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## DISTRIBUTION OF CATECHOLAMINERGIC AND PEPTIDERGIC CELLS IN THE GERBIL MEDIAL AMYGADALA, CAUDAL PREOPTIC AREA AND CAUDAL BED NUCLEI OF THE STRIA TERMINALIS WITH A FOCUS ON AREAS ACTIVATED AT EJACULATION

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### Abstract

The posterodorsal preoptic nucleus (PdPN), lateral part of the posterodorsal medial amygdala (MeApd) and medial part of the medial preoptic nucleus (MPNm) are activated at ejaculation in male gerbils as assessed by Fos expression. We sought to immunocytochemically visualize substance P (SP), cholecystokinin (CCK), oxytocin, vasopressin and tyrosine hydroxylase (TH), a catecholaminergic marker, in the mating-activated cells, but the need for colchicine precluded behavioral testing. Instead, we detailed distributions of cells containing these molecules in the medial amygdala, caudal preoptic area and caudal bed nuclei of the stria terminalis (BST) and quantified their densities in the PdPN, MPNm and lateral MeApd for comparison to densities previously assessed for mating-activated efferents from these sites. TH cells were as dense in the PdPN and lateral MeApd as activated efferents to the anteroventral periventricular nucleus. In the lateral MeApd, TH cells were grouped where cells activated at ejaculation are clustered and where CCK cells form a ball. Lateral MeApd CCK cells and PdPN SP cells were as dense as activated efferents to the principal BST. Oxytocinergic PdPN cells and SP cells in the MPNm were as dense as mating-activated efferents to the lateral MeApd. If some oxytocin cells in the PdPN project to the neurohypophysis, as in rats, they could be a source of the oxytocin secreted at ejaculation. Since gerbils are monogamous and biparental, it was also interesting that, unlike monogamous prairie voles, they had few TH cells in the MeApd or dorsal BST, resembling promiscuous rats, hamsters and meadow voles.

### Keywords

tyrosine hydroxylase; cholecystokinin; substance P; oxytocin; vasopressin; posterodorsal preoptic nucleus

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## 1. Introduction

Three areas of the gerbil forebrain contain cells activated specifically at ejaculation as assessed by Fos expression: the posterodorsal preoptic nucleus (PdPN), the lateral part of the posterodorsal medial amygdala (MeApd) and the medial part of the medial preoptic nucleus (MPN; Heeb and Yahr, 1996). After ejaculation, male gerbils, like rats (Baum and Everitt, 1992; Coolen et al., 1996), have a cluster of Fos-containing cells in the PdPN and lateral MeApd that is not seen in males that intromit equally often but are separated from the female before ejaculation. In the medial MPN (MPNm), cells activated at ejaculation are intermingled with cells activated when males re-enter the environment in which they gained sexual experience (Heeb and Yahr, 1996), perhaps in anticipation of sexual reward (Balfour et al., 2004). Whether the activation associated with ejaculation reflects sensory processing or the start of a pre-motor program for ejaculation or one of its sequelae, such as oxytocin secretion (Hughes et al., 1987), is not known.

To learn more about PdPN, MPNm and lateral MeApd cells activated with mating, we traced their projections (Heeb and Yahr, 1996; Simmons and Yahr, 2002) and identified some of their transmitters, including  $\gamma$ -aminobutyric acid (GABA), glutamate and nitric oxide (NO; Simmons and Yahr, 2003; Simmons and Yahr, unpublished). Cholecystokinin (CCK), substance P (SP), vasopressin (VP), oxytocin and catecholamines were also of interest. In hamsters, rats and voles, MeApd, PdPN and MPN cells containing CCK, SP and VP are sexually dimorphic and/or sensitive T (Simerly and Swanson, 1987; Micevych et al., 1988; Simerly et al., 1989; Swann and Newman, 1992; Wang, 1995; Wang and De Vries, 1995). In prairie voles, which, like gerbils, are monogamous and biparental, MeApd cells containing tyrosine hydroxylase (TH), a catecholaminergic marker, express Fos with mating, particularly ejaculation (Cavanaugh et al., 2010). In rats, oxytocin is found in cells of the caudal PdPN (Sofroniew, 1985; Yamada et al., 1996), previously thought to be the rostral anterior commissural nucleus (AC; Ju et al., 1989; Paxinos and Watson, 1986, 1997). Many of those cells project to the posterior pituitary (Vanhatalo and Soinila, 1995), and rats secrete oxytocin after ejaculation (Hughes et al., 1987).

In gerbils, colchicine treatment was needed to visualize cell bodies containing oxytocin, VP, SP, CCK and TH in the PdPN, MPNm and lateral MeApd, which precluded testing males for sex behavior. Since we could not study cellular colocalization of mating-induced Fos with these molecules, we detailed distributions of cells containing them in the caudal preoptic area (POA), caudal bed nuclei of the stria terminalis (BST) and medial amygdala (MeA) and quantified their densities in the PdPN, MPNm and lateral MeApd for comparison to densities of mating-activated efferents from these sites that we had identified previously (Heeb and Yahr, 2001; Simmons and Yahr, 2002).

