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Social signals increase monoamine levels in the tegmentum of juvenile Mexican spadefoot toads (*Spea multiplicata*)

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Abstract Monoamines are important neuromodulators that respond to social cues and that can, in turn, modify social responses. Yet we know very little about the ontogeny of monoaminergic systems and whether they contribute to the development of social behavior. Anurans are an excellent model for studying the development of social behavior because one of its primary components, phonotaxis, is expressed early in life. To examine the effect of social signals on monoamines early in ontogeny, we presented juvenile Mexican spadefoot toads (*Spea multiplicata*) with a male mating call or no sound and measured norepinephrine, epinephrine, dopamine, serotonin, and a serotonin metabolite, across the brain using high-pressure liquid chromatography. Our results demonstrate that adult-like monoaminergic systems are in place shortly after metamorphosis. Perhaps more interestingly, we found that mating calls increased the level of monoamines in the juvenile tegmentum, a midbrain region involved in sensory-motor integration and that contributes to brain arousal and attention. We saw no such increase in the auditory midbrain or in forebrain regions. We suggest that changes in monoamine levels in the juvenile tegmentum may reflect the effects of social signals on arousal state and could contribute to context-dependent modulation of social behavior.

Keywords Monoamines · Neuromodulator · Anuran · Acoustic communication · HPLC

Abbreviations

5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytryptamine (serotonin)
A-D di	Anterior-dorsal diencephalon
DA	Dopamine
DOPAC	3,4-Dihydroxyphenylacetic acid
E	Epinephrine
HVA	Homovanillic acid
ISO	Isoproterenol
MHPG	3-Methoxy-4-hydroxyphenylglycol
NE	Norepinephrine
P	Pallium
P-D di	Posterior-dorsal diencephalon
PoA	Preoptic area
P-V di	Posterior-ventral diencephalon
SC	Suprachiasmatic nucleus
SubP	Subpallium
Teg	Tegmentum
TS	Torus semicircularis

Introduction

Social behavior emerges from neural processing that translates sensory information deriving from social signals into meaningful behavioral responses. Although vertebrates may differ in their manifestations of social behavior, the principles of neural processing, as well as the neural and molecular mechanisms underlying social responses,

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are strikingly similar across species (O'Connell and Hofmann 2011a, b). Indeed, the neuromodulatory systems responsible for social arousal, including the monoamines, are highly conserved in both anatomy and function (Berridge 2008; Hall et al. 2010; Hurley and Hall 2011; O'Connell and Hofmann 2011a, b). Yet, we know very little about the ontogenetic development of such systems and how, if at all, they contribute to the development of social behavior. To begin to address these issues, we examined the effects of a key social signal—mating calls—on monoamine levels in the brains of juvenile Mexican spadefoot toads (*Spea multiplicata*).

Anurans (frogs and toads) are an excellent model for studying the development of social behavior. In most anurans, social behavior consists of simple, stereotyped responses to conspecific social signals that can be readily elicited in the laboratory (Baugh et al. 2012). In particular, a primary feature of anuran social behavior is phonotaxis: the movement toward attractive male calls. Phonotaxis is an innate (non-learned) behavior that depends on the integration of auditory input with motor output. Although this behavior primarily mediates reproductive behaviors, both adult males and females (Walkowiak et al. 1999; Pfennig et al. 2000; Endepols et al. 2003; Pfennig 2007; Bernal et al. 2009; Chakraborty and Burmeister 2009; Baugh and Ryan 2010; Pfennig and Stewart 2011) and juveniles (Baugh and Ryan 2010) exhibit phonotaxis. Indeed, adult-like auditory nuclei structure and connectivity are in place by the end of metamorphosis (Boatright-Horowitz and Simmons 1995, 1997; Kumaresan et al. 1998; Horowitz et al. 2007), and species-typical patterns of neural activation in response to calls can be seen quite early during post-metamorphic development (Baugh et al. 2012). These findings therefore suggest that the neural circuits underlying phonotaxis are in place and functional early in life.

Although we know a great deal about the expression and connection patterns of monoamines in the adult anuran brain (Endepols et al. 2000, 2006, Smeets and Gonzalez 2000; Sanchez-Camacho et al. 2001, 2003; Muhlenbrock-Lenter et al. 2005; Lopez et al. 2010; Maier et al. 2010; O'Connell et al. 2010), we know comparatively less about how these neuromodulatory systems respond to social signals and whether such responses are in place in the early post-metamorphic period. To gain a better understanding of how monoaminergic systems respond to social signals during the development of social behavior, we examined the effects of mating calls on monoamine levels of post-metamorphic, juvenile *S. multiplicata*. *Spea multiplicata* have a short tadpole stage followed by a longer juvenile phase, reaching sexual maturity in as little as 9–18 months post-metamorphosis (Forester 1975; K. Pfennig, unpublished observations). We focused on the early juvenile period (1-week post-metamorphosis) a time when we expect little phonotaxis behavior would be expressed (Baugh and Ryan 2010) even though both sensory and motor systems are in place. We presented juvenile toads with either a conspecific mating call or no sound and used high-pressure liquid chromatography with electrochemical detection (HPLC-ED) to quantify changes in norepinephrine (NE), epinephrine (E), dopamine (DA), serotonin (5-HT), and the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA). We measured monoamine and metabolite levels in tissue samples taken from the torus semicircularis, tegmentum, posterior-dorsal diencephalon, posterior-ventral diencephalon, anterior-dorsal diencephalon, suprachiasmatic nucleus, preoptic area, pallium, and subpallium (see Table 1; Fig. 1).

