The Role of the Dorsal Periaqueductal Grey in Risky Foraging Behavior

Melissa A. Reilly

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Jeansok J. Kim

Jaime Diaz

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Abstract

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Melissa A. Reilly

Chair of the Supervisory Committee:

Jeansok J. Kim

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Of all the behaviors a wild animal engages in on a daily basis, few are more important – or potentially more dangerous – than foraging for resources. To do so successfully, the fear system serves a modulatory role in foraging behavior. The periaqueductal gray (PAG) and has long been implicated in the generation of defensive behaviors as part of this fear system. The dorsal PAG in particular is involved in escape when a threat is imminent. In order to investigate more precisely the role of the PAG on foraging in an environment with varied levels of risk of predation, rats were subjected to PAG or SHAM lesions. Rats were then trained to venture into a foraging area to procure food pellets at various distances from the safety of their home nest, while a remote-controlled robot alligator "attacked" from the opposite end of the arena. There were few behavioral differences between SHAM

and PAG-lesioned rats, suggesting that the dorsal PAG may not be the only nucleus involved in mediating these types of behaviors.

Introduction

Of all the behaviors a wild animal engages in on a daily basis, few are more important – or potentially more dangerous – than foraging for resources. Hunger is an effective motivator, but this motivation must be weighed against the risks; choosing the optimal time and location to forage will lessen the risk of coming up empty-handed, or worse, predation. The mere presence of a predator in a smaller animal's territory has been shown to take a dramatic toll on normal foraging and exploratory behaviors; after a brief introduction of a cat into a colony of rats living in a visible burrow system, rats were observed to eat and drink less, show a greater preference for areas in which they would be concealed from view, and increased scanning of open areas, as well as changing the way they move through them (Blanchard & Blanchard, 1989). Without even having to encounter the predator directly, the fear of predation seems to be an effective motivator to change foraging patterns. The contribution of the fear system in making this change is not entirely understood.

Long implicated in the processing of fear, and even touted as the "switchboard for fear" (Gross & Canteras, 2012), is the amygdala – a major limbic structure located in the medial temporal lobe. The multiple subnuclei of the amygdala are involved to varying degrees in many different aspects of fear. The lateral amygdala (LA), for example, is considered the terminal for incoming sensory input, with damage to this area resulting in impairments in acquisition of Pavlovian fear conditioning (Campeau & Davis, 1995; Kim & Jung, 2006). The central amygdala (CeA) serves as the output center for the amygdala and projects to a number of hypothalamic and brainstem targets to mediate aspects of the fear response. Lesion or inactivation of the CeA can eliminate responses typically associated

with fear, such as conditioned freezing and ultrasonic vocalization (Choi & Brown, 2003) and conditioned bradycardia (Burhans & Schreurs, 2013).

A primary target of the CeA is the periaqueductal gray (PAG), a midbrain structure that surrounds the cerebral aqueduct. This structure has been implicated in a number of behaviors related to fear including pain and analgesia, vocalization, lordosis, and cardiovascular control (Behbehani, 1995). The execution of these varying behaviors within a single structure is perhaps enabled by the unique organization of the PAG: at least four longitudinal columns running rostrocaudally along the length of the aqueduct (Bandler, Carrive, & Depaulis, 1991; Vianna & Brandao, 2003). The PAG has been suggested to be the "coordinator" of defensive activities in response to fear-inducing stimuli (Fanselow M. , 1991), particularly its dorsal (dPAG) and ventral (vPAG) columns. Evidence suggests that these two columns mediate different stages of the defensive response.