## 2. Materials and methods

### 2.1 Subjects

Gerbils were purchased as adults (Harlan Sprague Dawley, Indianapolis, IN), housed in same-sex pairs and exposed to a 14:10-hr light:dark cycle with food and water freely available. All of the males used were sexually experienced, having copulated to ejaculation at least once during 1–3 pairings of 30–40 min with females made sexually receptive by subcutaneous implantation of a 5-mm Silastic capsule (1.57 mm i.d., 3.16 mm o.d.) of estradiol benzoate 5–10 days before use. All gerbils were anesthetized with sodium pentobarbital before surgery (50 mg/kg) or perfusion (10 mg/male). All procedures conformed to the guidelines of the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

## 2.2 Colchicine treatment

Males were given infusions of colchicine (50–400 µg; Sigma; St. Louis, MO) in 10 µl of distilled water, or 0.9% saline, 1–2 days before perfusion, by stereotaxically directing a pipette (50–80 µm tip) connected to a syringe pump at the right lateral ventricle (1.65 mm anterior and 0.85 mm lateral to Bregma; 2.7 mm below the skull surface; incisor bar set at +4.0 mm). Infusions lasted 8–12 min. Five min later, the pipette was removed and the wound closed. For CCK, 200–400 µg was needed. For the other peptides and TH, 50 µg proved adequate. Analgesic (0.03 mg/kg Torbutrol; Western Medical Supply, Arcadia, CA) was injected subcutaneously after surgery and then twice daily.

## 2.3 General immunocytochemistry (ICC) procedures

Unless otherwise stated in a specific protocol, the following procedures were followed. Males were perfused with saline followed by fixative in phosphate-buffered saline (PBS). Brains were removed and postfixed for 1.5–2 hr at 4 °C in the same fixative and cryoprotected by storage for 15–36 hr at 4 °C in the same PBS containing 20–30% sucrose. They were then frozen and cut coronally at 30 µm. Every section through the MPN, PdPN and MeA was collected in PBS. Some were collected as alternates or sets of three for processing with different antibodies or antibody dilutions.

Procedures were done at room temperature. Sections were rinsed initially, and after each incubation, except the preliminary blocking step. Rinses involved three changes over 15 min of the buffer from the previous incubation. Rinses and incubations involved gentle agitation. If acrolein was used for perfusion, residual aldehydes were removed by incubating sections for 15 min before the preliminary blocking step in PBS containing 1% NaBH<sub>4</sub>.

To block non-specific binding sites, sections were incubated for 1–1.5 hr in PBS containing 0.3% Triton X-100 and 20% normal serum (NS) from horse (TH) or goat (peptides). Then they were incubated for 48–72 hr at 4 °C in PBS containing 0.3% Triton X-100, 5% NS and either mouse monoclonal (TH) or rabbit polyclonal (peptides) IgG. Next, they were incubated for 1–1.5 hr in PBS containing 0.3% Triton X-100, 5% NS and a 1:200 dilution of biotinylated horse anti-mouse (TH) or goat anti-rabbit (peptides) IgG (Vector Labs; Burlingame, CA). Antibodies were visualized with Vectastain Elite ABC kits (Vector Labs), following kit instructions. The chromogen, 3,3'-diaminobenzidine (DAB), was used at 0.05% in Tris-buffered saline (TBS; pH 7.6) and developed with increasing concentrations (0.006–0.01%) of H<sub>2</sub>O<sub>2</sub>. Development was stopped with PBS. Sections were rinsed and stored in PBS until they were mounted onto slides coated with gelatin and chrom-alum. After air drying, the sections were dehydrated in graded alcohols, delipidized with xylene and coverslipped using DePeX (BDH Laboratory Supplies, Poole, UK). For TH and each peptide, some sections were processed without the primary antibody, a procedure that never produced any staining.

## 2.4 Oxytocin and Fos ICC

The fixative (150 ml over 10 min) was 4% paraformaldehyde (PFA) in NaPBS (pH 7.4). Postfixing and cryoprotection were combined by overnight storage at 4°C in PFA with 20% sucrose. NaPBS was used for ICC. Anti-oxytocin (Chemicon; Temecula, CA) was used at 1:5,000–1:7,000 and anti-c-Fos (Ab-5; Oncogene; Cambridge, MA) at 1:50,000. Males used for Fos ICC were not given colchicine and were retested for mating on the day of perfusion. They were perfused 1–1.5 hr after ejaculation [see Heeb and Yahr (1996) for details].

## 2.5 SP and CCK ICC

These antigens were often studied in alternate sections. Fixation was done over 12 min with either 200 ml of PFA + 2.5% acrolein (pH 6.8) or 50 ml of PFA + acrolein followed by 150

ml of PFA (pH 7.4) alone [all prepared in KPBS (0.05M; pH 7.4)]. PFA was used for postfixation. Either KPBS or NaPBS was used for ICC. KPBS gave better results for CCK. Anti-CCK (Diasorin; Stillwater, MN) was used at 1:5,000 – 1:10,000 and anti-SP (Diasorin) at 1:8,000 – 1:20,000.