We found that mating calls increased levels of all monoamines in the tegmentum, indicating that, even in very young juveniles, monoamine systems are responsive to socially arousing sounds. However, mating calls did not modify monoamine levels in any other brain region. In addition, our findings indicate that basal, unstimulated levels of monoamines varied across the brain. In the absence of sound, E and NE were highest in the anterior-dorsal diencephalon, whereas DA levels were highest in the preoptic area and posterior-ventral diencephalon. Our results also suggest that the monoamines with the highest basal concentrations across brain regions were E and 5-HT.

Materials and methods

Subjects

We obtained juveniles by breeding adult *S. multiplicata* that had been housed in a colony at the University of North Carolina, Chapel Hill. These adults had been collected from natural populations near Portal, Arizona, USA. We induced breeding of two pairs by injecting them with 0.07 mL 0.01 ug/ml GnRH agonist (Pfennig 2007). We housed the resulting tadpoles (approximately 30 per container) in plastic boxes (16 × 22 × 17 cm) filled with dechlorinated tap water. We fed the tadpoles ad libitum until metamorphosis. At metamorphosis, we transferred the juveniles to boxes (12 × 20 × 13 cm) where they were housed individually and fed crickets ad libitum for a week before we performed the sound treatment described below.

Sound treatment

We randomly assigned individuals from each sibship to either a mating call ($n = 6$) or no sound ($n = 6$) group. The mean mass and body length was the same for both groups (one-way ANOVAs: $F_{(1,10)} = 0.08$, $p > 0.78$;

Table 1 Tissue samples obtained from juvenile spadefoot toad brains

Sample (abbreviation)	Brain regions included	Monoaminergic elements included*							
		NE		E		DA		5-HT	
		C	F	C	F	C	F	C	F
Torus semicircularis (TS)	Principal, laminar, and magnocellular torus semicirularis	−	+	−	+?	−	+	−	+
Tegmentum (Teg)	Dorsal and ventral tegmentum, rostral part of locus coeruleus; rostral part of raphe nuclei	+	+	−	+?	−	+	+	+
Posterior-dorsal diencephalon (P-D di)	Posterior, central, lateroposterior, and ventromedial thalamic nuclei	−	+	−	+?	+	+	−	+
Posterior-ventral diencephalon (P–V di)	Ventral and dorsal hypothalamus, posterior tuberculum	−	+	−	+?	+	+	+	+
Anterior-dorsal diencephalon (A-D di)	Anterior, central, ventrolateral, and ventromedial thalamic nuclei, lateral amygdala	−	+	−	+?	+	+	−	+
Suprachiasmatic nucleus (SC)	Suprachiasmatic nucleus	−	+	−	+?	+	+	−	+
Preoptic area (PoA)	Preoptic area	−	+	−	+?	+	+	−	+
Pallium (P)	Medial, dorsal, and lateral pallium	−	+	−	+?	−	+	−	+
Subpallium (SubP)	Lateral septum, striatum, nucleus accumbens	−	+	−	+	−	+	−	+

Sample numbers correspond to Fig. 1

C immunoreactive cell bodies, *F* immunoreactive fibers

* Based on neuroanatomical studies in adult anurans; note that due to species differences, uncertainty exists as to which monoaminergic elements were actually included in our samples

$F_{(1,10)} = 0.06$ $p > 0.82$, respectively). Eighteen hours before the treatment, we placed each animal into a cylindrical mesh cup (8 × 10 cm) located in the center of a dark acoustic chamber (58 × 41 × 36 cm, Industrial Acoustics Company, New York, NY, USA) maintained at 25 °C and approximately 55 % relative humidity. The purpose of the acclimation period was to allow the animals to become accustomed to the novel surroundings and to minimize the effects of handling on our response measures. To prevent dehydration, we situated each cup on top of a petri dish with wet paper towels. We used the cup to minimize locomotion because locomotion could confound interpretation of our results and could introduce variation into the experience of the stimulus, as stimulus amplitude is influenced by proximity to the speaker. We positioned the cup holding the toad approximately 5 cm from a speaker (Pioneer TS-G1040R, Tokyo, Japan) that was connected to an M-Audio Firewire 410 8-channel audio playback unit (M-Audio, Arcadia, CA) and a Macintosh computer running Protocols M Powered playback software (version 7.1, M-Audio, Irwindale, CA, USA). After the 18-hour acclimation period, we presented juveniles with a continuous series of male *S. multiplicata* mating calls (peak amplitude = 78 dB SPL) or no sound for 40 min. We synthesized the male mating calls using the methods described in Pfennig (2007). The parameters of the mating calls (including call rate) consisted of mean values for *S. multiplicata* males from the populations in which the parents of the juveniles were collected (Pfennig 2000).

