UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM NEUROCIÊNCIAS

MORFOLOGIA DENDRÍTICA DE NEURÔNIOS DO NÚCLEO MEDIAL DA AMÍGDALA DE RATOS: UM ESTUDO PELA TÉCNICA DE GOLGI

Aline Dall'Oglio

Porto Alegre

2007

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM NEUROCIÊNCIAS

MORFOLOGIA DENDRÍTICA DE NEURÔNIOS DO NÚCLEO MEDIAL DA AMÍGDALA DE RATOS: UM ESTUDO PELA TÉCNICA DE GOLGI

Aline Dall'Oglio

Orientador: Prof. Dr. Alberto A. Rasia Filho

Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Neurociências da Universidade Federal do Rio Grande do Sul como requisito parcial para obtenção do título de mestre em Neurociências.

Porto Alegre

2007

Dedico este trabalho e agradeço aos meus pais Ernesto e Vera ("in memorian") pelo exemplo humano de caráter e pelo amor e dedicação sem palavras. Dedico também aos animais que, apesar da falta de escolha, contribuíram com a sua vida para que ele fosse possível.

"We hope that readers evaluating the work presented here will place less emphasis on the results themselves than on the time and effort that we have devoted to them. But above all, we hope that they will appreciate the unbiased and patriotic goals that served as our guides."

"Santiago Ramón y Cajal"

(*Histology of the Nervous System of Man and Vertebrates*, 1995; do original 1909)

Em um momento como esse, quando se está perto de concluir uma etapa importante da vida profissional, torna-se impossível, ao se olhar para trás e vendo o caminho percorrido, não avaliar tudo e todos que de maneira intencional ou não, contribuíram para isso acontecer. Gostaria de agradecê-los e em especial:

Ao meu orientador e amigo, Prof. Dr. Alberto Antônio Rasia Filho pela oportunidade e imensuráveis lições humanas e científicas. Agradeço por tua confiança, tua paciência, tua dedicação, e, principalmente, pelo teu adorável senso de humor.

Às minhas irmãs, Macheli e Emilly pela amizade, confiança e incentivo sempre. Amo vocês.

À minha nova família, Maurício e Musashi, agradeço pelo amor, carinho e paciência. Ao Maurício, especialmente, pelas aulas de Photoshop e Freehand. Vocês são a alegria da minha vida.

À amiga Simone Macuzzo por compartilhar comigo, no sentido mais literal da palavra compartilhar, a experiência do mestrado. Por me ouvir e ajudar sempre e por não me permitir desistir. Não posso deixar de incluir também o amigo Pedro Kalil, que embora tenha aparecido em tempo posterior, tem me auxiliado e torcido muito por mim.

Aos amigos Andréa Duarte, Antônio Miragem, Janaína Brusco e Rafaela de Araújo agradeço as horas de conversa e apoio. E à Mírian, por me auxiliar no encontro de mim.

A CAPES, à UFRGS e ao Programa de Pós Graduação em Neurociências pela oportunidade.

À Profª. Drª. Matilde Achaval pelas horas prestadas, pelos conselhos e por ter me recebido no Laboratório de Histofisiologia Comparada.

À Profª. Drª. Maria Flávia Marques Ribeiro pela dedicação e oportunidade no estágio didático e por me ajudar a vencer o medo de dar aula.

Ao Prof. e colega Günther Gehlen pela parceria estabelecida neste trabalho.

À Profª. Drª. Sidia Callegari Jacques pela disponibilidade e importantíssimo auxílio com a análise estatística.

Aos colegas do laboratório de Histofisiologia Comparada do Departamento de Ciências Morfológicas da UFRGS: Juliana de Castilhos, Érica Hermel, Jocemar Ilha, Patrícia do Nascimento, Giordano Viola, Aline Pagnussat, Maria Izabel Ugalde, Flávia Martinez, Márcio Dutra, Régis Mestriner, Letícia Rodrigues e às ex-colegas, Cláudia Forti, Cláudia Puperi e Alessandra Swarowsky, pela convivência agradável e pelas contribuições.

Aos colegas do laboratório de Fisiologia da FFFCMPA: Hélington Neckel, Édson Quagliotto, Carol Böettge, Alexandre Paim, Ramiro Barcos, Thiago Heck, Mauren Porto, Elisa de Leon, Nathália e Géferson pela parceria e amizade. Também aos professores da FFFCMPA: Pedro Dall'Ago, Cyntia Fin, Cíntia Gama, Maria Beatriz Kohek, Kátia

Rigatto, Ana Maria de Azevedo e Márcia Giovenardi, e aos técnicos de laboratório, Carmen Rocha, Rô Bjerk, Alexandre e Mário pela ajuda e consideração.

Aos colegas, professores e funcionárias do PPG Neurociências, especialmente aos colegas Ana Raquel Karkow, Lenir Orlandi, Camila Padilha, Leonardo Crema, Fernanda Cechetti, Lucas de Oliveira, Isabel Fossati, José Menna, Carol Perinazzo, Gisele Louvattel, Lígia Centenaro, ao Prof. Léder Xavier e a funcionária Andréa Antunes pelas contribuições e amizade.

SUMÁRIO

ABREVIATURAS

ACe	Núcleo central da amígdala
AMBl	Núcleo basilar lateral da amígdala
AVPV	Núcleo periventricular ântero-ventral do hipotálamo
BNST	Núcleo intersticial da estria terminal
BNST-IA	Porção intra-amigdaliana da estria terminal
ER-α	Receptor do tipo α para estrógeno
ER-β	Receptor do tipo β para estrógeno
GFAP	Proteína ácida fibrilar glial
GFAP-ir	Imunorreatividade à proteína ácida fibrilar glial
GnRH	Hormônio liberador de gonadotrofinas
Int A	Núcleos "intercalados" da amígdala
MeA	Núcleo medial da amígdala
MeAD	Núcleo medial ântero-dorsal da amígdala
MeAV	Núcleo medial ântero-ventral da amígdala
MePD	Núcleo medial póstero-dorsal da amígdala
MePDi	Porção intermediária do núcleo medial póstero-dorsal da amígdala
MePDl	Porção lateral do núcleo medial póstero-dorsal da amígdala
MePDm	Porção medial do núcleo medial póstero-dorsal da amígdala
MePV	Núcleo medial póstero-ventral da amígdala
SN	Sistema nervoso
ST	Estria terminal
TO	Trato óptico
VMH	Núcleo ventro-medial do hipotálamo

LISTA DE FIGURAS

ARTIGO

LISTA DE TABELAS

RESUMO

O núcleo medial da amígdala (MeA) de ratos contém receptores para hormônios gonadais em quantidades similares às encontradas em vários núcleos do hipotálamo e modula diversos comportamentos sociais, inclusive o reprodutivo. Os objetivos deste estudo foram: 1) detalhar a morfologia dendrítica geral de neurônios de ratos, machos e fêmeas adultos, dos subnúcleos do MeA, a saber: ântero-dorsal (MeAD), póstero-dorsal (MePD) e póstero-ventral (MePV); 2) quantificar e comparar os aspectos relevantes da morfologia dendrítica; 3) e, baseados em dados empíricos, elaborar dendrogramas descrevendo diâmetros e comprimentos dendríticos que possam auxiliar na descrição de sua morfologia e possível função. Para tanto se empregou a técnica de Golgi e desenhos em câmara clara foram mensurados em computador. Com base nisso, o número de ramos dendríticos (por nível de arborização, desde primário até quaternário) e o comprimento dendrítico total foram submetidos à análise da variância multivariada (MANOVA). A distribuição dos dendritos em função da distância do soma foi estudada aplicando-se a técnica dos círculos concêntricos de Sholl, seguida por uma análise da variância (ANOVA) para medidas repetidas, com critério de Roy para teste de interação entre as variáveis. A distribuição espacial preferencial dos ramos dendríticos (nas coordenadas dorsal, ventral, lateral, medial e suas interações e subdivisões) foi estudada pela técnica da sobreposição de neurônios sobre grades de quadrados e submetida a um teste de $\chi 2$. Em todos os casos, o nível de significância estatística foi estabelecido em 5%. Nos três subnúcleos estudados do MeA, neurônios multipolares foram classificados como estrelados ou bipenachados, apresentaram arborização dendrítica esparsa e seus ramos dendríticos tiveram comprimentos variados. Diferença

estatisticamente significante entre os sexos foi encontrada no número de ramos secundários no MeAD (maior em fêmeas, $p < 0,05$), mas principalmente, houve um padrão sexualmente dimórfico preferencial na distribuição dos ramos dendríticos nas coordenadas espaciais estudadas, nos três subnúcleos do MeA (como por exemplo, ramos preferentemente localizados medialmente em machos e em posição mais dorsal e ventro-medial em fêmeas, $p < 0,01$). Tais achados, que demonstram pela primeira vez uma diferença entre machos e fêmeas quanto à morfologia dendrítica, sugerem outra possível ação dos hormônios gonadais nos subnúcleos do MeA de ratos. Ademais, os resultados aqui apresentados podem ser integrados com dados hodológicos e com circuitos funcionalmente dinâmicos que são relevantes para a organização comportamental em ambos os sexos.

ABSTRACT

The medial nucleus of the amygdala (MeA) contains receptors for sex steroids and modulates several social behaviors. The aims of this study were: 1) to detail the general dentritic morphology of Golgi-impregnated neurons from the anterodorsal (MeAD), posterodorsal (MePD) and posteroventral (MePV) MeA subnuclei of adult male and female rats; 2) to describe, quantify and compare the morphology of their dendritic arbors; and, 3) to generate empirical dendrograms. Dendritic arborization level, number of branches in each level, and total dendritic length were compared by a multivariate analysis of variance (MANOVA). The distribution of the dendrites was submitted to the Sholl´s concentric circle technique and to an ANOVA for repeated measures followed by the Roy's test. The preferred spatial distribution of dendritic branches (main coordinates and subdivisions) was studied using the overlaid square technique and subjected to the $\chi 2$ test. In the MeA subnuclei multipolar neurons were rather classified as bitufted or stellate ones, their spiny dendrites showed variable lengths and branched sparingly. Statistical significance between sexes was found in the number of secondary dendrites in the MeAD (higher in females, $p<0.05$). Interestingly, the predominant dendritic spatial orientation was sexually dimorphic in the three MeA subnuclei (for example, rather medial in males and more dorsal and ventromedial in females, $p<0.01$). These results provide basic morphological data for the MeA subnuclei neurons and suggest that their dendrites are also affected by gonadal hormones actions. These findings can be integrated with hodological data and within functional dynamic circuits relevant for behavioral organization in both sexes.

1. INTRODUÇÃO

1. 1. Amígdala

O nome “amígdala” adveio de estudos anatômicos nos quais vários núcleos colocados rostralmente à cauda do núcleo caudado, ao hipocampo e ao corno temporal do ventrículo lateral pareciam, agrupadamente, ter uma conformação esferóide e alongada que lembrava a forma de uma amêndoa (do grego adveio seu nome, embora não tenha esse aspecto em todos os animais estudados até o momento; RASIA-FILHO e HILBIG, 2005). Há, no entanto, considerável debate sobre sua subdivisão em núcleos, em várias espécies animais e por isso são comuns referências ao núcleo inteiro como complexo amigdaliano [“amigdaliano” é algo relativo ou pertencente à amígdala, enquanto “amigdalóide” é semelhante à amígdala, embora seja o primeiro termo que esteja contemplado na Terminologia Anatômica (2001). Como uma liberdade e para ensejar boa discussão, usar-se-á o termo amigdaliano no presente trabalho].

Em ratos, a amígdala é uma das maiores estruturas da parte médio-caudal do telencéfalo basilar e compõe vias preservadas filogeneticamente, como as aferências que recebe do bulbo olfatório principal e acessório e as eferências que envia para vários núcleos e subsistemas funcionais do hipotálamo (BRUCE e NEARY, 1995; AIZAWA *et al*., 2004). Isso poderia sugerir a importância da amígdala para a sobrevivência e adaptação do animal em seu ambiente e, de fato ela integra atividades comportamentais, endócrinas, simpáticas e parassimpáticas importantes, inatas e aprendidas (RASIA-

FILHO *et al*., 2000). Foi a partir do trabalho pioneiro de Klüver e Bucy (1939), que se demonstrou que a lesão que envolvia o lobo temporal anterior de macacos fazia com que esses animais apresentassem agnosia visual, tivessem uma tendência a investigar com a boca toda sorte de objetos colocados a seu alcance, diminuíssem sua agressividade e as manifestações comportamentais relacionadas com medo, além de apresentarem um aumento anormal do comportamento sexual.

A amígdala, todavia, não é nem uma unidade morfológica nem funcional no encéfalo de ratos (SWANSON e PETROVICH, 1998). Por isso é muito importante estudar de forma individual e detalhada cada um de seus componentes e, por vezes, de seus subcomponentes, os quais formam circuitos específicos para gênese e modulação de respostas neurais envolvidas com comportamentos e ajustes diversos (CANTERAS *et al.*, 1995; NEWMAN, 1999; SHEEHAN *et al.,* 2001, PETROVICH *et al.,* 2001; RASIA-FILHO *et al.,* 2004; RONDINI *et al.,* 2004; CAVALCANTE *et al*., 2006). Dentre tais diferentes funções da amígdala de ratos estão às respostas a estímulos gerados por medo e ansiedade, percepções de estímulos olfatórios e hormonais (como o dos esteróides sexuais e os glicocorticóides), modulação dos comportamentos reprodutivo, maternal e defensivo e participação na aquisição do aprendizado e da memória condicionada (KLING e BROTHERS, 1992; LeDOUX, 1992; EVERITT, 1995; KONDO e ARAI, 1995; WOOD e NEWMAN, 1995; GLOOR, 1997; QUIRK *et al.*, 1995; SWANSON e PETROVICH, 1998; DAVIS, 2000; PITKÄNEN, 2000; RASIA-FILHO *et al*., 2000; 2004; de CASTILHOS *et al*., 2006).

Em ratos, apesar dos limites anatômicos precisos e a classificação de suas subdivisões permanecerem ainda controversos (SWANSON e PETROVICH, 1998;

CANTERAS *et al*.,1995; NEWMAN, 1999; PITKÄNEN, 2000; de OLMOS *et al*., 2004), há certo consenso em aceitar que, de acordo com suas características citoarquitetônicas, imunoistoquímicas e hodológicas, a amígdala seja dividida em quatro regiões principais: 1) amígdala "expandida", denominada assim por que se estende além de seus limites anatômicos e que é formada pelos núcleos medial (MeA) e central (ACe) da amígdala. O MeA, por exemplo, compartilha alguns aspectos estruturais com as partes anterior, ventral e posterior do núcleo intersticial da estria terminal (BNST), BNST intra-amigdaliano, divisão medial supracapsular do BNST e divisão sublenticular da amígdala "expandida" (ALHEID *et al*. 1995); 2) amígdala com características corticais, subdividida em porção basilar lateral (AMBl) e em porções que se ligam às vias olfatórias e vomeronasal; 3) áreas de transição, localizadas entre a porção ventral dos núcleos da base e a amígdala "expandida"; e 4) núcleos ainda não classificados, constituídos por um grande grupo de células dispersas na substância branca e no interior do BNST (ALHEID *et al*., 1995; SWANSON e PETROVICH, 1998; de OLMOS *et al*., 2004; Figura 1).

Maior atenção será dada ao MeA, tema desta dissertação e onde foram realizados os experimentos que serão descritos a seguir.

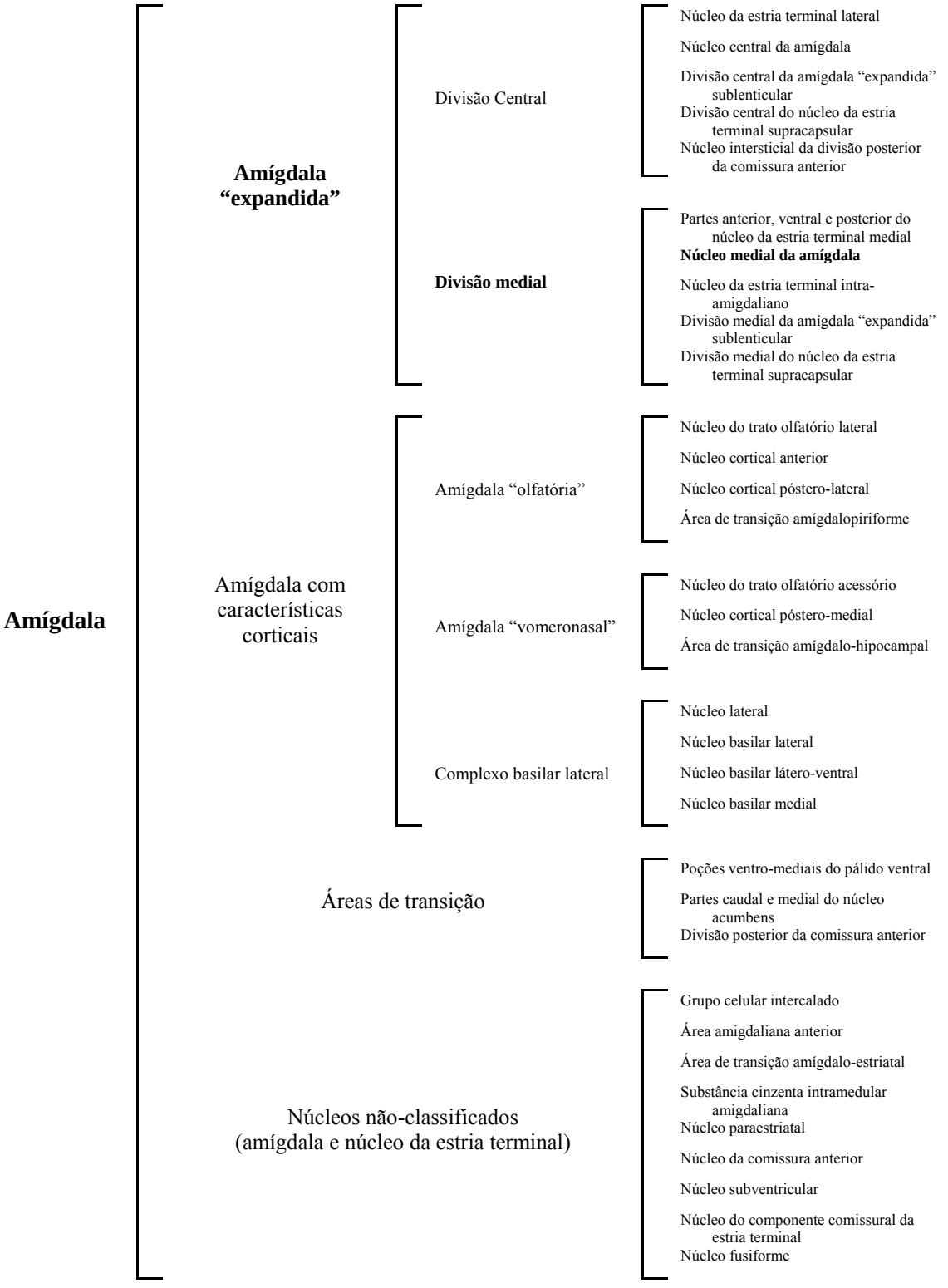


Figura 1. Diagrama da amígdala do rato com suas subdivisões anatômicas e seus componentes principais, conforme descrito por Alheid e colaboradores (1995) e modificado de Rasia-Filho e colaboradores (2000).

1. 2. Núcleo medial da amígdala

1. 2. 1. Localização, divisão e citoarquitetura

O MeA de ratos se situa rostromedialmente na porção superficial do complexo amigdaliano. É composto por células justapostas à porção lateral do trato óptico (TO), seguindo-o desde a posição rostral até caudal. Na parte rostral encontra-se em localização medial e posterior ao núcleo do trato olfatório; e se estende caudalmente até o surgimento da porção temporal do ventrículo lateral. Aí ele se situa dorsomedialmente ao pólo cefálico da área de transição amígdalo-hipocampal (ALHEID *et al*., 1995). Dorsolateralmente, o MeA é separado do ACe por uma região pobre em células, mas que compõe separadamente grupamentos específicos dos núcleos "intercalados" da amígdala (Int A), a porção intra-amigdaliana da estria terminal (BNST-IA). Em posição dorsal e mais caudalmente, grupos de fibras nervosas que ascendem ou descendem dentro da estria terminal estão interpostos entre o MeA e outros núcleos da amígdala. Caudalmente os subnúcleos do MeA são separados do AMBl pelos Int A. (BRODAL, 1947; ALHEID *et al*., 1995; PAXINOS e WATSON, 1998; de OLMOS *et al*., 2004).

Há diferentes sugestões de subdivisão do MeA segundo critérios de diferentes autores. Por exemplo, Pitkänen (2000) divide-o em três regiões: uma porção rostral, uma porção central (que compreende as porções dorsal e ventral) e uma porção caudal. Alheid et al. (1995) e de Olmos et al. (2004), por outro lado, dividem-no em quatro

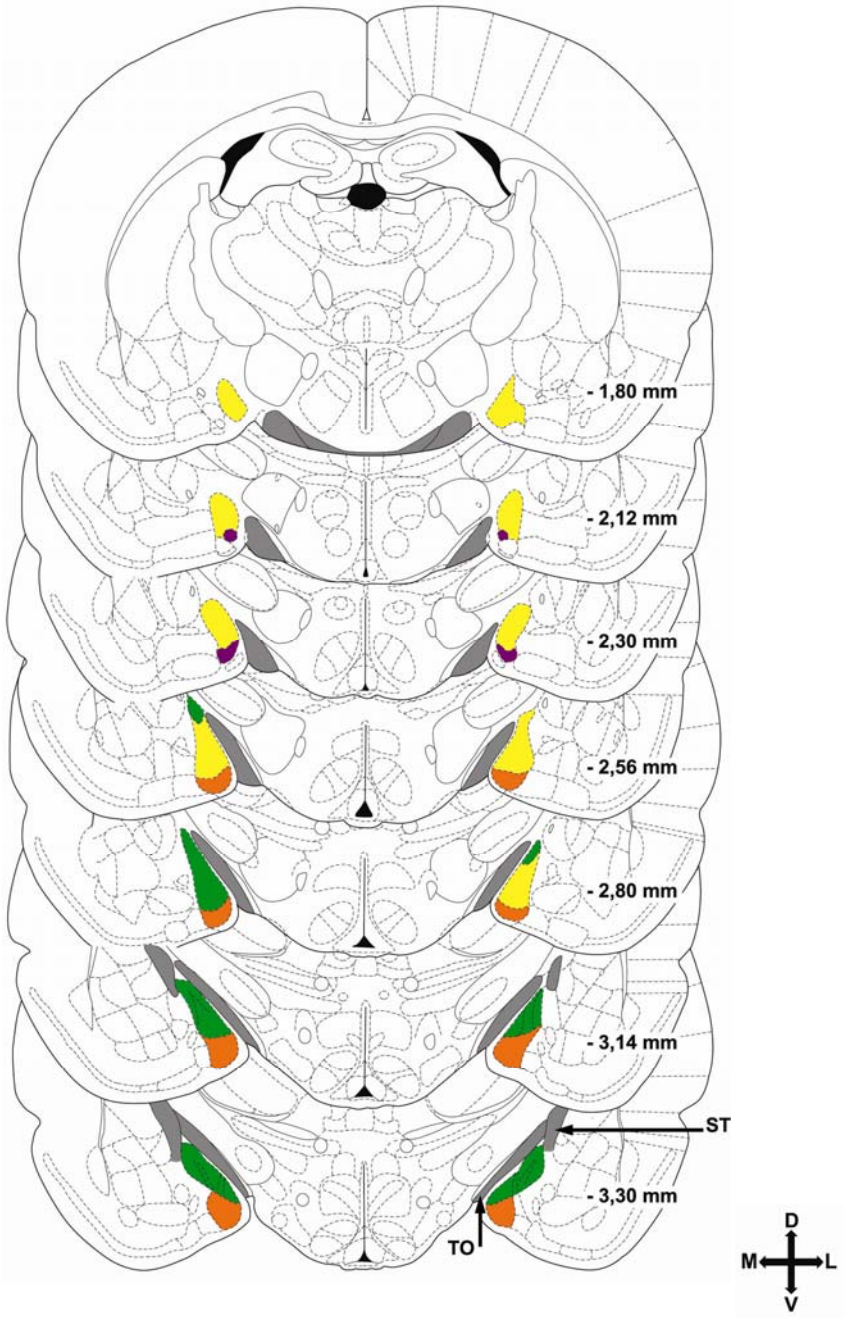


Figura 2. Representação esquemática de cortes coronais do encéfalo do rato onde se podem observar os quatro subnúcleos do núcleo medial da amígdala: ântero-dorsal (em amarelo), ântero-ventral (em roxo), póstero-dorsal (em verde) e póstero-ventral (em laranja). Os valores em mm colocados no lado direito das imagens referem-se à distância posterior ao bregma e as coordenadas dorsal (D), lateral (L), medial (M) e ventral (V), igualmente ao lado direito encefálico. Figuras baseadas no atlas do encéfalo do rato de Paxinos e Watson (1998). TO, trato óptico; ST, estria terminal.

subnúcleos: núcleo medial ântero-dorsal (MeAD), núcleo medial ântero-ventral (MeAV), núcleo medial póstero-dorsal (MePD) e núcleo medial póstero-ventral (MePV; Figura 2). Há ainda uma terceira subdivisão sugerida por Canteras *et al*., (1995) que, se baseando nas conexões eferentes dos subnúcleos do MeA, propõe que ele seja organizado em uma região anterior, da qual fazem parte o MeAD, o MeAV e o MePV, e uma região posterior, formada pelo MePD.

O MePD pode ainda ser subdividido conforme a disposição colunar de seus neurônios em uma porção mais superficial e medial (MePDm) uma intermediária com células menos agrupadas (MePDi), e uma porção lateral (MePDl; COOLEN *et* al., 1997; de OLMOS *et al.,* 2004; de CASTILHOS *et al*., 2006). A divisão adotada nesse trabalho corresponde àquela proposta por Alheid et al. (1995) e de Olmos et al. (2004), sendo considerados para estudo os subnúcleos MeAD, MePD e MePV. O MeAV foi excluída por dificuldades técnicas, devido ao seu tamanho diminuto.

Estudos morfológicos, empregando a clássica técnica de Golgi, para identificar os neurônios do MeA e realizados em gatos (HALL, 1972; TÖMBÖL; SZAFRANSKA-KOSMOL, 1972; PRICE, 1987), cães (MUKHINA; LEONTOVICH, 1970), camundongos (VALVERDE, 1962) e ratos (YU, 1969; de OLMOS et al., 1985; PRICE, 1987; RASIA-FILHO et al., 1999; 2004; de CASTILHOS, et al., 2005) têm demonstrado muitas semelhanças. São encontrados apenas neurônios multipolares, classificados como estrelados (Figura 3) ou bipenachados (tradução livre da expressão inglesa “bitufted”, Matilde Achaval e Leny A. Cavalcante, comunicações pessoais; Figura 4), de acordo com o número de prolongamentos dendríticos emergindo do soma neuronal. Quando apresentam três ou mais ramos primários são ditos estrelados e

quando apresentam dois ramos primários são denominados bipenachados (FERNANDEZ-GALAZ *et al*., 1997; RASIA-FILHO *et al*.,1999, 2004). Não se deve empregar a denominação "bipolar" para esses últimos (de acordo com Ramón y Cajal, 1909), embora seja encontrado na literatura (GOMEZ e NEWMAN, 1991; McDONALD, 1992). E nem aqui ambos os tipos celulares são semelhantes aos neurônios de tamanho médio e com espinhos dendríticos típicos do estriado, como impropriamente mencionado por outros autores (BENNUR *et* al., 2007).