CCK staining was sometimes amplified with biotinylated tyramine. For this, fixation was done with PFA and acrolein followed by PFA alone. KPBS was used for ICC. After incubation with the secondary antibody (1:5,000), sections were incubated (30 min) in a 1:8 dilution of ABC solution containing 0.3% Triton X-100, then 20 min in a 1:1,000 dilution of biotinylated tyramine (NEN Life Sciences; Boston, MA) in KPBS containing 0.005% H<sub>2</sub>O<sub>2</sub> and then 1 hr at 37 °C in a 1:16 dilution of ABC solution with 0.3% Triton X-100. Visualization involved DAB as above.

## 2.6 VP ICC

Fixation was done with 5% acrolein in NaPBS (150 ml/10 min). No postfixing was done. The ICC buffer was TBS. Preliminary blocking (15 min) used 2% NS and 0.3% Triton X-100. Incubation with anti-VP (1:1,000; ICN; Costa Mesa, CA) was done at 37 °C for 1–1.5 hr.

## 2.7 TH ICC

Fixation was done with PFA and acrolein followed by PFA alone and PFA postfixing (see 2.5 *SP and CCK*). The ICC buffer was TBS. Before exposure to NaBH<sub>4</sub>, sections were incubated for 15 min in 3% H<sub>2</sub>O<sub>2</sub> to exhaust endogenous peroxidase. Preliminary blocking used 10% NS. Anti-TH (Diasorin) was used at 1:10,000.

## 2.8 Histological analyses

Distributions of immunoreactive (IR) cells in the MeA, caudal POA and caudal BST were assessed at 40–125X with brightfield illumination. Cell groups were identified by landmarks using rat brain maps and atlases (Paxinos and Watson, 1997; Swanson, 1992) and a description of the gerbil POA (Commins and Yahr, 1984). At a caudal level of the MPN, cells IR for TH, CCK, SP and VP were charted. Using a drawing tube, they were traced as they appeared at 78X on one side of one section from one male per antigen. Landmarks were also traced, and outlines of the MPNm, central MPN (MPNc) and ventral BST from an archived, thionin-stained section were superimposed. Cells were considered to be IR if they had outlines consistent with neuronal soma that were distinct from the surround due to reaction product in the cytoplasm. A blue filter (Wratten 47A; Kodak, New York, NY) was sometimes used to make the brown reaction product appear darker. Oxytocin-IR cells, which were the least common in the MPNm, were not charted.

The gerbil MPNm and ventral BST were initially identified as parts of a sexually dimorphic area (SDA) in the hypothalamus (Commins and Yahr, 1984). Based on similarities between the lateral SDA and parts of the rat BST as discussed in Finn and Yahr (2005), we now refer to the lateral SDA as the ventral BST because it corresponds to the magnocellular subnucleus of the rat BST (BSTmg) plus the parts of the ventral subnucleus lying ventral and lateral to the BSTmg as described by Ju and Swanson (1989). It includes the dorsal parts of the preoptic BST subnucleus as described by Moga et al. (1989) and may correspond to the magnocellular medial preoptic nucleus (MPN mag) in hamsters (Wang and Swann, 2006). Based on homologies discussed in Finn et al. (1993), the medial SDA, the area lateral to it and the SDA *pars compacta* correspond to the MPNm, lateral MPN (MPNl) and MPNc of rats, respectively.

## 2.9 Quantification of IR cell densities

IR cells in the PdPN, MPNm and lateral MeApd were counted at 125X, 312X and 500X, respectively, in areas defined by boxes superimposed on the tissue with the drawing tube. The PdPN box ( $135 \times 135 \mu\text{m}$  subtended tissue) was positioned so that its lateral edge was just lateral to the fornix and its top edge was a third of the way down the third ventricle (below the anterior commissure), at or just caudal to the level where the fornix abuts the anterior commissure. The lateral MeApd box ( $160 \times 160 \mu\text{m}$ ) was placed at the edge of the MeApd, as revealed by the stria terminalis, where the edge is farthest from the optic tract and most concave. This was 30–150  $\mu\text{m}$  rostral to the lateral ventricle. For the MPNm, the third ventricle, optic chiasm and anterior commissure were used to position the box ( $120 \times 240 \mu\text{m}$ ), vertically, in the caudal MPNm, ventromedial to the MPNc.

Using the drawing tube, each IR cell body inside the box or on its edges was drawn. From the drawings, cells in the box or overlapping its top or right edge were counted. Those overlapping the left or bottom edge were counted if at least half of the cell body was inside the box. When possible, counts were done at two rostrocaudal levels per area, always using nonadjacent sections. The sides selected were those that appeared to have the most stained cells, which was usually the side of the colchicine infusion. If more than one hemisection was counted for an area, the total count for that male was divided by the number of hemisections used before the group mean was computed.