Preparation of tissue for microdissection

Immediately following the 40-min treatment period, we decapitated each animal and dissected it quickly to remove its brain intact in its cranium. We placed each brain (with the skull) in a 2-ml tube containing Tissue-Tek OCT embedding medium (Sakura, Finetek, Torrance, CA), froze it in liquid nitrogen and stored it at −80 °C until sectioning. We sectioned brains in a caudal-to-rostral direction, using a cryostat set at −15 °C and a juvenile anatomical atlas that we made to guide us through the sectioning process; our atlas was based on neuroanatomical studies of juvenile and adult anuran brains (Potter 1965; Neary and Northcutt 1983; Hall and Feng 1987; Neary 1990; Feng and Lin 1991; Endepols et al. 2005; Horowitz and Simmons 2010). We obtained five 300 µm-transverse sections for microdissection (see below), as well as the immediate 'before' and 'after' 15 µm-sections to verify the brain regions that were included in each microdissection (Fig. 1). We thaw-mounted each section on a glass slide by briefly warming the slide. Between sections, slides were kept on dry ice. Slides were stored at −80 °C until microdissection.

Microdissection of discrete brain regions

We microdissected target brain areas following a protocol published previously by Lowry et al. (1996) with slight modifications. Briefly, we placed each slide containing individual sections on a cold stage (Thermoelectric Cold

