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Neurochemical Mediation of Affiliation and Aggression Associated With Pair-Bonding

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ABSTRACT

BACKGROUND: The neuropeptides vasopressin and corticotropin-releasing factor facilitate, while serotonin inhibits, aggression. How the brain is wired to coordinate interactions between these functionally opposed neurotransmitters to control behavioral states is poorly understood.

METHODS: Pair-bonded male prairie voles (*Microtus ochrogaster*) were infused with a retrograde tracer, Fluoro-Gold, and tested for affiliation and aggression toward a female partner or novel female subject. Subsequent immunocytochemical experiments examined neuronal activation using Fos and neurochemical/neuroreceptor profiles on brain areas involved in these social behaviors. Finally, a series of behavioral pharmacologic and real-time in vivo brain microdialysis experiments were performed on male prairie voles displaying affiliation or aggression.

RESULTS: We localized a subpopulation of excitatory vasopressin neurons in the anterior hypothalamus that may gate corticotropin-releasing factor output from the amygdala to the anterior hypothalamus and then the lateral septum to modulate aggression associated with mate guarding. Conversely, we identified a subset of inhibitory serotonergic projection neurons in the dorsal raphe that project to the anterior hypothalamus and may mediate the spatiotemporal release of neuropeptides and their interactions in modulating aggression and affiliation.

CONCLUSIONS: Together, this study establishes the medial extended amygdala as a major neural substrate regulating the switch between positive and negative affective states, wherein several neurochemicals converge and interact to coordinate divergent social behaviors.

Keywords: Anterior hypothalamus, Corticotropin-releasing factor, Dopamine, Medial amygdala, Serotonin, Vasopressin

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A critical challenge in the psychiatry field is to determine the neurochemical circuitry underlying an individual's propensity to transition between prosocial emotional states to physical violence (1). Although preclinical neuroscience has largely focused on examining the function of individual neurochemicals, brain areas, and neuronal mechanisms therein, we know surprisingly little about the neuromodulatory microcircuits regulating emotion (2).

The posterior dorsal medial amygdala (MeAPD) projects to several subdivisions of the hypothalamus (3–5) to regulate various forms of social behavior (3–10). However, the circuitry remains largely undefined beyond these second-order projections. The integrating command centers that process sensory input and control descending motor output to program socioemotional behavior are unclear. Previous work has relied on using traditional laboratory rodents to dissect the neural circuitry involved. However, these animals do not readily display certain types of behavior and may not be appropriate for some investigations (11). For example, most laboratory animals do not exhibit strong social bonds between mates, and male animals typically do not display paternal behavior or female-directed aggression (12). Because mating naturally induces these behaviors in the socially monogamous prairie vole (*Microtus ochrogaster*), this rodent species represents a unique animal model to investigate neural circuitry programming pair-bonds (12,13).

Lesions of the vomeronasal organ (14) or MeAPD (15) impair partner preference formation and affiliation in prairie voles. In male prairie voles, parvocellular vasopressin (AVP) neurons in the nucleus circularis and medial supraoptic nucleus are both recruited during aggression (16) and release their contents in the anterior hypothalamus (AH) activating AVP 1A receptors (V1aR) to facilitate aggression selectively toward novel conspecifics but not toward a partner (17). Two weeks of sociosexual experience also induce structural plasticity of V1aR to mediate selective aggression (17). Furthermore, viral vector-mediated gene transfer of V1aR into the AH of sexually naïve male animals recapitulates pair-bonding-induced aggression (17). Finally, dopamine signaling in the rostral nucleus accumbens shell (NAcc) is also involved in selective aggression to maintain monogamous pair-bonds (18). However, despite these studies, we know little about how these brain regions, genes, and neurochemicals integrate into a network to control pair-bonding behavior (19).

Because recent work demonstrates regional overlap of molecularly specified neurons in the ventral medial hypothalamus

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that control properties characteristic of emotion states regulating social (20), sexual (21,22), and aggressive (21–25) behaviors, we investigated whether individual pair-bonding behaviors are encoded via similar or different neuronal systems. Here, we focused on examining the neurotransmitters AVP, corticotropin-releasing factor (CRF), and serotonin (5-HT) for their roles in regulating behavioral states. We proposed that AVP/CRF facilitate aggression, while 5-HT functions to inhibit the activity of the AVP/CRF systems in the AH to switch from aggression to affiliation. Our data provide necessary refinement steps toward understanding how multiple neurotransmitter systems interact within neuronal microcircuits to drive attachment.

METHODS AND MATERIALS

Subjects

Subjects were male prairie voles (90–120 days of age) that were either sexually naive or pair-bonded with a female subject for 2 weeks, which reliably induces partner preferences and selective aggression toward novel conspecifics (16–18) (Supplemental Experimental Procedures). All experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at the Florida State University.