## 2.10 Image preparation

Digitized images were acquired with a Zeiss AxioImager M2 light microscope using a 10X objective and AxioVision software v4.7. To better reproduce the appearance of the tissue at microscopy, images were adjusted for tonal and/or color qualities using Adobe Photoshop CS3 (levels, curves and color balance tools). Figures were assembled in Adobe Illustrator CS3.

# 3. Results and Discussion

## 3.1 Catecholaminergic neurons

In the caudal POA, many dark TH-IR cells corresponding to the dorsal A14 and A15 dopaminergic cells of rats (Hökfelt et al., 1984) were seen in the PeV and anterodorsal preoptic nucleus as shown in Figure 1. The A14 group formed a band below the ventral BST that extended diagonally from the SCN along the optic chiasm and through the lateral POA. TH-IR cells were also found in the MPNm (see Figure 2B), where they seemed denser than in rats (Simerly et al., 1986), and in the MPNI and MPNc. The PdPN had TH-IR cells (see Figure 3B) that were as dense as mating-activated efferents to the anteroventral periventricular nucleus (AVPv; Simmons and Yahr, 2002). The distributions of Fos-IR cells in the MPNm and PdPN of a mated male are shown in Figures 2A and 3A for comparison to the distributions of TH-IR cells at those sites. For TH and every peptide studied here, the density of MPNm, PdPN and lateral MeApd cells containing them is given in Table 1.

TH-IR cells were less common in the caudal BST than in the caudal POA, but a few were seen in the ventral BST (see Figure 1). Slightly more were noted in the BSTpr, where they were densest rostrally. This is shown in Figure 4D.

In the MeA, a few TH-IR cells were seen in the medial MeApd and anterodorsal MeA, but the posteroventral MeA had none. More were noted in the caudal MeApd, where most were scattered. However, in the lateral MeApd, TH-IR cells were clustered where the Fos-cell cluster appears at ejaculation, as shown in Figure 5A and D, and their density (see Table 1) matched that of mating-activated efferents to the AVPv (Simmons and Yahr, 2002). Like

TH cells at midrostral MeA levels in hamsters (Asmus et al., 1992), some TH cells in the lateral MeApd had prominent dendrites extending into the medial MeApd, where cells express Fos in response to odors (Kollack-Walker and Newman, 1997) and/or anticipation of sexual reward elicited by exposure to an environment associated with mating (Balfour et al., 200; Heeb and Yahr, 1996).

In rats, TH cells were not seen in the MeApd with *in situ* hybridization for TH mRNA or TH ICC using colchicine (Kiyama et al., 1990; Kosaka et al., 1987). A few TH-IR cells were detected without colchicine in the MeApd of meadow voles and hamsters (Northcutt et al., 2007; Vincent, 1988), but those in hamsters did not express Fos with mating (Asmus and Newman, 1994). In contrast, many TH-IR cells were seen without colchicine in the MeApd and dorsal BST of prairie voles (Northcutt et al., 2007), and they expressed Fos and another immediate-early gene, *erg-1*, with mating, particularly ejaculation (Cavanaugh and Lonstein, 2010).

Because the MeApd and dorsal BST are sites where chemosensory and hormonal inputs converge, they are foci for research on affiliative behaviors, such as pair bonding and biparental care of young, as well as male sex behavior. Prairie voles are a model for research on affiliative behaviors because they are monogamous and biparental and closely related to meadow voles, which are promiscuous, like hamsters and rats. Thus, it is interesting that gerbils, which are monogamous and biparental, resemble rats, hamsters and meadow voles, not prairie voles, in TH-cell density in the MeApd and dorsal BST. Gerbils also differ from many monogamous rodents in that T levels and aggressiveness toward male nest intruders do not decline when males become paternal (Juana et al., 2010). Thus, just as gene-behavior correlations among selected lines are prone to confounds if either direction of selection is represented by only one line (DeFries et al., 1974), brain-behavior correlations among rodents may be prone to confounds if selection for an adaptation such as monogamy and biparental care of young is represented by only one species. Comparing prairie voles to gerbils could be useful in this regard.

### 3.2 SP neurons

The distribution of SP-IR cells in the caudal POA and BST of gerbils resembled that in rats and hamsters (Ju et al., 1989; Neal and Newman, 1991; Simerly et al., 1986; Swann and Newman, 1992). In the caudal POA, SP-IR cells were seen primarily in the PeV but also in the MPNm (see Figure 2C), where they were mostly in its caudal aspect. Their density in the MPNm (see Table 1) matched that of mating-activated efferents to the lateral MeApd (Heeb and Yahr, 2001). SP-IR cells were also seen dorsal to the MPNm, in the cell bridge connecting the MPNm to the ventral BST, in the MPNI and in the suprachiasmatic nucleus (SCN), as shown in Figure 1. They were not seen in the MPNc, which stood out because it lacked both SP fibers and cells, whereas its surround had both. SP-IR cells were found in the PdPN, as shown in Figure 2C, and their density there (see Table 1) matched that of mating-activated efferents to the BSTpr (Simmons and Yahr, 2002).