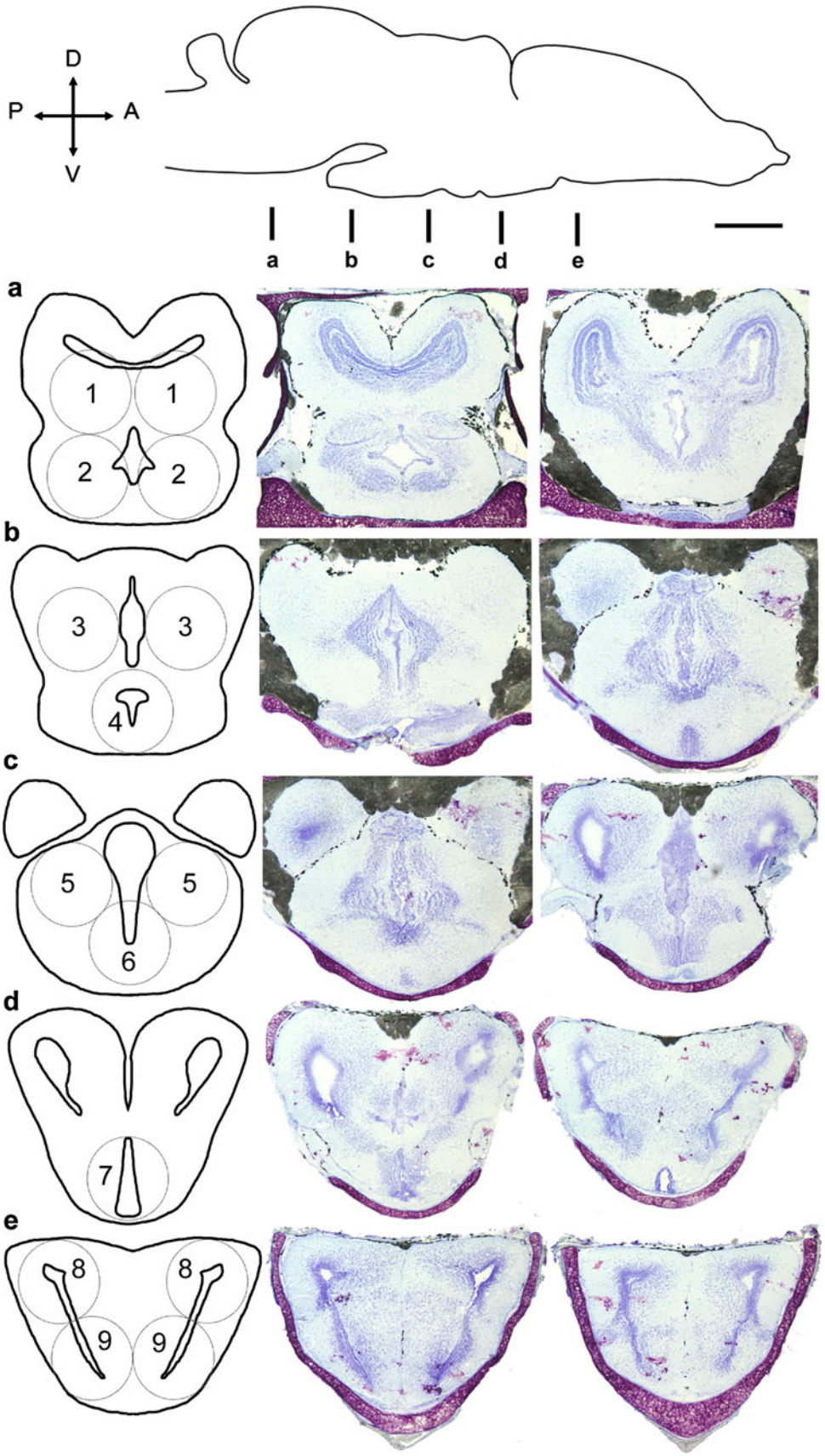

Fig. 1 Schema of the juvenile spadefoot brain and diagrams of transverse sections illustrating the brain regions studied, depicted in the direction of sectioning (caudal to rostral). *Vertical bars* indicate the starting point of each 300 µm-transverse section from which we micropunched the brain regions of interest, shown in *corresponding rows* (**a**–**e**). The *left* side of each *row* (**a**–**e**), shows schematic diagrams of the caudal position of each micropunch (indicated by *circles*), and the photomicrographs of the Thionin-stained sections represent the 'before' (*center*) and 'after' (*right*) sections of each punch. We obtained a total of nine punches (see Table 1 for brain regions included in each punch): *1* torus semicircularis, *2* tegmentum, *3* posterior-dorsal diencephalon, *4* posterior-ventral diencephalon, *5* anterior-dorsal diencephalon, *6* suprachiasmatic nucleus, *7* preoptic area, *8* pallium, *9* subpallium. *Scale bar* 300 µm

Plate, TCP-2; Thermoelectrics Unlimited, Inc.) that maintained the section at −20 °C during microdissection. With the help of a juvenile brain atlas and using a stereomicroscope (4× magnification), we identified brain regions of interest based on local landmarks and collected samples using chilled thin-walled stainless steel spring-loaded punch needles (350 µm i.d.; Fine Science Tools, Foster City, CA, USA). We obtained a total of nine tissue samples from each brain (Fig. 1; Table 1). We combined punches from the two hemispheres except for the posterior-ventral diencephalon, suprachiasmatic nucleus, and preoptic area, which were taken from the midline (Fig. 1). In all cases, we expelled the tissue punches into 1.9 ml polypropylene microcentrifuge tubes containing 100 µl of mobile phase (see below) and 0.1 pg/µl of internal standard (isoproterenol, Sigma) and rapidly froze them on dry ice. We stored all samples at −80 °C until analysis by HPLC-ED.

Quantification of monoamines and metabolites

We determined tissue concentrations of NE, E, DA, 5-HT, and 5-HIAA using HPLC-ED, which reflect the amount of monoamines that are stored (in cells and fibers) and released. Although our protocol also allowed us to separate DA and NE/E metabolites, their levels were too low for reliable detection (Fig. 2). We followed the procedure of Salvante et al. (2010) with a few modifications. Briefly, the mobile phase consisted of sodium acetate (3.1 g), monohydrate citric acid (8.84 g), disodium EDTA (5 mg), sodium octyl sulfonate (215 mg), HPLC grade methanol (200 ml) and double-distilled deionized water (800 ml). Immediately before the assay, we sonicated the samples for approximately 5–10 s and then we centrifuged them at 16,000*g* for 16 min at 4 °C.

We aspirated the supernatant and injected 100 µl from each sample into the HPLC system. The chromatographic system consisted of a HTEC-500 HPLC machine (EICOM Corporation, Kyoto, Japan), MIDAS Autosampler (Spark Holland, Emmen, Netherlands) and EPC-500 PowerChrom software Version 2.5 (EICOM Corporation, Kyoto, Japan) running on a PC. A precolumn (PC-04, 100 × 3.0 mm i.d., EICOM Corporation, Kyoto, Japan) was added to the system to avoid contamination of the separation column (EICOMPAK SC-30DS, EICOM Corporation, Kyoto, Japan). The flow rate was set at 350 µl/min and the electrode potential was maintained at 750 mV with respect to an Ag/AgCl reference electrode. We prepared three external standard solutions containing a fixed amount (10 µl) of the internal standard isoproterenol (ISO; Sigma) and proportional amounts of DA, NE, E, 5-HT, and their respective metabolites (see above) to reach final concentrations of 5, 1, and 0.5 pg/µl. Finally, choosing the height ratio option, Powerchrom automatically calculated each amine concentration based on standard calibration curves, and we subsequently used these values to calculate the total amount of amines and metabolites in each sample. We were not able to normalize monoamine levels per µg of protein as the protein content of each sample was too low to be detected by standard protein assays. This may have decreased our ability to detect differences in response to the mating calls because, without measures of protein content in each punch, we were unable to account for error variation due to variation in sectioning thickness or placement of the punch. In addition, because we could not account for variation in protein content, which likely varied among samples from different brain regions, when comparing samples from across the brain, we standardized by punch number (see "Statistical analysis", below).