A maior parte dos corpos neuronais nos subnúcleos do MeA são classificados como pequenos (8-10 μm de diâmetro médio) ou médios (com 10-15 μm de diâmetro médio), com valores de área em torno de 105 μm^2, muito embora alguns neurônios com corpos grandes também sejam encontrados (RASIA-FILHO *et al*., 1999). Os formatos desses somas parecem ser moldados pela quantidade e disposição de ramos primários e podem ser classificados em ovais, arredondados, fusiformes, triangulares, piriformes ou com formatos irregulares (RIGOTI, 2002; RASIA-FILHO *et al*., 2004). Os ramos dendríticos são descritos como sendo longos (GOMEZ e NEWMAN, 1991; McDONALD, 1992) ou de comprimento variável (NARKIEWICZ *et al*., 1978; RASIA-FILHO *et al*., 1999), grossos, razoavelmente retilíneos e pouco numerosos. Nos diferentes subnúcleos do MeA há a característica peculiar de existir arborização dendrítica esparsa e raramente os neurônios apresentam relativa profusão de ramos (McDONALD, 1992). Pela análise ultra-estrutural dos neurônios do MePD, sinapses axo-dendríticas no tronco dos dendritos são as mais freqüentemente observadas e, pelo aspecto morfológico, parecem ser principalmente excitatórias (HERMEL *et al.,* 2006). Os espinhos dendríticos, quando presentes, apresentam formas variadas e encontram-se de forma aparentemente homogênea ao longo de cada dendrito, porém igualmente em

alguns somas celulares e cones axonais (McDONALD, 1992; RASIA-FILHO *et al*., 1999, 2004; RIGOTI, 2002; HERMEL *et al*., 2006).

Os axônios dos neurônios dos subnúcleos do MeA dirigem-se às estruturas adjacentes ou a duas grandes vias de eferências, embora haja descrição de conexões recíprocas (TÖMBÖL; SZAFRANSKA-KOSMAL, 1972; KAMAL; TÖMBÖL, 1975). Ou seja, além de seguirem para a estria terminal, esses axônios dirigem-se para a alça lenticular. Essas projeções, especificamente as da porção posterior do MeA, que terminam na área medial da região posterior do BNST são conexões densas e bidirecionais em camundongos e ratos (VALVERDE, 1962; COOKE e SIMERLY, 2005). No interior dos subnúcleos do MeA, quando visíveis pela técnica de Golgi, os axônios são supostamente mielinizados, seguem um curso sinuoso e originam ramos colaterais com poucas ramificações, os quais se distribuem nas proximidades dos corpos celulares de origem (VALVERDE, 1962; KAMAL; TÖMBÖL, 1975).

Figura 3. Neurônio de tipo estrelado do subnúcleo póstero-dorsal do núcleo medial da amígdala (MePD) de rato adulto, impregnado pela técnica de Golgi e com típica arborização dendrítica esparsa. Fotomontagem por soma digital (Photoshop 7.0, EUA) de fotomicrografias de diferentes planos focais, permitindo a observação dos ramos dendríticos em toda sua extensão. Escala = 50 µm.

Figura 4. Neurônio de tipo bipenachado do subnúcleo póstero-dorsal do núcleo medial da amígdala (MePD) de rato adulto, impregnado pela técnica de Golgi e com típica arborização dendrítica esparsa. Fotomontagem por soma digital (Photoshop 7.0, EUA) de fotomicrografias de diferentes planos focais, permitindo a observação dos ramos dendríticos em toda sua extensão. Escala = 50 µm.

1. 2. 2. Hodologia e funções

O MeA possui uma ampla rede de conexões neurais entre seus subnúcleos, com outros núcleos da amígdala e também com outros núcleos extra-amigdalianos (CANTERAS *et al*., 1995; DONG *et* al., 2001; PETROVICH *et al*., 2001). Suas aferências principais estão listadas na Tabela 1 e são provenientes do córtex pré-frontal, do BNST e de vários núcleos do hipotálamo envolvidos com a modulação do sistema neuroendócrino e do comportamento reprodutivo (PITKÄNEN, 2000). Além disso, a maioria das aferências neuronais que chegam ao MeA são provenientes diretamente do bulbo olfatório acessório, que envolve estímulos dos feromônios, os quais atuam inicialmente no órgão vomeronasal (GUILLAMÓN e SEGOVIA, 1997) . Há ainda aferências mais modestas de regiões do telencéfalo basilar, as quais possuem conexões recíprocas com o MeA (OTTERSEN, 1980; GROVE, 1988); e vários núcleos do tálamo (OTTERSEN e BEN-ARI 1979; OHTAKE e YAMADA, 1989) e da região dorsal da ponte relacionada com informações somáticas e viscerais (HERBERT e SAPER, 1990); de grupos celulares da rafe mesencefálica (AZMITIA e SEGAL, 1978; OTTERSEN, 1981) e do *locus ceruleus* (JONES e MOORE, 1977). Por outro lado, os subnúcleos do MeA enviam suas projeções para o sistema olfatório (principal e acessório); para a formação hipocampal (ventralmente); para o estriado ventral, globo pálido ventral e BNST no telencéfalo basilar; para diversos núcleos hipotalâmicos; para o tálamo na porção medial; para a substância cinzenta periaquedutal e para os núcleos da rafe mesencefálica, por exemplo (CANTERAS *et al*., 1995; PETROVICH *et al*., 2001). Descrição mais ampla das eferências de cada subnúcleo do MeA está apresentada na Tabela 2.

Tabela 1. Aferências intra-amigdalianas e extra-amigdalianas para os subnúcleos do núcleo medial da amígdala, conforme McDonald (1998), Pitkänen (2000) e adaptado Hermel, (2006).

INTRA-AMIGDALIANAS	**CORTICAIS**
Área amígalo-hipocampal	Área pré-límbica
Córtex periamigdaliano	Córtex entorrinal
Núcleo basilar	Córtex infralímbico
Núcleo basilar acessório	Córtex perirrinal dorsal
Núcleo cortical anterior e posterior	Ínsula agranular posterior
Núcleo lateral	Ínsula agranular ventral
Subnúcleos componentes do núcleo medial	Subículo temporal distal e proximal
INTER-AMIGDALIANAS (contralaterais)	**TALÂMICAS**
Córtex periamigdaliano	Núcleo centromediano
Núcleo basilar acessório	Núcleo medial
Núcleo cortical posterior	Núcleo parafascicular
Núcleo do trato olfatório lateral	Núcleo paratenial
	Núcleo paraventricular
SISTEMA OLFATÓRIO	Núcleo posterior
Córtex piriforme	Núcleo de reunião
Bulbo olfatório	Núcleo subparafascicular
Bulbo olfatório acessório	Núcleo talâmico póstero-ventral
Bulbo olfatório anterior	
Núcleo endopiriforme	**TRONCO ENCEFÁLICAS**
	Núcleo retrobulbar [A8]
HIPOTALÂMICAS	Área tegmental ventral
Área hipotalâmica anterior	Células adrenérgicas da área postrema [C1]
Área pré-óptica medial e lateral	e células noradrenérgicas [A1] no bulbo
Área retroquiasmática	ventro-lateral
Núcleo arqueado	Núcleo central superior
Núcleo dorso-medial	Núcleos dorsais da rafe
Núcleo hipotalâmico posterior	Núcleo dorsal do lemnisco lateral
Área hipotalâmica lateral	Núcleo parabraquial
Núcleo pré-mamilar ventral	Núcleo peripeduncular
Núcleo supramamilar	Núcleo tegmental pedunculopontino
Núcleo supra-óptico	
Núcleo tuberal	**OUTRAS**
Núcleo ventromedial	Núcleo da banda diagonal de Broca
Núcleo túbero-mamilar	Núcleo próprio da estria terminal
	Substância inominada

Tabela 2. Eferências intra-amigdalianas e extra-amigdalianas dos subnúcleos ântero-dorsal (MeAD), póstero-dorsal (MePD) e póstero-ventral (MePV) do núcleo medial da amígdala de ratos, segundo Canteras e colaboradores (1995) e adaptado de Hermel (2006). As projeções foram classificadas em: +++ (densas); ++ (moderadas); + (fracas); - (ausentes).

PROJEÇÕES:		MeAD	MePD	MePV
INTRA-NUCLEARES:				
Núcleo medial	ântero-dorsal		++	+++
	ântero-ventral	+++	++	+++
	póstero-dorsal	++		++
	póstero-ventral	+++	++	
INTRA-AMIGDALIANAS:				
Área amigdalopiriforme		+++	+	+++
Área amigdaliana anterior		+++	+	+++
Núcleo basilar lateral	anterior	+	-	+
	posterior	+	+	+
Núcleo basilar medial	anterior	+++	+	+++
	posterior	+++	+	+++
Núcleo central	medial	++	+	+
	central	+++	++	++
	lateral	+	-	+
Núcleo cortical	anterior	+++	+	++
	póstero-lateral	++	+++	++
	póstero-medial	++	+++	++
Núcleo lateral		++	+	++
Núcleo posterior		++	++	+++
Núcleo próprio do trato olfatório acessório		+++	+	+++
CORTICAIS E OUTRAS ÁREAS TELENCEFÁLICAS				
Área de transição pós-piriforme		+++	++	+
Área insular agranular		+	-	+
Área piriforme		++	+	+
Área entorrinal	lateral	+++	++	++
	medial	+	+	+
	ventro-medial	+	+	-
Área infralímbica		+	+	+
Área pré-límbica		+	-	+
Bulbo olfatório acessório - camada mitral		+++	-	-
Região I do hipocampo próprio		+	+	+
Claustro		+	-	+
Estriado		++	-	+
Núcleo do trato olfatório lateral		+	-	+
Núcleo endopiriforme	dorsal	+	+	+
	ventral	++	+	++
Núcleo olfatório anterior	dorsal	+	-	-
	externo	+	-	-
	lateral	+	-	-
	medial	+	-	-
	póstero-ventral	++	-	-
Parasubículo		+	-	+
Subículo		+	+	+
Tubérculo olfatório		++	-	+
HIPOTALÂMICAS:				
Nível pré-optico:				
Área pré-óptica lateral		+	-	-
Área pré-óptica medial		+++	-	+++
Núcleo periventricular ântero-ventral		+	+++	-
Núcleo pré-óptico medial	medial	+++	+++	+
	central	+	+++	+
	lateral	+++	++	+
Nível hipotalâmico anterior:				
Núcleo anterior	anterior	+	+	+++
	central	++	++	+++
	dorsal	++	-	+
	posterior	++	++	+++

Tabela 2. (Continuação)

PROJEÇÕES:		MeAD	MePD	MePV
HIPOTALÂMICAS (continuação):				
Núcleo paraventricular	magnocelular anterior	+	-	-
	magnocelular póstero-medial	+	-	-
	parvocelular anterior	++	-	-
	parvocelular dorsal	+	-	-
	parvocelular dorso-medial	+	-	-
	periventricular	+	-	-
Núcleo periventricular	anterior	+	-	++
	intermediário	++	+	+++
	pré-óptico	+	-	++
Núcleo supraquiasmático		++	-	+++
Zona subparaventricular		+++	-	+++
Nível tuberal:				
Área hipotalâmica anterior		++	-	+
Núcleo arqueado		++	+	+
Núcleo dorso-medial	anterior	++	+	+
	posterior	+	+	+
	ventral	+	+	+
Núcleo ventro-medial	anterior	+++	-	+++
	central	+++	+	+++
	dorso-medial	+++	-	+++
	ventro-lateral	+++	+	+
Nível mamilar:				
Núcleo mamilar medial		+	+	+
Núcleo periventricular posterior		+++	+	+
Núcleo posterior		+++	+++	+++
Núcleo supramamilar		+	+	+
Núcleo pré-mamilar ventral		+++	+++	+++
TALÂMICAS:				
Núcleo dorso-medial	medial	++	-	-
	central	-	-	-
	lateral	-	-	-
Núcleo paratenial		+	-	-
Núcleo de reunião medial		+++	-	++
Núcleo subparafascicular	magnocelular	-	-	-
	parvocelular	+	-	-
TRONCO ENCEFÁLICO:				
Área tegmental ventral		+	+	+
Núcleos da rafe	dorsal	+++	-	+
	interfascicular	+	-	+
	linear rostral	+	-	+
	linear central	+	-	+
Substância cinzenta periaqueductal		+	+	+
OUTRAS:				
Núcleo acumbens		+	-	-
Núcleo da estria terminal				
Anterior	ântero-dorsal	+++	+++	+++
	ântero-lateral	++	+	++
	ântero-ventral	+++	+	+
	dorso-lateral	++	+	++
	dorso-medial	+++	+	+++
	fusiforme	+	-	–
	justacapsular	+	-	–
	magnocelular	++	-	++
	oval	+	+	+
	rombóide	++	-	+
	subcomissural	++	+	+
Posterior	interfascicular	+++	+	+++
	principal	+	+++	+
	transverso	+++	+	+++
Núcleo septal lateral	dorsal	+	-	-
	intermediário	+++	+	+
	ventral	+++	+	+
Núcleo septal medial		+	+	+
Núcleo septofimbrial		+	+	+
Substância inominada		+++	+++	+++
Zona incerta		++	-	+

Com base nisso, o MeA contribui para a interpretação de informações sensoriais interoceptivas e exteroceptivas (BRESSLER e BAUM, 1996; GUILLAMÓN e SEGOVIA, 1997; DIELEMBERG *et al*., 2001), para regulação de comportamentos sociais (BOLHUIS *et al*., 1984; NEWMAN, 1999; 2002), sexual de machos e fêmeas e maternal (RASIA-FILHO *et al*., 1991; COLLEN *et al*., 1997; NEWMAN, 1999; 2002; SHEEHAN *et al*., 2001), bem como para a modulação da memória condicionada e para aprendizado onde o componente emocional esteja envolvido (CANTERAS *et al*., 1995; ROOZENDAAL e McGAUGH, 1996; RASIA-FILHO *et al*., 2000).

Dentre esta gama de comportamentos, é relevante salientar o papel na gênese e regulação de comportamentos reprodutivos em ratos e hamsters (KLING; BROTHERS, 1992; KONDO, 1992; KONDO e ARAI, 1995; WOOD e NEWMAN, 1995). Devido ao fato de existirem aferências provenientes do bulbo olfatório, do órgão vomeronasal (TAKAHASHI e GLADSTONE, 1988; DOMINGUEZ *et al*., 2001) e de vários núcleos do hipotálamo (McDONALD *et al*., 1999), os subnúcleos do MeA parecem estar em posição estratégica para modular comportamentos que requeiram ativação "quimiossensorial" para sua ocorrência, como é o caso da atividade sexual (TAKAHASHI e GLADSTONE, 1988; DOMINGUEZ e HULL, 2001). Em ratos, o MeA (inicialmente estudado como um todo) facilita as respostas aos estímulos sexuais (KONDO, 1992) enquanto nas fêmeas parece ser um componente neural do sistema inibitório de regulação do comportamento de cópula (VOCHTELOO e KOOLHAAS, 1987; TAKAHASHI e GLADSTONE, 1988). Lesão nesta área de machos produziu diminuição na freqüência de ejaculações, aumento do número de intromissões penianas que precedem a primeira ejaculação e aumento do intervalo entre as intromissões quando comparados a animais não lesionados (KONDO, 1992; MEISEL e SACHS,

1994). Por outro lado, ratas submetidas à lesão no MeA, quando colocadas junto a ratos, demonstraram redução da ocorrência de atividade pré-copulatória (exploração olfatória direcionadas aos machos) e aumento na duração da cópula (LEHMAN e WINANS, 1982). Após o acasalamento, essas fêmeas buscavam menos freqüentemente seus companheiros de acasalamento quando comparadas às ratas submetidas à lesão fictícia e quando colocadas frente a outras ratas demonstraram uma diminuição de seu comportamento ofensivo (LEHMAN e WINANS, 1982). Esses dados sugerem que os neurônios do MeA ou os circuitos onde eles se encontram são possivelmente diferentes entre machos e fêmeas para gerar atividades que são sexualmente dimórficas. De fato, dimorfismo sexual pode ser encontrado também no sistema vomeronasal, no núcleo póstero-medial cortical da amígdala, no BNST, na área pré-óptica medial e no hipotálamo ventro-medial, todas as estruturas interconectadas com o MeA (CANTERAS *et al*., 1995; WOOD e NEWMAN, 1995; GUILLAMÓN e SEGOVIA, 1997).

Estudos mais detalhados têm sugerido que os diferentes subnúcleos do MeA possuem distintas funções na regulação do comportamento sexual de ratos (NEWMAN, 1999; DOMINGUEZ e HULL, 2001). Por exemplo, os subnúcleos MeAD e MePV, para a atividade reprodutiva feminina, enviam amplas projeções diretas para o núcleo ventro-medial do hipotálamo (VMH) podendo transmitir informações víscero-sensoriais advindas dos nervos pélvicos (COOPERSMITH *et al*. 1996; PFAUS e HEEB 1997; PETROVICH *et al*. 2001; SHELLEY e MEISEL 2005), além de quantidades moderadas a esparsas em direção ao núcleo arqueado (CANTERAS *et al*., 1995). Esses núcleos hipotalâmicos estão relacionados com o comportamento de exacerbação da lordose lombar para atividade copulatória ou com a ovulação em ratas (NELSON,

1999). O MeAD recebe projeções do córtex infralímbico, que pode ser a mais importante área capaz de integrar informações olfatórias com impulsos polimodais não-olfatórios (McDONALD, 1998) e pode estar envolvida em comportamentos reprodutivos e defensivos (PETROVICH *et al*. 2001). Ainda neste sentido, hamsters machos com lesão no MeAD apresentaram perda do comportamento de acasalamento, bem como perda da investigação anogenital frente às fêmeas (LEHMAN e WINANS, 1982; TAKAHASHI e GLADSTONE, 1988). O MePV também pode afetar a "zona motora neuroendócrina", a "rede geradora de padrão visceromotor" e parte do sistema de controle de comportamento defensivo no hipotálamo (DONG *et al*. 2001; PETROVICH *et al*. 2001).

Já o MePD, embora envie poucas eferências para o VMH, parece estar envolvido com funções neuroendócrinas ativadas por estimulação cérvico-vaginal em ratas (PFAUS *et al*., 1996). Ele envia fibras para a região periventricular ântero-ventral (AVPV) da região pré-óptica hipotalâmica para influenciar a secreção do hormônio liberador de gonadotrofinas (GnRH), bem como para modular atividades simpática e parassimpática, comportamentos agonistas, sexual e maternal (CANTERAS et al. 1995; PFAUS e HEEB, 1997; PETROVICH *et al*. 2001; DONG *et al*. 2001; SHEEHAN *et al*. 2001; SIMERLY, 1998; 2004; LEHMANN e ERSKINE, 2005; de CASTILHOS *et al*. 2006). Informações olfatórias para regulação neuroendócrina podem igualmente chegar ao núcleo pré-mamilar ventral hipotalâmico (BELTRAMINO e TALEISNIK, 1978; CAVALCANTE *et al*., 2006). Ratos e hamsters com danos no MePD apresentaram uma diminuição da investigação anogenital e um aumento da duração da atividade copulatória na presença de fêmea em estro (NEWMAN, 2002). Ademais, ratos com

lesão no MePD apresentaram uma redução do comportamento de cópula e perda de ereção peniana quando expostos ao odor da fêmea em estro (KONDO, 1992).

Esses subnúcleos do MeA também emitem axônios para a área entorrinal lateral ventral, em parte do subículo ventral, e uma parte ventral da região III do hipocampo próprio que poderiam representar uma rota alternativa para estímulos feromonais afetarem a atividade sináptica da região parahipocampal e hipocampal e, conseqüentemente, a formação de memória (PETROVICH *et al*. 2001, ver também FERGUSON *et al*., 2001).

1. 2. 3. Possível papel dos hormônios gonadais no dimorfismo sexual encontrado nos subnúcleos do MeA

As conexões sinápticas que os neurônios do bulbo olfatório principal e acessório estabelecem com os subnúcleos do MeA (BRESSLER e BAUM, 1996; GUILLAMÓN e SEGOVIA, 1997; MEREDITH e WESTBERRY, 2004) são sugestivos de um circuito gonadal que integra informações quimiosensórias para modular atividades em ambos os sexos (NEWMAN, 2002). De fato, os subnúcleos do MeA estão envolvidos de modos distintos com o processamento emocional deste tipo de informação e com a modulação de atividades sociais e sua decorrência em machos e em fêmeas (SHEEHAN *et al*. 2001; NEWMAN, 2002; LEHMANN *et al*. 2005). Tais subnúcleos podem ser afetados pela ação epigenética ou determinística dos hormônios gonadais tanto durante o início do desenvolvimento, como após, na puberdade ou na vida adulta (NISHIZUKA e ARAI, 1981, 1983a,b; MALSBURY e MCKAY, 1994; RASIA-FILHO *et al*. 2004; ZEHR *et al*. 2006; e ver NISHIZUKA e ARAI, 1982). Essas ações dos esteróides sexuais estão muito bem relatadas na literatura (FERNANDEZ-GUASTI e PICAZO, 1997; 1999; GORSKI, 2000; SILVA-GOMEZ, *et al*., 2003).

Desse modo, não é surpresa que no MeA de ratos encontre-se receptores para esteróides sexuais (SHERIDAN, 1979; SIMERLY *et al*., 1990; LI; *et al*., 1997; SHUGHRUE *et al*., 1997; ÖSTERLUND *et al*., 1998) e em quantidade comparável à observada em vários núcleos do hipotálamo (SIMERLY *et al*., 1990; SHUGHRUE *et al*., 1997). Especificamente, há mais receptores para andrógenos concentrados no MePD e no MePV de machos (SIMERLY *et al*., 1990; GRECÓ *et al*., 1998). E há mais

receptores dos tipos α e β (ER-α e ER-β) para estrógeno no MePD (LI *et al*., 1997; SHUGHRUE *et al*., 1997; ÖSTERLUND *et al*., 1998), embora sejam encontrados também no MeAD e no MePV, de fêmeas (LI *et al*., 1997; SHUGHRUE *et al*., 1997; ÖSTERLUND, 1998). O MeA também contém receptores para progesterona, especificamente no MePD e no MePV (McEWEN *et al*., 1983; KATO, 1985; SAR, 1988, ROMANO *et al*., 1989; HAGIHARA *et al*., 1992; De VRIES e SIMERLY, 2002). Do mesmo modo, a aromatase, enzima que transforma testosterona em estradiol, também é encontrada no MeA (SHINODA *et al*., 1994; WAGNER e MORRELL, 1996). Essa enzima tem alta atividade durante o período crítico neonatal de maturação do sistema nervoso (SN; SHINODA, *et al*., 1994). No MeA, entre 80 a 90% dos neurônios que possuem receptores para estrógenos também possuem receptores para andrógenos, enquanto o contrário acontece somente em cerca de 30% dos casos. (GRECÓ *et al*., 1998).

Alterações na estrutura e função do SN são parte das ações que os esteróides sexuais possuem para modulação da atividade neural de ratos adultos (GOMEZ e NEWMAN, 1991; LUQUIN *et al*., 1993; MALSBURY e McKAY, 1994; McEWEN *et al*., 1999). Em função do tempo, as ações desses hormônios podem ser classificadas em rápidas, intermediárias e lentas. As rápidas envolvem, tanto em latência como em duração, atividades relacionadas à abertura direta de canais iônicos ou a estimulação de exocitose. Os efeitos intermediários envolvem a fosforilação de enzimas, de proteínas componentes de canais iônicos e de receptores ou proteínas estruturais que podem perdurar de minutos a horas. Por último, os efeitos mais lentos e duradouros são aqueles que alteram a expressão gênica e promovem indução ou repressão de enzimas ou de

proteínas receptoras, respostas tróficas e como citado anteriormente, o remodelamento estrutural dos tecidos-alvos desses hormônios (McEWEN, 2006).

Como conseqüência, os hormônios gonadais podem alterar a atividade dos neurônios do MeA, como por exemplo, a implantação de estradiol em ratos machos castrados em idade adulta que é capaz de aumentar a atividade copulatória desses animais (RASIA-FILHO, *et al*., 1991). Da mesma forma, no MeA de hamsters machos, a implantação de estradiol, mas não de diidrotestosterona, aumentou o comportamento sexual masculino (WOOD e NEWMAN, 1995). Além disso, em termos estruturais, características sexualmente distintas ou mudanças morfológicas em função da manipulação de hormônios gonadais têm sido observadas em alguns parâmetros dos subnúcleos do MeA. Em ratos, o volume total do MeA é maior em machos do que em fêmeas (MIZUKAMI *et al*., 1983; HINES *et al*., 1992) e diminuiu em ratos adultos submetidos à castração (MALSBURY e McKAY, 1994). O neurópilo pode bem ser o local de atuação dos hormônios gonadais para afetar o volume estrutural, pois a castração também diminuiu o comprimento dendrítico total e o percentual de neurônios com ramos terciários em hamsters machos (GOMEZ e NEWMAN, 1991). A castração por 90, mas não por 8 dias diminuiu a densidade de espinhos no MePD de machos (FORTI, 2005). As variações hormonais que ocorrem durante o ciclo estral também parecem afetar os espinhos dendríticos nos diferentes subnúcleos do MeA. Ratas virgens em diestro tiveram maior densidade de espinhos dendríticos do que fêmeas em proestro, estro e metaestro. Além disso, machos apresentaram mais espinhos dendríticos que fêmeas no MeAD, MePD e MePV, exceto no MePD de fêmeas em diestro (RASIA-FILHO *et al*., 2004). Machos têm mais contatos sinápticos feitos diretamente nos ramos ou em espinhos dendríticos do que fêmeas (NISHIZUKA e ARAI, 1983a,b). O

conteúdo de alguns neuropeptídeos também parece ser distindo entre os dois sexos, como por exemplo maior quantidade de células contendo colecistoquinina, vasopressina e substância P foram vistas em machos do que em fêmeas (SIMERLY, 1990; MALSBURY e MCKAY, 1994; DeVRIES e SIMERLY, 2002), Outros parâmetros afetados pela ação dos hormônios gonadais no MeA são presença de receptores para opióides (WILSON *et al*., 2002) e a ligação de alfa-bungarotoxina em dendritos (ARIMATSU *et al*., 1981).

Com relação à neuroglia, no MePD e no MePV, mas não no MeAD, fêmeas apresentaram maior imunorreatividade à proteína ácida fibrilar glial (GFAP – ir), filamento intermediário do citoesqueleto de astrócitos maduros, do que machos (RASIA-FILHO *et al*., 2002). GFAP – ir foi maior em fêmeas em proestro do que fêmeas em outras fases do ciclo estral (MARTINEZ *et al*. 2006). Ainda, em fêmeas ovarectomizadas que receberam terapia hormonal substituta com estradiol ou estradiol mais progesterona tiveram aumento da GFAP – ir no MePD e no MePV, mas não no MeAD (MARTINEZ *et al*. 2006).

Está, com isso, aberta e embasada a possibilidade de se questionar de que forma os hormônios gonadais estariam modificando o neurópilo do MeA de ratos. Neste sentido, escolhemos estudar os aspectos da ramificação dendrítica, onde uma série de alterações em parâmetros morfométricos tem sido estudados em diferentes modelos experimentais (McDONALD, 1982; WOOLEY E McEWEN, 1994; AKULININ *et al*., 1997; VYAS *et* al., 2003; BLACK *et al*., 2004; FLYNN *et al*., 2004; MARTÍNEZ-TELLEZ *et al*., 2005; SHIMADA *et al*., 2006), o que têm ajudado a esclarecer questões sobre a plasticidade no SN.

1.3. Dendritos e o método de Golgi

Há mais de um século, Ramón y Cajal (1909), partindo da observação morfológica revelada pela técnica de Golgi, defendeu que a função dos dendritos ia além do transporte de nutrientes ao corpo celular e que eles tinham um papel importante na condução de impulsos neurais. De fato, hoje se sabe que arranjos dendríticos formam um substrato flexível extraordinário para propriedades passivas e ativas de geração de uma ampla variedade de tipos de processamento de informação (SHEPHERD, 1999b; SEGAL e ANDERSEN, 2000; LONDON e HÄUSSER, 2005). Os dendritos são capazes de prover uma polarização funcional, partindo de dendritos distais anterogradamente em direção ao cone axonal, tal como a propagação dos potenciais excitatórios pós-sinápticos vistos na maior parte dos exemplos de funcionamento neural. Também realizam operações funcionais localmente, em diferentes partes da árvore dendrítica, tais como geração, recepção, integração e saída de informação (SHEPHERD, 1999b; GOLDBERG e YUSTE, 2005; LONDON e HÄUSSER, 2005). Como unidades de processamento múltiplo em diversos níveis de organização e com ações coincidindo no tempo, geram compartimentalização de suas atividades. Eles podem ainda propagar retrogradamente potenciais de ação, o que desempenha um papel essencial na ativação de sinapses que partem dos dendritos e um papel crítico de contribuição para plasticidade de sinapses em resposta a impulsos subseqüentes (SHEPHERD, 1999b; GULLEDGE *et al*., 2005; HUMEAU, *et al*., 2005).