Stimulation of the PAG has been observed to cause fear responses in humans and animals alike. Stimulating electrodes placed in the human midbrain, including the PAG, as a treatment for central pain caused patients to experience very negative feelings of terror and discomfort, being "scared to death." These negative emotions were also accompanied by more peripheral effects, such as hyperventilation, vocalization, and the inability to speak (Nashold, Wilson, & Slaughter, 1969) Stimulation of the rodent PAG, though not verbally confirmed, has been observed to be aversive (Oliveira, Nobre, Brandao, & Landeira-Fernandez, 2004), and both chemical (Kincheski, Mota-Ortiz, Pavesi, Canteras, & Carobrez, 2012) and electrical (Kim, et al., 2013) stimulation of this area have served as an effective US for olfactory and auditory fear conditioning, respectively. Fanselow (1991) observed that placement of stimulating electrodes into the ventral rat PAG caused immediate

freezing behavior, while more dorsolateral placements caused what resembled a footshock-induced activity burst, followed immediately after by freezing. Microinjection of the excitatory amino acid DLH into the caudal portion of the feline PAG produced similar results: a flight response for more dorsolateral placements and hyporeactive immobility for ventrolateral placements (Carrive, 1993).

On the other hand, lesions of the dPAG eliminated defensive responses in (normally intensely fearful) wild-caught rats to a variety of tactile and auditory stimuli (Blanchard, Williams, Lee, & Blanchard, 1981). In a contextual fear conditioning paradigm, rats subjected to rostral dPAG lesions maintained high levels of freezing comparable to sham controls, whereas rostral vPAG lesions caused a robust decrease in freezing behavior (Kim, Rison, & Fanselow, 1993). Thus, the ventral and dorsolateral columns would appear to be functionally opposed, and indeed, it has been argued that the dlPAG mediates escape behaviors whereas the vPAG mediates freezing (Fanselow M. , 1991). The contributions of these columns have also been interpreted as mediating conditioned or cued danger responses (vPAG) and unconditioned or immediate danger responses (dPAG) (Vianna & Brandao, 2003). The present study seeks to elucidate the role of the dorsal periaqueductal grey in rats foraging against the threat of predation.

Materials and Methods

Subjects

Twelve naïve male Charles River Long-Evans rats arrived initially weighing 275-300 g. Rats were individually housed in the AAALAC-accredited Department of Psychology animal care facility at the University of Washington and maintained on a reverse 12 hour light/dark cycle (lights on at 19:00). Rats were given free access to water and were food

restricted to gradually reach and maintain a target body weight of 80-85% normal weight. All experiments were conducted during the dark cycle with strict adherence to University of Washington Institutional Animal Care and Use Committee guidelines.

Surgery

Rats were administered children's Tylenol (14ml into drinking water) 24 hours prior to surgery. Under anesthesia (30 mg/kg ketamine and 2.5 mg/kg xylazine, i.p.), rats were mounted in a stereotaxic instrument and received bilateral lesions of the PAG. Stereotaxic coordinates were as follows, referenced from bregma: (*i*) dorsal PAG group: anteroposterior (AP) -7.8, mediolateral (ML) ±0.5, dorsoventral (DV) -4.7 and ; (*ii*) dorsal and ventral PAG rats (anterior/posterior) AP -6.8/-7.8, ML ±0.5/±0.5, DV -6.0/-5.7. Lesions were made by passing constant current (1 mA, 10 s; Grass Medical Instruments) through epoxy-coated stainless steel insect pins (no. 00) except for ~0.5 mm exposed at the tip. For SHAM rats, lesions were lowered to 1mm above the DV target and no current was passed. A 0.1ml dose of diazepam was given subcutaneously immediately following surgery, and rats were given 7 d of surgical recovery before experimental testing began.