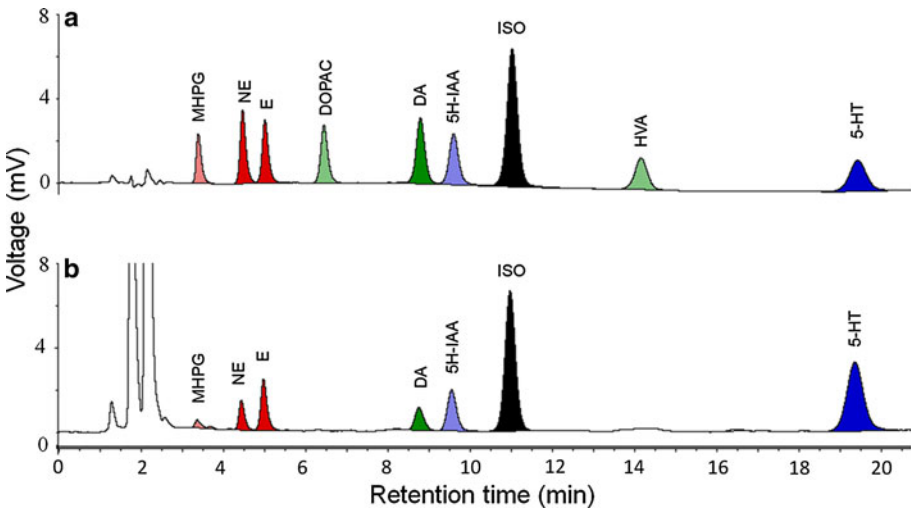

Fig. 2 HPLC output showing resolution of monoamines, metabolites, and the internal control (ISO) in a standard (**a**) and a sample (**b**) from juvenile brain

Statistical analysis

Before examining acoustically induced levels of monoamines, we first investigated the basal, unstimulated levels of monoamines among tissue samples. However, we could not compare tissue samples to one another directly because some tissue samples included only one punch, whereas other tissue samples included two punches (see Fig. 1). Therefore, to standardize the data for our analysis of basal monoamine levels, for each individual, we divided monoamine levels by the number of punches used, which was two for all tissue samples except the posterior-ventral diencephalon, suprachiasmatic nucleus, and preoptic area. Using these standardized data from the no sound group, we then used one-way repeated measures ANOVA with tissue sample as a factor.

Next, we examined the effect of acoustic stimulation on monoamine levels in the mesencephalon (samples 1–2), diencephalon (samples 3–7), and telencephalon (samples 8–9) using repeated measures ANOVA. For each brain subdivision, we performed separate ANOVAs with brain tissue sample as a within-subjects factor and stimulus as the between-subjects factor. In the diencephalon, because there were more than two tissue samples, we used a multivariate approach to the repeated measures ANOVA, because it does not assume sphericity. When we detected significant interactions between tissue sample and stimulus, we followed up with one-way ANOVAs to determine the source of the interaction. The level of significance α was 0.05 in all statistical tests used.

Results

Basal monoamine levels across the brain

Not surprisingly, we found that, with the exception of 5-HT, basal levels of monoamines varied across the brain (NE, $F_{8,40} = 8.93$, $p < 0.001$; E, $F_{8,40} = 15.53$, p 0.001; DA, $F_{8,40} = 4.40$, $p < 0.001$; 5-HT, $F_{8,40} = 1.57$, $p = 0.166$; 5-HIAA, $F_{8,40} = 5.85$, $p < 0.001$; Fig. 3). NE and E levels were highest in the anterior-dorsal diencephalon, which included mostly thalamic nuclei, in the suprachiasmatic nucleus, and the tegmentum, which included part of the locus coeruleus (Fig. 3a, b). DA levels were highest in the posterior-ventral diencephalon, which included the ventral hypothalamus and posterior tuberculum, and the preoptic area (Fig. 3c). The pallium showed low levels of all monoamines (Fig. 3). Interestingly, levels of E were higher than those of NE in all tissue samples (Fig. 3), similar to what has been observed in other anurans (Segura and Biscardi 1967; Cooney et al. 1985; Takeda 1997). When we analyzed basal levels of 5-HIAA across tissue samples, we found its highest levels in the tegmentum ($p < 0.05$; Fig. 3e).