Dendritos apresentam formas e tamanhos variados, estão presentes em todas as espécies com SN e continuam a se desenvolver após o nascimento, de acordo com o

estabelecimento de circuitos neurais (COLLIN *et al*., 1997; HÄUSSER *et al*., 2000). É nos dendritos que a maioria dos contatos sinápticos são estabelecidos (FERIA-VELASCO *et al*., 2002; LONDON e HÄUSSER, 2005) e isso não se dá aleatoriamente. Há uma organização específica nas fibras aferentes sobre zonas dendríticas-alvo, de acordo com a região neural de origem e da natureza do neurotransmissor liberado na fenda sináptica (FERIA-VELASCO *et al*., 2002). A diversidade de respostas aumenta com a sua variação morfológica dendrítica e com a distribuição de seus canais iônicos (HÄUSSER *et al*., 2000). De fato, o tipo, a quantidade e o funcionamento dos contatos sinápticos podem alterar a excitabilidade do neurônio pós-sináptico (CRILL, 1997; SEGEV e RALL, 1998). Desse modo, um neurônio pode desempenhar papéis específicos de acordo com seu arranjo geométrico, suas propriedades intrínsecas de membrana, sua atividade elétrica e suas conexões sinápticas (HILLMAN, 1979; MIGLIORE e SHEPHERD, 2005). A complexidade da morfologia dendrítica reflete o número de conexões sinápticas feitas por um neurônio que, juntamente com suas propriedades elétricas passivas e ativas, são relevantes para a integração sináptica espacial-temporal (RAMÓN-MOLINER, 1962; FIALA e HARRIS, 1999; SHEPHERD, 1999a,b; WEARNE *et al*. 2005). Estudos eletrofisiológicos, dendrogramas baseados em dados empíricos, e simulações com modelamento compartimental têm mostrado o quanto as ramificações dendríticas, a extensão dos seus ramos e a topologia local contribuem para o processamento neuronal da informação (RALL 1959; SEGEV e RALL, 1998; SEGEV e LONDON, 1999; ASCOLI, 2002; WEARNE *et al*. 2005).

Essas são igualmente razões importantes para se detalhar a morfologia dendrítica geral nos subnúcleos do MeA. Neste sentido, o método de Golgi pode prover amostras representativas dos tipos neuronais dentro de uma área encefálica e continua sendo uma

técnica de pesquisa relevante para o estudo da morfologia celular (RAMÓN y CAJAL, 1909; RAMÓN-MOLLINER, 1962; McDONALD, 1992; WOOLLEY e McEWEN, 1994; FAIRÉN, 2005; LARRIVA-SAHD, 2006). Com a técnica de Golgi, que é um método de impregnação argêntica, pode-se ter a visualização da célula nervosa inteira, a qual adquire uma coloração parda escura que contrasta com o restante do tecido em cor amarelo-alaranjado. Quando gera resultados satisfatórios, somente uma pequena proporção de células nervosas (quiçá 1-10%) presentes no tecido é impregnada pela prata e de uma maneira ainda tida como aleatória. Ademais, nem todas as regiões do sistema nervoso de diferentes espécies impregnam-se igualmente e, conforme avança a idade do animal, torna-se muito mais difícil obter bons resultados (RAMÓN Y CAJAL, 1909; VALVERDE, 1962; PETERS; SCHEIBEL; SCHEIBEL, 1978; WOOLLEY; McEWEN, 1993; PANNESE, 1996; DALL'OGLIO *et al*. no prelo). A técnica de Golgi oferece a vantagem de permitir a visualização de células mais isoladas para estudo e, ao mesmo tempo a desvantagem de que, por seu caráter imprevisível, nunca se saber ao certo quando algum tipo de neurônio ficará visível completamente. Se isto ocorre, os neurônios podem ter seus componentes (corpo celular, dendritos, espinhos e axônio) passíveis de identificação e mensuração (RASIA-FILHO *et al.*, 1999; 2002; 2004; DALL'OGLIO *et al*. no prelo). No presente trabalho, estudar-se-á a morfologia dos dendritos de neurônios dos subnúcleos do MeA de ratos valendo-se da técnica de Golgi para tanto.

2. OBJETIVOS

2. 1. Geral

Estudar a morfologia dendrítica de neurônios, impregnados pela técnica de Golgi, do MeAD, do MePD e do MePV de ratos machos e fêmeas adultos.

2. 2. Específicos

- Descrever, quantificar e comparar aspectos da arborização dendrítica relevantes, tais como: comprimento, número de ramificações, padrão de arborização e distribuição espacial, com a atenção voltada a identificar possíveis dimorfismos sexuais dos neurônios nesses subnúcleos do MeA;

- Ainda, baseados em dados empíricos, elaborar dendrogramas que possam ser usados para demonstração da organização dendrítica desses neurônios nos três subnúcleos do MeA, em ratos machos e fêmeas adultos.

3. MÉTODOS, RESULTADOS E DISCUSSÃO

De acordo com o novo modelo proposto pelo PPG em Neurociências da UFRGS, apresentar-se-ão esses itens na forma de artigo. É o que se trata a seguir.

3. 1. Artigo

Neurons from the medial amygdala subnuclei of male and female rats: a Golgi study of dendritic morphology

Aline Dall'Oglio[1], Günther Gehlen[1,2], Matilde Achaval[1,3], Alberto A. Rasia-Filho[1,4 *]

[1]*Programa de Pós-Graduação em Neurociências, Universidade Federal do Rio Grande do Sul, Porto Alegre 90050-170 RS, Brazil*

[2]*Centro Universitário Feevale, Novo Hamburgo, 93352-000 RS, Brazil*

[3]*Departamento de Ciências Morfológicas, Universidade Federal do Rio Grande do Sul, Porto Alegre 90050-170 RS, Brazil*

[4]*Departamento de Ciências Fisiológicas, Fundação Faculdade Federal de Ciências Médicas, Porto Alegre, 90050 RS, Brazil*

Running head: Dendritic morphology of medial amygdala neurons

Key words: dendrites, amygdaloid complex, sexual dimorphism.

Corresponding author: Alberto A. Rasia-Filho, Departamento, de Ciências Fisiológicas, Fundação Faculdade Federal de Ciências Médicas, Porto Alegre, 90050 RS, Brazil. Fax: +55 51 33038752. E-mail: rasiafilho@pesquisador.cnpq.br

Support: This work was supported by UFRGS and funding agencies CNPq and CAPES. M.A. and A.A.R.F are CNPq investigators.

3. 1. 1. Abstract

The medial nucleus of the amygdala (MeA) contains receptors for sex steroids and modulates several social behaviors. The aims of this study were: 1) to detail the general morphology of Golgi-impregnated neurons from the anterodorsal (MeAD), posterodorsal (MePD) and posteroventral (MePV) MeA subnuclei of adult male and female rats; 2) to describe, quantify and compare the morphology of their dendritic arbors; and, 3) to generate empirical dendrograms. Dendritic arborization level, number of branches in each level, and total dendritic length were compared by a multivariate analysis of variance (MANOVA). The distribution of the dendrites was submitted to the Sholl´s concentric circle technique and to an ANOVA for repeated measures followed by the Roy's test. The preferred spatial distribution of dendritic branches (main coordinates and subdivisions) was studied using the overlaid square technique and subjected to the $\chi 2$ test. In the MeA subnuclei multipolar neurons were rather classified as bitufted or stellate ones, their spiny dendrites showed variable lengths and branched sparingly. Statistical significance between sexes was found in the number of secondary dendrites in the MeAD (higher in females, $p<0.05$). Interestingly, the predominant dendritic spatial orientation was sexually dimorphic in the three MeA subnuclei (for example, medial in males and more dorsal and ventromedial in females, $p<0.01$). These results provide basic morphological data for the MeA subnuclei neurons and suggest that their dendrites are also affected by gonadal hormones actions. These findings can be integrated with hodological data and within functional dynamic circuits in both sexes. A proposed scheme is presented looking at the integrated neuroanamotomy and function of these MeA subnuclei within a neural circuitry relevant for reproduction.

3. 1. 2. Abbreviations

AOB, accessory olfactory bulb; AVPV, anteroventral periventricular hypothalamic nucleus; BAOT, bed nucleus of the accessory olfactory tract; BST, bed nucleus of the stria terminalis; BSTpr, principal nucleus of the bed nucleus of the stria terminalis; CoA, cortical amygdala; ER-α, estrogen receptors α; ER-β, estrogen receptors β; GnRH, gonadotrophin releasing hormone; IntA, intercalated amygdaloid nuclei; MeA, medial nucleus of the amygdala; MeAD, anterodorsal part of the medial nucleus of the amygdala; MeAV, anterodorsal part of the medial nucleus of the amygdala; MePD, posterodorsal part of the medial nucleus of the amygdala; MePV, posterodorsal part of the medial nucleus of the amygdala; mEPSC, miniature excitatory post-synaptic current; MOT, main olfactory tract; MPOA, medial preoptic area; PCA, posterior cortical amygdala; PMv, ventral premammillary hypothalamic nucleus; PRL, prolactin; ST, stria terminalis; VMH, ventromedial hypothalamic nucleus; OT, optic tract.

3. 1. 3. Introduction

The amygdala is not a morphological or functional unit in the rat brain (Swanson and Petrovich, 1998). Rather, it is composed of several interconnected nuclei with intra- and extra-amygdaloid connections that modulate behavioral, homeostatic and adaptive responses (Gloor, 1997; Pitkänen et al. 1997; Aggleton, 2000; Rasia-Filho et al. 2000). The medial nucleus of the amygdala (MeA) is considered a part of the medial division of the "extended amygdala" (Alheid et al. 1995; de Olmos et al. 2004) and shares some structural features with the anterior, ventral and posterior parts of the medial bed nucleus of the stria terminalis (BST), the intra-amygdaloid BST, the medial division of the supracapsular BST, and the medial division of the sublenticular extended amygdala (Alheid et al. 1995).

The MeA has been divided according to cytoarchitectonic, chemoarchitectonic, hodological and functional criteria into four main subnuclei: anterodorsal (MeAD), anteroventral (MeAV), posterodorsal (MePD), and posteroventral ones (MePV; Alheid et al. 1995; Canteras et al. 1995). Although there are extensive connections among MeA subregions, the MeAD receives projections from the infralimbic cortex, which may be a higher-order olfactory area able to integrate olfactory information with polymodal non-olfactory inputs (McDonald, 1998) and may be involved with reproductive and defensive behaviors (Petrovich et al. 2001). Output connections enable the MePD to affect neuroendocrine secretion of gonadotrophin releasing hormone (GnRH) by the anteroventral periventricular hypothalamic nucleus (AVPV), sympathetic and parasympathetic activities, agonistic, sexual and maternal behaviors (Canteras et al.

1995; Pfaus and Heeb, 1997; Petrovich et al. 2001; Dong et al. 2001; Sheehan et al. 2001; Simerly, 2004; Lehmann and Erskine, 2005; de Castilhos et al. 2006). In turn, the MePV may relay hormonal and pelvic viscero-sensorial information to the ventromedial hypothalamic nucleus (VMH) to regulate reproductive behavior (Coopersmith et al. 1996; Pfaus and Heeb 1997; Petrovich et al. 2001; Shelley and Meisel 2005), as well as to affect a "neuroendocrine motor zone", a "visceromotor pattern generator network" and parts of the defensive behavior control system in the hypothalamus (Dong et al. 2001; Petrovich et al. 2001). These MeA subnuclei also project to the ventral lateral entorhinal area, a field in the ventral subiculum/field CA1, and a ventral part in field CA3, which would represent an alternative route for pheromonal stimuli to affect parahippocampal and hippocampal synaptic activity and memory formation (Petrovich et al. 2001, see also Ferguson et al., 2001). These hodological data are relevant because they contribute to understand the demand that local MeA subnuclei neurons have to deal with constantly. In addition, some of these interconnected areas are clearly sexually dimorphic in rats (Gorski, 2000).

In this sense, the synaptic connections that the main and the accessory olfactory bulb (AOB) establishes with these MeA subnuclei (Bressler and Baum, 1996; Guillamón and Segovia, 1997; Meredith and Westberry, 2004) are suggestive of a gonadal steroid-responsive circuit that integrates chemosensory information and hormonal signals to modulate activities in both sexes (Newman, 2002). In fact, the MeA subnuclei are involved in distinct ways with the emotional processing of information and modulation of social activities that are different in males and females (Sheehan et al. 2001; Newman, 2002; Lehmann et al. 2005). These subnuclei can be affected by the actions of gonadal hormones either during the initial development, later in puberty or in adulthood

(Nishizuka and Arai, 1981, 1983a,b; Malsbury and McKay, 1994; Rasia-Filho et al. 2004; Zehr et al. 2006; and see Nishizuka and Arai, 1982). Androgen receptors appear to be more concentrated in the posterior MeA, mainly in the MePD than in the MePV (Simerly et al. 1990; Gréco et al. 1996); both estrogen receptors α and β (ER-α, ER-β) are detected in the MeAD and in the MePV, but are more found in the MePD (Simerly et al. 1990; Shughrue et al. 1997; Österlund et al. 1998); and, progesterone receptors can also be seen in the MePD and the MePV (DeVries and Simerly, 2002). Dendritic pruning occurs in the MeA during puberty (Zehr et al., 2006) and adult male castration decreased mean highest dendritic branching and the percentage of neurons with tertiary branch segments in the posterior, but not the anterior, aspect of the MeA of adult male hamsters (Gomez and Newman, 1991). Estrogen enhances the growth and differentiation of neurons and their processes (Toran Allerand, 1995) and also modulates constitutive or regulatory intracellular elements, an effect that exists in the MeA (Zhou et al., 2005).

Therefore, it is not surprising that sex steroids can affect the MeA volume (Mizukami et al. 1983; Hines et al. 1992), neuronal somatic volume (Hermel et al. 2006a), dendritic spine density (Rasia-Filho et al. 2004), synaptic connectivity (Nishizuka and Arai, 1983a,b), expression of glial fibrillary acidic protein (Rasia-Filho et al. 2002; Martinez et al. 2006), content of neuropeptides, such as cholecystokinin, vasopressin, substance P (Malsbury and McKay, 1994; DeVries and Simerly, 2002;) or presence of delta opioid receptors (Wilson et al. 2002), and the binding of alpha-bungarotoxin in dendrites (Arimatsu et al. 1981). Because some of these findings involve the MeA subnuclei neuropil, dendrites can be one of the targets for these hormonal actions (Blaustein et al., 1992; Lorenzo et al. 1992; Rasia-Filho et al. 2004; Cooke and Wolley, 2005a).

A neuron can perform specific roles according to its geometric design, intrinsic membrane properties, electrical activity, and synaptic connections (Hillman, 1979; Migliore and Shepherd, 2005). The complexity of dendritic morphology reflects the number of connections made by a neuron that, together with passive and active electrical properties, are relevant for space-temporal synaptic integration and its firing pattern (Ramón-Moliner, 1962; Fiala and Harris, 1999; Shepherd, 1999a,b; London and Häusser, 2005; Wearne et al. 2005). Electrophysiological studies, empirical data-based dendrograms, and compartmental modeling simulations have shown how much dendritic branching, arborization extent and local topology contribute to the ongoing neuronal processing of information (Rall 1959; Segev and Rall, 1998; Segev and London, 1999; Ascoli, 2002; Wearne et al. 2005). More elaborated inputs usually require the development of more complex dendrites (Jacobs et al. 2001). In addition, an increase in arbor size in a small brain can greatly change the extent and scope of the neuronal receptive field (Kaas and Preuss, 2003). Revealing if dendrites in a region have a characteristic planar orientation can also indicate the propensity of a neuron to make contacts with axons from a large number of cells or many connections with just a few of them in a parallel distribution (Ramón-Moliner, 1962). Such data can contribute to understand the functioning of neuronal circuitries and their projections (Larriva-Sahd, 2006). These are important reasons to detail the general dendritic morphology in the MeA subnuclei. In this regard, the Golgi method can provide representative samples of neuronal types within a brain area and continues to be a relevant research tool for the detection of cellular morphology (Ramón y Cajal, 1909; Ramón-Molliner, 1962; McDonald, 1992; Woolley and McEwen, 1994; Fairén, 2005; Larriva-Sahd, 2006).

The aims of the present report were threefold: a) to detail the general dendritic morphology of neurons from the different MeA subnuclei, as revealed by the Golgi impregnation procedure; b) to describe, quantify and compare relevant dendritic branching features, such as length, branching number, pattern, and spatial distribution in an attempt to identify possible sexual dimorphisms within these subnuclei; and, c) based on these empirical data, to elaborate dendrograms that would be of use in demonstrating the dendritic organization of the neurons in each MeA subnuclei of adult male and female rats. Ultimately, these data are relevant for further understanding the activities in which the MeA components are involved and the behavioral results that have been obtained. At the end, a proposed scheme is presented looking at the integrated neuroanamotomy and function of these MeA subnuclei within a neural circuitry relevant for reproduction. In this study, we adapted or used a similar methodology for collecting data as employed by other authors to demonstrate the cellular organization of several areas of the rat brain (McDonald, 1982; Woolley and McEwen, 1994; Vyas et al., 2003; Flynn et al., 2004; Martínez-Tellez et al., 2005).

3. 1. 4. Materials and methods

Animals

Wistar rats of both sexes (3-6 months of age) were housed in groups with free access to food and water. Temperature was maintained around 22^{o}C in a 12 h light:dark cycle (lights off at 5 p.m.). Vaginal smears were taken from virgin rats and normally cycling females were sacrificed in the afternoon of the diestrus phase of the estrous cycle. This arbitrary criterion was chosen primarily to avoid unpredictable mixed variations in the results due to different levels of circulating ovarian steroids. All efforts were made to minimize the number of animals used in the study and their suffering. In addition, all rats were manipulated according to international laws for the ethical care and use of laboratory animals (European Communities Council Directive of 24 November 1986, 86/609/EEC). Local ethics committee also approved the present study.

Histological procedure and data acquisition

The single-section Golgi method was used in accordance with its original description (Gabbott and Somogyi, 1984). All rats were anesthetized with sodium thiopental (50 mg/kg, i.p.), injected with heparin (1000 I.U.) and transcardially perfused with 4% paraformaldehyde and 1.5% picric acid in 0.1 M phosphate buffer (pH = 7.4)

using a peristaltic pump. Brains were post-fixed in the same fixative solution for no more than 24 h and, afterwards, were sectioned using a vibratome (Leica, Germany). Due to technical limitations to reliably determine the MeA subnuclei strict borders (see comments in Rasia-Filho et al. 2002), horizontal and sagittal brain sections could not be studied here. Coronal sections (200 μm thick) were received in a 3% potassium dichromate (Merck, Germany) dissolved in distilled and ion-free water and left in the same solution for 24 h. Sections were washed in distilled water, mounted on glass coverslips and impregnated in 1.5% silver nitrate (Merck, Germany), dissolved in distilled and ion-free water, for at least an additional 48 h in the dark. Sections were then rinsed in distilled water and unwanted crystals were removed. Finally, sections were dehydrated, cleared with xylene, mounted on slides and covered with non-acidic synthetic balsam and coverslips.

The location of each MeA subnuclei was based on the descriptions of Alheid et al. (1995), Canteras et al. (1995), and de Olmos et al. (2004). The direct apposition of the MeA to the lateral side of the optic tract (OT) was taken as a reference for the location of MeAD (Figure 1). Additionally to the OT, the dorsal position of the stria terminalis (ST) served to localize the MePD and the MePV (Figure 2). Caution was taken with the formerly called MePD “molecular layer” where there are rather efferent fibers from the bed nucleus of the accessory olfactory tract (BAOT) close to the OT and at the ventral and medial parts of the MePV (Scalia and Winans, 1975; de Olmos et al. 2004). The MeAV was not studied due to technical difficulties related to its small size. In addition, for the localization of the MeA subdivisions, microscopic images of the brain slices were projected onto the schematic drawings of coronal sections of the rat brain obtained from the atlas of Paxinos and Watson (1998). The sections that contained

the MeAD corresponded to a distance 1.80 to 2.30 mm posterior to the bregma (plates 26-29 of the atlas, Figure 1 for an example) and, for the MePD and the MePV, 3.14 to 3.30 mm posterior to the bregma (plates 32-33 of the atlas, Figure 2 for an example). In Figures 1 and 2, coronal sections processed for the classical Nissl staining are presented to partially show the location of each MeA subnuclei. Because the number of Golgi-impregnated neurons was variable from section to section, both sides of the brain were used. At least for neuronal somatic volume, there is no effect of laterality in this parameter (see comments in Hermel et al. 2006a). Brain sections of the same approximate size were used for all groups, whereas those sections that appeared "shrunken" after histological processing were not used for further study. No correction formula was employed in the present study.

To be selected for further analysis, neurons had to possess the following characteristics: a) have neuronal cell bodies undoubtedly located within the boundaries of each one of the MeA subnuclei and relatively distant from all their outer limits; b) be located near the middle third of the section; c) have well-impregnated dendrites, with defined borders that could be clearly distinguished from the background, and a tapered appearance toward their endings throughout the section; and, d) be relatively isolated from neighboring impregnated cells to avoid "tangled" dendrites that could not be individualized from adjacent neurons. Due to empirical findings and inherent to the section thickness and dendritic orientations within it, additional inclusion criteria were adopted: e) neurons must not have more than 1/3 of their dendritic branches showing signs of incomplete impregnation or cut by the vibratome, named here as "cut-off" branches. In other words, at least 2/3 of the dendritic branches in each neuron must be tapering towards their ends, suggesting completeness of their lengths. And, f) to be

considered as a tapering branch, the apparent dendritic diameter at the end should be at least 50% narrower than its initial portion and should tend to be a final segment. For the neurons selected for further study, there was no statistical difference between sexes in the number of "cut-off" branches (around 30% for all the selected neurons) in the MeAD [$F(1.34) = 1.297$; $p = 0.263$], in the MePD [$F(1.35) = 0.002$; $p = 0.967$] or in the MePV [$F(1.30) = 0.024$; $p = 0.877$]. This percentage is in the same range previously reported by other authors (Andrade et al. 2000).

The first neurons that fulfilled these aforementioned inclusion criteria were traced (400 X) using a camera lucida drawing tube coupled to an optic microscope (Olympus BX-41, Japan). By this procedure, all the selected dendritic branches were drawn in the three dimensions (including "z") and converted to a bi-dimensional ("x" and "y") final image (Figures 3-5). Any doubts regarding the neuronal morphology were solved by the observation of the selected cell at a higher magnification (1000 X). Following this, all neurons were scanned and measured using an image analysis system (Image Pro Plus 4.1., Media Cybernetics, USA). Traced dendrites had their estimated length calculated by placing a calibrated thread along the drawing and then measuring the thread length (Flynn et al. 2004). Because no morphological characteristics can reliably differentiate sex-steroid responding neurons from non-responding ones (Nabekura et al. 1986), and differences in neither the somatic area nor in the dendritic spine densities between the two types of multipolar neurons (bitufted and stellate ones) were consistently found in the MeA subnuclei (Rasia-Filho et al. 1999; de Castilhos et al. 2006), both cells provided data for the present study (Figures 3-5). Again, these neurons were obtained at random as they could pass the restrictive inclusion criteria. In the three MeA subregions, more stellate than bitufted neurons composed the final data

and the proportions of each were identical for males and females in the three subnuclei studied here (two-tailed Fisher´s exact test, $p > 0.5$ in all cases). Morphological descriptions were based on data obtained under these methodological conditions and it is likely that numbers generated from these Golgi-impregnated sections reflect results that under-represent the actual values for entire cells (as also described in Gomez and Newman, 1991; Woolley and McEwen, 1994; Rasia-Filho et al. 1999; Flynn et al. 2004). Notwithstanding, all experimental procedures and all measurements were rigorously the same for both sexes and for each MeA subnuclei studied.

The total number of neurons selected and studied were: in the MeAD, 14 from males and 21 from females (from n = 8 and 6 rats, mean $\pm$ SD = 1.7 $\pm$ 1.1 and 3.5 $\pm$ 1.8 cells per rat, respectively); in the MePD, 17 from males and 19 from females (from n = 6 and 7 rats, mean $\pm$ SD = 2.8 $\pm$ 1.8 and 2.7 $\pm$ 1.3 cells per rat, respectively); and, in the MePV, 16 from males and 15 from females (from n = 7 rats in both groups, mean $\pm$ SD = 2.2 $\pm$ 1.1 and 2.1 $\pm$ 1.4 cells per rat, respectively). These numbers of sampled cells are in accordance with other authors (Shimada et al. 2006). For each neuron, morphometric analyses included the following parameters: a) number of dendritic branches in each arborization level (Figure 6), determined by the order of centrifugal appearance of primary dendrites from the soma; b) number of branching points, i.e., total number of dendritic ramifications; c) total dendritic length, which represents the summed length of all dendritic segments; d) radial distribution of dendrites in relation to the distance from the center of the cell body, by the use of Sholl´s concentric circles technique, and the intersection of the dendritic branching field with each circle with 20 μm of radius (Figure 7, right side); and, e) the predominant spatial distribution of branches (in medial, lateral, dorsal, and ventral coordinates and their combinations, i.e., mediodorsal,

dorsolateral, lateroventral, and ventromedial), identified using the overlaid square (20 μm each side) technique (Figure 8). For this purpose, every selected neuron had its cell body set at the center of the figure and the number of dendritic branches that radiated in each square was counted afterwards. The spatial location of all dendrites, irrespective of the branch order, was assessed by the direct observation of their position and classified in the aforementioned coordinates. The overall number of dendrites in these coordinates served to indicate the dendritic preferred orientation per sex in each MeA subnuclei studied.

Finally, from another experimental set obtained under the same methodological conditions, animals provided dendrites from the three MeA subnuclei to elaborate representative dendrograms (n = 5 males and 5 females, 10 neurons from each MeA subnuclei in both sexes; mean $\pm$ SD = 2.2 $\pm$ 1.1 cells per rat in each studied subnucleus). These dendrites also had to fulfill the inclusion criteria in order to have their segment length and diameters measured at initial and final points. Digitized images of all dendritic segments per neuron were obtained using optic microscopy (400 X), reconstructed using computer software (Adobe PhotoShop 7.0, USA) and dendritic lengths and diameters were measured with the aid of an image analysis system (Image Pro Plus 4.1. Media Cybernetics, USA). Dendrograms were constructed using a vectorial program (Macromedia Free Hand 8.0.1, USA) based on a previous report (Clairborne et al. 1990). In Figures 9-11, values represent the length of the selected branches and dendritic diameters are the width of the image projection perceived using optic microscopy. Therefore, these data also probably represent conservative estimates of the changes produced by the action of sex steroids (Gomez and Newman, 1991; Woolley and McEwen, 1994; Rasia-Filho et al. 1999; Flynn et al. 2004).

Statistical analysis

Dendritic branches within each level of arborization were grouped as total numbers of primary, secondary, tertiary, and quaternary branches. Due to differences in the amount of neurons studied, total values were divided by the number of cells from which data were gathered in each MeA subnuclei of males and females. Then, for each neuron, it was calculated the relative values of the number of dendrites within each arborization level, the number of branching points and the total dendritic length. After a square root transformation, these data were compared between sexes in the MeAD, in the MePD, and in the MePV using the multivariate analyses of variance (MANOVA) test.

The distribution of dendritic branches in relation to the distance from the neuronal soma were compared between males and females in each MeA subnuclei using an analysis of variance (ANOVA) test for repeated measures. Afterwards, based on the results of matrices equality, the Mauchly´s test for sphericity and the Levene's test for the heterogeneity of variances, it was employed the Roy's largest root multivariate test.

For males and females, in each MeA subnuclei, the number of dendritic branches in a preferred spatial distribution, evaluated by their location in different spatial coordinates, were compared using the $\chi 2$ test employing a contingency table of 2x8 (i.e., 2 sexes and 8 subdivisions of spatial coordinates) followed by the residual analysis test. In all cases, the statistically significant level was set at 5% (Zar, 1998).

