredator defensive structures, probably via connections to the BNST.

The relatively robust responses of the amygdala following predator odor exposure were in contrast to the lack of observed...
responses toward the neutral odor of eugenol. Robust activity in amygdala nuclei similar to those examined here has been demonstrated toward other neutral odors (such as amyl acetate) in both awake (Saphier et al. 1988) and anesthetized (Cain and Bindra 1972) animals. However, this appears to be dependent on the type of odor presented, with many odors eliciting minimal or no responses in specific amygdala subnuclei (Cain and Bindra 1972). Collectively, these findings suggest that the lack of response seen toward eugenol in the present study is likely due to the properties of this specific odor rather than the use of anesthesia.

The study presented here increases our knowledge of the interamygdala circuitry involved in predator odor processing. The importance and involvement of the structurally heterogeneous amygdalar complex in processing the olfactory cues of a natural predator are yet to be fully established. Here we demonstrate, in the first in vivo electrophysiological examination of amygdala activity to controlled presentations of cat urine, that the amygdala selectively responds to cues present in a behaviorally identified predator odor. The multiunit approach adopted here provides a measure of mean population response, thereby providing a global assessment of amygdala subnucleus activity to a predator odor. The present findings converge with previous studies to solidify the role of the MeA as a crucial component of the antipredator defensive circuit and also implicate a previously undocumented involvement for the BMp and BSTIA in processing olfactory cues signaling predator threat. These novel findings provide an important foundation for understanding the underlying circuitry mediating unconditioned fear.

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REFERENCES