Foraging Apparatus

A custom-built "seminaturalistic" apparatus consisted of a nesting area (29.21-cm length × 57.12-cm width × 59.69-cm height; equipped with a water bottle; 16.2 Lux luminance) with a remotely controlled, vertically opening gateway to an adjacent foraging area (201.93-cm length × 58.42-cm width × 60.96-cm height; 56.7 Lux; 60-dB white noise, A scale) (Figure 1). The ANY-maze video tracking system (Stoelting), with video feed from an overhead ultradigital wireless camera (LW2101; Lorex Technology) connected to a Sony HD DVD recorder (RDR-HX900), was used to record and automatically track the animal's

movement (30 frames per s) from both nesting and foraging areas. On "Robot" trials, the end of the foraging area opposite the gate contained a Mindstorms robot (LEGO Systems), in a figure of Robogator on wheels (66.04 cm length, 17.78 cm width, 15.24 cm height). The Robogator was programmed to surge 23 cm (at a velocity of ~75 cm/s), snap its jaw once (at an angular velocity 44.4 rpm), and return to its starting position.

Foraging Procedures

Habituation. For 2 consecutive days, animals were placed in the nesting area and contained there for 30 min/d with 10 food pellets (F0173; Bio-Serv; grain-based, 1 g) to acclimatize them to the nesting area. Rats were given five of the food pellets in the home cage the day prior to habituation to reduce neophobia.

Baseline testing. After 2 min in the nesting area (without food), the gateway to the foraging area opened, and the animal was allowed to explore and search for a food pellet placed 25.4 cm from the nest area (first trial). As soon as the animal took the pellet back inside the nest, the gateway closed. Once the animal finished consuming the pellet, the second foraging trial (with the pellet placed 50.8 cm from the nest area) and then the third foraging trial (with the pellet placed 76.2 cm from the nest area) started in the same manner. All animals met the criterion of retrieving the pellets and returning back to the nest within 60 s for three successive trials between 3 and 5 consecutive days of baseline foraging.

Robot testing. The food pellet was placed at the 76.2-cm location on the first encounter with the Robogator. After the gateway opened, each time the animal approached the vicinity (~25 cm) of the pellet, the Robogator surged 23 cm toward the pellet, snapped its jaws once, and returned to its original position. Animals were permitted 3 min to

procure the pellet. If the rat was unsuccessful, the gate was closed with the animal inside the nesting area, and the food pellet was placed 25.4 cm closer to the nest on the following trial. The latency required for the animal to procure the pellet successfully (i.e., the time from the gate opening to the rat's returning to the nest with the pellet) served as the dependent variable.

Contextual Fear Conditioning

Contextual fear conditioning was conducted in a small rodent chamber (Coulbourn Instruments, Allentown, PA) placed in a sound-attenuating box. The chamber contained two aluminum side walls and two Plexiglas walls at the front and back. The floor consisted of stainless steel rods that were attached to a shock generator during training. The chamber house light as well as an additional light in the sound attenuating chamber were turned on with white light. Before each animal was brought in, the chamber was wiped down with a 70% alcohol solution.

On the day of conditioning, the rat was brought into the experimental room in an opaque transport box. After placement in the chamber, a three minute baseline period was followed by three 1-second unsignaled footshocks (1 mA) spaced 1-minute apart. At the conclusion of testing, the rats were removed from the chamber and returned to the homecage. On the day of testing, rats were returned to the same chamber and freezing behavior was monitored for 8-minutes. No shocks were given on the test day.

Data collection and stimulus presentations were controlled with an IBM-PC computer equipped with the Coulbourn LabLinc Habitest Universal Linc System. A 24-cell infrared activity monitor was mounted on top of each cage and was used to assess freezing behavior, with 3-seconds of immobility being the threshold for freezing.

Histology

At the completion of behavioral testing, animals were overdosed with Beuthanasia and perfused intracardially with 0.9% saline followed by 10% buffered formalin. The brains were removed and stored in 10% formalin overnight and then kept in 30% sucrose solution until they sank. Transverse 80-µm sections were taken through the extent of the lesion, mounted on gelatin-coated slides, and stained with cresyl violet and Prussian blue dyes.

Statistical Analysis

All statistics were conducted using SPSS. A repeated-measures ANOVA was used to analyze latency to retrieve the food pellet across days of testing as well as freezing behavior in the operant chamber. Pellet foraging distances were scored as ordinal data, and the furthest distance of successful procurement for each rat on each test day was used for a Mann-Whitney U test between groups.