3. 1. 5. Results

Qualitative description

Because of the great interest in research into the MePD, as shown by several recent publications (Gréco et al. 2003; Cooke and Woolley, 2005a,b; Lehmann et al. 2005; Hermel et al. 2006b; Zehr et al. 2006), we decided to begin the qualitative description of Golgi-impregnated neurons with this subnucleus and from coronally sectioned brain slices of male rats. Notwithstanding, some of these morphological features also apply to the MePV and to the MeAD, as well as for females. Based on this, and to avoid redundancies, the following description of the dendritic morphology refers to the three MeA subnuclei, whereas differences exhibited by one of these particular subdivisions will be pointed out when necessary. Cell body area and volume and dendritic spine density for the neurons of each MeA subnuclei are described elsewhere (Rasia-Filho et al. 1999, 2004; Hermel et al. 2006a).

Although empirically it appears that few neurons in the MeA are normally impregnated in each brain, within the MePD it was possible to observe several neurons lateral to the OT and ventral to the ST (Figure 2). Due to the characteristically random results of the Golgi-impregnation procedure, it was not be possible to establish different columns (medial, intermediate and lateral) of cells in the MePD, as clearly apparent with the Nissl staining (de Olmos et al. 2004). Additionally, the same occurred for the ultimate rostral, lateral and ventral borders of the MePD because the MeAD, the MePV

and the other amygdaloid nuclei (such as the central one) in their vicinity showed some similarities of their cellular types.

In those situations in which the Golgi method impregnated more cells at the same time in the MePD, it was observed neurons with a relatively packed distribution. Exception to this finding occurred close to the lateral border of the OT where few cell bodies were regularly found, which might well represent a rim where BAOT axons pass through (Scalia and Winans, 1975; de Olmos et al. 2004). Even in this cell sparse region, but remarkable along the adjacent tissue, there were small- to medium-sized cell bodies with ovoid, round, fusiform, piriform or irregular shapes. Irregular somatic shapes were determined by the number and location of primary dendrites (Figures 2 and 4). Although somatic size was rather homogeneous, neurons with larger diameters and with thicker dendrites could also be found in the MePD, lateral to the "molecular layer". In the MeAD, neurons appeared to be more loosely distributed within the parenchyma than in the MePD or in the MePV. In the MePV, cell bodies were not usually in close apposition to the ultimate ventral and medial borders of the MePV; rather, they were grouped and displayed a round to oval distribution within the internal aspect of this subnucleus.

On the basis of the number of primary dendrites, neurons throughout the MeA subnuclei were classified as multipolar and the nomenclature employed was based on an adaptation from Ramón y Cajal (1909). At principle, two types were recognized based on their general somatodendritic appearance. Bitufted neurons have two primary dendrites that give off successive branches, providing the dendritic arborization a "tufted" appearance, although branchpoints and branching appearance were typically not profuse. Stellate neurons have three or more primary dendrites that distort the soma into

irregular shapes, some of them resembling (but apparently only in this parameter) a pyramidal-like form. It was unusual to find more than 6 primary dendrites in these cells. Examples of them are shown in Figures 1-5. The appearance of bitufted and stellate neurons also permits the inclusion of these cells, with a slight adaptation, within the description of "radiate cells" provided by Ramón-Molliner (1962), as an attempt at classifying nerve cells on the basis of their dendritic patterns. Moreover, bitufted neurons can present cylindrical and biconical radiations (for example, see Figure 3). Stellate neurons tended to have spherical and partially spherical radiations (for example, see Figure 3), as depicted by Fiala and Harris (1999). Many of these neurons showed dendrites with coronal orientations, from the rostral to caudal aspects, some of them with an oblique orientation throughout the section thickness. Bitufted neurons appeared to provide more coronal dendrites while stellate cells generated more oblique ones.

Most neurons in the MePD were spiny, but the dendritic spine density varied among the different dendrites in each cell and among neurons within this subnucleus. On the other hand, spine distribution appeared to be relatively homogeneous along the extension of each dendrite. On the basis of their quantity, MePD neurons in the rat could be classified as moderately spiny cells. In some cases, even greater densities were observed in less frequently impregnated neurons. Differences in spine neck lengths and widths as well as in head diameters were evident along the whole extension of the dendrites. Apparently aspiny neurons were unusually found and, even in them, few dendritic protrusions could be detected that would pose doubts regarding whether they were or not stubby spines or dendritic irregularities.

These MePD spiny neurons have dendrites that generally branched sparingly and have few branchpoints. In the “molecular layer”, dendrites emanated from cell bodies that were observed in the adjacent lateral region. In the MePD, dendritic shafts were basically rectilinear and extended in different directions. Nevertheless, perpendicular, oblique or parallel orientation towards the OT was frequently observed (Figure 2, for an exemple). Thick and thin primary dendrites had their diameters generally directly related with the somatic volume, although initial branches with larger diameters were also observed in neurons with medium-sized cell bodies.

As a rule, the lengths of dendrites were heterogeneous, extending over a wide range from the soma (Figures 2, 4 and 10). From the sample showed in Figure 10, MePD primary branches had very different path lengths, ranging from around 2 μm to 240 μm and with initial apparent diameters varying from 2 μm to 6 μm. Secondary branches showed almost the same pattern and some presented close to 250 μm as a maximum value. Tertiary branches could be as long as 530 μm, as occurred for one of the highest values observed in the sample studied. Quaternary branches commonly represented the highest order of dendritic ramification. Quinquenary branches were less frequently observed and dendrites of sixth order were rarely seen. It was usual to find branches running along higher distances and, some of them, reaching adjacent areas such as the MePV and the amygdaloid intercalated nuclei.

For the MeAD, spiny neurons had basically rectilinear dendrites that extended in different directions (Figures 1 and 3), though some branches with a parallel orientation towards the ventral part of this subnucleus and others, perpendicular to the dorsal position of the OT were also observed. Dendrites crossing the MeAD and extending to

other adjacent amygdaloid areas could be seen as well. The appearance of some of these dendritic arborizations suggests that they could be collecting data in a more restricted local area of the neuropil as typical small interneurons (see the three bottom dendritic tree diagrams for male MeAD neurons in the Figure 9). Primary branches had path lengths ranging from as few as 9 μm to 300 μm and with initial diameters varying from approximately 2 μm to 4.5 μm. Secondary branches displayed almost the same pattern and 370 μm was one of the maximum values observed. Tertiary branches extended approximately 210 μm, as one of the greatest values observed in the sample studied. Quaternary, and less frequently quinquenary, branches also represented basically the highest order of dendritic ramification (Figure 9).

In the MePV, bitufted and stellate cells also had dendritic shafts with heterogeneous lengths extending from the soma over a wide range (Figures 2, 5 and 11). Primary branches showed path lengths ranging from as few as 4 μm to near 290 μm and with initial diameters varying from near 1.3 μm to 4.5 μm. Secondary branches displayed almost the same pattern and approximately 270 μm was one of the highest value observed. Tertiary branches extended until almost 360 μm as one of the highest values observed in the sample studied. Quaternary, and less frequently quinquenary, branches also represented basically the highest order of dendritic ramification, while dendrites of sixth order or more were seldom observed (Figure 11). Interestingly, although these spiny dendrites could be oriented in multiple directions, it became evident that many dendrites located more superficially displayed a radial orientation that accompanied the ventral and the medial borders of the MePV (Figure 2), close to the OT and where the “molecular layer” of the MePD merges with and continues dorsally. In the deeper part of the MePV, dendrites appeared to have an oblique orientation in relation to

the OT and, near the border with the MePD, they showed a more perpendicular orientation towards the OT. In addition, sometimes dendrites projected far away the lateral and the dorsal boundaries of the MePV.

In the three MeA subnuclei, whenever visible, only one process that resembled an axonal cone was seen per neuron and arising from the soma or, likewise, from a primary dendrite. As a rule, with the technique employed, the majority of these axons were not completely impregnated, which would indicate that they are rather myelinated ones, although some other thin axons could be traced for short distances (Figures 3-5 for some few examples).

Quantitative description

In the MePD, there were no statistically significant differences between males and females in the relative number of dendrites of primary [$F(1,35) = 0.939$; $p = 0.339$], secondary [$F(1,35) = 0.017$; $p = 0.896$], tertiary [$F(1,35) = 2.845$; $p = 0.101$] or quaternary levels [$F(1,35) = 0.466$; $p = 0.499$; Figure 6]. Neither was there a sexual dimorphism in the number of branching points [mean $\pm$ sem, 2.9 ± 0.5 and 3.6 ± 0.6 in males and females, respectively; $F(1,35) = 0.587$; $p = 0.499$] nor in the total dendritic length [mean $\pm$ sem, 700.0 ± 49.7 μm and 686.8 ± 64.4 μm in males and females, respectively; $F(1,35) = 0.207$; $p = 0.652$]. The comparison of the distribution of dendritic branches between males and females showed that there was a significant difference in the number of branches related with the increase in the distance away from the soma

(their number decreasing with the distance, $p < 0.001$), but there was no interaction between sex and distance ($p = 0.963$), i.e., the differences observed among the different concentric circles were the same for both sexes (Figure 7). Nevertheless, sexual dimorphism was found in the distribution of dendritic branches in the MePD. In males, more dendritic branches were oriented medially ($p < 0.001$) whereas in females they were seen to take predominantly dorsal ($p = 0.002$) and ventromedial ($p = 0.001$) orientations (Figure 8).

In the MeAD, there was a sexual dimorphism in the relative number of second order dendrites [higher in females; $F(1,34) = 8.347$; $p = 0.007$], but there were no statistically significant differences between males and females in the values obtained for dendrites of primary [$F(1,34) = 0.029$; $p = 0.867$], tertiary [$F(1,34) = 0.140$; $p = 0.711$] or quaternary orders [$F(1,34) = 0.080$; $p = 0.778$; Figure 6]. There was no sexual dimorphism in the number of branching points [4.0 ± 0.5 and 5.0 ± 0.5 in males and females, respectively; $F(1,34) = 1.702$; $p = 0.201$] nor in the total dendritic length [766.5 ± 70.2 μm and 716.3 ± 51.3 μm in males and females, respectively; $F(1,34) = 0.174$; $p = 0.679$]. The comparison between sexes of the distribution of dendritic branches showed that there was a significant difference in the number of branches related to the increase in the distance away from the soma (the total number decreasing along the distance, $p < 0.001$), but there was no interaction between sex and distance ($p = 0.699$; Figure 7). On the other hand, sexual dimorphism was found in the distribution of dendritic branches in the MeAD and males displayed more dendritic branches oriented medially ($p = 0.023$) and laterally ($p = 0.013$) than females, in which dendrites were predominantly orientated ventromedially ($p = 0.001$; Figure 8).

In the MePV, there were no statistically significant differences between males and females in the relative number of dendrites of primary [$F(1,30) = 0.981$; $p = 0.330$], secondary [$F(1,30) = 0.233$; $p = 0.633$], tertiary [$F(1,30) = 0.213$; $p = 0.648$] or quaternary orders [$F(1,30) = 1.110$; $p = 0.301$; Figure 6]. As occurred with the other two MeA subregions, there was no sexual dimorphism in the number of branching points [3.6 ± 0.7 and 3.2 ± 0.3 in males and females, respectively; $F(1,30) = 0.089$; $p = 0.768$] nor in the total dendritic length [816.0 ± 94.1 μm and 800.5 ± 87.2 μm in males and females, respectively; $F(1.30) = 0.004$; $p = 0.950$]. Again, the comparison of the distribution of dendritic branches between males and females showed that there was a significant difference in the number of branches affected by the increase in the distance away from the soma (the greater the distance, the fewer the number of branches, $p < 0.001$), but there was no interaction between sex and distance ($p = 0.229$; Figure 7). Highly statistically significant differences between sexes were found in the distribution of dendritic branches in the MePV. In males the dendrites were predominantly orientated medially ($p < 0.001$) and mediodorsally ($p = 0.004$) whereas in females they were in the ventral position ($p < 0.001$; Figure 8).

3. 1. 6. Discussion

The neuronal general morphology was relatively homogeneous in the three MeA subnuclei, although with some particularities in terms of dendritic orientation. Neurons resembled those found in the medial subdivision of the central nucleus of the amygdala and in connected parts of the BST**,** but are simpler than those found in other amygdaloid areas not belonging to the "extended amygdala", such as the basolateral nucleus, for example (see data in McDonald, 1982, 1992). Being treated as a cell group, present morphological description does not permit to undoubtedly classify Golgi-impregnated MeA multipolar neurons as similar to striatal medium-spiny ones in rats (see a parallel discussion in Millhouse, 1986). Our qualitative description agrees with and expand previous observations that studied more than one MeA subnucleus altogether in rats, mice, hamsters and cats (Valverde, 1967; Kamal and Tömböl, 1975; de Olmos et al., 1985; Gomez and Newman, 1991; McDonald 1992). And the quantitative data on the dendritic trees and their spatial arrangement add new morphological information and another sexual dimorphic finding for each MeA subnuclei.

As occurs with other techniques, the Golgi method presents advantages and disadvantages for descriptive and morphometric studies (Scheibel and Scheibel, 1978; Fairén, 2005; and see other elegant comments in Crossland et al., 1994). This procedure has been employed in recent studies of the rat brain (Vyas et al., 2003; Flynn et al., 2004; Martínez-Tellez et al., 2005; see also a methodological comment on Shimada et al., 2006). It does not differentiate those neurons that concentrate from those that do not concentrate sex steroids and it is possible that intra-animal and inter-animal variability

can occur due to this unavoidable mixing (Gomez and Newman, 1991; Rasia-Filho et al., 1999). For example, not all cells possess receptors for gonadal hormones (Gréco et al., 1996, 2001, 2003) and those neurons that are sensitive to these steroids are not specifically identifiable by their general morphology in the MeA (Nabekura et al., 1986). In this sense, whereas the percentages of cells that co-express different sex steroid receptors and those that are also Fos-ir following sexual stimuli were already reported (Gréco et al. 1996, 2001, 2003), it is not known the relative percentages of steroid-receptor versus non-steroid-receptor expressing cells and the total number of neurons in each MeA subnuclei evaluated by stereological approaches. On the other hand, the Golgi method has the advantage that it can impregnate several neurons at the same time and in a random manner, and either larger neurons or interneurons. If rigorous care is taken to define selecting criteria, these neurons can provide data for studying representative cells within their circuitries (Peters et al., 1991; McDonald, 1992; Larriva-Sahd, 2006). The possibility of comparison between morphometrical results from 3-D reconstructions and those from camera lucida drawings depends on the cell class and cell dendrite type (see Figure 2A-C in DeVoogd et al., 1981). There are no available 3-D studies of male and female MeA subnuclei neurons with intracellular injection of biocytin, HRP or Luciffer Yellow for comparisons with the present data. Neither are there clues that could help us to generate correction formulae to control for some modifications in the neuronal morphology related with the histological procedure employed in other brain areas (Desmond and Levy, 1982; Claiborne et al., 1990; Bannister and Larkman, 1995).

The present results indicated that dendrites in the MeA subnuclei radiate near the soma but in different orientations, for which there was a sexual dimorphism in their preferred localization. That is, although no sexual dimorphism was detected in other

dendritic morphometric parameters, there were differences in the relative number of second-order dendrites in the MeAD (more in females) and, remarkably, in the spatial distribution of branches in all the MeA subnuclei studied. Both dendritic shape and distribution can represent neural plasticity and highlight how neurons can process afferent coded information to transform it into outputs in integrated circuits, but there are no other empirical results about this issue in the MeA subnuclei of males and females. Therefore, some hypotheses have to be raised and can be useful to direct further investigation. And more studies are needed to reveal the dendritic ionic channel types, kinetics and distribution; possible compartimentalized processing of information in dendrites; neurochemical characterization of synaptic transmitters and their receptors; and, neuro-glial dynamic interactions for MeA subnuclei bitufted and stellate cells. Presently, it cannot be determined if a distinct dendritic branching pattern or orientation is determined genetically, due to another epigenetic mechanism or the result of gonadal hormones effects directly in the MeA during early or late development. Likewise, it has to be determined how much a different dendritic orientation within each MeA subnuclei of male and females is the result of synaptic inputs arriving to these regions from other gonadal hormone-sensitive and sexually dimorphic areas. It is possible that MeA subnuclei local receptors for sex steroids (Gréco et al., 1996, 2001), together with bi-directional connections with other sex steroid-sensitive areas (Coolen and Wood, 1998; Cooke and Simerly, 2005), are determining neuronal morphology and tailoring them to perform specific roles and process synaptic inputs in distinctive ways (Bannister and Larkman, 1995).

Nevertheless, dendrites are involved with the direction of information flow and their extent and complexity of branching patterns affect the neuronal activity (Shepherd,

1999b). Thus, it is interesting to note that in the MePD and in the MePV there are dendrites oriented towards the “molecular layer” through which axons from the BAOT run. In the MePD there were also dendrites extending towards axons in the ST and in the most lateral part of this subnucleus. Ultrastructural studies specifically in these regions revealed part of the nature of the synaptic contacts that occur in these regions. Following lesion of the posterior cortical amygdala (PCA), intact synapses on dendritic shafts of the medial “molecular layer” and those on dendritic spines of the ventral “molecular layer” significantly decreased in number in male rats, a finding not found in females (Nishizuka and Arai, 1983a). Indeed, a sexual dimorphism was reported for the number of dendritic shaft and spine synapses in the medial and ventral parts of the “molecular layer”, being higher in males than in females (Nishizuka and Arai, 1981,1983b). These data indicate the existence of a sexually dimorphic neuronal circuitry in the “molecular layer” that medially surrounds the MePD and the MePV. Differences in the dendritic orientation within these subnuclei suggest that males and females may have different hodological and functional organization and are gathering synaptic information with a different spatial distribution. Accordingly, dendritic spine densities in other subnuclear parts of these MeA components are different in both sexes (Rasia-Filho et al., 2004), which suggest that different synaptic inputs can be occurring in other discrete portions besides the “molecular layer”.

Again taking the MePD as a reference, Fos studies can also help us to comprehend the functional need for a differential dendritic spatial distribution in both sexes. In male rats, the medial column of cells are involved with the occurrence of intromission behavior during mating behavior with a receptive female, whereas the lateral column is mainly involved with the neural circuit responsible for ejaculation

(Coolen et al., 1997). As these are interrelated behaviors that occur as a sequence of events during the sexual behavior of male rats, it is plausible to suppose that information should be processed by neurons synaptically interconnected with dendrites oriented in a rather horizontal position, as was in fact found here. Likewise, pheromonal stimuli are sent to the MePD by the intercalated amygdaloid nuclei (IntA; Meredith and Westberry, 2004), and they probably enter the MePD coming horizontally via its lateral aspect. In females, dorsal to ventral distribution of ER-α and ER-β are involved with the perception of vaginocervical stimulation during mating behavior (Gréco et al., 2003) and, additionally, in the neural control of endocrine secretion and reproduction (Pfaus and Hebb, 1997; Lehmann and Erskine, 2005). Notably, females showed a rather vertical distribution of MePD dendrites. This dorsoventral gradient in ER distribution is related to the number of cell bodies (Gréco et al. 2003), but estradiol also enhanced dendritic complexity in the MeA at least *in* vitro (Lorenzo et al. 1992). Present morphological data regarding a sexual dimorphism in the orientations of MePD dendrites in males (more medially) and in females (more dorsally and ventromedially) deserve additional functional studies although, at this moment, it is technically difficult to develop electrophysiological studies to test the implications of these morphological findings.

Finally, taking the data in the literature and considering that synaptic activity is relevant to co-ordinate the expression of complex stereotyped sexually dimorphic behaviors and precisely timed physiological events (Coopersmith et al., 1996; Ferguson et al., 2001; Simerly et al., 2004), there is an implicit anatomical and functional correlation for the morphological data described here and the hodology of each MeA subnuclei. Descriptions of the MeA subnuclei connections can be found in Canteras et al. (1995), McDonald (1998), and Petrovich et al. (2001) and a more extensive revision

of them is out of the present scope. But a contribution to clarify the MeA subnuclei functional neuroanatomy can be made and is presented in Figure 12. A minimal neural circuit is proposed, linking the different available experimental findings and expanding a previous proposition presented by Swanson and Petrovich (1998).

Although not all the details are fully described, let us begin with male data and with the idea that relevant olfactory and pheromonal pathways are excitatory when they reach end targets, including the MeA (Quaglino et al., 1999; and see Polston et al., 2004). The MeAD receives interspecific olfactory input and, by establishing the social relevance of it, sends this information to the IntA (Meredith and Westberry, 2004). From them, inhibitory GABAergic efferences are sent to the MePD (Meredith and Westberry, 2004), which shows more Fos immunoreactivity when animals are exposed to sex-related pheromonal stimuli (Bressler and Baum, 1996). At the same time, direct axons from the AOB reach the MePD (Ichikawa, 1988) or olfactory and vomeronasal information also act upon other amygdaloid nuclei (for example, the cortical amygdala) that can provide additional inputs to the MePD (Nishizuka and Arai, 1983a; Canteras et al., 1992). It is not completely known how these incoming axons terminate within MePD neurons, i.e., if upon interneurons or directly upon projecting cells. However, if dendritic spines near the cell body are receiving inhibitory synaptic input, the greater the activity coming to the MePD neurons, the lower their action potential firing output. MePD projects directly towards different hypothalamic nuclei involved with reproductive behavior, for example (Canteras et al., 1995; Petrovich et al., 2001; Cavalcante et al., 2006) or, indirectly, via some components of the BST (Dong et al., 2001). Sexual dimorphism can be found in the projections that originate from the principal nucleus of the bed nucleus of the stria terminalis (BSTpr) and are much more dense in males than in

females (Polston et al., 2004). Moreover, some of these connections are reciprocal (Cooke and Simerly, 2005) and, being bidirectional, might serve to determine feedback or feedforward controls. The next step in this circuit would involve the final destination of MePD fibers within specific hypothalamic nuclei. As evidenced by *in situ* hybridization, some MePD outputs use GABA as a neurotransmitter (Swanson and Petrovich, 1998; and see Polston et al., 2004).

In the present model, when males are faced with receptive females, it is the inhibition of the MePD neurons (which have inhibitory projections) ending into the hypothalamus that would occur when using a direct pathway. Besides, if an indirect pathway with more cells is involved before MePD outputs reach the hypothalamus, BSTpr neurons would have more activity. As these BSTpr neurons are also GABAergic (Polston et al., 2004), they can be inhibiting local interneurons in their end-target hypothalamic nuclei. In both cases, the resultant could be a disinhibitory/facilitatory effect and some hypothalamic nuclei would become more prone to generate the display of reproductive behavior, for example. This proposition has a high level of correlation with electrical and chemical MePD stimulations on the male rat sexual behavior (Dominguez and Hull, 2001; Newman, 2002; de Castilhos et al., 2006, and see also Stark et al., 1998; Stark, 2005).

This functional scheme can also help to elucidate why MePD neurons acting on parallel and overlapping circuits can have several modulatory functions and be involved with many different social behaviors (as proposed by Newman, 2002). Indeed, MePD neurons are located within circuitries that receive different somatosensorial pathways and intra-MeA subnuclei connections. Considering that it is the organized spatial

distribution of timely synaptic inputs that are being processed by the dendrites and their spines that will determine the MePD firing pattern, the resultant will be a code that each integrated MePD end-targets will receive. This integrated code leads the animal to perceive what is happening to it, correlating these stimuli with the demand imposed by its ongoing situations, and selecting the best (or the possible) innate, learned or adaptive choice among the behavioral repertoire (for example, see Yoshida et al., 1994; Abe et al., 1998; Ferguson et al., 2001; Sheehan et al., 2001; Newman, 2002; Rasia-Filho et al., 2004). Alternatively, this idea could be also applied to specific subpopulations of neurons within the MePD responsible for distinct functions (Coolen et al., 1997; but see also de Castilhos et al., 2006).

In females, where cyclic variations in the levels of ovarian steroids impose that dynamic changes occur in integrated gonadal hormone-sensitive brain areas, neuroendocrine secretion and behavioral adjustments have to be related with sexual receptiveness and ovulation. MeA subnuclei can affect the emotional response of females caused by male presence, paced mating and olfactory cue preference (Kondo and Sakuma, 2005). However, the variation in the density of dendritic spines in the MePD across the estrous cycle points to another higher level of complexity. If in females there are also more axonal contacts in the dendritic shafts than in dendritic spines as occurs in males (Hermel et al., 2006b), the changeable number of dendritic spines and their synapses would rather represent the entry of phasic inputs while synapses upon dendritic shafts would serve for providing tonic afferent activity. Accordingly, the density of the proximal dendritic spines decreases from diestrus to proestrus in the female MePD (Rasia-Filho et al., 2004). If these spines are also receiving inhibitory afferences, a reduction in their number would lead to a greater activation of MePD

neurons. Output connections of the female MePD could be sent towards hypothalamic nuclei and would act upon inhibitory interneurons at these end-targets. Notably, the GABAergic activity of BSTpr neurons is almost exclusive of males (Polston et al., 2004), but it has been reported that estradiol can increase GABAergic activity in the MPOA probably through local cells (see comment in Polston et al., 2004). Thus, during proestrus, MePD neurons would be providing a higher inhibitory phasic activity onto local hypothalamic interneurons. By decreasing the activity of local GABAergic cells, the outcome would be a greater activation of the hypothalamic nuclei that would be prone to execute the neuroendocrine secretion and reproductive behavior necessary for this phase of the estrous cycle.

This proposition also fits well with the finding that MePD serves to transmit pheromonal stimuli relevant for gonadotrophin (GnRH) secretion acting via the ventral premammillary hypothalamic nucleus (PMv), the MPOA and the AVPV (Simerly, 2004) or for prolactin release by the arcuate nucleus as well (Gu and Simerly, 1997). Moreover, direct MePD electrical stimulation induces GnRH secretion (Kalra and McCann, 1975; Beltramino and Taleisnik, 1978) and appears to be involved with the occurrence of ovulation in rats (Bagga et al., 1984; Sanchez and Dominguez, 1995). Other brain areas would also contribute to this reproductive adjustment during the proestrus as the number of dendritic spines increases in the female VMH, an area relevant for lordosis behavior (Frankfurt et al., 1990). The interconnected MePV also changes its activity due to both tactile and nontactile social components of mating stimulation and, thereafter, sexual behavior and pseudopregnancy induction in hamsters (Shelley and Meisel 2005). Clearly other sources of social information could be affecting the dynamic of this circuit and should be included in this proposed scheme, as

well as with other integrated brain areas (Yoshida et al 1994; Sheehan et al., 2001; De Vries and Simerly, 2002; Newman, 2002; Simerly, 2004).

Two contrary arguments to this proposition could be set forth. First, that spines in male MePD neurons appear to receive more excitatory than inhibitory afferences (Hermel et al., 2006b). However, their exact location on the dendrites along the distance from the cell body was not directly studied. Second, pre-pubertal males have nearly 80% more excitatory synapses per left MePD neuron and greater miniature excitatory post-synaptic current (mEPSC) frequency than in females due to a difference in excitatory synapse number (Cooke and Woolley, 2005b). In contrast, the number of symmetric synapses, the frequency and the amplitude of miniature inhibitory post-synaptic currents were not sexually dimorphic in the MePD of these same rats (Cooke and Woolley, 2005b). Nevertheless, it remains to be established what occurs in relation to connectivity and electrophysiology in the MePD after puberty in males and in adult females with variable levels of endogenous estradiol and progesterone (a cue for this complexity is presented in Isgor et al., 2002).

The present descriptions of the dendritic morphology in the MeA subnuclei indicate a relevant difference in males and females. Altogether, these neurons are integrated within a hodological and functional context, which involves the perception of sensorial cues, neuroendocrine adjustments and the organization of the animal behavior display.

Acknowledgements

Authors are highly indebted to Dr. Sidia Callegari-Jacques (UFRGS, Brazil) for her expertise and outstanding contribution in the statistical analysis of the present experimental data. This work was supported by UFRGS and funding agencies CNPq and CAPES. M.A. and A.A.R.F are CNPq investigators

3.1.7. Literature Cited

Abe K, Noguchi K, Saito H (1998) Medial amygdala-induced spike potentiation in the rat dentate gyrus is dependent on N-methyl-D-aspartate receptors and subcortical afferents. *Neurosci Lett* **24**, 85-88.