Behavioral Assays

Subjects' aggressive behaviors were examined using the resident-intruder test (RIT), a well-characterized and ethologically valid model of offensive aggression (26). Briefly, a conspecific intruder was introduced into the home cage of the subject (resident), and the resident was scored for 10 minutes for aggressive responses, including the frequency of lunges, bites, and chases, as well as the duration of affiliative side-by-side contact and anogenital investigation, as previously described (16,17) (Supplemental Experimental Procedures).

Monosynaptic Tracer Injection Parameters

Subjects were stereotaxically injected into the AH (coordinates from bregma: posterior 0.55 mm, lateral ± 0.75 mm, ventral 6.1 mm), rostral NAcc shell (anterior 1.60 mm, lateral ± 1.0 mm, ventral 4.5 mm), lateral septum (LS) (anterior 0.80 mm, lateral ± 0.61 mm, ventral 4.1 mm), or MeAPD (posterior 1.30 mm, lateral ± 2.70 mm, ventral 7.0 mm), respectively, with glass capillary micropipettes (A-M Systems, Inc., Carlsborg, WA) filled with 2% Fluoro-Gold (FG) (Fluorochrome, Englewood, CA) and 0.5% cresyl violet dye in 0.01 mol/L phosphate buffer solution (PBS) (pH 7.4) under sodium pentobarbital (0.1 mg/10 g body weight). Injection placement was evaluated by processing sections spanning the target area for FG immunocytochemical detection and cresyl violet dye spread. Data from the subjects with correct injection placement were included in neuroanatomical mapping (Supplemental Figure S3 and Supplemental Experimental Procedures).

Brain Microdissection and High-Performance Liquid Chromatography With Electrochemical Detection Analysis

Coronal brain sections (300 μm) were cut on a cryostat and frost mounted onto microscope slides. Bilateral tissue punches were taken using a 1-mm-diameter scalpel under 20× magnification on a Leica DMRB dissection microscope (Leica Biosystems Inc., Buffalo Grove, IL). Tissue samples were localized to the AH, medial preoptic area, and paraventricular nucleus of the hypothalamus and stored at −80°C. Subsequently, 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were measured using high-performance liquid chromatography with electrochemical detection (Supplemental Experimental Procedures).

Intra-AH Stereotaxic Cannulation and Drug Microinfusion

Subjects were anesthetized with sodium pentobarbital (0.1 mg/10 g body weight) and then stereotaxically implanted with guide cannula aimed at the AH, as described previously (17,18). All injections were made using a Hamilton syringe connected to an automatic micropump. Immediately after a 10-minute RIT test, subjects were overdosed with sodium pentobarbital and rapidly decapitated and their brains were sectioned for histologic verification of cannula placement. Subjects with correct cannula placement were included in data analysis (Supplemental Figure S2 and Supplemental Experimental Procedures).

Brain Preparation, Immunocytochemistry, and Image Analysis

Subjects were anesthetized with sodium pentobarbital and then perfused through the ascending aorta with 0.9% saline, followed by 4% paraformaldehyde in 0.1 mol/L PBS. Brains were dissected, postfixed for 2 hours in 4% paraformaldehyde, and then stored in 30% sucrose in PBS. Brains were cut into 30-μm coronal sections on a freezing microtome, and floating sections were stored in 0.1 mol/L PBS with 1% sodium azide at 4°C until immunostaining.

Different sets of floating brain sections at 150-μm intervals were processed for single- or double-immunoreactive (ir) labeling of FG, Fos, FG/Fos, FG/tyrosine hydroxylase (TH), FG/AVP, FG/5-HT, or FG/CRF. AH sections were processed for double- or triple-ir labeling for AVP, V1aR, 5-HT, 5-HT_{1A} receptors (5-HTr1a), CRF, CRF_2 receptors (CRFR2), FG, and Fos.

We quantified the colocalization of 5-HTr1a, V1aR, CRFR2, and 5-HT on AVP-, CRF-, and/or FG-expressing neurons in the AH. Leica imaging software (Leica Biosystems Inc.) profile methods of cell counting were employed and area measurements (square millimeters) were taken on each section analyzed to determine cell densities. Photomicrographs were captured by using a Zeiss Axioskop 2 (Carl Zeiss NTS, LCC, Peabody, MA) microscope with a SPOT RT Slider (Diagnostic Instruments, Sterling Heights, MI) camera and SPOTTM (version 3.0.6; Diagnostic Instruments) software. Image files were then stored and subsequently analyzed (Supplemental Experimental Procedures).

Real-Time In Vivo Brain Microdialysis With Neurochemical Analyses

Microdialysis probe construction, cannulation, and dialysate collection were previously described (17,27,28) (Supplemental Experimental Procedures). Immediately after RIT, subjects were overdosed with sodium pentobarbital and rapidly decapitated and their brains were sectioned for histologic verification