In the caudal BST, SP-IR cells were seen in and dorsal to the ventral BST as well as in the rostral BSTpr, near the fornix, and, further caudally, lateral to the BSTpr, next to the internal capsule. They were densest, though, in the dorsal BSTpr, just below the lateral ventricle. This distribution is shown in Figure 4B.

SP-IR cells were seen throughout the MeApd but were densest caudally. Their densities were similar in the medial MeApd, anteroventral and posteroventral MeA, and lateral MeApd, where they were densest ventrally (see Figure 5B).

### 3.3 VP neurons

Many darkly stained VP-IR cells were seen in the SCN and supraoptic nucleus. There were a few VP-IR cells in the PeV but none in the MPNm, the bridge connecting it to the ventral BST, the MPNc (see Figure 1) or PdPN. VP has not been studied in PdPN cells of other species. VP-IR cells were moderately dense ventral and lateral to the ventral BST.

In the caudal BST, VP-IR cells were moderately dense in the ventral BST and BSTpr. In the BSTpr, they were particularly dense rostrally (see Figure 4C), as they are in voles, jerboas and Siberian, but not Syrian, hamsters (De Vries et al., 1985; Sofroniew, 1985).

VP-IR cells were not seen in the MeA, but a few were interspersed with fibers of the stria terminalis between the MeApd and the central nucleus of the amygdala. In lacking VP cells in the MeA, gerbils resemble Syrian hamsters (Ferris et al., 1995) and differ from rats (Caffe and van Leeuwen, 1983; De Vries et al., 1985; Sofroniew, 1985), mice (Rood et al., 2008), voles (Wang, 1995), jerboas (Lakhdar-Ghazal et al., 1995) and Siberian hamsters (Dubois-Dauphin et al., 1994). In having VP cells interspersed with fibers of the stria terminalis just outside the MeApd, gerbils resemble voles, jerboas and Siberian hamsters, and differ from Syrian hamsters.

### 3.4 CCK neurons

In the caudal POA, caudal BST and MeA, CCK-IR cells were distributed as they are in rats (Ju et al., 1989; Micevych et al., 1987, 1988; Roberts et al., 1982; Simerly et al., 1986; Simerly et al., 1989; Simerly and Swanson, 1987). They were dense in the MPNc, sparse in the MPNm, absent from the PdPN (see Table 1 and Figure 1) and common in the dorsal BSTpr, especially just below the lateral ventricle (see Figure 4A).

In the MeA, CCK-IR cells were densest in the MeApd caudal to the ejaculation-related site but seemed less dense than in the MeApd of rats. Some were seen in the medial MeApd and in the anteroventral and posteroventral MeA. In the lateral MeApd, their density (see Table 1) matched that of mating-activated efferents to the BSTpr (Simmons and Yahr, 2002). Moreover, at the rostrocaudal level of the MeApd where a cluster of Fos cells appears after ejaculation, CCK-IR cells were seen only at the site of that cluster, where they formed a distinct ball. This is shown in Figure 5A and C.

### 3.5 Oxytocin neurons

In the caudal POA, oxytocin-IR cells were densest in the PeV, SON and PdPN. They were very sparse in the MPNm and ventral and lateral to the ventral BST. The few seen in the MPNl were part of a group that continued into the area below the caudal PdPN. In the PdPN (see Figure 3D), oxytocin-IR cells had a density (see Table 1) matching that of mating-activated efferents to the lateral MeApd (Heeb and Yahr, 2001).

Oxytocin cells are also seen in the rat PdPN (Sofroniew, 1985), from which many project to the posterior pituitary (Vanhatalo and Soynila, 1995). When tracing PdPN efferents in gerbils (Simmons and Yahr, 2002), we did not study the pituitary. We did, however, note short, continuing, vertically oriented fibers along the top of the optic chiasm in the POA and rostral hypothalamus. Those fibers may have been en route to the posterior pituitary. If oxytocin cells in the PdPN of rats, and possibly gerbils, that project to the posterior pituitary are among the PdPN cells activated at ejaculation, they could be a source of the oxytocin released into the circulation after ejaculation (Hughes et al., 1987).



The caudal BST had fewer oxytocin-IR cells than the caudal POA. Those seen were in the ventral BST and BSTpr. The latter are shown in Figure 5C. No oxytocin-IR cells were seen in the MeA.