Aggleton JP (2000) *The Amygdala*. New York: Oxford University Press.

Alheid GF, de Olmos JS, Beltramino CA (1995) Amygdala and extended amygdala. In *The Rat Nervous System* (ed. Paxinos G), pp 495-578. San Diego: Academic Press.

Andrade JP, Madeira MD, Paula-Barbosa MM (2000) Sexual dimorphism in the subiculum of the rat hippocampal formation. *Brain Res* **875**, 125-137.

Arimatsu Y, Seto A, Amano T (1981) Sexual dimorphism in α-bungarotoxin binding capacity in the mouse amygdala. *Brain Res* **213**, 432-437.

Ascoli GA (2002) Neuroanatomical algorithms for dendritic modelling. *Network; Comput Neural Syst* **13**, 247-260.

Bagga N, Chhina GS, Kumar VM, Singh B (1984) Cholinergic activation of medial preoptic área by amygdala for ovulation in rat. *Physiol Behav* **32**, 45-48.

Bannister NJ, Larkman AU (1995) Dendritic morphology of CA1 pyramidal neurones from the rat hippocampus: I. Branching patterns. *J Comp Neurol* **360**, 150-160.

Beltramino C, Taleisnik S (1978) Facilitatory and inhibitory effects of electrochemical stimulation of the amygdala on the release of luteinizing hormone. *Brain Res* **144**, 119-124.

Blaustein JD, Lehman MN, Turcotte JC, Greene G (1992) Estrogen receptors in dendrites and axon terminals in the guinea pig hypothalamus. *Endocrinology* **131**, 281-290.

Bressler SC, Baum MJ (1996) Sex comparison of neuronal Fos immunoreactivity in the rat vomeronasal projection circuit after chemosensory stimulation. *Neuroscience* **71**, 1063-1072.

Canteras NS, Simerly RB, Swanson LW (1992) Connections of the posterior nucleus of the amygdala. *J Comp Neurol* **324**, 143-179.

Canteras NS, Simerly RB, Swanson LW (1995) Organization of projections from the medial nucleus of the amygdala: a PHAL study in the rat. *J Comp Neurol* **360**, 213-245.

Cavalcante JC, Sita LV, Mascaro MB, Bittencourt JC, Elias CF (2006) Distribution of urocortin 3 neurons innervating the ventral premammilary nucleus in the rat brain. *Brain Res* **1089**, 116-125.

Claiborne BJ, Amaral DG, Cowan WM (1990) Quantitative, three-dimensional analysis of granule cell dendrites in the rat dentate gyrus. *J Comp Neurol* **302**, 206-219.

Cooke BM, Simerly RB (2005) Ontogeny of bi-directional connections between the medial nucleus of the amygdala and the principal bed nucleus of the stria terminalis in the rat. *J Comp Neurol* **489**, 42-58.

Cooke BM, Woolley CS (2005a) Gonadal hormone modulation of dendrites in the mammalian CNS. *J Neurobiol* **64**, 34-46.

Cooke BM, Woolley CS (2005b) Sexually dimorphic synaptic organization of the medial amygdala. *J Neurosci* **25**, 10759-10767.

Coolen LM, Peters HJPW, Veening JG (1997) Distribution of Fos immunoreactivity following mating versus anogenital investigationin the male rat brain. *Neuroscience* **77**, 1151-1161.

Coolen LM, Wood RI (1998) Bidirectional connections of the medial amygdaloid nucleus in the Syrian hamster brain: simultaneous anterograde and retrograde tract tracing. *J Comp Neurol* **399**, 189-209.

Coopersmith C, Gans SE, Rowe DW, Erskine MS (1996) Infusions of lidocaine into the amygdala, but not the preoptic area, block pseudopregnancy in the rat. *J Neuroendocrinol* **8**, 259-266.

Crossland WJ, Hu XJ, Rafols JA (1994) Morphological study of the rostral interstitial nucleus of the medial longitudinal fasciculus in the monkey, *Macaca mulatta*, by Nissl, Golgi, and computer reconstruction and rotation methods. *J Comp Neurol* **347**, 47-63.

de Castilhos J, Marcuzzo S, Forti CD, Frey RM, Stein D, Achaval M, Rasia-Filho AA (2006) Further studies on the rat posterodorsal medial amygdala: Dendritic spine density and effect of 8-OH-DPAT microinjection on male sexual behavior. *Brain Res Bull* **69**, 131-139.

de Olmos J, Alheid GF, Beltramino CA (1985) Amygdala. In *The Rat Nervous System* (ed. Paxinos G), pp 223-234. Sydney: Academic Press.

de Olmos J, Beltramino CA, Alheid GF (2004) Amygdala and extended amygdala of the rat: a cytoarchitectonical, fibroarchitectonical, and chemoarchitectonical survey. In *The Rat Nervous System* (ed. Paxinos G), pp 509-603. Amsterdam: Elsevier Academic Press.

De Vries GJ, Simerly RB (2002) Anatomy, development, and function of sexually dimorphic neural circuits in the mammalian brain. In *Hormones, Brain and Behavior* (eds. Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT), pp 137-191. San Diego: Academic Press.

Desmond NL, Levy WB (1982) A quantitative anatomical study of the granule cell dendritic fields of the rat dentate gyrus using a novel probabilistic method. *J Comp Neurol* **212**, 131-145.

DeVoogd TJ, Chang F-LF, Floeter MK, Jencius MJ, Greenough WT (1981) Distortions induced in neuronal quantification by camera lucida analysis: comparisons using a semi-automated data acquisition system. *J Neurosci Methods* **3**, 285-294.

Dominguez JM, Hull EM (2001) Stimulation of the medial amygdala enhances medial preoptic dopamine release: implication for male rat sexual behavior. *Brain Res* **917**, 225-229.

Dong H-W, Petrovich G, Swanson LW (2001) Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Res Rev* **38**, 192-246.

Fairén A (2005) Pioneering a golden age of cerebral microcircuits: the births of the combined Golgi-electron microscope methods. *Neuroscience* **136**: 607-614.

Ferguson JN, Aldag JM, Insel TR, Young LJ (2001) Oxytocin in the medial amygdala is essential for social recognition in the mouse. *J Neurosci* **21**: 8278-8285.

Fiala JC, Harris KM (1999) Dendrite structure. In *Dendrites* (eds. Stuart G, Spruston N, Häusser M), pp 1-34. New York: Oxford University Press.

Flynn C, Monfils MH, Kleim JA, Kolb B, McIntyre DC, Teskey GC (2004) Differential neuroplastic changes in neocortical movement representations and dendritic morphology in epilepsy-prone and epilepsy-resistant rat strains following high-frequency stimulation. *Eur J Neurosci* **19**, 2319-2328.

Frankfurt M, Gould E, Woolley CS, McEwen BS (1990) Gonadal steroids modify dendritic spine density in ventromedial hypothalamic neurons: A Golgi study in the adult rat. *Neuroendocrinol* **51**, 530-535.

Gabbott PL, Somogyi J (1984) The "single" section Golgi-impregnation procedure: methodological description. *J Neurosci Methods* **11**, 221-230.

Gloor P (1997) *The Temporal Lobe and Limbic System*. New York: Oxford University Press.

Gorski RA (2000) Sexual differentiation of the nervous system. In *Principles of Neural Science* (ed. Kandel ER, Schwartz JH, Jessell TM), pp. 1131-1148. New York: McGraw-Hill.

Gomez DM, Newman SW (1991) Medial nucleus of the amygdala in the adult Syrian hamster: a quantitative Golgi analysis of gonadal hormonal regulation of neuronal morphology. *Anat Rec* **231**,498-509.

Gréco B, Allegretto EA, Tetel MJ, Blaustein JD (2001) Coexpression of ERβ with ERα and progestin receptor proteins in the female rat forebrain: effects of estradiol treatment. *Endocrinology* **142**, 5172-5181.

Gréco B, Edwards DA, Michael RP, Clancy AN (1996) Androgen receptor immunoreactivity and mating-induced Fos expression in forebrain and midbrain structures in the male rat. *Neuroscience* **75**, 161-171.

Gréco B, Blasberg ME, Kosinski EC, Blaustein JD (2003) Response of ERα-IR and ERβ-IR cells in the forebrain of female rats to mating stimuli. *Horm Behav* **43**, 444-453.

Gu GB, Simerly RB (1997) Projections of the sexually dimorphic anteroventral periventricular nucleus in the female rat. *J Comp Neurol* **384**: 142-164.

Guillamón A, Segovia S (1997) Sex differences in the vomeronasal system. *Brain Res Bull* **44**: 377-382.

Hermel EE, Ilha J, Xavier LL, Rasia-Filho AA, Achaval M (2006a) Influence of sex and estrous cycle, but not laterality, on the neuronal somatic volume of the posterodorsal medial amygdala of rats. *Neurosci Lett* **405**, 153-158.

Hermel EES, Faccioni-Heuser MC, Marcuzzo S, Rasia-Filho AA, Achaval M (2006b) Ultrastructural features of neurons and synaptic contacts in the posterodorsal medial amygdala of adult male rats. *J Anat* **208**, 565-575.

Hillman DE (1979) Neuronal shape parameters and substructures as a basis of neuronal form. In *The Neurosciences* (eds. Schmitt FO, Worden FG), pp. 477-498. Cambridge: The MIT Press.

Hines M, Allen LS, Gorski RA (1992) Sex differences in subregions of the medial nucleus of the amygdala and the bed nucleus of the stria terminalis of the rat. *Brain Res* **579**, 321-326.

Ichikawa, M (1988) Plasticity of intra-amygdaloid connections following the denervation of fibers from accessory olfactory bulb to medial amygdaloid nucleus in adult rat: immunohistochemical study of anterogradely transported lectin (Phaseolus vulgaris leucoagglutinin). Brain Res **451**, 248-254.

Isgor C, Huang GC, Akil H, Watson SJ (2002) Correlation of estrogen β-receptor messenger RNA with endogenous levels of plasma estradiol and progesterone in the female rat hypothalamus, the bed nucleus of stria terminalis and the medial amygdale. *Mol Brain Res* **106**, 30-41.

Jacobs B, Schall M, Prather M, Kapler E, Driscoll L, Baca S, Jacobs J, Ford K, Wainwright M, Treml M (2001) Regional dendritic and spine variation in human cerebral cortex: a quantitative Golgi study. *Cereb Cortex* **11**, 558-571.

Kaas JH, Preuss TM (2003) Human brain evolution. In *Fundamental Neuroscience* (eds. Squire LR, Bloom FF, McConnell SK, Roberts JL, Spitzer NC, Zigmond MJ), pp. 1147-1166. San Diego: Academic Press.

Kalra PS, McCann SM (1975) The stimulatory effect on gonadotropin release of implants of estradiol or progesterone in certain sites in the central nervous system. *Neuroendocrinol* **19**: 289-302.

Kamal AM, Tömböl T (1975) Golgi studies on the amygdaloid nuclei of the cat. *J Hirnforschung* **16**, 175-201.

Kondo Y, Sakuma Y (2005) The medial amygdala controls the coital access of female rats: a possible involvement of emotional responsiveness. *Jap J Physiol* **55**, 345-353.

Larriva-Sahd J (2006) Histological and cytological study of the bed nuclei of the stria terminalis in adult rat. II. Oval nucleus: extrinsic inputs, cell types, neuropil, and neuronal modules. *J Comp Neurol* **497**, 772-807.

Lehmann ML, Erskine MS (2005) Glutamatergic stimulation of the medial amygdala induces steroid dependent *c-fos* expression within forebrain nuclei responsive to mating stimulation. *Neuroscience* **136**, 55-64.

Lehmann ML, McKellar H, Erskine MS (2005) Coding for the initiation of pseudopregnancy by temporally patterned activation of amygdalar NMDA receptors. *J Neurosci* **25**: 8696-8703.

London M, Häusser M (2005) Dendritic computation. *Annu Rev Neurosci* **28**: 503-532.

Lorenzo A, Diaz H, Carrer H, Caceres A (1992) Amygdala neurons in vitro: neurite growth and effects of estradiol. *J Neurosci Res* **33**: 418-435.

Malsbury CW, McKay K (1994) Neurotrophic effects of testosterone on the medial nucleus of the amygdala in adult male rats. *J Neuroendocrinol* **6**: 57-69.

Martinez FG, Hermel EE, Xavier LL, Viola GG, Riboldi J, Rasia-Filho AA, Achaval M (2006) Gonadal hormone regulation of glial fibrillary acidic protein immunoreactivity in the medial amygdala subnuclei across the estrous cycle and in castrated and treated female rats. *Brain Res* **1108**, 117-126.

Martínez-Tellez R, Gomez-Villalobos Mde J, Flores G (2005) Alteration in dendritic morphology of cortical neurons in rats with diabetes mellitus induced by streptozotocin. *Brain Res* **1048**, 108-115.

McDonald AJ (1982) Cytoarchitecture of the central amygdaloid nucleus of the rat. *J Comp Neurol* **208**, 401-418.

McDonald AJ (1992) Cell types and intrinsic connections of the amygdala. In *The Amygdala* (ed. Aggleton JP), pp. 67-96. New York: Wiley-Liss.

McDonald AJ (1998) Cortical pathways to the mammalian amygdala. *Prog Neurobiol* **55**, 257-332.

Meredith M, Westberry JM (2004) Distinctive responses in the medial amygdala to same-species and different-species pheromones. *J Neurosci* **24**, 5719-5725.

Migliore M, Shepherd G (2005) An integrated approach to classifying neuronal phenotypes. *Nature Rev Neurosci* **6**, 810-818.

Millhouse OE (1986) The intercalated cells of the amygdala. *J Comp Neurol* **247**, 246-271.

Mizukami S, Nishizuka M, Arai Y (1983) Sexual difference in nuclear volume and its ontogeny in the rat amygdala. *Exp Neurol* **79**, 569-575.

Nabekura J, Oomura Y, Minami T, Mizuno Y, Fukuda A (1986) Mechanism of the rapid effect of 17α-estradiol on medial amygdala neurons. *Science* **233,** 226-228.

Newman SW (2002) Pheromonal signals access the medial extended amygdala: one node in a proposed social behavior network. In *Hormones, Brain and Behavior* (eds. Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT), pp 17-31. San Diego: Academic Press.

Nishizuka M, Arai Y (1981) Sexual dimorphism in synaptic organization in the amygdala and its dependence on neonatal hormone environment. *Brain Res* **212**, 31-38.

Nishizuka M, Arai Y (1982) Synapse formation in response to estrogen in the medial amygdala developing in the eye. *Proc Natl Acad Sci USA* **79**, 7024-7026.

Nishizuka M, Arai Y (1983a) Male-female differences in the intra-amygdaloid input to the medial amygdala. *Exp Brain Res* **52**, 328-332.

Nishizuka M, Arai Y (1983b) Regional difference in sexually dimorphic synaptic organization of the medial amygdala. *Exp Brain Res* **49**, 462-465.

Österlund M, Kuiper GG, Gustafsson J-A, Hurd YL (1998) Differential distribution and regulation of estrogen receptor-alpha and -beta mRNA within the female rat brain. *Mol Brain Res* **54**: 175-180.

Paxinos G, Watson C (1998) *The Rat Brain in Stereotaxic Coordinates*. 4th Edition. San Diego:Academic Press.

Peters A, Palay S, Webster H (1991) Dendrites. In *The Fine Structure of the Nervous System* (eds. Peters A, Palay S, Webster H), pp 70-100. New York: Oxford University Press.

Petrovich GD, Canteras NS, Swanson LW (2001) Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. *Brain Res Rev* **38**: 247-289.

Pfaus JG, Hebb MM (1997) Implications of immediate-early gene induction in the brain following sexual stimulation of female and male rodents. *Brain Res Bull* **44**, 397-407.

Pitkänen A, Savander V, LeDoux JE (1997) Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. *Trends Neurosci* **20**, 517-523.

Polston EK, Gu G, Simerly RB (2004) Neurons in the principal nucleus of the bed nucleus of the stria terminalis provide a sexually dimorphic GABAergic input to the anteroventral periventricular nucleus of the hypothalamus. *Neuroscience* **123**, 793-803.

Quaglino E, Giustetto M, Panzanelli P, Cantino D, Fasolo A, Sassoe-Pognetto M (1999) Immunocytochemical localization of glutamate and gamma-aminobutyric acid in the accessory olfactory bulb of the rat. *J Comp Neurol* **408**, 61-72.

Rall W (1959) Branching dendritic trees and motoneuron membrane resistivity. *Exp Neurol* **1**, 491-527.

Ramón y Cajal S (1909) *Histologie du Système Nerveux de l´Homme & des Vertébrés*. Paris: Maloine.

Ramón-Moliner E (1962) An attempt at classifying nerve cells on the basis of their dendritic patterns. *J Comp Neurol* **119**, 211-227.

Rasia-Filho AA, Fabian C, Rigoti KM, Achaval M (2004) Influence of sex, estrous cycle and motherhood on dendritic spine density in the rat medial amygdala revealed by the Golgi method. *Neuroscience* **126**, 839-847.

Rasia-Filho AA, Londero RG, Achaval M (1999) Effects of gonadal hormones on the morphology of neurons from the medial amygdaloid nucleus of rats. *Brain Res Bull* **48**, 173-183.

Rasia-Filho AA, Londero RG, Achaval M. 2000. Functional activities of the amydala: an overview. *J Psychiatry Neurosci* **25,** 14-23.

Rasia-Filho AA, Xavier LL, dos Santos P, Gehlen G, Achaval M (2002) Glial fibrillary acidic protein immunodetection and immunoreactivity in the anterior and posterior medial amygdala of male and female rats. *Brain Res Bull* **58**, 67-75.

Sanchez MA, Dominguez R (1995) Differential effects of unilateral lesions in the medial amygdala on spontaneous and induced ovulation. *Brain Res Bull* **38**, 313-317.

Scalia F, Winans SS (1975) The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. *J Comp Neurol* **161,** 31-55.

Scheibel ME, Scheibel AB (1978) The methods of Golgi. In *Neuroanatomical Research Techniques* (ed. Robertson RT), pp. 89-114.New York: Academic Press.

Segev I, Rall W (1998) Excitable dendrites and spines: earlier thereotical insights elucidate recent direct observations. *Trends Neurosci* **21**, 453-460.

Segev I, London M (1999) A theoretical view of passive and active dendrites. In *Dendrites* (eds. Stuart G, Spruston N, Häusser M), pp 205-230.New York: Oxford University Press.

Sheehan T, Paul M, Amaral E, Numan MJ, Numan M (2001) Evidence that the medial amygdala projects to the anterior/ventromedial hypothalamic nuclei to inhibit maternal behavior in rats. *Neuroscience* **106,** 341-356.

Shelley DN, Meisel RL (2005) The effects of mating stimulation on c-Fos immunoreactivity in the female hamster medial amygdala are region and context dependent. *Horm Behav* **47**, 212– 222.

Shepherd GM (1999a) Electrotonic properties of axons and dendrites. In Fundamental Neuroscience (eds. Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR), pp 107-127. San Diego: Academic Press.

Shepherd GM (1999b) Information processing in dendrites. In Fundamental Neuroscience (eds. Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR), pp 363-388. San Diego: Academic Press.

Shimada A, Tsuzuki M, keino H, Satoh M, Chiba Y, Saitoh Y, Hosokawa M (2006) Apical vulnerability to dendritic retraction in prefrontal neurones of ageing SAMP10 mouse: a model of cerebral degeneration. *Neuropathol Appl Neurobiol* **32**, 1-14.

Shughrue PJ, Lane MV, Merchenthaler I (1997) Comparative distribution of estrogen receptor-α and -β mRNA in the rat central nervous system. *J Comp Neurol* **388**, 507-525.

Simerly RB (2004) Anatomical substrates of hypothalamic integration. In *The Rat Nervous System* (ed. Paxinos G), pp. 335-368. San Diego: Academic Press.

Simerly RB, Chang C, Muramatsu M, Swanson LW (1990) Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an *in situ* hybridization study. *J Comp Neurol* **294,** 76-95.

Stark CP (2005) Behavioral effects of stimulation of the medial amygdala in the male rat are modified by prior sexual experience. *J Gen Psychol* **132**, 207-224.

Stark CP, Alpern HP, Fuhrer J, Trowbridge MG, Wimbish H, Smock T (1998) The medial amygdaloid nucleus modifies social behavior in male rats. *Physiol Behav* **63**, 253-259.

Swanson LW, Petrovich GD (1998) What is the amygdala? *Trends Neurosci* **21**, 323-331.

Toran Allerand, CD (1995) Developmental interactions of estrogens with neurotrophins and their receptors. In *Neurobiological Effects of Sex Steroid Hormones* (eds. Micevych PE, Hammer Jr RP), pp 391-411. Cambridge: New York.

Valverde F (1967) Intrinsic organization of the amygdaloid complex. A Golgi study in the mouse. *Trab Inst Cajal Invest Biol* **54**, 291-314.

Vyas A, Bernal S, Chattarji S (2003) Effects of chronic stress on dendritic arborization in the central and extended amygdala. *Brain Res* **965**, 290-294.

Yoshida M, Suga S, Sakuma Y (1994) Estrogen reduces the excitability of the female rat medial amygdala afferents from the medial preoptic area but not those from the lateral septum. *Exp Brain Res* **101**, 1-7.

Wearne SL, Rodriguez A, Ehlenberger DB, Rocher AB, Henderson SC, Hof PR (2005) New techniques for imaging, digitization and analysis of three-dimensioanl neural morphology on multiple scales. *Neuroscience* **136**, 661-680.

Wilson MA, Mascagni F, McDonald AJ (2002) Sex differences in delta opioid receptor immunoreactivity in rat medial amygdala. *Neurosci Lett* **328**, 160-164.

Woolley CS, McEwen BS (1994) Estradiol regulates hippocampal dendritic spine density via an N-methyl-D-aspartate receptor-dependent mechanism. *J Neurosci* **14**, 7680-7687.

Zar, JH (1998) *Biostatistical Analysis*. Upper Saddle River, Prentice Hall.

Zehr JL, Todd BJ, Schulz KM, McCarthy MM, Sisk CL (2006) Dendritic pruning of the medial amygdala during pubertal development of the male Syrian hamster. *J Neurobiol* **66**, 578-590.

Zhou J, Zhang H, Cohen RS, Pandey SC (2005) Effects of estrogen treatment on expression of BDNF and CREB expression and phosphorylation in rat amygdaloid and hippocampal structures. *Neuroendocrinol* **81**, 294-310.

3. 1. 8. Legends

Figure 1. **A (left)**: Cresyl violet staining of a coronal brain section showing the location of the rat anterodorsal medial amygdala (MeAD) from where part of the present data was obtained (in this case, 2.30 mm posterior to the bregma). **A (right)**: Schematic diagram of a matched coronal brain section showing the MeAD, adapted from Paxinos and Watson (1998). **B**: Reconstructed digitized microscopic image showing the cellular organization of the rat MeAD revealed by the Golgi method. Note the presence of neurons with bitufted (arrows heads) and stellate (arrows) morphologies. Only background contrast was adjusted. MeAV, anteroventral medial amygdala; OT, optic tract. Scale bar = 800 μm in A and 50 μm in B.

Figure 2. **A (left)**: Cresyl violet staining of a coronal brain section showing the location of the rat posterodorsal medial amygdala (MePD) and the posteroventral medial amygdala (MePV) from where part of the present data was obtained (in this case, 3.30 mm posterior to the bregma). **A (right)**: Schematic diagram of a matched coronal brain section showing the MePD and MePV, adapted from Paxinos and Watson (1998). B: Reconstructed digitized microscopic image showing the cellular organization of the MePD and the MePV revealed by the Golgi method. Note the presence of neurons with bitufted (arrows) and stellate (arrows heads) morphologies in both subnuclei. Only background contrast was adjusted. OT, optic tract; ST, stria terminalis. Scale bar = 800 μm in A and 50 μm in B.

Figure 3. Camera lucida drawings of representative Golgi-impregnated neurons from coronal sections through the anterodorsal medial amygdala (MeAD) of adult male (top) and female (bottom) rats. For a matter of correctness, spine distribution was not drawn for these spiny neurons. Note the morphology of the cell body shapes of these multipolar neurons classified as bitufted or stellate ones and their typical dendritic arborization. When visible, "ax" refers to an axon . Scale bar = 50 μm.

Figure 4. Camera lucida drawings of representative Golgi-impregnated neurons from coronal sections through the posterodorsal medial amygdala (MePD) of adult male (top) and female (bottom) rats. For a matter of correctness, spine distribution was not drawn for these spiny neurons. Note the morphology of the cell body shapes of these multipolar neurons classified as bitufted or stellate ones and their typical dendritic arborization. When visible, "ax" refers to an axon . Scale bar = 50 μm.

Figure 5. Camera lucida drawings of representative Golgi-impregnated neurons from coronal sections through the posteroventral medial amygdala (MePV) of adult male (top) and female (bottom) rats. For a matter of correctness, spine distribution was not drawn for these spiny neurons. Note the morphology of the cell body shapes of these multipolar neurons classified as bitufted or stellate ones and their typical dendritic arborization. When visible, "ax" refers to an axon. Scale bar = 50 μm.

Figure 6. Mean (± sem) of the number of dendritic branches at each level of arborization (from primary to quaternary), classified according to their order of centrifugal appearance from the cell body, of Golgi-impregnated neurons obtained from the anterodorsal (MeAD, top), posterodorsal (MePD, middle) and posteroventral

(MePV, bottom) medial amygdala of male and female rats. No statistical difference was found between sexes, with the exception of the number of secondary branches in the MeAD (higher in females, $p < 0.05$).

Figure 7. Number and distribution of dendritic branches along increasing distances from their origin in the neuronal cell body, as revealed by the use of the concentric circles technique (schematically presented in the right) applied to each Golgi-impregnated neuron obtained from the anterodorsal (MeAD, top), posterodorsal (MePD, middle) and posteroventral (MePV, bottom) medial amygdala of male (black line) and female (gray line) rats. No statistical difference was found between sexes in each medial amygdala subnuclei.

Figure 8. Spatial orientation of dendritic branches from Golgi-impregnated neurons obtained from the anterodorsal (MeAD, top), posterodorsal (MePD, middle) and posteroventral (MePV, bottom) medial amygdala of male and female rats. Neuronal cell body was set at the center of the figure and the number of dendritic branches in each square was counted afterwards. The different colors represent the density of branches in each one of the spatial coordinates studied (legend at the bottom right), ranging from 1-5 of them (yellow), passing through intermediate values (from orange to brown), and reaching the highest ones (31 or more of them, black). Sexual dimorphisms were observed in the preferred dendritic spatial orientation in the three subnuclei. On the righ of the figure, the statistically significant differences in this parameter are schematically shown as large lines and represent where males (dark gray) and females (light gray)

differed. D, dorsal; DL, dorsolateral; L, lateral; LV, lateroventral; M, medial; MD, mediodorsal; V, ventral; VM, ventromedial.

Figure 9. Computer-generated dendritic tree diagrams from ten different Golgi-impregnated neurons obtained from the anterodorsal medial amygdala (MeAD) of male (top) and female (bottom) rats. For each dendritic segment, the number in the initial and above each box represents the apparent dendritic diameter measured. The numbers in the middle and below each line are proportional measures and represent the apparent dendritic length measured in μm. Scale bar = 20 μm.

Figure 10. Computer-generated dendritic tree diagrams from ten different Golgi-impregnated neurons obtained from the posterodorsal medial amygdala (MePD) of male (top) and female (bottom) rats. For each dendritic segment, the number in the initial and above each box represents the apparent dendritic diameter measured. The numbers in the middle and below each line are proportional measures and represent the apparent dendritic length measured in μm. Scale bar = 20 μm.

Figure 11. Computer-generated dendritic tree diagrams from ten different Golgi-impregnated neurons obtained from the posteroventral medial amygdala (MePV) of male (top) and female (bottom) rats. For each dendritic segment, the number in the initial and above each box represents the apparent dendritic diameter measured. The numbers in the middle and below each line are proportional measures and represent the apparent dendritic length measured in μm. Scale bar = 20 μm.