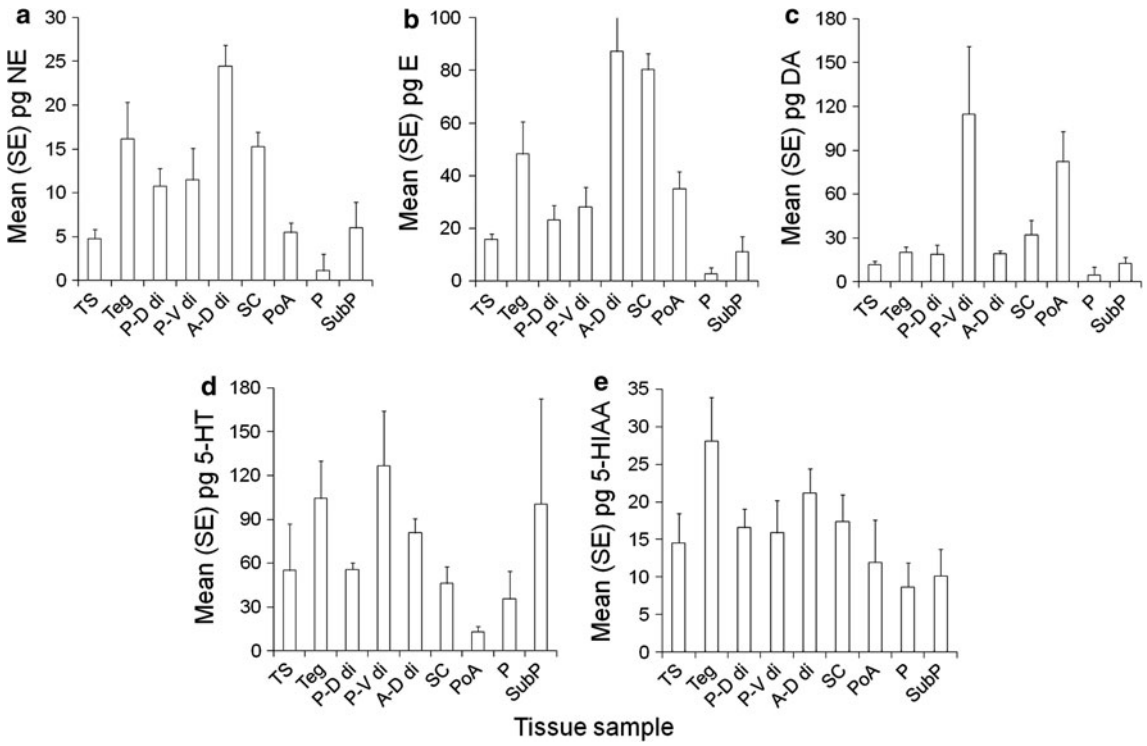

Fig. 3 Levels of monoamines across the brain in unstimulated juvenile spadefoot toads. Data were derived from the no sound group only and were standardized by punch number (see "Methods" for details)

Mating calls increased monoamine levels in the tegmentum

The mating calls did not affect monoamine levels in the telencephalon or diencephalon (no significant effects of stimulus or sample × stimulus interactions; Fig. 4). The one exception was a marginal effect of stimulus on NE levels in tissue samples in the diencephalon ($F_{1,10} = 4.5$; $p = 0.063$; Fig. 4a) suggesting the possibility of a modest, overall increase in NE levels.

In contrast, within the mesencephalon, the mating calls affected monoamine levels differentially in the tegmentum and torus semicircularis (sample × stimulus: NE, $F_{1,10} = 8.75$; $p = 0.014$; E, $F_{1,10} = 9.23$; $p = 0.013$; DA, $F_{1,10} = 6.71$; $p = 0.027$; 5-HT, $F_{1,10} = 5.39$; $p = 0.043$; 5-HIAA, $F_{1,10} = 13.21$; $p = 0.005$). Follow-up analyses demonstrated that the mating calls caused an increase in NE, E, and DA in the tegmentum but not the torus semicircularis (Fig. 4; Table 2). Increased concentrations of NE, E, and DA could reflect changes in synthesis and/or release. In the case of 5-HT, however, measures of its metabolite, 5-HIAA, enable us to make direct inferences about 5-HT release because changes in 5-HIAA reflect degradation following release. Thus, because mating calls caused an increase in 5-HIAA levels, we can conclude that mating calls increased 5-HT release, even though the effect of mating calls on 5-HT concentrations itself was only marginally significant (Fig. 4; Table 2).

Discussion

We investigated whether male mating calls, an important social signal, alter monoamines early in ontogeny, and explored how those responses, if any, varied across brain regions. We found that male mating calls elicited a robust increase in monoamine concentrations in the tegmentum in young juvenile toads. Perhaps surprisingly, acoustic stimulation with mating calls did not change monoamine levels in the juvenile torus semicircularis, the main auditory region in anurans, or in any of the forebrain regions studied. Because we measured concentrations of monoamines from tissue punches, our results reflect a combination of changes in monoamine synthesis, storage and/or release. Changes in monoamine concentrations in this context reflect changes in the underlying monoaminergic tone, while changes in 5-HIAA specifically reflect changes in 5-HT release. Thus, the mating calls elicited widespread changes in monoaminergic systems in juvenile spadefoot toads. Are the effects of the mating calls we describe specific to the social signal we used, or could other sounds elicit a similar response? On the one hand, monoaminergic systems in mammals have been shown to respond to a