### 3.6 Summary and conclusions

TH, SP, CCK, VP and oxytocin were each found in at least one forebrain area activated at ejaculation, *i.e.*, the PdPN, lateral MeApd or MPNm, and each of these areas had cells containing three or more of these molecules. At all three sites, cells containing TH, SP, CCK, VP or oxytocin were less abundant than cells that make GABA and/or NO (Simmons and Yahr, 2003; Simmons and Yahr, unpublished). Except in the PdPN, they are also less abundant than putatively glutamatergic neurons. Of course, the peptide-containing cells visualized here could be cells that also produce GABA, glutamate or NO. Similarly, TH could coexist with NO. Expressing more than one molecule for intercellular communication is a common way for neurons to be subdivided into subsets serving different functions. Alternatively, or in addition, TH- or peptide-containing cells may be interspersed with the more abundant types. In either case, catecholaminergic cells, and cells containing CCK, SP, VP or oxytocin, may play a role in integrating the activities or outputs of the PdPN, MPNm and lateral MeApd. This includes the possibility that they could be among, or interact with, cells activated at ejaculation. Similarities in some of their densities to the densities of mating-activated efferents of the PdPN, MPNm and lateral MeApd are consistent with this possibility.

#### Research Highlights

The posterodorsal preoptic nucleus (PdPN) contains TH, SP and oxytocin cells.  
 The lateral posterodorsal medial amygdala (MeApd) contains TH, SP and CCK cells.  
 The medial MPN has more TH and SP cells than CCK or oxytocin cells.  
 TH, CCK, ejaculation-related cells are grouped at the same site in the lateral MeApd.  
 The PdPN may be a source of the oxytocin that males secrete at ejaculation.

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### Abbreviations

<b>3v</b>	third ventricle
<b>ac</b>	anterior commissure
<b>fx</b>	fornix
<b>lv</b>	lateral ventricle
<b>oc</b>	optic chiasm
<b>ot</b>	optic tract
<b>sm</b>	stria medullaris
<b>AC</b>	anterior commissural nucleus
<b>AVPv</b>	anteroventral periventricular nucleus

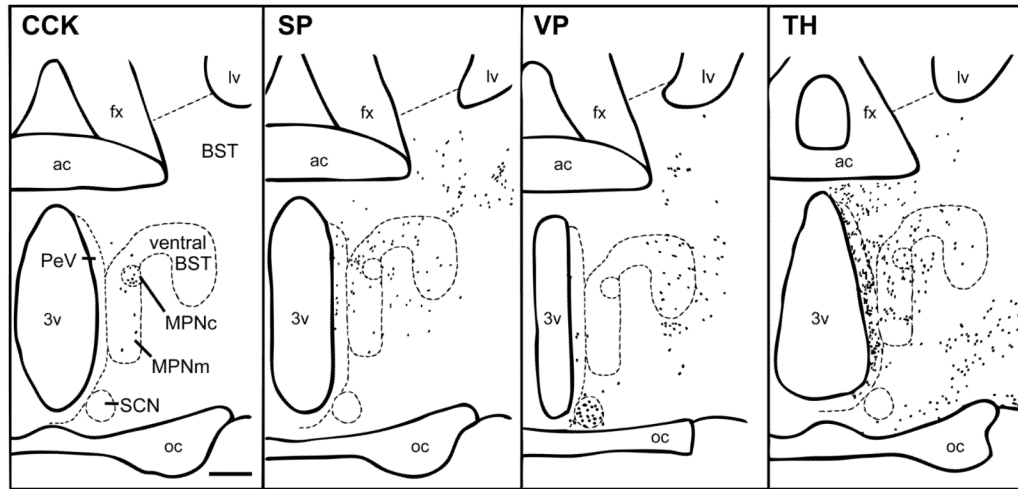
<b>BST, mg, pr</b>	bed nuclei of the stria terminalis, magnocellular subnucleus, principal subnucleus
<b>MeA, pd</b>	medial nucleus of the amygdala, posterodorsal
<b>MPN, l,m,c,</b>	medial preoptic nucleus, lateral, medial, central
<b>MPN mag</b>	magnocellular medial preoptic nucleus (hamsters)
<b>PdPN</b>	posterodorsal preoptic nucleus
<b>PeV</b>	periventricular nucleus
<b>POA</b>	preoptic area
<b>SCN</b>	suprachiasmatic nucleus