Figure 12. Proposed model for the anterodorsal (MeAD) and posterodorsal (MePD) medial amygdala subnuclei participation in a neural system for reproductive behavior. Although other functions and regions could be additionally included, vomeronasal and olfactory stimuli reaches the MeAD via the accessory olfactory tract (AOT) or the main olfactory tract (MOT). From the MeAD, relevant social information is relayed to the intercalated amygdaloid nuclei (IntA) to the MePD, or come to it from the cortical amygdala (CoA). From the MePD, direct outputs or, alternatively, involving indirect pathways such as that coming from the principal nucleus of the stria terminalis (BSTpr), reach several hypothalamic nuclei to modulate neuroendocrine secretion and sexual behavior display. Only for the indirect pathway, dashed lines represent additional projecting or local interneurons. Inhibitory activity is shown as "-". Some anatomical, morphological and functional evidence includes results obtained with MePD electrical stimulation, change in dendritic spine density across the estrous cycle and bilateral lesions. ARC, arcuate hypothalamic nucleus; AVPV, anteroventral periventricular hypothalamic nucleus; GnRH, gonadotropin releasing hormone; MPOA, medial preoptic area; PMV, ventral premammilary hypothalamic nucleus; PRL, prolactin; VMH, ventromedial hypothalamic nucleus.

Figure 1

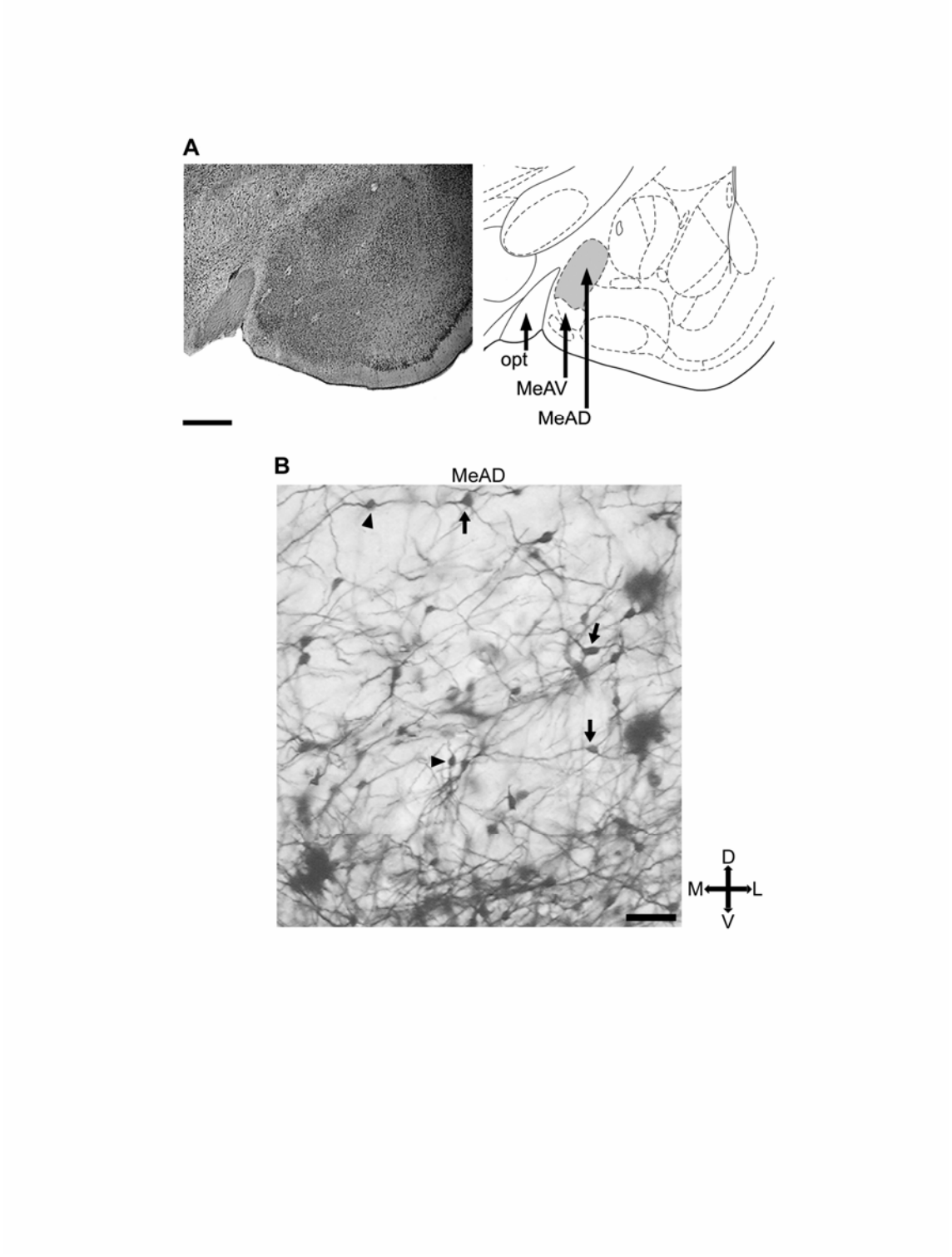


Figure 2

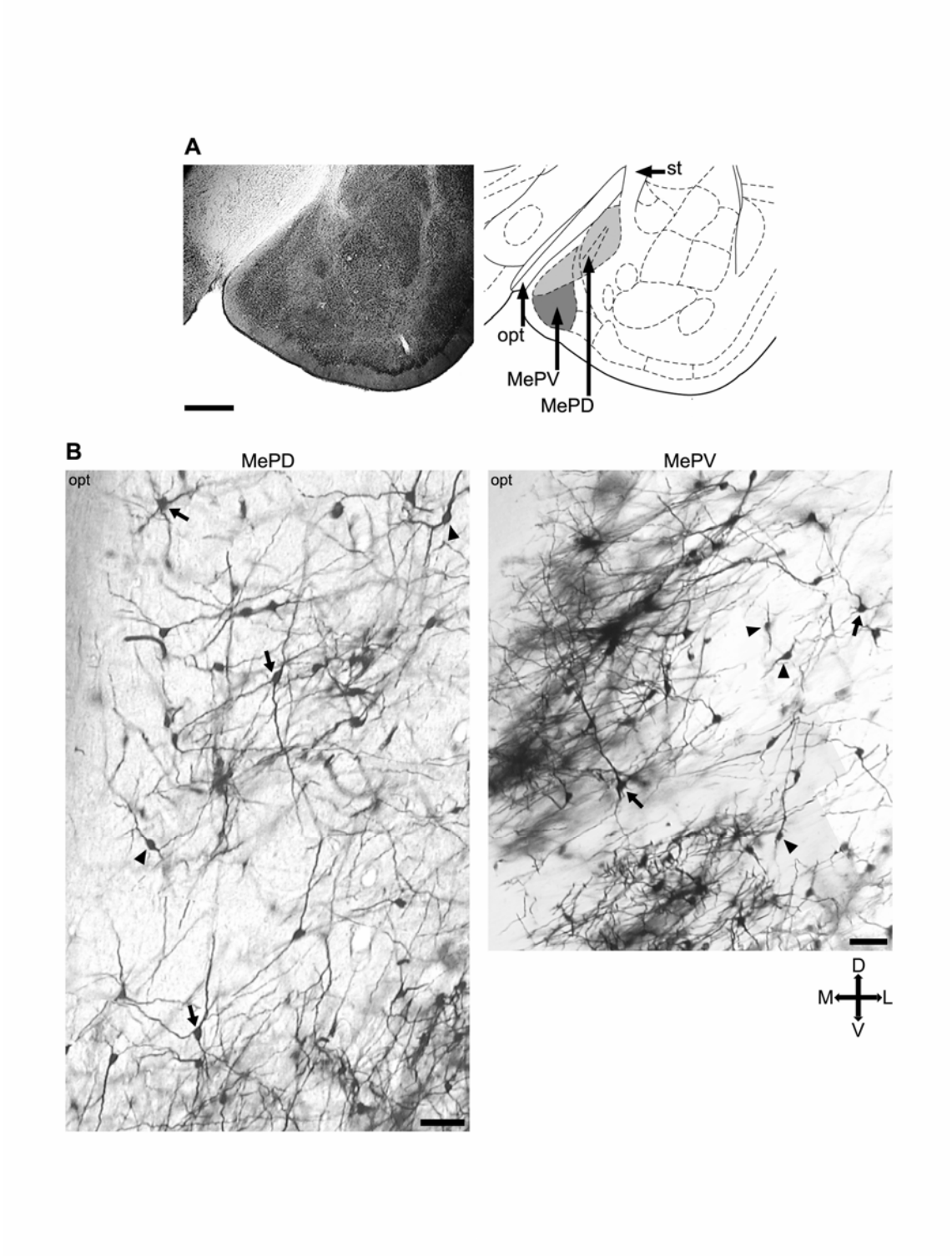


Figure 3

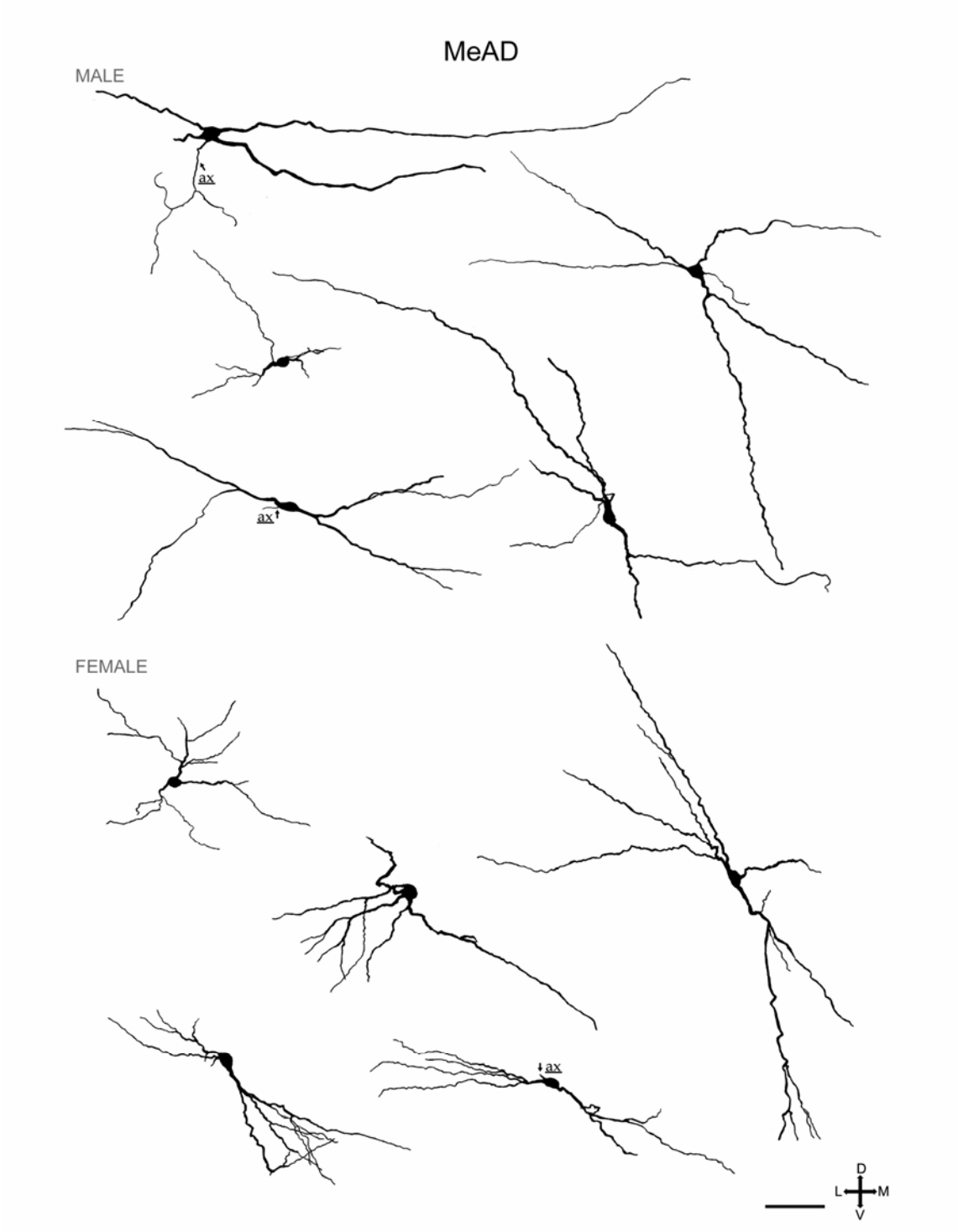


Figure 4

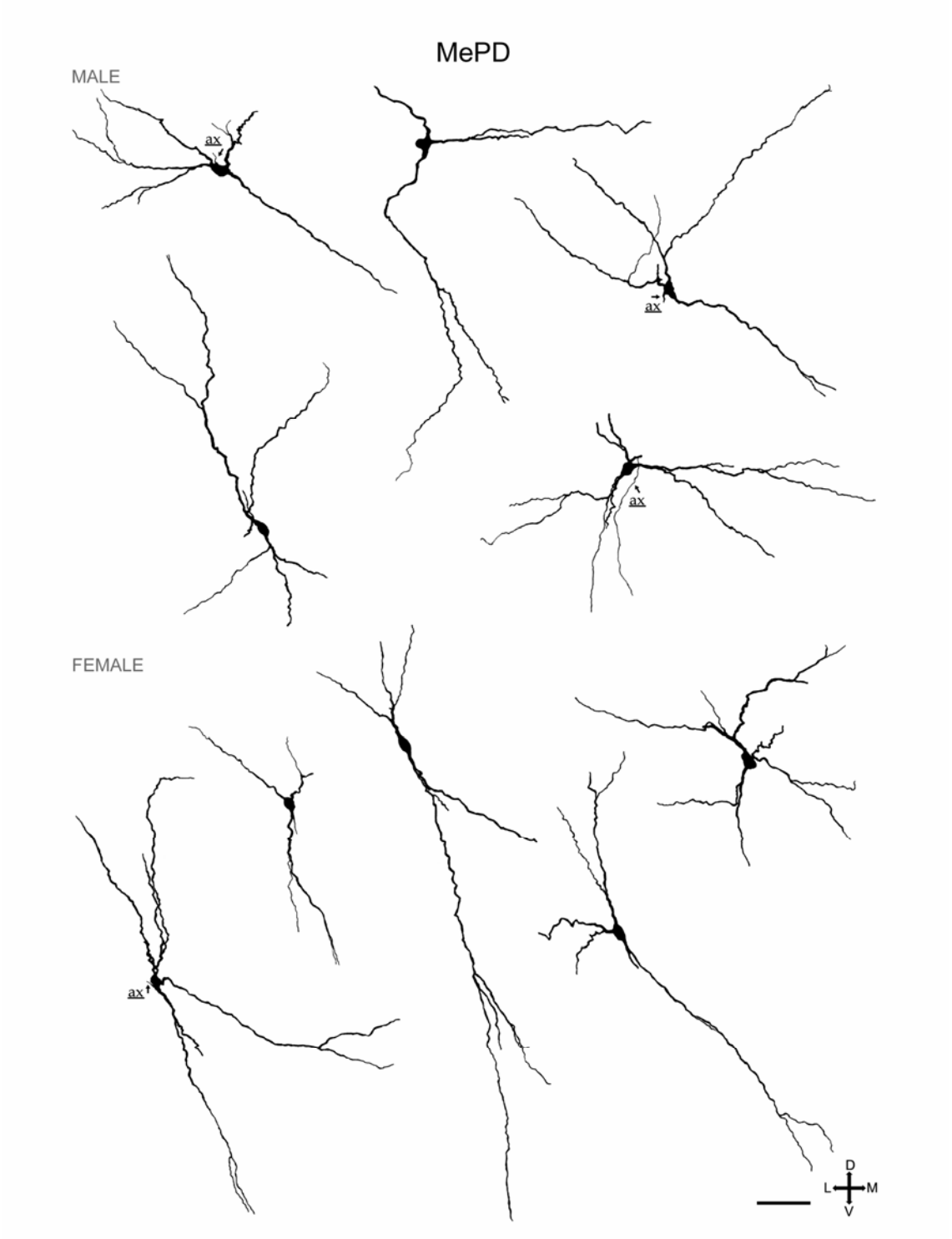


Figure 5

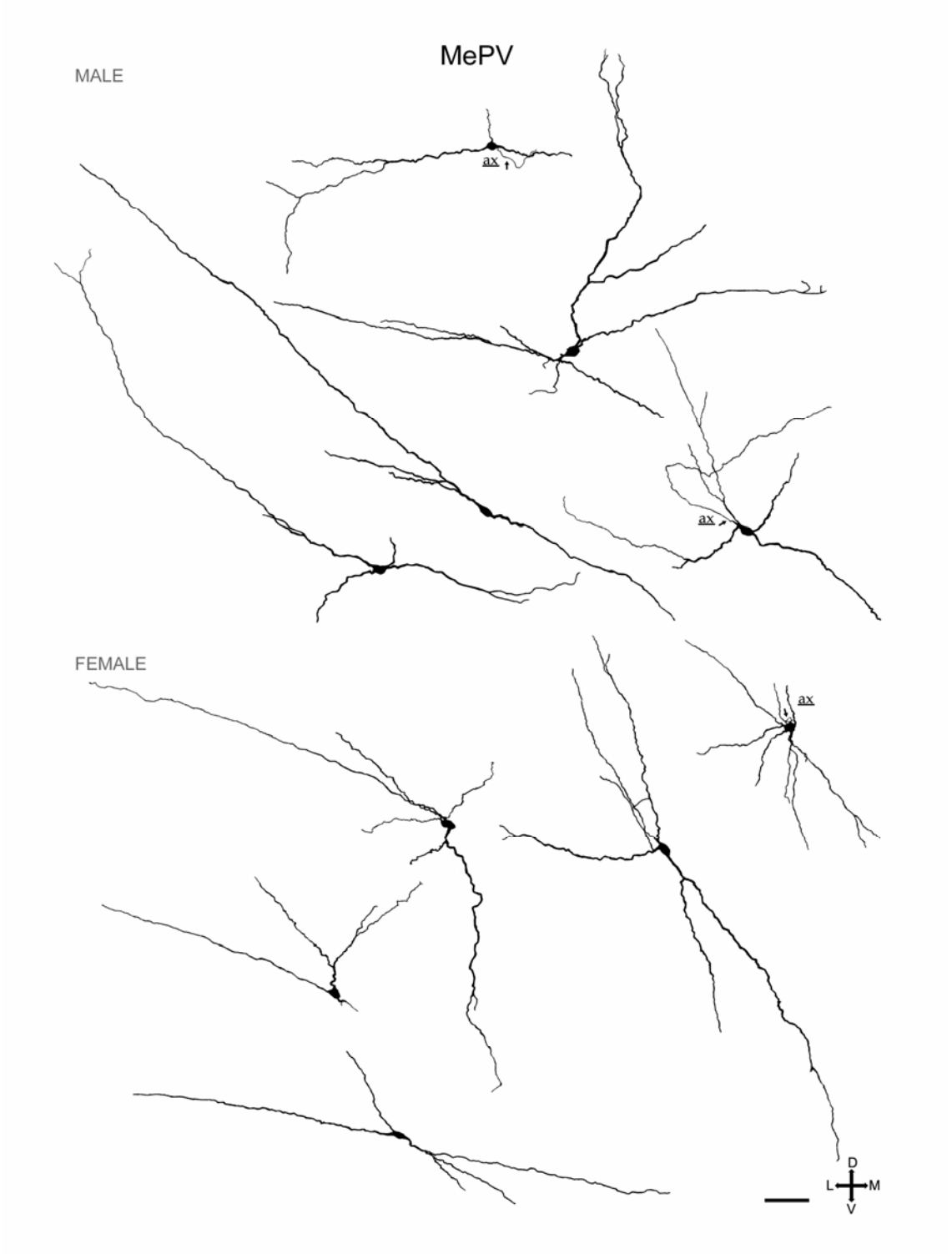


Figure 6

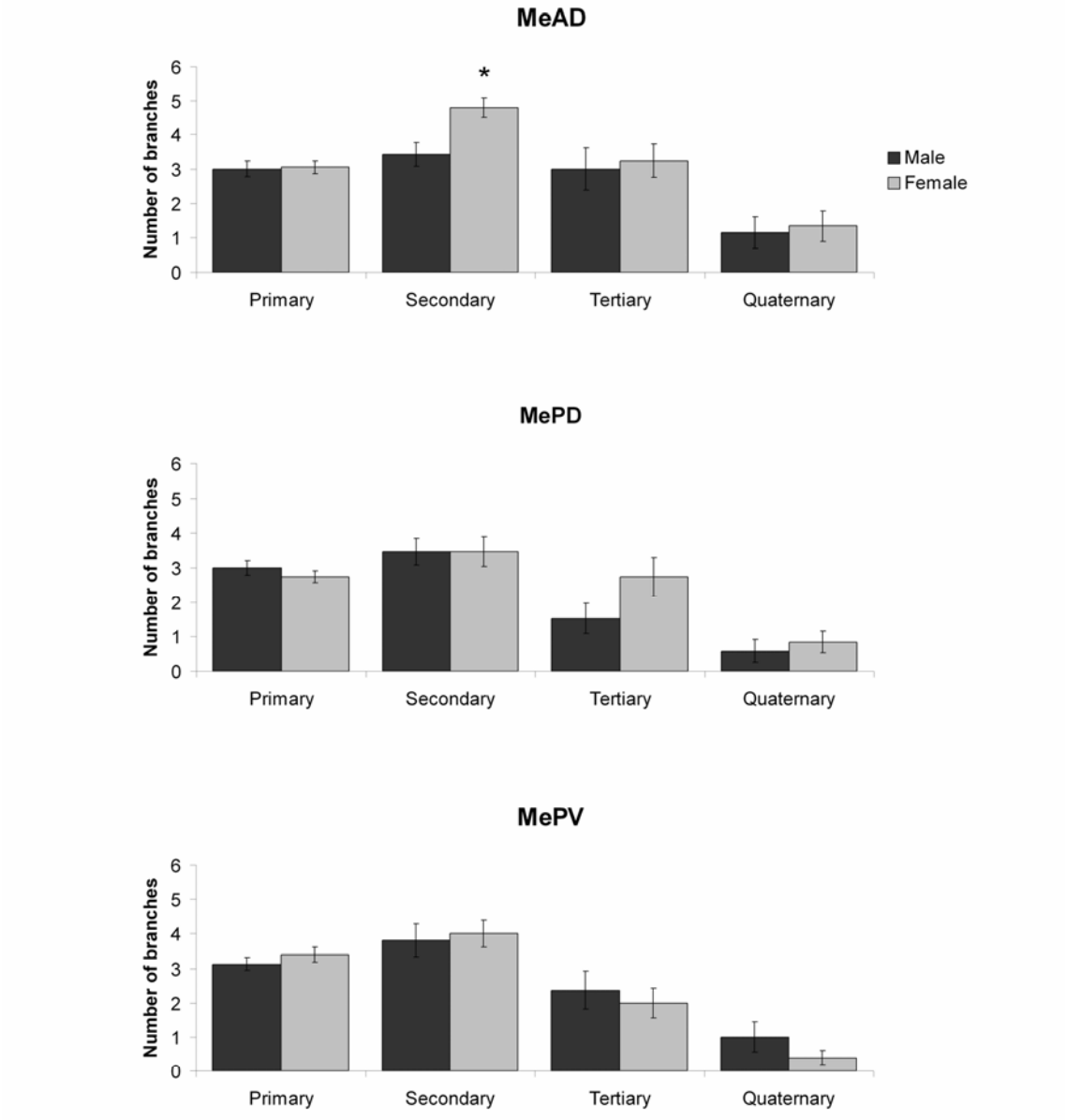

MeAD
Number of branches
0
1
2
3
4
5
6
*
Primary
Secondary
Tertiary
Quaternary
Male
Female
MePD
Number of branches
Primary
Secondary
Tertiary
Quaternary
MePV
Number of branches
Primary
Secondary
Tertiary
Quaternary

Figure 7

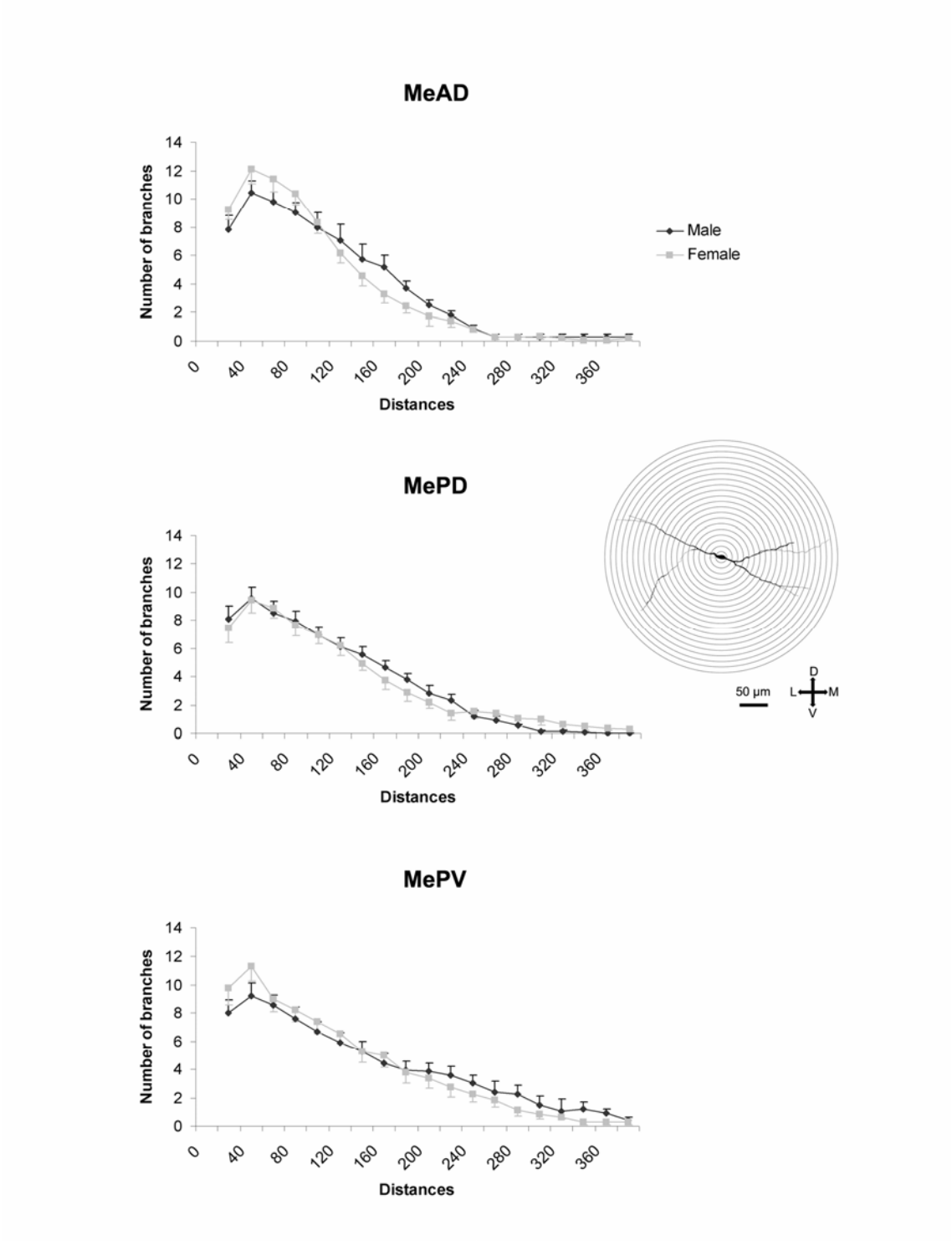

MeAD
Number of branches
Distances
Male
Female
MePD
Number of branches
Distances
50 µm
D
L
M
V
MePV
Number of branches
Distances