Results

Histology

After mounting and staining the slides, it became apparent that the extent of the lesions varied greatly (Figure 2). Though the lesion target was the dPAG, there were animals with additional damage to the ventral PAG. The final group divisions were: SHAM (n = 4), dPAG (n = 3), MISS (n = 3), ant. d/vPAG (n = 1), post. d/v PAG (n = 1). Lesions that missed the target were not included in the analysis. Behavioral data seems to suggest a difference in effect between combined dorsal and ventral lesions of the PAG depending on where they fell along the anterior-posterior axis; thus, these rats were not grouped

together, and were also not included in statistical analysis. They will be discussed only anecdotally (advPAG: lesions of both dorsal and ventral anterior PAG; pdvPAG: lesions of both dorsal and ventral posterior PAG) and have been included in graphs where appropriate.

Foraging Behavior

Food-restricted rats quickly learned to search for food pellets placed 25.4, 50.8 and 76.2cm away from the nesting area in a large open field (Figure 3A). Upon reaching the pellet, rats instinctively brought it back to the nesting area and consumed it there. After 3-5 days of baseline testing, the dPAG (M = 5.63 sec) and SHAM (M = 8.2 sec) groups were similar in their pellet retrieval time at the long distance, $t(5) = 0.39$, $p > .05$., and thus began robot testing. On the first day of testing there were no successes in procuring the pellet at the long distance within the 3-minute limit except for the sole rat with advPAG lesions, which retrieved the pellet in 10 seconds (Figure 3B). As testing progressed through the medium and short trials, both groups showed modest improvements in their retrieval time, though at no point did the groups significantly differ, thus dPAG lesions did not seem to dampen the fearful response to the Robogator. The rat with pdvPAG lesions did not succeed in procuring the pellet at any distance. Looking at the furthest distance at which a rat successfully retrieved the pellet showed that the SHAM and dPAG groups did not differ in their willingness to get close to the Robogator, $U = 5.50$, $z = -.20$, $p > .05$.

At the 24hr retest, rats repeated the exact task as the day before to determine if the lesions would have any effect on habituation to the Robogator. At all distances, the latency to retrieve the pellet was faster than the day before (Figure 4), indicating some degree of habituation to the robot by the second day, however there was no significant difference

between groups. There was a significant main effect of test day, $F(2,10) = 19.15$, $p = .00$. Contrasts revealed that latency to retrieve the pellet in both the robot test, $F(1,5) = 12758.27$, r = .99, p = .00, and the 24hr retest, $F(1,5) = 12.243$, $r = .84$, $p = .02$, were significantly greater than at baseline, reinforcing that the presence of the robot has a robust and persistent effect on foraging behavior. There was no effect of group, suggesting that dPAG lesions do not alter the rate of habituation to a fearful stimulus. The rats with dorsal and ventral PAG lesions performed almost exactly the same as the day before, with the advPAG rat successfully retrieving the pellet at all three distances at an average of 44.33 seconds per trial, and the pdvPAG rat failing to get the pellet at all. Though not significantly so, the mean pellet retrieval times were consistently lower for the SHAM group relative to those with dPAG lesions. Much like the first robot exposure, after the second day of testing was complete, there was no difference between groups in how far they were willing to go to retrieve the pellet in the presence of the Robogator, $U = 4.50$, $z = -.56$, $p > .05$

Fear Conditioning

After undergoing contextual fear conditioning, rats were returned to the same context in which they had previously been conditioned and freezing behavior was monitored for eight minutes. A repeated-measures ANOVA revealed there was no main effect of group, $F(1,5) = .291$, $p > .05$. There was a main effect of time, $F(7,35) = 8.052$, $p = .00$, with time freezing increasing over time and peaking in the 5th minute (Figure 5). The pdvPAG rat froze the least, 18% of the total 8 minutes.