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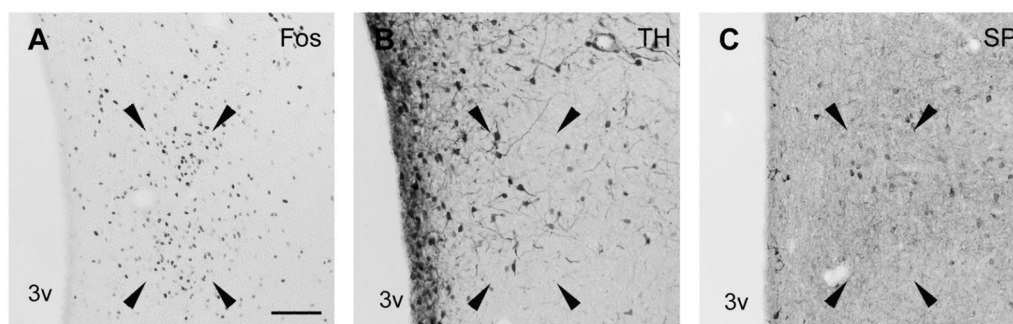
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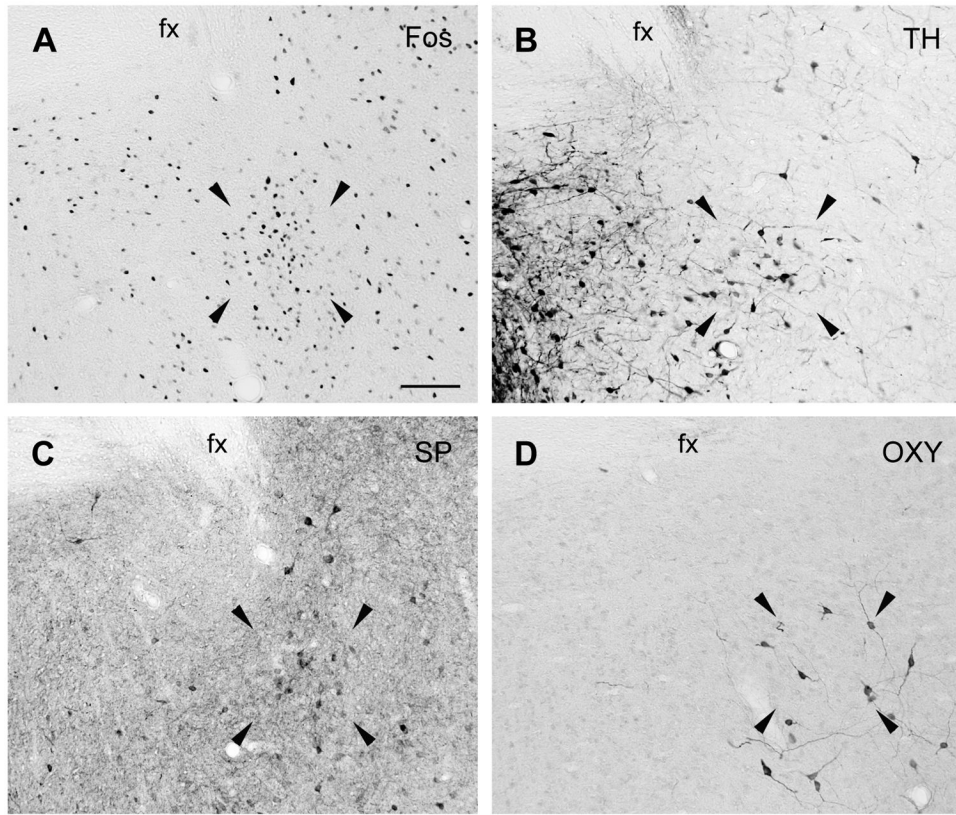
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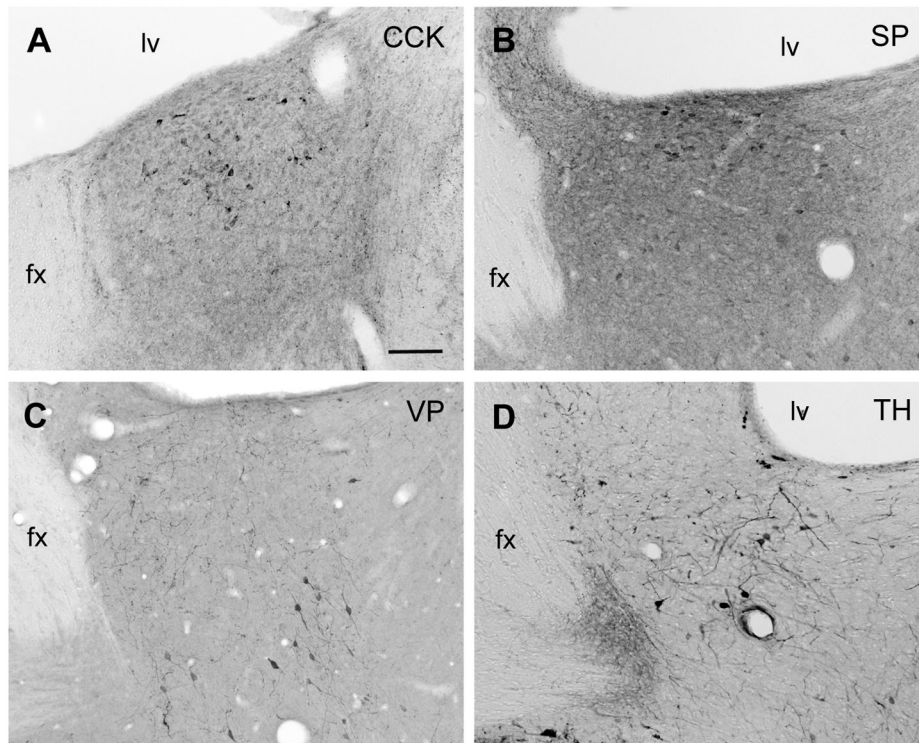
**Figure 1.** Camera lucida drawings of coronal sections through the caudal POA of male gerbils showing the distributions of cells IR for CCK, SP, VP and TH. Each dot represents one cell. Scale bar = 100  $\mu\text{m}$ . ac = anterior commissure; fx = fornix; lv = lateral ventricle; 3v = third ventricle; oc = optic chiasm.