Figure 8

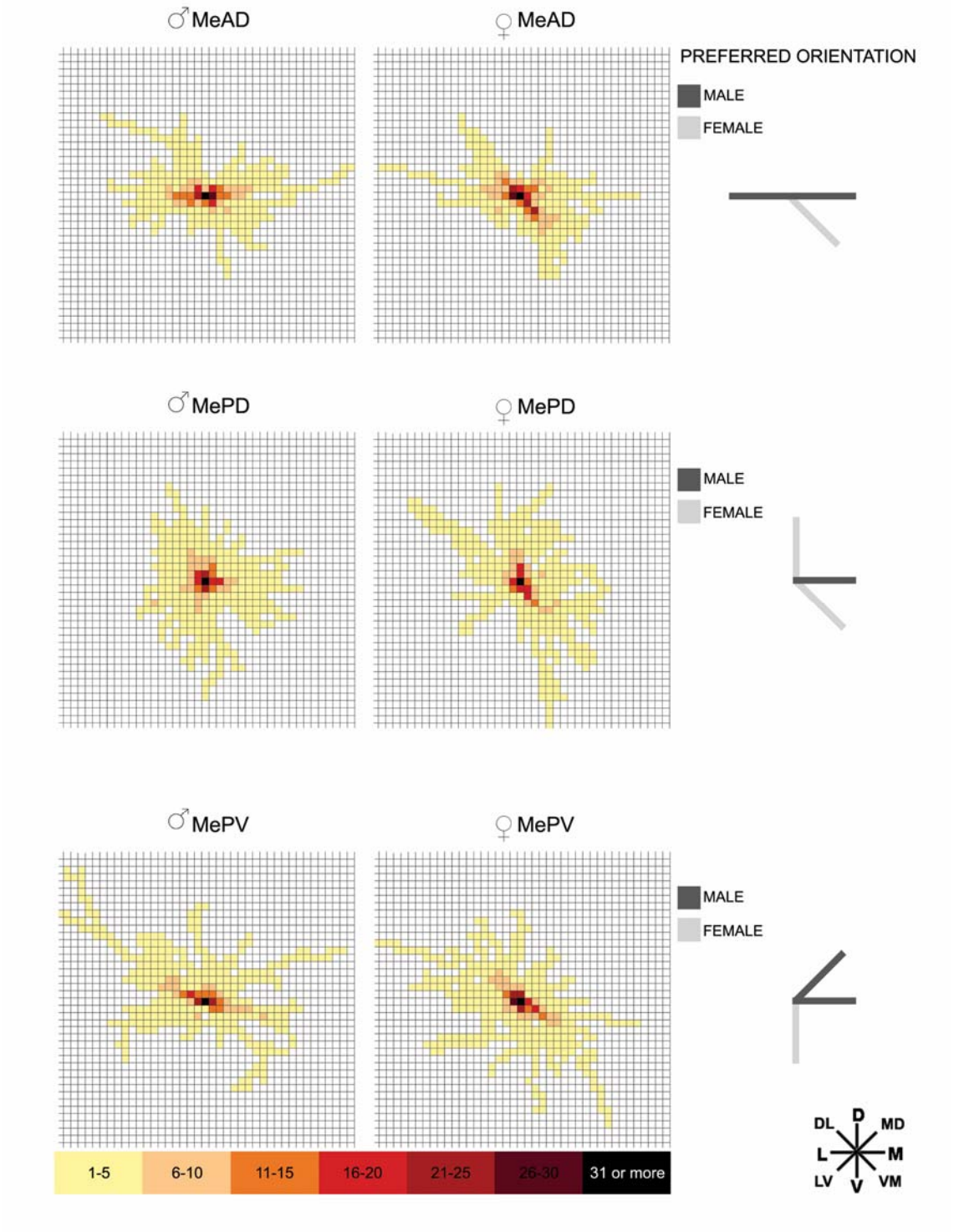

♂MeAD
♀MeAD
PREFERRED ORIENTATION
MALE
FEMALE
♂MePD
♀MePD
MALE
FEMALE
♂MePV
♀MePV
MALE
FEMALE
1-5
6-10
11-15
16-20
21-25
26-30
31 or more
D
DL
MD
L
M
LV
V
VM

Figure 9

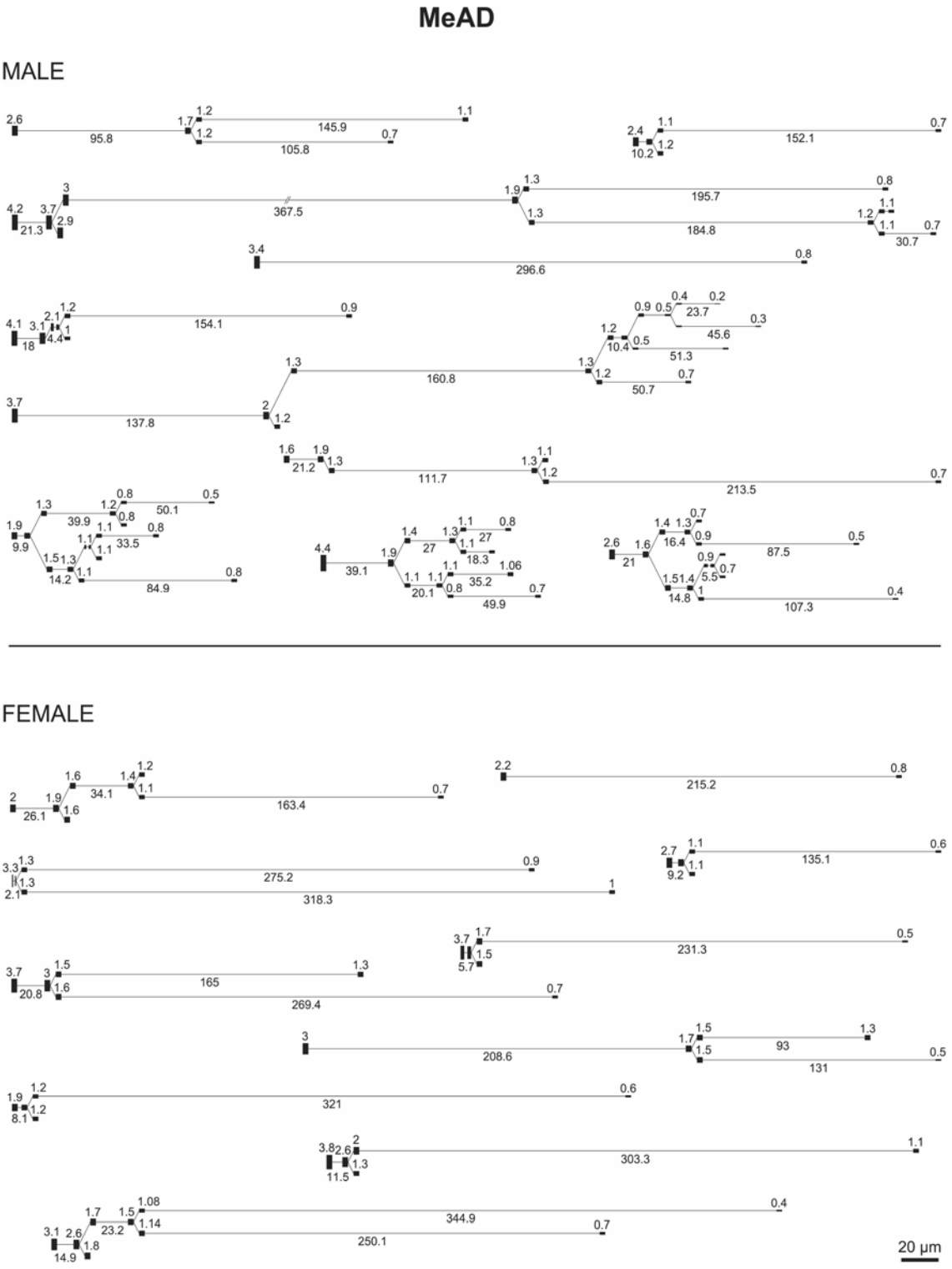


Figure 10

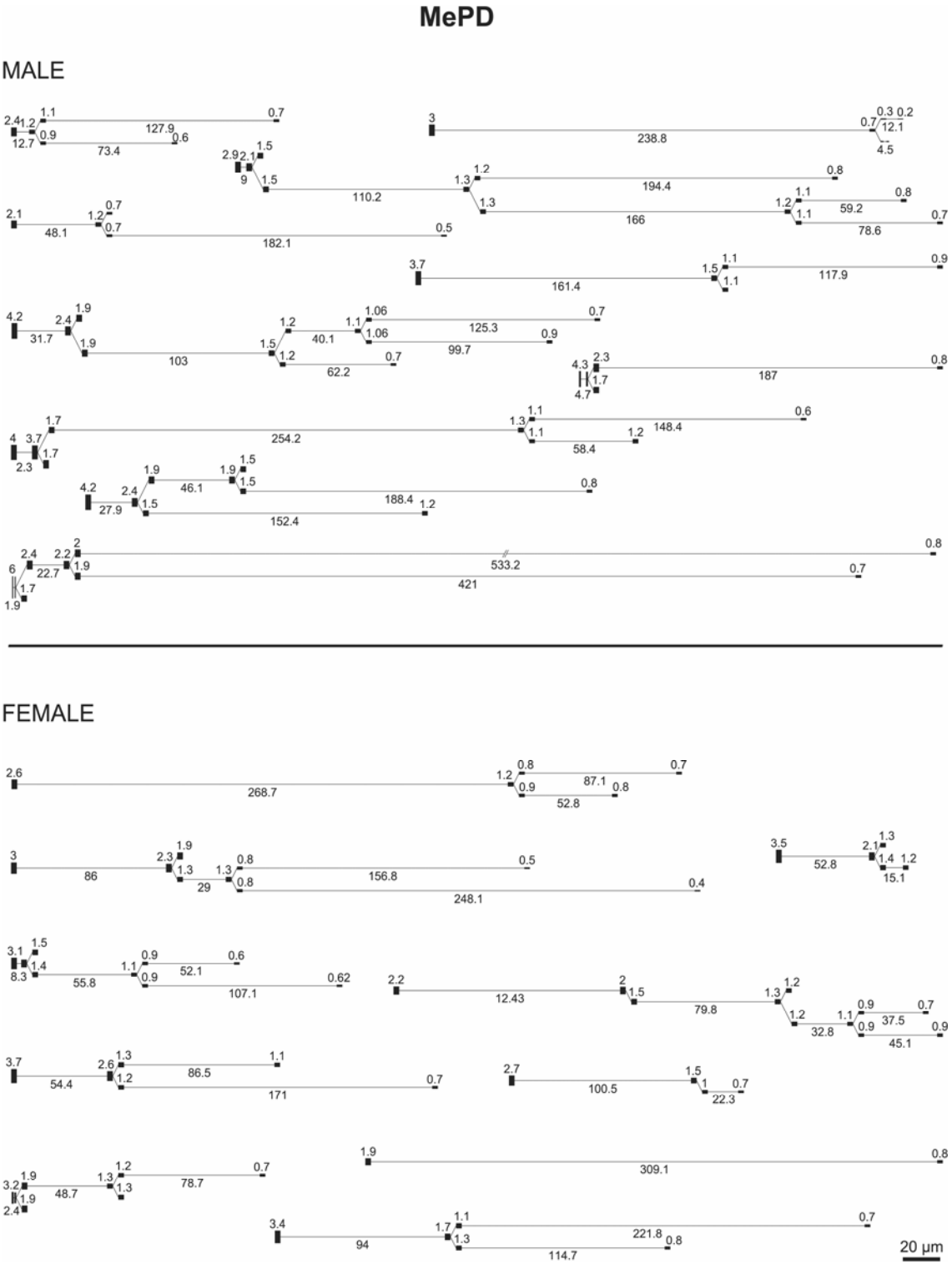


Figure 11

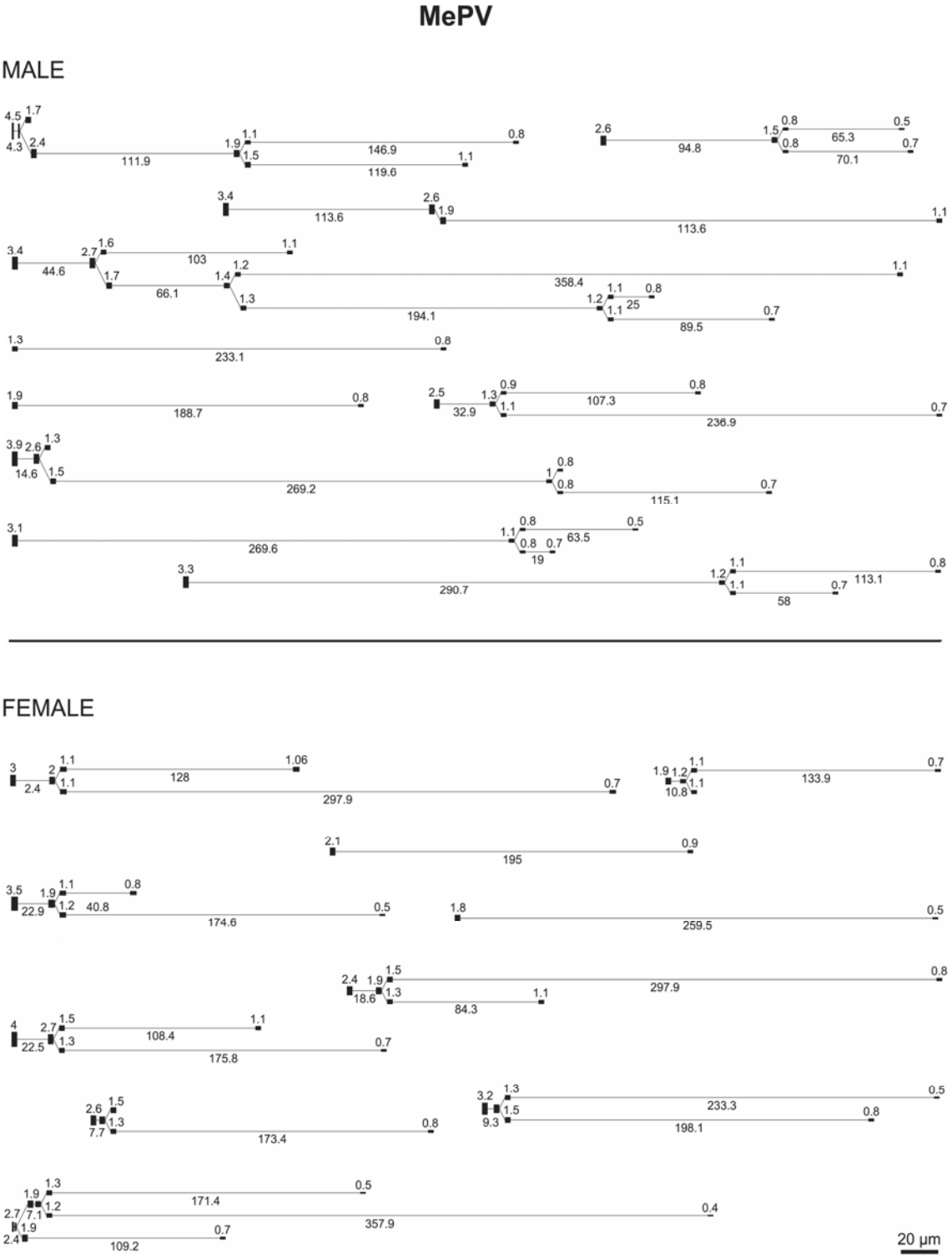
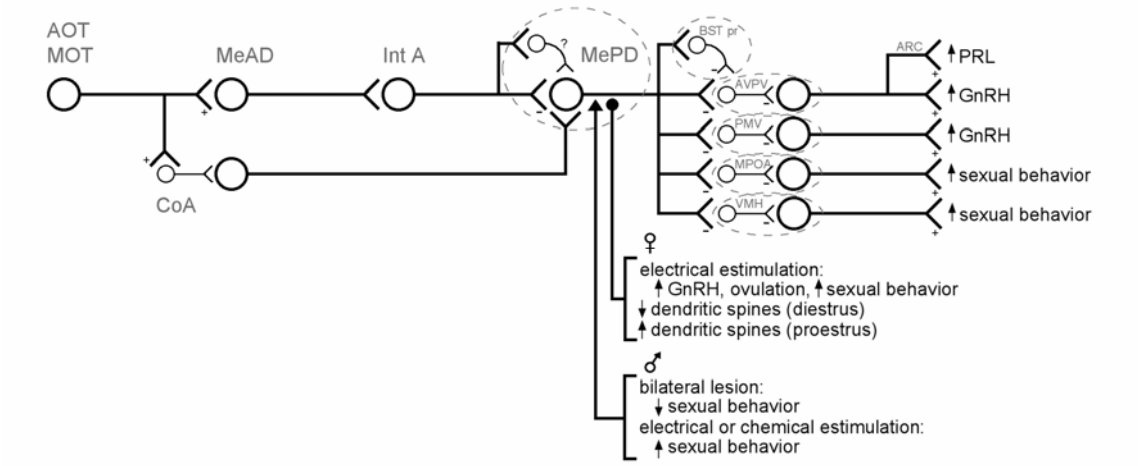


Figure 12


AOT
MOT
MeAD
Int A
?
MePD
BST pr
AVPV
PMV
MPOA
VMH
ARC
PRL
GnRH
GnRH
sexual behavior
sexual behavior
CoA
♀
electrical estimulation:
GnRH, ovulation, sexual behavior
dendritic spines (diestrus)
dendritic spines (proestrus)
♂
bilateral lesion:
sexual behavior
electrical or chemical estimulation:
sexual behavior

4. CONCLUSÕES

A morfologia dendrítica, como observada em neurônios impregnados pela técnica de Golgi, é basicamente homogênea nos subnúcleos do MeA de ratos. Nos três subnúcleos estudados (MeAD, MePD e MePV), neurônios multipolares foram classificados como estrelados ou bipenachados, apresentaram arborização dendrítica esparsa e seus ramos dendríticos tiveram comprimentos variados. Não obstante, fêmeas tiveram mais ramos secundários no MeAD e nos três subnúcleos ocorreu um padrão de distribuição espacial diferente em ambos os sexos. Sugere-se que a morfologia desses dendritos possa estar sendo modulada pela ação dos hormônios gonadais e que esse dimorfismo sexual afete o funcionamento do neurônio em circuitos neurais diferentes entre machos e fêmeas.

5. PERSPECTIVAS

- Para complementar e dar continuidade a esses achados, pretende-se estudar os mesmos parâmetros dendríticos descritos anteriormente em fêmeas ao longo das outras demais fases do ciclo estral. É um experimento que já está em andamento e em fase final de análise de dados. Seria interessante também estendê-lo a modelos experimentais de castração e reposição hormonal, para se comprovar a ação epigenética ou não, dos hormônios gonadais. Estudos imunocitoquímicos para determinação do(s) transmissor (s) químico(s) envolvido(s) nos contatos sinápticos em dendritos auxiliariam na interpretação da funcionalidade neuronal integrada.

- Além disso, partindo dos dados dos dendrogramas, pode-se fazer uma simulação em computador do funcionamento dos ramos dendríticos, admitindo para eles propriedades passivas e/ou ativas e dispondo contatos sinápticos mais proximais ou mais distais em relação ao soma neuronal. Este tipo de experimento pode servir de base teórica para respostas eletrofisiológicas intracelulares.

Todos esses dados morfológicos são relevantes para o entendimento das atividades que envolvem os componentes do MeA e podem permitir correlacionar os padrões de recepção e processamento de informações sinápticas com a modulação de comportamentos em ambos os sexos. Conjugá-los com experimentos que testem emissão de comportamentos específicos é ainda de alto grau de dificuldade técnica, mas seria de grande relevância para ratificar ou retificar as hipóteses sobre a aplicabilidade dos presentes resultados.

6. REFERÊNCIAS BIBLIOGRÁFICAS

AIZAWA, H.; SATO, Y., MAEKAWA, M.; FUJISAWA, H.; HIRATA, T.; YUASA, S. Development of the amygdalohypothalamic projection in the mouse embryonic forebrain. **Anatomy and Embryology**, v. 208, p. 249-264, 2004.

AKULININ, V. A.; STEPANOV, S. S. SEMCHENKO, V. V. BELICHENKO, P. V. Dendritic changes of the pyramidal neurons in layer V of sensory-motor cortex of the rat brain during postresuscitation period. **Resuscitation**, v. 35, p. 157-164, 1997.

ALHEID, G. F.; DE OLMOS, J. S.; BELTRAMINO, C. A. Amygdala and extended amygdala. In: PAXINOS, G. **The Rat Nervous System**. San Diego: Academic Press, p. 495-498, 1995.

ARIMATSU, Y.; SETO, A.; AMANO, T. Sexual dimorphism in α-bungarotoxin binding capacity in the mouse amygdala. **Brain Research**, v. 213, n. 2, p. 432-437, 1981.

ASCOLI G. A. Neuroanatomical algorithms for dendritic modelling. **Network; Computacional Neural System**, v 13, p. 247-260, 2002.

AZMITIA, E. C.; SEGAL, M. An autoradigraphic analysis of the differential ascending projections of the dorsal and median raphé in the rat. **The Journal of Comparative Neurology**, v. 179, p. 641-668, 1978.

BELTRAMINO, C.; TALEISNIK, S. Facilitatory and inhibitory effects of electrochemical stimulation of the amygdala on the release of luteinizing hormone. **Brain Research**, v. 144, p. 119-124, 1978.

BENNUR, S.; SHANKARANARAYANA RAO, B. S.; PAWLAK, R.; STRICKLAND S.; McEWEN, B. S.; CHATTARJI, S. Stress-induced spine loss in the medial amygdala is mediated by tissue-plasminogen activator. **Neuroscience**, v.144, p. 8-16, 2007.

BLACK, J. E.; KODISH, I. M.; GROSSMAN, A. W.; KLINTSOVA, A. Y.; ORLOVSKAYA, D.; VOSTRIKOV V.; URANOVA, N.; GREENOUGH, W. T. Pathology of Layer V Pyramidal Neurons in the Prefrontal Cortex of Patients With Schizophrenia. **American Journal of Psychiatry,** v. 161, p. 742–744, 2004.

BOLHUIS, J. R. The corticomedial amygdala and learning in an agonistic situation in the rat. **Physiology and Behavior**, v. 32, p. 575-579, 1984.

BRESSLER, S. C.; BAUM, M. J. Sex comparison of neuronal Fos immunoreactivity in the rat vomeronasal projection circuit after chemosensory stimulation. **Neuroscience**, v. 71, p. 1063-1072, 1996.

BRODAL, A. The amygdaloid nucleus in the rat. **The Journal of Comparative Neurology**, v.87, p. 1-17, 1947.

BRUCE L. L.; NEARY T. J. The limbic system of tetrapods: acomparative analysis of cortical and amygdalar populations. **Brain, Behavior and Evolution**, v. 46, p. 24–234, 1995.

CANTERAS, N. S.; SIMERLY, R. B.; SWANSON, L. W. Organization of projections from the medial nucleus of the amygdala: A PHAL study in the rat. **The Journal of Comparative Neurology**, v. 360, p. 213-245, 1995.

CAVALCANTE J. C.; SITA, L.V.; MASCARO M. B.; BITTENCOURT J. C.; ELIAS, C. F. Distribution of urocortin 3 neurons innervating the ventral premammilary nucleus in the rat brain. **Brain Research**, v. 1089, p. 116-125, 2006.

COLLIN, C.; MIYAGUCHI, K.; SEGAL, M. Dendritic spine density and LTP induction in cultured hippocampal slices. **The Journal of Neurophysiology**, v. 77, p.1614-1623, 1997.

COOKE, B. M.; SIMERLY, R. B. Ontogeny of bi-directional connections between the medial nucleus of the amygdala and the principal bed nucleus of the stria terminalis in the rat. **The Journal of Comparative Neurology**, v. 489, p. 42-58, 2005.

COOKE, B. M.; WOOLLEY, C. S.; Sexually dimorphic synaptic organization of the medial amygdala. **Journal of Neuroscience**, v. 25, n. 46, p. 10759-67, 2005.

COOLEN, L. M.; PETERS, H. J. P. W.; VEENING, J. G. Distribution of Fos immunoreactivity following mating versus anogenital investigationin the male rat brain. **Neuroscience**, v. 77, p. 1151-1161, 1997.

COOPERSMITH, C.; GANS, S. E.; ROWE, D. W.; ERSKINE, M. S. Infusions of lidocaine into the amygdala, but not the preoptic area, block pseudopregnancy in the rat. **Journal of Neuroendocrinology** , v. 8, p. 259-266, 1996.

CRILL, W. E.; The efectiveness of distal synaptic imputs on neurons. **Annals of the New York Academy of Sciences**, v. 855, p 77-82, 1997.

DALL'OGLIO, A.; MARCUZZO, S.; DE CASTILHOS, J.; RASIA-FILHO, A. A. Método de Golgi. In: ELIAS, C.; BITTENCOURT, J. **Métodos em Neurociências.** Ed: Rocco, (no prelo).

DAVIS, M. The role of the amygdala in conditioned fear. In: AGGLETON, J. P. **The Amygdala**. New York: Wiley-Liss, p. 255-306, 2000.

de CASTILHOS, J. Estudo sobre a ação dos hormônios gonadais na densidade de espinhos dendríticos na amígdala medial póstero-dorsal de ratas. 2005. **Dissertação** (Mestrado em Ciências Biológicas: Fisiologia) – Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul, Porto Alegre, 2005.

de CASTILHOS, J.; MARCUZZO, S.; FORTI, C. D.; FREY, R. M.; STEIN, D., ACHAVAL, M.; RASIA-FILHO, A. A. Further studies on the rat posterodorsal medial amygdala: Dendritic spine density and effect of 8-OH-DPAT microinjection on male sexual behavior. **Brain Research Bulletin,** v. 69, n. 2, p. 131-139, 2006.

de OLMOS, J. S.; ALHEID, G. F.; BELTRAMINO, C. A. Amygdala. In: PAXINOS, G. **The Rat Nervous System**, Sydney: Academic Press, p. 223-234, 1985.

de OLMOS, J.; BELTRAMINO, C. A.; ALHEID, G. F. Amygdala and extended amygdala of the rat: a cytoarchitectonical, fibroaschitectonical, and chemoarchetectonical survey. In: PAXINOS, G. **The Rat Nervous System**, Amsterdam: Elsevier Academic Press, p. 509-603, 2004.

De VRIES, G. J.; SIMERLY, R. B. Anatomy, development, and function of sexually dimorphic neural circuits in the mammalian brain. In: PFAFF, D.W.; ARNALD, A. P.; ETGEN, A. M.; FAHRBACH, S. E.; RUBIN, R. T. **Hormones, brain and behavior**. San Diego: Academic Press, p. 137-191, 2002.

DIELENBERG, R. A.; HUNT, G. R.; McGREGOR, L. S. When a Rat Smells a Cat: The distribution of FOS immunoreactivity in the rat brain following exposure to a predatory odor. **Neuroscience**, v. 104, n. 4, p. 1085-1097, 2001.

DOMINGUEZ, J. M.; HULL, E. M. Stimulation of the medial amygdala enhances medial preoptic dopamine release: implications for male rat sexual behavior. **Brain Research**, v. 917, p. 225-229, 2001.

DONG, H-W.; PETROVICH, G.; SWANSON, L. W. Topography of projections from amygdala to bed nuclei of the stria terminalis. **Brain Research Review**, v. 38, p. 192-246, 2001.

EVERITT, B. Limbic lobe and olfatory pathways. In: BERRY, M. M.; BANNISTER, L. H.; STANDRING, S. M. **Gray's Anatomy**. London: Churchill Livingstone, p.1115-1141, 1995.

FAIRÉN, A. Pioneering a golden age of cerebral microcircuits: The births of combined Golgi-electron microscope methods. **Neuroscience,** v. 136, p. 607-614, 2005.

FERGUSON, J. N.; ALDAG, J. M.; INSEL, T. R.; YOUNG, L. J. Oxytocin in the medial amygdala is essential for social recognition in the mouse. **Journal of Neuroscience**, v. 21, p. 8278-8285, 2001.

FERIA-VELASCO, A.; ROSA del ANGEL, A.; GONZALEZ-BURGOS, IGNACIO. Modification of dendritic development. **Brain Research,** v. 136, chapter 11, p. 135-143, 2002.