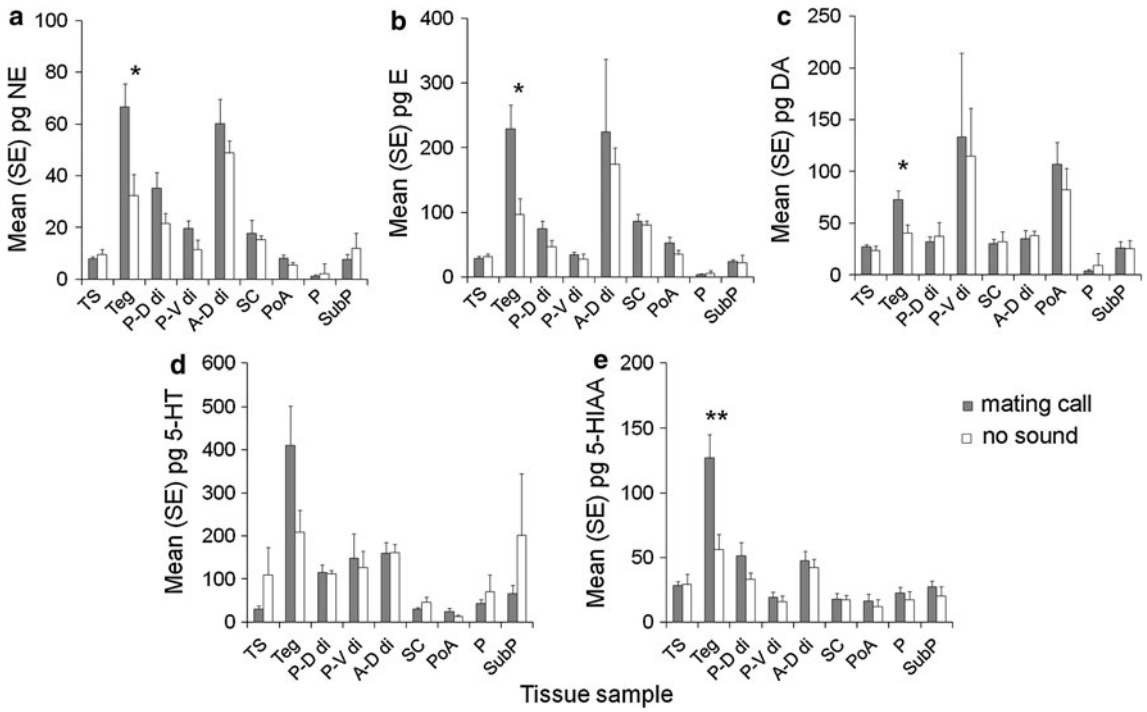

Fig. 4 Effects of acoustic stimulation (mating calls versus no sound) on levels of monoamines across the brain of juvenile spadefoot toads. Tissue samples were not standardized by the number of punches (see "Methods" for details). *Asterisks* indicate level of significance (*$p < 0.05$; **$p < 0.01$)

Table 2 Statistical results showing effects of acoustic stimulation (mating call versus no sound) on monoamine levels in the mesencephalon

Tissue sample	Monoamine									
	NE		E		DA		5-HT		5-HIAA	
	$F_{(1,10)}$	p	$F_{(1,10)}$	p	$F_{(1,10)}$	p	$F_{(1,10)}$	p	$F_{(1,10)}$	p
Torus semicircularis	0.618	0.450	0.210	0.657	0.501	0.495	1.538	0.243	0.015	0.905
Tegmentum	7.930	0.018*	9.015	0.013*	7.760	0.019*	3.749	0.082	11.037	0.008**

Single asterisks indicate $p < 0.05$ and double asterisks indicate $p < 0.01$

range of sounds, including sounds that are not biologically relevant (Cransac et al. 1998; Niu and Canlon 2002). On the other hand, in songbirds, monoamine systems are sensitive to male social signals (e.g., conspecific but not heterospecific songs; Bharati and Goodson 2006) and to variation in the quality of male songs (Sockman and Salvante 2008). In anurans, like songbirds, male social signals are highly salient signals that elicit robust immediate early gene responses (Chakraborty et al. 2010) suggesting, perhaps, that the changes in monoamines that we report are specific to the social signal we used.