Discussion

Despite lesions to the dPAG, rats reacted to the Robogator in the same way as those with sham lesions – an effect we did not anticipate. It was expected that rats with lesions of

the dPAG would be undeterred by the robot and would retrieve the pellet about as quickly as they had in baseline trials. Following dPAG lesions, Blanchard and colleagues (1981) observed experimental rats to be positively tame, exhibiting minimal aggressive or defensive behavior in several tasks that would certainly be stressful to a rodent – being picked up by an experimenter, having their whiskers touched, and being exposed to an anaesthetized conspecific. Moreover, the rats in that study were wild-caught, making these tasks all the more unnatural and, one would assume, fear-provoking. The present study found that dPAG lesioned rats were as fearful as the SHAM controls; in fact, though the small sample size limits any definitive conclusions, SHAM controls seemed to out-perform dPAG lesioned rats in both overall retrieval time and individual trial success. A recent study (Mota-Ortiz, et al., 2012) examining the effects of rostrolateral PAG lesions on predatory hunting in male rats revealed that damage to this area completely abolished the rats' desire to chase or attack prey. Though regular food intake and hunting are believed to be mediated by different neural circuits, it is possible that the unique demands of this task recruit both. Thus, lesions extending ventrally from dPAG into the lateral column may explain the poor performance exhibited in this task by lesioned rats.

In the conditioning chamber, rats with dPAG lesions reacted similarly to the SHAM group, maintaining a freezing response for the 8-minute context test, as was expected given the literature: rats with dPAG lesions have not been observed to perform differently from SHAM controls in contextual fear conditioning (Kim, Rison, & Fanselow, 1993) and the dPAG is not generally believed to be important for conditioned freezing behavior. Lesions to the vPAG (Kim, Rison, & Fanselow, 1993; Fanselow M. , 1991) or the PAG as a whole (Amorapanth, Nader, & LeDoux, 1999), however, have been shown to greatly decrease

conditioned freezing in contextual fear conditioning tasks. In the present study, the two rats with lesions encompassing dorsal and ventral PAG froze considerably less than the dPAG and SHAM controls, and would seem to support those findings. It is noteworthy that the rat with lesions to the caudal dvPAG showed inconsistent fear behavior in the foraging apparatus (complete failure to procure pellet) and in the contextual fear conditioning chamber (minimal freezing throughout the 8-minute test), a finding difficult to explain in view of its rather complete dorsal and ventral PAG destruction.

The realization that the PAG is more than just a "primitive reticular structure" has been a critical turning point in the advancing of our understanding of this structure and its complex organization (Bandler & Shipley, 1994), but the specificity of the columns of the PAG based on anatomy and function still falls somewhat short in predicting behavioral outcomes of brain manipulations. Stimulating more rostral parts of the dorsolateral column with kainic acid has been observed to cause backward defense responses while more caudal sites caused forward defense responses and occasional jumping in the rat (Bandler & DePaulis, 1991). In addition to the column of interest and the location along the rostrocaudal axis within the brain, the physical context of the experiment can also change the behaviors observed. In our own lab we have observed different responses to dPAG stimulation if the rat was in a fear conditioning chamber or in the foraging apparatus (Kim, et al., 2013), suggesting that context is an important consideration to be made in exploring the function of the PAG.

Examining these results through the lens of the Predatory Imminence Continuum (Fanselow & Lester, 1988), it is possible that the Robogator foraging apparatus does not adequately elicit the full spectrum of defensive behaviors. After the initial encounter, rats

do seem to remain in the "pre-encounter defense phase," characterized by reorganized meal patterns and a cautious stretch-approach behavior in the nest area. In future studies, it would be interesting to close the gate to the nest after the rat exits, disallowing the run-and-hide defense, to see whether the rat would adopt a cower-and-freeze strategy or try more aggressively to obtain the pellet, and to see what effect dPAG lesions would have in this case.

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