FERNANDEZ-GALAZ, M. C.; PARDUCZ, A.; NAFTOLIN, G; TORRES-ALEMAN, J.; GARCIA-SEGURA, L. M. Interaction of gonadal steroids and growth factors in the brain sex differentiation. **Biomedical Reviews**, v. 7, p. 67-74, 1997.

FERNANDEZ-GUASTI, A. PICAZO, O. Anxiolytic actions of diazepam, but not of buspirone, are influenced by gender and the endocrine stage. **Behavior and Brain Research**, v. 88, P. 213-218, 1997.

FERNANDEZ-GUASTI, A.; PICAZO, O. Sexual differentiation modifies the allopregnanolone anxiolytic actions in rats. **Psychoneuroendocrinology**, v. 24, p. 251-267, 1999.

FIALA, J. C.; HARRIS, K. M. Dendrite structure. In: **Dendrites**. STUART, G.; SPRUSTON, N.; HÄUSSER, M. p. 1-34. New York: Oxford University Press, 1999.

FLYNN, C.; MONFILS, M. H.; KLEIM, J. A.; KOLB, B.; McINTYRE, D. C.; TESKEY, G. C. Differential neuroplastic changes in neocortical movement representations and dendritic morphology in epilepsy-prone and epilepsy-resistant rat strains following high-frequency stimulation. **European Journal of Neuroscience**, v.19, p. 2319-2328, 2004.

FORTI, C. D. Estudo da Densidade dos espinhos dendríticos na região póstero-dorsal da amígdala medial de ratos intactos e orquiectomizados. **Dissertação** (Mestrado em Ciências Biológicas: Neurociências), Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2005.

GLOOR P. The amygdaloid system. In: GLOOR, P. **The temporal lobe and limbic system**, New York: Oxford University Press, p. 591-721, 1997.

GOLDBERG, J. H.; YUSTE, R. Space matters: local and global dendritic Ca2+ compartmentalization in cortical interneurons. **Trends in Neurosciences**, v 28, p. 158-167, 2005.

GOMEZ, D. M.; NEWMAN**,** S. W. Medial nucleus of the amygdala in the adult Syrian hamster: A quantitative Golgi analysis of gonadal hormones regulation of neuronal morphology. **The Anatomical Record**, v. 231, p. 498-509, 1991.

GORSKI, R. Sexual differentiation of the nervous system. In: **Principles of Neural Sciences**. KANDEL, E.; SCHWARTZ, J.H. & JESSEL, T.M. New York, MGraw Hill., p. 1131-1148, 2000.

GRECÓ, B.; EDWARDS, D. A.; MICHAEL, R. P.; CLANCY, N. A. Androgen receptors and estrogen receptors are colocalized in male rat hypothalamic and limbic neurons that express Fos immunoreactiviy induced by mating. **Neuroendocrinology**, v. 67, p. 18-28, 1998.

GROVE, E. A. Eferent connections of the substantia innominata in the rat. **The Journal of Comparative Neurology**, v. 277, p. 347-364, 1988.

GUILLAMÓN, A.; SEGOVIA, S. Sex Differences in the Vomeronasal System. **Brain Research Bulletin**, v. 44, n. 4, p. 377-382, 1997.

GULLEDGE, AT.; KAMPA, BM.; STUART, G. J. Synaptic integration in dendritic trees. **Journal of Neurobiology**, v 65, p 205-206, 2006.

HAGIHARA, K.; HIRATA, S.; OSADA, T.; HIRAY, M.; KATO, J. Distribution of cells containing progesterone receptor mRNA in the female rat di- and telencephalon: an in situ hybridization study. **Brain Research Mollecular Brain Reseach**, v. 14, n. 3, p. 239-249, 1992.

HALL, E. Some aspects of the structural organization of the amygdale. In: ELETHERIOU B. E. **The Neurobiology of the Amygdala**. Plenum Press, New York, p. 95-121, 1972.

HÄUSSER, M.; SPRUSTON, N.; STUART, G. J. Diversity and dinamics of dendritic signaling. Science, v. 290, p. 739-744, 2000.

HERBERT, H.; SAPER, C. B. Cholecystokinin- , galanin-, and coritcotropin-releasing factor-like immunoreactive projections from the núcleos of solitary tract to parabrachial nucleus in the rat. **The Journal of Comparative Neurology**, v 293, p. 581-598, 1990.

HERMEL, E.; MARCUZZO, S.; HEUSER, M. C. F.; RASIA-FILHO, A. A.; ACHAVAL, M. Ultrastructural features of neurons and synaptic contacts in the posterodorsal medial amygdala of adult male rats. **Journal of Anatomy**, v. 208, p. 565-575, 2006.

HILLMAN, D. E. Neuronal shape parameters and substructures as a basis of neuronal form. In: **The Neurosciences**. SCHMITT, F. O.; WORDEN, F. G. Cambridge: The MIT Press, p. 477-498, 1979.

HINES, M.; ALLEN, L. S.; GORSKI, R. A. Sex differences in subregions of the medial nucleus of the amygdala and the bed nucleus of stria terminalis of the rat. **Brain Research**, v. 579, p. 321-326, 1992.

HUMEAU, Y.; HERRY, C.; KEMP, N.; SHABAN, H.; FOURCAUDOT, E.; BISSIERE, S.; LUTHI, A Dendritic spine heterogeneity determines afferent-specific Hebbian plasticity in the amygdala. **Neuron**, v. 45, p. 119-131, 2005.

JONES, B. J.; MOORE, R. Y. Ascending projections of the *locus ceruleus* in the rat. II. Autoradigraphic study. **Brain Research**, v. 127, p. 23-53, 1977.

KAMAL, A. M.; TÖMBÖL, T. Golgi studies on the amygdaloid nucleiof the cat. **Journal Hirnforschung**, v. 16, p. 175-201, 1975.

KATO, J. Progesterone receptor in brain ad hypophysis. In: D. W. PFAFF**. Current Topics in Neuroendocrinology, Vol. V.**. Springer Verlag,Berlin, p. 31-81, 1985.

KERCHNER, M.; MALSBURY, C. W.; WARD, O. B.; WARD, I. L. Sexually dimorphic areas in the rat medial amygdala: resistance to the demasculinizing effect of prenatal stress. **Brain Research**, v. 672, p. 251-260, 1995.

KLING, A. S.; BROTHERS, L. A. The amygdala and social behavior. In: AGGLETON, J. P. **The Amygdala**. New York: Wiley-Liss, p. 353-377, 1992.

KLÜVER, H.; BUCY, P.C. Preliminary analysis of the temporal lobes in monkeys. **Archives in Neurology and Psychiatry**, v. 42, p. 979-1000, 1939.

KONDO, Y. Lesions of the medial amygdala produce severe impairment of copulatory behavior in sexually inexperience male rats. **Physiology and Behavior**, v. 51, p. 939-943, 1992.

KONDO, Y.; ARAI, Y. Funcional association between the medial amygdala and the medial preoptic area in regulation of mating behavior in the male rat. **Physiology and Behavior**, v. 57, p. 69-73, 1995.

LARRIVA-SAHD, J. Histological and cytological study of the bed nuclei of the stria terminalis in adult rat. II. Oval nucleus: extrinsic inputs, cell types, neuropil, and neuronal modules. **The Journal of Comparative Neurology**, v. 497, p. 772-807, 2006.

LeDOUX, J. E. Emotion and amygdala. In: AGGLETON, J. P. **The Amygdala**. New York: Wiley-Liss, p. 339-352, 1992.

LEHMAN, M. N.; WINANS, S. S. Vomeronasal and olfactory pathways to the amygdala controlling male hamster sexual behavior. Autoradiographic and behavioral analyses. **Brain Research**, v. 240, n. 1, p. 27-41, 1982.

LEHMANN, M. L.; ERSKINE, M. S. Glutamatergic stimulation of the medial amygdala induces steroid dependent c-fos expression within forebrain nuclei responsive to mating stimulation. **Neuroscience**, v. 136, p. 55-64, 2005.

LEHMANN, M. L.; McKELLAR, H.; ERSKINE, M. S. Coding for the initiation of pseudopregnancy by temporally patterned activation of amygdalar NMDA receptors. **Journal of Neuroscience**, v. 25, p.8696-8703, 2005.

LI, X.; SCHWARTZ, P. E.; RISSMAN, E. F. Distribution of estrogen receptor-β-like immunoreactivity in rat forebrain. Neuroendocrinolology, v. 66, p. 63-67, 1997.

LONDON, M.; HÄUSSER, M. Dendritic computation. **Annual Review of Neuroscience**, v. 28, p. 503-532, 2005.

LUQUIN, A. B.; NAFTOLIN, F.; GARCIA-SEGURA, L. M. Natural fluctuation and gonadal hormone regulation of astrocyte immunoreactivity in dentate gyrys. **Journal of Neurobiology**, v. 27, n. 7, p. 913-924, 1993.

MALSBURY, C. W.; McKAY, K. Neurotrophic effects of testosterone on the medial nucleus of the amygdala in adult male rats. **Journal of Neuroendocrinology**, v.6, n. 1, p. 57-69, 1994.

MARTINEZ, F. G.; HERMEL, E. E.; XAVIER, L. L.; VIOLA, G. G.; RIBOLDI, J.; RASIA-FILHO, A. A.; ACHAVAL, M. Gonadal hormone regulation of glial fibrillary acidic protein immunoreactivity in the medial amygdala subnuclei across the estrous cycle and in castrated and treated female rats. **Brain Research**, v. 1108, p. 117-126, 2006.

MARTÍNEZ-TELLEZ, R.; GOMEZ-VILLALOBOS, M. de J.; FLORES, G. Alteration in dendritic morphology of cortical neurons in rats with diabetes mellitus induced by streptozotocin. **Brain Research**, v. 1048, p. 108-115, 2005.

McDONALD, A. J. Cytoarchitecture of the central amygdaloid nucleus of the rat. **The Journal of Comparative Neurology**, v. 208, p. 401-418, 1982.

McDONALD. A. J. Cell types and intrinsic connections of the amygdala. In: AGGLETON, J. P. **The Amygdala**. New York, Wiley-Liss., p. 67-92, 1992.

McDONALD. A. J. Cortical pathways to the mammalian amygdala. **Progress in Neurobiololy**, v. 55, n. 3, p. 257-332, 1998.

McDONALD. A. J.; SHAMMAH-LAGNADO, S. J.; SHI, C.; DAVIS, M. Cortical afferents to the extended amygdala. **Annals of New York Academy of Science**, v. 877, p. 309-338, 1999.

McEWEN, B. S. Endocrine effects on the brain and their relationship to behavior. In: SIEGEL, G.; ALBERTS, R. W.; BRADY, S.; PRICE, D. **Basic Neurochemistry**, Academic Press, p. 843-858, 2006.

McEWEN, B. S. Gonadal steroid influences on brain development and sexual differentiation. **International Reviews of Physiology**, v. 27, p. 99-145, 1983.

McEWEN, B. S. Stress and hippocampal plasticity. **Annual Review of Neuroscience**, v. 22, p. 105-122, 1999.

MEISEL, R.L.; SACHS, B.S. The physiology of male sexual behavior. In:**The Physiology of Reproduction.** KNOBIL, E.; NEILL, I.D. New York: Raven Press, p. 3-105, 1994.

MEREDITH, M.; WESTBERRY, J. M. Distinctive responses in the medial amygdala to same-species and different-species pheromones. **Journal of Neuroscience**, v. 24, p. 5719-5725, 2004.

MIGLIORE, M.; SHEPHERD, G. An integrated approach to classifying neuronal phenotypes. **Nature Review Neuroscience**, v. 6, p.810-818, 2005.

MIZUKAMI, S.; NISHIZUKA, M.; ARAI, Y. Sexual difference in nuclear volume and its ontogeny in the rat amygdala. **Experimental Neurology**, v. 79, p. 569-575, 1983.

MUKHINA, L.; LEONTOVICH, T. A. Neuronal structure of some amygdaloid nuclei in dogs. **Arkev Anatomii Gistologii Embriologii (Moscow)**, v. 59, p. 62-70, 1970.

NARKIEWICZ, O.; JURANIEC, J.; WRZOLKOWA, T. The distribution of axon terminals with flattened vesicles in the nuclei of the amygdaloid body of the cat. **Journal fur Hirnforschung**, v. 19, p. 133-143, 1978.

NELSON, R. J. **An introduction to behavioral endocrinology**. Sunderland: Sianuer Associates, 2º ed., 1995.

NEWMAN, S. W. Pheromonal signals access the medial extended amygdala: one node in a proposed social behavior network. In: **Hormones, Brain and Behavior.** PFAFF, D. W.; ARNOLD, A. P.; ETGEN, A. M.; FAHRBACH, S. E.; RUBIN, R. .T. San Diego: Academic Press, p 17-31, 2002.

NEWMAN, S. W. The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network. **Annals of the New York Academy of Sciences**, v. 877, p. 242-257, 1999.

NISHIZUKA, M.; ARAI, Y. Male-female differences in the intra-amygdaloid input to the medial amygdale. **Experimental Brain Research,** v. 52, p. 328-332, 1983a.

NISHIZUKA, M.; ARAI, Y. Regional difference in sexually dimorphic synaptic organization of the medial amygdala. **Experimental Brain Research**, v. 49, p. 462-465, 1983b.

NISHIZUKA, M.; ARAI, Y. Sexual dimorphism in synaptic organization in the amygdala and its dependence on neonatal hormone environment. **Brain Research**, v. 212, p. 31-38, 1981.

NISHIZUKA, M.; ARAI, Y. Synapse formation in response to estrogen in the medial amygdala developing in the eye. **Proceedings in National Academy of Sciences of the USA**, v. 79, n. 11, p. 7024-7026, 1982.

OHTAKE, T.; YAMADA, H. Efferent connections of the núcleo reuniens and the rhomboid nucleus in the rat: Na anterograde PHA-L tracing study. **Neuroscience Research**, v. 6, p. 556-568, 1989.

ÖSTERLUND, M. Differencial distribuition and regulation of estrogen receptor-alpha and -beta mRNA within the female rat brain. **Molecular Brain Research**, v. 54, p. 175-180, 1998.

ÖSTERLUND, M.; KUIPER, G. G.; GUSTAFSSON, J.-A.; HURD, Y. L. Differential distribution and regulation of estrogen receptor-alpha and -beta mRNA within the female rat brain. **Molecular Brain Research**, v. 54, p. 175-180, 1998.

OTERSSEN, O. P. Afferent connections to the amygdaloid complex of the rat with some observations in the cat. III. Aferents from the lower brainstem. **The Journal of Comparative Neurology**, v. 202, p. 335-356, 1981.

OTTERSEN, O. P. Afferent connections to the amygdaloid complex of the rat and cat: II. Afferents from the hypothalamus and the basal telencephalon. **The Journal of Comparative Neurology**, v.194, p. 267-289, 1980.

OTTERSEN, O. P.; BEN-ARI, Y. Afferent connections to the amygdaloid complex of the rat and cat: I. Projections from the thalamus. **The Journal of Comparative Neurology**, v.187, p. 401-424, 1979.

PANNESE, E. The black reaction. **Brain Research Bulletin**, v. 41, n. 6, p. 343-349, 1996.

PAXINOS, G.; WATSON, C. **The Rat Brain in Stereotaxic Coordinates**. San Diego: Academic Press, 1998.

PETERS, A.; KAISERMAN-ABRAMOF, I. R. The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines. **The American Journal of Anatomy,** v. 127, p. 321-356, 1970.

PETROVICH, G. D.; CANTERAS, N. S.; SWANSON, L. W. Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. **Brain Research Reviews**, v. 38, p. 247-289, 2001.

PFAUS, J. G.; HEEB, M.M. Implications of immediate-early gene induction in the brain following sexual stimulation of female and male rodents. **Brain Research Bulletin**, v. 44, n. 4, p. 397-407, 1997.

PFAUS, J. G.; MARCANGIONE, C.; SMITH, W, J.; MANITT, C. ABILLAMMA, H. Differential induction of Fos in the female rat brain following different amounts of vaginocervical stimulation: Modulation by steroid hormones. **Brain Research**, v. 741, p. 314-330, 1996.

PITKÄNEN, A. Connectivity of the rat amygdaloid complex. In: AGGLETON, J. P. **The Amygdala**. Oxford University-Press, p. 31-115, 2000.

PRICE, C. The Limbic Reegion II: The Amygdaloid complex. In: **Handbook of Chemical Neuroanatomy.** BJÖKLUND, A; HÖKFELT, T.; SWANSON, L.W. Amsterdan: Eslevier, v. 5, p. 279-388, 1987.

QUIRK, G. J.; REPA, C.; LeDOUX, J. E. Fear conditioning enhances short latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. **Neuron**, v. 15, p. 1029-1039, 1995.

RALL, W. Branching dendritic trees and motoneuron membrane resistivity. **Experimental Neurology**, v. 1, p. 491-527, 1959.

RAMÓN y CAJAL, S. Neurons: size and general morphology. In: SWANSON, N.; SWANSON, S. W. **Histology of the Nervous System of Man and Vertebrates**, New York: Oxford University Press, p. 46-57, 1995. (traduzido da edição francesa de 1909)

RAMÓN-MOLINER, E. An attempt at classifying nerve cells on the basis of their dendritic patterns. **The Journal of Comparative Neurology**, v. 119, p. 211-227, 1962.

RASIA-FILHO A. A.; HILBIG, A. Papel da amígdala e do hipocampo no transtorno do estresse pós-traumático. Em: **Transtornos do estresse pós traumático**. CAMINHA, R. M. Editora Casa do Psicólogo, parte I, p. 37-53, 2005.

RASIA-FILHO, A. A.; FABIAN, C.; RIGOTI, K.; ACHAVAL, M. Influence of sex, estrous cycle and motherhood in dendritic spine density in the rat medial amygdala revealed by the Golgi methods. **Neuroscience**, v. 126, p. 839-847, 2004.

RASIA-FILHO, A. A.; LONDERO, R. G.; ACHAVAL, M. Effects of gonadal hormones on the morphology of neurons from the medial amygdaloid nucleus of rats. **Brain Research Bulletin**, v. 48, p. 173-183, 1999.

RASIA-FILHO, A. A.; LONDERO, R. G.; ACHAVAL, M. Funcional activites of the amygdala: an overview. **Journal of Psychiatry and Neuroscience**, v. 25, n. 1, p. 14-23, 2000.

RASIA-FILHO, A. A.; PERES, T. M.; CUBILLA-GUTIERREZ, F. H.; LUCION, A. B. Effect of estradiol implanted in the corticomedial amygdala on the sexual behavior of castrated male rats. **Brazilian Journal of Medical Biology Research**, v. 24, p. 1041-1049, 1991.

RASIA-FILHO, A. A.; XAVIER, L. L.; SANTOS, P.; GEHLEN, G.; ACHAVAL, M. Glial fibrillary acidic protein immunodetection and immunoreactivity in the anterior and in the posterior medial amygdala of male and female rats. **Brain Research Bulletin**, v 58, p. 67-75, 2002.

RIGOTI, K. M. Análise morfológica neuronal e da densidade de espinhos dendríticos da amígdala medial de ratas durante o ciclo estral. 2002. **Dissertação** (Mestrado em Ciências Biológicas: Neurociências), Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2002.

ROMANO, G. J.; KRUST, A.; PFAFF, D. W. Expression and estrogen regulation of progesterone receptor mRNA in neurons of the mediobasal hypothalamus: an in situ hybridization study. **Molecular Endocrinology**, v. 3, n. 8, p. 1295-1300, 1989.

RONDINI, T. A.; BADDINI, S. P.; SOUSA, L. F.; BITTENCOURT, J. C.; ELIAS, C. F. Hypothalamic cocaine- and amphetamine-regulated transcript neurons project to areas expressing gonadotropin releasing hormone immunoreactivity and to the anteroventral periventricular nucleus in male and female rats. **Neuroscience,** v. 125, p. 735-748, 2004.

ROOZENDAAL, B.; McGAUGH, J. L. Amigdaloid nuclei lesions differentially affect glucocorticoid-induced memory enhancement in na inhibitory avoidance task. **Neurobiology of Learning and Memory**, v. 65, p. 1-8, 1996.

SAR, M. Distribution of progestin-concentrating cells in rat brain: colocalization of [3H] ORG.2058, a synthetic progestin, and antibodies to tyrosine hydroxylase in hypothalamus by combined autoradiography and immunocytochemistry. **Endocrinology**, v. 123, n. 2, p. 1110-1118, 1988.

SCHEIBEL, M. E.; SCHEIBEL, A. B. The methods of Golgi. In: ROBERTSON, R. T. **Neuroanatomical Research Techniques**, New York: Academic Press, p. 89-114, 1978.

SCHEIBEL, M. E.; SCHEIBEL, A. B. **The methods of Golgi**. In: ROBERTSON, R. T. Neuroanatomical Research Techniques, New York: Academic Press, p. 89-114, 1978.
SEGAL, M.; ANDERSEN, P. Dendritic spines shaped by synaptic activity. Current Opinion in Neurobiology, v. 10, p. 582-586, 2000.

SEGEV, I.; LONDON, M. A theoretical view of passive and active dendrites. In: **Dendrites**. STUART, G.; SPRUSTON, N.; HÄUSSER, M. New York: Oxford University Press, p 205-230, 1999.

SEGEV, I.; RALL, W. Excitable dendrites and spines: earlier thereotical insights elucidate recent direct observations. **Trends in Neuroscience**, v. 21, p. 453-460, 1998.

SHEEHAN, T. P.; AMARAL, E.; NUMAN, M. J.; NUMAN, M. Evidence that the medial amygdala projects to the anterior/ventromedial hypotalamic nuclei to inhibit maternal behavior in rats. **Neuroscience**, v. 106, p. 341-356, 2001.

SHELLEY, D. N.; MEISEL, R. L. The effects of mating stimulation on c-Fos immunoreactivity in the female hamster medial amygdala are region and context dependent. **Hormones and Behavior**, v. 47, p. 212– 222, 2005.

SHEPHERD, G. M. Eletrotonic properties of axons and dendrites. In: ZIGMOND, J. M.; BLOOM, F. E.; LANDIS, S. C.; ROBERTS, J. L. **Fundamental Neuroscience**. Academic Press. San Diego, California, p. 107-127, 1999a.

SHEPHERD, G. M. Information processing in dendrites. In: ZIGMOND, J. M.; BLOOM, F. E.; LANDIS, S. C.; ROBERTS, J. L. **Fundamental Neuroscience**. Academic Press. San Diego, California, p. 363-388,1999b.

SHERIDAN, P. J. The nucleus intersticialis stria terminalis and the nucleus amygdaloideus medialis: prime targets for androgen in the rat forebrain. Endocrinology, v. 104, n. 1, p. 130-136, 1979.

SHIMADA, A.; TSUZUKI, M.; KEINO, H.; SATO, H. M.; CHIBA, Y.; SAITOH, Y.; HOSOKAWA, M. Apical vulnerability to dendritic retraction in prefrontal neurones of ageing SAMP10 mouse: a model of cerebral degeneration. **Neuropathology and Applied Neurobiolog y**, v. 32, p. 1-14, 2006.

SHINODA, K.; NAGANO, M.; OSAWA, Y. Neuronal aromatase expression in preoptic, strial, and amygdaloid regions during late prenatal and early postnatal development in the rat. **The Journal of Comparative Neurology**, v. 343, p. 113-129, 1994.

SHUGHRUE, P. J.; LANE, M. V.; MERCHENTHALER, I. Comparative distribution of estrogen receptor-α and β mRNA in the rat central nervous system. **Journal of Comparative Neurology**, v. 388, p. 507-525, 1997.

SILVA-GOMEZ, A. B.; BERMUDEZ, M.; QUIRION, R.; SRIVASTAVA, L. K.; PICAZO, O.; FLORES, G. Comparative behavioral changes between male and female postpubertal rats following neonatal excitotoxic lesions of the ventral hippocampus. **Brain Research**, v. 973, p. 285-292, 2003.

SIMERLY, R. B. Anatomical substrates of hypothalamic integration. In: **The Rat Nervous System**. PAXINOS, G. Amsterdam: Elsevier Academic Press, p.335-368., 2004.

SIMERLY, R. B. Organization and regulation of sexually dimorphic neuroendocrine pathways. **Behavioral Brain Research**, v. 92, p. 195-203, 1998.

SIMERLY, R. B. Wired for reproduction: organization and development of sexually dimorphic circuits in the mammalian forebrain. **Annual Review Neuroscience**, v. 25, p.507-536, 2002.

SIMERLY, R. B.; CHANG, C.; MURAMATSU, M.; SWANSON, L. M. Distribution of androgen and estrogen receptor mRNA containing cells in the rat brain: an in situ hydrydization study. **Journal of Comparative Neurology**, v. 294, p. 76-95, 1990.

SIMERLY, R.G. Hormonal control of neuropeptide gene expression in sexually dimorphic olfactory pathways. **Trends in Neurosciences**, v. 13, p. 104-110, 1990.

SOCIEDADE BRASILEIRA DE ANATOMIA. **Terminologia Anatômica**, ed. Manole, 2001.

SWANSON, L. M.; PETROVICH, G. D. What is the amygdala? **Trends in Neurosciences**, v. 21, p. 323-331, 1998.

TAKAHASHI, L.K.; GLADSTONE, C.D. Medial Amygdaloid Lesions and the Regulation of Sociosexual Behavioral Patterns Across the Estrous Cycle in Female Golden Hamsters. **Behavioral Neuroscience**, v. 102, n. 2, p. 268-275, 1988.

TÖMBÖL, T.; SZAFRANSKA-KOSMOL, A. A Golgi study of amygdaloid complex in the cat. **Acta Neurobiologiae Experimentalis**, v. 32, p. 835-848, 1972.

VALVERDE, F. Intrinsic organization of the amygdaloid complex. A Golgi study in the mouse. **Trabajos del Instituto Cajal de Investigaciones Biológicas**, v. 54, p. 291-314, 1962.

VOCHTELLO, J.D.; KOOLHAAS, J.M. Medial amygadala lesions in male rats reduce aggressive behavior: interference with experience. **Physiology and Behavior**, v. 41, p. 99-102, 1987

VYAS, A.; BERNAL, S.; CHATTARJI, S.; Effects of chronic stress on dendritic arborization in the central and extended amygdala. **Brain Research,** v. 965, p. 290-294, 2003.

WAGNER, C. K.; MORREL, J. I. Distribution and steroid hormone regulation of aromatase mRNA expression in the forebrain of adult male and female rats: a cellular-level analysis using in situ hybridization. **The Journal of Comparative Neurology**, v. 370, p. 71-84, 1996.

WEARNE, S. L.; RODRIGUEZ, A.; EHLENBERGER, D. B.; ROCHE, A. B.; HENDERSON, S. C.; HOF, P. R. New techniques for imaging, digitization and analysis of three-dimensional neural morphology on multiple scales. **Neuroscience,** v. 136, p. 661-680, 2005.

WILSON, M. A.; MASCAGNI, F.; McDONALD, A. J. Sex differences in delta opioid receptor immunoreactivity in rat medial amygdala. **Neuroscience Letters**, v. 328, p.160-164, 2002.

WOOD, R. L.; NEWMAN, S. W. Hormonal influence on neurons of the mating behavior pathway in male hamster. In: **Neurobiological Effects of Sex Steroid Hormones.** MICIVYCH, P. E.; HAMMER Jr, R. P. New York: Cambridge, p. 3-39, 1995.

WOOLLEY, C. S.; McEWEN, B. S. Estradiol regulates hippocampal dendritic spine density via na N-methyl-D-aspartate receptor-dependent mechanism. **The Journal of Neuroscience**, v. 14, n. 12, p. 7680-7687, 1994.

WOOLLEY, C. S.; McEWEN, B. S. Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. **Journal of Comparative Neurology**, v. 336, n. 2, p. 293-306, 1993.

YU, H. The amygdaloid complex in the rat. **Masters Thesis**. University of Otawa, Otawa, 1969.

ZEHR, J. L.; TODD, B. J.; SCHULZ, K. M.; McCARTHY, M. M.; SISK, C. L. Dendritic pruning of the medial amygdala during pubertal development of the male Syrian hamster. **Journal of Neurobiology**, v. 66, p. 578-590, 2006.