Our results indicate that the tegmentum is part of an important arousal system that responds to social cues. Although little is known about its function in anurans, it can be considered part of the midbrain reticular formation, which includes both arousal systems and sensory-motor areas. For example, the tegmentum may regulate visually guided behaviors by modulating tectal activity and controlling the passage of visual information to pre-motor centers in the spinal cord (Wu and Wang 2007). The tegmentum is also a major target of monoaminergic input. For example, in adult anurans, the tegmentum contains dense 5-HT immunoreactive fibers (Walkowiak and Luksch 1994; Endepols et al. 2000) that most likely derive from the nucleus raphe of the medulla oblongata (Walkowiak and Luksch 1994). The tegmentum also receives significant catecholaminergic input, as evidenced by the presence of tyrosine hydroxylase elements (Gonzalez and Smeets 1991; Endepols et al. 2000). In the case of NE, our tegmentum tissue samples included both a source of NE (i.e., rostral part of the locus coeruleus) and the tegmentum itself, which is a target of the NE fibers originating in the locus coeruleus (Sanchez-Camacho et al. 2001). The dopaminergic nuclei that are known to project to the tegmentum are the preoptic area and the posterior thalamic nucleus (Hall and Feng 1987; Walkowiak et al. 1999; Endepols et al. 2000). The anatomical basis of E input to the tegmentum is unclear, as the site of central E in amphibians has remained somewhat ambiguous (Yoshida et al. 1983; Gonzalez and Smeets 1995; Sanchez-Camacho et al. 2003). Consistent with these previous results, we found that the tegmentum had high concentrations of all monoamines and we further showed that these monoamines respond to a social signal.

The tegmentum is not typically associated with responding to mating calls in anurans, possibly because prior work has focused on the torus semicircularis. However, the tegmentum shares many similarities with the torus semicircularis. Besides forming massive connections with one another (Walkowiak and Luksch 1994), both neuronal groups project to sensory, pre-motor, and motor regions (Luksch and Walkowiak 1998; Walkowiak et al. 1999), receive similar neuromodulatory input (Walkowiak and Luksch 1994; Endepols et al. 2000), have similar afferent connectivity with other brain regions (e.g., striatum, posterior thalamus) (Vesselkin et al. 1980; Wilczynski and Northcutt 1983; Hall and Feng 1987; Lazar and Kozicz 1990; Marin et al. 1997a, b; Marin et al. 1999; Walkowiak et al. 1999; Wilczynski and Endepols 2007; Maier et al. 2010), and show comparable patterns of axonal projections of individual neurons (Walkowiak and Luksch 1994). Indeed, tegmental neurons have been shown to respond to auditory stimulation (Luksch and Walkowiak 1998), and previous in vitro experiments demonstrated that electrical stimulation of lower brainstem auditory nuclei evoked activity not only in the torus semicircularis, but also in the tegmentum (Walkowiak and Luksch 1994). Furthermore, the tegmentum has been implicated in the detection and response to release calls (Schmidt 1990). Thus, while the two brain regions share many similarities, our present results highlight a potentially important role for the tegmentum in social arousal. Taken together, the emerging picture is that the torus semicircularis and the tegmentum belong to a highly parallel and divergent efferent system that contributes to the audiomotor integration necessary for responding to social cues.

We also sought to characterize the general pattern of basal monoamine levels across juvenile brain regions. Our results were generally consistent with immunohistochemical studies in adults, which have shown high concentrations of monoamines in areas with cell bodies or dense fiber staining. For example, DA levels were highest in tissue samples that included the preoptic area, suprachiasmatic nucleus, the hypothalamus, and the posterior tuberculum,

brain regions that contain the majority of dopaminergic cell bodies. In contrast, E and NE levels were highest in tissue samples that included thalamic nuclei, which are areas that tend to have high fiber density. Although comparing tissue samples with different amount of brain tissue may introduce uncontrolled variation to our measurements (e.g., punches that included the ventricles had less brain tissue), our findings are consistent with general patterns of monoaminergic expression in adult anurans (Yoshida et al. 1983; Ueda et al. 1984; Gonzalez and Smeets 1991, 1993, 1995; Endepols et al. 2000). Thus, our results suggest that these systems are well developed in post-metamorphic juveniles.

We also found that E was the predominant amine relative to NE, as has been found in other amphibians (Segura and Biscardi 1967; Juorio 1973; Fuller and Hemrick-Luecke 1983; Cooney et al. 1985; Takeda 1997). Such a pattern contrasts with mammals, where central levels of E are low compared to NE, particularly in the cerebral cortices (Vogt 1954; Carlsson 1959; Gunne 1962; Mefford et al. 1982). Although the significance of these observations are still unclear, the predominance of NE over E in the mammal CNS might be related to the extensive development of the locus coeruleus and hypertrophy of one of its major targets, the isocortex (Levitt and Moore 1979).

In summary, we found that social signals caused an increase in monoamines in the tegmentum, a sensory-motor brain region that contributes to arousal and attention. Our results are consistent with a role for the tegmentum in state-dependent and/or context-dependent modulation of social behavior. The fact that we found such an effect in juveniles suggests that these systems are in place early in ontogeny. Future studies will allow us to determine whether these acoustically induced changes in tegmental monoamine levels represent socially relevant responses and whether these effects change after sexual maturity. Indeed, such studies could provide a functional framework for extending our knowledge of how monoamines act to regulate critical social decisions such as mate choice.

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