**Figure 2.** Brightfield photomicrographs of coronal sections through the MPN of male gerbils showing the distributions of cells IR for Fos after ejaculation (A), TH (B) and SP (C). Arrowheads point to the corners of the area in which MPNm cells were counted. Scale bar = 100  $\mu$ m. 3v = third ventricle.

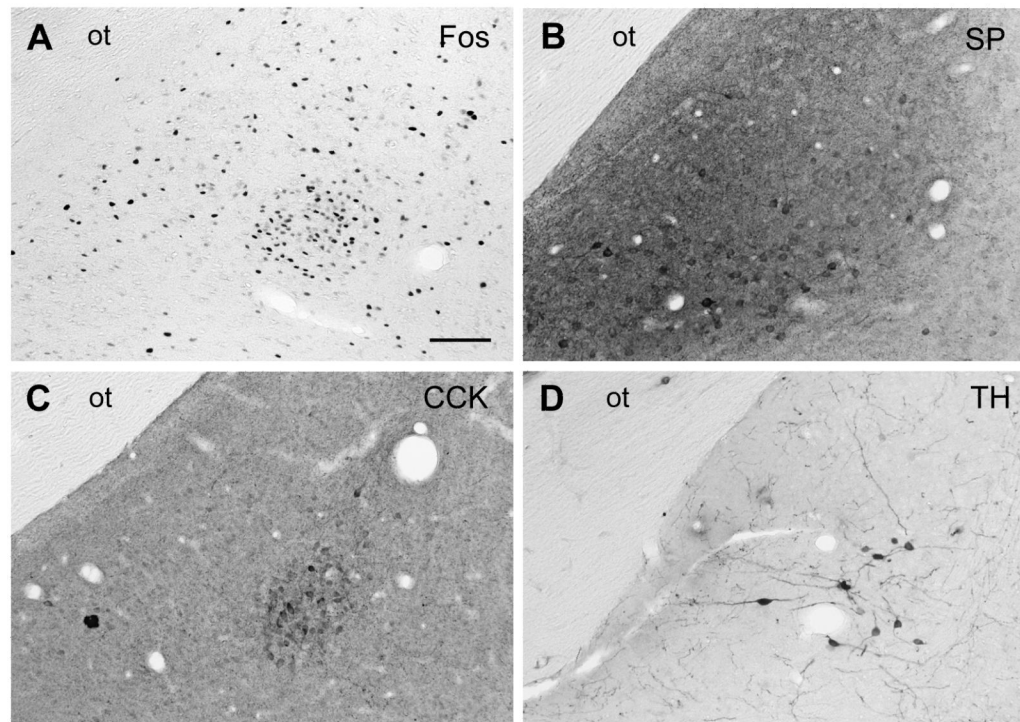


**Figure 3.** Brightfield photomicrographs of coronal sections through the PdPN of male gerbils showing the cluster of Fos-IR cells seen after ejaculation (A) in relation to the distributions of cells IR for TH (B), SP (C) and oxytocin (OXY; D). Arrowheads point to the corners of the area in which PdPN cells were counted. Scale bar = 100  $\mu$ m. fx = fornix.



**Figure 4.** Brightfield photomicrographs of coronal sections through the caudodorsal BSTpr (top row) or rostral BSTpr (bottom row) of male gerbils showing the distributions of cells IR for CCK (A), SP (B), VP (C), or TH (D). Scale bar = 100  $\mu$ m. fx = fornix; lv = lateral ventricle.





**Figure 5.** Brightfield photomicrographs of coronal sections through the MeApd of male gerbils showing the cluster of Fos-IR cells seen after ejaculation (A) in relation to the distributions of cells IR for SP (B), CCK (C) and TH (D). Scale bar = 100  $\mu$ m. ot = optic tract.

**Table 1**

Mean ( $\pm$  SEM) Number of MPNm, PdPN and lateral<sup>1</sup> Immunoreactive for TH, CCK, SP, Vasopressin or Oxytocin

	N	MPNm	PdPN	Lateral MeApd
TH	7	12 $\pm$ 1	9 $\pm$ 1	6 $\pm$ 1 <sup>2</sup>
CCK	11	4 $\pm$ 1 <sup>3</sup>	0	17 $\pm$ 2
SP	8	9 $\pm$ 1	6 $\pm$ 1	11 $\pm$ 2
Vasopressin	5	0	3 $\pm$ 1	0
Oxytocin	6	1 $\pm$ 0 <sup>2</sup>	2 $\pm$ 0	0

<sup>1</sup> Per 0.029 (MPNm), 0.018 (PdPN) and 0.026 (lateral MeApd) mm<sup>2</sup>.

<sup>2</sup> N = 6;

<sup>3</sup> N = 5